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Note

Assay of *m*-chlorophenylpiperazine in plasma and brain of rat by capillary gas chromatography–mass spectrometry

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Central nervous system (CNS)-active compounds are often hydrophilic whereas the blood–brain barrier is permeable to lipophilic molecules [1]. This permeability may be increased in hypertension [1], or via an endogeneous factor whose nature and physiological role are not yet well known. The activity of a lipophilic drug could be due to the molecule itself and to one of its metabolites, as is the case with trazodone, etoperidone and mepiprazole. These psychotropic molecules are metabolized to give *m*-chlorophenylpiperazine (mCPP) [2], which has high affinity for 5-hydroxytryptamine (5-HT) binding sites *in vitro* [3].

In previous pharmacokinetic studies of trazodone and mCPP in plasma and brain tissue, mCPP levels were quantified by gas chromatography (GC) with electron-capture detection [4,5]. These authors observed that the levels of mCPP were more than twenty-fold greater in cerebral tissue than in plasma. The sensitivity of the method was then 10 ng/ml in plasma or 50 ng/g in brain.

With a view to carrying out extended kinetics studies with low doses of mCPP or its precursors, we developed a highly sensitive and specific method of assay, using capillary GC with a mass-selective detection.

EXPERIMENTAL

Reagents

mCPP and the internal standard (I.S.), *m*-trifluoromethylphenylpiperazine, were gifts from R. L. Cerm (Riom, France). All reagents and solvents were ana-

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lytical-reagent grade purity RS: hexane, ethyl acetate, trifluoroacetic anhydride (Merck, Darmstadt, F.R.G.) and methanol (Carlo Erba, Milan, Italy).

Standard solutions of mCPP and the I.S. were prepared in methanol (1 mg/ml). Working solutions (0.1–100 μg/ml) were freshly prepared before analysis, by dilution in methanol.

Gas chromatography–mass spectrometry (GC–MS)

The determinations were carried out on a quadrupolar GC–MS system (Hewlett-Packard, 5971 A) in the electron-impact (EI) mode. The column (15 m × 0.25 mm I.D.) was a CP-SIL-19 CB fused-silica capillary (Chrompack France,

