Journal of Pharmaceutical & Biomedical Analysis
Vol. 9, No. 4, pp. 345–349, 1991
Printed in Great Britain

0731-7085/91 $3.00 + 0.00
Pergamon Press plc

Short Communication

Liquid chromatographic analysis of the adenosine agonist PD 117519 in dog plasma

DONALD L. REYNOLDS*† and LAWRENCE A. PACHLA‡

Pharmacokinetics/Drug Metabolism Department, Parke–Davis Pharmaceutical Research Division, Warner–Lambert Company, Ann Arbor, MI 48105, USA

Keywords: *Adenosine agonist PD 117519; antihypertensive; solid-phase extraction; dog plasma; assay validation; reversed-phase liquid chromatography; pharmacokinetics.*

Introduction

PD 117519 (**I**) [(*R*)-*N*-(2,3-dihydro-1H-inden-1-yl)adenosine, CI-947] is an adenosine agonist which has shown oral antihypertensive activity in pharmacological animal models [1, 2]. Physicochemical properties of this compound include an aqueous solubility of 0.21 mg ml^{-1}, a pK_a value of 2.4 (in 50% methanol), and an apparent octanol–water partition coefficient (log *P*) of 1.99 at pH 7. A sensitive and selective assay was required to quantitate PD 117519 in dog plasma to support pre-clinical pharmacokinetic studies.

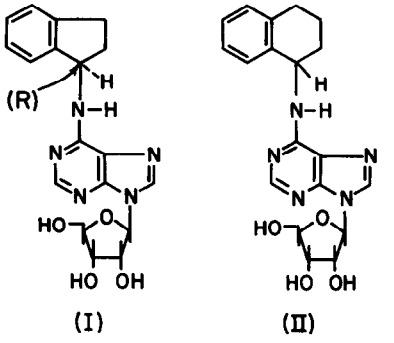

Figure 1
Structures of PD 117519 (**I**) and internal standard (**II**).

Experimental

Reagents

PD 117519 (**I**) and internal standard [*N*-(1,2,3,4-tetrahydro-1-napthalenyl)adenosine, PD 117973] (**II**) [Fig. 1] were synthesized at Parke–Davis Pharmaceutical Research Division (Ann Arbor, MI, USA) and were used as received. Acetonitrile, methanol, and water (EM Industries, Gibbstown, NJ, USA) and glacial acetic acid (J.T. Baker, Phillipsburg, NJ, USA) were HPLC grade. Citrated dog plasma (Pelfreez, Rogers, AK, USA), hydrochloric acid (1N, Acculute, Anachemia Chemicals, Champlain, NY, USA), and Bond–Elut C-18 cartridges (1-ml capacity, Analytichem International, Harbor City, CA, USA) were used as received. All other reagents were analytical reagent grade.

Apparatus

The chromatographic system consisted of a Spectra-Physics 8700 pump (San Jose, CA, USA), a Waters 710B WISP (Milford, MA, USA), and a Waters 440 absorbance detector. Separation was obtained using a C-8 column (3 μm, 100 × 4.6 mm i.d., Microsorb Short-Ones, Rainin, Woburn, MA, USA) maintained at 35°C (Bioanalytical Systems, W. Lafayette, IN, USA). The analytical column was protected with a pre-column (20 × 2 mm i.d., Upchurch Scientific, Oak Harbor, WA, USA) which was drypacked with Perisorb RP-2 sorbent (30–40 μm, MCB, Cincinnati, OH,

* Author to whom correspondence should be addressed.
† Present address: Clinical Pharmacology Department, Marion Merrell Dow Inc., Kansas City, MO 64137, USA.
‡ Present address: Biotechnology Department, Sterling Drug, Malvern, PA 19355, USA.

USA). The pre-column was repacked weekly. Peak–area ratios were determined using a Hewlett–Packard 3390A integrator (Avondale, PA, USA). Data were transmitted directly to an HP A-600 microcomputer for subsequent analysis. Method specificity was evaluated using a Hewlett–Packard 1040A rapid scan photodiode array detector.

Chromatographic conditions

Separation was achieved using binary gradient elution (A:B, v/v), where solvent A was acetonitrile and solvent B was an aqueous buffer containing 12.5 mM ammonium sulphate, 2.5 mM tetrabutylammonium hydrogen sulphate, and 0.33% (v/v) glacial acetic acid. An initial composition of 24% A was maintained until 3.0 min after injection, when a linear gradient reaching 29% A at 5.0 min was initiated. These conditions were maintained until 6.0 min after injection when a linear gradient reaching 40% A at 8.0 min was started. The pump was returned to initial conditions at 8.1 min. A flow rate of 1.0 ml min^{-1}, and a run time of 12 min was maintained throughout the analyses. The column effluent was monitored spectrophotometrically at 280 nm.

