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# Sensitive high-performance liquid chromatographic method for a dopamine receptor agonist, CI-1007, and its metabolite PD 147693 in monkey plasma

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**Abstract**

A sensitive gradient high-performance liquid chromatographic (HPLC) method for the simultaneous quantitation of a dopamine autoreceptor agonist CI-1007 (I) and its metabolite PD 147693 (II) is described. Monkey plasma samples were purified by liquid–liquid extraction using hexane. Liquid chromatographic separation was achieved on two $C_{18}$ analytical columns (installed in series) using gradient elution. Column effluent was monitored using a fluorescence detector programmed to change wavelengths at specified times. Minimum quantitation limits of I and II were 3.0 and 5.0 ng/ml, respectively, for a plasma sample volume of 0.100 ml. Linearity was demonstrated up to 300 ng/ml. The assay has been applied to the analysis of I and II in plasma from monkeys following intravenous and oral doses of I.

## 1. Introduction

CI-1007 (PD 143188, I), 1,2,3,6-tetrahydro-4-phenyl-1-[(3-phenyl-3-cyclohexen-1-yl)methyl]-pyriine, (*R*)-(+) enantiomer, is a potent dopamine autoreceptor agonist [1–4], currently being developed as an antipsychotic agent. The therapeutic potential of I for the treatment of schizophrenia is encouraging because of its potency and novel mechanism of action. At the early stage of its development concentrations of I in rat and monkey plasma were determined using isocratic HPLC assays [5]. Subsequent in-vivo and in-vitro metabolism studies indicated that I is extensively metabolized in rat and monkey following oral administration, and a gradient HPLC assay was then developed for the optimum separation of I and its metabolite peaks in plasma. Results from preliminary pharmacology studies indicated that a monohydroxy derivative, PD 147693 (II), is an active metabolite [6], which rapidly appears in monkey plasma after oral administration of I. Therefore a gradient HPLC method has been developed and validated for the simultaneous quantitation of I and II in monkey plasma. The chemical structures of I, II, the internal standard PD 145773 (I.S.), and PD 149394 (III) and PD 155144 (IV), two other hydroxy derivatives and possible metabolites of I [6], are shown in Fig. 1. The method involves a

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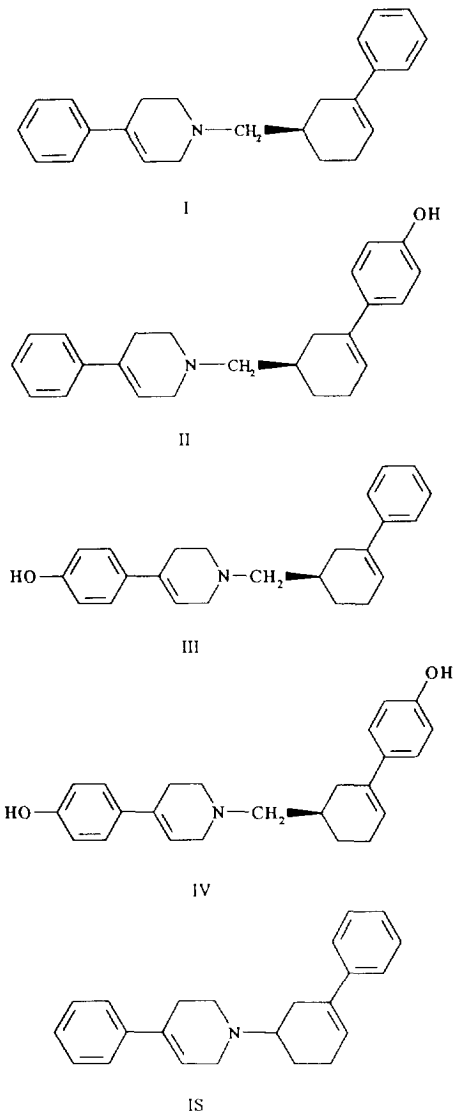


Fig. 1. Structures of I, II, III, IV and I.S.

liquid–liquid extraction of I, II, and I.S. from monkey plasma using hexane. Liquid chromatographic separation of the analytes was achieved on two $C_{18}$ analytical columns (installed in series) using gradient elution to attain optimum separation. The column effluent was monitored using a fluorescence detector programmed to change wavelengths at specified times to allow for optimum sensitivity.

