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Note

Determination of 1-(4-methoxybenzoyl)-5-oxo-2-pyrrolidinepropanoic acid, a potential nootropic agent, in human plasma and urine by reversed-phase high-performance liquid chromatography

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1-(4-Methoxybenzoyl)-5-oxo-2-pyrrolidinepropanoic acid (I, CI-933, Fig. 1) is a new potential nootropic agent currently undergoing Phase I clinical investigation. It has been shown to enhance performance of learned behaviors in many animal models of cognition [1, 2]. This work describes simple and reproducible methods to determine I in human plasma and urine by high-performance liquid chromatography (HPLC). Urine concentrations were determined by direct injection while plasma levels of I were quantified by the use of an internal standard. The methods were applied to pharmacokinetic studies of I in normal human volunteers.

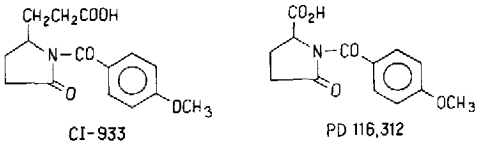

Fig. 1. Chemical structure of I (CI-933) and internal standard (PD 116,312).

EXPERIMENTAL

Chemicals

I and the internal standard (PD 116,312) (Fig. 1) were synthesized at Warner-Lambert/Parke-Davis Research Labs. Methylene chloride and methanol,

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distilled in glass, were obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) and tetrabutylammonium hydrogen sulfate was purchased from Aldrich (Milwaukee, WI, U.S.A.). All other chemicals were of analytical grade. Water used in reagent preparations was glass-distilled.

Stock solutions

A stock solution of I (100 μg/ml) was prepared in 0.05 *M* phosphate buffer (pH 7). This was diluted to 10, 2 and 1 μg/ml for plasma assay calibration standards. The internal standard solution (100 μg/ml) was prepared in 0.05 *M* phosphate buffer and diluted to 10 μg/ml.

Preparation of samples

Plasma assay. Plasma (1 ml) and 50 μl (0.5 μg) of the internal standard solution were pipetted into 12-ml conical centrifuge tubes. This was followed by 5 ml of methylene chloride and 0.5 ml of 1 *M* hydrochloric acid. The tubes were capped and shaken for 10 min. After centrifugation (900 *g*) the organic phase was transferred to a 12 × 75 mm disposable tube and evaporated to dryness under a stream of nitrogen. Mobile phase (100 μl) was added to each tube, vortexed, and 25 μl were injected.

Urine assay. An aliquot of urine (1.0 ml) was placed in a disposable glass vial and precisely 20 μl were injected directly onto the column via an autosampler with a fixed-volume loop.

Calibration and precision

Six plasma calibration standards containing 0.1, 0.25, 0.5, 1.0, 2.5 and 5 μg/ml I in 1 ml control human plasma were processed daily with each set of unknowns. Plasma calibration curves were constructed by plotting peak-height ratios of I to internal standard as a function of the concentration of I. The best-fit straight line was determined by least-squares linear regression. Plasma I concentrations in unknown samples were calculated by interpolation from the calibration curve.

From a stock solution of 1000 μg/ml I in drug-free human urine, nine additional standards of 1, 2, 5, 10, 25, 50, 100, 250 and 500 μg/ml in urine were made by dilution. These were processed with each set of unknowns. Urine calibration curves were constructed by plotting peak height as a function of the concentration of I. The best-fit straight line was determined by least-squares linear regression and a weighting factor of $1/y$. Urine I concentrations in unknown samples were calculated by interpolation from the calibration curve.

To determine precision of the assay procedures, triplicate plasma and urine standards were analyzed on three separate days to yield nine replicate values for each of the plasma and urine concentrations.

Recovery in plasma

Recovery of I and the internal standard from plasma was determined by extraction of high, medium, and low concentrations in triplicate from plasma and comparing it to aqueous standards injected directly.

