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Short Communication

Measurement of CI-979 (a candidate drug for the treatment of age-related disorders of cognition) in human plasma by capillary gas chromatography with nitrogen-selective detection

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ABSTRACT

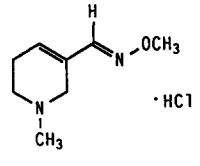
A specific and highly sensitive method for the measurement of CI-979 in human plasma is described. The compound and internal standard were extracted from alkalinized plasma with methyl *tert.*-butyl ether and analyzed by capillary gas chromatography with nitrogen-selective detection. The method was demonstrated to be accurate and precise. Since the limit of quantitation was 0.10 ng/ml, this method was suitable for clinical pharmacokinetic studies in which subjects received repeated administration of 0.5–2.5 mg CI-979 every 6 h.

INTRODUCTION

CI-979, (*E*)-1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde O-methyloxime monohydrochloride (Fig. 1), is being investigated for use in the treatment of age-related disorders of cognition, such as Alzheimer's disease or senile dementia of the Alzheimer's type.

In vivo studies in rodents and monkeys have confirmed the cholinomimetic activity of CI-979 [1,2]. CI-979 decreases spontaneous swimming activity, decreases body temperature, increases local cortical blood flow, and enhances cortical electrophysiological arousal in rodents at doses of 0.32 mg/kg or higher. In rhesus monkeys, CI-979 increases neocortical arousal as measured by electroencephalographic procedures, with central cholinomimetic activity seen at 0.01 mg/kg or higher. The action of CI-979 in rodents and

CI-979 MONOHYDROCHLORIDE



CI-844 SULFATE

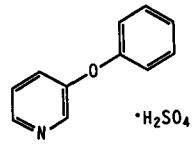


Fig. 1. Structures of CI-979 and I.S.

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monkeys can be blocked by the centrally acting anticholinergic scopolamine but not the primarily peripherally acting methylscopolamine, thus supporting a central cholinergic mechanism of action in these species.

We present here a gas chromatographic (GC) method with nitrogen–phosphorus detection (NPD) which allows determination of plasma concentrations of CI-979 as low as 0.10 ng/ml. Solvent evaporation was avoided due to the volatile nature of the drug and internal standard.

EXPERIMENTAL

Reagents

CI-979 and CI-844, 3-phenoxypyridine sulfate (internal standard, I.S.), were synthesized at Parke-Davis Pharmaceutical Research Labs. All reagents and solvents were of HPLC grade purity, where available. Methyl *tert.*-butyl ether and hexane were obtained from Burdick and Jackson (Muskegon, MI, USA). Standard solutions of CI-979 and I.S. were prepared in 0.1 *M* NaOH and stored at 4°C for up to one month. No special handling procedures were required for the stock solutions or actual study samples. Working solutions (0.10–10.0 ng per 100 μl) were freshly prepared before daily analysis by dilution in 0.1 *M* NaOH.

Gas chromatography

Chromatography was carried out on a megabore column (15 m × 0.53 mm I.D., 1.0 μm film thickness, 50% phenyl, 50% methylsilicone (DB-17); J & W, Folsom, CA, USA) in a Hewlett-Packard Model 5890 gas chromatograph (Hewlett Packard Instruments, Sunnyvale, CA, USA) equipped with a nitrogen–phosphorus detector. Helium was used as the carrier gas, at a flow-rate of 3 ml/min. Hydrogen and air flow-rates were 3.0 and 30 ml/min, respectively. The column was maintained at 100°C for 1 min, then programmed at 8°C/min to 190°C, at 60°C/min to 220°C, and at 50°C/min to 100°C. Injector and detector temperatures were 190 and 300°C, respectively. Run time was 15 min.

Extraction procedure

Aliquots of plasma (1.0 ml) were pipetted into 100 mm × 16 mm screw-cap glass tubes containing 500 ng of I.S. (100 μl of a 500 ng/100 μl working solution), 200 μl of 1 *M* NaOH, and 5 ml of methyl *tert.*-butyl ether. Tubes were shaken horizontally for 15 min. After centrifugation for 10 min at 1600 *g*, solvent phase was transferred to clean 100 mm × 16 mm tubes containing 200 μl of 0.1 *M* HCl. Tubes were again shaken and centrifuged. The solvent layer was discarded and the aqueous layer transferred to 1-ml micro-extraction vials (assembled from commercially available components: vials 200-492, inserts 200-756, and caps/septums 200-596 from Sun Brokers, Wilmington, NC, USA; silicone pads 73008 from Waters, Milford, MA, USA) containing 20 μl of 50% NaOH and 300 μl of methyl *tert.*-butyl ether–hexane (1:1). After horizontal shaking for 15 min and centrifugation for 10 min at 1600 *g*, 200 μl of the solvent layer were transferred to Hewlett-Packard autosampler vials containing limited-volume inserts. Injection volume was 5 μl.