## 2. Experimental

### 2.1. Reagents and chemicals

Compounds I, II, III, IV, and I.S., were synthesized at Parke-Davis Pharmaceutical Research (Division of Warner–Lambert Company, Ann Arbor, MI, USA). HPLC grade acetonitrile and methanol, and potassium phosphate dibasic were from Mallinckrodt (Paris, KY, USA). Reagent grade triethylamine was from MCB Manufacturing Chemists (Cincinnati, OH, USA). HPLC grade water, hexane, and other reagents were from EM Science (Gibbstown, NJ, USA). Control heparinized monkey plasma was from Lampire Biological Laboratories (Pipersville, PA, USA).

### 2.2. Preparation of solutions

*Compounds I and II*

Stock solution A, 100 μg/ml (free-base equivalents) of I and II, was prepared by dissolving accurately weighed quantities of I and II in 20.0 ml of methanol, then diluting with acetonitrile to a final volume of 50.0 ml. Stock solution B, 10.0 μg/ml (free-base equivalents) of I and II, was prepared by diluting a 5.00-ml aliquot of stock solution A to 50.0 ml with acetonitrile. Stock solution B was further diluted with acetonitrile to prepare 8 working solutions: 3.0, 5.0, 10.0, 25.0, 50.0, 100, 150, and 300 ng/ml. Aliquots (0.100 ml) of the working solutions were added to blank monkey plasma (0.100 ml) to prepare calibration standards.

*Internal standard*

A 100 μg/ml stock solution was prepared by dissolving an accurately weighed quantity of I.S. in 50.0 ml of acetonitrile. This solution was diluted 1:1000 with acetonitrile to give a working internal standard solution of I.S. at a concentration of 100 ng/ml. Aliquots (0.100 ml) of the working I.S. solution were added to the monkey plasma for assay.

*Quality control samples*

Monkey plasma quality control standards, which contained 12.0, 50.0, and 250 ng/ml of I and II (free-base equivalents) were prepared by diluting a 0.060-ml aliquot of an independently weighed 10 μg/ml stock solution, and 0.025- and 0.125-ml aliquots of an independently weighed 100 μg/ml stock solution to 50.0 ml with blank monkey plasma, respectively. These solutions were subdivided into 0.5-ml aliquots and were stored at approximately −70°C.

### 2.3. Instrumentation

The chromatographic system consisted of an SP8800 Spectra-Physics Pump and an SP Chromjet Model 4400 (Spectra-Physics) integrator (San Jose, CA, USA); an LC-240 Perkin-Elmer fluorescence detector and an ISS-200 Perkin-Elmer autosampler (Norwalk, CT, USA). Separation was achieved on two Hewlett-Packard $C_{18}$ columns (3 μm particle size, 60 × 4.6 mm I.D.), installed in series (Wilmington, DE, USA). The reason for using two columns is to increase the total number of theoretical plates to achieve better separation of the analytes from unknown metabolites and other plasma impurities [7].

### 2.4. Chromatographic conditions

A gradient was used for optimum peak separation. The mobile phase consisted of solvent A (0.05 *M* ammonium phosphate–0.1% triethylamine, pH 3.5) and solvent B (acetonitrile) and was pumped at a flow-rate of 1.0 ml/min from 0 to 7 min and 1.5 ml/min from 7 to 18 min. The proportion of solvent B was linearly increased after 7 min from 47% to 70% at 18 min. The column effluent was monitored using a fluorescence detector programmed to change wavelengths at specified times. The excitation wavelengths were 252, 245, and 245 nm, and the corresponding emission wavelengths were 340, 320, and 320 nm for II, I.S., and I, respectively. Shifting of the excitation and emission wavelengths does cause a drift of baseline as shown in Figs. 2 and 3, which are fairly representative chromatograms from actual plasma samples.

