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An HPLC assay utilizing solid-phase extraction for CI-1010, an alkylating radiosensitizer, in rat plasma

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Abstract

CI-1010, a 2-nitroimidazole, is a chiral prodrug for the active moiety PD 146923 and is under development as an alkylating radiosensitizer to be used as an adjuvant to radiotherapy. Because CI-1010 has an estimated half-life ≤ 2 min under physiological conditions its metabolites/degradation products PD 146415, an inactive moiety, and PD 146923 were assayed to support rat toxicology studies. The method involves the processing of plasma samples through phenyl solid-phase extraction cartridges followed by chromatography on CN columns with UV detection at 325 nm. The assay appears linear over the range 0.050–100 μg ml^{-1} for both PD 146415 and PD 146923. Interrun accuracy and precision estimates for PD 146415 and PD 146923 were within ± 6.50 and $\leq 3.27\%$, respectively, and ± 12.8 and $\leq 4.06\%$, respectively, for quality controls containing nominal concentrations of 0.400, 4.00 and 40.0 μg ml^{-1}. The absolute recovery of CI-1010, PD 146415 and internal standard, PD 126675, were approximately 40, 96 and 95%, respectively. The recovery of PD 146923 appeared concentration dependent and ranged from 68 to 92%. PD 146145 and PD 146923 were both stable in rat plasma at 4°C and −77°C for at least 7 h and 154 days, respectively. CI-1010 was not stable in rat plasma at 4°C. CI-1010, PD 146145 and PD 126675 were stable for at least 63 days in 10 mM phosphate buffer at pH 3.0 and 4°C. Under identical conditions PD 146923 was stable for only 8 days. The applicability of this method to determine concentrations of PD 146145 and PD 146923 in rat plasma is reported in this paper. © 1997 Elsevier Science B.V.

Keywords: CI-1010; PD 146923; PD 146415; RSU 1069; RB 6145; 2-nitroimidazoles; Alkylating radiosensitizer; Solid-phase extraction; Rat plasma; HPLC

1. Introduction

CI-1010, a 2-nitroimidazole, is a chiral prodrug for the active moiety PD 146923 and is under development as an alkylating radiosensitizer to be used as an adjuvant to radiotherapy. CI-1010 contains both a 2-nitro imidazole group, which is responsible for its radiosensitization properties, and a 2-bromoethylamino group, which is the pro-moiety of the alkylating aziridine group in PD 146923. CI-1010 was chosen for development as a prodrug in order to circumvent the instability and high emetic potential of PD 146923. PD

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146923 is slightly more effective than CI-1010 as a radiosensitizer [1]. CI-1010, the *R*-enantiomer, and its *S*-enantiomer have similar hypoxia-selective cytoxicity in vitro and radiosensitizing efficiencies [2]. The *R*-enantiomer was selected for development over the *S*-enantiomer or the racemate due to its lower emetic potential [3].

Because CI-1010 has an estimated half-life ≤ 2 min under physiological conditions [4], its metabolites/degradation products, PD 146415 and PD 146923 were assayed. A validated achiral liquid chromatographic assay was required for the quantitation of PD 146415 and PD 146923 in rat plasma to support toxicology studies. The structures of CI-1010, PD 146415, PD 146923, PD 151745, PD 153235 and PD 126675, the internal standard (IS), are shown in Fig. 1. PD 151745 and PD 153235 are known metabolites or degradation products of CI-1010. RB 6145, RSU 1069, RSU 1137 and RSU 1111 are racemates containing CI-1010, PD 146923, PD 151745 and PD 153235, respectively, as one of the enantiomeric pair. The authors are unaware of a racemate containing PD 146415. The IS, PD 126675 is a racemate.

