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QUANTITATIVE ANALYSIS OF SULPIRIDE IN BODY FLUIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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SUMMARY

A high-performance liquid chromatographic method for the analysis of sulpiride, N-ethyl-2-(2-methoxy-5-sulphonamido-benzamido-methyl)-pyrrolidine, in body fluids is described. A structurally related compound, N-ethyl-2-(2,4-dimethoxy-benzamido-methyl)-pyrrolidine, was used as internal standard.

A fluorescence detector with excitation maximum at 299 nm and emission maximum at 342 nm was used for the quantitation. The detection limit was about 10 ng/ml in serum and cerebrospinal fluid and about 200 ng/ml in urine. The experimental error was 5—10% in the concentration range 25—100 ng/ml. Some preliminary data from a pharmacokinetic study in healthy volunteers are presented. The half-life for sulpiride in serum was about 8 h. Sulpiride was also measured in cerebrospinal fluid from five drug-treated psychotic patients.

INTRODUCTION

Sulpiride, N-ethyl-2-(2-methoxy-5-sulphonamido-benzamido-methyl)-pyrrolidine (Fig. 1), is a neuroleptic drug with a structure that is different from other antipsychotic agents [1]. Like neuroleptics, phenothiazines and butyrophenones it markedly accelerates dopamine synthesis and metabolism in the brain but it has only a weak effect on dopamine stimulated adenylate cyclase. The frequency of extrapyramidal side effects is reported to be low compared to other neuroleptics [2, 3]. This makes sulpiride a theoretically and clinically interesting drug. The pharmacokinetics of sulpiride in man has not previously been systematically studied.

All the available analytical methods for sulpiride have some disadvantages for pharmacokinetic studies. A very specific mass fragmentographic method has

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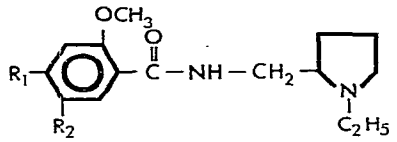
been developed [4], but it is not sensitive enough. A spectrophotofluorometric method with high sensitivity has been described [5], but interference from other fluorescent compounds with similar extraction qualities cannot be excluded. A high-performance liquid chromatography (HPLC) separation method has also been developed [6], but this technique has not been used for the quantitative analysis of sulpiride.

To make pharmacokinetic studies of sulpiride possible, we have developed a sensitive and specific analytical method for sulpiride in body fluids using HPLC with fluorescence detection. To compensate for erratical loss of the compound during the extraction procedure an internal standard with a chemical structure and fluorescence qualities very similar to those of sulpiride was used (Fig. 1).



R_1 = H, R_2 = SO_2NH_2 — Sulpiride

R_1 = OCH_3, R_2 = H — Internal standard

Fig. 1. Chemical structures of sulpiride and the internal standard.

EXPERIMENTAL

Materials

Acetonitrile for liquid chromatography was HPLC grade obtained from Rathburn Chemicals (Walkerburn, Great Britain). Other solvents and chemicals were of p.a. quality. Sulpiride was a gift from Delagrange (Paris, France). The internal standard, N-ethyl-2-(2,4-dimethoxy-benzamido-methyl)-pyrrolidine (Fig. 1), was a gift from Astra (Södertälje, Sweden).

HPLC was performed with a Spectra Physics System 3500B consisting of two solvent delivery pumps, a solvent programmer and a rotary valve injector with a 10-μl loop for syringe injection.

The fluorescence detector was a SFM 22 Model from Kontron (Stockholm, Sweden), equipped with a 10-μl flow-through cell. The recorder was a W+W 600 Tarkan.

A 15 cm × 4 mm reversed-phase column packed with 5 μm C_{18} Nucleosil (Macherey, Nagel & Co., Düren, G.F.R.) was used. The packing of the column was performed principally according to Bristow et al. [7].

