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Note

Capillary gas chromatographic assay with nitrogen—phosphorus detection for *trans*-6-(2-chlorophenyl)-1,2,3,5,6,10*b*-hexahydropyrrolo-[2,1-*a*] isoquinoline hydrobromide, a new antidepressant drug, in plasma

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trans-6-(2-Chlorophenyl)-1,2,3,5,6,10*b*-hexahydropyrrolo-[2,1-*a*] isoquinoline hydrobromide (1:1) (McN-5707-14, I) is a novel, potential antidepressant which is currently under preclinical evaluation. As an antidepressant, compound I has attributes in common with tricyclic antidepressants, the atypical antidepressant nomifensine, and various 5-hydroxytryptophan (5-HT) uptake inhibitors [1, 2].

The present paper reports the development of a sensitive and reproducible capillary gas chromatographic (GC) assay with nitrogen—phosphorus detection (NPD) for I in plasma. As an application of the assay, concentrations of I were determined in plasma from rats which had received a 20 mg/kg oral solution dose of the drug.

EXPERIMENTAL

Instrumentation

Gas chromatography. A Hewlett-Packard 5880A capillary gas chromatograph equipped with a Hewlett-Packard 7672A autosampler and a nitrogen–phosphorus thermionic detector was used. A DB-5 fused-silica capillary column, 15 m × 0.32 mm I.D., 0.25 μm film thickness (J & W Scientific, Rancho Cordoba, CA, U.S.A.), was used with helium carrier gas at a flow-rate of 3 ml/min at 160°C. The injector and detector temperatures were 300°C and oven temperature programming was employed from 160 to 200°C at 10°C/min.

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Splitless injection with a purge at 0.3 min was used in conjunction with the autosampler.

Data acquisition. A Hewlett-Packard 3354C laboratory automation system with software developed in-house was used for data acquisition and processing.

Reagents and supplies

Solvents. Nanograde methanol, isopropanol, toluene and hexane were obtained from Mallinckrodt (Paris, KY, U.S.A.) and used without further purification. Triply purified distilled water was obtained from Ephrata Mountain Water (Manheim, PA, U.S.A.).

Reagents. Hydrochloric acid (10 *M*) and sodium hydroxide, reagent ACS or certified ACS grade, were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.).

Other supplies. Compound I and the internal standard were obtained in-house (McNeil Pharmaceutical, Spring House, PA, U.S.A.). Structures for these compounds are given in Fig. 1. All concentrations of I in this report refer to the free base unless otherwise noted.

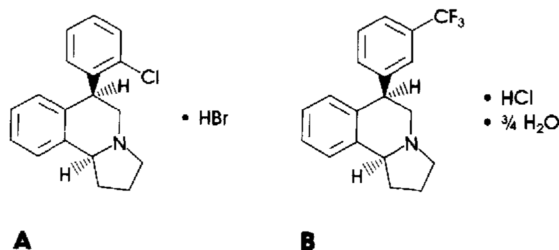

Fig. 1. Structural formulae for I (A) and the internal standard (B).

Extraction procedure

The isolation method of I, a three-step extraction of the drug from plasma, is described in the following paragraphs. To each 1-ml plasma sample (human or rat), 0.1 ml of methanol containing 30 ng/ml internal standard, 0.5 ml of 0.1 *M* sodium hydroxide, 1 ml of water and 6 ml of hexane were added. The samples were shaken for 15 min on a mechanical shaker and centrifuged for 5 min at 681 *g*. The aqueous layer was then frozen on a bed of dry ice, and the organic layer was decanted into a centrifuge tube containing 1.5 ml of 0.1 *M* hydrochloric acid. After mixing for 10 s using a Vortex-Genie® mixer (Scientific Industries, Springfield, MA, U.S.A.), the samples were shaken for 10 min on a mechanical shaker. The organic layer was aspirated, followed by the addition of 0.5 ml of 0.5 *M* sodium hydroxide and 6 ml of hexane to the aqueous layer. The samples were again mixed vigorously for 10 s and shaken for 10 min. The aqueous layer was frozen on a bed of dry ice, and the organic phase was decanted into another centrifuge tube and evaporated to dryness using a gentle stream of nitrogen.

The dried residue was reconstituted with 50 μl of a toluene–methanol (90:10) solution and the sample was transferred to autoinjector vials. A 2.5-μl aliquot was injected into the capillary gas chromatograph for analysis.

Standard curves

To establish a calibration curve, a series of nine standard solutions of I (1—100 ng/ml) containing 30 ng/ml internal standard were prepared in methanol using silanized glassware. A 0.1-ml aliquot of these solutions was added to 1 ml of plasma (instead of the 0.1 ml of methanol containing internal standard alone added to study samples) and the samples were extracted according to the procedure above. Duplicate standard curves were run on three consecutive days prior to sample analysis. The peak-height ratios (PHR) of I and the internal standard were weighted by 1/variance of the peak-height ratios. Linear regression analysis gave a calibration line which was used to calculate concentrations of I in the frozen seeded controls.

