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IMPROVED DETERMINATION OF SULPIRIDE IN PLASMA BY ION-PAIR LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

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SUMMARY

A specific and sensitive high-performance liquid chromatographic method for the measurement of sulpiride in plasma is described. The internal standard used was veralipride, a structurally related substituted benzamide. A fluorescence detector with maximum excitation at 300 nm and maximum emission at 365 nm was used for quantitation. After an alkaline extraction procedure, the benzamides were separated on a 5-μm ODS column using a large organic counter ion in the mobile phase. The detector response was linear from 10 to 1000 ng/ml and the detection limit was 10 ng/ml, which is sensitive enough for pharmacokinetic studies. The suitability of the method for the analysis of biological samples was tested by studying the variation with time of plasma concentrations of sulpiride in normal human volunteers after a single therapeutic 200-mg oral dose of three different formulations of sulpiride.

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INTRODUCTION

Sulpiride, N-(1-ethylpyrrolidin-2-ylmethyl)-2-methoxy-5-sulphamoylbenzamide, belongs to a pharmacological series with potent antipsychotic properties. Its peculiar affinity for the D2 and D4 brain dopamine receptors [1] makes sulpiride a clinically interesting drug, with a low frequency of extrapyramidal side-effects. Although it is very common, some pharmacokinetic data are still being investigated, especially its bioavailability. For clinical design, a quantitation method that is sensitive and reliable is required.

As sulpiride is not a recent drug, many methods for its determination have been published. However, the colorimetric, spectrophotometric [2] and spectrofluorimetric techniques [3] that have been described are not sensitive and specific enough for clinical studies. A gas chromatographic method has also been proposed [4], but unless it is associated with mass spectrometric detection, it has similar disadvantages. A radioimmunoassay with good sensitivity has been developed [5], but this technique is not feasible in common medical practice. Few high-performance liquid chromatographic (HPLC) studies have been reported. Two of them gave good results in the separation of substituted benzamides, but did not work with biological fluids [6, 7]. Some others, used in clinical assays, present the major drawbacks of being time-consuming and using large amount of plasma [8—10]. We present here an original HPLC method involving ion-pair partition under reversed-phase conditions.

EXPERIMENTAL

Chemicals

Diethylamine, sodium hydroxide (8 mol/l), chloroform (all analytical-reagent grade) and ethyl acetate (spectroscopy grade) were obtained from E. Merck (Darmstadt, F.R.G.). Phosphoric acid (Normapur), glacial acetic acid (analytical-reagent grade) and methanol (HPLC grade) were provided by Prolabo (Paris, France). Heptanesulphonic acid (sodium salt) from Kodak was supplied by Touzart et Matignon (Vitry, France). The aqueous reagents were all prepared using distilled water.

Sulpiride was supplied by Milanese Pharmaceutico (Milan, Italy). Veralipride, N-(1-allylpyrrolidin-2-ylmethyl)-2,3-dimethoxy-5-sulphamoylbenzamide, used as an internal standard, was extracted from the French commercial form Agreal® (Delagrange, Paris, France). Structures of the benzamides are given in Table I.

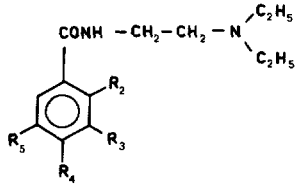
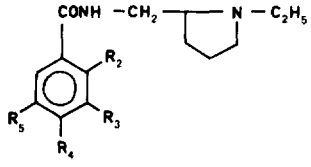
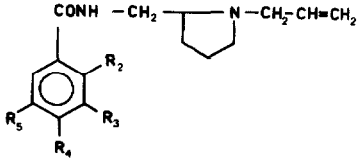
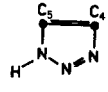
Standard solutions

A stock solution of sulpiride (1 mg/ml) was prepared by dissolving 20 mg of sulpiride in 20 ml of distilled water containing 50 μl of glacial acetic acid.

