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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF PIPOTIAZINE IN HUMAN PLASMA AND URINE

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SUMMARY

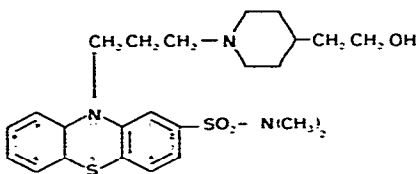
A high-performance liquid chromatographic method has been developed for the determination of pipotiazine in human plasma and urine. After selective extraction, pipotiazine and the internal standard (7-methoxypipotiazine) are chromatographed on a column packed with Spherosil XOA 600 (5 μ m) using a 7:3 (v/v) mixture of diisopropyl ether—isoctane (1:1, v/v) + 0.2% triethylamine and diisopropyl ether—methanol (1:1, v/v) + 0.2% triethylamine + 2.6% water. The eluted compounds are measured by fluorescence detection. The sensitivity of the method was established at 0.25 ng/ml pipotiazine in plasma and 2 ng/ml pipotiazine in urine (C.V. < 5%). The method has been successfully applied to a pharmacokinetic study following a single oral administration of 10 mg of pipotiazine.

INTRODUCTION

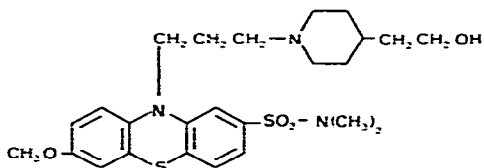
Pipotiazine, 10-[3-[4-(2-hydroxyethyl)-1-piperidinyl]propyl]-N,N-dimethyl-10H-phenothiazine-2-sulphonamide (Fig. 1, I), is a polyvalent neuroleptic according to Deniker's classification [1]. Therapeutically it is available in the form of the hydrochloride, for oral use to produce rapid action (Piportil[®]) and as the undecylenic ester (Piportil M₂[®]) or palmitic ester (Piportil L₄[®]), all from Laboratoires Specia, Paris, France, for use via the intramuscular route to produce a long-lasting action.

The scarcity of pharmacokinetic data concerning this drug in man is attributable to the lack of analytical techniques of sufficient sensitivity and specificity to be able to assay pipotiazine in biological media, notably in the blood or urine of patients under treatment with the drug.

Hitherto, only two methods have been available. The first [2] is based on tritium-labelling of the molecule, and is of limited application; the second [3]



I



II

Fig. 1. Structures of pipotiazine (I), and 7-methoxypipotiazine (II; internal standard).

is a gas chromatographic method which is sufficiently sensitive to assay pipotiazine during a course of repeated administration of the long-acting esters.

In this paper, we suggest a high-performance liquid chromatographic assay technique with a sensitivity and selectivity which enable it to monitor the pharmacokinetics of the unchanged pipotiazine both after a single dose and during a course of treatment using usual therapeutic dose schedules for both the oral form and the long-acting injectable form. An example of the application of this technique in a pharmacokinetic study in the healthy subject after the administration of 10 mg of pipotiazine is presented.

EXPERIMENTAL

Chemical and reagents

Apart from pipotiazine itself (I) and 7-methoxypipotiazine (II) as an internal standard (Fig. 1), the following compounds which are known as the three main metabolites of pipotiazine in urine were used in this study to check the selectivity of the technique: pipotiazine sulfoxide, pipotiazine N-oxide and 7-hydroxypipotiazine. All the standard solutions were made up in dilute hydrochloric acid ($10^{-3} M$) and kept at $4^{\circ}C$ away from direct light. Such solutions are stable for at least a month.

The pH 10 buffer (Titrisol; Merck, Darmstadt, G.F.R.), four times the usual strength, was chosen according to the best yields of extraction. The solvents — *n*-heptane, dichloromethane, diethyl ether and isoamyl alcohol — were all of analytical quality.

The mobile phase for the chromatography was a mixture of the isohydric solvents A and B (7:3, v/v) suggested by Thomas et al. [4], having the follow-

ing compositions. Solvent A: diisopropyl ether (Chromosol; SDS, Peypin, France), 50 volumes, isooctane (Chromosol; SDS), 50 volumes; triethylamine (Merck), 0.2%. Solvent B: diisopropyl ether (Chromosol; SDS), 50 volumes; methanol (Prolabo, Paris, France), 50 volumes; triethylamine (Merck), 0.2%; water, 2.6%.

Apparatus

The high-performance liquid chromatographic apparatus used comprised a Chromatem 380 pump, a Touzart et Matignon pulsation damper, a Waters automatic injector (WISP 710 B), a Schoeffel spectrofluorimeter (F 5970) and a Kipp and Zonen (BD 8) recorder. The column (10 cm × 4.6 mm I.D.) was prepared with Spherosil (XOA 600 5 μm; Prolabo) under a pressure of 400 bars.