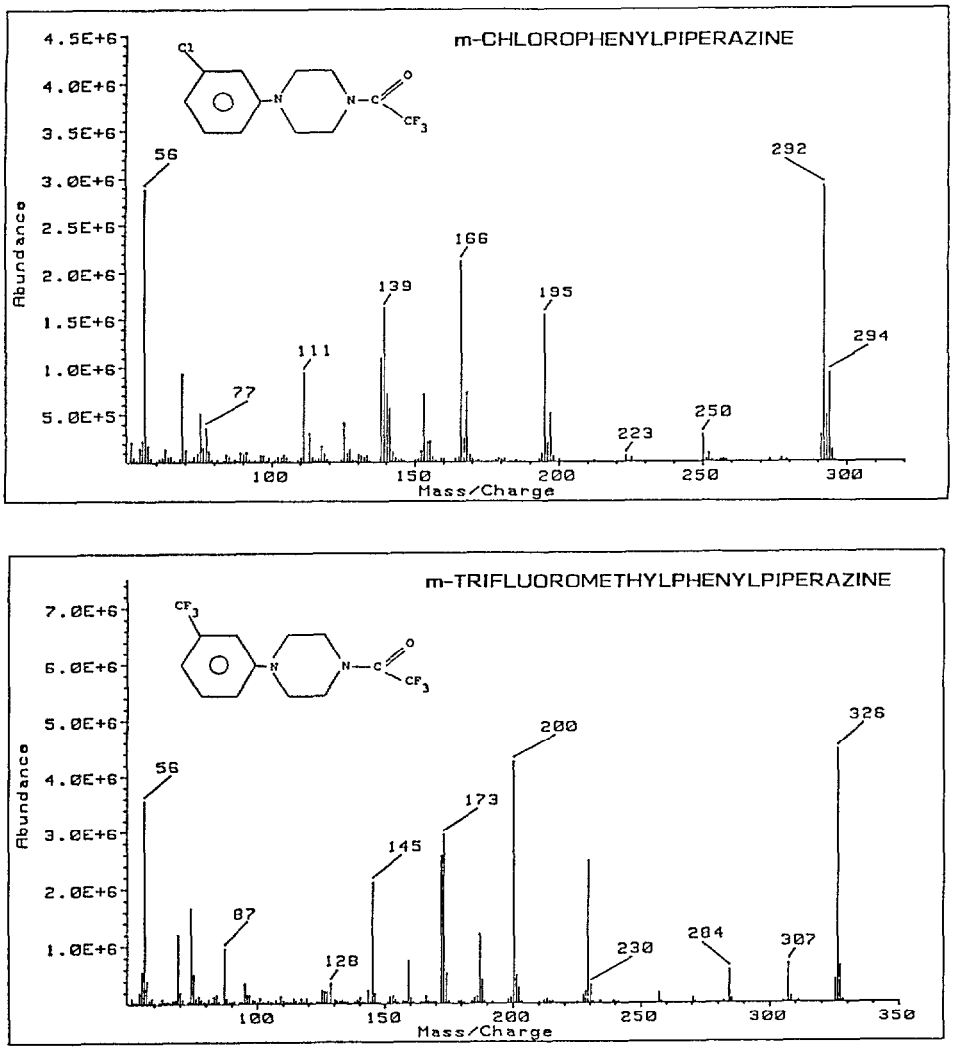

Fig 1 Electron-impact mass spectra of N-trifluoroacetyl derivatives of mCPP and the I.S

Les Ulis, France). Helium was used as carrier gas, at a pressure of *ca.* 0.4 bar. The column was maintained at 80°C for 0.5 min, then programmed at 35°C/min to 220°C and finally to 240°C at 8.5°C/min. The injector and transfer line temperatures were set at 250 and 280°C, respectively.

Selected-ion monitoring

Mass spectra of the trifluoroacetyl derivatives of mCPP and the I.S. were recorded (Fig. 1). Quantitative analysis was performed on the basis of molecular ions, *i.e* m/z 326 for the I.S. and m/z 292 for mCPP.

Selected-ion monitoring recording of plasma and brain samples are shown in Figs. 2 and 3.

Animals and sample collection

The experiments were carried out on male Wistar rats (280–320 g). The animals used in acute experiments were deprived of food for 18 h before each experiment, but they had free access to water.

The rats were treated with mCPP · 2 HCl (3 mg/kg intravenously) and killed at various times. Blood was collected on a citrated tube (75 µl of a 190 mg/ml solution) and centrifuged for 10 min at 1600 *g*. The collected plasma and brains were kept frozen at −20°C until analysed.

Extraction from plasma

Plasma samples (0.25 ml), spiked with 20 ng of the I.S. (20 µl at 1 µg/ml), were placed into a 15-ml glass centrifuge tube with 25 µl of 2 *M* sodium hydroxide solution and extracted twice with 2 ml of isoamyl alcohol–hexane (2:98, v/v) by mechanical shaking for 10 min. After centrifugation (10 min at 1600 *g*) the organic layer was evaporated to dryness, using a Speed Vac concentrator.

Extraction from brain

Brains were homogenized in 0.1 *M* acetic acid (5 ml/g), and 1 ml of this homogenate was transferred to a 15-ml glass centrifuge tube, spiked with 300 ng of the I.S. (30 µl of a 10 µg/ml solution) and 750 µl of a 2 *M* sodium hydroxide solution, and extracted twice with 3 ml of hexane. The organic layer was evaporated to dryness with a Speed Vac concentrator.

Derivatization procedure

To the residue were added 40 µl of ethyl acetate and 100 µl of trifluoroacetic anhydride. The vials were tightly capped and allowed to stand at 60°C for 30 min. After cooling at room temperature, the derivatization mixture was evaporated to dryness using a Speed Vac concentrator. The residue was then dissolved in 20 µl of methanol for plasma or 100 µl for brain, and 2 µl were injected for GC–MS analysis.

