Journal of Chromatography, 222 (1981) 291—296
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 737

Note

Gas—liquid chromatographic determination of pipotiazine in plasma of psychiatric patients

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(First received July 21st, 1980; revised manuscript received September 9th, 1980)

Pipotiazine palmitate [dimethylsulphamido-3-{(hydroxyethyl-4-piperidino)-3-propyl}-10-phenothiazine palmitate] is a neuroleptic drug of the phenothiazine family (Fig. 1, IA). It is used as an efficacious antipsychotic agent in the

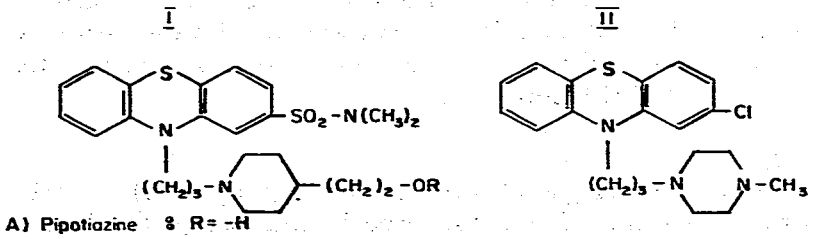

Fig. 1. Structural formulae of pipotiazine (IA), pipotiazine palmitate (IB) and prochlorperazine (II).

treatment of schizophrenia [1—6]. It is available in the long-acting preparation (in sesame oil) as an ester of palmitic acid (Fig. 1, IB) which is intended for intramuscular injection. Another available neuroleptic of this series, viz. pipo-

0378-4347/81/0000—0000/$02.50

tiazine undecylenate, results from the esterification of pipotiazine by undecylenic acid. The principles of depot preparations are (1) slow release of the molecule from the injection site, (2) gradual enzymatic hydrolysis of the ester bond, (3) slow metabolism and (4) its high concentration in the brain.

Recently, we have also found that pipotiazine palmitate is at least as effective or in some cases even superior to fluphenazine enanthate, which is another long-acting injectable neuroleptic in maintaining chronic hospitalized schizophrenic patients [7]. Quantitation of pipotiazine after therapeutic doses requires an analytical method sensitive enough to measure low concentrations in plasma. There is only one study, which describes pipotiazine pharmacokinetics after oral and intravenous administration of tritiated pipotiazine in man. This ratioisotope labelling method was adopted in order to achieve the required sensitivity and accuracy [8]. To our knowledge this is the first report on the specific measurement of pipotiazine in plasma of schizophrenic patients under intramuscular treatment of unlabelled pipotiazine palmitate.

The present study describes a sensitive and specific technique for pipotiazine determination in plasma by electron-capture gas—liquid chromatography (GLC) following acylation of the hydroxyl group with pentafluoropropionyl imidazole (PFPI). The method was applied to quantitative determinations in the plasma of patients treated with pipotiazine palmitate.

EXPERIMENTAL

Reagents

The following reagents were used: Spectroanalyzed grade toluene, isobutyl alcohol and methanol, analytical grade triethylamine (Fisher Scientific, Fair Lawn, NJ, U.S.A.), and a specially purified grade of pentafluoropropionyl imidazole (PFPI) (Pierce Chemicals, Rockford, IL, U.S.A.). The inorganic reagents were made up in doubly distilled water.

Standards

Pipotiazine base and pipotiazine palmitate for chromatographic standards as well as pipotiazine palmitate formulation for intramuscular administration to patients were supplied by Poulenc (Montreal, Canada). Prochlorperazine was used as an internal standard for GLC (Fig. 1, II).

Gas—liquid chromatography

A Hewlett-Packard Model 5830A gas chromatograph equipped with a ^{63}Ni (15 mCi) electron-capture detector (ECD) was used in this study; the instrument was linked to a digital integrator (HP 18850A). The stationary phase was 1% OV-17 on high-performance Chromosorb W (100—120 mesh) packed into a 1.8-m coiled glass column (I.D. 3.5 mm; O.D. 6 mm). The column was conditioned at 275°C for 24 h with an argon—methane (19:1) carrier gas flow-rate of 50 ml/min. The column and the injection port were operated at 265°C and the detector at 300°C. The flow-rate of carrier gas was 50 ml/min. Under these conditions, the relative retention time of pipotiazine to the internal standard prochlorperazine was 1.51 (Fig. 2B).

