Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 1015–1021

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Determination of cidofovir in human plasma after low dose drug administration using high-performance liquid chromatography–tandem mass spectrometry

Jeremiah D. Momper[a], Shimin Zhang[a], Parmjeet S. Randhawa[c], Ron Shapiro[d], Kristine S. Schonder[b], Raman Venkataramanan[a,c,*]

[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, USA
[b] Department of Pharmacy and Therapeutics, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, USA
[c] Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA
[d] Department of Surgery, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

ARTICLE INFO

Article history:
Received 29 April 2010
Received in revised form 18 June 2010
Accepted 26 June 2010
Available online 6 July 2010

Keywords:
Cidofovir
BK virus
Liquid chromatography–mass spectrometry

ABSTRACT

A sensitive and specific method for the determination of cidofovir (CDV) in human plasma using high-performance liquid chromatography with tandem mass spectrometry (LC–MS/MS) was developed and validated. Plasma samples were processed by a solid phase extraction (SPE) procedure using Varian® SAX extraction cartridges prior to chromatography. The internal standard was ^{13}C5-Folic acid (^{13}C5-FA). Chromatography was performed using a Luna C8(2) analytical column, 5 μm, 150 mm × 3.0 mm, using an isocratic elution with a mobile phase consisting of 43% methanol in water containing 12 mM ammonium acetate, at a flow rate of 0.3 mL/min. The retention times of CDV and ^{13}C5-FA were 2.1 min and 1.9 min, respectively, with a total run time of 5 min. The analytes were detected by a Micromass Quattro Micro triple quadrupole mass spectrometer in positive electron spray ionization (ESI) mode using multiple reaction monitoring (MRM). The extracted ions monitored following MRM transitions were m/z 280.0 → 262.1 for CDV and m/z 447.0 → 294.8 for ^{13}C5-FA (IS). The assay was linear over the range 20–1000 ng/mL. Accuracy (101.6–105.7%), intra-assay precision (4.1–5.4%), and inter-assay precision (5.6–6.8%) were within FDA limits. No significant variation in the concentration of CDV was observed with different sample storage conditions. This method is simple, adaptable to routine application, and allows easy and accurate measurement of CDV in human plasma.

1. Introduction

Cidofovir (VISTIDE®, CDV) is a nucleotide analog of deoxycytidine monophosphate with *in vitro* and *in vivo* activity against herpesviruses, adenoviruses, poxviruses, and polyomaviruses [1–4]. Intravenous CDV is approved by the US Food and Drug Administration (FDA) for systemic treatment of cytomegalovirus retinitis in patients with AIDS. In addition, a low dose CDV regimen of 0.25–0.5 mg/kg weekly is empirically used at many institutions for the treatment of BK polyomavirus nephropathy (BKVN) in kidney transplant recipients [5–8]. Although the pharmacokinetics of CDV have been described in patients with normal renal function and renal insufficiency [9,10], no data is available regarding the disposition of this drug in patients with a single transplanted kidney. In order to elucidate the pharmacokinetics and pharmacodynamics of CDV used for the treatment of BKVN in kidney transplant recipients, it was necessary to develop a sensitive and specific assay method for the determination of CDV in human plasma. To date, four HPLC methods and one LC–MS/MS method have been described in the literature [11–15]. The HPLC methods require a large blood volume making intensive sampling difficult, or involve a laborious pre-column fluorescence derivatization process. Further, due to the low dose of CDV used in the renal transplant population, the published LC–MS/MS method, with a selective detection in the range of 78.125–10,000 ng/mL requiring 300 μL serum, cannot be used. [15]. This method is also difficult to reproduce as it employs an internal standard that is not commercially available. Therefore, the objective of this study was to develop a sensitive, specific and reproducible LC–MS/MS analytical method to estimate CDV concentrations in human plasma following low dose intravenous administration to kidney transplant patients.

Abbreviations: CDV, cidofovir; BKVN, BK polyomavirus nephropathy; ^{13}C5-FA, ^{13}C5-Folic acid.

