Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 11, pp. 1273–1280, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00
Pergamon Press plc

Determination of pipothiazine in human plasma by reversed-phase high-performance liquid chromatography

D. A. OGDEN,* A. A. MCKECHNIE,† D. A. BOLTON,* R. B. RANKIN,* S. E. DOBBINS‡ and J. A. CLEMENTS*§

* *Department of Pharmacy, Heriot-Watt University, Edinburgh, UK*
† *Bangour Village Hospital, Broxburn, West Lothian, UK*
‡ *Rhône-Poulenc Limited, Dagenham, Essex, UK*

Abstract: A method is described for the determination of pipothiazine in human plasma, based on reversed-phase HPLC. The method has been applied in a pharmacokinetic study of pipothiazine in six psychiatric patients receiving repeated depot intramuscular injections for six months. A number of compounds likely to be taken concurrently by patients were tested for potential to interfere with the assay. There was no evidence of "dose-dumping" in the period following injection. Comparison of the pharmacokinetic profiles after the first and sixth injections showed no evidence of drug accumulation.

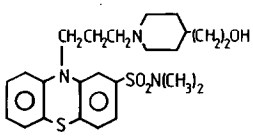
Keywords: *Pipothiazine; pharmacokinetics; depot therapy; HPLC analysis.*

Introduction

A number of liquid chromatographic methods have been described for the determination of phenothiazines [1–3]. For the phenothiazine compound pipothiazine le Roux *et al.* [4] have developed a normal-phase HPLC method and applied it to pharmacokinetic studies after oral administration of the hydrochloride salt to volunteers. Kaye *et al.* [5] have reported a reversed-phase method for its measurement in plasma.

Pipothiazine palmitate (Piportil®, Rhône-Poulenc Limited) is administered to psychiatric patients in doses of 20–250 mg as depot injections, usually at intervals of 4 weeks. Plasma concentrations of pipothiazine following a single oral dose (10 mg) of

Structure of pipothiazine (10-[3-[4-(2-hydroxyethyl)-1-piperidinyl]propyl]-*N*,*N*-dimethyl-10H-phenothiazine-2-sulphonamide)



PIPOTHIAZINE

§Present address: Clinical Services Department, Inveresk Clinical Research Limited, Research Park, Riccarton, Edinburgh EH14 4AP.

pipothiazine hydrochloride are reported to be less than 10 ng ml^{-1} [4]. In a preliminary report, the concentrations of pipothiazine after depot injections of 100 mg of the palmitate ester are given as 0.6–3.4 ng ml^{-1} [6].

A reversed-phase HPLC method with fluorescence detection suitable for use in a clinical study of the pharmacokinetics of pipothiazine after depot injection of pipothiazine palmitate has been developed. With the method pipothiazine is determined in plasma in the concentration range 0.1–12.5 ng ml^{-1} with promethazine hydrochloride as an internal standard. Preliminary pharmacokinetic data are presented for six patients undergoing six months' therapy.

Experimental

Drugs

Pipothiazine, pipothiazine-*N*-oxide, pipothiazine sulphoxide, 7-hydroxypipothiazine and promethazine hydrochloride were kindly supplied by Rhône-Poulenc Limited, Dagenham.

Reagents

HPLC grade methanol, hexane, dichloromethane and acetonitrile (Rathburn Chemicals) were used. Ammonium carbonate (BDH Limited) was Analar grade and sodium hydroxide (BDH Limited) was laboratory grade.

Chromatography

A 250 × 4.6 mm stainless steel column packed with SAS-Hypersil 5 μm (Shandon), protected by a guard column containing Partisil-20, was maintained at 45°C with a thermal jacket (Jones Chromatography). The eluent was acetonitrile–ammonium carbonate 0.05 M 50:50 (v/v). Flow was maintained with a Gilson Model 802 Pump and flow rate was 1.4 ml min^{-1}. Detection was with a Kratos FS970 fluorometer set at 263 nm excitation and 470 nm emission wavelengths. Detector output was monitored using a Gallenkamp Euroscribe recorder set to 10 mV.

Procedure

To 2.0 ml plasma was added 200 μl of a solution of the internal standard (promethazine hydrochloride solution, 6.25 μg ml^{-1} freshly prepared and protected from light). To the mixture was added, with mixing 500 μl of methanol, 100 μl of 7 M sodium hydroxide solution and 8 ml of freshly prepared hexane–dichloromethane mixture (50:50 v/v).