The mass spectrometer used to check the selectivity of the assay was a Finnigan 4000 coupled with a gas chromatographic unit and computerised.

Extraction procedure

Plasma. A 2-ml sample of plasma and 0.5 ml of the aqueous solution of the internal standard, containing either 20 ng or 10 ng of 7-methoxypipotiazine depending on the concentration to be determined, are measured into a 20-ml conical centrifuge tube fitted with a ground-glass stopper and containing 1 ml of pH 10 buffer and 10 ml of diethyl ether—dichloromethane (2:1, v/v). The stoppered tube was shaken for 15 min on an Infors shaker, and then centrifuged at 3000 *g* for 10 min. The organic phase was transferred to a 10-ml conical glass tube and then evaporated to dryness under a stream of nitrogen at 40°C.

Urine. A 2-ml sample of urine, diluted if necessary, was treated as above, but in this case the solvent mixture used was *n*-heptane—isoamyl alcohol (95:5, v/v). The organic phase was transferred to a centrifuge tube containing 1 ml of 0.1 *N* sodium hydroxide solution. The tube was stoppered, shaken for 1 min and centrifuged as before; the organic phase was evaporated to dryness under a stream of nitrogen at a temperature of 40°C.

CHROMATOGRAPHY

The evaporation residues were dissolved in 200 μl of the mobile phase and placed on the revolving platform of the automatic injector set for injections of 50–200 μl depending on the concentrations of pipotiazine expected. Figs. 2 and 3 show some examples of chromatograms obtained with a flow-rate of 1 ml/min, a recorder chart-speed of 2 mm/min, and wavelengths of 270 nm for excitation and 470 nm for emission. The choice of wavelengths is based on the UV spectral data of pipotiazine and the fluorescence maximum. The capacity ratio values were 7.5 for pipotiazine and 9 for the internal standard (retention times: 10.5 min, and 13.5 min, respectively). The average length of chromatographic run was 15 min. The threshold of detection estimated as twice the background was about 0.10 ng/ml (sensitivity of the detector: about 0.20 ng).

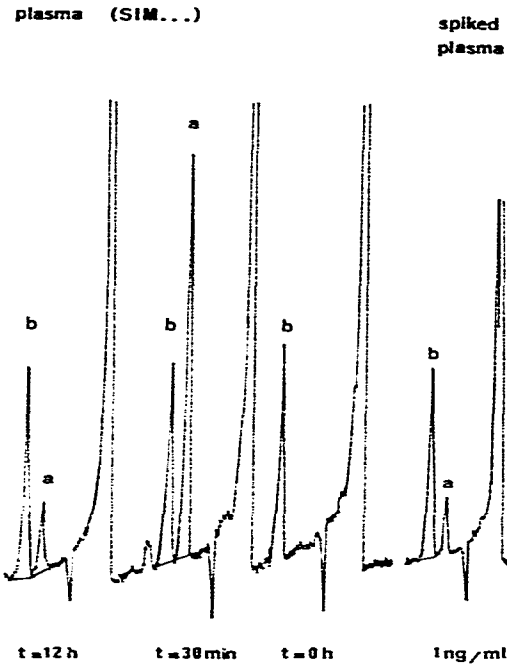


Fig. 2. Right: chromatogram obtained with plasma sample spiked with pipotiazine. Left: chromatograms of plasma sample extracts from a healthy subject (SIM) following oral administration of 10 mg of pipotiazine (sampling times: 0, 0.50, 12 h). Peaks: a = pipotiazine; b = internal standard.

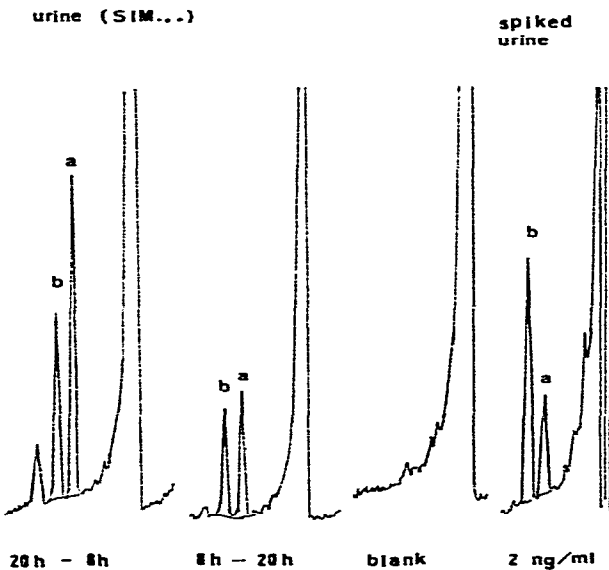


Fig. 3. Right: chromatogram obtained with urine sample spiked with pipotiazine and of a urine blank. Left: chromatograms of urine sample extracts from a healthy subject (SIM) following oral administration of 10 mg of pipotiazine (urine collected between 0 and 12 h, and between 12 and 24 h after administration). Peaks: a = pipotiazine; b = internal standard.