* Corresponding author at: University of Pittsburgh, School of Pharmacy, Department of Pharmaceutical Sciences, 718 Salk Hall, 3501 Terrace Street, Pittsburgh, PA 15261, USA. Tel.: +1 412 648 8547; fax: +1 412 383 7671.
E-mail address: rv@pitt.edu (R. Venkataramanan).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.06.034

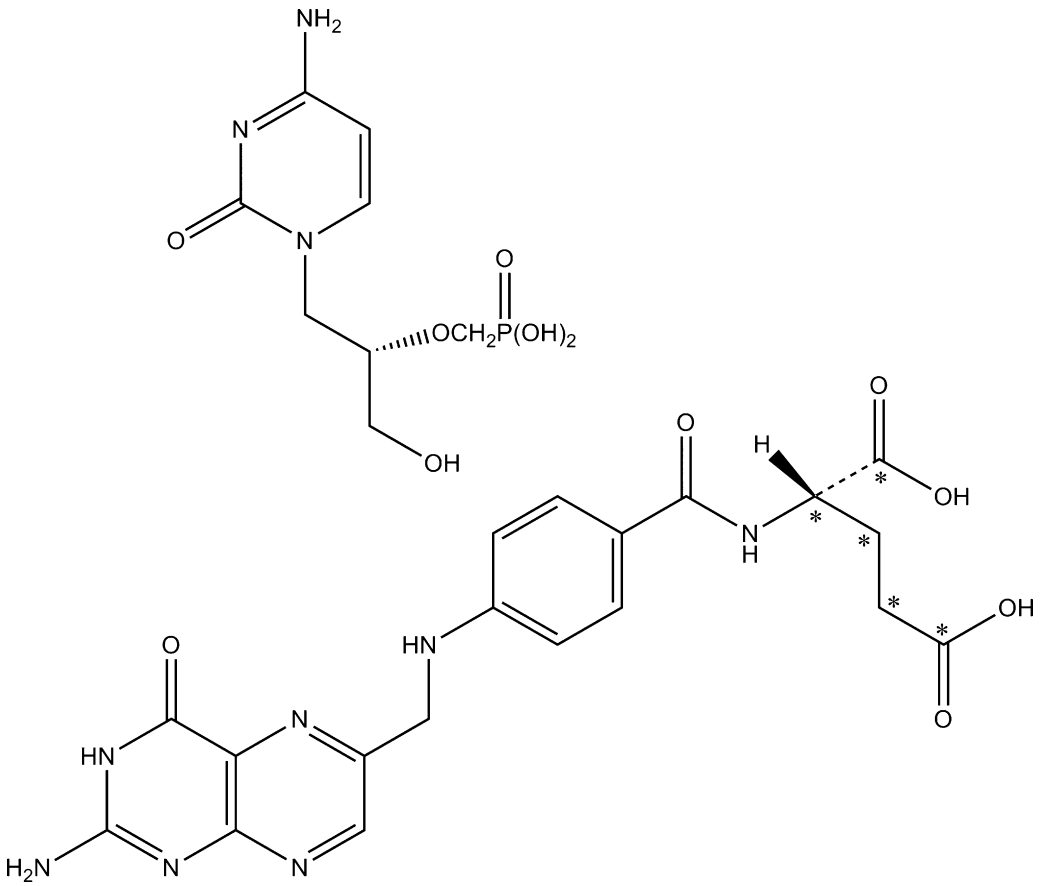

Fig. 1. Chemical structures of CDV (top, molecular weight: 279.19) and ^{13}C5-FA (internal standard, bottom, molecular weight: 446.4).

2. Materials and methods

2.1. Chemicals and materials

The chemical structures of CDV and the internal standard, ^{13}C5-Folic acid (^{13}C5-FA), are represented in Fig. 1. CDV reference standard was graciously supplied by Gilead Sciences, Inc. (Foster City, CA, USA). ^{13}C5-FA was purchased from Merck Aprova AG (Schaffhausen, Switzerland). Varian® Bond Elut SAX 1 mL (100 mg) extraction cartridges were purchased from Varian, Inc. (Lake Forest, CA, USA). Luna C8(2) column (150 mm × 3.0 mm, 5 μm, 100 Å) and C8 SecurityGuard cartridge (4.0 mm × 2.0 mm) were purchased from Phenomenex (Torrance, CA, USA). Optima HPLC grade methanol and HPLC grade water were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Aliquots of blank human plasma used for preparation of spiked standards were obtained from the central blood bank (Pittsburgh, PA, USA).

2.2. Preparation of standards and quality control samples

Stock solutions of CDV were prepared at 1 mg/mL in water and used for a maximum of 6 months, while being stored at 4 °C in the dark. On assay days, the working solution was diluted in human plasma to produce the following CDV concentrations: 20, 50, 100, 200, 350, 700, and 1000 ng/mL. The stock internal standard solution (50 μg/mL) was prepared in 20 mmol/L phosphate buffer (pH 7.2) and diluted to the working standard solution (1 μg/mL) in mobile phase.

