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Selective determination of sultopride in human plasma using high-performance liquid chromatography with ultraviolet detection and particle beam mass spectrometry

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Abstract

We developed a sensitive and selective method for determining levels of sultopride, a neuroleptic drug of the substituted benzamide, in human plasma using high-performance liquid chromatography (HPLC) combined with UV detection and particle beam mass spectrometry (PBMS). Sultopride was extracted with *tert.*-butylmethyl ether using a salting-out technique. Tiapride served as an internal standard (I.S.). Sultopride and I.S. were separated by HPLC on a silica column with a mobile phase of acetonitrile–0.1 *M* ammonium acetate (94:6, v/v). The calibration curves were linear over the concentration range from 5 to 1000 ng/ml by HPLC with UV detection and from 10 to 1000 ng/ml with PBMS detection. The limit of quantitation was 5 ng/ml with UV detection and 10 ng/ml with PBMS detection. The absolute recovery was 92% and the within-day coefficients of variation were 2.9–7.1% at plasma concentrations from 50 to 500 ng/ml, determined by HPLC with UV detection. Using this method, we measured the plasma concentrations of sultopride with replicate analyses in four hospitalized patients and steady-state plasma levels were determined to be 161.6±30.8, 321.1±93.7, 726.5±143.1 and 1273.6±211.2 ng/ml, respectively.

Keywords: Sultopride

1. Introduction

Sultopride, N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-(ethylsulphonyl)-2-methoxybenzamide (Fig. 1), is an antagonist of cerebral dopamine receptors and has one chiral center at its pyrrolidine ring. This drug is clinically used as a racemate, but it was reported that in rats (−)-sultopride was neuroleptically more active than (+)-sultopride [1]. Sultopride, as well as tiapride (Fig. 1) and sulpiride, is structurally classified as a substituted benzamide and is prescribed as a neuroleptic. Substituted benzamides are relatively

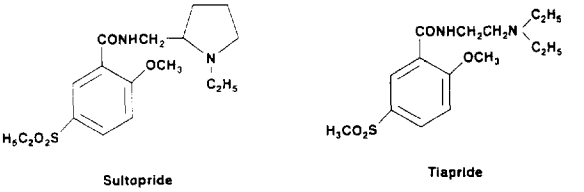

Fig. 1. Structures of sultopride and tiapride.

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safe in therapeutic doses, however, fatal poisoning can occur [2–4].

Several methods have been used to determine levels of sultopride in human biological fluids, including high-performance liquid chromatography (HPLC) [5–7], radioimmunoassay [8] and gas chromatography (GC) [9]. However, the specificity of the established methods is unsatisfactory, compared with determination using mass spectrometry (MS), in cases where there is mixing or contamination with other components.

We therefore deemed it important to design a more sensitive and specific method to determine levels of sultopride in human plasma; HPLC combined with UV and particle beam mass spectrometry (PBMS) proved valuable. Plasma concentrations of sultopride in hospitalized patients are described here.

2. Experimental

2.1. Reagents

Sultopride was provided by Dainippon (Osaka, Japan) and tiapride hydrochloride was from Fujisawa (Osaka, Japan). *tert.*-Butylmethyl ether and acetonitrile were of analytical-reagent grade and were purified by distillation. A Hypersil silica column (5 μm, 150×2.1 mm I.D.) was purchased from Hewlett-Packard (Palo Alto, CA, USA).

2.2. Biological samples

Plasma, used for the control samples and collected by venipuncture from healthy volunteers who had not ingested any drug, was kept at −20°C until analysis. All plasma samples were confirmed to contain no drugs.

We tested plasma samples obtained from four Japanese men who had been admitted to a psychiatric hospital and who were treated on a long-term basis with sultopride. Ages ranged from 29 to 65 years and body weights ranged from 56 to 70 kg. Daily doses of sultopride given to these patients were 150, 300, 600 and 1200 mg, respectively. Blood samples were taken by venipuncture in the morning, once a month over a six-month period and before ingestion of food or drug. At least 10 h had elapsed after administration of the last dose before blood was taken. All samples were centrifuged immediately at 2000 *g* for 3 min and stored at −20°C until analysis.