Extraction procedure

To 3 ml of plasma in a 13-ml conical glass-stoppered centrifuge tube were added 50 μl of the methanolic solution of prochlorperazine (100 μg/ml) as the internal standard. The sample was made alkaline by the addition of 0.5 ml of 5% sodium hydroxide and extracted with 5 ml of toluene—isobutyl alcohol (90:10) by shaking for 15 min in a mechanical shaker. After centrifuging at 600 *g* for 10 min, the organic layer was transferred to another 13-ml tube and the aqueous layer was re-extracted with 5 ml of the same solvent mixture for 15 min. After centrifuging, the organic phases were combined in the previous tube and the aqueous layer was discarded. The organic layer was back-extracted with 2 ml of 0.05 *N* hydrochloric acid for 15 min. The sample was centrifuged as before and the organic layer was removed and discarded. The sample was made alkaline with 0.2 ml of 5% sodium hydroxide and extracted with 2 ml of toluene for 15 min, then centrifuged. The aqueous layer was rejected. The organic layer was transferred into a 3-ml conical glass-stoppered tube and evaporated to dryness at 60°C with a slow stream of nitrogen. The walls of the tube were rinsed with 0.2 ml of methanol by vibrating with a Vortex mixer for 1 min. The solution was evaporated to dryness as before. To the residue were added 50 μl of a solution of 2% (v/v) PFPI in toluene and 20 μl of 0.1 *M* triethylamine in toluene. The mixture was vortexed for 1 min and then heated for 1 h at 65°C. To the mixture were added 100 μl of 0.1 *M* phosphate buffer (pH 6.0) for hydrolyzing the excess of PFPI. The mixture was again vortexed for 1 min, centrifuged at 600 *g* for 3 min and about 2—3 μl of the toluene phase were injected into the gas chromatograph.

Calibration curve

To 3 ml of heparinized blank human plasma in 13-ml glass-stoppered centrifuge tubes were added 10, 15, 20, 25 and 30 μl of methanolic solution of pipotiazine (45 μg/ml) and 50 μl of prochlorperazine solution in methanol (100 μg/ml). The samples were then carried through the complete extraction procedure described above. Quantitation was achieved using the ratio of the peak area of pipotiazine to that of the internal standard prochlorperazine. Peak area ratios were plotted against weight to obtain the calibration curve, which was linear up to 60 ng pipotiazine on injection.

Human studies

Five out of forty chronic ambulatory schizophrenics participating in a large-scale double-blind clinical study of pipotiazine palmitate vs. fluphenazine decanoate were the subjects of this experiment. One of the aims of the study was to establish the effective prophylactic dose of antipsychotic medication for these two drugs. The initial dose of the medication was 12.5 mg of pipotiazine palmitate for one month intramuscularly and then the second injection was in an increment of 12.5 mg. Adjustments during the course of treatment were based on the requirements of each patient and considering the pre-study requirements of neuroleptic of each patient. At the end of the last monthly injection 10-ml blood samples were drawn by venipuncture of the antecubital vein before the next dose. Samples were immediately centrifuged; the plasma was aspirated into a second aluminium foil-wrapped tube and was deep-frozen at

−20°C until analysis. This procedure was followed in order to minimize contact time with the evacuated tube's tip which causes contamination of the sample.

RESULTS AND DISCUSSION

Selectivity

Analytical studies indicate that extracts from blank human plasma do not show peaks that could interfere with the quantitative determination of pipotiazine. This is exemplified by a typical chromatogram resulting from blank plasma and plasma with added pipotiazine (Fig. 2) carried through the extraction procedure. However, under the present GLC conditions, pipotiazine could not be resolved from its chemically and pharmacologically related neuroleptic fluphenazine.