After vortexing the sample for 1 min and centrifugation at 750*g* for 5 min, the organic layer was transferred to a clean tube. The solvent was evaporated under a stream of nitrogen at 35–40°C. The dry tubes were washed down with a further 1 ml of the extraction solvent which was evaporated under a flow of nitrogen. The samples were reconstituted in 100 μl of eluent and vortexed for 5 s before injection of 20 μl on to the column.

Human plasma samples prepared to contain pipothiazine in the concentration range 1.25–12.5 ng ml^{-1} were assayed. Patient plasma samples were analysed on the same day as plasma standards of pipothiazine (2.5 and 12.5 ng ml^{-1}) previously prepared and stored in ampoules at −20°C. All plasma samples were analysed in duplicate and the mean concentrations calculated.

Subjects

Six patients (3 male) aged 35–61 years and requiring depot neuroleptic therapy gave informed consent to participate in the study which was approved by an ethics review committee. Five patients were receiving concurrent medication but which excluded compounds known to interfere with pipothiazine analysis. All patients regularly smoked >10 cigarettes per day. Each patient received 4-weekly depot intramuscular injections of pipothiazine palmitate at a constant dose (range 5–62.5 mg per week) decided by the physician. Blood samples were taken by venepuncture at 1, 4, 7, 14, 21 and 28 days during the first month and at 0 (predose), 7, 10, 14, 21 and 28 days during the sixth months of treatment. Plasma was separated and stored at −20°C until required for analysis.

Pharmacokinetics

Peak plasma concentrations were found by inspection. The area under the pipothiazine plasma concentration–time curve (AUC) was calculated by the linear trapezoidal rule over the dose interval. Mean concentration was calculated as AUC/dose interval. Accumulation indices (R) were calculated as:

$$R_1 = \frac{\text{Peak concentration after last dose}}{\text{Peak concentration after first dose}} \quad \text{(i)}$$

and

$$R_2 = \frac{\text{AUC for last dose}}{\text{AUC for first dose}}. \quad \text{(ii)}$$

Results

Analytical method

The retention times for pipothiazine and promethazine were 7.25 and 14.2 min, respectively (Fig. 1). Peak height ratios (pipothiazine/promethazine) were rectilinearily related to pipothiazine concentration ($r = 0.999$). The relative standard deviation for analysis of a plasma standard containing 5 ng ml^{-1} was 3.1% ($n = 8$).

Determination of three possible metabolites of pipothiazine namely, 7-hydroxypipothiazine, pipothiazine-*N*-oxide and pipothiazine sulphoxide was also investigated. The retention times, phase capacity ratios, relative fluorescence intensities and extraction efficiencies of pipothiazine, 7-hydroxypipothiazine, pipothiazine-*N*-oxide and pipothiazine sulphoxide are as shown in Table 1.

Whereas the 7-hydroxy metabolite was not extracted efficiently, probably due to formation of a phenoxide ion at high pH values, the sulphoxide and *N*-oxide derivatives were well extracted. Although the polarities of all three compounds were greater than that of pipothiazine, peaks were adequately separated (Fig. 2). However, no measurable concentrations of metabolites were found in samples of plasma from any patients receiving pipothiazine.

The following pure compounds were shown not to interfere with the assay method: aspirin, benzhexol, benztropine, caffeine, chlorpromazine, diazepam, diphenhydramine, droperidol, haloperidol, lorazepam, nitrazepam, orphenadrine, paracetamol, procyclidine, sulpiride and temazepam.