2.3. Standard solutions

Sultopride hydrochloride (11.0 mg) was dissolved in methanol and the volume was adjusted to 10 ml to give a concentration of 1000 ng/μl, as free sultopride. This solution was further diluted to the required concentrations. A standard solution of tiapride, used as an internal standard (I.S.), was prepared in the same manner.

2.4. Extraction procedure

A 1-ml volume of plasma was mixed with 4 ml of 0.5 *M* sodium hydroxide, 2 g of sodium chloride and 1 μl of I.S. solution (tiapride, 100 ng) in a 30-ml centrifuge tube. After adding 10 ml of *tert.*-butylmethyl ether, the preparation was shaken for 10 min and centrifuged at 850 *g* for 10 min (extraction). The solvent layer was put into a 30-ml centrifuge tube containing 2.5 ml of 0.1 *M* hydrochloric acid. The mixture was then shaken and centrifuged at 850 *g* for 10 min (back-extraction). The aqueous layer was transferred to a 10-ml centrifuge tube and made alkaline by adding 1 ml of 0.5 *M* sodium hydroxide and 1.5 g of sodium chloride. To the solution was added 2 ml of *tert.*-butylmethyl ether and the preparation was shaken for 10 min (re-extraction). After centrifugation, the solvent layer was evaporated to dryness under a stream of nitrogen. The residue was dissolved in 50 μl of acetonitrile and a 10-μl aliquot of the solution was injected onto a high-performance liquid chromatograph with UV and PBMS detectors.

2.5. HPLC conditions

HPLC was performed on a Hewlett-Packard 1090M HPLC system. The column was a Hypersil silica column (5 μm particle size, 150×2.1 mm I.D.; Hewlett-Packard). The mobile phase was acetonitrile–0.1 *M* ammonium acetate (94:6, v/v) and the flow-rate was 0.4 ml/min. The column temperature was maintained at 40°C. This high-performance liquid chromatograph was combined with both an UV detector and a PBMS detector, in series.

2.6. Conditions of UV detection

The Hewlett-Packard 1090M HPLC system includes a photodiode-array detector. To detect sultopride, the wavelength of the photodiode-array detector was set at 240 nm.

2.7. Conditions of PBMS detection

The HPLC system was connected to a HP 59980A particle beam interface (Hewlett-Packard). The HPLC eluent was converted to an aerosol in a nebulizer with a helium pressure of 35 p.s.i. and was sprayed into a desolvation chamber that was maintained at a low pressure and at 60°C. The beam of solvent-free particles was introduced into the ion source of a Hewlett-Packard 5989A MS engine. The MS system was operated in the negative ion chemical ionization (NICI) mode. The reagent gas for NICI was methane, with a source pressure of 1 torr. The temperature of the ion source was maintained at 250°C. The mass spectrometer was operated either in the full scan mode for qualification or in the selected-ion monitoring (SIM) mode for quantitation. The ions m/z 339 (for sultopride) and m/z 313 (for the I.S.) were selected for quantitation.

2.8. Preparation of the calibration curve

Plasma samples were prepared by spiking sultopride to control plasma at concentrations of 5–1000 ng/ml for HPLC with UV detection and 10–1000 ng/ml for PBMS detection, each containing 100 ng/ml of I.S. These samples were extracted in the same manner as described in Section 2.4. Calibration curves were obtained by plotting the peak-area ratio of sultopride to I.S. versus the amount of sultopride.

3. Results and discussion

3.1. Extraction procedure

To efficiently extract the drug and I.S., the presence of sodium chloride during the extraction as well as the re-extraction step was required. Compared with extracts not subjected to the salting-out technique, recovery of sultopride and I.S. increased 3.5-fold and 5.3-fold, respectively. A single extraction is a simple technique and usually yields a higher recovery of drugs. However, we used the three-step solvent extraction procedure, as the obtained extract gave fewer interfering peaks on the chromatogram. In forensic practice, plasma is not always available, and whole blood and solid tissues are the samples analyzed. When we applied our extraction procedure to whole blood, liver and skeletal muscle samples, no endogenous interfering peaks appeared on the chromatograms. Therefore, our extraction procedure can be used for the analysis of various tissues in regular forensic studies.