The contents of one Agreal capsule (containing 100 mg of veralipride) were mixed vigorously with 50 ml of distilled water and 2 ml glacial acetic acid. After centrifugation for 10 min at 1000 *g*, the supernatant was filtered and distilled water was added to give a final (and theoretical) concentration of 1 mg/ml.

TABLE I

BENZAMIDE STRUCTURES

General structure	Substituents				Compound name
	R_2	R_3	R_4	R_5	
	OCH_3	H	H	SO_2CH_3	Tiapride
	OCH_3	H	NH_2	Cl	Metoclopramide
	OCH_3	H	H	SO_2NH_2	Sulpiride
	OCH_3	H	H	$SO_2C_2H_5$	Sultopride
	OCH_3	OCH_3	H	SO_2NH_2	Veralipride
	OCH_3	H			Alizapride

When stored at 4°C the stock solution of sulpiride remained stable for more than one month, whereas that of veralipride became contaminated within two weeks. Daily working standard solutions (1 μg/ml) were prepared by dilution of the stock solutions with distilled water.

HPLC equipment and operating conditions

The HPLC system consisted of a Chromatem 380 pump equipped with a pulse damper (Touzart et Matignon) coupled to a Shimadzu RF 530 fluorescence detector, which was itself linked to a recorder (Kipp and Zonen BD 40), both supplied by Touzart et Matignon. The samples were injected with a Rheodyne 7125 50-μl loop injector (Rheodyne, Berkeley, CA, U.S.A.). Separations were carried out on a 5-μm Spherisorb ODS column (150 × 4.6 mm I.D.) (SFCC, Gagny, France).

The mobile phase was prepared by diluting 700 ml of sodium heptanesulphonate solution (2.5 m*M*) with 300 ml of methanol and 1 ml of diethylamine. This solution was adjusted to an apparent pH of 3.5 with orthophosphoric acid, then filtered through a Whatman No. 2 filter and degassed ultrasonically (B12 Bioblock apparatus, Bransonic, Strasbourg, France) before use.

The flow-rate was 0.8 ml/min, while the column was kept at room temperature. The detector was set at 300 nm for excitation and 365 nm for emission, the respective excitation and emission maxima of the drugs in the mobile phase being 291—350 nm for sulpiride and 295—380 nm for veralipride.

Extraction

Plasma (1 ml) was spiked with 0.2 ml of internal standard solution (1 μg/ml) and with 0.2 ml of sodium hydroxide solution (0.5 mol/l). After gentle mixing, two 10-min extractions with 6 ml of chloroform—ethyl acetate (3:1) were carried out using a laboratory shaker (Toulemonde, Paris, France). The lower organic layers obtained after centrifugation at 1000 *g* were combined and evaporated under a stream of air until completely dry, while the glass tubes were kept in a thermostated bath (50°C).

The residue was dissolved in 150 μl of mobile phase, homogenized on a vortex mixer for 10 s and 50 μl were injected into the chromatograph.

Calibration

A calibration graph was obtained by adding known amounts of working sulpiride solution to human blank plasma in the range 25—1000 ng/ml. Extraction was carried out as described above. Peak-height ratios (sulpiride/veralipride) were used to generate the least-squares regression line.

RESULTS

Chromatographic separation

Fig. 1 shows chromatograms obtained with 1 ml of blank plasma and 1 ml of standard plasma (100 and 500 ng/ml) and Fig. 2 those obtained with 1 ml of

