ELSEVIER

Journal of Chromatography B, 668 (1995) 141–151

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

High-performance liquid chromatographic assay for CI-980, a novel 1-deaza-7,8-dihydropteridine anticancer agent, in human plasma and urine

William W. Bullen*, Lloyd R. Whitfield, Gary A. Walter, Joanne I. Brodfuehrer

Parke-Davis Pharmaceutical Research, Department of Pharmacokinetics and Drug Metabolism, Division of Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105, USA

First received 26 September 1994; revised manuscript received 31 January 1995; accepted 1 February 1995

Abstract

CI-980, a 1-deaza-7,8-dihydropteridine, is a novel anticancer agent that is a potent mitotic inhibitor acting as a tubulin binder similar to the vinca alkaloids. CI-980 has shown equivalent or superior anticancer activity in vitro compared to vincristine and retains full activity against vincristine resistant tumors in vitro. A high-performance liquid chromatographic (HPLC) assay was developed and validated for human plasma and urine to support Phase 1 clinical trials. CI-980 and PD 080658, internal standard, were isolated from 2-ml samples of human plasma and urine by solid-phase extraction with Bond-Elut C_{18} cartridges. Urine samples must be pretreated with bovine serum albumin (BSA) to minimize the binding of CI-980 to glass and some plastics. The eluate from the cartridges for both matrices was evaporated to dryness and taken up in mobile phase. Zorbax RX C_{18} columns, mobile phase buffer of 10 m*M* ammonium dihydrogen phosphate at pH 7.5 and a flow-rate of 0.75 ml/min were used for both matrices. Column dimensions, column temperature and mobile phase acetonitrile–buffer ratio were 300 mm × 4.6 mm I.D., 30°C and 38:62 (v/v), respectively, for the plasma assay and 250 mm × 4.6 mm I.D., 35°C and 40:60 (v/v), respectively, for the urine assay. Column effluent was monitored fluorometrically for the plasma method using excitation and emission wavelengths of 388 nm and 473 nm, respectively. Ultraviolet detection at 380 nm was used for the urine method. Peak-area ratios were proportional to CI-980 concentrations from 0.2 to 25 ng/ml and 1 to 100 ng/ml for plasma and urine, respectively. CI-980 in water will bind to glass and plastics but not PTFE or stainless steel. Urine calibration standards were frozen prior to use in order to compensate for loss of CI-980 due to freezing in this matrix. The accuracy of the assay was within 4.7%, with a precision of 5.6% for both matrices. Recoveries ranged from 93.8 to 102% and 90.7 to 92.3% for plasma and urine, respectively. CI-980 was stable in plasma and urine for at least 275 and 217 days, respectively, when stored at −70°C. The assay is suitable for studying the clinical pharmacokinetics of CI-980.

1. Introduction

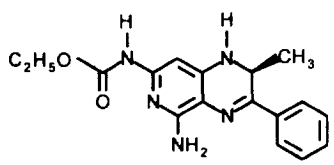
CI-980, a novel anticancer agent (I), has shown superior or equivalent anticancer activity in vitro compared to vincristine and retains full activity against vincristine resistant tumors in vitro [1–5]. I was first synthesized as one of a series of potential folate antagonists [6,7]. How-

* Corresponding author.

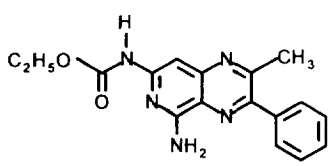
0378-4347/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0378-4347(95)00067-4

ever, I did not act as a folic acid analogue but as a potent mitotic inhibitor similar to the vinca alkaloids which act by binding to tubulin and thereby prevent tubulin polymerization [1,2,8].

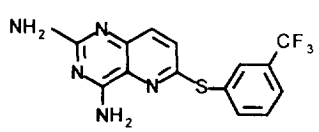
I, ethyl (*S*)-(5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl)carbamate 2-hydroxyethanesulfonate (1:1), is a potent compound [9]. The first doses in man would be 0.12 $mg/m^2/d$, and thus the expected plasma concentrations would likewise be low [10]. Therefore, in order to support pharmacologically guided dose escalation in Phase 1 clinical trials, a method with picogram sensitivity was needed to assay in plasma. This report describes the development and validation of liquid chromatographic assays for I in human plasma and urine. The method described separates I from PD 132182 (II), a known degradation product of I and a possible metabolite, but does not quantify II. The structures of I, II and the internal standard, PD 080658 (III) are shown in Fig. 1.



