ELSEVIER

Journal of Chromatography B, 693 (1997) 159–166

JOURNAL OF CHROMATOGRAPHY B

Determination of two CI-1007 sulfate metabolites in monkey plasma and urine

M. Rose Feng*, J. Atherton, S. Knoll, Carol A. Strenkoski, D. Scott Wright

Parke-Davis Pharmaceutical Research, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor MI 48 105, USA

Received 4 October 1996; revised 21 November 1996; accepted 5 December 1996

Abstract

Two HPLC assays were developed and validated for simultaneous quantitation of two sulfate metabolites, PD 163637 (VI) and PD 163639 (VIII), of an investigational antipsychotic drug CI-1007 (I) in monkey plasma and urine. VI and VIII were identified as major metabolites in monkey plasma, and both were excreted in urine. Monkey plasma samples were directly injected after deproteinization, and urine samples were analyzed after a clean-up procedure using methyl-*tert.*-butyl ether. Liquid chromatographic separation was achieved on a Zorbax RX C_8 analytical column using gradient elution. Column effluent was monitored using fluorescence detection with excitation and emission wavelengths of 254 and 330 nm, respectively. Minimum quantitation limit was 50 ng/ml in plasma and 100 ng/ml in urine. Linearity was demonstrated up to 3000 ng/ml in plasma and urine. Recoveries of the analytes from plasma and urine were greater than 85%. The assay has been applied to the determination of VI and VIII in plasma and urine samples from monkeys receiving oral administration of I.

Keywords: CI-1007; CI-1007 sulfates

1. Introduction

CI-1007 (PD 143188, I), is an investigational antipsychotic drug [1,2] currently under clinical evaluation. I is extensively metabolized in rats and monkeys following oral administration [3–5]. Chemical structures of I, VI, VIII, and the other metabolites are shown in Fig. 1. One monohydroxy metabolite PD 147693 (II) was identified in rat plasma, and one dihydroxy (PD 155144, IV) and two monohydroxy (II and PD 149394, III) metabolites were identified in monkey plasma [6–8]. A sensitive HPLC assay with fluorescence detection was published in 1995 for simultaneous quantitation of I and the active metabolite II in monkey plasma [8]. Afterward, a gradient HPLC method was developed for the detection of I, II, III, IV and five sulfate conjugates PD 163249 (V), PD 163637 (VI), PD 163638 (VII), PD 163639 (VIII), and PD 163640 (IX) in monkey plasma and urine (Fig. 2). Since VI and VIII were identified as the major metabolites in monkey plasma and both were excreted in urine, two assays with fluorescence detection were further developed and validated for simultaneous quantitation of VI and VIII in monkey plasma and urine.

*Corresponding author.

PII S0378-4347(97)00015-7

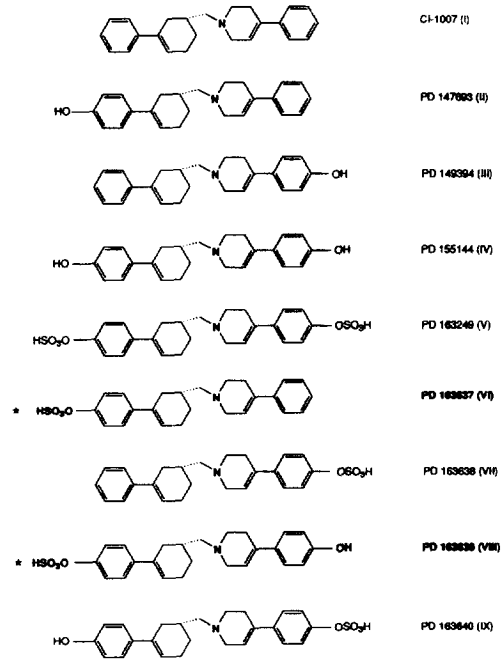

Fig. 1. Structures of I, II, III, IV, V, VI, VIII, VIII and IX.