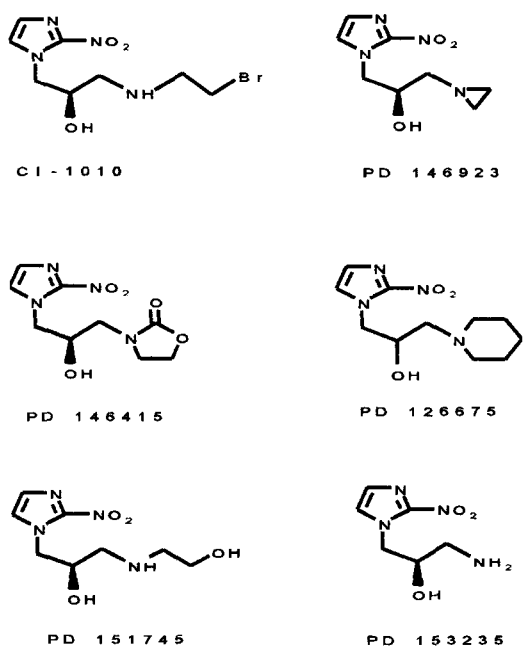

Fig. 1. Structures of CI-1010, PD 146923, PD 146415, PD 126675, PD 151745 and PD 153235.

A normal phase enantioselective assay for CI-1010 in formulations has been published [3] but was found unsuitable for use with the plasma procedure described in this report. An enantioselective assay for CI-1010 in biological matrices will be the subject of another paper [5].

2. Materials and methods

2.1. Materials

CI-1010, PD 146415, PD 146923, PD 151745, PD 153235 and PD 126675 were supplied by Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, (Ann Arbor, MI). Acetonitrile and methanol were obtained from Mallinckrodt (Paris, KY). Water, KH_2PO_4, H_3PO_4, and trifluoroacetic acid were purchased from EM Science (Cherry Hill, NJ). $NaClO_4$ (HPLC grade), $HClO_4$ and NaH_2PO_4, were obtained from Fisher Scientific (Fair Lawn, NJ). All solvents were HPLC grade and all reagents were analytical grade unless noted otherwise. Bond-Elut™ phenyl cartridges, 500 mg sorbent and 2.8 ml cartridge volume, were purchased from Varian (Harbor City, CA). Heparinized rat plasma was supplied by Pelfreeze Biologicals (Rogers, AR). Teflon stopcocks were purchased from Scientific Products (McGaw Park, IL). A 24 position vacuum manifold was obtained from Alltech (Deerfield, IL).

2.2. Liquid chromatography

The liquid chromatographic (LC) system consisted of an SP8800 pump, an SP8490 UV detector and Chromjet integrator obtained from Thermal Separation Products, formerly Spectra-Physics, (San Jose, CA). The automated sample injector was a Perkin-Elmer ISS-200 (Norwalk, CT). A thermally jacketed sample tray was used in the ISS-200 and chilled at 4 ± 2°C using a Polystat circulating water bath from Cole-Parmer (Chicago, IL).

Chromatographic conditions published for a mouse assay [6] were modified as follows for rat

plasma extracts. Two Resolve CN, 8 × 100 mm columns and 10 μm particle size, were placed serially in a Waters radial compression module (RCM) fitted with a single extension tube. The CN columns and RCM were purchased from Millipore Corporation, formerly Waters Chromatography, (Milford, MA). The CN columns were preconditioned prior to use by flushing with ≈100 ml methanol–water (50:50, v/v). The columns were equilibrated with mobile phase until successive injections of a standard containing 5 μg ml^{-1} of each of the six compounds gave adequate resolution for quantitation. The RCM was held at ambient room temperature (22 ± 2°C) and protected from temperature fluctuations by placement in a styrofoam box. The solvent system was methanol–$NaClO_4$ (25:75, v/v; pH 3.0; 10 mM) with a flow rate of 2.0 ml min^{-1}. UV detection was at 325 nm.

2.3. Preparation of calibration standards and quality controls

All reagents, buffers, plasma calibration standards and plasma quality controls were kept chilled in ice baths, unless noted otherwise, to prevent the degradation of PD 146923. A standard containing 1.0 mg ml^{-1} of both PD 146415 and PD 146923 was prepared in KH_2PO_4 buffer (pH 3.0; 10 mM). The 1.0 mg ml^{-1} standard was serially diluted in buffer to produce working standards. Aliquots of 0.1 ml of the appropriate working standards were added to 0.35 ml rat plasma to make calibration standards. The calibration standards ranged from 0.050 to 100 μg ml^{-1}. Fresh standards were prepared for each batch run beginning with the weighing of compounds.