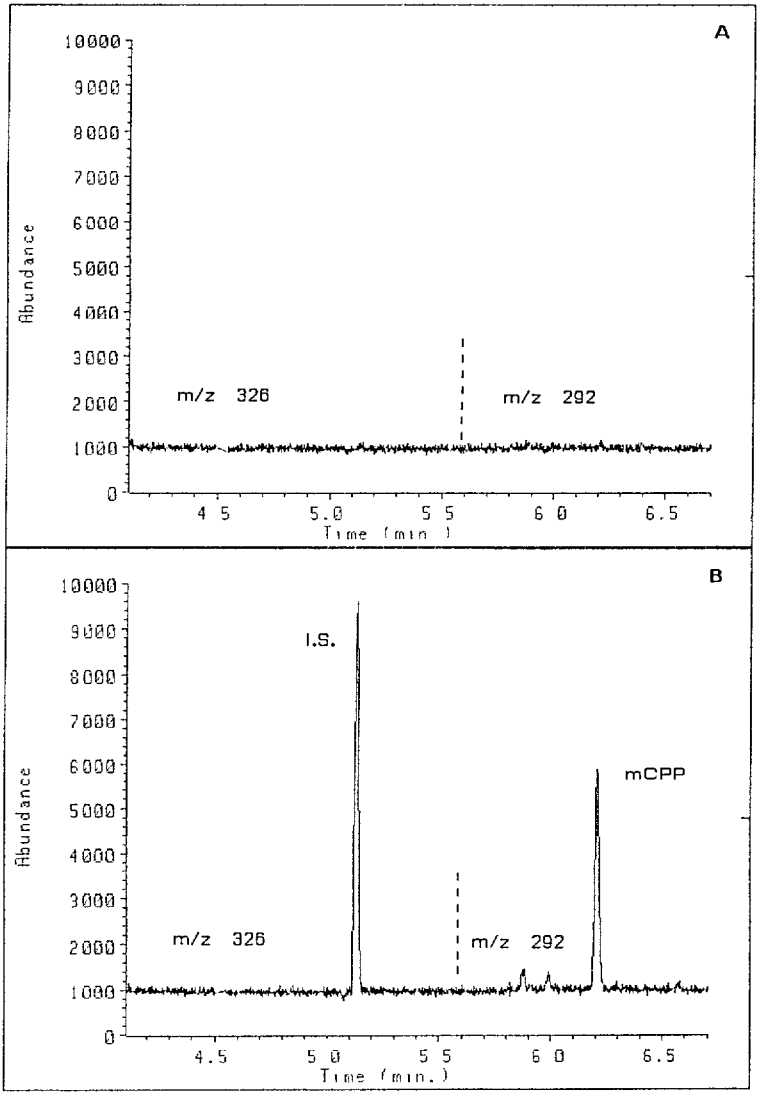

Fig 2 (A) Selected-ion monitoring record of a blank rat plasma extract (0 25 ml) (B) Selected-ion monitoring record of a rat plasma extract (0 25 ml) spiked with 20 ng of mCPP and the I S

RESULTS

Linearity

Over the range of concentrations studied, *i.e.* from 12 ng/g to 10 μg/g for brain and from 4 ng/ml to 0.64 μg/ml for plasma, the linearity was satisfactory and the correlation coefficients were >0.990. The linear regression equations of the mean plots ($n = 7$) were: $y = 0.0027x - 0.0035$ ($r = 0.993$) for brain and $y = 0.047x -$