### 2.5. Extraction procedure

To 100 μl of heparin-treated monkey plasma, 100 μl of I.S. solution (100 ng/ml in acetonitrile) or 100 μl of acetonitrile (for blank), and 100 μl of I and II working solution in acetonitrile or 100 μl of acetonitrile (for blank, quality controls, and samples) were added. After mixing, 200 μl of 1 *M* potassium phosphate (pH 10) and 4.0 ml of hexane were added and the mixture was vigorously shaken for a few minutes and centrifuged at 1600 *g* for 10 min. An aliquot (3.5 ml) of the hexane phase was evaporated to dryness in a 40°C waterbath under a gentle stream of nitrogen. The residue was redissolved in 300 μl of reconstitution solvent (acetonitrile–0.01 *M* phosphoric acid, 50:50, v/v) and 100 μl of hexane was added. After mixing and centrifugation at 1600 *g* for 10 min, 210 μl of the aqueous phase was mixed with 5 μl of 0.1 *M* diammoniumhydrogen phosphate buffer, and an aliquot was injected for HPLC assay.

Several solvents were screened for the extraction procedure, but no significant differences in recoveries were obtained. HPLC grade hexane was chosen for its selectivity. The optimal percent of acetonitrile in the reconstitution solvent was 50%, as final recoveries of I and II decreased with a lower percent of acetonitrile, and interfering peaks appeared when a higher percent of acetonitrile was used.

### 2.6. Recovery from monkey plasma

Recoveries of I and II from monkey plasma were determined at concentrations of 10.0, 50.0, and 150 ng/ml. Recovery of I.S. from monkey plasma was determined at 100 ng/ml. The peak heights of I, II, and I.S. obtained from monkey plasma samples assayed using this method were compared with the peak heights of standards that were prepared in acetonitrile.

### 2.7. Storage stability

The storage stability of I and II in monkey plasma was evaluated by analysis of quality control samples stored at −70°C following assay validation and sample analysis. Additional stability data were obtained by subjecting quality control samples to 3 freeze–thaw cycles.

### 2.8. Data analysis

The method was calibrated for each run by regressing peak-height ratios (I/I.S. or II/I.S.) against concentration ($C$) of the calibration standards (I or II). The best-fit line was determined by the linear least-squares method using a weighting factor of $1/C^2$. Concentrations of I and II in unknown samples were calculated using the regression equation.

## 3. Results and discussion

The method was validated for I and II in monkey plasma over the concentration range 3.0–300 and 5.0–300 ng/ml, respectively, in compliance with FDA regulations. Specificity, system parameters, recovery, linearity, precision, and accuracy of the method were determined.

### 3.1. Specificity

Representative chromatograms are shown in Fig. 2. No endogenous components in monkey