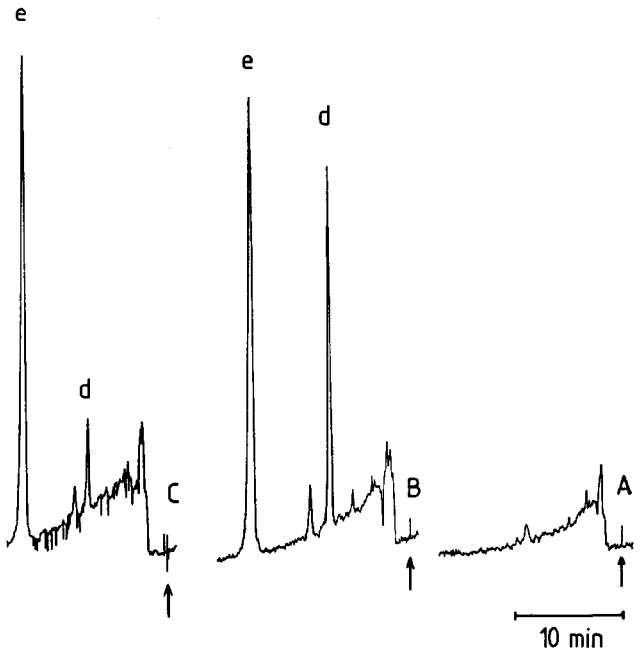


Figure 1
Chromatogram obtained after extraction of (A) 2.0 ml blank plasma, (B) 2.0 ml plasma sample containing pipothiazine (5.0 ng ml^{-1}) (peak d) with added internal standard (200 µl of promethazine HCl solution, 6.6 µg ml^{-1}) (peak e) and (C) patient sample containing 1.26 ng ml^{-1} pipothiazine. Injection volume 20 µl; sensitivity 500 nA.

Table 1
Phase capacity ratios, retention times, relative fluorescence intensities and extraction efficiencies of promethazine, pipothiazine, metabolites and related compounds

Compound	Capacity ratio k'	Retention time (min)	Relative fluorescence	Extraction efficiency (%)
Pipothiazine	2.02	7.25	1.00	84
Pipothiazine-*N*-oxide	0.35	3.23	1.49	71
Pipothiazine sulphoxide	0.62	3.88	0.22	86
7-Hydroxypipothiazine	1.08	4.98	0.02	<1
Promethazine	4.95	14.2	0.01	77
Mesoridazine	4.76	13.8	—	—
Sulforidazine	4.67	13.6	—	—
Thioridazine	16.1	41.0	—	—
Thioradazine ring sulphoxide	3.34	11.1	—	—

To test for interference from other antipsychotic agents, samples of blood plasma obtained from patients receiving such drugs were analysed by this method. Figure 3 shows the chromatogram from the plasma of a patient receiving thioridazine and chlorpropamide. Multiple peaks were observed and those due to thioridazine and two of its metabolites, mesoridazine and sulforidazine, were identified by comparison with chromatograms of the pure compounds; mesoridazine and sulforidazine were not resolved under these conditions. On this basis, the following compounds or their metabolites (or both) were shown to interfere with the assay: clopenthixol, flupenthixol, fluphenazine, methotrimeprazine, mesoridazine, perphenazine, piperacetazine, sulforidazine, thioridazine and trifluoperazine.

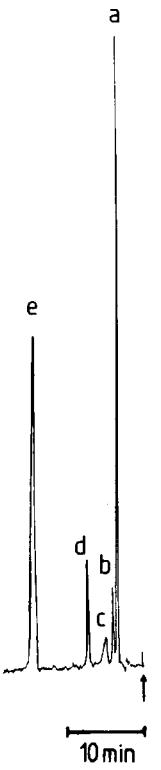


Figure 2
Chromatogram for aqueous standard containing (a) pipothiazine-*N*-oxide (3.3 ng on column), (b) pipothiazine sulphoxide (2.7 ng), (c) 7-hydroxypipothiazine (8.1 ng), (d) pipothiazine (0.8 ng) and (e) promethazine HCl (200 ng); sensitivity 500 nA.

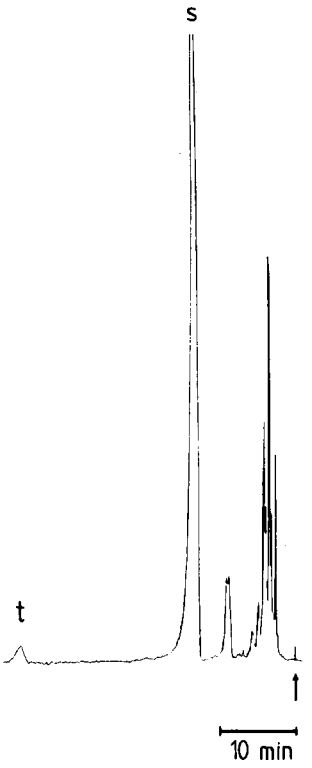


Figure 3
Chromatogram of plasma extract from patient taking thioridazine (100 mg three times daily) and chlorpropamide (500 mg daily). (s) mesoridazine and sulforidazine, (t) thioridazine. Injection volume 2 μl; sensitivity 1000 nA.

Pharmacokinetic data

Five patients completed the study. One female patient withdrew from the study after the first 28-day study period. Pipothiazine plasma concentrations in the five patients who completed the study are shown for the first and sixth months of treatment in Fig. 4. Peak concentrations occurred at one to two weeks after injection and were approximately proportional to the administered dose (Table 2). There was no evidence for rapid release of a proportion of the dose ("dose-dumping"). Based on peak concentrations, mean concentrations and AUC, the degree of accumulation over the six months was small.