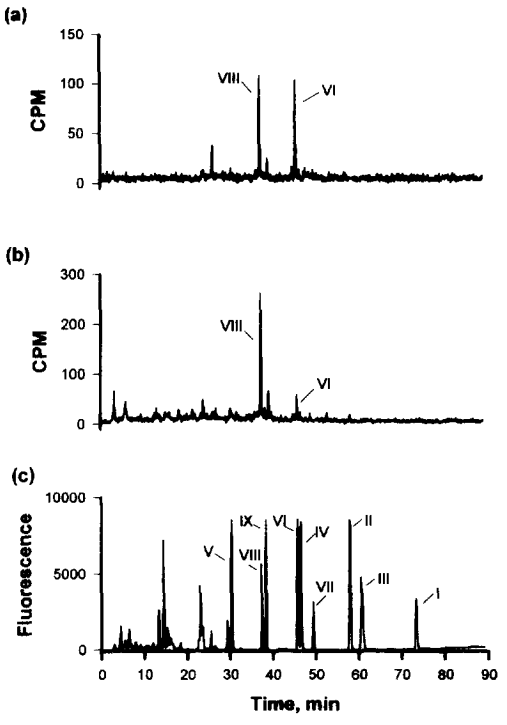

Fig. 2. Representative chromatograms of (a) 5-h postdose plasma sample from a monkey receiving a 75 mg/kg single oral administration of [^{14}C]I, (b) 8–24 h postdose urine sample from a monkey receiving a 25 mg/kg single oral administration of [^{14}C]I, and (c) a standard of I, II, III, IV, V, VI, VII, VIII and IX. A 90-min gradient HPLC method with dual radiometric and fluorescence detection was used.

2. Experimental

2.1. Reagents and chemicals

Compound I, pyridine, 1,2,3,6-tetrahydro-4-phenyl-1-[(3-phenyl-3-cyclohexen-1-yl)methyl]-(*R*)-(+) enantiomer, [^{14}C]I, and the metabolites were synthesized at Parke-Davis Pharmaceutical Research, Division of Warner-Lambert (Ann Arbor, MI, USA). HPLC grade water, methanol, acetonitrile, and other reagents were from EM Science (Gibbstown, NJ, USA). Ammonium acetate and ammonium phosphate monobasic were from Fisher Scientific (Fair Lawn, NJ, USA). Control heparinized monkey plasma was from Lampire Biological Laboratories (Pipersville, PA, USA).

2.2. Instrumentation

The chromatographic system consisted of Perkin-Elmer 200 LC pump, ISS-200 autosampler, and LC-240 fluorescence detector (Norwalk, CT, USA). A Flo-One series A-200 detector (Radiomatic) was used for ^{14}C radiometric detection. Separation was achieved on a Zorbax RX C_8 column (5 μm particle size, 250×4.6 mm I.D., MAC-MOD Analytical, Chadds Ford, PA, USA).

2.3. Chromatographic conditions

Twenty-five minute gradient: this method was used for quantitation of VI and VIII in monkey plasma. Mobile phase consisted of solvent A (0.02 *M* ammonium acetate buffer pH 6.5–acetonitrile, 90:10, v/v) and solvent B (0.02 *M* ammonium acetate buffer pH 6.5–acetonitrile 25:75, v/v) and was pumped at a flow-rate of 1.0 ml/min. The proportion of solvent B was increased linearly from 25% at 0 min to 40% at 20 min, and to 60% at 25 min. The time for re-equilibration was 10 min before the next injection. Column effluent was monitored using fluorescence detection with excitation and emission

wavelengths of 254 and 330 nm, respectively. Column was operated at room temperature.

Thirty-two minute gradient: this method was used to quantitate VI and VIII in monkey urine. Mobile phase consisted of solvent A [0.025 *M* ammonium phosphate buffer pH 7.0 (containing 0.1% triethylamine)–acetonitrile, 90:10, v/v] and solvent B [0.025 *M* ammonium phosphate buffer (containing 0.1% triethylamine) pH 7.0–acetonitrile, 25:75, v/v] and was pumped at a flow-rate of 1.0 ml/min. The proportion of solvent B was increased linearly from 25% at 0 min to 45% at 32 min. The time for re-equilibration was 15 min before the next injection. Column effluent was monitored using fluorescence detection with excitation and emission wavelengths of 254 and 330 nm, respectively. Column temperature was maintained at 40°C.

Ninety minute gradient: this method was used for detection of I, II, III, IV, V, VI, VII, VIII, and IX in monkey plasma and urine. Mobile phase consisted of solvent A (0.02 *M* ammonium acetate buffer pH 4.0–acetonitrile, 90:10, v/v) and solvent B (0.02 *M* ammonium acetate buffer pH 4.0–acetonitrile, 25:75, v/v). The proportion of solvent A was 100% at 0 time, and the proportion of solvent B was increased linearly from 0% at 0 min to 85% at 90 min. The time for re-equilibration was 15 min before the next injection. Column effluent was monitored with dual radiometric and fluorescence detection (excitation and emission wavelengths of 245 and 320 nm, respectively). Column was operated at room temperature.