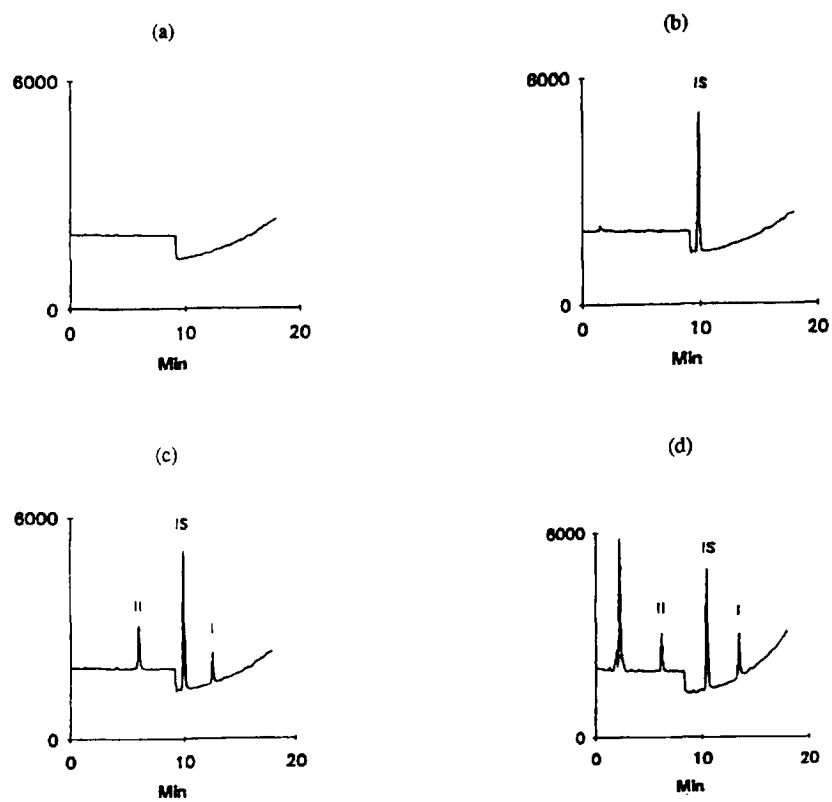


Fig. 2. Representative chromatograms of (a) monkey plasma without drug or I.S., (b) monkey plasma with I.S., (c) 25 ng/ml I and II calibration standard in monkey plasma, (d) plasma sample from a monkey at 12 h after receiving a single 25 mg/kg oral dose of I (with I.S.).

plasma eluted at the retention times of II, I.S., and I in blank control monkey plasma. During chromatographic method development, isocratic conditions were tried first, however the hydroxy derivatives II, and III, and IV were not well resolved from each other nor from endogenous components, and I did not appear until 20 min after the injection with a wide peak and low sensitivity. Using the present gradient condition, II was well resolved from the other two hydroxy derivatives and from endogenous components in monkey plasma. Optimum sensitivity was achieved for both I and II. Gradient elution also provides sufficient separation of I, II, and other metabolite peaks in plasma samples from a monkey having received a 75 mg/kg oral dose of I as shown in Fig. 3. M1, M2, and M3 peaks coeluted with II, III, and IV, respectively. The structure of I and the three metabolite peaks corresponding to the standards was confirmed by HPLC-UV diode-array purity spectra and reconstructed electrospray ionization (ESI)-MS chromatograms [6].

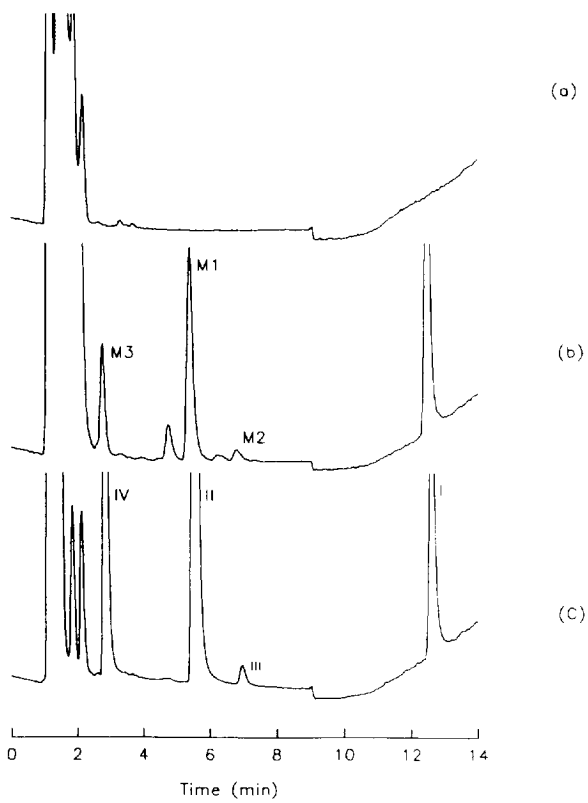


Fig. 3. Representative chromatograms of plasma samples from a monkey (a) predose, (b) 3 h after a 75 mg/kg oral dose of I, and (c) a control plasma sample spiked with 100 ng/ml of I, II, III, and IV. M1, M2, and M3 coeluted with II, III, and IV, respectively.

### 3.2. System parameters

Mean ( ± S.D.) retention times for II, I.S., and I were 6.0 ( ± 0.2), 10.2 ( ± 0.3), and 13.0 ( ± 0.4) min, respectively. Corresponding capacity factors were 1.8, 3.7, and 5.0; and asymmetry factors were 1.1, 1.2, and 1.0, respectively. Separation factors were 2.1 for II and I.S. and 1.4 for I.S. and I, respectively. The number of theoretical plates ranged from 3400 for II to 11 900 for I.