3.2. HPLC conditions

To obtain high sensitivity for PBMS detection, the following HPLC conditions were required; a 2.1-mm I.D. column, a flow-rate of 0.4 ml/min, volatile buffers and a high proportion of organic solvent in the mobile phase.

A method that makes use of the reversed-phase column [5] was first applied. However, peak shape and separation of sultopride and I.S. were unsatisfactory. Law et al. [10,11] analyzed 84 basic drugs and 69 mono-functional aryl–alkyl amines on a silica column with an aqueous methanol eluent. They stated that the addition of ammonium salt to the eluent would be effective in attaining separation and peak shape of the drugs being investigated. Using their method, we found that the separation of drugs with good peak shape was feasible using a silica column with an aqueous acetonitrile eluent containing ammonium acetate. As the higher concentration of ammonium acetate in the eluent reduced the retention times of sultopride and I.S., 0.1 *M* ammonium acetate was used. Addition of ammonium acetate, a volatile buffer, was also suitable for PBMS detection. The established HPLC conditions were considered to be useful for sensitive determination of sultopride, with both UV and PBMS detection.

3.3. Determination of sultopride by HPLC with UV detection (HPLC–UV)

The HPLC–UV chromatograms of the extracts from blank plasma and spiked plasma containing 100 ng/ml each of sultopride and I.S. are shown in Fig.

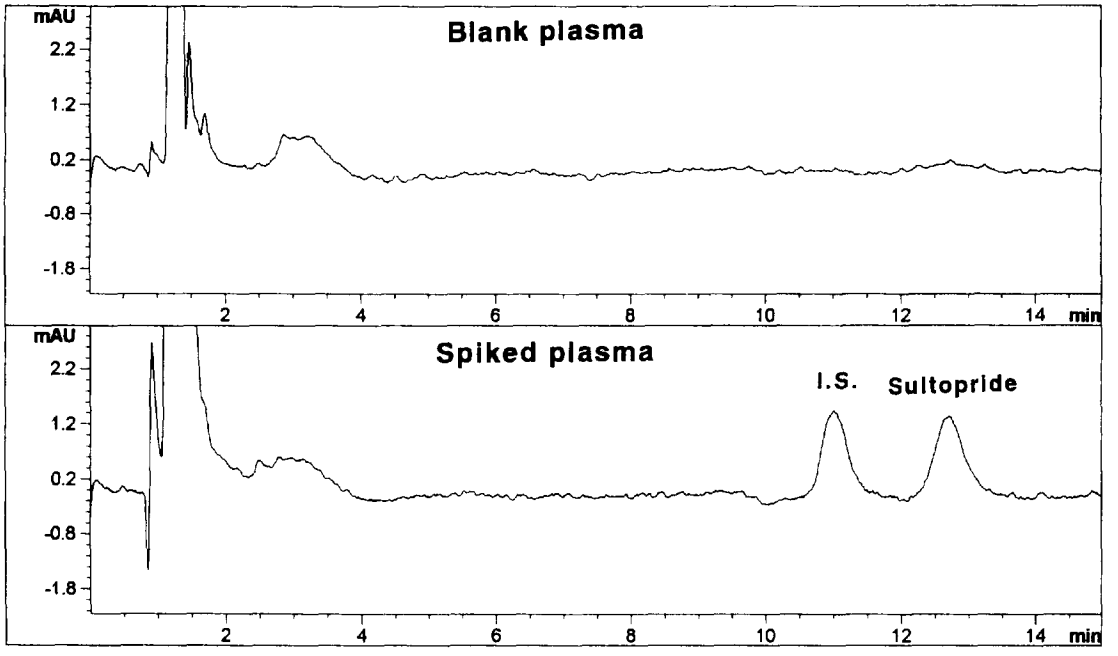


Fig. 2. HPLC–UV chromatograms of extracts from blank plasma and spiked plasma containing 100 ng/ml each of sultopride and I.S.. The extraction procedure and chromatographic conditions are detailed in Section 2.