CI-980 (I)



PD 132182 (II)



PD 080658 (III)

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

2. Materials and methods

2.1. Chemicals

All chemicals were HPLC or reagent-grade unless noted otherwise. Acetonitrile was obtained from Fisher Scientific (Fair Lawn, NJ, USA). Water, methanol and ammonium hydroxide were purchased from EM Science (Gibbstown, NJ, USA). Acetone was supplied by Mallinckrodt (Paris, KY, USA). Ammonium acetate and ammonium dihydrogen phosphate were purchased from Aldrich Chemical Co. (Milwaukee WI, USA). Heparinized human plasma was obtained from Interstate Blood Bank (Memphis, TN, USA). Control human urine was donated by healthy volunteers. Bovine serum albumin (fraction V) and human serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bond-Elut C_{18} cartridges (100 mg sorbent, 1.0 ml cartridge volume) and Vac-Elut, 10 place vacuum manifold, were purchased from Varian (Sunnyvale, CA, USA), formerly Analytichem International (Harbor City, CA, USA). I, [^{14}C]-I, II and III were obtained from Parke-Davis (Ann Arbor, MI, USA).

2.2. Chromatographic equipment and conditions for the plasma method

The HPLC system consisted of a SP 8800 solvent delivery system from Spectra Physics (San Jose, CA, USA), a ISS-100 automated sample injector and a LC-240 fluorescence detector from Perkin-Elmer (Norwalk, CT, USA). The system used an SP 4400 integrator from Spectra Physics and TCM column oven from Waters. Zorbax RX C_{18} columns, 5 μm particle size, 150 × 4.6 mm I.D. were purchased from MacMod Analytical (Chadds Ford, PA, USA). The plasma method requires two 150 × 4.6 mm I.D. columns connected in tandem with a 10 cm × 0.01 in. I.D. (1 in. = 2.45 cm) piece of

stainless steel tubing. The column with higher factory determined efficiency was placed first in line. Mobile phase buffer was 10 m*M* ammonium dihydrogen phosphate adjusted to pH 7.5 with concentrated ammonium hydroxide. Column temperature and mobile phase acetonitrile–buffer ratio were 30°C and 38:62 (v/v), respectively. The flow-rate was 0.75 ml/min. Detection was carried out using fluorescence, with excitation at 388 nm and emission at 473 nm. Sample injection volume was 150 μl of matrix extracts reconstituted in mobile phase.

2.3. Chromatographic equipment and conditions for the urine method

The HPLC system for urine analysis consisted of a Model 590 pump and a Model 712 automatic injector from Waters Chromatography Division (Milford, MA, USA); and a Spectra 200 UV detector from Spectra Physics. The column oven and integrator were identical to the plasma method. Zorbax RX C_{18} columns, 5 μm particle size, 250 × 4.6 mm were purchased from MacMod Analytical. Mobile phase buffer was identical to the plasma method. Column temperature and mobile phase acetonitrile–buffer ratio were 35°C and 40:60 (v/v), respectively. The flow-rate was 0.75 ml/min. Column effluent was monitored with UV detection at 380 nm. Sample injection volume was 150 μl of matrix extracts reconstituted in mobile phase.

2.4. Standards

All standards were protected from light by wrapping in foil or placing in drawers when not in use. A 500 μg/ml stock solution of I was prepared in methanol monthly and stored in single-use aliquots at −70°C. A 500 ng/ml working solution of I in methanol, prepared on the assay day, was serially diluted with plasma to make a calibration curve ranging from 0.2 to 25 ng/ml. Urine calibration standards, 1 to 100 ng/ml, were prepared by the addition of 25 μl of appropriate dilutions of I in methanol to 4.0 ml of urine in 15 ml PTFE vials. Order of addition was critical. Urine must be added to the PTFE vial first and then followed by I. Urine calibration standards were stored over night at −70°C prior to use. Working solutions of 120 ng/ml and 40 μg/ml of III in methanol were prepared for the plasma and urine assays, respectively, from a 400 μg/ml stock solution. A 250 μg/ml working solution of II was prepared in acetone. The stock solutions of II and III were stored in the same fashion as the I stock solution.