Rat plasma quality controls containing 0.400, 4.00 and 40.0 μg ml^{-1} of PD 146415 and PD 146923 were prepared by adding 2.0 ml 10.0, 100 and 1000 μg ml^{-1} standards, respectively to 48 ml chilled blank rat plasma. As quickly as possible, the quality controls were subdivided into 1.0 ml aliquots, placed in chilled 1.5 ml centrifuge tubes, quick frozen in a dry ice–isopropanol bath at approximately −65°C, and stored at approximately −77°C. The weighing used to prepare quality controls was separate from the weighings used to prepare calibration standards.

2.4. Extraction procedure

Phenyl extraction cartridges were preconditioned, at room temperature, with 3 ml each of methanol, acetonitrile, elution solvent (methanol–water–trifluoroacetic acid, 70:30:0.1, v/v/v) and water. Extraction cartridges were chilled by passing 3.0 ml ice cold NaH_2PO_4 extraction buffer (pH 6.0; 50 mM) just prior to sample addition.

Calibration standards were prepared as described earlier. Quality controls were thawed in a water bath at 33 to 37°C for 90 s. Thawed quality controls were placed immediately in an ice bath and mixed for 30 s. Aliquots of 0.35 ml were placed in culture tubes. IS, 0.1 ml 100 μg ml^{-1} PD 126675 in methanol, was added to all samples and the samples were mixed for 1–3 s. Extraction buffer (1 ml) was added to each sample and thoroughly mixed for 30 s. Samples were aspirated through the preconditioned and chilled extraction cartridges. Cartridges were washed twice with 3.0 ml water followed by 1.0 ml acetonitrile–water (10:90, v/v) and dried at full vacuum for 30 s at approximately 60 kPa. Compounds were eluted into prechilled 1.5 ml centrifuge tubes with elution solvent. Cartridges were dried for 30 s at a vacuum of ≈35 kPa. Analytes were stabilized by the addition of 0.025 ml KH_2PO_4 (pH 3.0; 100 mM), placed in an ice bath and thoroughly mixed for 30 s. Samples were centrifuged for 22 min at 2400 × *g* at 1–4°C. Supernatants were placed in prechilled amber crimp-top injection vials and placed in the thermostated sample injector tray. A 0.2 ml aliquot was injected into the LC system.

2.5. Quantitation

The assay method was validated over the concentration range 0.050–100 μg ml^{-1} for PD 146415 and PD 146923 by assaying 11 calibration standards and 3 quality control samples in triplicate in three separate batch runs. The best-fit line was determined by least-squares linear regression of the calibration data from each batch run using a weighting factor of $1 \times \text{concentration}^{-2}$ [7].

Concentrations of PD 146415 and PD 146923 in quality controls were calculated using peak-area ratios of analyte:IS and the regression parameters.

2.6. Selectivity

Assay selectivity was assessed by testing blank rat plasma from ten different sources. The assay was considered adequately selective if no endogenous rat plasma components eluted at the retention times of PD 146923, PD 146923 and the IS.

2.7. Accuracy and precision

Quality control samples were assayed in triplicate at three concentrations on three separate occasions. Assay accuracy was expressed as the percent relative error (%RE), the percentage of the deviation of the mean observed concentration from the nominal value. Assay precision was expressed as the percentage of the relative S.D. (R.S.D.) of the mean observed concentrations.

2.8. Recovery

Recovery of CI-1010, PD 146415 and PD 146923 from rat plasma was determined at concentrations of 0.400, 4.00 and 40.0 μg ml^{-1}. Recovery of IS was determined at 28.6 μg ml^{-1}. Peak areas for CI-1010, PD 146415, PD 146923 and IS from extracted rat plasma samples were compared to the mean peak area of nonextracted standards in the eluate from extracted blank plasma to calculate percent recoveries.

2.9. System repeatability

Repeatability of chromatographic response, given as %R.S.D. of mean peak area ratios, was determined for nine replicate injections of extracted rat plasma quality control samples at three concentrations of PD 146415 and PD 146923.

