Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 861 (2008) 1–9
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Quantification of cidofovir in human serum by LC–MS/MS for children

André Breddemann, Linda Hsien, Edith Tot, Stephanie Läer *

Department of Clinical Pharmacy and Pharmacotherapy, Heinrich-Heine-University of Düsseldorf, Universitätsstrasse 1, 40225 Düsseldorf, Germany

Received 16 July 2007; accepted 26 November 2007
Available online 4 December 2007

Abstract

A new method for the quantification of cidofovir (CDV), an acyclic nucleotide analogue of cytosine with antiviral activity against a broad-spectrum of DNA viruses, in human serum, using high-performance liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) has been developed. A strong anion exchange (SAX) solid-phase extraction procedure was applied for the sample preparation. The tandem mass spectrometer was tuned in the multiple reaction monitoring mode to monitor the *m/z* 278.1 → 234.9 and the *m/z* 288.1 → 133.1 transitions for CDV and the internal standard 9-(2-phosphonylmethoxyethyl)guanine (PMEG), respectively, using negative electrospray ionization. The MS/MS response was linear over the concentration range from 78.125 ng/ml to 10,000 ng/ml, with a lower limit of quantification of 78.125 ng/ml. The intra- and inter-day precisions (relative standard deviation (%)) for CDV were less than 7.8% and the accuracies (% of deviation from nominal level) were within ±12.1% for quality controls. The novel LC–MS/MS method allowed a specific, sensitive and reliable determination of CDV in human serum and was applied to investigate the yet unknown pharmacokinetic properties of CDV in a paediatric cancer patient.

Keywords: Cidofovir; PMEG; LC–MS/MS; Pharmacokinetic study; Children

1. Introduction

Cidofovir (CDV, HPMPC, VISTIDE®) is an acyclic nucleotide analogue of cytosine with antiviral activity against a broad-spectrum of DNA viruses including herpes viruses, poxviruses, adenoviruses, and papovaviruses. CDV is approved for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients, but the spectrum of its therapeutic use nowadays includes also the pre-emptive therapy and the treatment of disease in CMV-infected paediatric cancer patients [1–3]. Paediatric cancer patients are at considerable risk to develop these life-threatening viral infections due to intensive immunosuppressive therapy [4]. In contrast to adult patients, information about the pharmacokinetic properties of CDV in paediatric patients is still lacking [5–9]. However, particularly in paediatric patients, it is crucial to determine the individual drug exposure and the resulting concentration of a therapeutic compound to avoid sub-therapeutic or toxic dosing, since pharmacokinetic properties in paediatric patients may vary significantly from those in adults [10]. In order to investigate these pharmacokinetic properties, our purpose was to develop an LC–MS/MS method for the quantification of CDV in human serum as an alternative to the established HPLC methods. So far, there are four HPLC methods described in the literature by Naiman et al. [14], Eisenberg and Cundy [11], Yuan et al. [13] and Cundy et al. [6,12]. Comparing the already established HPLC methods with the present LC–MS/MS method, the required blood volume is of particular interest because of the restricted blood sampling in paediatric patients. The required serum or plasma volumes for one single analysis range from 100 μl plasma [11] to 500 μl serum [6]. However, the former method [11] employed a time-consuming precolumn fluorescence derivatization to increase the sensitivity and selectivity of the detection. The developed LC–MS/MS method facilitates a quick and selective detection requiring 300 μl serum. The novel method is therefore suitable as an alternative analytical method and can be integrated into pharmacokinetic studies in paediatric patients.

