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Determination of 1-[5-(2-cyclopropyl-5,7-dimethyl-imidazo[4,5-b]pyridin-3-ylmethyl)thiophen-2-yl]cyclopent-3-enecarboxylic acid (CP-191,166), an angiotensin II antagonist, in dog and rat plasma by high-performance liquid chromatography

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Abstract

A simple and precise high-performance liquid chromatographic (HPLC) assay was developed and validated for the determination of a novel angiotensin II antagonist, 1-[5-(2-cyclopropyl-5,7-dimethyl-imidazo[4,5-b]pyridin-3-ylmethyl)thiophen-2-yl]cyclopent-3-enecarboxylic acid (CP-191,166, I), in dog and rat plasma. The internal standard (II, a saturated derivative of I) and analyte were extracted by liquid–liquid extraction using methyl *tert.*-butyl ether. Samples were analyzed by reversed-phase HPLC using a Zorbax C_8 narrow-bore column with ultraviolet detection at 289 nm. The quantitation limit of I was 10 ng/ml and the calibration curve was linear over the range of 0.01–10.0 μg/ml (r^2>0.99). In dog and rat plasma, intra- and inter-assay precision ranged from 0.00 to 3.36% and 0.00 to 4.95%, respectively. The average recoveries were similar (73%) for both I and II and the upper limit of quantification of I can be as high as 500 μg/ml. The method described has been successfully applied to the quantification of I in about 2000 dog and rat plasma samples over a nine-month period.

Keywords: 1-[5-(2-cyclopropyl-5,7-dimethyl-imidazo[4,5-b]pyridin-3-ylmethyl)thiophen-2-yl]cyclopent-3-enecarboxylic acid; CP-191,166

1. Introduction

Inhibition of the renin-angiotensin system (RAS) is indicated for hypertension and congestive heart failure (CHF). There are presently three methods of inhibiting the renin-angiotensin system: renin inhibition (RI), angiotensin-converting enzyme inhibition (ACE-I), and angiotensin II (AII) receptor antagonism. An AII receptor subtype AT_1 antagonist has been reported to offer potential therapeutic advantages over existing ACE inhibitors by more effectively blocking the detrimental end-organ actions of AII (generated via non-ACE pathways) that contribute to cardiac hypertrophy and heart failure [1,2]. To facilitate studies of the pharmacokinetics of novel Pfizer AT_1-selective AII receptor antagonists [3], we developed a sensitive and selective method for the determination of the drug in dog and rat plasma

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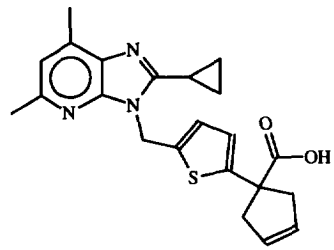
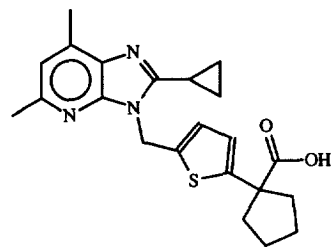


CP-191,166, I



CP-217,302, II

Fig. 1. Structures of CP-191,166 (I) and the internal standard, CP-217,302 (II).

using high-performance liquid chromatography (HPLC) with ultraviolet detection. The HPLC assay for 1-[5-(2-cyclopropyl-5,7-dimethyl-imidazo[4,5-b]pyridin-3-ylmethyl)thiophen-2-yl]cyclopent-3-enecarboxylic acid (CP-191,166, I, Fig. 1) was validated and characterized over the range of 0.01–10.0 μg I/ml of plasma.

2. Experimental

2.1. Materials

CP-191,166 (I) and CP-217,302 (II), a saturated derivative internal standard, were synthesized at Pfizer Central Research (Groton, CT, USA) (Fig. 1). HPLC-grade methyl *tert.*-butyl ether (MTBE) was purchased from Burdick and Jackson (Muskegon, MI, USA). HPLC-grade acetonitrile (CH_3CN) and 85% phosphoric acid (H_3PO_4) were obtained from Fisher Scientific (Fair Lawn, NJ, USA) and 37% hydrochloric acid (HCl) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Triethylamine (TEA) was purchased from Eastman Kodak (Rochester, NY, USA). The water used for preparing mobile phase and aqueous solutions was obtained from a Millipore Milli-EQ System (Bedford, MA, USA).

