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Note

Gas chromatographic assay of pramiracetam in human plasma using nitrogen specific detection

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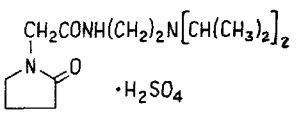
Pramiracetam, N-[2-[bis(1-methylethyl)amino]ethyl]-2-oxo-1-pyrrolidine-acetamide sulfate (1:1), is a new orally active cognition activator [1] currently undergoing clinical evaluation. To facilitate pharmacokinetic and bioavailability studies, a simple and sensitive gas chromatographic method has been developed for the determination of pramiracetam at therapeutic concentrations. The assay entails a one-step extraction with chloroform and subsequent quantitation using a nitrogen—phosphorus detector.

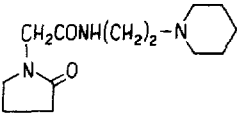
EXPERIMENTAL

Materials

Pramiracetam (I) and the internal standard (II) were synthesized in the Warner-Lambert/Parke-Davis Research Labs. (Ann Arbor, MI, U.S.A.). Glass distilled chloroform with 1% ethanol as preservative was purchased from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.). Triethylamine (TEA) was obtained from Matheson, Coleman and Bell (Cincinnati, OH, U.S.A.); 3% OV-225 on 100—120 mesh Gas-Chrom Q (Applied Science Labs., State College, PA, U.S.A.). Working standards containing 2, 1.5, 1, and 0.5 μg/ml of



I



II

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pramiracetam free base were prepared in 0.05 *N* hydrochloric acid. An internal standard solution (2 μg/ml) was likewise prepared.

Extraction procedure

Aliquots of plasma (0.5—1 ml) and 0.5 ml of internal standard solution (1 μg) were pipetted into 150 × 16 mm glass-stoppered tubes containing 8 ml of the extraction solvent, chloroform—TEA (99.5:0.5). After the addition of 0.3 ml of 1 *N* sodium hydroxide and ca. 0.5 g of Na_3PO_4, the tubes were shaken for 10 min in a mechanical shaker. Following centrifugation, the aqueous phase and the plasma protein plug were aspirated off and discarded. The tubes were centrifuged again briefly, and the organic extract was transferred into 100 × 13 mm tubes by pouring smoothly, avoiding any transfer of the residual aqueous phase. The samples were evaporated to dryness under a stream of nitrogen and the residue was taken up in 25 μl of isopropanol—methanol (80:20) containing 0.5% TEA.

Calibration standards containing 0.125, 0.25, 0.5, 0.75, and 1 μg of pramiracetam in 1 ml of drug-free control human plasma were processed as described above with each set of unknown samples.

Apparatus

A Perkin-Elmer Sigma 2 gas chromatograph equipped with a nitrogen—phosphorus detector and a coiled glass column (1.22 m × 2 mm I.D.) packed with 3% OV-225 on 100—120 mesh Gas-Chrom Q were used. The injector, oven, and detector temperatures were maintained at 275, 225, and 275°C, respectively. The gas flow-rates were: hydrogen, 2.5 ml/min; air, 100 ml/min; nitrogen (carrier), 28 ml/min. The peak areas and retention times of pramiracetam and the internal standard were obtained using a Perkin-Elmer Sigma 10 data systems.

Calculations

A calibration curve was constructed by plotting the ratio of the peak area or peak height of pramiracetam to that of the internal standard as a function of the amount of pramiracetam added to control human plasma. The best fit straight line was determined using the method of least squares. The concentrations of pramiracetam in the unknown samples were calculated by interpolation from the standard curve.

RESULTS AND DISCUSSION

The retention times of pramiracetam and the internal standard were 4.65 and 6.76 min, respectively. The chromatograms of a control human plasma extract and of the same plasma spiked with 1 μg each of pramiracetam and the internal standard are shown in Fig. 1. There were no interferences from the normal components of the plasma extract. The total analysis time required for each run was less than 12 min.

The extraction recovery of pramiracetam from control human plasma was 98%. Triethylamine was added to the extraction solvent to minimize drug adsorption to glassware.