2. Each peak was clearly separated on the chromatogram and no interfering peaks were observed for blank human plasma. The calibration curves were linear in the concentration range 5–1000 ng/ml, with correlation coefficients of 0.999. The limit of quantitation was 5 ng/ml and the absolute recovery of sultopride was 92% for 100 ng/ml plasma samples. The within- and between-day reproducibility of this method was checked at three plasma concentrations, 50, 100 and 500 ng/ml of sultopride. The within- and between-day coefficients of variation for sultopride were 2.9–7.1% and 2.6–4.8%, respectively. The results are given in Table 1.

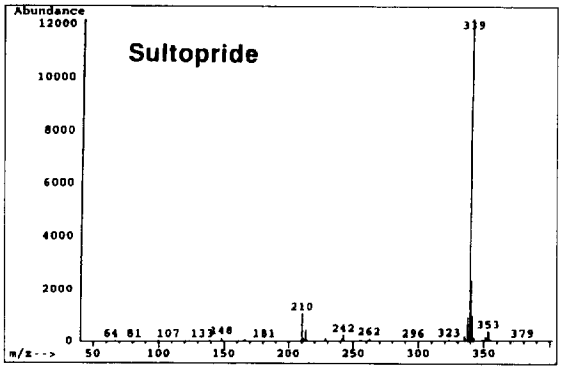
3.4. Determination of sultopride by HPLC with PBMS detection (HPLC–PBMS)

The most favorable feature of HPLC–PBMS is that both electron impact ionization (EI) and chemical ionization (CI) mass spectra can be obtained. In the EI mode, the base peak at m/z 98 for sultopride and at m/z 86 for tiapride were detected. On the mass chromatograms of the extracts from biological samples at these ions, there were many interfering peaks. In the positive ion CI (PICI) mode, quasi-molecular ions at m/z 355 ($[M+H]^+$) for sultopride and m/z 329 ($[M+H]^+$) for I.S. were observed. The mass spectra of sultopride and I.S. obtained by HPLC–PBMS with NICI mode are shown in Fig. 3. Sultopride and I.S. showed ions at m/z 339 ($[M-CH_3]^-$) and at m/z 313 ($[M-CH_3]^-$), respectively. Among three ionization modes, the NICI mode showed the highest sensitivity, without endogenous interference, therefore, the quantitation was done using the NICI mode.

Table 1
Precision and accuracy of sultopride data determined by HPLC–UV

Added concentration (ng/ml)	Within-day ($n=5$)		Between-day ($n=5$)	
	Found concentration (mean±S.D.) (ng/ml)	C.V. (%)	Found concentration (mean±S.D.) (ng/ml)	C.V. (%)
50	51.0±1.5	2.9	50.3±1.3	2.6
100	104.8±7.5	7.1	102.1±4.9	4.8
500	509.1±23.8	4.7	509.9±17.6	3.5

S.D.=standard deviation; C.V.=coefficient of variation.



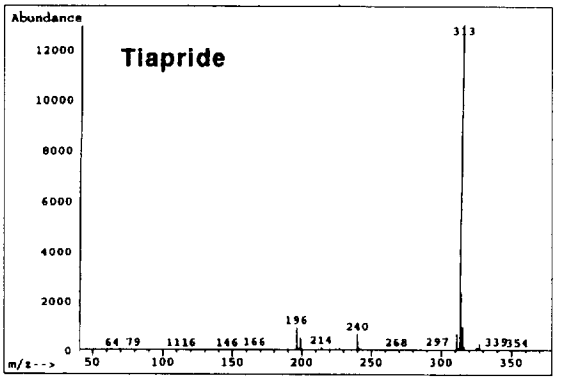


Fig. 3. NICI mass spectra of sultopride and I.S. (tiapride) using HPLC–PBMS. Chromatographic conditions are detailed in Section 2.

The SIM chromatograms of extracts from the blank plasma and the plasma spiked with 100 ng each of sultopride and I.S. are shown in Fig. 4. The peaks of sultopride and I.S. were clearly separated on chromatograms at m/z 339 and m/z 313, and no interfering peaks appeared on the chromatogram of blank plasma. The calibration curve was linear in the concentration range 10–1000 ng/ml, with a correlation coefficient of 0.999. The limit of quantitation was 10 ng/ml. The within-day precision of this method for 100 ng/ml plasma samples was examined. The determined sultopride concentration was 99.06±7.74 ng/ml (mean±S.D.), with a coefficient of variation (n=5) of 7.8%.