Conclusions

A method for the determination of pipothiazine plasma concentrations has been described and preliminary pharmacokinetic results from six patients maintained on depot pipothiazine palmitate are presented. The plasma concentrations are within the ranges expected for therapeutic doses of the drug [4, 6]. These results suggest that the depot injection of pipothiazine palmitate in the dose range of 20–250 mg at 4-weekly intervals

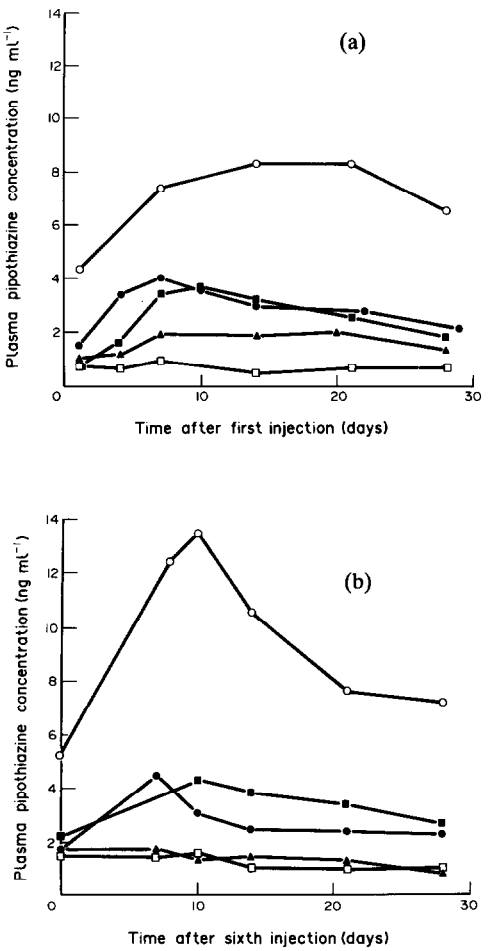


Figure 4
Plots of pipothiazine plasma levels at intervals during the 28-day treatment period: (a) the first month of therapy and (b) the sixth month. Each line represents a separate patient, receiving the same monthly dose throughout the experimental period. The doses were: □ = 20 mg; ▲ = 37.5 mg; ■ = 75 mg; ● = 100 mg; ○ = 250 mg.

Table 2
Pharmacokinetic data for six patients

		First dose				Last dose			
Patient No.	IM dose (mg/4 weeks)	Peak concentration ($ng\ ml^{-1}$)	Time to peak (days)	Mean concentration ($ng\ ml^{-1}$)	AUC 0–28 day $ng\ ml^{-1} \times day$	Peak concentration ($ng\ ml^{-1}$)	Time to peak (days)	Mean concentration ($ng\ ml^{-1}$)	AUC 0–28 day $ng\ ml^{-1} \times day$
1	20	1.0	7	0.8	21.5	1.4	10	1.2	33.4
2	37½	2.0	7	1.7	48.3	1.7	7	1.4	38.3
3	75	3.7	10	2.5	73.2	4.3	10	3.4	94.9
4	100	4.0	7	2.6	81.8	4.4	7	2.7	77.4
5	150	5.1	14	4.3	120	—	—	—	—
6	250	8.3	14	6.9	205	13.5	10	8.6	260

has the appropriate kinetic behaviour for an effective depot neuroleptic with no evidence of "dose-dumping".

References

[1] J. A. F. deSilva, in *GLC and HPLC Determination of Therapeutic Agents* (K. Tsuki, Ed.), Chap. 17, pp. 581–636. Marcel Dekker, New York (1978).
[2] S. H. Curry, E. A. Brown, O. Y.-P. Hu and J. Perrin, *J. Chromatogr.* **231**, 361–376 (1982).
[3] K. K. Midha, J. K. Cooper, I. J. McGilveray, A. G. Butterfield and J. W. Hubbard, *J. Pharm. Sci.* **70**, 1043–1046 (1981).
[4] Y. le Roux, J. Gaillot and A. Bieder, *J. Chromatogr.* **230**, 401–408 (1982).
[5] C. M. Kaye, P. Mitchell and C. Stanley, *Br. J. Clin. Pharmac.* **19**, 576P–577P (1985).
[6] M. Girard, F. Grainer, L. Schmitt, J. Cotonat, M. Escade and M. Blanc, *L'Encephale* **10**, 171–176 (1984).

[*Received for review 11 August 1988; revised manuscript received 7 April 1989*]