2.2. Equipment

The HPLC system consisted of an LDC constametric 3200 pump (Riviera Beach, FL, USA) and an ISS-200 autosampler (Perkin Elmer, Norwalk, CT, USA) connected to an LDC spectromonitor 3200 variable wavelength spectrophotometer. A Zorbax RX-C_8 column (150×2.1 mm I.D., 5 μm) from DuPont (Wilmington, DE, USA) was used for the HPLC separations. The signal was acquired by a Perkin Elmer Nelson 1020 integrator/computer operating to collect peak heights.

2.3. Chromatographic conditions

The flow-rate was set to 0.4 ml/min and effluent was monitored at 289 nm. The HPLC mobile phase composition was 78% water, 22% CH_3CN (v/v) with 22 m*M* TEA and 17 m*M* H_3PO_4 (i.e., 780 ml H_2O+220 ml CH_3CN+3 ml TEA+1 ml H_3PO_4). All analyses were performed at ambient temperature.

2.4. Sample preparation

After addition of 20 μl of internal standard, a 200-μl aliquot of plasma was acidified to pH 3.0 with 10 μl of 2 *M* HCl. The samples were extracted with 3 ml of MTBE by shaking for 10 min at 100 rpm. After centrifugation for 5 min at 2000 *g* the organic solvent was transferred to clean tubes and evaporated to dryness. The samples were then reconstituted in mobile phase and transferred to injection vials for HPLC analysis. A 20-μl aliquot out of a total of 100 μl was then injected onto the LC system for analysis.

2.5. Quantification

Calibration standards were prepared by adding a known amount of I (2–2000 ng) and 200 ng of II to

200 μl of control plasma. The sample extraction and HPLC analysis were carried out as described above. Concentrations of I were calculated from the linear least-squares fitted line of peak-height ratios of I to II versus standard concentrations, with reciprocal weighting on the standard concentrations. The $1/x$ weighting scheme was found to reduce residuals and improve the fit of the data to a straight line. The linear range of the standard curve was from 0.01 to 10 μg/ml in plasma of both dogs and rats.

3. Results and discussion

3.1. Intra-day assay calibration

Calibration curves consisting of ten concentrations from 0.01 to 10.0 μg/ml ($n=2$/concentration) were used to quantify dog plasma concentrations of I. Nominal calibration curve concentrations (mean found concentration) were as follows for the intra-day assay calibration: 0.01 (0.01), 0.02 (0.02), 0.05 (0.05), 0.10 (0.10), 0.25 (0.27), 0.50 (0.53), 1.00 (1.06), 2.50 (2.60), 5.00 (5.09), and 10.0 (9.72) μg/ml. The correlation coefficient, slope, and y-intercept were >0.99, 1.78, and 0.004, respectively. Intra-day accuracy and precision of the method was determined with 0.05, 1.00, and 5.00 μg/ml independently fortified dog plasma samples ($n=5$/concentration). Plasma fortified at each of these concentrations was analyzed, and mean found concentrations (±S.D.) were 0.05 (±0.00), 0.97 (±0.01) and 4.56 (±0.03) μg/ml, respectively. Mean accuracy (±precision) for the three quality control concentrations was 100% (±0.00%), 97% (±1.03%), and 91% (±0.66%), respectively.

3.2. Selectivity

Representative chromatograms of the analytes in dog and rat plasma are shown in Fig. 2 and Fig. 3, respectively. No peaks in the window necessary for analysis interfered with the detection of I or II in dog or rat plasma. Under the chromatographic conditions described, the retention times of I and II were approximately 9 and 12 min, respectively.

3.3. Inter-day assay accuracy and precision

Four calibration curves consisting of ten concentrations from 0.01 to 10.0 μg/ml were run on separate occasions and used to quantify dog plasma concentrations of I. The mean (±S.D.) correlation coefficient, slope and y-intercept for the curves were >0.99 (±0.00), 1.40 (±0.25), and 0.003 (±0.001), respectively. The quality control dog plasma samples fortified with I at 0.05, 1.00, and 5.00 μg/ml were assayed on these four separate days ($n=5$/concentration). The accuracy and precision of the method over the four days are noted in Table 1. Inter-day accuracy for the low, mid, and high range was 100%, 95%, and 90%, respectively. Inter-day assay precision for the same three concentrations was ±0.00%, ±2.11%, and ±1.33%, respectively.