2.5. Processing of plasma

Unless noted otherwise, polypropylene vials and tubes were used in order to minimize binding of I to glass. Frozen samples were thawed as needed in a water bath at 30 ± 2°C for 10 min, vortexed for 30 s then centrifuged for 15 min at 2200 *g*. A 50-μl aliquot of II was added to each 2.0-ml sample in 12 × 75 mm culture tubes, except quality control blanks and patient pretreatment samples. C_{18} cartridges were conditioned with two 1-ml volumes of acetonitrile followed by two 1-ml of volumes water. Vacuum pressure was maintained at approximately 10 to 20 kPa, unless noted otherwise. The 2-ml sample was added to the cartridge in two 1-ml aliquots and aspirated. An aliquot of 1 ml of water was used to wash the sample tube and then applied to the cartridge. Cartridges were washed with 1 ml acetonitrile–2% (w/v) ammonium acetate, pH 8.0 (30:70, v/v) followed by 1 ml of the acetate buffer. Cartridges were not permitted to dry until this step. Cartridges were dried for 30 s at 50–70 kPa. The manifold was removed and probes wiped with absorbent tissue and collection tubes inserted. The compounds were then eluted with two 0.5-ml aliquots of acetonitrile–acetate buffer (60:40, v/v). The cartridges were dried for 20 s at full vacuum, 50–70 kPa, following each 0.50-ml elution solvent addition. Samples were evaporated to dryness under a vacuum of 50–70 kPa at ca. 40°C, reconstituted in 0.2 ml of mobile phase, vortexed for 30 s and centrifuged for 15 min at 2200 *g*. Supernatant was transferred to injector vials.

2.6. Processing of urine

Urine samples were collected in PTFE beakers or stainless steel bedpans and stored at −70°C in 15 ml PTFE vials. Frozen samples were thawed as needed by sitting at room temperature for 1.5 h in the dark. Urine samples were not permitted to come in contact with glass or plastics other than PTFE until after albumin was added (see results). Bovine serum albumin, 50% (w/v), was added to the urine samples while still in the PTFE vials in the ratio of 1 ml albumin per 10 ml of urine. The volume of urine in the PTFE vial was estimated by determining the weight of urine in the vial and converting the weight to volume assuming: the weight of an empty vial was 29.04 g, the specific gravity of urine was 1.017 [11] and the density of water at 20°C was 0.9982 [12]. The samples were then vortexed for 30 s. Aliquot of 2 ml of the albumin-treated urine sample and 25 μl of III were transferred to 12 × 75 mm culture tubes. Samples were vortexed for 30 s immediately after addition of III. PTFE luer stopcocks were used in the extraction manifold. The samples were processed through C_{18} solid-phase extraction cartridges in the same fashion as plasma.

2.7. Data collection and calculation

Calibration curves were characterized by assaying each calibration standard in triplicate on three separate occasions. Linear regression analysis of calibration standard data was performed by regressing peak-area ratios of I on concentrations of drug in matrix. Slopes, intercepts and coefficients of determination were determined and evaluated for linearity and reproducibility.

2.8. Selectivity

Selectivity was assessed by the lack of endogenous materials eluting at the same retention time of I. The retention time of II, a known degradation product and potential metabolite, was determined.

2.9. Accuracy and precision

Plasma (1.5, 9.0 and 20 ng/ml) and urine (4.0, 20 and 70 ng/ml) quality control samples were prepared and stored at −70°C. The quality control samples were assayed in triplicate on three separate occasions. Accuracy was expressed as the percent deviation of the mean observed concentration ($n = 9$) from the theoretical value. Precision was expressed as the mean percentage of the relative standard deviation (%R.S.D.) of the observed concentration. These samples were also assayed in duplicate for each assay during the Phase 1 trials in order to validate each assay and assess long-term stability.

2.10. Lower limit of quantification

The lower limit of quantification was determined by evaluating calibration standards during the validation process and selecting the lowest concentration of the calibration standards tested that possessed acceptable accuracy and precision, generally 15%.

2.11. Recovery

Recovery of I from human plasma was determined at concentrations of 0.5, 1.5, 9.0 and 20 ng/ml. Recovery of III from plasma was determined at 3.0 ng/ml. Recovery of I from human urine was determined at concentrations of 4, 20, and 70 ng/ml. Recovery of III from urine was determined at 500 ng/ml. Peak areas of I and III obtained from extracted samples were compared with peak areas of standards which were prepared in mobile phase and injected directly.

2.12. System repeatability

System repeatability was determined by injection of nine replicates of the high and low quality controls for both matrices. System repeatability was expressed as the %R.S.D. of the mean peak area ratios.

2.13. Stability

The stability of the I, II and III stock solutions at 500, 250 and 400 μg/ml, respectively, was assessed by comparing the peak areas of freshly prepared samples to samples stored at −70°C after 1, 3, 8, 10, 15, 22, 24, and 41 days. Long term stability of the quality control samples at −70°C was monitored at each of the three concentrations by assessing the concentration of the quality controls versus time during the period study samples were assayed. Stability was further assessed by subjecting the quality controls to three freeze–thaw cycles. The samples were analyzed after the third freeze–thaw cycle and concentrations were compared to mean concentrations obtained for the samples during the validation. Samples were considered stable if their assayed concentrations were within 10% of the mean concentration value determined during validation.