Quality control (QC) stock solution was prepared independently from a separate weighing of CDV and stored at 4 °C in the dark. This solution was diluted in control human plasma to produce the following QC samples: QC low (QCL) 60 ng/mL; QC mid (QCM) 400 ng/mL; and QC high (QCH) 800 ng/mL. Additionally, plasma samples were prepared from the QC stock solution at 20 ng/mL, which was the lower limit of quantitation (LLOQ).

2.3. Sample preparation

Routine daily calibration curves, controls, and clinical samples were thawed at room temperature. Exactly 400 μL of plasma was diluted with 500 μL of water and passed through Varian® SAX 1 mL (100 mg) extraction cartridges, previously conditioned with methanol and water. After washing with 2 mL of water, CDV was eluted with 2 mL of 5% acetic acid in methanol and the eluent was evaporated to dryness under air at 38 °C. The residue was reconstituted in 50 μL of 10% ammonium hydroxide in 65% methanol and 50 μL of internal standard (1 μg/mL). Following 5 min of centrifugation at 10,000 rpm at ambient temperature, 10 μL of the solution was injected into the LC–MS/MS system.

2.4. Chromatographic and mass spectrometer conditions

The HPLC system was a Waters 2759 model (Waters Corporation, MA, USA). Separation was performed with a Luna C8(2) column (150 mm × 3.0 mm, 5 μm, 100 Å) with a C8 SecurityGuard cartridge (4.0 mm × 2.0 mm). An isocratic mobile phase was used consisting of 43% methanol in water containing 12 mM ammonium acetate. The total run time was 5 min at a flow rate of 0.3 mL/min.Analysis was performed on a Micromass Quattro Micro triple quadrupole mass spectrometer (Waters Corporation, MA, USA) with positive electrospray ionization mode using multiple reaction monitoring (MRM). For the analyte and internal standard,

Table 1
The cone and collision energy set in LC–MS for CDV and ^{13}C5-FA (IS).

	Parent m/z	Daughter m/z	Dwell (s)	Cone energy (V)	Collision energy (V)
CDV	280.0	262.1	0.1	28	14
^{13}C5-FA	447.0	294.8	0.1	22	11

MRM settings used were as follows: capillary voltage 3.2 kV; source temperature 100 °C; desolvation temperature 500 °C; cone gas flow 50 L/h; desolvation gas flow 550 L/h; argon pressure 20 ± 10 psig; nitrogen pressure 100 ± 20 psig. Cone and collision energy are presented in Table 1. The extracted ions following MRM transitions were monitored at m/z 280.0 → 262.1 for CDV and m/z 447.0 → 294.8 for ^{13}C5-FA (IS). The LC–MS system was controlled by the Masslynx® software version 4.1, and data were collected with the same software.

2.5. Validation procedures

2.5.1. Calibration curve and lower limit of quantitation (LLOQ)

Decreasing concentrations of CDV in human plasma, prepared as previously described, were injected into the analytical system to achieve a signal-to-noise ratio of at least 5:1. Calibration standards, blank, and zero samples were analyzed in triplicate to establish the calibration range with acceptable accuracy and precision. The response for each sample was calculated by dividing the area of the CDV peak by the area of the internal standard peak. Standard curves of CDV were constructed by plotting the analyte-to-internal standard ratio *versus* the nominal concentration of CDV in each sample. Standard curves were fit by linear regression with weighting by $1/y^2$, without forcing the line through the origin, followed by the back calculation of concentrations. The deviations of these back-calculated concentrations from the nominal concentrations, expressed as percentage of the nominal concentration, reflected the assay performance over the concentration range.

2.5.2. Accuracy and precision

The accuracy and precision of the developed method were determined by analyzing plasma samples with CDV at the LLOQ, QCL, QCM, and QCH concentrations in a minimum of five replicates in 3 analytical runs together with an independently prepared, triplicate calibration curve. Accuracy was calculated at each test concentration as:

$$\frac{\text{Mean measured concentration}}{\text{Nominal concentration}} \times 100\%$$

The precision of the assay was expressed using % coefficient of variation (CV). Intra-assay and inter-assay precision were assessed by replicate analysis of specimen aliquots on a single day or successive days, respectively.

2.5.3. Selectivity and specificity

To investigate whether endogenous matrix constituents interfered with the assay, six individual batches of control, drug-free human plasma were processed and analyzed according to the described procedures. Responses of CDV at the LLOQ concentration were compared with the response in the blank samples.

