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Note

Analysis of trazodone and *m*-chlorophenylpiperazine in plasma and brain tissue by high-performance liquid chromatography

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Trazodone is a triazolopyridine derivative compound available as a treatment for depressive illness [1]. Although its mechanism of action is unknown, trazodone possesses serotonin antagonist activity while its major active metabolite, 1-*m*-chlorophenylpiperazine (m-CPP) possesses agonist activity [2—4]. Of interest in elucidating the central effects of trazodone is knowledge of the drug's disposition and that of its metabolite in brain tissue compared to plasma. Previous methods for analysis of trazodone have only described application to plasma [5], have ignored m-CPP [6—9] or have depended upon gas—liquid chromatography requiring derivatization of m-CPP for analysis [10]. We have developed a precise and reproducible method using high-performance liquid chromatography (HPLC) for measurement of trazodone and m-CPP in either plasma or brain tissue. This method is suitable for the determination of these antidepressant compounds in single- or multiple-dose pharmacokinetic studies in animals or man.

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EXPERIMENTAL

Apparatus

Chromatography was performed with a Waters (Milford, MA, U.S.A.) Model 6000A pump fitted with a Rheodyne 7126 injection valve (Rheodyne, Cotati, CA, U.S.A.) and a 50-μl sample loop. A 25 cm × 4.6 mm reversed-phase column (IBM Instruments, Wellingford, CT, U.S.A.) was used containing 5-μm particle size trimethyl silane as the sorbent. An IBM 9523 variable-wavelength UV detector was operated at 214 nm. An IBM CS9000 laboratory computer was used to record, store and analyze chromatograms.

Reagents and chemicals

Analytical-grade phosphoric acid, monobasic potassium phosphate, sodium borate, sodium carbonate triethylamine, heptane sulfonic acid, acetonitrile, hexane and methyl-*tert*.-butyl ether were obtained from Fisher Scientific (Fairlawn, NJ, U.S.A.). Trazodone and m-CPP were gifts from Mead-Johnson (Evansville, IN, U.S.A.) while bupropion hydrochloride (internal standard) was obtained from Burroughs-Wellcome (Research Triangle Park, NC, U.S.A.). Water was passed through a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.) before use.

Mobile phase and buffers

The mobile phase was a mixture of acetonitrile—phosphate buffer, pH 3.0 (27:73) and contained 0.02 *M* heptane sulfonic acid and 0.04 *M* triethylamine. A flow-rate of 1.5 ml/min at ambient laboratory temperature (22°C) was used.

Standards for calibration graphs

Stock solutions of trazodone, m-CPP and bupropion (internal standard) were prepared by dissolving the appropriate amounts in methanol to make 1 mg/ml free base solutions. Standards for calibration curves were prepared by spiking 1.0-ml aliquots of plasma with diluted stock solutions to make m-CPP standards ranging from 10 to 100 ng/ml and trazodone standards ranging from 100 to 1000 ng/ml. For assay of brain tissues, standards in brain for both trazodone and m-CPP ranged from 20 to 500 ng/ml. Six standards within the ranges stated were used for each calibration curve. The concentration of the internal standard was 200 ng/ml in each plasma sample and 100 ng/ml for brain samples. Calibration graphs of the recovered standards were prepared for each day of analysis to establish the linearity and reproducibility of the HPLC system. Graphs were constructed of the peak-height ratio of each compound to internal standard against drug concentration.

Extraction procedure for plasma and brain

For plasma, 1.0 ml of standard or unknown sample was pipetted into a polypropylene tube and 10 μl (200 ng) of internal standard, 0.5 ml of saturated sodium borate solution and 5.0 ml of methyl-*tert*.-butyl ether were added. Each sample was mixed briefly following each addition using a Vortex-type mixer. The tubes were stoppered and mixed on a reciprocating shaker (Eber-

bach, Ann Arbor, MI, U.S.A.) for 10 min. After centrifuging for 10 min at 220 *g*, the ether phase was transferred with a pasteur pipet into a clean test tube and 75 μl of 10 m*M* phosphoric acid were added for back-extraction. Following vortex-mixing, the samples were centrifuged and the ether layer was removed. A 50-μl aliquot of the remaining aqueous phase was injected onto the HPLC column.