2.10. Stability in rat plasma

Short term stabilities of CI-1010, PD 146415 and PD 146923 at concentrations of 0.400, 4.00 and 40.0 μg ml^{-1} were evaluated in rat plasma at $\approx 4°C$ for up to 7 h. Long term stability of PD 146415 and PD 146923 was assessed at approximately $-77°C$ using the plasma quality control samples. Stability was further assessed by subjecting the quality controls to three freeze-thaw cycles. Each freeze-thaw cycle consisted of thawing the sample for 90 s at 33–37°C, mixing for 30 s and quick freezing in a dry ice–isopropanol bath. In all cases stability was evaluated by comparing initial concentrations with subsequently determined concentrations. Analytes were considered stable if the responses were within 10% of initial values.

2.11. Stability in solvents

The stabilities of 50 μg ml^{-1} standards of CI-1010, PD 146415, PD 146923, PD 153235, PD 151745 and IS were evaluated in KH_2PO_4 buffer (pH 3.0; 10 mM) at $\approx 4°C$ serially over a 63 day period. The stabilities of CI-1010, PD 146415, PD 146923 and IS were also evaluated in the injection solvent used in this assay procedure at approximately 4°C over a 76 h period. Samples were considered stable if the responses were within 10% of initial values.

2.12. Applicability of method

The suitability of the method for investigating the pharmacokinetics of CI-1010 in rats was assessed by analyzing plasma samples from an intravenous toxicity study in male and female rats. Animals were administered 80 mg kg^{-1} of CI-1010 as a single daily i.v. bolus for 5 consecutive days. On the 5th day heparinized blood samples were taken by cardiac puncture at predose, immediately postdose, and 15 min, 1, 3 and 6 h after drug administration. Plasma was harvested by centrifugation at $\approx 4°C$, frozen in a dry ice–isopropanol bath and stored at $-77°C$ until analysis.

3. Results

3.1. Quantitation

The peak area ratios of PD 146415 and PD 146923 were proportional to concentration over

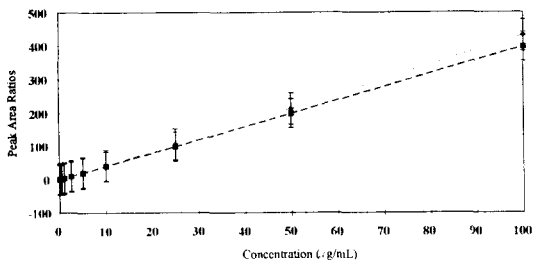

Fig. 2. Calibration plots of the mean peak area ratios of extracted plasma calibration standards from three batch analyses versus concentration for PD 146415 (■—■) and PD 146923 (◆-◆). Error bars represent the S.E.M.

the ranges tested (Fig. 2). The calibration curves were well described by least-squares linear regression lines, with mean coefficients of determination ($N = 3$) of 0.9984 and 0.9938 for PD 146415 and PD 146923, respectively. The mean slopes and mean intercepts for PD 146415 and PD 146923 were 3.97 and −0.0173, respectively, and 4.02 and −0.0737, respectively. Calibration curve reproducibility was evaluated by the deviation of individual back-calculated standards from the regression line. Interrun R.S.D. for PD 146415 calibration standards ranged from 0.567 through 7.00% over the three batch runs with interrun relative errors between −2.47 and 1.21%. Interrun R.S.D. for PD 146923 calibration standards ranged from 1.34 through 6.21% over the three batch runs, with interrun relative errors of −9.96 through 7.44%. Based on the accuracy and precision of calibration standards, the method demonstrates sufficient adherence to a linear model over the concentration range 0.050–100 μg ml^{-1} for PD 146415 and PD 146923.

