Journal of Chromatography, 419 (1987) 363–367
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3714

Note

Gas chromatographic determination of chlorproethazine in plasma

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(First received December 19th, 1986; revised manuscript received March 18th, 1987)

Chlorproethazine [3-chloro-10-(3-diethylaminopropyl)phenothiazine] is a phenothiazine used as neuromuscular myorelaxant. Its pharmacokinetics has not yet been studied because of the lack of an analytical method for determination of chlorproethazine in blood samples.

In this report a gas chromatographic (GC) assay for chlorproethazine in plasma is described. The assay (extraction procedure and chromatographic conditions) is derived from previously reported methods for analysis of chlorpromazine [1–3] and methotrimeprazine [4] in blood samples.

EXPERIMENTAL

Chemicals

Chlorproethazine hydrochloride and chlorpromazine hydrochloride [3-chloro-10-(3-dimethylaminopropyl)phenothiazine] (Laboratoires Genevrier, Neuilly, France) were used without further purification. All solvents were analytical-reagent grade.

Chromatographic conditions

The gas chromatograph (Girdel 3000) was equipped with an Ni electron-capture detector. The column was a 40 m$\times$0.32 mm fused-silica capillary column (WCOT) coated with CP^{TM} Sil 8 CB (Chrompack). Purified helium and methane were used as carrier gas. Operating conditions were as follows: isothermal column temperature, 230°C; injection port temperature, 280°C; detector temperature, 300°C; helium flow-rate, 9.5 ml/min.

0378-4347/87/$03.50

Injections (1 μl) were made by an automatic sampler (Precision Sampling 311 H, Coultronics) directly on-column. Chromatograms were recorded and peak-height ratios and concentrations calculated using a recording integrator (LTT Enica 10, Delsi).

Preparation of stock solutions and calibration curves

A stock solution of the internal standard (chlorpromazine hydrochloride, 25 μg/ml) was prepared in ethanol. The solution was stored in the dark at 4 °C, and 100 μl were added to each plasma sample (clinical samples, calibration curve samples and calibration standard).

An ethanolic solution of chlorproethazine hydrochloride (25 μg/ml) was prepared and stored as described for the internal standard. This solution was used as stock solution for the preparation of the calibration curves and standards.

Extraction procedure

To a glass test-tube (20 ml) were added 2 ml of plasma, 100 μl of the stock solution of chlorpromazine and 10 ml of hexane. The sample was mixed for 10 min and the upper organic layer was transferred to a clean glass tube. The extraction was repeated three times. The organic extracts were combined and evaporated (under a nitrogen stream) to ca. 500 μl at 37 °C and further adjusted to exactly 500 μl. An aliquot of 1 μl was injected into the chromatograph.

Calibration curve

The calibration curve was prepared with blank plasma spiked with 10–100 μl of the stock solution of chlorproethazine and 100 μl of the stock solution of chlorpromazine. After extraction, final concentrations of 500–5000 ng/ml for chlorproethazine and 5000 ng/ml for chlorpromazine were obtained.

Plasma levels study

As an application of this assay procedure, chlorproethazine has been administered to one monkey (*Cynomolgus macaca fascicularis*). The monkey received an intravenous infusion (1 ml/min) and one week later an intramuscular injection of 25 mg of chlorproethazine. Blood samples were collected from the femoral artery at 0 (prior to infusion), 2, 5, 15, 30 and 45 min and 1, 2 and 3 h (post-infusion), and at 0 (prior to injection), 15, 30 and 45 min and 1, 2, 3, 4, 6 and 8 h (post-injection). Samples were collected into tubes containing sodium fluoride and centrifuged. Plasma was separated and stored at −19 °C until assayed.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms of an extract of blank plasma (A), of a calibration standard (blank plasma spiked at 5000 ng/ml of chlorproethazine and 5000 ng/ml of chlorpromazine) (B), and of plasma collected 30 min after intramuscular injection of 25 mg of chlorproethazine to a monkey (C). Chlorproethazine and the internal standard gave well separated sharp peaks with retention