Standard solutions

Stock solutions of drug (50.2 mg PD 117519/50 ml) and internal standard (5.0 mg PD 117973/50 ml) were prepared daily in 0.025 N HCl. Standard solutions containing 0.1, 0.2, 0.4, 1.0, 2.0, 4.0, 8.0, 12.0 and 16.0 μg ml^{-1} PD 117519 were prepared by diluting the stock solution with water. Internal standard solution was diluted with water to a final concentration of 1 μg ml^{-1}.

Quality control standards

Quality control standards containing 0.15, 1.25 and 2.5 μg ml^{-1} PD 117519 were prepared by diluting 0.15-, 1.25-, and 2.5-ml aliquots, respectively, of a 100 μg ml^{-1} stock solution to a final volume of 100 ml with control dog plasma. Controls were sub-divided into 1.5-ml aliquots and were stored frozen (−20°C).

Sample preparation

To a 1.0-ml dog plasma sample in a 12 × 75 mm disposable tube was added 0.25 ml internal standard solution and 0.25 ml water (or PD 117519 standard). Samples containing >4 μg ml^{-1} PD 117519 were diluted appropriately with dog plasma prior to analysis. Tube contents were well mixed and samples randomized prior to extraction.

Bond–Elut C-18 cartridges were preconditioned by rinsing with methanol (5 ml) followed by water (5 ml). The plasma mixture was introduced and pulled through the cartridge with a gentle vacuum (approximately 5–10 in. Hg). The cartridge was rinsed sequentially with water (2.5 ml), 15% aqueous acetonitrile (1.0 ml), water (1.0 ml) and 0.1 N HCl (1.0 ml). Drug and internal standard were eluted from the cartridge with 25% acetonitrile in 0.5 N HCl (1.0 ml). The eluate pH was adjusted to ≈pH 3 by addition of 0.5 M dibasic ammonium phosphate in 2.5% aqueous ammonium hydroxide (0.1 ml). An aliquot (0.25 ml) was analysed directly by liquid chromatography.

Assay validation

The assay was validated over the concentration range 0.025–4.0 μg ml^{-1} PD 117519 by analysing nine calibration standards and three quality control standards in triplicate on 4 separate days. The best-fit straight line was determined daily by least-squares linear regression analysis using a weighting factor of reciprocal concentration. Concentrations of PD 117519 in quality control standards were determined using the regression equation.

Plasma recovery

Recovery of PD 117519 from dog plasma was determined at concentrations of 0.05 and 3.0 μg ml^{-1} by assaying six samples at each concentration. Peak areas of extracted standards were compared to peak areas of identical standards prepared in pH-adjusted elution solvent.

Results and discussion

The analysis of PD 117519 in dog plasma consisted of isolation from plasma by solid-phase extraction, separation by reversed-phase liquid chromatography, and detection by UV spectrophotometry. The validity of this procedure was established by investigation of extraction efficiency, selectivity, linearity, precision and accuracy of calibration curves, and assay precision and accuracy.

Solid-phase isolation

PD 117519 and internal standard were iso-

lated from dog plasma by solid-phase extraction using Bond–Elut C-18 cartridges. Sample clean-up on the cartridge was optimized using a previously reported strategy [1]. The influence of pH on the secondary amine group (pK_a = 2.4) was used to optimize separation selectivity. Analytes were isolated from untreated dog plasma and proteins and hydrophilic components were removed with water. A wash solvent containing 15% aqueous acetonitrile was used to remove weakly retained components from the sorbent without removing drug or internal standard. Analytes were then protonated and removed from the sorbent with 30% acetonitrile in 0.05 N HCl, the weakest solvent which totally removed compounds. Hydrophobic components that were strongly retained on the cartridge were discarded with the sorbent. The elution solvent was adjusted to approximately pH 3 to facilitate liquid chromatographic analysis.

Extraction efficiency

The absolute recovery of PD 117519 from dog plasma was evaluated at concentrations of 0.05 and 3.0 μg ml^{-1} PD 117519. Recoveries (±SD) were 85.5 ± 3.1% (N = 6) and 85.5 ± 4.8% (N = 6), respectively. Extraction precision (RSD) was 3.6 and 5.7%, respectively.

