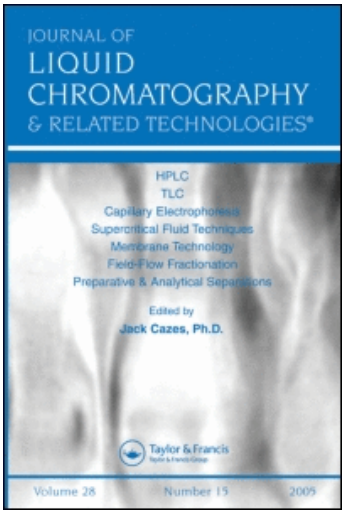

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A Simultaneous Determination of Trazodone and Its Metabolite 1-m-Chlorophenylpiperazine in Plasma by Liquid Chromatography with Electrochemical Detection

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A SIMULTANEOUS DETERMINATION OF TRAZODONE AND ITS METABOLITE 1-m-CHLOROPHENYLPIPERAZINE IN PLASMA BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

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ABSTRACT

A liquid chromatographic method coupled with electrochemical detection was developed to measure plasma trazodone and its metabolite 1-m-chlorophenylpiperazine (m-CPP). Following extraction from 1 ml of alkaline plasma with methyl-t-butyl ether, the extracts were chromatographed on a reversed phase trimethylsilyl bonded column using a 0.05 M phosphate buffer and acetonitrile (90:10) with n-nonylamine and sodium heptane sulfonate added to the mobile phase. The compounds were detected via a thin layer electrochemical transducer with glassy carbon electrodes at a potential of +1.15V vs Ag/AgCl reference electrode. The recovery of trazodone ranged from 91-97% and the coefficient of variation was less than 5% for between run and within-run analyses. The recovery of m-CPP ranged from 82-86% and the coefficient of variation was less than 8% for between run and within-run analysis. Steady state plasma concentration data are presented from several patients.

INTRODUCTION

Trazodone is a triazolopyridine derivative with antidepressant activity that is chemically unrelated to other currently available antidepressant agents (Fig. 1a). In animals, trazodone exhibits

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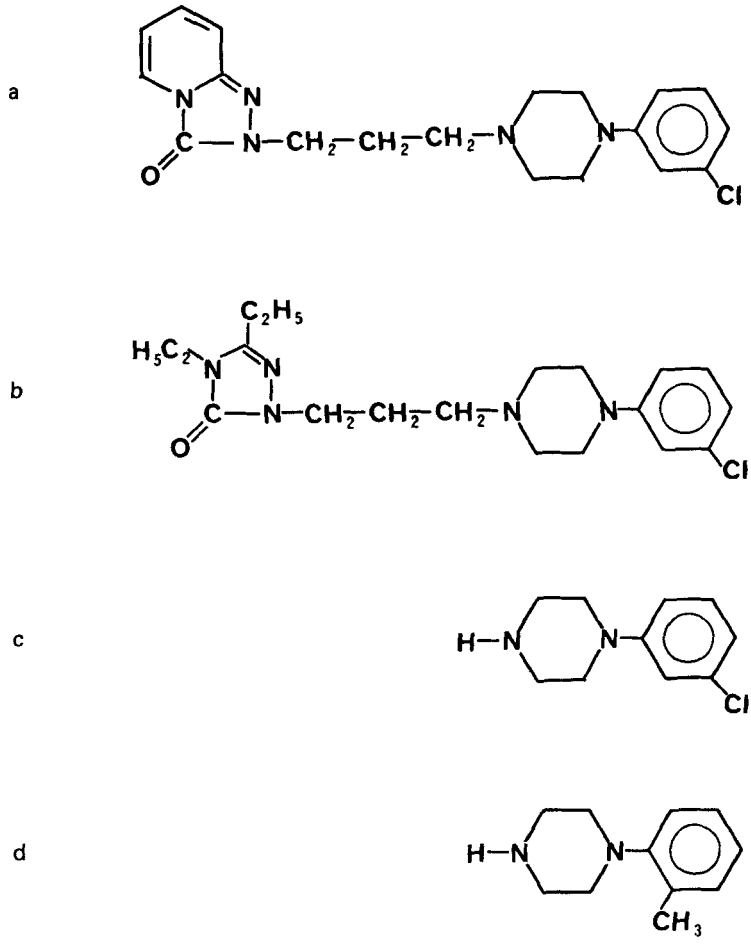