2.5.4. Extraction recovery and matrix effect

The extraction recovery of CDV from human plasma was determined by comparing the absolute response of an extract of control plasma to which CDV had been added after extraction with the absolute response of an extract of plasma to which the same amount of CDV had been added before extraction. The matrix effect of plasma on CDV was defined as the effect on the signal when comparing the absolute response of an extract of control plasma to which CDV had been added after the extraction with the absolute response of reconstitution solvent to which the same amount of CDV had been added. Experiments were performed at the QCL, QCM, and QCH concentrations in triplicate.

2.5.5. Stability

The stability of CDV in plasma was evaluated at the QCL, QCM, and QCH concentrations in triplicate under different conditions. The control plasma samples were stored for either 24 h at room temperature, 7 days at 4 °C, 1 month at −20 °C, or 3 months at −80 °C. Additionally, three freeze–thaw cycles of plasma samples prior to extraction were assessed. The reference concentration was calculated from freshly spiked plasma injected immediately post-extraction. Stability was expressed in terms of percentage of nominal concentration. The acceptance criterion for % relative recovery was set at 100 ± 10%.

2.6. Application to clinical sample analysis

To demonstrate the applicability of the method, we quantitated concentrations of CDV in the plasma of a renal transplant recipient treated intravenously with 0.3 mg/kg CDV for BKVN without concomitant probenecid. Prior to participating in the study, the subject gave written, informed consent as approved by the University of Pittsburgh Institutional Review Board. Blood samples were collected into EDTA tubes before CDV dosing and at 0.5, 1, 1.5, 2, 4, 6, 8, and 12 h after administration. Each sample was centrifuged at approximately 3000 rpm for 10 min and the resulting plasma was stored at −80 °C until analyzed using the procedure described above.

3. Results

3.1. Mass spectrometry and chromatography

When CDV and ^{13}C5-FA were injected directly into the mass spectrometer with a positive ion ESI interface, the protonated molecules $(MH)^+$ were seen in abundance. The mass to charge transition from parent ions to product ions was observed to have m/z 280.0 → 262.1 for CDV and m/z 447.0 → 294.8 for ^{13}C5-FA. The instrument parameters were selected to optimize specificity and selectivity of both parents and product ions and included capillary voltage of 3.2 kV, source temperature of 100 °C, desolvation temperature of 500 °C, cone gas flow of 50 L/h, desolvation gas flow of 550 L/h, argon pressure of 20 ± 10 psig, and nitrogen pressure of 100 ± 20 psig. The daughter scan mass spectra (m/z) for CDV and ^{13}C5-FA are displayed in Fig. 2.

The retention times for CDV and ^{13}C5-FA were 2.1 min and 1.9 min, respectively, with a total run time of 5 min. Typical chromatograms of human blank plasma, plasma spiked with 20 ng/mL CDV, and a clinical sample collected 30 min after initiation of 0.3 mg/kg intravenous CDV infusion are shown in Fig. 3.

3.2. Calibration curve and lower limit of quantitation (LLOQ)

Triplicate standard curves were performed in plasma on five sequential days. The ratio of peak area of CDV to ^{13}C5-FA was linearly related to the concentration of CDV in the concentration range of 20–1000 ng/mL in plasma. A regression coefficient