### 3.3. Recovery from monkey plasma

Recoveries of I and II from monkey plasma were determined by assaying 6 samples at each concentration from within-day validation data. Mean ( ± S.D.) recoveries ranged between 95.5 ( ± 11.6) and 103 ( ± 5)% for I, and 88.4 ( ± 5.5) and 93.2 ( ± 7.6)% for II, respectively. Mean ( ± S.D.) I.S. recovery was 104 ( ± 6%) (Table 1).

### 3.4. Storage stability

Storage stability of the quality control samples was evaluated following assay validation and sample analysis. Measured concentrations of I and II in quality control samples following approximately 3 months storage at −70°C were within ± 10.0% of the original values, indicating that I and II are stable for at least 3 months in frozen monkey plasma. Additional storage stability of the quality control samples was evaluated after subjecting them to 3 freeze–thaw cycles. Measured concentrations of I and II in quality control samples after 3 freeze–thaw cycles were within ± 6.0% of the original values, indicating that I and II are stable in monkey plasma after repeated freezing and thawing.

Table 1
Recovery of I, II, and I.S. from monkey plasma at different concentrations

| Compound | Recovery (mean ± S.D., $n = 6$) (%) | | | |
|---|---|---|---|---|
| | 10.0 ng/ml | 50.0 ng/ml | 150 ng/ml | 100 ng/ml |
| I | 95.5 ± 11.6 | 98.4 ± 6.6 | 103 ± 5 | – |
| II | 93.2 ± 7.6 | 88.4 ± 5.5 | 89.8 ± 4.2 | – |
| I.S. | – | – | – | 104 ± 6 |

### 3.5. Linearity

Eight plasma calibration standards and 3 quality control samples were analyzed in triplicate in 3 separate batch runs. Peak-height ratios (I/I.S. and II/I.S.) were proportional to the amount of I and II added to monkey plasma over the range 3–300 ng/ml for I and 5–300 ng/ml for II, respectively. Best-fit lines were determined by the method of least-squares using a weighting factor of $1/C^2$. Calibration-curve reproducibility was determined as the variation of individual standards from the regression line. Relative standard deviations of calculated values for calibration standards of I ranged from 2.0 to 8.0% with relative errors ranging from −6.6 to 5.0% (Table 2). Relative standard deviations of calculated values for calibration standards of II ranged from 1.5 to 3.0% with relative errors ranging from −2.0 to 2.0% (Table 3). The minimum quantitation limits of I and II were 3.0 and 5.0 ng/ml, respectively, for a 0.100 ml-plasma sample volume. Correlation coefficient ($r$) values were higher than 0.996 during assay validation.

### 3.6. Assay precision and accuracy

Assay precision and accuracy were evaluated using the monkey plasma quality controls. Precision (R.S.D.) of the concentrations found in the monkey quality controls ranged from 2.4 to 3.9% for I (Table 2) and from 2.5 to 3.2% for II (Table 3). Accuracy, expressed as percent rela-

Table 2
Assay precision and accuracy of I

| Added (ng/ml) | Found (ng/ml) | Relative standard deviation (%) | Relative error (%) |
|---|---|---|---|
| *Calibration standards* | | | |
| 3.0 | 3.1 | 8.0 | 5.0 |
| 5.0 | 4.7 | 5.3 | −6.6 |
| 10.0 | 9.7 | 5.2 | −2.7 |
| 25.0 | 24.3 | 2.3 | −2.8 |
| 50.0 | 49.6 | 2.2 | −0.8 |
| 100 | 103 | 2.0 | 3.0 |
| 150 | 154 | 3.7 | 2.7 |
| 300 | 309 | 2.1 | 3.0 |
| *Quality controls* | | | |
| 12.0 | 12.1 | 3.9 | 0.8 |
| 50.0 | 50.7 | 2.4 | 1.4 |
| 250 | 247 | 3.6 | −1.2 |

Calibration standards and quality controls were analyzed in triplicate on 3 separate days.