The plasma or urinary concentrations of pipotiazine were calculated from fluorimetric results, the appropriate peak height ratios being compared with a calibration curve.

The calibration curves for plasma and urine, calculated by linear regression from the data of six assays for each concentration of pipotiazine added, are as follows.

Plasma: $Y = -0.0030 + 0.2549X$, $r = 0.9982$

Confidence intervals at 95%: ordinate at the origin = -0.0030 ± 0.00431 ;
slope = 0.2549 ± 0.0049 .

Urine: $Y = -0.0346 + 0.2230X$, $r = 0.9989$

Confidence intervals at 95%: ordinate at the origin = -0.0346 ± 0.0543 ;
slope = 0.2230 ± 0.0047 .

Precision and accuracy of the method

The reliability characteristics of the method are given in Table I.

The scatter of concentration of pipotiazine used in constructing the calibration curves was evaluated by means of the method of variation coefficients. The sensitivity threshold of the method was established at 0.25 ng pipotiazine per ml of plasma; at this level, the variation coefficient is 11%. At higher plasma levels the coefficients are below 10%. Study of the scatter of urine determinations between 2 and 20 ng pipotiazine indicated that the variation coefficients were below 5%.

The average divergence between the amount of pipotiazine actually added

TABLE I

PRECISION AND RECOVERY IN THE DETERMINATION OF PIPOTIAZINE IN SPIKED HUMAN PLASMA AND URINE SAMPLES

Sample volume = 2 ml, for both urine and plasma. $n = 6$ in all cases.

Pipotiazine added (ng/ml)	Pipotiazine recovered (ng/ml)	S.D.	C.V. (%)	Absolute recovery (%)	Precision (%)
<i>Plasma</i>					
0.25	0.28	0.03	11.00	112.0	+12.0
0.50	0.53	0.02	4.04	106.0	+6.0
1.00	1.14	0.07	6.05	114.0	+14.0
2.00	2.03	0.04	1.91	101.5	+1.5
5.00	4.64	0.33	7.21	92.8	-7.2
10.0	10.11	0.15	1.46	101.1	+1.1
20.00	20.03	1.03	5.12	100.2	+0.2
<i>Urine</i>					
2.0	2.1	0.1	4.3	105	+5
5.0	4.9	0.0	0.9	98	-2
10.0	10.0	0.2	2.0	100	0
20.0	20.0	0.7	3.4	100	0

and the measurement obtained (accuracy) was $3.9 \pm 7.3\%$ for plasma and $1 \pm 3\%$ for urine. The average recovery of pipotiazine added was $104 \pm 7\%$ for plasma and $100 \pm 3\%$ for urine.

Selectivity

The selectivity of the method was established first of all with regard to the major known metabolites of pipotiazine in man (7-hydroxy, sulfoxide and N-oxide derivatives) [5]. In fact, as can be seen from the chromatogram (Fig. 4) obtained by direct injection of large amounts, greater than $1 \mu\text{g}$, of these metabolites in 1 ml of the mixture of solvents A and B, the retention times of the metabolites (23.75 min, 67 min and 126.5 min, respectively) differed widely from those of pipotiazine and internal standard. Furthermore, under the conditions used for extraction of pipotiazine and internal standard from plasma and urine samples of a healthy subject, following oral administration of 10 mg of pipotiazine, no interference was observed. Therefore, because of the lack of interference with the assay of drug, injections were made every 15 min.

The selectivity of the method was also checked with regard both to minor metabolites and to unidentified impurities. With this aim, a urinary extract from a patient who had received a 10-mg oral dose of pipotiazine was tested on a mass spectrometer after chromatography. The experimental conditions were: electronic ionization 70 eV, temperature of the source 200°C , temperature of the probe 280°C . Comparison of the spectrum obtained with the reference spectrum (spectrum No. 143 An Pci) showed them to be identical and with the same m/e ratio: 142 (main peak), 170, 246, 345, 367, 475 (molecular peak).

Samples containing 3.25 ng/ml pipotiazine were kept away from the light at -20°C for three months; no degradation of the pipotiazine was observed.