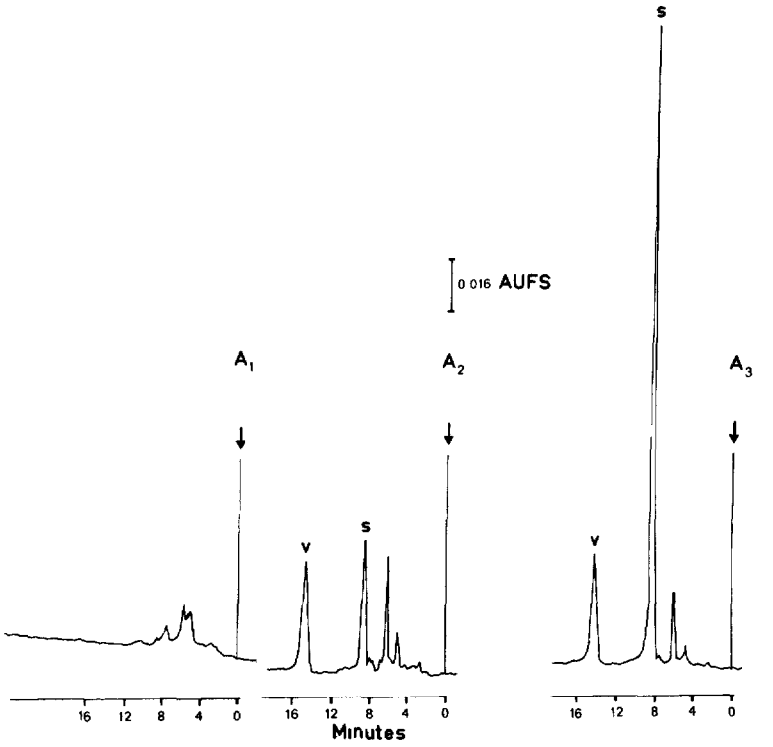


Fig. 1. Chromatograms of extracts from blank plasma (A_1), standard plasma containing 100 ng/ml sulpiride with 200 ng of veralipride (A_2) and standard plasma containing 500 ng/ml sulpiride with 200 ng of veralipride (A_3). Peaks: S = sulpiride; V = veralipride.

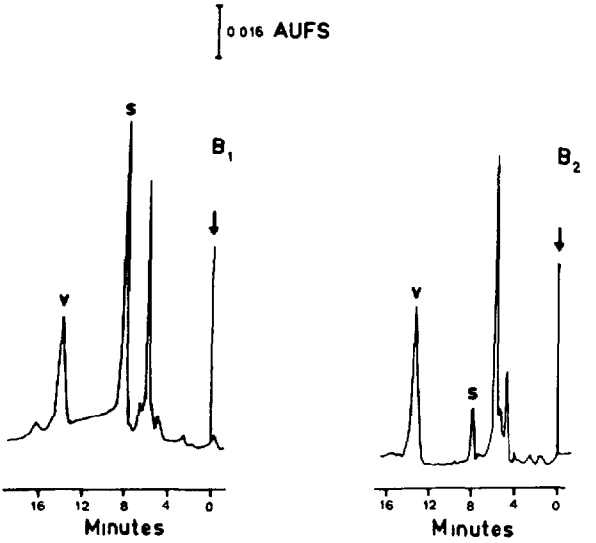


Fig. 2. Chromatograms of extracts from volunteer plasma, 2.5 h (B_1, 240 ng/ml sulpiride with 200 ng of veralipride) and 36 h (B_2, 34 ng/ml sulpiride with 200 ng of veralipride) after a 200-mg sulpiride oral dose. Peaks: S = sulpiride; V = veralipride.

a volunteer's plasma (2.5 and 36 h after a single 200-mg oral dose). The different substances are well separated without any interferences. The retention time of sulpiride and veralipride are 8.0 and 14.0 min, respectively (capacity factor $k' = 4.33$ and 8.33, respectively).

Linearity and sensitivity

The detector response is linear over the range 10—1000 ng/ml. The mean equation of the calibration graph is $y = 0.0109x + 0.03$ ($r = 0.999$, $n = 15$), where y is the peak-height ratio (sulpiride/veralipride) and x is the plasma sulpiride concentration.

The coefficient of variation (C.V. = S.D./mean · 100) of the slope is 6.9%. Under the experimental conditions used, 10 ng/ml represents a detection limit (baseline noise level × 2) that can easily be achieved.

Reproducibility

The within-day reproducibility of the method was checked for three plasma concentrations (50, 200 and 500 ng/ml), ten measurements being made at each concentration. The coefficients of variation are 6.46, 4.31 and 4.09%, respectively ($n = 10$).