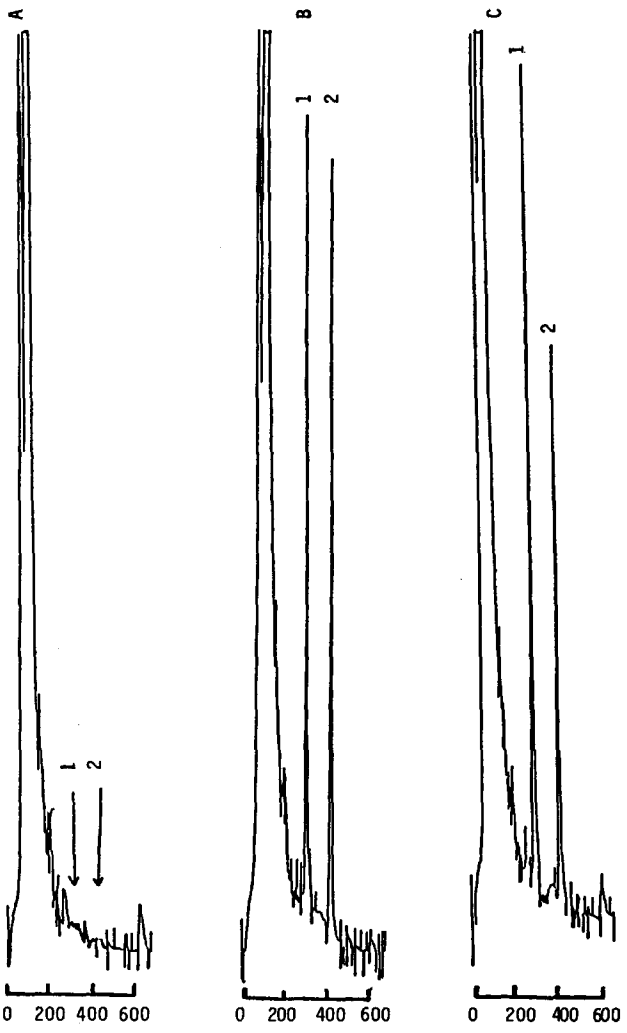

Fig. 1. Gas chromatograms of (A) blank plasma, (B) calibration standard (blank plasma spiked with 5000 ng/ml chlorproethazine and chlorpromazine) and (C) plasma collected 30 min after intramuscular injection of 25 mg of chlorproethazine to a monkey. Peaks: 1 = chlorpromazine; 2 = chlorproethazine. Time in seconds.

times of 426 ± 3 and 305 ± 3 s, respectively. The estimated concentration of the 30-min post-dose plasma sample (2 ml) was 969.9 ng/ml.

Assay precision, accuracy, linearity and recovery

The precision and accuracy of the chlorproethazine assay procedure were assessed by analysis of replicate plasma samples to which known amounts (500–5000 ng/ml) of chlorproethazine had been added. The within-day assay precision and accuracy are summarized in Table I. The accuracy for all concentrations was within $\pm 2\%$ of the actual fortified chlorproethazine concentrations.

The day-to-day reproducibility was determined by analysis within each exper-

TABLE I

GC ESTIMATION OF CHLORPROETHAZINE ADDED TO PLASMA

Concentration added (ng/ml)	n	Concentration calculated (mean ± S.D.) (ng/ml)	C.V. (%)	Error (%)
5000	3	4906 ± 41	0.83	−2
4500	3	4560 ± 64	1.40	+1
3000	3	2947 ± 45	1.52	−2
2500	3	2422 ± 49	2.02	−3
1500	3	1517 ± 26	1.7	+1
1000	3	1075 ± 39	3.6	+1
500	3	491 ± 14	2.85	−2