2.4. Preparation of calibration standards

Plasma: stock solution A, 100 μg/ml of VI and VIII, was prepared by dissolving accurately weighed quantities of VI and VIII in 10 ml of dimethyl sulfoxide (DMSO). Stock solution A was further diluted with DMSO to prepare a 5 μg/ml stock solution B of VI and VIII. Stock solutions A and B were used to prepare seven working solutions in DMSO: 50, 100, 300, 500, 1000, 1500, and 3000 ng/ml. Volumes (0.050 ml) of the working solutions were added to 0.050 ml of blank monkey plasma to prepare calibration standards.

Urine: stock solution A, 25 μg/ml of VI and VIII, was prepared by dissolving accurately weighed quantities of VI and VIII in 100 ml of DMSO. Stock solution A was further diluted with a mixture of DMSO–methanol (1:6, v/v) to prepare eight working solutions: 100, 150, 200, 300, 500, 1000, 1500, and 3000 ng/ml. Volumes (0.200 ml) of the working solutions were added to 0.700 ml modified monkey urine to prepare calibration standards.

Modified urine was prepared by mixing 250 ml of blank monkey urine with 125 ml of DMSO and 750 ml of methanol and was used to prepare quality control samples. Compounds VI and VIII are well dissolved in DMSO, but have limited solubility in water. During sample collection, DMSO and methanol were added to actual urine samples to prevent bacteria growth and possible precipitation of VI and VIII during storage.

2.5. Preparation of quality controls

Plasma: monkey plasma quality control standards, which contained 100, 500, and 1500 ng/ml of VI and VIII were prepared by diluting 0.500-ml aliquots of a 5 μg/ml stock solution or 0.125 ml or 0.375 ml of a 100 μg/ml stock solution of VI and VIII to 25.0 ml with blank monkey plasma, respectively. These solutions were subdivided into 0.200-ml volumes and stored at approximately −20°C.

Urine: urine quality control standards, which contained 250, 800, and 2000 ng/ml of VI and VIII were prepared by diluting 0.500, 1.60, and 4.00-ml volumes of a 25 μg/ml stock solution to 225 ml with modified monkey urine, respectively. These solutions were subdivided into 3.00-ml volumes and stored at approximately −20°C.

2.6. Preparation of samples

Plasma: to 0.050 ml of heparin-treated monkey plasma, 0.050 ml DMSO (for blank, quality controls, and samples) or 0.050 ml of the working solutions of VI and VIII in DMSO (for calibration standards) and 0.400 ml of methanol were added. Samples were mixed and centrifuged at 1600 *g* for 10 min, and the resulting supernatant was removed and evaporated to near dryness (some DMSO remains) in a 50°C waterbath under nitrogen. To the residue, 0.400 ml of mobile phase solvent A were added and mixed

thoroughly, and aliquots (0.100 ml) were injected for HPLC assay.

Urine: to 0.200 ml of blank monkey urine, 0.700 ml of a mixture of DMSO–methanol (1:6, v/v) (for blank) or 0.200 ml of the working solutions of VI and VIII in DMSO–methanol (1:6, v/v) and 0.500 ml of a mixture of DMSO–methanol (1:6, v/v) (for calibration standards) were added. The samples were thoroughly mixed.

To 0.800 ml of the above prepared blank or calibration standards (or 0.800 ml of the quality controls or samples), 0.400 ml of a 0.005 *M* H_3PO_4 solution were added. Samples were vortexed for 15 s, and 7.0 ml of methyl *tert.*-butyl ether were added. Samples were vortexed for another 5 min and centrifuged for 10 min at 1600 *g*. The organic layer was removed and discarded, and 0.200 ml of aqueous phase were transferred into injection vials containing 0.800 ml of mobile phase A. After mixing, volumes (0.050 ml) were injected for HPLC assay. Several solvents were screened for the clean-up procedure, and methyl-*tert.*-butyl ether was used for the assay since it selectively extracted most of the endogenous interferences from the urine samples.

2.7. Recovery

Recoveries of VI and VIII from monkey plasma or urine were determined at concentrations of 200, 1000 and 3000 ng/ml. HPLC response of VI and VIII obtained from assayed monkey plasma or urine sample was compared with response of standards that were prepared in solvent.