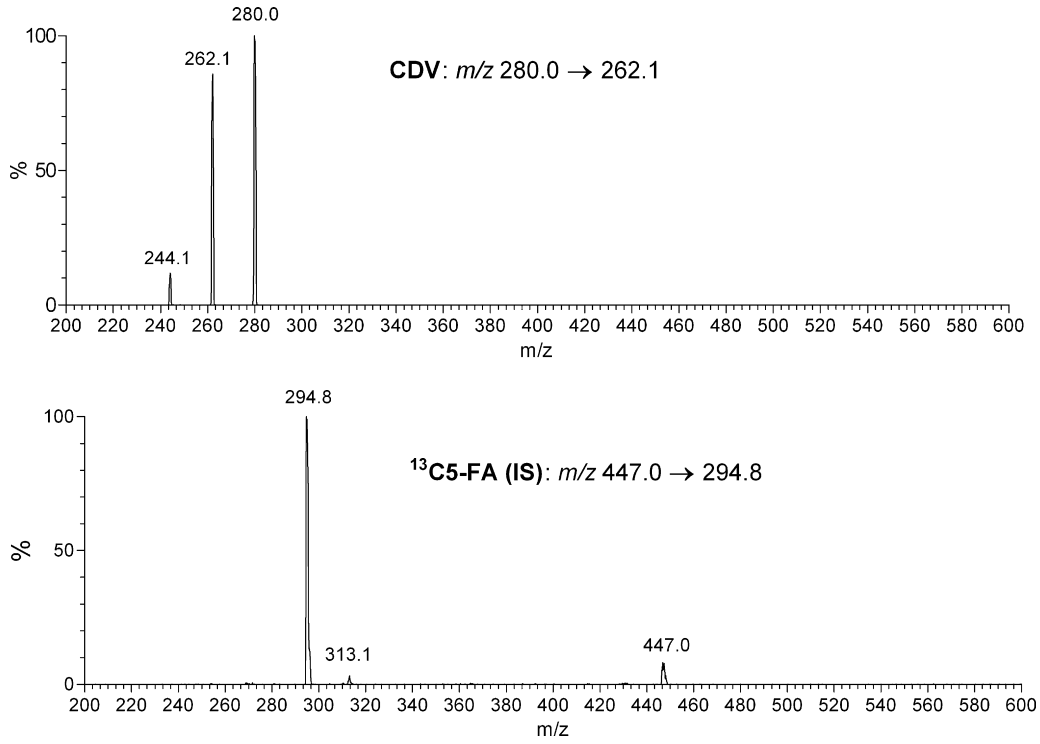

Fig. 2. Representative positive ion electrospray ionization MS/MS spectrums for CDV (top) and ^{13}C5-FA (internal standard, bottom).

of >0.99 was obtained in all analytical runs, with an equation of $y = 0.0016x + 0.0002$, where x = CDV concentration in ng/mL and y = CDV area/IS area. The LLOQ was 20 ng/mL using a plasma volume of 400 μL, at a signal-to-noise ratio of >10. The individual values for the mean of the back-calculated values at each nominal concentration used in the standard curve and the accuracies calculated from those values are displayed in Table 2.

3.3. Accuracy and precision

The accuracies for all tested concentrations should be within ±15%, except for the LLOQ, in which case these parameters should not exceed 20%. The accuracies and intra- and inter-assay precisions for the tested concentrations (LLOQ, QCL, QCM, QCH) were all within these pre-defined acceptance criteria (Table 3).

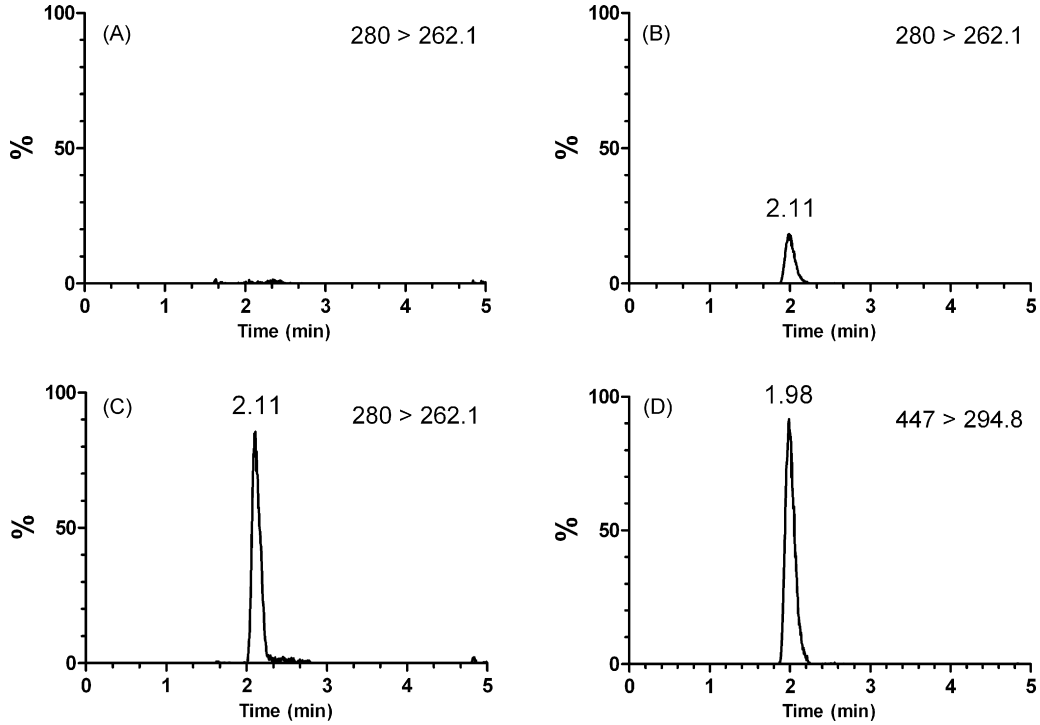

Fig. 3. Representative MRM chromatograms of CDV and ^{13}C5-FA in human plasma. (A) Blank human plasma without CDV. (B) CDV spiked 20 ng/mL in blank plasma. (C) Clinical plasma sample 30 min after initiation of 0.3 mg/kg intravenous CDV infusion. (D) Internal standard spiked blank plasma sample.

