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Note

High-performance liquid chromatographic method for the analysis of plasma *m*-chlorophenylpiperazine

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In preclinical studies, *m*-chlorophenylpiperazine (m-CPP, Fig. 1) has been shown to cause changes in serotonin (5-hydroxytryptamine, 5-HT) synthesis and turnover consistent with post-synaptic receptor agonist activity [1]. In animals and man m-CPP produces classic behavioral, neuroendocrine and physiological changes readily reversed by 5-HT antagonists [2–4]. Recently, this compound has been used experimentally in humans in assessing its anxiogenic and neuroendocrine properties [4–6], as well as in Alzheimer's disease [7]. m-CPP, also a metabolite of the antidepressant trazodone [8], has been

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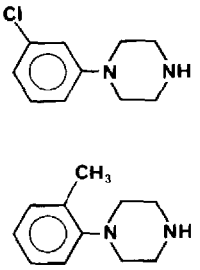

Fig. 1. Structures of *m*-chlorophenylpiperazine (m-CPP, top) and *o*-tolylpiperazine (OTP, bottom)

found to significantly increase obsessional symptoms in many obsessive compulsive patients. Increases in the severity of these symptoms following m-CPP administration was significantly correlated with a corresponding increase in peak m-CPP blood levels [9].

Determination of the plasma pharmacokinetic profile of the m-CPP challenge dose therefore requires a sensitive and specific analytical procedure. Several methods for m-CPP analysis have been reported. Caccia et al. [10] used gas chromatography with electron-capture detection (GC–ECD) following derivatization. Several high-performance liquid chromatographic (HPLC) methods using UV detection at 254 nm [11], 214 nm [12] or electrochemical detection [13] have been reported. Inadequate sensitivity and lengthy chromatographic analysis time inherent in these procedures are two main factors that initiated development of a more specific assay for m-CPP.

EXPERIMENTAL

Reagents and standards

Phosphoric acid, potassium phosphate monobasic, heptanesulfonic acid sodium salt, triethylamine and acetonitrile were all HPLC grade (Fisher Scientific, Fairlawn, NJ, U.S.A.). Methyl *tert.*-butyl ether was obtained from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.).

Both analytical standards, 1-(3-chlorophenyl)piperazine hydrochloride (m-CPP·HCl) and 1-(*o*-tolyl)piperazine dihydrochloride (OTP·2HCl) were obtained from Aldrich (Milwaukee, WI, U.S.A.). Stock solutions (1 mg/ml) and working standards (1 ng/μl) of each were prepared in 0.01 *M* hydrochloric acid.

Apparatus

Chromatography was performed using a Model 510 solvent delivery pump, an Ultra WISP Model 715 autosampler and a Model 440 UV detector with an extended-wavelength module operating at 214 nm (Waters Assoc., Milford, MA, U.S.A.). Separations were carried out using a 25 cm × 4.6 mm I.D. column

packed with a 5 μm particle size trimethylsilyl-bonded silica (LC-1, Supelco, Bellefonte, PA, U.S.A.). Chromatograms were recorded on a Model B5217-5 Omniscribe recorder (Houston Instruments, Austin, TX, U.S.A.). Chromatographic data were collected and analyzed on a Model 3203 chromatography laboratory automation system (Perkin-Elmer, Norwalk, CT, U.S.A.). UV scans were performed using a Model 1040M photo-diode array detector with a Series 300 controller (Model HP79996, Hewlett-Packard, Palo Alto, CA, U.S.A.) operating software under the same chromatographic conditions described below.

Extraction

Blood was collected from human subjects and immediately transferred to specially prepared glass tubes containing balanced oxalate as the anticoagulant. The plasma was separated and transferred to clean tubes and stored frozen at −20 °C until processed.

Five-point standard curves are processed with each analytical run. These curves comprise drug-free human plasma spiked with known amounts of m-CPP. Aliquots (1 ml) of these standards were processed exactly as the unknown samples. For assay, 1 ml of standard or unknown sample was pipetted into a clean borosilicate glass tube and 75 μl (75 ng) of internal standard OTP were added. A 1-ml volume of 0.6 *M* carbonate buffer (pH 9.8) and 6.0 ml of methyl *tert.*-butyl ether were then added and the mixture was gently shaken for 15 min, then centrifuged at 1500 *g* for 15 min. The organic layer was then transferred to a 15-ml tapered glass centrifuge tube containing 250 μl acidic phosphate buffer (pH 2.2), mixed for 10 min and centrifuged for 10 min at 1500 *g*. The organic layer was aspirated and discarded and the aqueous portion transferred to small glass inserts suitable for automatic injection by the WISP 715.