2.8. Storage stability

The storage stability of VI and VIII in monkey plasma and urine was evaluated by analysis of quality control samples stored at −20°C following assay validation and sample analysis. Additional stability data were obtained by subjecting quality controls to three freeze–thaw cycles.

2.9. Data analysis

The method was calibrated for each run by regressing peak height of each analyte against concentration (C) of the calibration standards (VI or VIII). The best-fit lines were determined by the linear least-squares method using a weighting factor of $1/C^2$. Compounds VI and VIII concentrations in unknown samples were calculated using the regression equations.

3. Results and discussion

Two assays were validated for simultaneous quantitation of the sulfate metabolites VI and VIII in monkey plasma and urine over the concentration range of 50–3000 ng/ml and 100–3000 ng/ml, respectively. In compliance with FDA regulations, specificity, recovery, stability, linearity, precision, and accuracy of the method were determined.

3.1. Specificity

A 25-min HPLC gradient was used for the plasma assay, and a 32-min gradient was used for the urine assay. Representative HPLC chromatograms with fluorescence detection are shown in Figs. 3 and 4. During chromatographic method development, isocratic conditions were first tried, however, the hydroxy and sulfate metabolites were not well resolved from each other nor from endogenous components. Gradient HPLC was used and mobile phase pH was adjusted to achieve optimum separation. The retention time of I, II, III, and IV became longer when mobile phase pH increased. But the sulfate metabolites are highly ionized at almost any pH, and the retention times of V, VI, VII, VIII, and IX only slightly moved when mobile phase pH was changed. Using the present gradient conditions, VI is well resolved from the hydroxy derivative IV, and VIII from the sulfate IX (Figs. 3 and 4d), and both VI and VIII are well separated from the endogenous components in monkey plasma or urine. Mobile phase buffer was switched to ammonium phosphate in the urine assay to further improve the resolution since more endogenous peaks were detected in this matrix. Six different sources of monkey plasma and urine were screened and no endogenous components eluted at the retention times of VI and VIII in control plasma or urine.

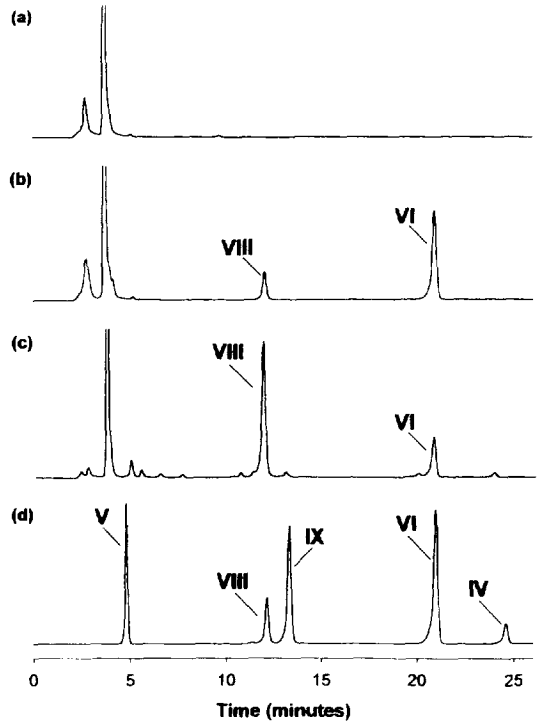

Fig. 3. Representative chromatograms of (a) control monkey plasma, (b) 1000 ng/ml calibration standard of VI and VIII in control monkey plasma, (c) 12-h postdose plasma sample from a monkey receiving a single 75 mg/kg oral dose of I, (d) a standard of IV, VI, VIII and IX. A 25-min gradient with fluorescence detection was used.