Sample preparation

Serum and cerebrospinal fluid samples. The extraction was performed partly according to Kleimola et al. [5]. The internal standard (1 μg in 100 μl methanol) was added to 4.0 ml serum or cerebrospinal fluid (CSF) (or when high

levels were expected 2.0 ml serum) followed by 240 μl or 120 μl 0.5 *N* sodium hydroxide. The sample was mixed and 10.0 ml of chloroform was added. The test-tube was gently turned 10 times manually and centrifuged at 2000 *g* for about 5 min. A 9.0-ml aliquot of the chloroform layer was transferred to another test-tube. The water phase was extracted once more with 5.0 ml chloroform, 4.0 ml of which was combined with the first chloroform phase. If the test-tube with chloroform was contaminated by the water layer, the chloroform was transferred to a clean test-tube. The chloroform was evaporated nearly to dryness in a stream of nitrogen. During the evaporation the test-tube was heated with a hair drier. The residue in the tube was dissolved in about 1 ml methanol and transferred to a small conical tube. The methanol was evaporated to dryness and the residue was dissolved in 50 μl methanol and stored at −20° pending analysis.

Urine samples. To 1.0 ml urine about 1 g sodium chloride was added. The internal standard (5.0 μg in 50 μl methanol) was added and the pH was adjusted to 9.5—10.0 with 0.5 *M* sodium hydroxide (about 100 μl). The sample was extracted twice with 5.0 ml chloroform in the same manner as with the serum. The residue was dissolved in 100 μl methanol.

Preparation of standard curves

Standard curves were prepared by adding internal standard and known amounts of sulpiride in methanol to pool samples of the body fluid to be analysed. Several dilutions of sulpiride were made to keep the volume of methanol added to each sample small, between 25 and 100 μl. The range of the standard concentrations of sulpiride in serum were 10 ng/ml to 2500 ng/ml and for the corresponding urine samples 0.5 μg/ml to 100 μg/ml.

Liquid chromatography

The solvent in pump A consisted of water—acetic acid (99:1) and in pump B of acetonitrile—acetic acid—water (50:1:49). A linear program from 12–60% B was run in 10 min. The flow-rate was adjusted so that the pressure over the column should not exceed 400 p.s.i. (1.2—1.6 ml/min). Ten microliters of the sample were injected and the program was started. When the internal standard had been eluted (10—16 min) the composition of the solvent was changed to 12% B in 1 min. After 5 min equilibration, the next sample could be injected. The detector settings were: excitation at 299 nm and emission at 342 nm.

Sampling of serum

Serum was collected by venipuncture from three healthy male volunteers who had received 100 mg sulpiride by intravenous (i.v.) injection and 4—6 weeks later an oral (p.o.) dose. The compound was given at 9 a.m. with the subjects fasting. The time schedule for sampling is indicated in Fig. 2.

Sampling of cerebrospinal fluid

CSF was collected from psychotic patients by lumbar puncture. Samples were taken before the morning dose after the patients had been treated for 4 weeks with sulpiride, 800 mg/day. All samples were frozen to −20° within an hour.