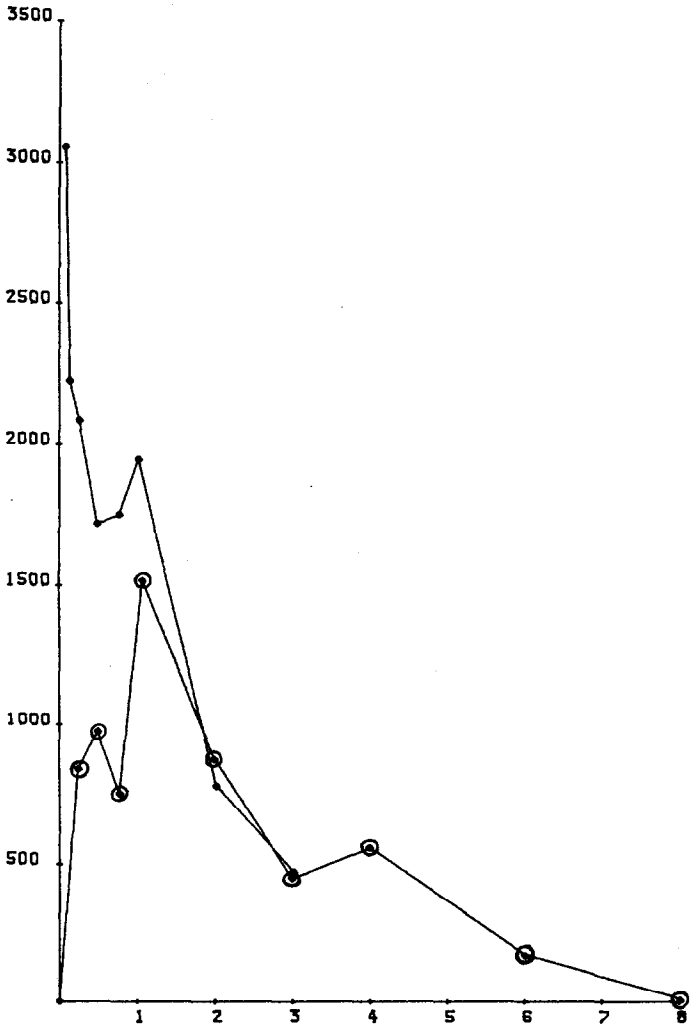

Fig. 2. Plasma levels in a monkey given 25 mg of chlorproethazine as an intravenous infusion (♦) and an intramuscular injection (⊙). Time in hours; concentration in ng/ml.

iment of replicate plasma samples spiked with 5000 ng/ml of chlorproethazine; the coefficient of variation (C.V.) was 1.84%.

The calibration curve was linear in the range 500–5000 ng/ml; the correlation coefficient was 0.994.

For the determination of the recovery of chlorproethazine and the internal standard, blank plasma was spiked at 5000 ng/ml with both substances. The samples were extracted as described above, and the peak heights were compared with those obtained for absolute injection of the same amounts in hexane. Results based on six determinations showed mean recoveries of 85.09 ± 2.79 and $94.91 \pm 6.45\%$ for chlorproethazine and the internal standard (chlorpromazine), respectively.

Plasma level study

An application of this method is shown in Fig. 2, where plasma concentration–time profiles are shown for the monkey. The animal received an intravenous infusion of 25 mg of chlorproethazine followed by an intramuscular injection (25 mg) one week later. Comparison of the areas under the curve shows that the bioavailability after intramuscular injection was close to the absolute bioavailability (after intravenous infusion): 4136 and 4118 ng/ml h, respectively.

The half-life was calculated according to Wagner [5] by the least-squares method. After intramuscular injection the apparent half-life calculated from the last six points of the semi-logarithmic plot of plasma concentration–time was 1 h (confidence interval 0.6–3.5). The apparent half-life estimated (three points) after intravenous infusion was 0.9 h (confidence interval 0.5–1.3).

The described method is simple and permits pharmacokinetics or bioavailability studies of chlorproethazine.

ACKNOWLEDGEMENT

The authors thank Dr. Rougeot (Laboratoires Genevrier) for his helpful discussions and for the gift of pure chlorproethazine and chlorpromazine.

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