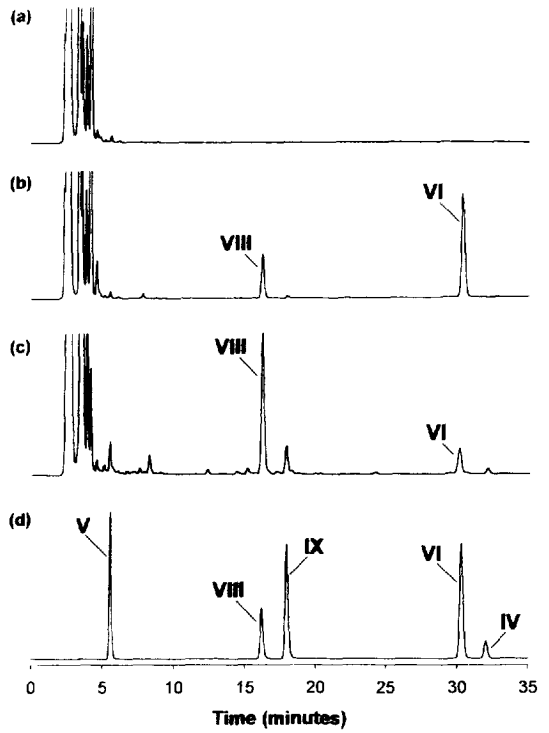

Fig. 4. Representative chromatograms of (a) control monkey urine, (b) 3000 ng/ml calibration standard of VI and VIII in monkey urine, (c) 0–8 h postdose urine sample from a monkey receiving a 10 mg/kg oral dose of I, (d) a standard of IV, VI, VIII and IX. A 32-min gradient with fluorescence detection was used.

3.2. Recovery

Recoveries of VI and VIII from monkey plasma or urine were determined by assaying six samples at each concentration from within-day validation data. Mean (±S.D.) recoveries from plasma were 92.0 (±3.2)%, 91.3 (±5.3)% and 86.0 (±2.3)% for VI and 92.7 (±5.4)%, 93.7 (±5.8)% and 91.1 (±4.3)% for VIII at concentrations of 200, 1000 and 3000 ng/ml, respectively. Mean (±S.D.) recoveries from urine were 93.0 (±1.7)%, 95.1 (±1.6)%, and 93.3 (±1.7)% for VI, and 101 (±1.6)%, 96.3 (±1.6)%, and 95.9 (±1.0)% for VIII at concentrations of 200, 1000 and 3000 ng/ml, respectively.

3.3. Storage stability

The storage stability of the quality control samples was evaluated following assay validation and sample analysis. Measured concentrations of VI and VIII in quality control samples following approximately 2 months storage at −20°C were within ±15.0% of the original values, indicating that VI and VIII are stable for at least 2 months in frozen monkey plasma and urine. Additional stability data were obtained by subjecting quality controls to three freeze–thaw cycles. Measured concentrations of VI and VIII in quality control samples after three freeze–thaw cycles were within ±10.0% of the original values, indicating that VI and VIII are stable in monkey plasma and urine after repeated freezing and thawing.

3.4. Linearity

Seven plasma calibration standards and three plasma quality control samples, or eight urine calibration standards and three urine quality control samples were analyzed in three separate batch runs.

Table 1
Assay precision and accuracy of VI in monkey plasma from three separate runs

Added (ng/ml)	Found (ng/ml)	Relative standard deviation (%)	Relative error (%)
Calibration standards[a]			
50.0	49.8	2.2	−0.4
100	101	1.9	1.0
300	298	3.6	−0.7
500	498	2.4	−0.4
1000	1020	3.1	2.0
1500	1490	2.2	−0.7
3000	2970	1.8	−1.0
Quality controls[b]			
100	101	6.9	1.0
500	514	6.9	2.8
1500	1480	4.1	−1.3

[a] $n=3$.
[b] $n=9$.

Peak height was proportional to the amount of VI and VIII added to monkey plasma over the range of 50–3000 ng/ml or to monkey urine over the range of 100–3000 ng/ml. 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. Correlation coefficient (r) values were higher than 0.994 during assay validation for both methods.

In the plasma assay, relative standard deviations of calculated values for calibration standards of VI ranged from 1.8 to 3.6% with relative errors within ±2.0% (Table 1), and relative standard deviations of calculated values for calibration standards of VIII ranged from 2.0 to 6.0% with relative errors within ±5.6% (Table 2). In the urine assay, relative standard deviations of calculated values for calibration standards of VI ranged from 0.3 to 6.1% with relative errors within ±6.7% (Table 3), and relative standard deviations of calculated values for calibration standards of VIII ranged from 1.7 to 9.4% with relative errors within ±4.7% (Table 4). Minimum quantitation limit for VI and VIII was 50 ng/ml in plasma and 100 ng/ml in urine with signal-to-noise ratios higher than 5.0.

Table 2
Assay precision and accuracy of VIII in monkey plasma from three separate runs

Added (ng/ml)	Found (ng/ml)	Relative standard deviation (%)	Relative error (%)
Calibration standards[a]			
50.0	51.5	5.7	3.0
100	94.4	5.3	−5.6
300	289	6.0	−3.7
500	497	2.2	−0.6
1000	1030	2.7	3.0
1500	1520	2.2	1.3
3000	3070	2.0	2.3
Quality controls[b]			
100	99.8	6.8	−0.2
500	514	8.4	2.8
1500	1560	6.9	4.0

[a] $n=3$.
[b] $n=8$–9.