3.2. Assay selectivity

No rat plasma components eluted at the retention times for PD 146415, PD 146923 and IS in blank rat plasma samples from 10 independent sources. Fig. 3A is a chromatogram of a rat plasma extract without any analytes added. Fig. 3B is a chromatogram of a 5 μg ml^{-1} nonextracted standard of CI-1010, PD 146415, PD 146923, PD 151745, PD 153235 and PD 126675 in KH_2PO_4 buffer (pH 3.0; 10 mM) illustrating the ability of the chromatographic system to adequately separate the compounds of interest. This standard was routinely injected prior to beginning any analysis in order to verify performance of the LC system. Fig. 4 is a chromatogram of an extract of a 5.00 μg ml^{-1} PD 1464215 and PD 146923 rat plasma calibration standard. It can be seen from this data that the method has adequate selectivity.

3.3. Intrarun accuracy and precision

Intrarun (within run) precision and accuracy were determined by assaying quality controls in triplicate, at each of three levels, in three separate batch runs. At nominal concentrations of 0.400, 4.00, and 40.0 μg ml^{-1}, PD 146415 intrarun accuracy (%RE) estimates were ±9.25, ±5.42 and ±6.92%, respectively, and intrarun precision (%R.S.D.) estimates were ≤2.25, ≤1.10 and ≤1.55%, respectively (Table 1). PD 146923 intrarun accuracy estimates were ±15.8, ±4.17 and ±1.25%, respectively, and intrarun precision esti-

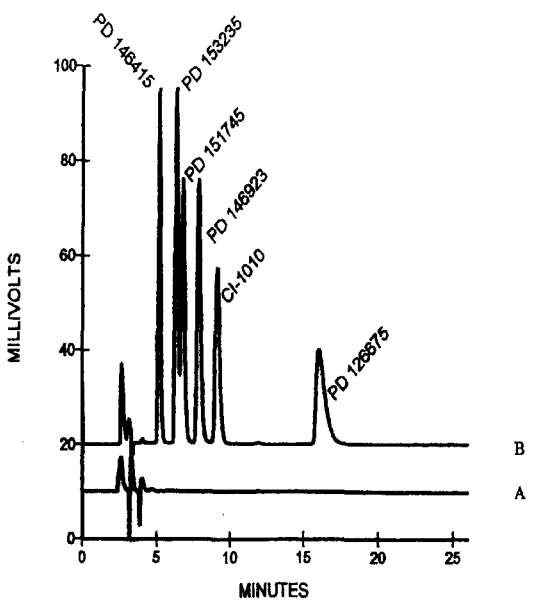

Fig. 3. Chromatogram of control (blank) rat plasma without any analytes added (A). Chromatogram of a 5 μg ml^{-1} nonextracted standard in KH_2PO_4 buffer (pH 3.0; 10 mM) of CI-1010, PD 146415, PD 146923, PD 153235, PD 151745 and PD 126675 (B). (A is offset by −10 mV.)

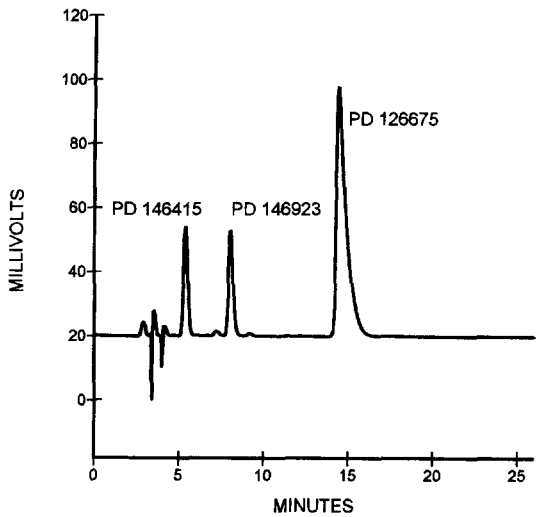

Fig. 4. Chromatogram of an extract of a 5 μg ml^{-1} PD 146415 and PD 146923 rat plasma calibration standard. Concentration of IS is 28.6 μg ml^{-1}.

mates were ≤1.70, ≤2.06 and ≤2.36%, respectively, (Table 1). These data indicate the assay has adequate intrarun precision and accuracy.