Figure 1: Chemical structures of (a) trazodone, (b) etoperidone, (c) m-chlorophenylpiperazine (m-CPP) and (d) 1-o-tolyl-piperazine (o-TP)

antiserotonin activity, but its mechanism of action in depressive illness in humans is not clear. Trazodone also possesses α-adrenergic blocking activity, but has very little anticholinergic effects as demonstrated in animal studies as well as in clinical trials in depressed patients. Several reviews of trazodone, its

pharmacology, chemistry, and therapeutic efficacy have been presented (1,2,3,4).

The biotransformation of trazodone can occur through oxidative and hydrolytic reactions causing the formation of hydroxy derivatives on both the pyridine and benzene nucleus and a diol derivative on the triazolopyridine ring. The hydrolytic reactions result in the formation of oxotriazolopyridinepropionic acid and its conjugate (5). The remaining fragment of the molecule resulting from hydrolytic cleavage is m-chlorophenylpiperazine (m-CPP) (Fig. 1c).

Recently, it has been suggested that m-CPP contributes to, or even accounts for the antidepressant action of the parent drug (6). Studies revealed that this metabolite acts as a direct serotonin receptor agonist (7). After oral administration of trazodone, m-CPP accumulates in the rat brain at a concentration comparable to that found after pharmacologically and biochemically effective doses of m-CPP (8). Maj et al. (9) reported that trazodone at low doses has antiserotonin properties while at higher doses, it acts as a central serotonin agonist. Furthermore, m-CPP was shown to be a pharmacologically active metabolite common to two structurally related psychotropic drugs etoperidone (Fig. 1b) and mepiprazole (10). In rats, the concentration of m-CPP in brain tissue reached several times that in body fluids (11).

To date, two methods have been reported for the determination of trazodone in plasma. Ankier et al. (12) used reversed-phase high-performance liquid chromatography with UV detection (254 nm) and achieved a lower limit of detection of 20 ng/ml for trazodone

only. Caccia et al. (11) quantitated both trazodone and its metabolite (mCPP) in plasma and brain tissue by gas-liquid chromatography. The latter method is complicated by the fact that after extraction the extracts were divided so that m-CPP could be derivatized and measured by electron capture while trazodone was quantitated by a nitrogen selective detector.

MATERIALS

Reagents

Acetonitrile (UV grade) and methyl-tert-butyl ether were obtained from Burdick and Jackson Laboratories (Muskegon, MI), sodium heptane sulfonate (Eastman Kodak Co., Rochester, NY) and n-nonylamine(Aldrich Chemical Co., Milwaukee, WI) were used without further purification. Reagent grade potassium phosphate monobasic and phosphoric acid were obtained from Fisher Scientific Co. (Fairlawn, NJ). Distilled water was passed through a water purification system before use (Milli-Q, Millipore Corp., Bedford, MA).

Trazodone HCl, etoperidone HCL, and m-chlorophenylpiperazine HCl (m-CPP) were kindly supplied by Dr. Keith Wheeler (Mead Johnson & Co., Evansville, IN). 1-(o-tolyl)piperazine (o-TP) 2 HCl was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Standards

Stock solutions of 1 mg/ml of trazodone, etoperidone m-CPP and 1-(o-totylpiperazine) were prepared in 0.01N HCl and stored refrigerated. Working standards were prepared in 0.01N HCl in

concentrations of 10 ng/ul, for trazodone and etoperidone and and 1 ng/ul for m-CPP and 1-(o-tolyl)piperidone (o-TP).