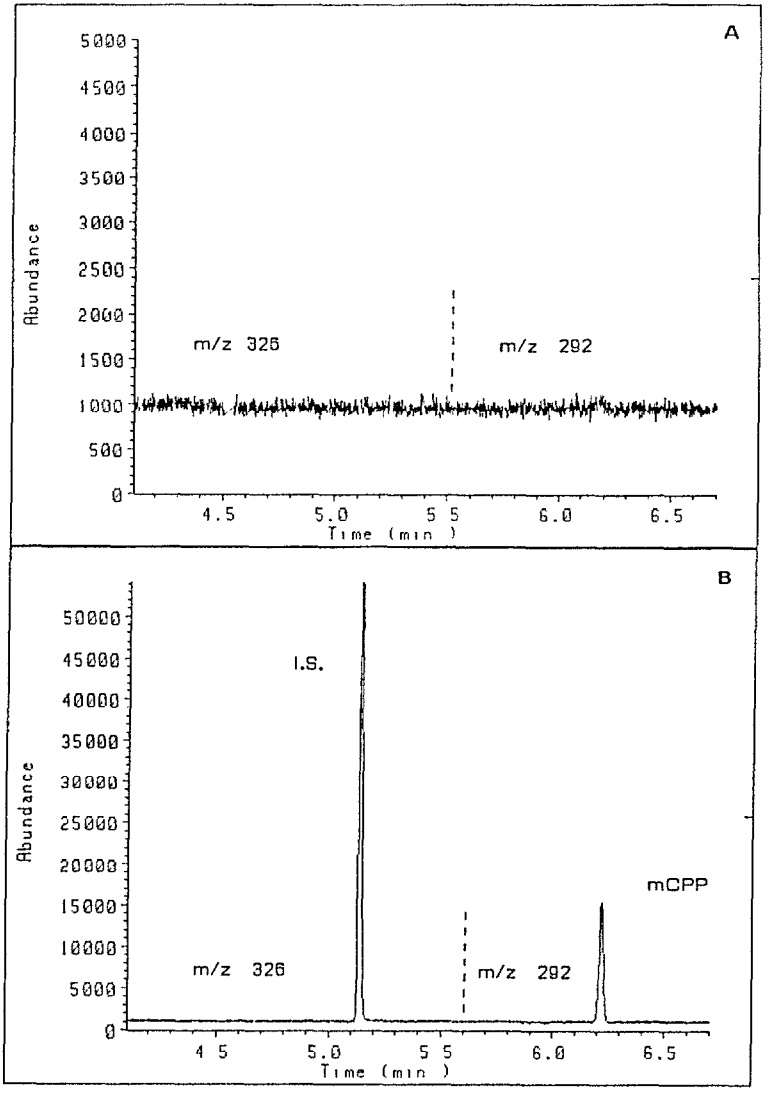

Fig. 3 (A) Selected-ion monitoring record of a blank rat brain homogenate (1 ml) (B) Selected-ion monitoring record of rat brain homogenate (1 ml) spiked with 150 ng of mCPP and the I S

0.020 ($r = 0.994$) for plasma, where y is the peak-area ratio of mCPP to the I.S., x the concentration of mCPP and r the correlation coefficient.

Precision and accuracy

The intra-day precision was checked by determining seven plasma and brain samples spiked with different concentrations of mCPP. The inter-day precision

was determined by analysing plasma and brain samples on seven different days over a period of two months.

The relative standard deviation (R.S.D.) was used as a measure for the precision, and the relative difference between found and added amounts as a measure for the accuracy. The results obtained are given in Tables I–IV.

TABLE I

INTRA-DAY PRECISION AND ACCURACY FOR mCPP IN SPIKED PLASMA SAMPLES

Amount added (ng/ml)	Amount recovered (mean ± S D) (ng/ml)	R S D [a] (%)	Relative error (%)
4	4 5 ± 0 5	10 8	+13 0
8	8 1 ± 0 7	8.3	+1.0
80	80 8 ± 5 2	6.0	+1.0
320	320 8 ± 13 6	4.2	+0.2

[a] R S D = (S D./mean) × 100%, $n = 7$

TABLE II

INTRA-DAY PRECISION AND ACCURACY FOR mCPP IN SPIKED BRAIN SAMPLES

Amount added (ng/g)	Amount recovered (mean ± S D) (ng/g)	R S.D [a] (%)	Relative error (%)
12	13 3 ± 1 9	14 1	+11 0
900	902 5 ± 59 4	6 6	+0.3
12 000	12 035 4 ± 1040 7	8.6	+0.3

[a] $n = 7$.

TABLE III

INTER-DAY PRECISION AND ACCURACY FOR mCPP IN SPIKED PLASMA SAMPLES

Amount added (ng/ml)	Amount recovered (mean ± S D) (ng/ml)	R S.D [a] (%)	Relative error (%)
4	4.3 ± 0 6	14 2	+6 5
8	7 9 ± 0 7	9 4	−1 5
80	79 9 ± 6 4	8 0	−0 2
320	321 4 ± 21.8	6.8	+0.4

[a] $n = 7$.

TABLE IV

INTER-DAY PRECISION AND ACCURACY FOR mCPP IN SPIKED BRAIN SAMPLES

Amount added (ng/g)	Amount recovered (mean ± S D) (ng/g)	R S D [a] (%)	Relative error (%)
12	12 9 ± 1 3	10.2	+7.3
900	938 7 ± 88 8	9 4	+4.3
12 000	11 730 2 ± 1117 0	9 5	−2 2

[a] $n = 7$

Limit of quantitation

The limits of quantitation under the experimental conditions described were 4 ng/ml for plasma (sample size of 0.25 ml) and 12 ng/g for brain. Therefore, the sensitivity of the method in plasma may be improved by performing the assay on 1 ml. The limit was then 1 ng/ml (mean ± S.D. = 1.03 ± 0.17, C.V. = 16.2%, $n = 7$).

In all cases, the limit of quantitation value was above the limit of detection, which was defined as three times the baseline noise.

Extraction recovery

The extraction recoveries were found to be 86.2 ± 8.03% at 80 ng/ml of plasma and 62.7 ± 6.4% at 900 ng/g of brain ($n = 6$). The yield of brain tissue appears to be rather low. This could be related to the extraction solvent used for brain and plasma. The yield of the analyte was better when extracted with isoamyl alcohol–hexane. However, the associated increase of polarity involved the co-extraction of endogenous compounds and produced poor chromatograms.