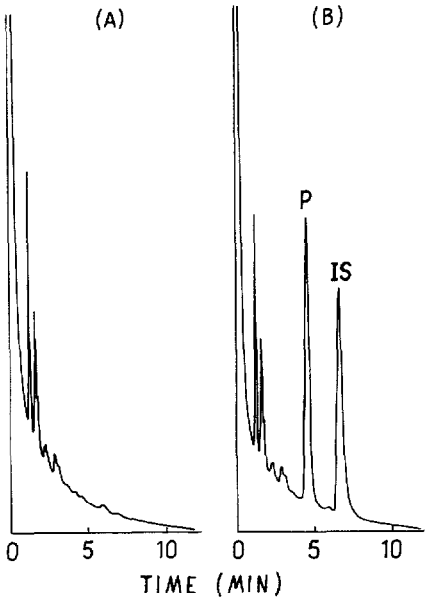

Fig. 1. Chromatograms of human plasma extracts. (A) Control human plasma; (B) control plasma containing 1 μg each of pramiracetam (P) and internal standard (IS).

The peak area or peak height ratios were linearly related to drug concentrations over a range of 0.125—1.0 μg/ml. The best fit linear regression line was $y = 0.9725x - 0.0198$ ($r = 0.9999$) for the peak area ratios, or $y = 1.3356x - 0.0272$ ($r = 0.9997$) for the peak height ratios, where y is the peak area or peak height ratio, and x is the amount of pramiracetam in human plasma. The slopes of four calibration curves constructed over a two-month period showed a relative standard deviation (R.S.D.) of 1.4% and 5% for the peak area and peak height methods, respectively. The lower limit of detection was estimated to be 0.02 μg/ml.

The precision and accuracy of the method were evaluated by extracting and analyzing replicate control human plasma samples containing 0.125—1 μg/ml of pramiracetam. The R.S.D. ranged from 0.95 to 3.8% for the peak area ratios, and from 1.4 to 4.9% for the peak height ratios (Table I). The average measured drug concentrations showed close agreement with the expected values by either the peak area or peak height methods. Column reproducibility following five sequential injections of the same sample had a relative standard deviation of 2.8% and 1.9% for peak area ratios and peak height ratios, respectively.

A number of nootropic drugs, including piracetam, hydergine, and vincamine, were chromatographed under the same conditions to test the specificity of the method, and no interferences were observed.

To demonstrate the utility of the method, plasma samples obtained from a human volunteer receiving a single 400-mg oral dose of pramiracetam capsule were assayed, and the resulting plasma concentration versus time profile is shown in Fig. 2. The apparent elimination half-life was 4.9 h. Details of the clinical pharmacokinetic studies will be reported elsewhere. Recent studies also indicate that the same procedure can be applied to human urine without further modification. However, it should be noted that pramiracetam concen-

TABLE I

PRECISION AND ACCURACY OF REPLICATE ANALYSIS OF PRAMIRACETAM ADDED TO CONTROL HUMAN PLASMA (n = 4)

Expected value (μg/ml)	Peak area ratios		Peak height ratios	
	Assayed value (μg/ml)	Relative standard deviation (%)	Assayed value (μg/ml)	Relative standard deviation (%)
1.0	1.007	0.95	1.001	1.4
0.75	0.743	2.1	0.734	1.7
0.50	0.492	3.1	0.491	1.5
0.25	0.250	2.4	0.249	1.6
0.125	0.132	3.8	0.125	4.9

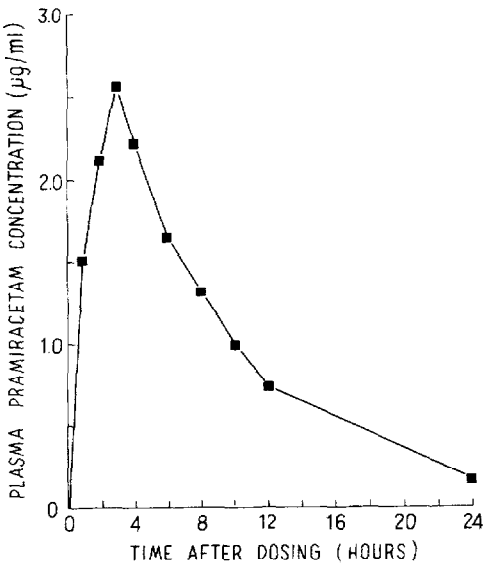

Fig. 2. Plasma concentrations of pramiracetam in a human volunteer following a single 400-mg oral dose.

tration in human urine is far greater than in plasma, and appropriate calibration standards covering a higher concentration range should be used.

ACKNOWLEDGEMENTS

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REFERENCE

1 B.P.H. Poschel, J.G. Marriott and M.I. Gluckman, Psychopharmacol. Bull., (1983) in press.