Instrumentation

Chromatography was performed with a Model 6000A solvent delivery system and a Model U6K injector or WISP 710B automatic injector (Waters Associates, Milford, MA). The column was 4.6 mm i.d. x 25 cm packed with 5 μ particle size trimethylsilyl material (LC-1, Supelco, Bellafonte, PA). A Model LC-4B amperometric detector with a TL-5A (Bioanalytical Systems Inc., W. Lafayette, IN) thin layer transducer was used to monitor the compounds of interest. The current response was recorded by a dual pen Omniscribe Recorder (Houston Instruments, Austin, TX) at 1 V and 10 V inputs full scale preceded by a signal amplifier (gain = x1).

Cyclic voltammetry was carried out using a Model CV-1B cyclic voltammetry instrument (Bioanlytical Systems, W. Lafayette, IN) and a Model 7034A x-y recorder (Hewlett-Packard, Palo Alto, CA). The potential was scanned from 0.0V to + 1.3 V vs Ag/AgCl reference electrode at 180 mv/sec. Glassy carbon was used as the working electrode.

Sample Extraction

To 1 ml of plasma, 60 ul (600 ng) of internal standard etoperidone, 25 ul (25 ng) of the internal standard 1-(o-tolyl) piperazine, 1.0 ml carbonate buffer (0.6 M pH 9.5) and 8 mls of methyl-tert-butyl ether were added. The mixture was shaken for 10 minutes and centrifuged for 10 minutes. The organic layer was then transferred

to 15 ml tapered centrifuge tube containing 1.2 ml 0.1N HCl. After mixing and centrifuging for 10 minutes, the top layer was aspirated and the aqueous portion transferred to a 3 ml tapered glass-stoppered centrifuge tube. The contents were made alkaline with 0.5ml carbonate buffer (0.6 M pH 9.5) and extracted with 0.7 ml of methyl-tert-butyl ether. After mixing and centrifuging for 5 minutes, the lower layer was discarded and the ether layer transferred to small 1.0 ml vials or "low volume inserts" if automatic sampling was desired. After evaporation to dryness in a vacuum concentrator, Model SVC-100 M Speed Vac Concentrator, (Savant Instruments, Hicksville, NY), the residue was redissolved in 100 ul of mobile phase and injected on column.

Chromatographic Conditions

The mobile phase consisted of 90% phosphate buffer (0.05 M pH 3.0) and 10% acetonitrile with 0.005 M sodium heptane sulfonate and 0.005 M n-nonylamine added. The flow rate was 2.2 ml/min and temperature ambient. The effluent was monitored via a thin-layer flow through electrochemical transducer having a potential of +1.15 V vs Ag/AgCl reference electrode.

Quantitation

The peak-height ratios of trazodone to the internal standard etoperidone and m-CPP to the internal standard o-TP were plotted against concentration. A least squares linear regression analysis of these data were used to calculate the parameters slope, x-intercept, correlation coefficient and standard errors.

RESULTS AND DISCUSSION

Trazodone and its metabolite m-CPP were extracted from 1 ml of plasma, separated by reversed phase liquid chromatography, and quantitated by an electrochemical detector. The use of a divided signal from the controller and a dual recorder set at different attenuations permitted the simultaneous determination of trazodone and m-CPP without changing attenuation during each chromatogram. Thus, this procedure could be adapted to automatic sample processing.