This is apparently the first report of the analysis of sultopride in human plasma, using MS. Several GC–MS methods have been used to determine substituted benzamides and each required a derivatization step [12,13]. The use of HPLC–PBMS means that derivatization is not required. Therefore, this system can be used to analyze a wide range of substituted benzamides. HPLC combined with UV and PBMS detection can be separated into two techniques, HPLC–UV and HPLC–PBMS. Thus, each can be efficiently used, depending on the purpose. HPLC–UV is useful for routine analysis of sultopride, while HPLC–PBMS is convenient for qualitative and quantitative analysis of sultopride, for both clinical and forensic purposes.

4. Practical application

Our newly developed method was used for qualitative and quantitative determinations of sultopride in plasma obtained from psychotic, hospitalized patients.

As shown in Fig. 5, a sharp and symmetrical peak was obtained with a retention time of 12.9 min with UV detection on the chromatogram of extract from plasma of a hospitalized patient who had been treated with 600 mg of sultopride per day. This peak proved to be sultopride by NICI mass spectra obtained by PBMS detection, as described above. Fig. 6 shows the concentrations of sultopride determined by HPLC–UV in plasma collected from patients who had been administered 150, 300, 600 and 1200 mg of sultopride, respectively, on a daily basis over a six-month period. Each concentration was measured by replicate analysis. The concentration in each patient was fairly stable and steady-state plasma levels of sultopride were determined to be 161.6±30.8, 321.1±93.7, 726.5±143.1 and 1273.6±211.2 ng/ml, respectively.

5. Conclusion

A sensitive and selective method for the determination of sultopride in human plasma was developed using HPLC with UV and PBMS detection. This system can be used to monitor levels of drugs ingested by patients and for toxicological analysis in forensic cases.

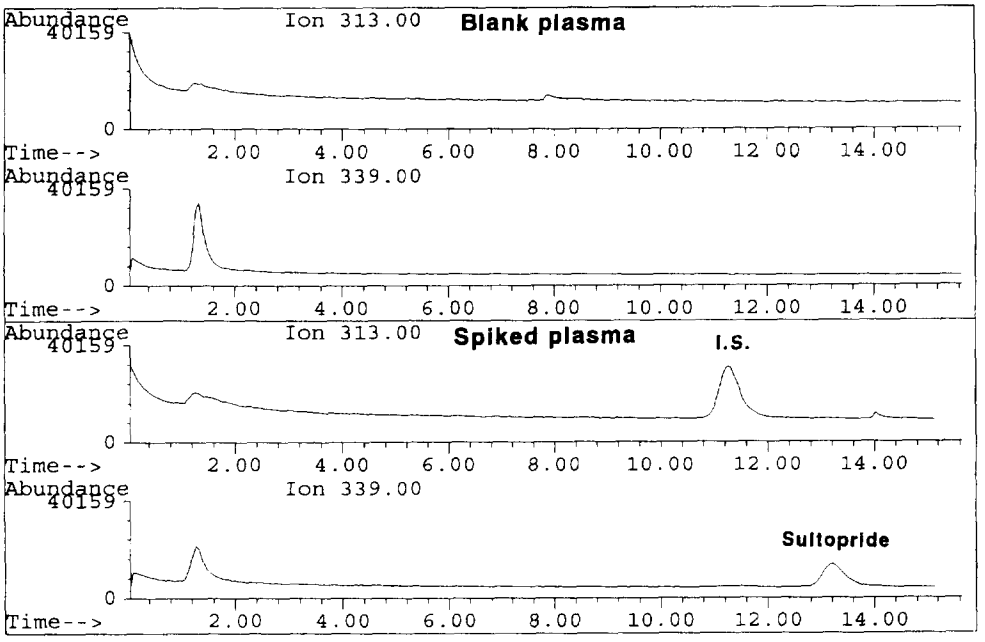


Fig. 4. SIM chromatograms of extracts from blank plasma and spiked plasma containing 100 ng/ml each of sultopride and I.S. The extraction procedure and chromatographic conditions are detailed in Section 2.