Table 2
Assay performance data of the calibration samples for CDV in human plasma.

Nominal concentration (ng/mL)	Mean measured concentration (ng/mL)	Accuracy (%)	Intra-assay precision (%)	Inter-assay precision (%)
20	21.5	107.5	3.7	7.3
50	50.8	101.6	4.8	5.8
100	105.7	105.7	2.8	4.1
200	194.2	97.1	4.5	5.3
350	352.1	100.6	3.3	4.5
700	702.8	100.4	5.5	6.1
1000	992.1	99.2	3.6	4.1

Table 3
Assay performance data for the quantitation of LLOQ, QCL, QCM and QCH CDV concentrations in human plasma.

Plasma concentration (ng/mL)	Mean measured concentration (ng/mL)	Accuracy (%)	Intra-assay precision (%)	Inter-assay precision (%)
20 (LLOQ)	19.6	97.9	2.7	3.8
60 (QCL)	61.9	103.3	5.4	6.2
400 (QCM)	422.8	105.7	4.9	6.8
800 (QCH)	812.8	101.6	4.1	5.6

3.4. Selectivity and specificity

To test for interference, six different sources of plasma were analyzed as blanks and after addition of CDV at the LLOQ (20 ng/mL). The responses in blank plasma were always less than 5% of the signal at the LLOQ.

3.5. Recovery and ion suppression

The extraction recovery of CDV was determined by comparing the absolute response of an extract of control plasma to which CDV had been added after extraction with the absolute response of an extract of plasma to which the same nominal concentration of CDV had been added before extraction [16,17]. Recovery data and relative response when tested for matrix effects are displayed in Table 4.

3.6. Storage stability data

There was not a significant difference in the estimated concentrations of CDV in plasma samples maintained and analyzed under different stability conditions as compared to freshly spiked plasma samples (Table 5).

3.7. Application to clinical sample analysis

The developed method was applied to a set of plasma samples collected in a clinical study evaluating CDV for the treatment of BKVN in renal transplant recipients. The assay was capable of quantitating CDV concentrations in all post-treatment samples with all QC samples within 15% of their respective nominal value in the analytical run. A representative plasma concentration *versus* time profile is displayed in Fig. 4 and pharmacokinetic parameters are presented in Table 6.

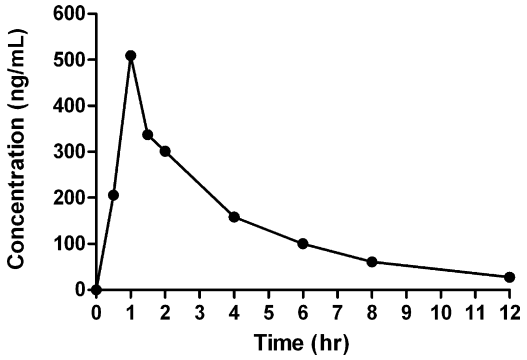

Fig. 4. A representative plasma concentration *versus* time profile of a renal transplant recipient treated with 0.3 mg/kg CDV intravenously with a 1 h infusion.

4. Discussion

Cidofovir has shown promise in the management of BK virus nephropathy in renal transplant recipients [5–8]. Low doses of CDV (representing 5–10% of the FDA approved dose for CMV retinitis) are typically used in this population due to the drug's nephrotoxicity. Although an LC–MS/MS assay has been published previously, its suitability for clinical pharmacokinetic studies of low dose CDV is decreased by the lack of a commercially available internal standard and insufficient sensitivity [15]. The method described in the current manuscript is adequately sensitive to characterize systemic drug exposure following low dose CDV administration and capable of being implemented in laboratories with standard LC–MS instrumentation. Additionally, the reported assay has been validated according to the most recent FDA guidelines [16].

Preliminary analysis of CDV was performed using both positive and negative ion modes. Enhanced sensitivity was observed with positive ionization and consequently selected for the assay. We next established a lack of endogenous interference in blank human plasma using HPLC and MS techniques. Additionally, because transplant patients take a regimen of immunosuppressants

Table 4
Total and ion suppression recovery of CDV in human plasma.