In addition to the large difference in concentrations between plasma trazodone and m-CPP, other problems such as peak tailing and poor resolution due to possibly other as yet unidentified metabolites of trazodone were initially encountered. After testing several bonded reverse-plase columns (C_{18}-C_8) a trimethylsilyl bonded reverse phase column was found to adequately resolve the peaks of interest. The addition of n-nonylamine to the mobile phase and maintaining the pH at 3 improved peak symmetry and reduced overall retention times. Fig. 2 shows a dual chromatogram of a spiked plasma sample. The selection of two internal standards was obviously necessary because of the aforementioned concentration differences in plasma trazodone and m-CPP. Etoperidone (Fig. 1b) closely resembles trazodone chemically while o-tolylpiperazine (o-TP) (Fig. 1d) is chemically related to m-CPP. Because of the presence of the phenyl piperazine moiety, all of these compounds displayed similar cyclic voltammograms (Fig. 3). The scans indicate an irreversible oxidation reaction at ca +1.1V vs Ag/AgCl reference electrode.

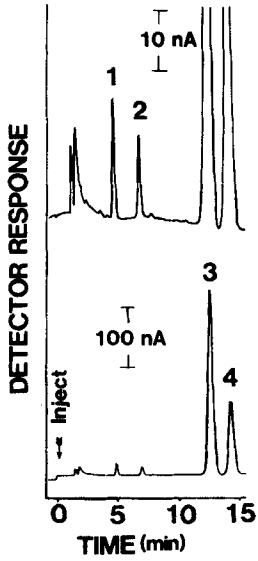

Figure 2: A dual chromatogram of a 1 ml spiked plasma sample containing 20 ng of m-CPP (2) and 1000 ng of trazodone (3). The entire reconstituted extract was injected. Etoperidone (4) and o-TP (1) are internal standards.

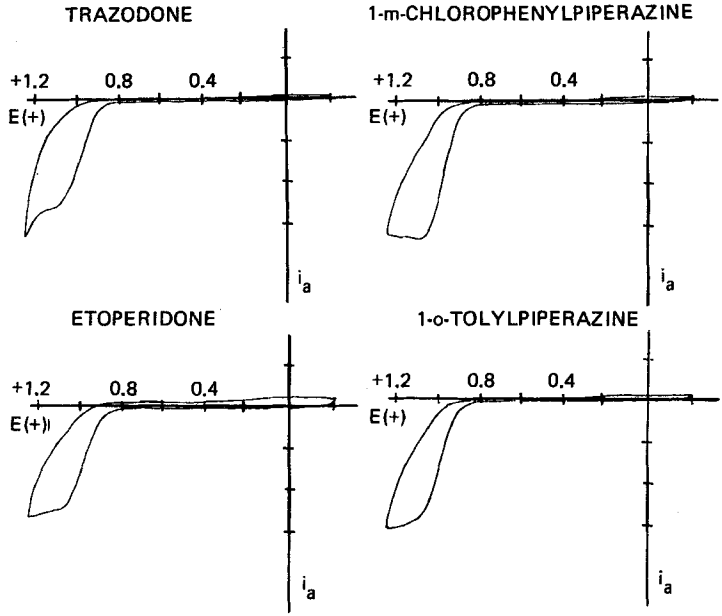

Figure 3: Cyclic voltammograms of trazodone, etoperidone, m-chlorophenylpiperazine (m-CPP) and 1-o-tolylpiperazine (o-TP). Concentrations for all compounds were 2 mg/10 ml of mobile phase.

Therefore, the detector was set at the the optimum potential +1.15 V vs Ag/AgCl reference electrode.

Even at this high potential, the chromatograms did not indicate any interfering endogenous peaks as shown by a plasma blank (Fig. 4). Therefore the minimum quantifiable level of detection was 5 ng/ml for m-CPP, while for trazodone 100 ng/ml was the lower limit because of the relatively high level of the internal standard etoperidone. If single dose studies were necessary then this limit would be reduced to ca 10 ng/ml by addition of 10% of the internal standard. With a signal to noise ratio = 3, 1 ng of m-CPP or trazodone injected directly can easily be detected. Fig. 5 shows a dual chromatogram of an actual patient plasma sample. The presence of "x" between peaks 1 and 2 is probably an induction of one or more as yet unidentified metabolites.