The extraction procedure for brain tissue varied slightly from that of plasma. Whole brains which had been removed from untreated rats and rats previously treated with trazodone were weighed and placed in 50-ml polypropylene centrifuge tubes. To each tube were added 10 ml of 0.34 *M* perchloric acid containing EDTA ($1 \cdot 10^{-5}$ *M*) and the brains were homogenized for 20 s using a Brinkman Model PT 10/35 homogenizer (Brinkman Instruments, Westbury, NY, U.S.A.). To a 1.0-ml aliquot in a polypropylene tube, 5 μl (100 ng) of internal standard, 0.5 ml of 0.6 *M* sodium carbonate and 3.0 ml of 2% isoamyl alcohol in hexane were added. The tubes were stoppered and mixed on a reciprocating shaker for 10 min, then centrifuged for 10 min at 220 *g*. The hexane phase was transferred to a clean test tube. A second aliquot of hexane was added to the sample and the extraction repeated. The hexane phases were combined and extracted into 75 μl of 10 m*M* phosphoric acid as described for plasma. A 50-μl aliquot was used for injection onto the HPLC column.

Recovery and assay validation

Recovery and within-day precision were determined by comparing the peak heights from extracted samples without internal standard with those obtained from a direct injection of the same amount of drug in methanol. Day-to-day variability was assessed by extracting an aliquot of frozen spiked sample for ten consecutive assay days. A standard curve was prepared daily and the calculated sample concentration compared to the theoretical value for the spiked sample.

RESULTS

The chromatographic procedure separated trazodone, its major metabolite, m-CPP, and bupropion (internal standard) with a short overall run time. Retention times for m-CPP, internal standard and trazodone were 7.1, 10.6 and 12.8 min, respectively. Calibration curves were consistently linear and passed through the origin. Fig. 1 illustrates typical chromatograms of a methanol stock solution, extract from brain tissue of an untreated animal, extract from a spiked brain sample and extract of a brain from a rat previously treated with trazodone. Extracts of plasma samples resulted in chromatograms with similar appearance.

Table I illustrates the recovery and within-run precision data obtained by chromatographing spiked batch plasma and brain tissue homogenates. Recoveries of trazodone from plasma and tissue and *m*-CPP from plasma were greater than 83%. The recovery of m-CPP from brain tissue at 50 ng/g was 72% and decreased over a ten-fold increase in concentration into 58% at 500 ng/g. The variability coefficients were low, less than 3.5% in all cases. Table II shows between-day precision data for trazodone and m-CPP in spiked plasma. The coefficients of variation were between 5.8 and 10%.

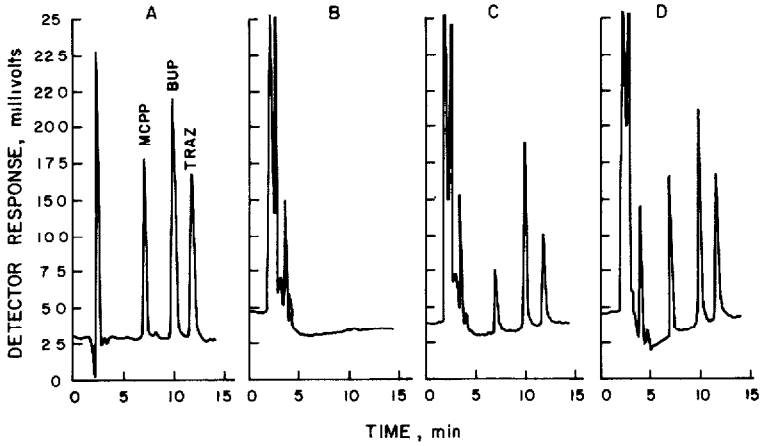

Fig. 1. Chromatograms of (A) methanol stock solution containing 100 ng of trazodone (TRAZ), 100 ng of bupropion (BUP, internal standard) and 100 ng of m-CPP (MCPP); (B) extract from blank brain; (C) an aliquot of an extract from brain spiked with 100 ng of each compound; (D) extract of brain from a rat previously treated with 10 mg/kg trazodone. The concentration of trazodone in this sample was 851 ng/g while the m-CPP concentration was 804 ng/g.

TABLE I

RECOVERY OF TRAZODONE AND m-CPP FROM TISSUES AND WITHIN-RUN ASSAY REDUCTION (n = 6)

Compound	Tissue	Concentration (ng/ml or ng/g)	Recovery (%)	Coefficient of variation (%)
Trazodone	Plasma	250	89.6	2.3
		500	89.6	2.0
		1000	90.4	1.9
	Brain	50	84.0	3.3
		100	83.0	1.7
		500	80.0	2.8
m-CPP	Plasma	25	98.8	3.0
		50	98.5	2.0
		100	90.0	2.1
	Brain	50	72.0	2.6
		100	65.0	2.3
		500	58.0	3.4

TABLE II

DAY-TO-DAY VARIABILITY FOR CALIBRATION GRAPHS AND ALIQUOTS OF SPIKED PLASMA ASSAYED FOR TEN DAYS

Standards were prepared daily in the ranges of 100—1000 ng/ml for trazodone and 10—100 ng/ml for m-CPP.