Quality-control and stability studies

Plasma quality-control pools of 0.4, 0.80 and 4.0 μg/ml were prepared in drug-free human plasma and aliquots were stored frozen in disposable glass vials until use. Urine quality controls of 4, 75 and 750 μg/ml were prepared in drug-free human urine and stored as described above. The quality-control samples were analyzed daily with each set of unknowns to determine the reliability of each day's analysis.

Instrumentation

For plasma analysis an HPLC system comprised of an Altex Model 110A pump, a Kratos Model 783 ultraviolet (UV) detector operated at 282 nm and a Perkin Elmer Model 1SS 100 sample processor was used.

Analysis of urine was performed with an HPLC system that consisted of a Perkin Elmer Model 10 pump, UV detector by Hitachi (Model 655A) at 282 nm and a Gilson Model 230 autosampler with a 20-μl fixed loop.

A 5-μm C_{18} Nova Pack column (150 × 4.6 mm I.D.) from Waters (Milford, MA, U.S.A.) was used in both analyses. Samples were eluted isocratically at ambient temperature with a mobile phase of methanol—0.05 *M* phosphate buffer (pH 7) (25:75) containing 0.008 *M* tetrabutylammonium hydrogen sulfate. Mobile phase flow-rate was 1.0 ml/min (15.17 MPa) and 0.5 ml/min (6.89 MPa) for plasma and urine analysis, respectively.

RESULTS AND DISCUSSION

Representative chromatograms obtained from plasma and urine analyses are depicted in Figs. 2 and 3, respectively. Retention times of the internal standard and I in plasma were 3.4 and 5.0 min. In urine samples the retention time of I was 8.0 min. There were no interfering peaks from normal components of plasma or urine.

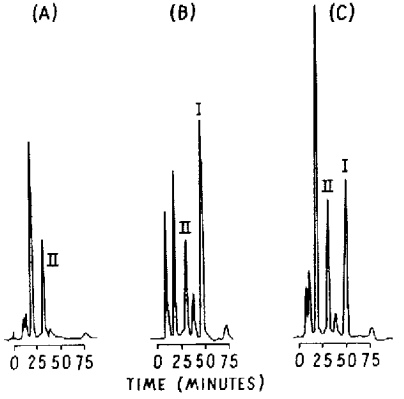

Fig. 2. Chromatograms of an extract of (A) drug-free human plasma with internal standard, (B) 2.50 μg/ml plasma calibration standard and (C) 4-h post dose human plasma sample (500 mg oral dose) containing 1.20 μg/ml I. Peaks: I = CI-933; II = internal standard.

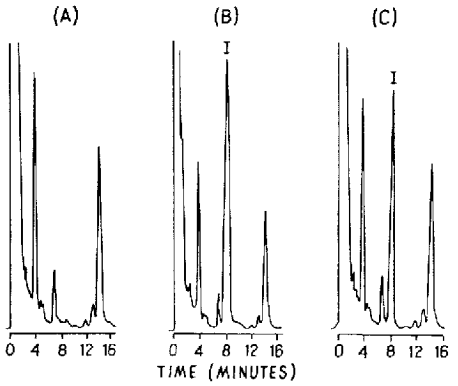

Fig. 3. Chromatograms of (A) drug-free human urine, (B) 25 μg/ml urine calibration standard and (C) 12-h post dose human urine sample (600 mg oral dose) containing 22 μg/ml I. Peak I = CI-933.

Based on peak-height ratios of three replicate sequential injections of 0.25 and 2.5 μg/ml plasma standards, the HPLC system reproducibility had a relative standard deviation (R.S.D.) of 4.9 and 1.4%, respectively.

Recovery of I from plasma at concentrations of 0.4, 1.0 and 5.0 μg/ml averaged 95% while recovery of the internal standard was 85%.