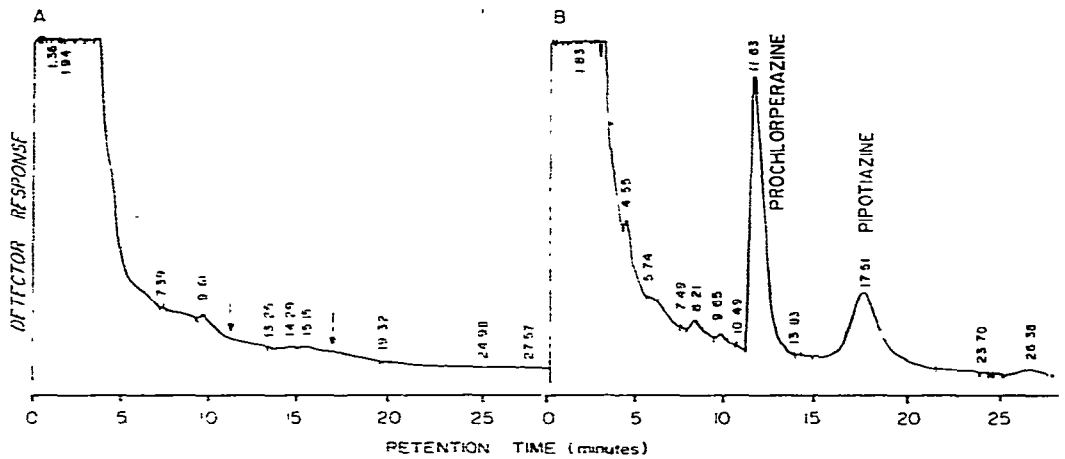

Fig. 2. (A) Chromatogram obtained from an extract of 3 ml of blank human plasma. The arrows show the absence of signals at the retention times of prochlorperazine (internal) standard) and pipotiazine. (B) Chromatogram obtained from an extract of 3 ml of human plasma containing the internal standard prochlorperazine and pipotiazine.

Recovery studies

The absolute recoveries of pipotiazine from spiked plasma were determined using the same internal standardization method as described previously. The peak area ratio of pipotiazine and the internal standard prochlorperazine was used as the index of detector performance and overall efficiency of the analytical procedure. Quadruplicate plasma samples, spiked with pipotiazine at different concentrations were analysed and the results are presented in Table I.

Sensitivity

The method was sensitive enough to measure pipotiazine down to a level of 10 ng/ml of plasma. The lower detection limit was fixed to the minimum response of the ECD to pipotiazine with peak areas up to 40,000 counts at an attenuation of × 128.

TABLE I

ABSOLUTE RECOVERY OF PIPOTIAZINE FROM PLASMA BY GLC

Pipotiazine added (μg/ml)	Pipotiazine recovered (μg/ml)	Absolute recovery* (%)	Coefficient of variation (%)
0.050	0.042	84.00	9.41
0.100	0.083	83.00	6.08
0.150	0.121	80.67	8.72
0.225	0.176	78.22	2.36
0.300	0.230	76.67	5.20
0.375	0.275	73.33	7.63
0.450	0.319	70.89	4.14

*Each value is the mean of four determinations. Mean absolute percentage recovery in plasma is 78.11 ± 4.87%.

Application of the method to human studies

The plasma levels of pipotiazine in psychiatric patients are presented in Table II. The plasma concentrations of pipotiazine measured in patients after a monthly intramuscular injection show a marked individual variation. However, these patients did not receive a uniform dose and had different periods of treatment. The therapeutic levels of pipotiazine are not yet well defined. This method can be useful in clinical therapeutic monitoring of patients.

TABLE II

PLASMA LEVELS OF PIPOTIAZINE IN PATIENTS RECEIVING PIPOTIAZINE PALMITATE THERAPY

Patient No.	Duration of pipotiazine palmitate therapy (weeks)	Last dose of pipotiazine palmitate (mg)	Plasma level of pipotiazine (ng/ml)
1	18	25	28.7
2	19	112.5	18.1
3	22	87.5	39.3
4	27	50	57.9
5	20	112.5	45.5

ACKNOWLEDGEMENTS

The authors express their gratitude to Poulenc, Montreal, Canada for providing them with Piportil$_{L4}$® (pipotiazine palmitate: intramuscular formulation) for clinical use and authentic standards of pipotiazine, pipotiazine palmitate and prochlorperazine for laboratory use. They also acknowledge the technical assistance of Miss Angelique Bordeleau.

REFERENCES

1 G. Chouinard, L. Annable and M. Kropsky, J. Clin. Pharmacol., 18 (1978) 148.
2 A. Schlosberg and M. Shadmi, Curr. Ther. Res. Clin. Exp., 23 (1978) 642.
3 P. Simon, J. Farmanian, D. Ginestet, M.A. Goujet and P. Péron-Magnan, Arch. Gen. Psychiat., 35 (1978) 893.
4 A.N. Singh and B. Saxena, Curr. Ther. Res. Clin. Exp., 25 (1979) 121.
5 R.E. Johnston and F. Niesink, J. Int. Med. Res., 7 (1979) 187.
6 A. Villeneuve, Curr. Ther. Res. Clin. Exp., 27 (1980) 411.
7 J.M. Albert, R. Elie and S.F. Cooper, Curr. Ther. Res. Clin. Exp., 27 (1980) 897.
8 P.J. De Schepper, C. Vranckx, R. Verbeeck and M.L.O. Van den Berghe, Arzneim.-Forsch., 29 (1979) 1056.