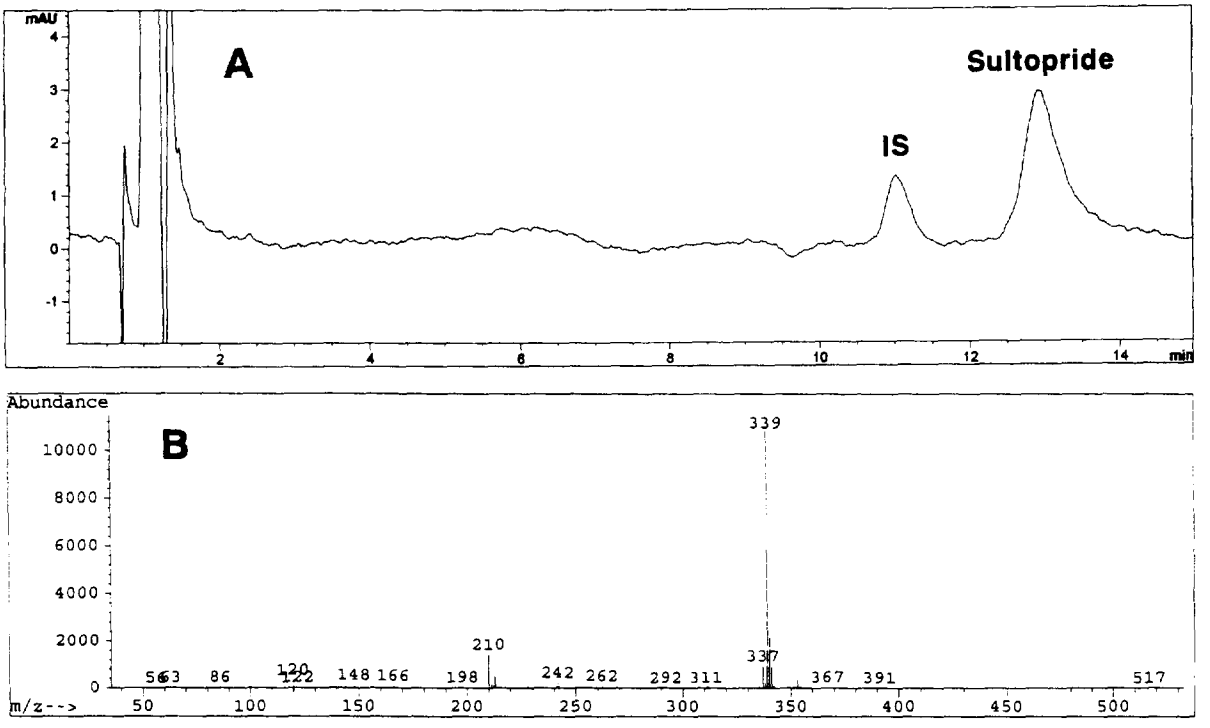


Fig. 5. HPLC–UV chromatogram and NICI mass spectra of an extract from plasma obtained from a hospitalized patient who had been prescribed 600 mg of sultopride orally, on a daily basis.

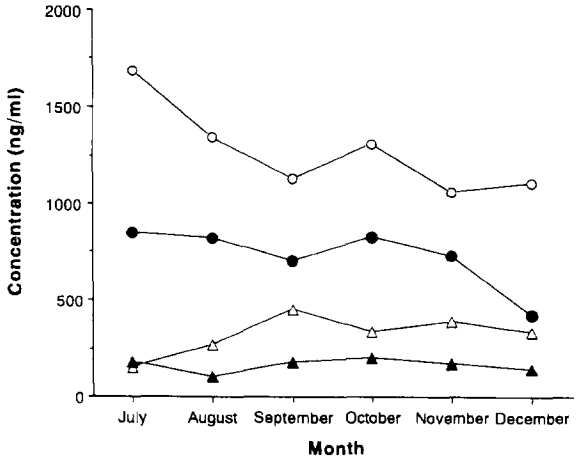


Fig. 6. Concentration–time curves of sultopride in plasma of the four hospitalized patients who had been treated with 150 (▲), 300 (△), 600 (●) and 1200 mg (○) per day of sultopride, respectively. Detailed background on the patients is given in Section 2.

Acknowledgments

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