3.4. Lower and upper limits of quantification

Based on an intra-assay precision of less than 15% and an accuracy within 15%, the lower and upper limits of quantification (LLOQ and ULOQ, respectively) were determined to be 0.01 and 10.0 μg/ml, respectively. Plasma fortified at each of these concentrations ($n=5$) was analyzed, and mean found concentrations (±S.D.) were 0.01 (±0.00) and 9.58 (±0.23) μg/ml, respectively. Accuracy (±precision) for the LLOQ and ULOQ was 100% (±0.00%) and 96% (±2.40%), respectively.

3.5. Integrity of dilution

Dog plasma was fortified at 5.00 μg/ml and diluted with control matrix by factors of two and fifty. These samples were assayed in triplicate and found to be both accurate (94% and 97%, respectively, for the two dilution factors) and precise (±1.27% and ±6.00%, respectively).

3.6. Recovery

Compound I recovery samples were analyzed at 0.05, 1.00 and 5.00 μg/ml. The recovery was determined by comparing peak heights from five unextracted standards with those of five extracted standards at the same three concentrations. Mean % recovery (±S.D.) for I at the low, mid, and high

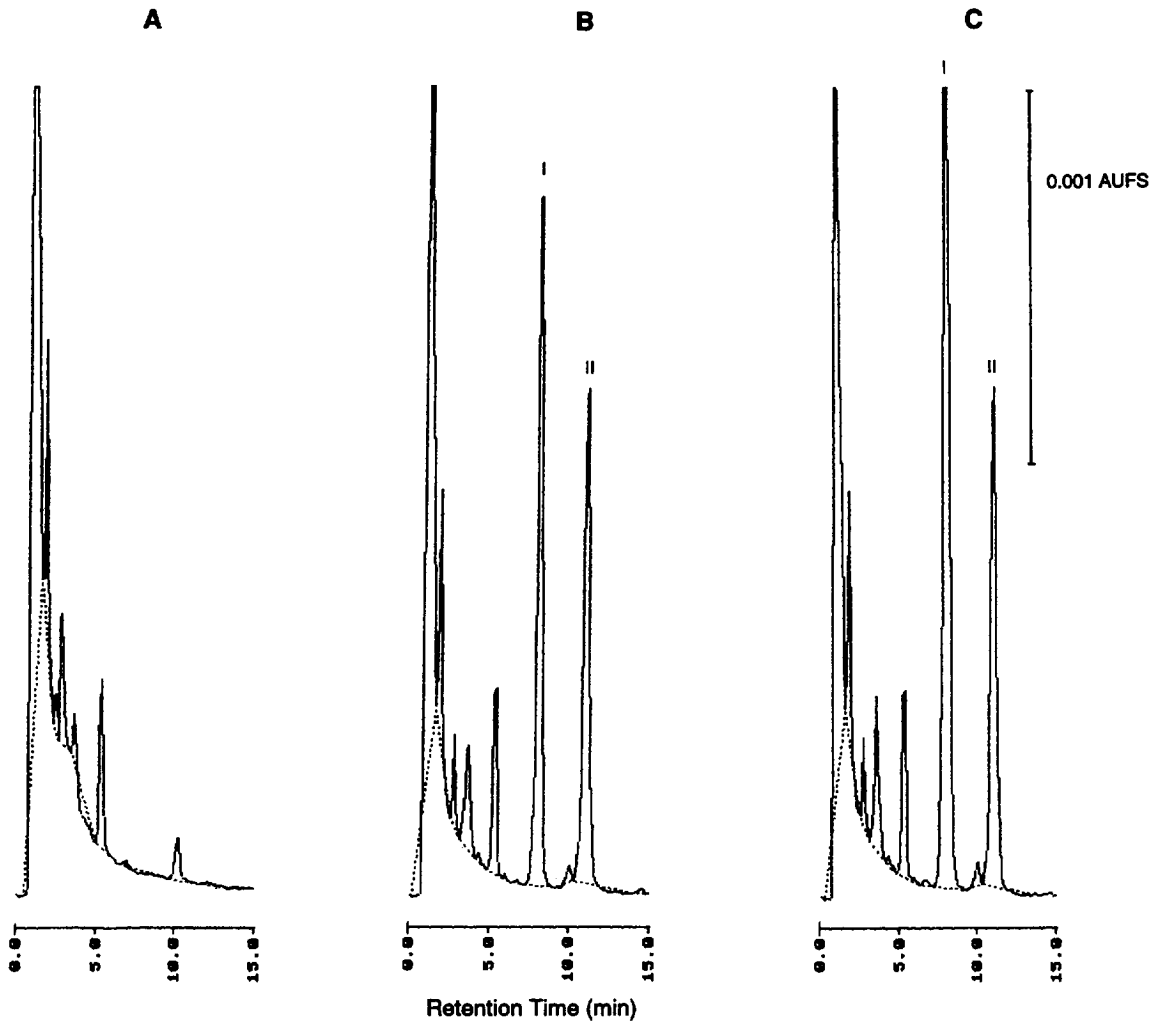