2.14. Binding of I to glass, plastics, PTFE and stainless steel

The binding of I in water and urine to glass, polyethylene, polystyrene, polypropylene, PTFE and stainless steel surfaces was assessed at concentrations of 10, 100 and 1000 ng/ml using ^{14}C labelled I (252 μCi/mg).

2.15. Applicability of the method

The suitability of the method for investigating the pharmacokinetics of I was assessed by analyzing plasma and urine samples from patients with metastatic cancer who had received a 6 h i.v. infusion of 4.35 mg/m^2 I on three consecutive days as part of Phase 1 clinical trials. Heparinized blood samples were obtained prior to and serially during the 24-h period after the first dose, prior to the second infusion and prior to and serially up to 48 h after the third infusion. Plasma was harvested by centrifugation and stored in polypropylene screw-cap vials. Urine was collected prior to the first infusion and in 12 h intervals thereafter. All samples were stored at −70°C until analysis. Plasma concentration–time data were analyzed using noncompartmental methods implemented with the LAGRAN computer program [13].

3. Results and discussion

3.1. Data collection and calculation

Peak-area ratios of calibration standards were proportional to the concentration of I in both matrices over the ranges tested. The calibration curves appeared linear and were well described by least squares regression lines with mean (n = 3) coefficients of determination of 0.998 and 0.999 for plasma and urine, respectively, and mean ± standard deviation (S.D.) slopes of 39.5 ± 1.7 and 7.77 ± 0.20 for plasma and urine, respectively. A weighting factor of 1/concentration [14] was chosen for the calibration curves to achieve homogeneity of variance. The mean concentrations of the calibration standards (n = 9) were within 5.0–10% of nominal values for plasma and urine, respectively, with R.S.D.s less than or equal to 15 and 6.1% for plasma and urine, respectively.

3.2. Selectivity

The assay was adequately selective for I in both matrices. The retention times of I, II and III were 23.0 ± 1.4, 24.9 ± 1.5 and 28.3 ± 1.9 min, respectively, for plasma and 16.5 ± 1.5, 18.3 ± 1.6 and 20.2 ± 1.8 min, respectively, for urine. Chromatograms of extracts of plasma and urine samples obtained from more than 30 cancer patients prior to the administration of I were free of peaks at the retention time of I. Chromatograms of extracts of human plasma obtained both prior to dosing and 5 h after administration of I and a 2.5 ng/ml plasma calibration standard are shown in Figs. 2A, B and C, respectively. Chromatograms of extracts of human urine obtained both prior to dosing and 12–24 h after the second administration of I and a 5.0 ng/ml urine calibration standard are shown in Figs. 2D, E and F, respectively.

Screening of human plasma revealed endogen-

ous components sometimes eluted at the retention time of III. Therefore, the amount of III was adjusted during method development to make the area of this endogenous component less than 1% of the area of III. Human plasma also has endogenous components that elute at approximately 35 and 90 min. These components are present in Fig. 2B at 15 min and in 2C at 35 min. Therefore, predose samples were injected first in order to monitor these peaks. If necessary, the run time and/or the delay between injections was adjusted so the component at 90 min eluted between injections or in the early portions of subsequent injections. Approximately 1 patient in 25 required some adjustment in the run times and/or in the delay between the injections.