3.4. Interrun accuracy and precision

Interrun (between-run) precision and accuracy were determined by pooling all individual assay results of triplicate quality controls over 3 separate batch runs. Interrun accuracy estimates for PD 146415 were ±6.50, ±4.00 and ±5.75% and interrun precision estimates were ≤3.27, ≤1.57 and ≤1.49% for quality controls containing nominal concentrations of 0.400, 4.00 and 40.0 μg ml^{-1} PD 146415, respectively, (Table 1). Interrun accuracy estimates for PD 146923 were ±12.8, ±2.75 and ±0.25% and interrun precision estimates were ≤4.06, ≤2.29 and ≤1.54%, respectively, (Table 1). These data indicate the assay has adequate intrarun precision and accuracy.

3.5. Extraction recovery

The extraction efficiencies of PD 146415 and PD 146923 from rat plasma, expressed as percent recoveries, were determined by assaying seven samples at each of three concentrations and comparing the results to nonextracted standards assayed in triplicate. The extraction efficiency of CI-1010 was estimated in the same fashion using triplicates for both the extracted and nonextracted standards. At concentrations of 0.400, 4.00 and 40.0 μg ml^{-1} in rat plasma the mean (%R.S.D.) recoveries of PD 146415 were 95.7 (3.71), 95.8 (1.95) and 97.9% (1.87%), respectively, the mean recoveries of PD 146923 were 68.0 (6.16), 84.2 (2.02) and 91.9% (2.71%), respectively, and the mean recoveries of CI-1010 were 36.6, 43.4 and 43.9%, respectively. The mean recovery of the IS at 28.6 μg ml^{-1} was 95.0% (2.51%).

3.6. System repeatability

HPLC system repeatability was 1.66, 1.31 and 0.66% for PD 146415 and 3.25, 1.81 and 1.70% for PD 146923 at concentrations of 0.400, 4.00 and 40.0 μg ml^{-1}, respectively. System repeatability was 2.96% for IS. These data indicate adequate HPLC system repeatability.

3.7. Stability in rat plasma

The mean percent remaining in rat plasma controls at nominal concentrations of 0.400, 4.00 and 40.0 μg ml^{-1} held for 7 h at ≈4°C were 96.4, 96.0 and 99.3%, respectively, of PD 146923 initial values and 97.7, 97.0 and 99.5%, respectively, of PD 146415 initial values (Table 2). These data suggest PD 146923 and PD 146415 were stable in rat plasma for at least 7 h under the conditions tested. CI-1010 was not stable under similar conditions with less than 1% remaining after 2 h (Table 2).

PD 146415 and PD 146923 rat plasma quality controls at nominal concentrations of 0.400, 4.00, 40.0 μg ml^{-1} held at approximately −77°C for 154 days were within 10% of mean initial values obtained during validation indicating long term stability (data not shown). The concentrations of PD 146415 and PD 146923 rat plasma quality controls, after three freeze-thaw cycles, were within 2.5% of mean initial values, indicating PD 146415 and PD 146923 were stable in rat plasma to three freeze-thaw cycles (data not shown).

Table 1
Intrarun and interrun accuracy and precision of PD 146415 and PD 146923 in rat plasma quality controls for three separate batch runs

Compound	PD 146415			PD 146923		
Concentration added (μg ml^{-1})	0.400	4.00	40.0	0.400	4.00	40.0
Batch Run 1	0.390	3.93	38.5	0.372	3.97	41.2
	0.390	3.92	38.2	0.369	4.01	40.2
	0.385	3.86	37.4	0.360	3.99	39.3
Intrarun mean	0.388	3.90	38.0	0.367	3.99	40.2
Intrarun %R.S.D.	0.743	0.970	1.50	1.70	0.501	2.36
Intrarun %RE	−3.00	−2.50	−5.00	−8.25	−0.25	0.50
Batch run 2	0.369	3.83	37.6	0.336	3.87	39.7
	0.368	3.77	37.1	0.338	3.76	39.2
	0.377	3.75	37.0	0.336	3.87	39.6
Intrarun mean	0.371	3.78	37.2	0.337	3.83	39.5
Intrarun %R.S.D.	1.33	1.10	0.863	0.343	1.66	0.670
Intrarun %RE	−7.25	−5.50	−7.00	−15.8	−4.25	−1.25
Batch run 3	0.365	3.83	38.2	0.347	3.92	39.8
	0.370	3.87	37.1	0.342	3.89	39.6
	0.354	3.83	38.0	0.342	3.77	40.3
Intrarun mean	0.363	3.84	37.8	0.344	3.86	39.9
Intrarun %R.S.D.	2.25	0.601	1.55	0.840	2.06	0.904
Intrarun %RE	−9.25	−4.00	−5.50	−14.0	−3.50	−0.25
N	9	9	9	9	9	9
Grand mean	0.374	3.84	37.7	0.349	3.89	39.9
Interrun %R.S.D.	3.27	1.57	1.49	4.06	2.29	1.54
Interrun %RE	−6.50	−4.00	−5.75	−12.8	−2.75	−0.25