Liquid chromatography

PD 117519 and the internal standard were separated using binary gradient reversed-phase liquid chromatography. A linear acetonitrile gradient was used to improve resolution and decrease overall analysis time. Following elution of the analytes, a linear gradient to 40% acetonitrile was implemented to clean the analytical column prior to the next injection. An overall run time of 12 min was required for each sample.

Chromatograms of control dog plasma, a 0.5 μg ml^{-1} PD 117519 calibration standard in dog plasma, and a plasma sample obtained from a dog after receiving a 5 mg kg^{-1} dose of PD 117519 are shown in Fig. 2. No interfering peaks were observed near the drug or internal standard peaks in control dog plasma [Fig. 2(c)]. Peaks eluting at 4.4 and 6.6 min in the plasma samples of a dog that had received the drug [Fig. 2(c)] are possible PD 117519 metabolites. Analytes are suitably resolved from all sample components.

The system selectivity was evaluated further using rapid scan photodiode array detection.

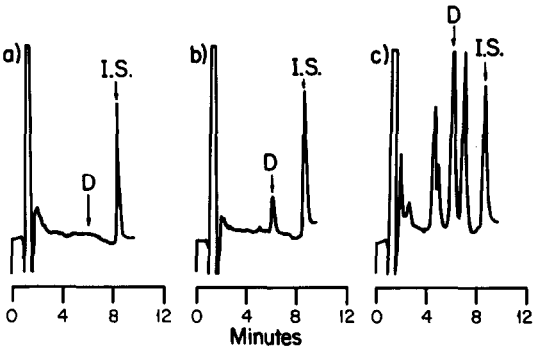

Figure 2
Chromatograms of plasma samples. Key: (a) control dog plasma containing internal standard; (b) calibration standard in dog plasma containing 0.050 μg ml^{-1} PD 117519; (c) plasma sample from a dog after receiving a 5 mg kg^{-1} dose of PD 117519. D = PD 117519; I.S. = internal standard. Attenuation = 8.

Ultraviolet spectra obtained at the apex of the PD 117519 peaks from an aqueous standard, a quality control standard, and an actual dog plasma sample were superimposable, confirming peak identity (Fig. 3). Additionally, spectra obtained at the up-slope, apex, and down-slope of the PD 117519 peaks in a quality control standard and plasma sample from a dog that had received the drug were superimposable, implying peak homogeneity (Fig. 4). Over 500 plasma extracts were assayed using this method. No interfering plasma constituents or metabolites were observed. In addition, chromatographic performance did not change throughout these analyses.

Linearity, precision and accuracy of the calibration curves

The assay was validated by assaying nine calibration standards and three quality control standards in triplicate on 4 separate days. Peak–area ratios were proportional to the amount of PD 117519 added to plasma over the concentration range 0.025–4.0 μg ml^{-1}. The best-fit straight line was determined daily by least-squares linear regression analysis. A weighting factor of reciprocal concentration was used to ensure homoscedasticity over the concentration range [3]. Mean (±SD) regression results were described by the following equation: peak–area ratio = 3.92 (±0.08) × [PD 117519 concentration] + 0.009 (±0.006).

Reproducibility of the calibration curve was determined as the variation of standards from the regression line. Calibration curve precision (RSD) ranged from 3.7 to 13% over 4 days,

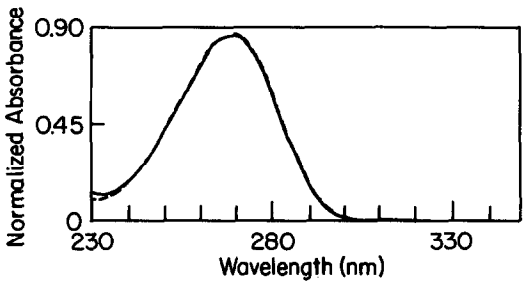

Figure 3
Liquid chromatographic UV spectra obtained at the apex of the PD 117519 peaks from an aqueous standard (---), a quality control standard (———), and an actual dog plasma sample (·····).

with an accuracy (relative error) of ±4.4% (Table 1). The minimum quantitation limit was 0.025 μg ml^{-1} PD 117519 using a 1-ml sample.

Assay precision and accuracy

Assay precision and accuracy was determined by analysing three quality control standards in triplicate on 4 separate days with the calibration standards. PD 117519 concentrations in control standards were calculated using the regression parameters. Assay precision was ±8.2%, based on RSDs of 8.2, 4.5 and 7.8% for quality control standards containing 0.15, 1.25 and 2.50 μg ml^{-1} PD 117519, respectively (Table 2). Assay accuracy was ±6.1% based on relative errors of 6.1, 2.2 and 3.9% for the same three standards.