As an additional control, seeded plasma pools were prepared at three concentrations (3, 10, and 50 ng/ml I), separated into 1-ml aliquots and frozen. Two samples from each seeded control pool were analyzed with each calibration curve to assess the precision of the assay procedure.

Assay application

Eighteen Wistar CD rats weighing between 130 and 220 g received 20 mg/kg I as the hydrobromide salt in a 2% polyethylene glycol solution by gavage. Individual blood samples (ca. 5 ml) were obtained by cardiac puncture following ether anesthesia at predetermined intervals following dose administration. Three rats were sacrificed at each of the following time points after dosing: 0.5, 1, 2, 4, 7 and 17 h. Blood samples were heparinized and plasma was harvested by centrifugation at 681 *g* for 20 min. Each plasma sample (eighteen in total) was analyzed separately by the assay procedure.

A 1-ml aliquot of plasma was analyzed except where concentrations exceeded 100 ng/ml. In these cases, either 0.5 or 0.2 ml were analyzed as needed. Plasma standards at nine concentrations as well as frozen seeded controls at three concentrations were analyzed with each set of rat plasma samples. Linear regression analysis with 1/variance weighting factors (obtained from the composite of three days of duplicate standard curves) was used to determine the concentrations in the rat plasma samples.

RESULTS AND DISCUSSION

Gas chromatography

Chromatograms of extracted human and rat plasma samples are shown in Fig. 2. No significant interfering peaks appear in the chromatogram of blank plasma and the two compounds are well separated.

Additionally, owing to the extraction procedure or the resolution of the capillary column, none of the following drugs interfere with the determination of compound I in plasma: amitriptyline, cimetidine, diazepam, phenobarbital, aspirin, acetaminophen, haloperidol, ibuprofen, chlorothiazide, nifedipine and propranolol.

Standard curves, precision and accuracy

Duplicate calibration curves run on three consecutive days and calibration curves run during samples analysis (three days) were linear over the concentra-

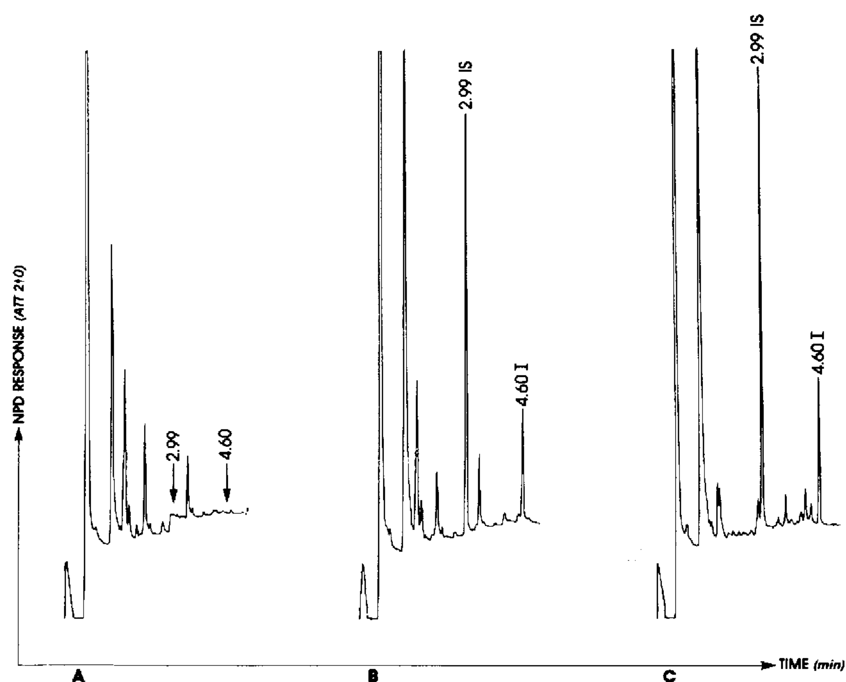

Fig. 2. Chromatograms of (A) blank human plasma, (B) extracted human plasma spiked with I (10 ng/ml) and internal standard (I.S.) (30 ng/ml) and (C) extracted rat plasma sample (measured concentration, 11.4 ng/ml).

TABLE I

SUMMARY OF CALIBRATION CURVE DATA FOR THE ANALYSIS OF COMPOUND I IN PLASMA

Composite data from three separate days of duplicate standard curve analysis and calibration curves from three days of sample analysis. Linear regression analysis of a six-day composite gave a slope of 0.027 ± 0.0003, a y-intercept of 0.005 ± 0.001 and a correlation coefficient of 0.99.