Calculations

A calibration curve was constructed by plotting the peak-height ratios (CI-979/I.S.) as a function of the amounts of CI-979 added to control human plasma. The best fit line was determined using least-squares regression with a weighting factor of $1/(\text{concentration})^2$. Concentrations of CI-979 in the unknown samples were calculated by interpolation from the standard curve.

RESULTS

Validation was performed by assaying nine standards in replicate ($n = 3$) on three separate days. Precision, expressed as relative standard deviation (R.S.D., %), and accuracy, expressed as relative error (R.E., %) between found and added amounts of drug, are given in Table I. Retention times for CI-979 and I.S. were 5.0 and 10.0 min, respectively. CI-979 was well resolved from the known demethylated metabolite,

TABLE I

PRECISION AND ACCURACY OF THE MEASUREMENT OF CI-979 IN HUMAN PLASMA SAMPLES

Concentration added (ng/ml)	Concentration measured (mean, n = 9) (ng/ml)	R.S.D.[a] (%)	R.E.[b] (%)
0.100	0.101	6.1	1.4
0.300	0.290	4.3	−3.4
0.500	0.493	4.9	−1.3
0.700	0.701	1.7	0.1
1.00	1.00	3.2	0.0
2.00	1.99	3.2	−0.4
3.00	3.02	1.4	0.7
5.00	5.04	2.9	0.9
10.0	10.2	3.2	2.1

[a] Relative standard deviation.
[b] Relative error.

1,2,5,6-tetrahydro- 3-pyridinecarboxaldehyde O-methoxime, with a retention time of 5.8 min. Peak-height ratios (CI-979/I.S.) were linearly related to drug concentration over a range of 0.10–10.0 ng/ml CI-979. Extraction recovery of CI-979 from human plasma relative to aqueous (0.1 M NaOH) was 99.2 ± 3.1, 93.9 ± 6.1, and 98.9 ± 2.0% for 0.5, 1.0, and 5.0 ng/ml, respectively. Extraction recovery of I.S. from human plasma relative to aqueous was 99.7 ± 8.9% for 500 ng/ml.

Stability in plasma was determined by analyzing replicate (n = 3) samples at 0.389, 1.52, and 4.31 ng/ml following exposure to three freeze–thaw cycles. Relative error was 0.1, −1.1, and −0.1% for 0.389, 1.52, and 4.31 ng/ml, respectively.

Limit of quantitation under the experimental conditions described was 0.10 ng/ml based on R.E. and R.S.D. values of <10%. Representative chromatograms of plasma samples with and without CI-979 and I.S. are shown in Fig. 2. Due to the required assay sensitivity and detection limit of the gas chromatograph, the baseline drift observed in the representative chromatograms is not uncommon and does not affect quantitation of CI-979 at picogram levels.

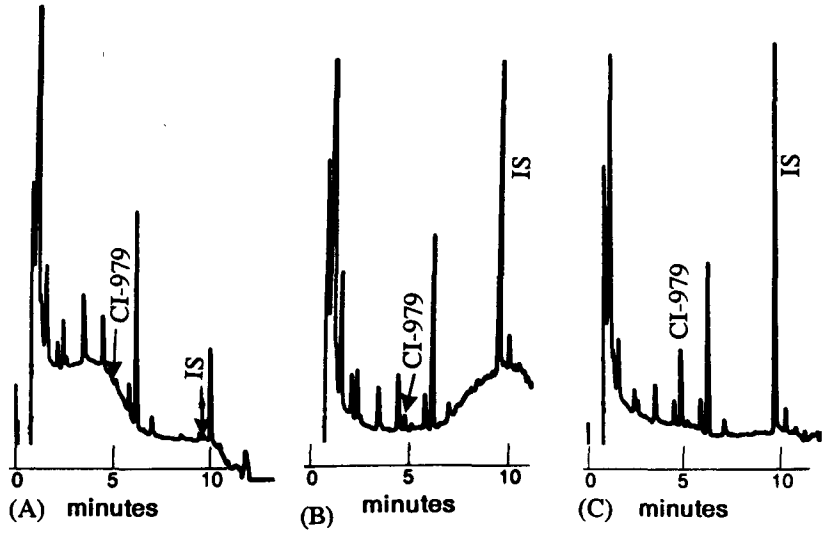

Fig. 2. Representative chromatograms of extracted human plasma. (A) Blank Plasma; (B) plasma with 0.56 ng/ml CI-979 and I.S.; (C) plasma with 2.99 ng/ml CI-979 and I.S.

CONCLUSION

This highly sensitive method is suitable for pharmacokinetic analysis of CI-979 clinical trials.

REFERENCES

1 R. E. Davis, C. Raby, R. Schwarz, D. Dudley, P. Reece, J. C. Jaen and H. Tecle, *Prog. Brain Res.*, (1993), in press.
2 E. Toja, C. Bonetti, A. Butti, P. Hunt, M. Fortin, F. Barzaghi, M. L. Formento, A. Maggioni, A. Nencioni and G. Galliani, *Eur. J. Med. Chem.*, 26 (1991) 853.