3.8. Stability in solvents

The peak area responses CI-1010, PD 146923, PD 146415, PD 153235, PD 151745 and IS, 50 μg ml^{-1} in KH_2PO_4 buffer (pH 3.0; 10 mM) at ≈4°C were assessed at 0.18, 0.34, 0.84, 1.87, 3, 4, 7, 8, 10, 11, 14, 18, 25, 31, 51 and 63 days. CI-1010, PD 146415, IS, PD 153235 and PD 151745 peak area responses at 63 days were 98.7, 101, 98.9, 100 and 104% of initial peak area responses, respectively, indicating these compounds were stable for at least this time period under the conditions tested (data not shown). PD 146923 peak area response was 94.4% on day 8 and was less than 90% for all time periods after 8 days indicating this component was stable for at least 8 days under conditions tested.

The peak area ratios of PD 146415 and PD 146923 to IS in rat plasma extracts in injection solvent after 74–76 h at ≈4°C were within 10% of initial responses at 0.400, 4.00 and 40.0 μg ml^{-1}, indicating stability for at least this time period (data not shown). The peak area of CI-1010 added to injection solvent at concentrations of 0.400, 4.00 and 40.0 μg ml^{-1} was within 1% of initial values for 49 h at ≈4°C (data not shown). The peak area of IS in rat plasma extracts in injection solvent after 76 h at ≈4°C was within 3% of the initial response indicating stability for at least this time period. At 39 h the peak area ratios of PD 146415 and PD 146923 were approximately 95% of initial values. Therefore, samples in injection solvent were assayed within 39 h.

Table 2
Percent of CI-1010, PD 146415 and PD 146923 remaining in rat plasma stored at 4°C

Compound	Concentration (μg ml^{-1})	Time (h)			
		0.5	1	2	7
CI-1010	0.400	NT	NT	0	NT
	4.00	NT	NT	0	NT
	40.0	NT	NT	0.6	NT
PD 146415	0.400	100	98.2	97.9	97.7
	4.00	101	100	95.9	97.0
	40.0	102	100	100	99.5
PD 146923	0.400	103	101	100	96.4
	4.00	102	100	96.0	96.0
	40.0	104	99.8	98.6	99.3

NT, not tested.

3.9. Applicability of method

Fig. 3A is a chromatogram of an extract of rat plasma taken prior to administration of CI-1010. Fig. 5 is a chromatogram of an extracted plasma sample containing 62.6 μg ml^{-1} PD 146415 and 31.4 μg ml^{-1} PD 146923 collected from a female rat 15 min after administration of a fifth consecutive 80 mg kg^{-1} single daily i.v. bolus of CI-1010. Fig. 6 is a concentration–time profile in male rats on day 5 following single daily 80 mg kg^{-1} i.v. bolus doses of CI-1010 for 5 consecutive days plotted on a semilog scale. These data indicate the successful application of the method.