During method development the authors

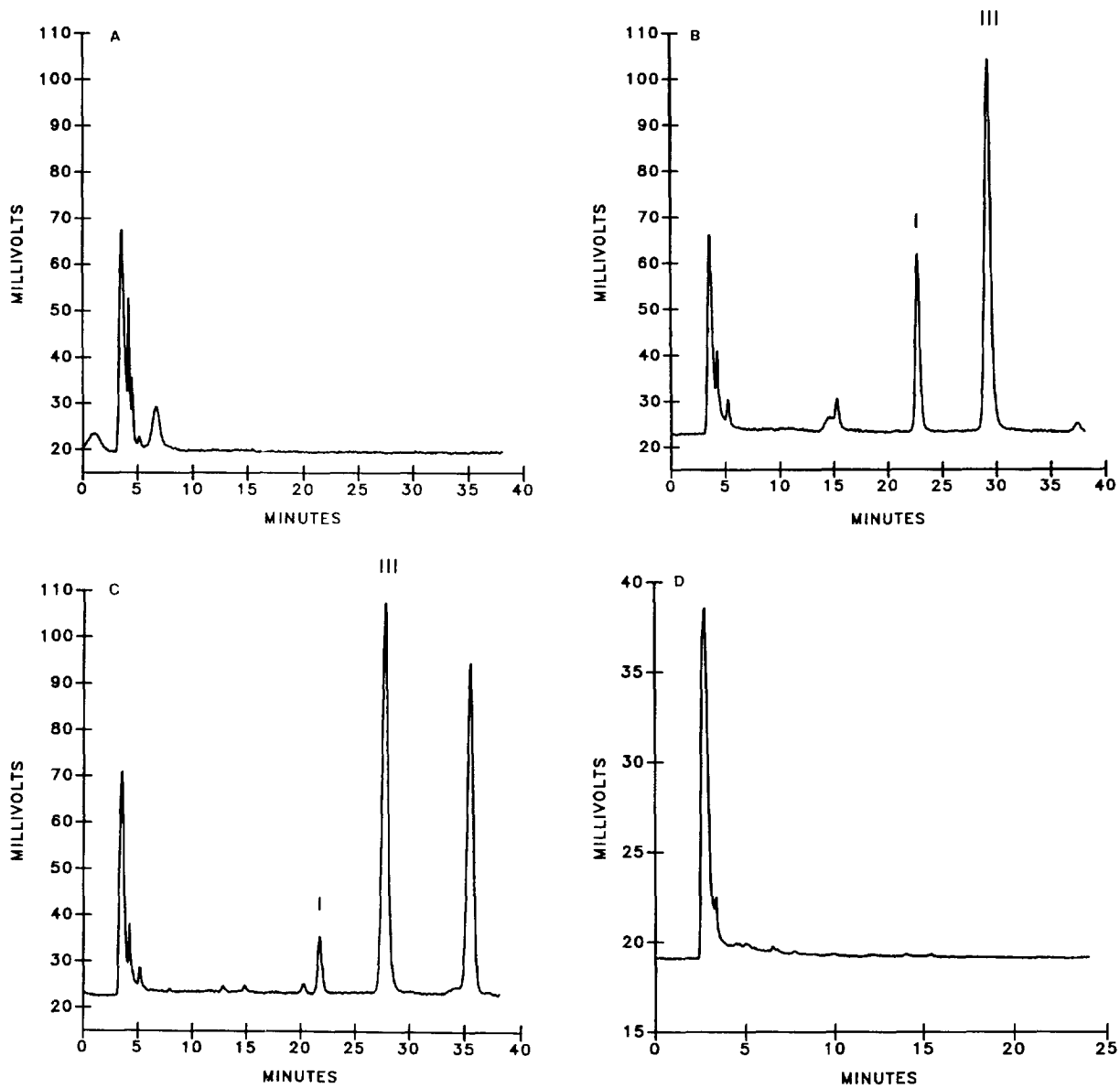


Fig. 2.