Concentration (ng/mL) ($n=4$)	Total recovery		Ion suppression relative recovery	
	Mean ± S.D. (%)	CV (%)	Mean ± S.D. (%)	CV (%)
60 (QCL)	53.7 ± 2.7	4.9	107.7 ± 4.2	3.8
400 (QCM)	54.9 ± 2.6	4.7	110.1 ± 9.5	8.4
800 (QCH)	51.5 ± 2.4	5.6	94.4 ± 4.6	4.9

Table 5
Stability of CDV under varying conditions.

Storage condition	Concentration (ng/mL)		Stability (%)	CV (%)	Replicates
Stock solution 6 months, 4 °C	1,000,000		97.4	4.1	3
Plasma 24 h, ambient temp.	QCL	60	105.5	2.3	3
	QCM	400	96.4	3.3	3
	QCH	800	101.8	5.5	3
Plasma three freeze–thaw cycles, −80 °C	QCL	60	93.2	6.2	3
	QCM	400	92.2	5.8	3
	QCH	800	93.5	6.7	3
Plasma 1 month, 20 °C	QCL	60	104.7	3.7	3
	QCM	400	101.9	9.6	3
	QCH	800	101.1	3.6	3
Plasma 7 days, 4 °C	QCL	60	105.7	3.3	3
	QCM	400	108.1	1.2	3
	QCH	800	103	0.9	3
Plasma 3 months, −80 °C	QCL	60	93.8	3.4	3
	QCM	400	94.3	5.5	3
	QCH	800	95.2	3.8	3

and anti-infectives, plasma from kidney transplant recipients not administered CDV was tested and no interference was observed.

An anion exchange solid phase extraction procedure was used to process plasma samples. Hydrophilic molecules such as CDV typically have low extraction recoveries from biological matrices. Accordingly, total recovery from the plasma extraction procedure averaged 53%. However, the extraction method was consistent and allowed for minimal ion suppression. Extraction recovery of all tested internal standard candidates was inconsistent and therefore the IS was added before injection to adjust for variation of LC–MS/MS analysis.

Separation of CDV from other components in plasma was performed using an analytical column with an isocratic profile. The selected column and mobile phase provided the most well separated and sharp peaks. Numerous compounds were evaluated as potential internal standards, including other nucleotide analogs and lipid ester analogs of CDV. However, ^{13}C5-FA provided the most consistent response under the conditions utilized in this method. It also eluted close to the analyte of interest and facilitated a short run time.

In pharmacokinetic studies plasma samples are stored at −20 or −80 °C until analysis and exposed to various temperatures during assay procedures. As a result, it was necessary to understand the stability of CDV at the conditions that samples would be subjected to prior to analysis. Stability was determined by the comparison of estimated concentrations of fresh samples to samples kept for 24 h at room temperature, 7 days at 4 °C, 1 month at −20 °C, and 3 months at −80 °C. Different sample processing conditions did not affect estimated CDV concentrations indicating stability under the conditions evaluated.

The majority of an intravenous dose of CDV is excreted unchanged in the urine. Therefore, in some instances it may be necessary to determine concentrations in urine samples. We applied the present method to both spiked blank urine and clinical samples collected in a pharmacokinetic study. In all cases, large variability (>25%) was observed in the matrix effect in urine from different subjects. Given that CDV concentrations in urine are generally substantially higher than in plasma, previously published HPLC–UV methods can be used to readily quantitate CDV in urine [11–14].

Table 6
Pharmacokinetic parameters of CDV in a renal transplant recipient following a 0.3 mg/kg i.v. dose.

Parameter	
C_{max} (ng/mL)	509
$t_{1/2}$ (h)	2.39
$AUC_{0-\infty}$ (mg h/L)	1.75
MRT (h)	4.0
CL (mL/h/kg)	174
V_{area} (L/kg)	0.61

In summary, we have developed and validated a sensitive LC–MS/MS method for quantitative assessment of CDV in human plasma that is useful in clinical pharmacokinetic studies in renal transplant recipients treated with low dose CDV.

Role of the funding source

Supported by NIH/NCRR/CTSA Grant UL1 RR024153. This grant supports the University of Pittsburgh Clinical and Translational Science Institute and played no role in study design, collection, analysis, interpretation of data, writing, or the decision to submit for publication.

Conflict of interest

The authors have no actual or potential conflicts of interest to disclose.

Acknowledgements

The authors gratefully acknowledge Robert Parise, Thomas Nolin, and Michael Zemaitis for constructive suggestions regarding this manuscript.