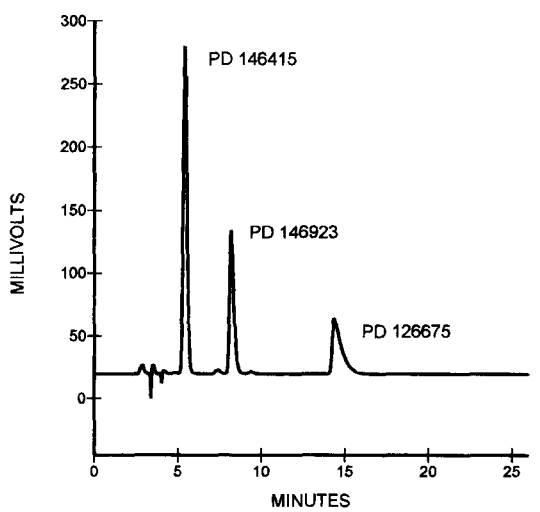

Fig. 5. Chromatogram of an extracted plasma sample containing 62.6 μg ml^{-1} PD 146415 and 31.4 μg ml^{-1} PD 146923 collected from a female rat 15 min after administration of a 5th consecutive 80 mg kg^{-1} single daily i.v. bolus of CI-1010.

4. Discussion

The authors would like to note that during method development for this assay the recovery of PD 146923, PD 146415 and PD 126675 from the phenyl cartridges varied considerably from lot to lot. In order to compensate for these changes the composition of the rinses and/or the elution solvent used in the solid-phase extraction steps were frequently modified. In order to avoid frequent

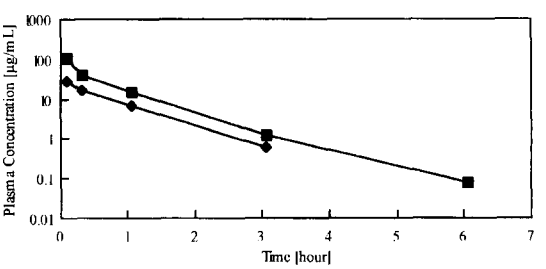

Fig. 6. Mean plasma PD 146415 (■–■) and PD 146923 (◆–◆) concentration–time profile in male rats on day 5 following single daily 80 mg kg^{-1} i.v. bolus doses of CI-1010 for 5 consecutive days plotted on a semilog scale. The C_{max}, T_{max}, half-life and AUC(0–6 h) were 105 μg ml^{-1}, 0.100 h, 0.739 h and 57.7 μg·h ml^{-1}, respectively, for PD 146415 and 27.0 μg ml^{-1}, 0.100 h, 0.577 h and 22.3 μg·h ml^{-1}, respectively for PD 146923.

modifications of the extraction procedure only one lot of phenyl cartridges was used for the validation of the rat plasma assay and subsequent sample analysis.

The authors were disappointed with the lifetime of the Waters Resolve CN columns, typically 250 injections, and with the batch-to-batch variability in column performance. These points were confirmed in a private conversation with another investigator (personal communication with Paul Workman). Typically the resolution of the PD 151745 and PD 146923 peaks as well as the PD 146923 and CI-1010 peaks deteriorated with column age. Curiously, the resolution of the PD 153235 and PD 151745 peaks improved with column age even to the point of baseline resolution. Published reports of assays for CI-1010 or PD 146923 used columns such as Waters Radial Pak μBondapak CN [8], Alltech CN [9], Supelcosil LC-8 [3], Beckman Spherisorb Phenyl [10] and Waters Resolve CN Radial Pak [4,6]. In an attempt to find a more rugged column, the authors tested a variety of columns including: MacMod Stable Bond Phenyl, CN and C18, Beckman ODS and Spherisorb Phenyl, Waters μBondapak CN and Phenyl, Supelcosil LC-8, and Interaction ACT-2. In general these columns did not offer adequate retention or selectivity for the compounds and matrix of interest. Therefore in order to minimize batch to batch variability, columns were screened prior to use for adequate resolution using an unextracted standard containing 5 μg ml^{-1} of each of the compounds of interest.

5. Conclusions

A liquid chromatographic method utilizing solid-phase extraction to quantitate CI-1010 metabolites/degradation products PD 146415 and PD 146923 in rat plasma has been validated. No endogenous rat plasma components interfered with quantitation of PD 146415 or PD 146923. The method was suitable for routine quantitation of PD 146415 and PD 146923 in rat plasma at concentrations for both analytes ranging from 0.050 to 100 μg ml^{-1}.

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