Fig. 2. Representative chromatograms of I (CP-191,166) and II (internal standard) in dog plasma. (A) Drug-free control dog plasma extract. (B) Control dog plasma extract containing 1.0 μg/ml I and 200 ng II. (C) A 1-h sample from a dog given a single 15 mg/kg oral dose of I (3.36 μg/ml I and 1.0 μg/ml II).

concentrations noted was 81% (±4.3), 75% (±1.9), and 73% (±0.9), respectively. Recovery (±S.D.) of II was monitored at 1.00 μg/ml (n=15) and determined to be 79% (±3.5).

3.7. Cross species validation

In order to validate the assay of I in rat plasma, this matrix was fortified at 0.05, 1.00, and 5.00 μg/ml and assayed (n=5/concentration) against a dog plasma standard curve. Mean found concentrations were 0.05, 1.01, and 4.90 μg/ml. Mean accuracy for the low, mid, and high concentrations was 100%, 101%, and 98%, respectively, and mean precision was ±0.00%, ±4.95%, and ±2.65%, respectively, for the three concentrations tested.

3.8. Stability

The stability of I in dog and rat plasma was evaluated at 0.05, 1.00, and 5.00 μg/ml. Compound I was stable in dog plasma after storage at −20°C for six weeks, after being frozen and thawed on three separate occasions, and after 24 h at room tempera-