The relationship between peak-height ratio (I/internal standard) and concentration of I in plasma was linear over the concentration range studied (0.1—5 μg/ml) and yielded a correlation coefficient of 0.998 or greater. For urine assay the relationship between peak height to concentration of I was linear over 1—1000 μg/ml with a correlation coefficient of 0.999 or greater.

Assay precision expressed as R.S.D. of nine replicate analyses varied from 1.8 to 4.9% for plasma assays and from 0.5 to 6.2% for urine analyses (Tables I and II). The measured values were at least 92 and 96% of theoretical values in urine and plasma. The limit of quantitation was 0.1 μg/ml for plasma and 1.0 μg/ml for urine.

Quality-control samples containing 4.0, 0.8 and 0.4 μg/ml I in plasma, and

TABLE I

PRECISION AND ACCURACY OF PLASMA CALIBRATION STANDARDS

$n = 9$.

Concentration of I (μg/ml)		R S.D. (%)
Added	Found	
0.10	0.10	3.3
0.25	0.24	4.9
0.50	0.50	4.4
1.00	1.00	4.3
2.50	2.53	2.9
5.00	4.97	1.8

TABLE II

PRECISION AND ACCURACY OF URINE CALIBRATION STANDARDS

$n = 9$.

Concentration of I (μg/ml)		R.S.D. (%)
Added	Found	
1	1.08	6.2
2	2.06	4.4
5	4.94	3.1
10	9.90	5.3
25	23.90	2.3
50	49.90	3.4
100	96.50	1.8
250	255.00	2.6
500	503.00	2.3
1000	997.00	0.5

TABLE III

STABILITY OF I IN PLASMA QUALITY-CONTROL SAMPLES OVER A ONE-MONTH PERIOD

$n = 15$.

Concentration of I (μg/ml)		Percentage of theoretical	R.S.D. (%)
Added	Found		
4.0	3.87	97	5.68
0.8	0.78	98	4.22
0.4	0.39	98	3.84

TABLE IV

STABILITY OF I IN URINE QUALITY-CONTROL SAMPLES OVER A ONE-MONTH PERIOD

$n = 8$.

Concentration of I (μg/ml)		Percentage of theoretical	R.S.D. (%)
Added	Found		
750	735.4	95	4.70
75	75.9	99	2.06
4	3.9	98	6.01

750, 75 and 4 μg/ml in urine were stored frozen and assayed over a one-month period. The mean plasma concentrations (Table III) obtained were within 97% of theoretical with an R.S.D. of less than 6%. Urine quality controls

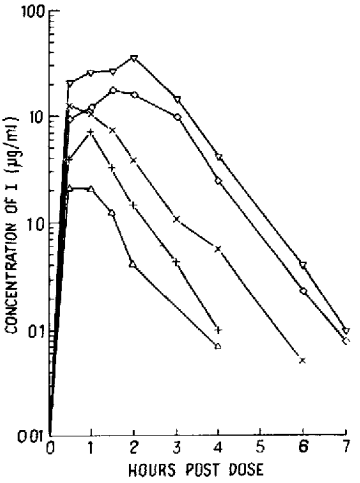

Fig. 4. Mean plasma concentrations of I—time profiles in human volunteers after single rising oral doses. (△) 125 mg; (+) 250 mg; (×) 500 mg; (◇) 1000 mg; (▽) 1500 mg.

(Table IV) were within 95% of theoretical with an R.S.D. less than 7%. Aqueous solutions of I were unstable at extreme pH ranges and temperatures; however, under physiological conditions no changes in concentrations of I were found in plasma and urine quality controls frozen over a one-month period.

The described methods have been successfully applied to a pharmacokinetic study [3] in normal human volunteers receiving single oral doses of I. Results showed that I was rapidly absorbed and excreted in the urine unchanged (Fig. 4).

In summary, the methods for analysis of I in urine and plasma were selective, reproducible and sufficiently sensitive for pharmacokinetic studies.

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