Compound	Added concentration (ng/ml)	Measured concentration (mean ± S.D.) (ng/ml)	Coefficient of variation (%)
Trazodone	250	255 ± 14.8	5.8
m-CPP	25	25.6 ± 2.6	10.0

DISCUSSION

Previous HPLC methods have been described for quantifying trazodone in plasma [5, 8, 10] and one method included m-CPP [5], the active metabolite. A major objective of the present study was to develop a method that could also be applied to brain tissue as this is the presumed site of antidepressant action for trazodone.

The most frequently used solvents for trazodone extraction have been diethyl ether [7, 9], 2% isoamyl alcohol in hexane [8], and methyl-*tert.*-butyl ether [5]. Of these, diethyl ether has the disadvantage of peroxide formation which results in extraction loss of trazodone [9]. While methyl-*tert.*-butyl ether has a higher flash point than diethyl ether, it does not form peroxides and extracts trazodone better from plasma (see Table I and ref. 5) than isoamyl alcohol—hexane, we found it unsuitable for brain tissue extraction. It was necessary to use isoamyl alcohol—hexane for this tissue because use of methyl-*tert.*-butyl ether resulted in emulsion formation and gave generally poor recovery of trazodone. By combining the use of two extraction methods good recovery of both trazodone and m-CPP was possible across a broad concentration range (Table I). At the higher brain concentration for m-CPP (500 ng/g) its recovery was decreased (Table I); however, the variability was small (3.4%). This problem could be overcome by diluting brain homogenates as necessary to lower measurable concentrations. By including a calibration curve of standards prepared from blank brain tissue, quantification of all unknowns are referenced to standards treated in an identical manner. This is a desirable procedure as we have found the recovery of centrally acting drugs to vary widely according to tissue type [11, 12].

Plasma concentrations of trazodone and m-CPP from humans being treated with trazodone for depression, and both plasma and brains from animals previously injected with trazodone are shown in Table III. These results

TABLE III

CONCENTRATIONS OF TRAZODONE AND m-CPP IN HUMANS TREATED FOR DEPRESSION AND IN ANIMALS UNDER EXPERIMENTAL CONDITIONS

Patients 1 and 2 had been chronically receiving 250 and 300 mg of trazodone per day, respectively, and had a blood sample collected 12 h after a previous dose. Rats had been treated 1 h previously with an intraperitoneal injection of trazodone, 25 mg/kg, calculated as free base. All measured brain concentrations were within the limits of the calibration curves and were normalized to amount per 1 g of tissue.

Sample	Trazodone		m-CPP	
	Plasma (ng/ml)	Brain (ng/g)	Plasma (ng/ml)	Brain (ng/g)
Patient No. 1	1212	—	18	—
Patient No. 2	387	—	42	—
Rat No. 1	386	804	27	851
Rat No. 2	230	566	22	667
Rat No. 3	439	939	25	924
Rat No. 4	477	1140	41	1209

illustrate that the present method is applicable to samples across a broad concentration range. These data also illustrate the potential importance of m-CPP as it was generally the predominant compound in brain tissue even though its concentration in plasma was far less than trazodone. Some caution should be exercised in interpreting this data as drug concentrations were only determined at a single point in time 1 h after the dose.

While maximum sensitivity was not a goal of the present study, the lower limit of sensitivity of either trazodone or m-CPP is 5 ng/ml from 1-ml sample extractions. This detection limit with UV absorbance monitoring at 214 nm is an improvement over a previous assay sensitivity of 20 ng/ml which used monitoring at 254 nm [10]. The lower limit of sensitivity could be further improved by extraction of larger sample volumes. UV absorption was linear up to 2000 ng/ml (largest sample tested).

CONCLUSIONS

The present method should prove useful for studying trazodone pharmacokinetics in humans or animals. The extraction method avoids the use of an evaporation stage and is suitable for brain tissue. We feel it could be easily adapted to other animal tissues. In our laboratory, column life has been excellent with no decrease in performance over a six-month period of approximately 1000 injections.

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