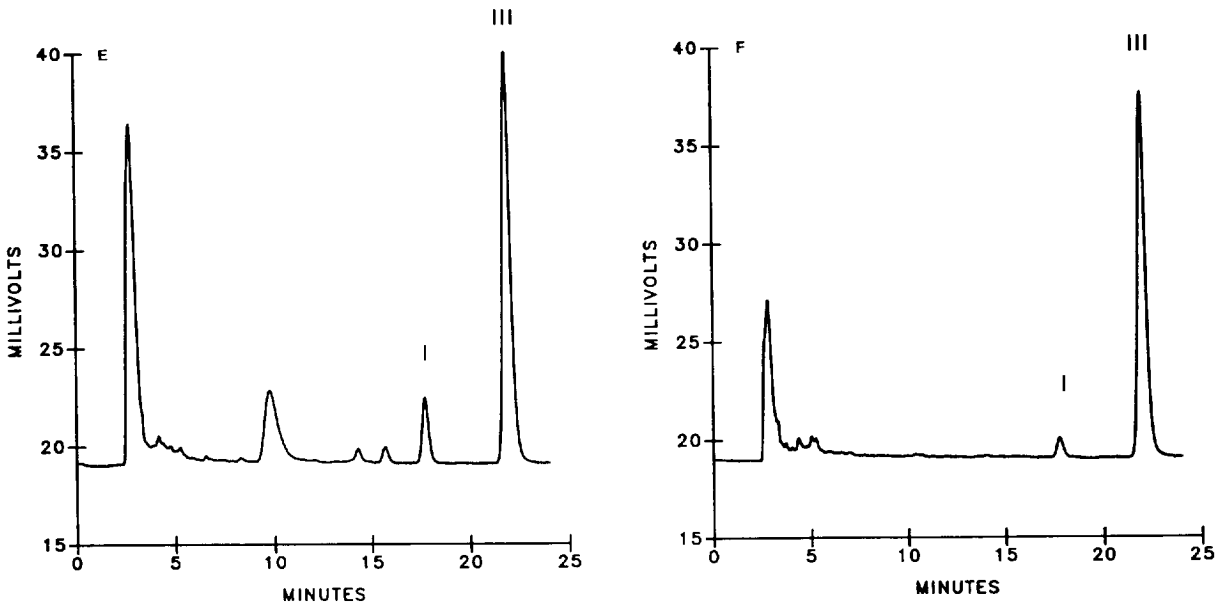


Fig. 2. Chromatograms of extracts of human plasma obtained both prior to dosing and 5 h after administration of I and a 2.5 ng/ml plasma calibration standard are shown in panels A, B and C, respectively. Chromatograms of extracts of human urine obtained both prior to dosing and 12–24 hours after the second administration of I and a 5.0 ng/ml urine calibration standard are shown in panels D, E and F, respectively. Peaks: I = CI-980; III = Internal standard. PD 080658.

noted the resolution between I and II was sensitive to column temperature and stationary phase while the resolution between II and III was more sensitive to the percent acetonitrile present in the mobile phase than the other two parameters. The resolution of I and II varied from column-to-column but was easily controlled by increasing the column temperature to 35°C. It is interesting to note that when RX C_8 columns were substituted for RX C_{18} columns, under similar chromatographic conditions, II eluted before I. RX C_{18} columns were chosen for the I method because they provided better separations between the compounds of interest and endogenous materials.

3.3. Accuracy and precision

Assay accuracy (relative error) was within ±4.7% and ±1.3% for plasma and urine quality controls, respectively (Table 1). Assay precision (%R.S.D.) was 5.6% and 4.3% for plasma and urine quality controls, respectively (Table 1).

Table 1
Accuracy and precision for I in human plasma and urine quality controls

Matrix	n	Mean concentration of I (ng/ml)		%R.S.D.	Percent deviation from nominal
		Nominal	Observed		
Plasma	9	1.5	1.43	5.6	−4.7
	9	9.0	8.35	5.4	−2.9
	9	20	19.4	2.5	0.5
Urine	9	4.0	3.95	4.3	−1.2
	9	20	19.7	3.6	−1.3
	9	70	70.2	2.2	0.2

3.4. Lower limit of quantification

Using 2-ml samples, the lower limit of quantification based on acceptable accuracy and precision was 0.2 ng/ml and 1 ng/ml for plasma and urine, respectively.

3.5. Recovery

Recovery (mean ±S.D.) of I from human plasma was 99.8 ± 11.6%, 102 ± 11.3%, 93.8 ± 4.0% and 93.8 ± 4.6% at concentrations of 0.5, 1.5, 9.0, and 20 ng/ml, respectively. Mean recovery of III from plasma was 88.3 ± 3.1% at 3.0 ng/ml. Recovery of I from human urine was 92.3 ± 4.1%, 90.7 ± 3.3% and 91.2 ± 1.9% at concentrations of 4.0, 20, and 70 ng/ml, respectively. Recovery of III from urine was 95.7 ± 2.2% at 500 ng/ml.

3.6. System repeatability

System repeatability (%R.S.D.) was 4.1% and 3.0% for plasma samples containing 1.5 and 20 ng/ml, respectively, and 3.9% and 0.7% for urine samples containing 4.0 and 70 ng/ml, respectively.

3.7. Stability of stock solutions

Peak areas of stock solutions of I, II and III stored at −70°C were within 11% of freshly prepared controls on all days tested, suggesting stability for at least 41 days.

3.8. Stability in plasma and urine

Concentrations of the I quality control samples stored at −70°C were still within 10% of validated values for at least 275 and 217 days for plasma and urine, respectively. Observed concentrations of the I quality controls after three freeze–thaw cycles were within 2.9 and 5.4% of validated values for plasma and urine, respectively.