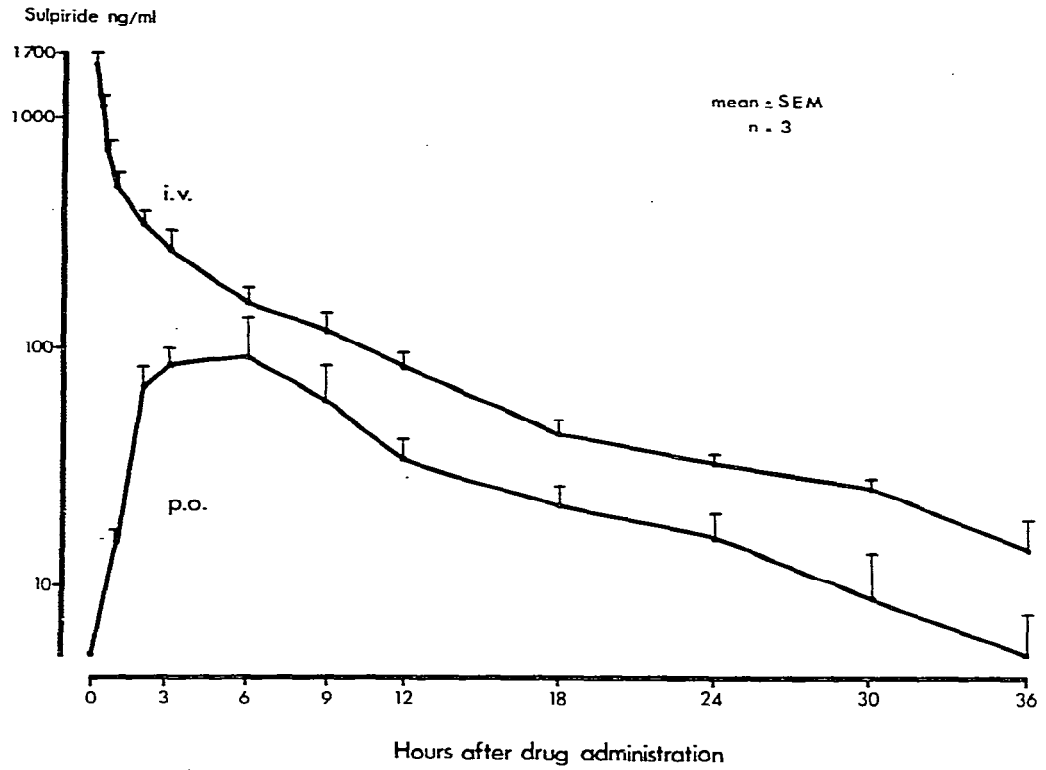


Fig. 2. Drug concentrations in serum from 3 male volunteers after i.v. and p.o. administration of 100 mg of sulpiride.

The experiments were approved by the Ethics Committee of the Karolinska Institute, Stockholm, Sweden.

RESULTS AND DISCUSSION

The recovery of sulpiride added to human serum was almost quantitative (Table I). The chromatographic procedure is very reproducible as the sampling valve delivers exactly 10 μl at each injection and the compound did not seem to be destroyed in the column. These circumstances make it possible to measure sulpiride also without the use of an internal standard.

TABLE I

RECOVERY OF SULPIRIDE AFTER EXTRACTION FROM SERUM

Amount of sulpiride added to 4 ml serum (ng)	Peak height (mm)	Sulpiride* peak height without extraction (mm)	Recovery (%)
200	13	13	100
400	32	31	103
1000	67	79	85
		mean $\bar{X}$	96

*Sulpiride dissolved in methanol to a concentration corresponding to 100% recovery.

The advantage of using an internal standard is that accidental loss of the drug during the extraction and vaporization of the final methanol solutions during storage would then be compensated for. The disadvantage of using the standard is noticeable in the chromatographic part of the analytical procedure. An ideal internal standard should have about the same elution qualities as sulpiride and also have its fluorescence maximum at about the same wavelength. The methoxy analogue used fulfills the fluorescence criterium having an excitation maximum of 293 nm and an emission maximum of 338 nm, values from the uncalibrated Kontron detector (sulpiride: excitation max. 300 nm, emission max. 342 nm). However, it is not as polar as sulpiride, which makes the retention time longer and consequently fewer samples can be run during a day. The experimental error, with and without the standard, was of about the same magnitude (5—10% for 25—100 mg sulpiride added to serum).

The standard curves were linear from the limit of detection (ca. 10 ng/ml) to at least 2 μg/ml (Fig. 3). The correlation coefficients were greater than 0.99. From several experiments we have noticed a tendency to somewhat higher correlation coefficient with the use of the internal standard.

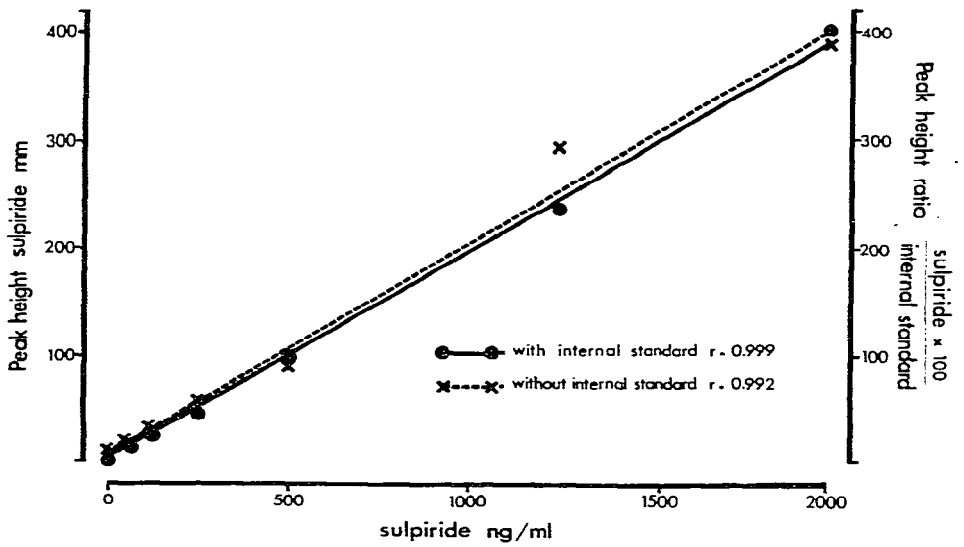


Fig. 3. Standard curves for the analysis of sulpiride in serum with and without the use of internal standard.