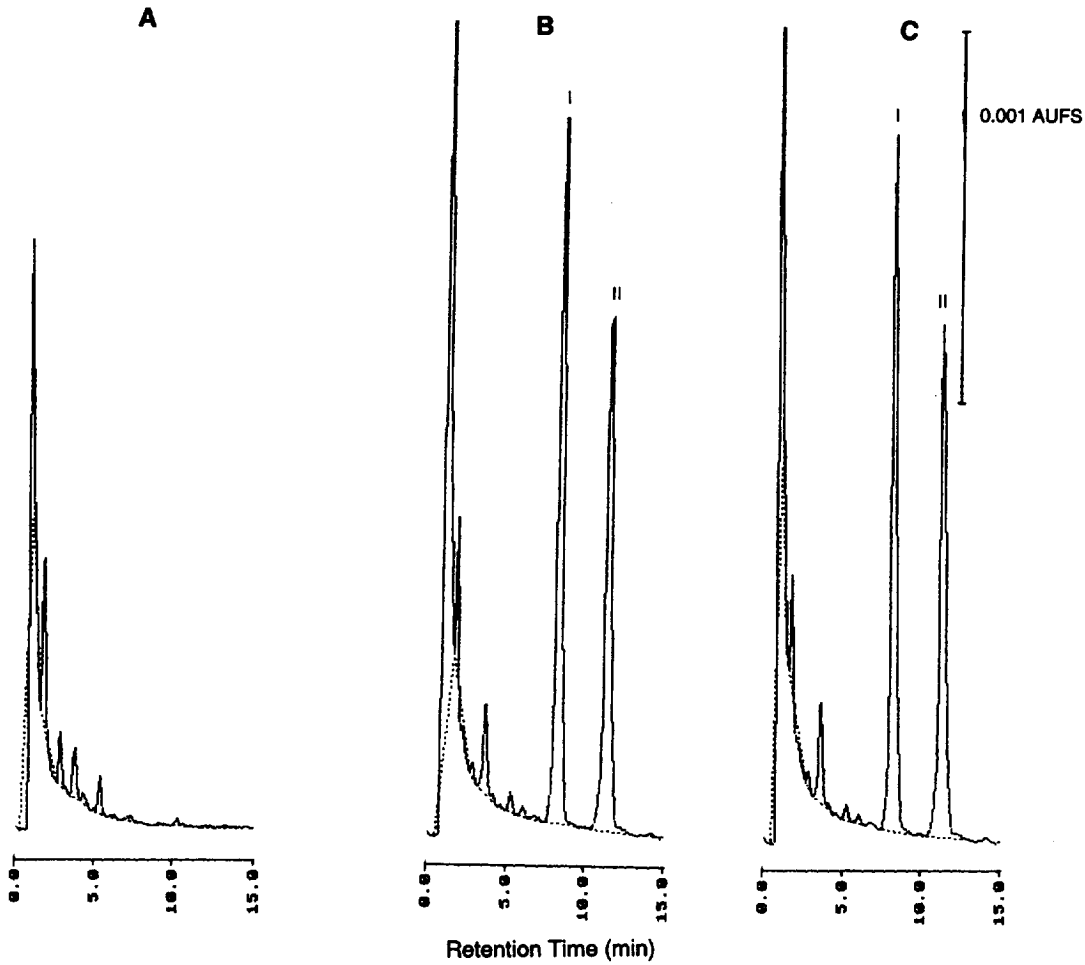


Fig. 3. Representative chromatograms of I (CP-191,166) and II (internal standard) in rat plasma. (A) Drug-free control rat plasma extract. (B) Control rat plasma extract containing 1.0 μg/ml I and 200 ng II. (C) A 1-h sample from a rat given a single 10 mg/kg oral dose of I (0.94 μg/ml I and 1.0 μg/ml II).

ture (Table 2). Compound I was demonstrated to be stable in rat plasma after storage at −20°C for four weeks (Table 3). Compound I was stable in HPLC injection solvent for up to 24 h at room temperature.

3.9. Analysis of animal pharmacokinetic study samples

The method described has been successfully applied to the quantification of I in about 2000 rat and dog plasma samples over a nine-month period. The data from a representative rat and dog given an oral dose of 10 and 15 mg/kg of I, respectively, are shown in Fig. 4.

4. Conclusion

A simple and reproducible procedure for the quantification of an AII antagonist in dog or rat plasma has been developed. The use of narrow-bore HPLC is documented to increase mass sensitivity and improve peak shape over conventional 4.6 mm I.D. columns [4,5]. In the case of I, a 2.1 mm I.D. HPLC Zorbax column allows the analysis to be cost