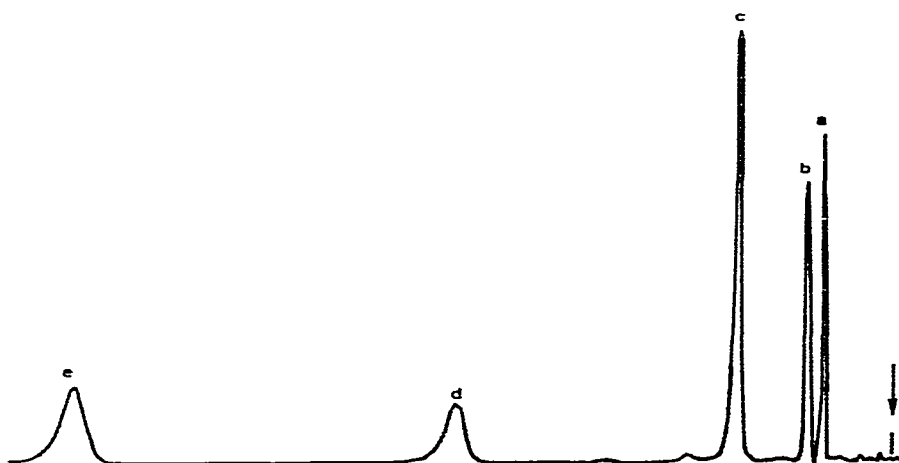


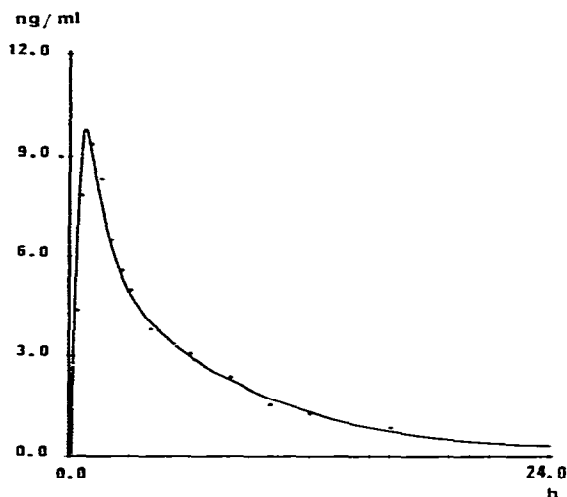
Fig. 4. Chromatogram of the major known metabolites of pipotiazine in man obtained by direct injection in the mixture of solvents A and B. Peaks: a = pipotiazine; b = internal standard; c = 7-hydroxypipotiazine; d = pipotiazine sulfoxide; e = pipotiazine N-oxide.

VALIDATION OF THE METHOD: PHARMACOKINETIC PROFILE IN MAN

Using the method suggested, the plasma levels and urinary excretion of pipotiazine were monitored in a healthy subject who had received 10 mg of Piportil® in solution form. Samples were taken at time 0 (just before administration of the drug), then at 0.25, 0.50, 0.75, 1.00, 2.00, 2.50, 3.00, 4.00, 6.00, 8.00, 10.00, 12.00, 16.00 and 24 h after ingestion of the solution. Blood samples (10 ml) were collected over heparin and, after centrifugation, the plasma was immediately frozen and stored at -20°C until analysis. Urines were collected during 24 h before, then between 0 and 12 h, and between 12 and 24 h, after administration of the drug. All determinations were performed in duplicate.

The chromatograms obtained from the plasma samples taken before, then 30 min and 12 h after administration are shown in Fig. 2; Fig. 3 shows those obtained from the urine samples collected before, then during, the two intervals after administration.

The curve of plasma concentration of unchanged pipotiazine versus time after a single oral administration of 10 mg of pipotiazine shown in Fig. 5 gives both plasma levels measured and the best fit to the experimental data [6,7]. These data were recently checked in a pharmacokinetic study carried out on five healthy subjects who had also ingested 10 mg of pipotiazine [8]; the plasma concentration peak (about 10 ng/ml) achieved at 0.78 h after oral intake is followed by a biphasic decreasing curve ending approximately 24 h



equation of the best fitting curve :

$$\begin{aligned}
 C(t) &= 6.6845 - \exp(-0.1362 - t) \\
 &\quad + 16.9342 - \exp(-1.3691 - t) \\
 &\quad - 23.6183 - \exp(-3.0353 - t) \\
 \text{lag } t &= 0.15 \text{ h}
 \end{aligned}$$

Fig. 5. Pharmacokinetic profile of pipotiazine in a healthy subject treated with 10 mg of pipotiazine. (■) experimental data points; (—) simulated curve.

later. The amount of unchanged pipotiazine excreted in the urine is about 1% of the administered dose.

The proposed method for the high-performance liquid chromatographic assay of pipotiazine would therefore seem to be highly suitable to study clinical pharmacokinetics and bioavailability of Piportil® in its various presentations.

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