Fig. 4 demonstrates a typical chromatogram from the analysis of sulpiride in serum from a subject given 100 mg of sulpiride orally. A small unknown peak is usually seen after the sulpiride peak. In some serum samples there are also peaks just before sulpiride. This is not a problem when analysing samples from sulpiride-treated patients as their serum levels are usually high. However, when the concentration is low, as in the late phase of excretion, after single-dose administration, gradient elution, starting with a low percentage acetonitrile is necessary to get a good separation of the peaks.

The detection limit for sulpiride in CSF was the same as in serum, about 10 ng/ml in a 4-ml sample. In the CSF samples no interfering peaks were observed.

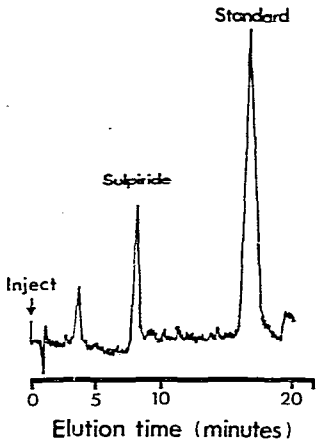


Fig. 4. Chromatogram of a serum sample from a volunteer. The sample was taken 2 h after p.o. administration of sulpiride, 100 mg. The drug concentration in serum was 51 ng/ml.

The chromatograms of urine samples showed several large unknown peaks. The detection limit for sulpiride in urine was about 200 ng/ml due to such interference. It is possible that an improved extraction procedure could lower this limit. However, we have not found it necessary to develop such a procedure as the amounts of sulpiride in urine from drug-treated subjects are very high. The addition of sodium chloride to the urine samples before the extraction is important, as the recovery otherwise could be very variable.

The precision of the method was high as demonstrated from duplicate analyses of 2-ml serum samples from a healthy volunteer after an i.v. injection of 100 mg sulpiride (Table II). The mean percent deviation was 3.3 ± 3.3%.

A pharmacokinetic study of sulpiride in healthy volunteers has recently been initiated. Some preliminary results are shown in Fig. 2. The interindividual variations in serum sulpiride levels were greater after p.o. than after i.v. administration probably due to differences in the absorption and first pass effect. The concentration in serum reached a maximum between 3 to 6 h after

TABLE II

CONCENTRATIONS OF SULPIRIDE IN SERUM FROM A HEALTHY VOLUNTEER AFTER AN I.V. INJECTION OF 100 mg SULPIRIDE

Time from injection		Sulpiride duplicate analysis		Deviation from mean (%)
min	h	ng/ml	ng/ml	
10		2662	2744	1.5
20		1332	1444	4.0
30		1178	966	9.5
	1	713	703	0.7
	2	548	546	0.2
	6	271	294	4.2
	9	159	141	6.0
	12	90	90	0

the intake of tablets. The serum levels found after p.o. administration were in the same range as those found by Kleimola et al. [5]. The half-life of sulpiride in plasma after i.v. administration was about 8 h, which is also in agreement with earlier findings [6].

Drug levels in CSF from a few schizophrenic patients treated with sulpiride have also been analysed (Table III). It is clear that sulpiride passes into the central nervous system and that quite high drug levels are present in the CSF during drug treatment.

The present method will be used in pharmacokinetic and clinical studies on sulpiride in healthy volunteers and schizophrenic patients. The correlation between serum and CSF levels of the drug and the relationships to biochemical and clinical variables will also be investigated.

TABLE III

DRUG LEVELS IN CSF FROM 5 FEMALE SCHIZOPHRENIC PATIENTS TREATED WITH SULPIRIDE (800 mg/DAY) FOR 4 WEEKS

Samples were taken at 8 a.m. before the first daily dose of sulpiride.

Patient No.	Sulpiride in CSF (ng/ml)
1	83
2	88
3	57
4	48
5	50

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