Seeded concentration (ng/ml)	n	Mean measured concentration (ng/ml)	Relative standard deviation (%)	Mean percentage deviation from seeded concentration
1	8	1.06	10.2	5.5
2	9	2.05	9.7	2.2
3	8	3.02	8.7	0.6
5	9	4.73	5.3	5.4
10	8	9.76	8.3	2.5
20	9	19.8	6.3	0.8
30	9	29.5	8.0	1.5
50	9	52.1	6.8	4.1
100	9	108	7.1	8.1

tion range (1—100 ng/ml) studied here (Table I). Regression analysis of the peak-height ratio versus concentration data gave the following equation: $y = 0.027x - 0.001$ (y = peak-height ratio drug/internal standard and x = concentration of I). The correlation coefficient for the six-day composite curve was 0.99. The

precision of the assay, as measured by the relative standard deviations at each concentration, was within 10.2%. The average back-calculated concentration was within 10% of the seeded value at each concentration. The average measured concentrations of the frozen seeded control plasma samples were within 10% of their theoretical (spiked) concentrations with relative standard deviations of less than 10% (data not shown).

Recovery and stability

The choice of hexane as an extraction solvent was based on two important considerations in assay development. First, hexane extracts compound I from plasma with high efficiency; secondly, owing to its relative weakness as an organic solvent, it provides an extract that is largely free of chromatographic interferences. The extraction efficiencies for I (at 5 and 50 ng/ml) and the internal standard (at 30 ng/ml) were estimated by measuring the peak heights of the compounds after extraction from spiked plasma. These peak heights were compared to peak heights obtained from blank plasma extracts which had been spiked at the same concentrations immediately prior to injection (n = 6 for each concentration). The extraction efficiencies of both I and the internal standard were estimated at $\geqslant$ 80% by this method. Compound I was stable in frozen plasma for at least one month based on data from the frozen seeded controls. However, the dried plasma extracts should be injected within 12 h after extraction since decreases in recovery (peak height) and precision (peak-height ratio reproducibility) were noted after samples were stored at 5°C for 18–24 h.

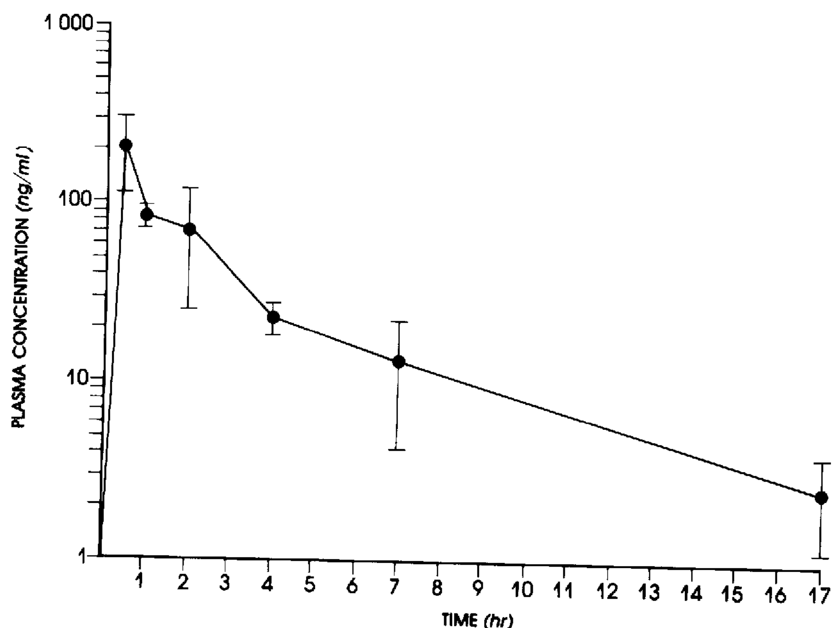

Fig. 3. Mean plasma concentrations obtained following the oral administration of 20 mg/kg equivalents of I to male CR Wistar rats (three rats at each time point; a total of eighteen rats was used).

Assay application

Plasma concentrations of I were determined in each of the rat samples using the capillary GC—NPD assay procedure. No interfering peaks were encountered. The mean plasma concentration data of I are plotted versus time in Fig. 3. Following the oral solution dose, the mean maximal plasma concentration of 212 ng/ml occurred at 30 min following dose administration. Plasma levels of I fell to 3 ng/ml by 17 h after oral dosing. Estimated values for disposition half-life ($t_{1/2}$) and area under the plasma concentration curve (AUC) for the oral solution dose were 4.1 h and 434 ng · h/ml, respectively.

In summary, a capillary GC—NPD assay for I in plasma has been developed with a minimum quantitation limit of 1 ng of I in 1 ml of plasma or 20 ng/ml using a 50-µl plasma aliquot. Application of the assay to an exploratory pharmacokinetic study in rats demonstrates the utility of the assay.

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REFERENCES

1 M.A. Dougherty, M.D. Brannan, B. Maryanoff, D. McComsey and S.P. Sit, Fed. Proc., Fed. Am. Soc. Exp. Biol., 44 (1985) 887.
2 J.L. Vaught, J.F. Gardocki, R.P. Shank, P.E. Setler and B. Maryanoff, Fed. Proc., Fed. Am. Soc. Exp. Biol., 44 (1985) 888.