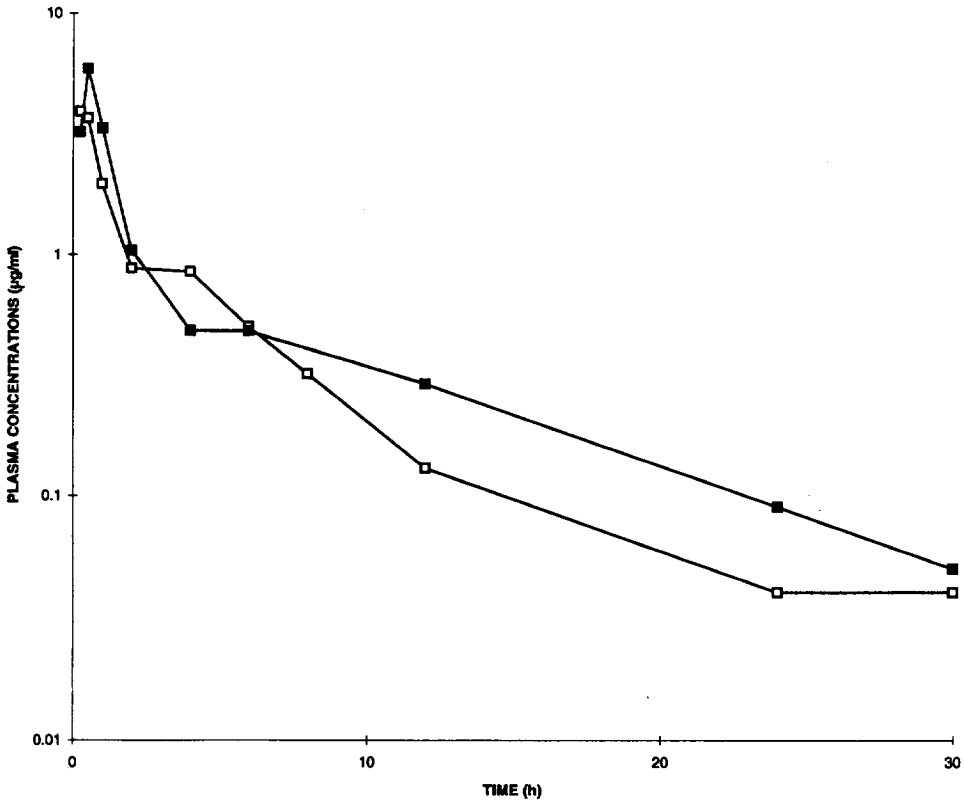


Fig. 4. Plasma concentrations of I following a single 10 mg/kg oral dose to a representative rat (□) and a single 15 mg/kg oral dose to a representative dog (■).

Table 1
Inter-day assay validity

	Day 1	Day 2	Day 3	Day 4	Inter-day
Nominal concentration 0.05 μg/ml					
Mean found (μg/ml)	0.05	0.05	0.05	0.05	0.05
S.D.	0.00	0.00	0.00	0.00	0.00
Accuracy (%)	100	100	100	100	100
Precision (±%)	0.00	0.00	0.00	0.00	0.00
Nominal concentration 1.00 μg/ml					
Mean found (μg/ml)	0.97	0.93	0.94	0.97	0.95
S.D.	0.01	0.03	0.01	0.01	0.02
Accuracy (%)	97	93	94	97	95
Precision (±%)	1.03	3.23	1.06	1.03	2.11
Nominal concentration 5.00 μg/ml					
Mean found (μg/ml)	4.56	4.47	4.45	4.58	4.52
S.D.	0.03	0.15	0.14	0.03	0.06
Accuracy (%)	91	89	89	92	90
Precision (±%)	0.66	3.36	3.15	0.66	1.33

Table 2
Stability of I in dog plasma

	0.05 μg/ml	1.00 μg/ml	5.00 μg/ml
24-h room temperature stability			
Mean found (μg/ml)	0.05	0.91	4.61
S.D.	0.00	0.02	0.18
Accuracy (%)	100	91	92
Precision (±%)	0.00	2.20	3.90
Six-week frozen stability (stored at −20°C)			
Mean found (μg/ml)	0.05	1.00	5.00
S.D.	0.00	0.02	0.16
Accuracy (%)	100	96	93
Precision (±%)	0.00	2.08	3.45
Freeze–thaw samples (thawed three times)			
Mean found (μg/ml)	0.05	0.97	4.58
S.D.	0.00	0.01	0.03
Accuracy (%)	100	97	92
Precision (±%)	0.00	1.03	0.66

Table 3
Stability of I in rat plasma

	0.05 μg/ml	1.00 μg/ml	5.00 μg/ml
24-h room temperature stability			
Mean found (μg/ml)	0.05	1.04	5.01
S.D.	0.01	0.05	0.02
Accuracy (%)	100	96	100
Precision (±%)	20.00	4.81	0.40
Four-week frozen stability (stored at −20°C)			
Mean found (μg/ml)	0.06	0.96	4.98
S.D.	0.00	0.03	0.09
Accuracy (%)	120	96	99
Precision (±%)	0.00	3.13	1.81

effective with regard to solvent use and waste disposal, while increasing the sensitivity required for measurement of three or more half-lives in animal pharmacokinetic studies.

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