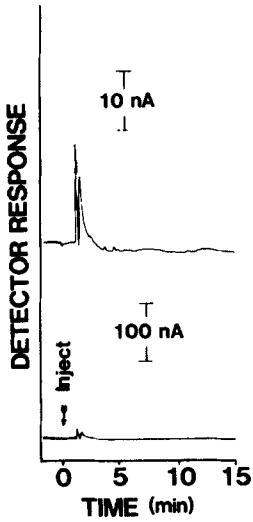

Figure 4: A dual chromatogram of a 1 ml blank plasma extract. Entire reconstituted extract was injected.

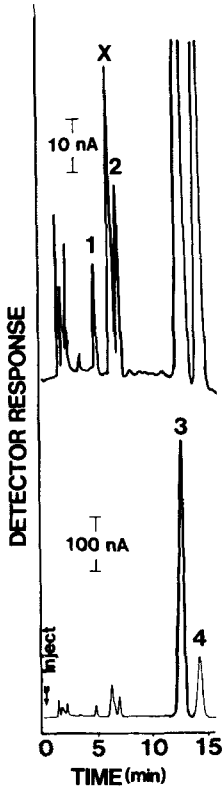

Figure 5: A dual chromatogram of a 1 ml plasma extract from a patient receiving trazodone daily for 3 weeks. Trazodone (3) levels were found to be 1797 ng and m-CPP (2) was at a concentration of 54 ng. The peak "x" probably represents other as of yet unidentified metabolites of trazodone.

The absolute recovery of trazodone and m-CPP was determined by spiking 1 ml of plasma with various concentrations of each compound. The extraction was carried out quantitatively. The internal standards were added after evaporation of the extract and again brought to dryness. The residue was reconstituted with mobile phase and injected. The same standards (non-processed and internal

standards) were also brought to dryness and reconstituted with mobile phase and injected directly on column. The difference between the ratio of the standards and internal standard in the porcessed samples compared to the direct injection sample gave a measure of the overall recovery (Table 1).

The precision of the reported procedure was determined by spiking eight 1 ml aliquots of drug-free plasma with various concentrations of both trazodone and m-CPP. The samples were processed and chromatographed as described. The results appear in Table 2.

A measure of the stability of the assay is demonstrated by the variability in the slope of the linear regression curves on 6 different days (Table 3).

Steady-state plasma concentrations were analyzed for trazodone and m-CPP (Table 4).

TABLE 1

Recovery of Trazodone and m-CPP from Plasma (n=8).

Compound	Concentration (ng/ml)	Percent Recovery	C.V. %
Trazodone	2000	91	2.4
	500	97	2.8
m-CPP	50	82	4.2
	10	86	8.3

TABLE 2

Within-run Precision of Assay (n=8).

Compound	Concentration (ng/ml)	C.V. %
Trazodone	2000	1.5
	1000	1.5
	100	1.5
m-CPP	50	6.6
	20	2.3
	5	2.8

TABLE 3

Day-to-day Stability of Assay Based Upon Slope of the Linear Regression Curves (n=6)

	Slope (ng/ml)	+ S.D.	C.V.%
Trazodone	378.5	17.05	4.5
m-CPP	28.0	2.03	7.3

TABLE 4

Steady-State Plasma Concentrations in Five Depressed Patients

Patient	Trazodone (ng/ml)	m-CPP (ng/ml)
1	824	8
2	496	20
3	875	10
4	1212	28
5	618	22

The metabolite m-CPP ranged from 1 to 4% of the parent drug in plasma. In a single dose kinetic study (6), m-CPP reached 10 ng/ml at C_{max}, amounting to about 1% of the parent drug plasma concentrations. In another study (11), m-CPP was found to be present in significant quantities in rat brain after oral administration.

This method should facilitate the quantitation of trazodone and m-CPP in biological fluids and aid in the further eludication of the role of this metabolite in clinical efficacy and side effect relationships in man.

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