References

[1] J.J. Bronson, L.M. Ferrara, M.J. Hitchcock, H.T. Ho, K.L. Woods, I. Ghazzouli, E.R. Kern, K.F. Soike, J.C. Martin, (S)-1-(3-hydroxy-2-(phosphonylmethoxy) propyl)cytosine (HPMPC): a potent antiherpesvirus agent, Adv. Exp. Med. Biol. 278 (1990) 277–283.
[2] C.B. de Oliveira, D. Stevenson, L. LaBree, P.J. McDonnell, M.D. Trousdale, Evaluation of cidofovir (HPMPC, GS-504) against adenovirus type 5 infection in vitro and in a New Zealand rabbit ocular model, Antiviral Res. 31 (1996) 165–172.
[3] N.A. Farasati, R. Shapiro, A. Vats, P. Randhawa, Effect of leflunomide and cidofovir on replication of BK virus in an in vitro culture system, Transplantation 79 (2005) 116–118.
[4] E. Bernhoff, T.J. Gutteberg, K. Sandvik, H.H. Hirsch, C.H. Rinaldo, Cidofovir inhibits polyomavirus BK replication in human renal tubular cells downstream of viral early gene expression, Am. J. Transplant. 8 (2008) 1413–1422.
[5] D.R. Kuypers, A.K. Vandooren, E. Lerut, P. Evenepoel, K. Claes, R. Snoeck, L. Naesens, Y. Vanrenterghem, Adjuvant low-dose cidofovir therapy for BK polyomavirus interstitial nephritis in renal transplant recipients, Am. J. Transplant. 5 (2005) 1997–2004.
[6] A. Vats, P.S. Randhawa, R. Shapiro, Diagnosis and treatment of BK virus-associated transplant nephropathy, Adv. Exp. Med. Biol. 577 (2006) 213–227.

[7] V. Cabello, N. Margarit, M. Diaz Pedrero, G. Bernal, P. Pereira, M.A. Gentil, Treatment of BK virus-associated nephropathy with cidofovir in renal transplantation, Transplant. Proc. 40 (2008) 2930–2932.
[8] F. Lamoth, M. Pascual, V. Erard, J.P. Venetz, G. Nseir, P. Meylan, Low-dose cidofovir for the treatment of polyomavirus-associated nephropathy: two case reports and review of the literature, Antiviral Ther. 13 (2008) 1001–1009.
[9] K.C. Cundy, Clinical pharmacokinetics of the antiviral nucleotide analogues cidofovir and adefovir, Clin. Pharmacokinet. 36 (1999) 127–143.
[10] S.R. Brody, M.H. Humphreys, J.G. Gambertoglio, P. Schoenfeld, K.C. Cundy, F.T. Aweeka, Pharmacokinetics of cidofovir in renal insufficiency and in continuous ambulatory peritoneal dialysis or high-flux hemodialysis, Clin. Pharmacol. Ther. 65 (1999) 21–28.
[11] E.J. Eisenberg, K.C. Cundy, High-performance liquid chromatographic determination of cytosine-containing compounds by precolumn fluorescence derivatization with phenacyl bromide: application to antiviral nucleosides and nucleotides, J. Chromatogr. B 679 (1996) 119–127.
[12] L.C. Yuan, G.J. Samuels, G.C. Visor, Stability of cidofovir in 0.9% sodium chloride injection and in 5% dextrose injection, Am. J. Health Syst. Pharm. 53 (1996) 1939–1943.
[13] B.B. Ba, M.C. Saux, Separation methods for antiviral phosphorus-containing drugs, J. Chromatogr. B: Biomed. Sci. Appl. 764 (2001) 349–362.
[14] A.N. Naiman, G. Roger, M.C. Gagnieu, J. Bordenave, S. Mathaut, S. Ayari, R. Nicollas, J.B. Bour, N. Garabedian, P. Froehlich, Cidofovir plasma assays after local injection in respiratory papillomatosis, The Laryngoscope 114 (2004) 1151–1156.
[15] A. Breddemann, L. Hsien, E. Tot, S. Laer, Quantification of cidofovir in human serum by LC–MS/MS for children, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 861 (2008) 1–9.
[16] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), and Center for Veterinary Medicine (CVM), Guidance for Industry—Bioanalytical Method Validation, 2001, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf.
[17] H. Rosing, W.Y. Man, E. Doyle, A. Bult, J.H. Beijnen, Bioanalytical liquid chromatographic method validation. A review of current practices and procedures, J. Liq. Chromatogr. Relat. Technol. 23 (2000) 329–354.