3.9. Binding of I to glass, plastics, PTFE and stainless steel

I in human urine bound to glass surfaces with losses ranging from 5 to 26% (Table 2). I in water bound to glass and polyethylene surfaces with losses ranging from 3 to 9% and 23 to 86%, respectively. The losses appear to be concentration dependent but may be due to additional exposure to these surfaces caused by serial dilutions. In addition, plastic tips, typically polypropylene or polyethylene, commonly used for many pipetting devices also cause a substantial loss of I. The loss of I, in urine or water, to these surfaces was minimized by the addition of BSA or human serum albumin (HSA). In addition, there was no substantial loss of I in urine to either PTFE or stainless steel surfaces. For these reasons, clinical urine samples were collected in PTFE or stainless steel containers and BSA was added to urine specimens prior to analysis.

3.10. Light sensitivity

I is a light-sensitive compound. Precautions taken to minimize exposure to light include: dissolving I in an amber bottle, storing all working solutions of I, II and III in a drawer when not in use, storing the I plasma standards in a drawer when not in use, lining the ISS-100 cover with aluminum foil, turning off half the

Table 2
Percent of I remaining in urine, water, 5% bovine serum albumin (BSA), or 5% human serum albumin (HSA) solutions in glass, polyethylene, PTFE and stainless steel containers

Medium	Material	Concentration of I (ng/ml)		
		10	100	1000
Urine	Glass	74.4	84.0	95.1
Urine	PTFE	106	103	101
Urine	Stainless steel	98.3	99.1	98.4
Water	Polyethylene	14.1	33.8	77.3
Water	Glass	90.7	92.8	97.3
5% BSA	Glass	103	102	102
5% HSA	Glass	101	101	100

Table 3
Effect of the order of addition on the preparation of 4.0 ng/ml I urine quality controls

	Concentration of I (ng/ml)	
Reagent added first	I	Urine
	2.57	4.29
	2.61	3.93
	3.24	4.13
	2.73	4.26
	2.91	3.69
	3.11	3.96
	2.58	4.13
	2.27	3.77
	2.98	3.96
	2.49	3.67
n	10	10
Mean	2.75	3.98
%R.S.D.	11.0	5.53
%Deviation from nominal	−31.2	−0.50

overhead lights in the lab whenever working with the compounds on the lab bench or weighing the compounds, turning the lights off in the hood during solid-phase extraction, storing the compounds in vials wrapped with aluminum foil in a refrigerated desiccator, covering the syringe containing III with aluminum foil.

3.11. Effect of order of addition

During method development, the order of addition was noted as critical in the preparation of urine quality control samples. Urine must be added to the PTFE vials first followed by I in order to avoid a 31% loss of I (Table 3).

3.12. Effect of freezing

Urine quality control sample concentrations were consistently less than theoretical values, suggesting a loss due to freezing. Since patient samples were frozen prior to analysis, urine calibration standards were frozen prior to analysis in order to compensate for the loss. A comparison of quality control samples assayed on three separate occasions with freshly prepared and frozen calibration standards in the same analysis are listed in Table 4. Quality control samples assayed with a fresh calibration curve were significantly different ($p \leq 0.001$) from quality control samples assayed with a

Table 4
Comparison of I quality control samples assayed with fresh and frozen calibration standards

	Concentration of I (ng/ml)					
	4.0		20.0		70.0	
	Fresh	Frozen	Fresh	Frozen	Fresh	Frozen
	3.76	4.08	19.4	20.1	67.3	69.2
	3.21	3.51	17.3	17.9	68.7	70.7
	3.67	3.99	18.3	19.0	67.8	69.8
	3.64	3.80	20.0	20.7	65.9	68.1
	3.85	4.01	19.1	19.8	68.7	71.1
	3.81	3.98	19.7	20.4	64.8	67.0
	3.88	4.22	19.7	21.5	69.2	75.5
	3.79	4.12	19.8	21.6	71.1	77.6
	4.08	4.44	19.1	20.8	66.9	73.0
Mean	3.74	4.02	19.2	20.2	67.8	71.3
%R.S.D.	6.42	6.47	4.48	5.84	2.77	4.85
%Deviation	−6.50	0.50	−4.0	1.00	−3.14	1.86

frozen calibration curve using the "General Linear Model" procedure in SAS [15].

3.13. Effect of evaporator

An N-Evap *can not* be used in place of the vacuum evaporator. There was a substantial loss of I using an N-Evap rather than a Haake–Buchler vacuum evaporator (data not shown).

3.14. Applicability of method

Fig. 3 depicts a plasma concentration–time profile of I obtained after a cancer patient was administered a 4.35 mg/m^2 i.v. dose of I by a 6-h constant rate infusion. Dosing was repeated on each of three consecutive days and plasma samples for pharmacokinetic analysis were taken on the first and third days. Less than 1% of the dose was excreted as unchanged drug in urine in the first 24 h.