Chromatographic conditions

The mobile phase consisted of 0.05 *M* potassium phosphate monobasic (pH adjusted to 3.2 with phosphoric acid) and acetonitrile (82:18). Triethylamine (1.2 ml/l) and 20% heptanesulfonic acid solution (5 ml/l) were added. The flow-rate was 1.8–2.0 ml/min and the temperature ambient. UV detection was optimized by using a zinc lamp with a discrete wavelength filter at 214 nm.

Quantitation

All determinations of plasma samples were calculated based upon the peak-height and peak-area ratios using the internal standard method. Calibration standards and quality control samples were run with each batch of samples.

RESULTS AND DISCUSSION

Analysis of plasma m-CPP can be routinely performed using column liquid

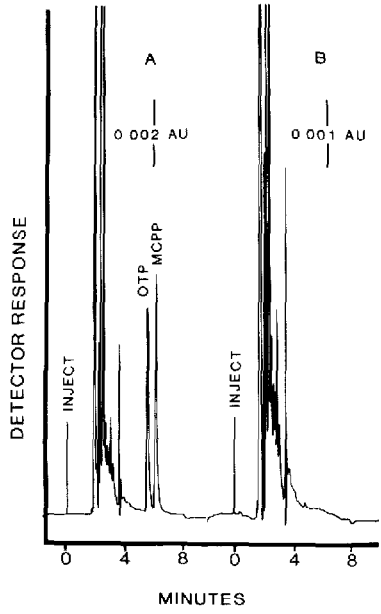

Fig. 2. (A) Chromatogram of a 1-ml plasma standard spiked with 50 ng of m-CPP. (B) Chromatogram of a 1-ml plasma blank. Chromatographic conditions are described in text.

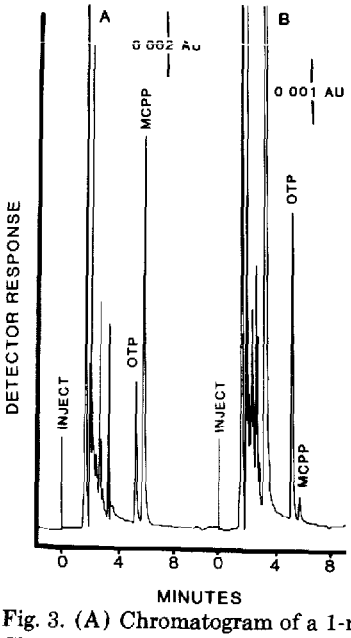

Fig. 3. (A) Chromatogram of a 1-ml patient plasma sample containing 122 ng/ml m-CPP. (B) Chromatogram of a 1-ml patient plasma sample containing 4 ng/ml m-CPP.

chromatography coupled with UV detection at 214 nm. Chromatographic analysis time is usually less than 8 min per sample. The system is very stable which permits long unattended analyses and adequate sensitivity (< 3 ng/ml) to allow for pharmacokinetic studies. Typical chromatograms appear in Figs. 2 and 3. Drug-free plasma contains no interfering endogenous material (Fig. 2B). Least-squares linear regression analysis of the standard curve data showed high linearity ($r \geq 0.9995$) with consistently low intercepts. Maximum sensitivity was achieved by utilizing a UV detector set at 214 nm, which is very near the UV maximum for m-CPP as well as OTP (Figs. 4 and 5) at these chromatographic conditions. Peak enhancement using the amine modifier triethylamine also contributed to increased sensitivity.

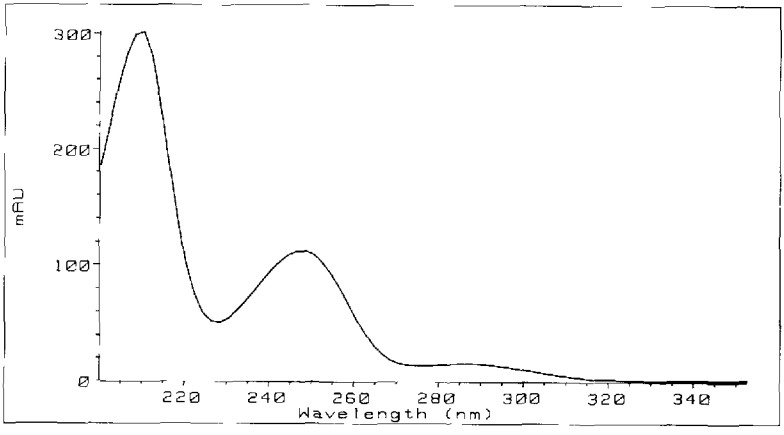

Fig. 4. UV absorbance plot of m-CPP obtained from a diode-array detector. Conditions are as described under Experimental.