Table 3
Assay precision and accuracy of II

| Added (ng/ml) | Found (ng/ml) | Relative standard deviation (%) | Relative error (%) |
|---|---|---|---|
| *Calibration standards* | | | |
| 5.0 | 5.0 | 2.3 | 0.8 |
| 10.0[a] | 9.9 | 2.3 | −0.7 |
| 25.0 | 24.5 | 3.0 | −2.0 |
| 50.0 | 49.4 | 1.6 | −1.2 |
| 100 | 102 | 2.4 | 2.0 |
| 150 | 149 | 1.5 | −0.7 |
| 300 | 306 | 2.1 | 2.0 |
| *Quality controls* | | | |
| 12.0 | 12.7 | 2.5 | 5.8 |
| 50.0 | 50.5 | 3.2 | 1.0 |
| 250 | 243 | 2.8 | −2.8 |

[a] $n = 8$.
Calibration standards and quality controls were analyzed in triplicate on 3 separate days.

tive error, was within ± 1.4% for I (Table 2) and within ± 5.8% for II (Table 3).

### 3.7. Applications

Plasma concentration–time profiles of I and II in one cynomolgus monkey following single 5 mg/kg intravenous and 25 mg/kg oral doses of I, are shown in Fig. 4. Corresponding AUC (area under the plasma concentration–time curve) values were 4520 and 130 ng h/ml after the intravenous dose, and 1540 and 324 ng h/ml after the oral dose, for I and II, respectively.

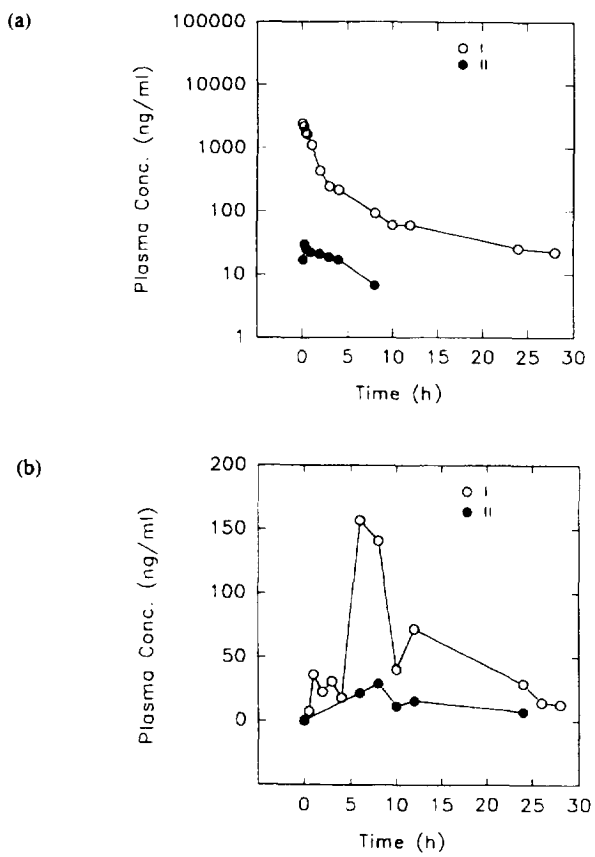


Fig. 4. Plasma concentration–time profiles of I and II in a cynomolgus monkey following (a) 5 mg/kg intravenous dose, and (b) 25 mg/kg oral dose of I.

Systemic plasma clearance of I calculated as dose divided by AUC, was 18.4 ml/min/kg. Absolute oral bioavailability of I based on the ratio of oral and intravenous AUC values normalized for dose is 6.8%. Following the 25 mg/kg oral dose, the plasma concentration–time curve of I showed multiple peaks suggesting a prolonged absorption.

## 4. Conclusions

A gradient liquid chromatographic assay with fluorescence detection for the simultaneous determination of I and II in monkey plasma is described. The method provides optimum separation and sensitivity for both I and II with minimum quantitation limits of 3.0 and 5.0 ng/ml, respectively. No monkey plasma components interfered with the quantitation of I, II, or I.S. The method is suitable for routine analysis of I and II in monkey plasma at concentrations between 3.0 and 300 ng/ml and 5.0 and 300 ng/ml, respectively.

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