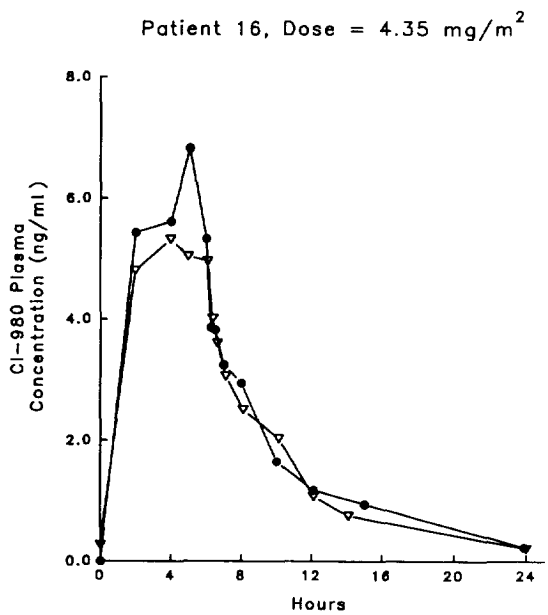


Fig. 3. I plasma concentration–time profile obtained after a cancer patient was administered 4.35 mg/m^2 i.v. dose of I by a 6-h constant rate infusion on each of three consecutive days with samples for pharmacokinetic analysis taken on the first (●) and third (▽) day.

4. Conclusion

An HPLC assay for I in human plasma and urine has been developed and validated. The assays are selective, precise, accurate and linear over the concentration ranges studied. This method was successfully used for pharmacologically guided dose escalations in Phase 1 clinical trials and is currently in use for the study of the clinical pharmacokinetics of I.

Acknowledgement

We would like to posthumously acknowledge Elvin L. Johnson for his assistance with optimizing the solid-phase extraction.

References

[1] G.P. Wheeler, B.J. Bowdon, C. Temple Jr., D.J. Adamson and J. Webster, *Cancer Res.*, 43 (1983) 3567.
[2] B.J. Bowdon, W.R. Waud, G.P. Wheeler, R. Hain, L. Dansby and C. Temple Jr., *Cancer Res.*, 47 (1987) 1621.
[3] W.R. Waud, W.R. Leopold, W.L. Elliot, et al., *Cancer Res.*, 50 (1990) 3239.
[4] G.P. Griswold, C.G. Temple Jr., M.W. Trader, W.R. Leopold III, W.R. Laster Jr. and D.J. Dykes, *Proc. Am. Assoc. Cancer Res.*, 27 (1986) 306.
[5] W. Klohs, R. Steinkampf, A. McMichael, J. Nelson, W. Leopold, J. Wolverton and W. Beck, *Proc. Am. Assoc. Cancer Res.*, 30 (1989) 523.
[6] C. Temple Jr., G.P. Wheeler, R.D. Elliot, J.D. Rose, R.N. Comber and J.A. Montgomery, *J. Med. Chem.*, 26 (1983) 91.
[7] G.P. Wheeler, B.J. Bowdon, J.A. Werline, D.J. Adamson and C.G. Temple Jr., *Cancer Res.*, 42 (1982) 791.
[8] G.P. Wheeler, B.J. Bowdon, J.A. Werline and C. Temple Jr., *Biochem. Pharmacol.*, 16 (1981) 2381.
[9] C. de Ines, D. Leynadier, I. Barasoin, V. Peyrot, P. Garcia, C. Briand, G.A. Rener and C. Temple, Jr. *Cancer Res.*, 54 (1994) 75.
[10] J. Brodfuehrer, L.R. Whitfield, L.R. Kowal, W. Grove, W. Bullen, D. Waterhouse and R. Natale, *Proc. Am. Assoc. Cancer Res.*, 33 (1992) 529.

[11] K. Diem and C. Lentner (Editors), *Documentsa Geigy Scientific Tables*, Geigy Pharmaceuticals, Ardstey, New York, 7th ed., 1974, p. 661.
[12] R.C. Weast (Editor), *CRC Handbook of Chemistry and Physics*, CRC Press, Cleveland, 58th ed., 1977–1978, p. F11.
[13] M.L. Rocci and W.J. Jusko, *Comput. Methods Programs Biomed.*, 16 (1983) 203.
[14] P.R. Bevington, *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York, 1969, p. 105.
[15] SAS Institute, *SAS User's Guide: Statistics*, Version 5 Edition, 1985, pp. 433–506.