An inter-day reproducibility was also calculated using a blank human plasma spiked with sulpiride at a theoretical concentration of 150 ng/ml. The concentration found is 153.9 ± 8.0 ng/ml (mean ± S.D., $n = 7$) with a coefficient of variation of 5.24%.

Selectivity

Blank plasma samples from different humans were tested for the absence of interfering endogenous components. The use of an internal standard assumes

TABLE II

COMPARATIVE ELUTION OF BENZAMIDES AND BENZODIAZEPINES WITH ION-PAIR PARTITION

Drug	Retention time* (min)	Capacity factor, k'
Acetazolamide	3.5	1.33
Sulpiride	8.0	4.33
Veralipride	14.0	8.33
Sultopride	21.0	13.0
Metoclopramide	>120	>79
Alizapride	>120	>79
Tiapride	>120	>79
Bromazepam	39.0	25.0
Nitrazepam	66.0	43.0
Flunitrazepam	85.0	55.66
Diazepam	>120	>79
Nordiazepam	>120	>79
Clonazepam	>120	>79
Oxazepam	>120	>79

*UV detection at 242 nm.

the same extraction and elution qualities as sulpiride and, in the same way, close UV or fluorescence maxima. Usually this aim can be achieved by using a structurally related compound.

Two families of drugs, benzamides and benzodiazepines, were investigated using a Varian 2050 UV detector (Varian, Les Ulis, France) set at 242 nm, the mutual absorption wavelength for these substances. Table II shows that this method is highly selective, especially because fluorescence detection is actually used.

Although sulpiride metabolites have been found in the rat, dog [11] and monkey [12], there is no evidence of their presence in man [8, 13, 14]. However, in our clinical study, it has been noticed with some subjects that the presence of an unknown peak becomes detectable around 5 h after a single 200-mg oral dose of sulpiride. This peak is eluted just after the internal standard. Its kinetics indicate a metabolite but obviously further investigations are required before any conclusion can be drawn.

DISCUSSION

The improvements in this assay concern the extraction procedure and the mobile phase composition. As the pK_a of sulpiride is 8.9 (data given by Delagrange, Paris, France), a quantitative extraction of molar form must be carried out at a pH > 10.9, which nearly precludes the use of buffer solutions because of their too high molarity if added in a small volume. This appears to contradict the procedure proposed by Nishihara et al. [7] and Bressolle and Bres [10]. Hence, by diluting 0.2 ml of sodium hydroxide solution (0.5 mol/l) in 1 ml of plasma, we ensure that the pH of the aqueous phase is always above 10.9 before and after the extraction step. Then, using chloroform as the extraction solvent, as described by Alfredsson et al. [8] and Bressolle and co-workers

[9, 10], the reproducibility and sensitivity were very poor. A recovery study with some popular solvents was therefore undertaken. The absolute percentage of extraction was determined by comparing the peak heights obtained after direct injection of a pure solution and another injection of plasma extracts containing the same amount of sulpiride. The recovery was zero with diethyl ether and toluene, ca. 15% with methylene chloride and chloroform and greater than 98% with ethyl acetate. However, although ethyl acetate was the best extraction solvent, it could not be used alone because of the poor HPLC profile of blank human plasma. Finally, chloroform–ethyl acetate (3:1) was found to be the best compromise, giving a recovery greater than 85% for sulpiride and greater than 93% for veralipride and with a very clean HPLC profile (see Fig. 1).

To give a sulpiride retention time long enough to avoid interferences of early or non-chromatographed components together with a total analysis time compatible with pharmacokinetic studies, an optimized mobile phase was determined. First, mobile phases proposed by Verbiese-Genard et al. [6], Nishihara et al. [7] and Bressolle and co-workers [9, 10] were tested. We obtained the same result, that is, a sulpiride retention time of about 4 min (k' = 1.66), with no real possibility of achieving a slower elution. Such an early retention time seems nearly unsuitable in our case for extraction of biological fluids. Table II shows how different the elution of six benzamides under the analytical conditions indicated above can be. For instance, sulpiride and sultopride, which differ in one substituent only (see Table I), are eluted with a 13 min gap.