Table 3
Assay precision and accuracy of VI in monkey urine from three separate runs

Added (ng/ml)	Found (ng/ml)	Relative standard deviation (%)	Relative error (%)
Calibration standards[a]			
100	99.0	3.2	−1.0
150	147	3.4	−2.0
200	208	6.1	4.0
300	306	0.3	2.0
500	492	4.4	−1.6
1000	1030	1.9	3.0
1500	1550	2.3	3.3
3000	2800	3.4	−6.7
Quality controls[b]			
250	246	6.3	−1.6
800	793	2.7	−0.9
2000	1930	6.5	−3.5

[a] $n=3$.
[b] $n=9$.

3.5. Assay precision and accuracy

Assay precision and accuracy were evaluated using quality controls. Precision (R.S.D.) of the concentrations found in the monkey plasma quality controls ranged from 4.1% to 6.9% for VI and from 6.8% to 8.4% for VIII (Tables 1 and 2). Accuracy, expressed as percent relative error, was within ±2.8% for VI and within ±4.0% for VIII.

Precision (R.S.D.) of the concentrations found in the monkey urine quality controls ranged from 2.7% to 6.5% for VI and 2.2% to 5.1% for VIII (Tables 3 and 4). Accuracy, expressed as percent relative error, was within ±3.5% for VI and within ±5.5% for VIII.

4. Conclusions

Two gradient HPLC assays using fluorescence detection were developed and validated for quantitation of VI and VIII in monkey plasma and urine. The methods provide optimum separation and sensitivity

Table 4
Assay precision and accuracy of VIII in monkey urine from three separate runs

Added (ng/ml)	Found (ng/ml)	Relative standard deviation (%)	Relative error (%)
Calibration standards[a]			
100	97.9	4.8	−2.1
150	156	2.3	4.0
200	195	9.4	−2.5
300	306	2.8	2.0
500	493	4.8	−1.4
1000	1020	1.7	2.0
1500	1540	2.3	2.7
3000	2860	4.5	−4.7
Quality controls[b]			
250	247	4.9	−1.2
800	774	2.2	−3.3
2000	1890	5.1	−5.5

[a] $n=3$.
[b] $n=9$.

for both VI and VIII with minimum quantitation limits of 50 and 100 ng/ml in monkey plasma and urine, respectively. No monkey plasma or urine components interfered with quantitation of VI and VIII. The method is suitable for routine analysis of VI and VIII in monkey plasma at concentrations between 50 and 3000 ng/ml and in monkey urine at concentrations between 100 and 3000 ng/ml.

References

[1] T. Pugsley, D. Davis, C. Akunne, L. Cooke, S. Whetzel, R. Mackenzie, Y. Shih, D. Van Leeuwen, L. Demattos, B. Caprathe, J. Wright, J. Jean, L. Wise and T. Heffner, J. Pharmacol. Exp. Ther., 274 (1995) 898–911.

[2] L. Meltzer, C. Christoffersen, A. Corbin, F. Ninteman, K. Serpa, L. Wise and T. Heffner, J. Pharmacol. Exp. Ther., 274 (1995) 912–920.

[3] M. Rose Feng, S. Bezek, A. Gandhi, C. Strenkoski, T. Woolf and D.S. Wright, Pharm. Res., 10 (Suppl.) (1994) S-347.

[4] J. Wright, D. Downing, M. Rose Feng, R. Hayes, T. Heffner, R. Mackenzie, L. Meltzer, T. Pugsley and L. Wise, J. Med. Chem., 38 (1995) 5007–5014.

[5] M. Sinz, B. Michniewicz and T. Woolf, Poster for 4th International ISSX, Seattle, WA, 1995.

[6] M.R. Feng, C. Strenkoski, T. Parker and D.S. Wright, Pharm. Res., 10 (Suppl.), (1993) S-320.

[7] M.R. Feng, A. Corbin, Y. Wang, C. Christoffersen, C. Strenkoski, E. Tucker, T. Parker, J. Wiley, F. Ninteman, T. Heffner and D.S. Wright, Pharm. Res., 9 (Suppl.) (1995) S-399.

[8] M. Rose Feng, P. Siersma, C. Strenkoski and D.S. Wright, J. Chromatogr. B, 665 (1995) 193–199.