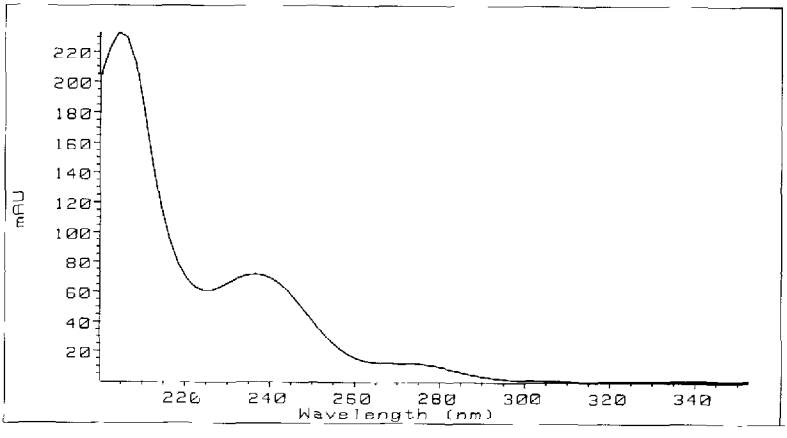

Fig. 5. UV absorbance plot of OTP. Conditions the same as in Fig. 4

The absolute recovery of plasma m-CPP at 25 ng/ml yielded 96 ± 7.5% ($n = 10$). The intra- and inter-assay variation is shown in Table I. The quality control samples at 25 ng/ml (inter-assay control) that were included in each day's run demonstrates the stability and reliability of the method over a three-year period. We have not observed any interfering peaks in any of the samples tested to date. This was not unexpected since the protocol for the m-CPP challenge tests requires the subjects to be drug-free for at least two to four weeks [5,6]. While most neuroleptics and antidepressant drugs (e.g. haloperidol, chlorpromazine, amitriptyline, imipramine) would elute beyond 10 min under these chromatographic conditions, it is quite conceivable that their respective polar metabolites could interfere with OTP and/or m-CPP. This was found to be the case with the 10-hydroxy metabolites of amitriptyline and nortriptyline and the major metabolite of bupropion (BW306U).

Fig. 6 illustrates a plasma concentration–time profile of a patient receiving

TABLE I

INTRA- AND INTER-ASSAY VARIATION FOR PLASMA m-CPP

Concentration added (ng/ml)	Concentration found (mean ± S.D.) (ng/ml)	n	Coefficient of variation (%)
Intra-assay			
3	2.99 ± 0.202	11	6.8
50	52.0 ± 1 82	10	3.5
Inter-assay			
25	24 7 ± 1.69	103	6.8

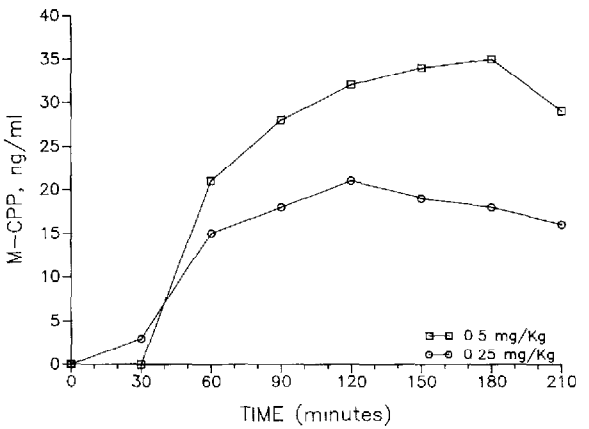

Fig. 6. Plasma concentration-time curve of a patient receiving two different oral doses of m-CPP.

two different single doses, each dose separated by six weeks. Establishing a profile such as this is essential in determining the availability of m-CPP at the 5-HT receptor site. Adequate sensitivity and precision enables meaningful correlations with other endocrine measures such as prolactin, ACTH and cortisol. Preliminary results using this analytical procedure have been presented [6,9,14].

In terms of analysis time and sensitivity, this specific assay for m-CPP represents an improvement over the more complex GC–ECD method [10] which requires derivatization. The previous HPLC methods [11–13] included the simultaneous quantitation of trazodone, the parent drug, causing prolonged chromatographic analysis times. This method has been in use in our laboratory for over four years during which time several thousand samples have been processed. It is reliable, rapid and can be adapted to any research laboratory with the basic HPLC equipment and technical ability.

ACKNOWLEDGEMENT

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