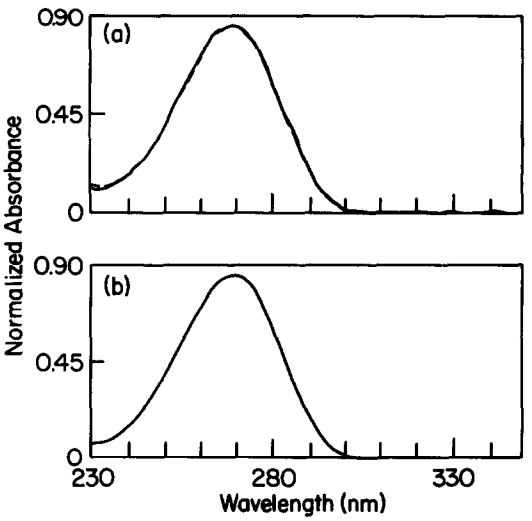

Figure 4
Liquid chromatographic UV spectra obtained at the up-slope (———), apex (·····), and down-slope (---) of the PD 117519 peak in (a) a quality control standard and (b) an actual dog plasma sample.

Application

This procedure was used to quantitate PD 117519 in dog plasma to support pharmacokinetic studies in this species. Over 200 plasma samples were assayed using this method, and no apparent interferences were observed throughout the analyses. Figure 5 shows the PD 117519 plasma concentration–time profile observed in a fasted beagle dog following

Table 1
Precision and accuracy of PD 117519 calibration standards in dog plasma assayed during a 4-day period

Concentration added (μg ml^{-1})	*N*	Mean concentration found (μg ml^{-1})	RSD* (%)	Relative error (%)
0.025	11	0.026	13	4.4
0.050	12	0.049	6.6	−2.0
0.100	12	0.099	5.4	−1.3
0.250	12	0.247	3.7	−1.3
0.500	12	0.510	7.7	2.0
1.00	11	1.01	4.1	0.86
2.00	12	1.97	6.2	−1.6
3.00	12	3.02	5.0	0.81
4.00	12	3.99	5.1	−0.15

* Relative standard deviation.

Table 2
Precision and accuracy of PD 117519 quality control standards in dog plasma assayed during a 4-day period

Concentration added (μg ml^{-1})	*N*	Mean concentration found (μg ml^{-1})	RSD* (%)	Relative error (%)
0.150	11	0.159	8.2	6.1
1.25	12	1.28	4.5	2.2
2.50	12	2.60	7.8	3.9

* Relative standard deviation.

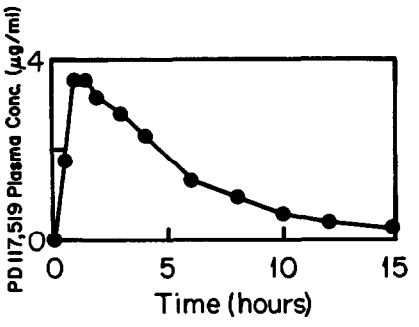

Figure 5
Plasma concentration–time profile of PD 117519 in a fasted beagle dog after receiving a 5 mg kg^{-1} P.O. dose of PD 117519.

administration of 5 mg kg^{-1} PD 117519 as a solid in a capsule. The assay is sufficiently sensitive to determine PD 117519 concentrations for up to 5 elimination half-lives at this dose.

Conclusions

A sensitive and selective liquid chromatographic method has been developed and validated to quantify the adenosine agonist PD 117519 in dog plasma. The method is suitable to quantitate PD 117519 over the concentration range 0.025–4.0 μg ml^{-1}. The method has been successfully used to quantitate PD 117519 in dog plasma samples from pharmacokinetic studies.

References

[1] D. Taylor, R. Bruns, F. Bjork, D. Cohen, R. Singer, B. Olszewski, M. Ryan, B. Trivedi and H. Kaplan *FASEB J.* **2**, A606 (1988).
[2] M. Ryan, T. Mertz, R. Potoczak, D. Taylor and H. Kaplan, *FASEB J.* **2**, A606 (1988).
[3] E.L. Johnson, D.L. Reynolds, D.S. Wright and L.A. Pachla, *J. Chrom. Sci.* **26**, 372–379 (1988).

[Received for review 14 February 1989; revised manuscript received 21 December 1990]