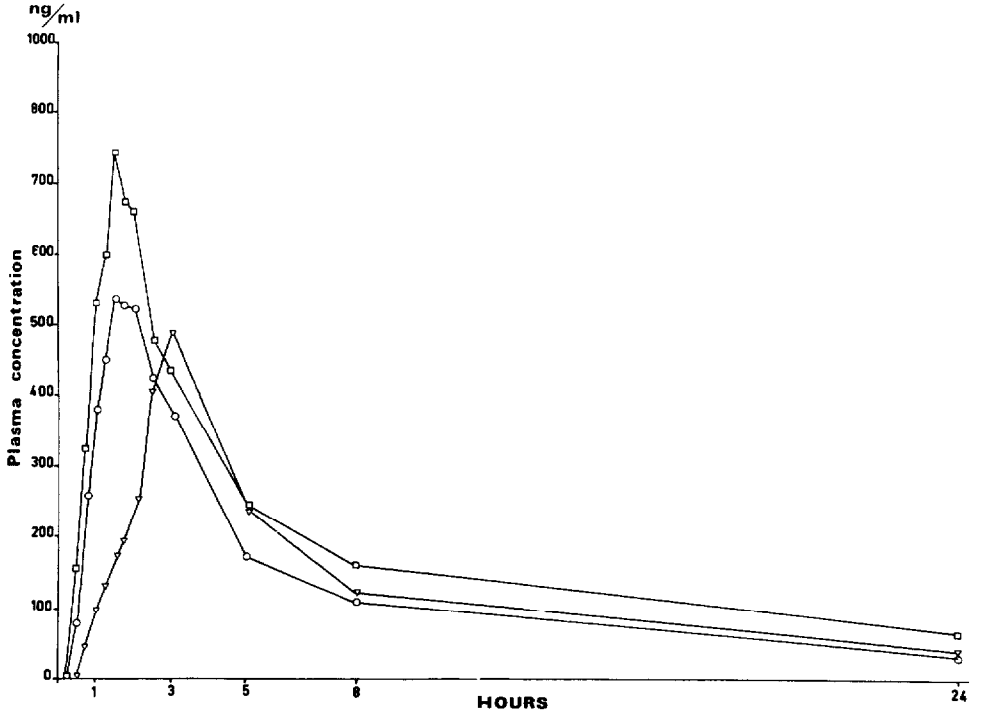


Fig. 3. Representative (one subject) pharmacokinetic profile of sulpiride following administration of a Dogmatil Fort 200-mg tablet (○), four Dogmatil 50-mg capsules (▽) and a sulpiride 200-mg capsule from a new formulation (□).

Benzamides are basic compounds with a very high affinity for reversed-phase columns. This may explain why Bressolle et al. [14] and Coulais et al. [15], working on a new benzamide and alizapride, respectively, needed large amounts of methanol for elution.

The use of sodium heptanesulphonate as a large counter ion solved this problem. However, the molarity of the counter ion and the final concentration of diethylamine must be carefully adjusted, on the one hand to obtain good retention times and on the other to avoid large tailing peaks, especially found here with the internal standard. Linked in an ion pair, benzamides are easier to work with using C_{18} columns. Increasing the elution strength of the mobile phase (with a higher methanol percentage or replacing it with acetonitrile or tetrahydrofuran) will decrease the capacity factor of, for instance, metoclopramide or alizapride and allow the effective chromatography of these drugs.

Clinical application

The bioavailability of sulpiride, after oral dosage, is weak (20—30%) with great inter- and intra-individual variability [9, 13, 16]. We carried out a bioequivalence study of three different formulations of sulpiride with healthy volunteers, each of whom received a single 200-mg oral dose. The sensitive and reliable assay described here successfully determined sulpiride. Fig. 3 shows the plasma sulpiride concentration—time curve for one subject.

A paper regrouping all the data of this cross-over design is currently being prepared for publication.

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