Application

The present method was used to determine the plasma and brain concentrations of mCPP after intravenous administration of mCPP · 2HCl (3 mg/kg) to rats. Fig.4 shows the curves of mean plasma and brain concentrations of mCPP over 10 h. Each point is the mean of six rats. Plasma and brain elimination half-lives of mCPP were *ca.* 1.1 and 1.2 h, respectively.

CONCLUSION

The proposed technique permits the quantitative assay of mCPP in rat plasma and brain, at concentrations down to 1 ng/ml and 12 ng/g, respectively. It is specific, reproducible and sensitive enough for determination of mCPP in pharmacokinetic, bioavailability and pharmacology studies

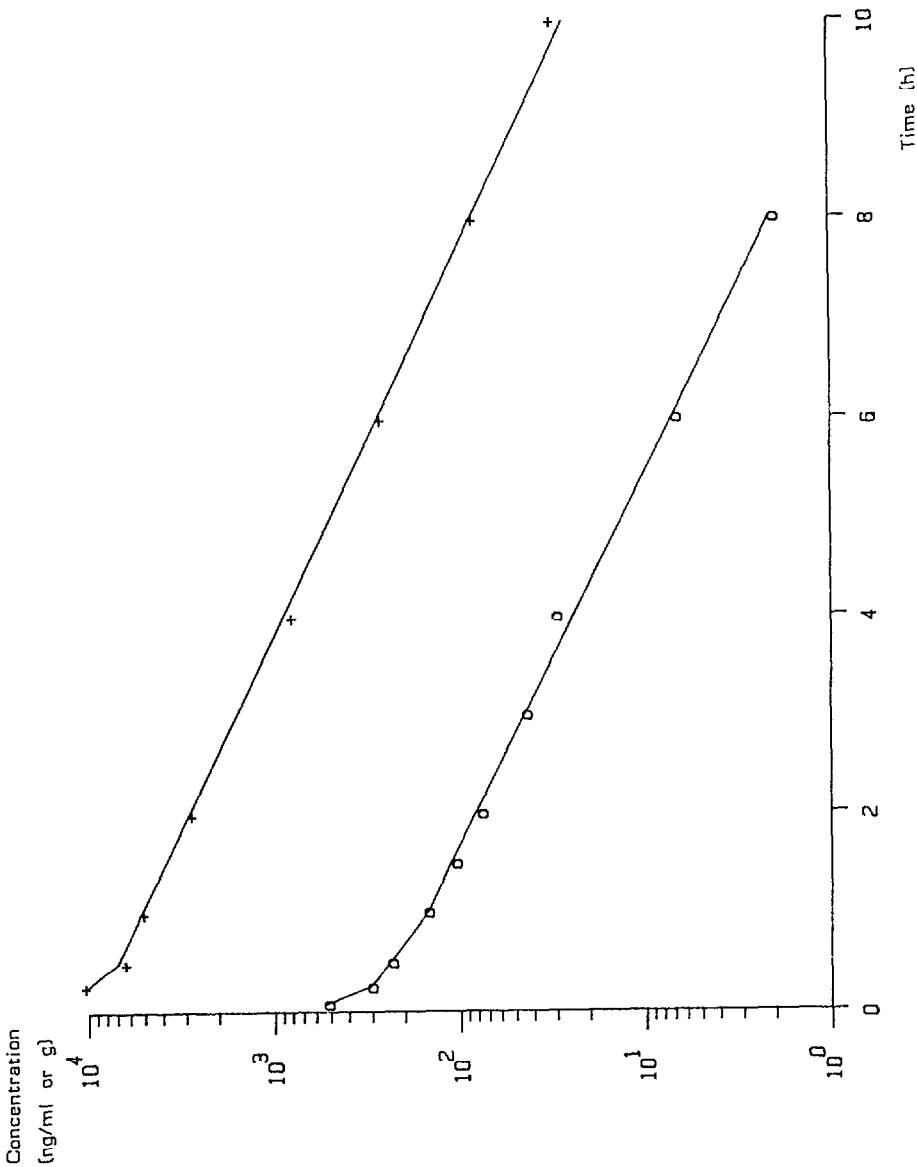

Fig 4 Mean plasma (○) and brain (+) concentrations of mCPP after intravenous administration of mCPP (3 mg/kg) Each point is the mean of six rats

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