2. Experimental

2.1. Chemicals and materials

Cidofovir reference standard and the internal standard (IS), 9-(2-phosphonylmethoxyethyl)guanine (PMEG), were synthe-

* Corresponding author. Tel.: +49 211 81 13664; fax: +49 211 81 13847.
E-mail address: stephanie.laeer@uni-duesseldorf.de (S. Läer).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.11.029

sized and supplied by Gilead Sciences, Inc. (Foster City, USA) according to a material transfer agreement. HPLC-grade methanol and water were purchased from VWR International (Fontenay sous Bois, France). Hydrochloric acid (37%) and ammonium hydroxide solution (ammonia in aqueous solution) were purchased from Sigma–Aldrich Laborchemikalien (Seelze, Germany). Ringer's solution was purchased from Baxter Deutschland GmbH (Ringer solution pfrimmer, Unterschleißheim, Germany). The SAX Varian Bond Elut solid-phase extraction (SPE) cartridges (100 mg, 1 ml) were purchased from Varian, Inc. (Lake Forest, USA).

2.2. Preparation of calibration and quality control samples

The CDV standard solution was prepared by dissolving the accurately weighed reference standard in Ringer's solution to obtain a final concentration of 100 μg/ml. The standard solution was then serially diluted with Ringer's solution to receive eight working solutions. The concentrations of the resulting dilutions were as follows: 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 6.25 μg/ml, 3.125 μg/ml, 1.563 μg/ml and 781.25 ng/ml. The calibration samples were then prepared by spiking 270 μl blank human serum with 30 μl of the working solution with the required concentration to obtain the final concentrations: 10.0 μg/ml, 5.0 μg/ml, 2.5 μg/ml, 1250 ng/ml, 625 ng/ml, 312.5 ng/ml, 156.25 ng/ml and 78.125 ng/ml. All dilutions were prepared daily except the CDV standard solution. The CDV standard solution was stored at 4 °C for the maximum time period of 1 month and was brought to room temperature before use. Furthermore, there was an independent weighing of the required quality control (QC) stock solution for the assessment of the accuracy and the precision of this method. Similarly to the preparation of the calibration samples, the QC stock solution was serially diluted with Ringer's solution.

2.3. Sample extraction

Analogue to the CDV standard solution, the IS standard solution was stored at 4 °C for the maximum time period of 1 month and was brought to room temperature before use. For the sample extraction, 30 μl of the internal standard solution (100 μg/ml PMEG in water) was added to 300 μl human serum and then diluted with water to a final volume of 1000 μl. The prepared sample was vortexed for 1 min and then transferred onto a conditioned Varian Bond Elut strong anion exchange (SAX) solid-phase extraction (SPE) cartridge. The SAX cartridges were conditioned with 2 ml methanol and 2 ml water. After sample application, the cartridges were rinsed twice with 1 ml water. Subsequently, the samples were eluted twice with 1 ml methanol containing 3% (v/v) hydrochloric acid. The extracts were collected in collection tubes (centrifuge glass type A, 15 ml, VWR International, Fontenay sous Bois, France) under the cartridges and then evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted with 300 μl of a methanol/water solution (50:50, v/v) modified with 3% (v/v) ammonia in aqueous solution to obtain the final extract. The final extract was vortexed for 30 s within the collection glasses and transferred into disposable plastic vials (safe-seal micro-tube, 2 ml, PP, Sarstedt AG, Nürnbrecht, Germany). Finally, these vials were centrifuged for 20 min at 3220 × *g* (4000 rpm) and 4 °C (Centrifuge Eppendorf 5810R, Eppendorf AG, Hamburg, Germany) to separate particulate material. A 10-μl aliquot of the supernatant of the final extract was injected onto the LC–MS/MS system for analysis.

2.4. LC–MS/MS conditions

The modular HPLC system consisted of the following components: Shimadzu controller SCL10Avp, two separate Shimadzu pumps LC10Avp with a standard analytical mixing device for low- and high-pressure gradient configurations (Shimadzu Deutschland GmbH, Duisburg, Germany), a four-channel online degasser Knauer K-5004 (Knauer GmbH, Berlin, Germany) and a VWR/Hitachi Column Oven L-2300 (VWR International, Fontenay sous Bois, France). The HPLC was connected in series with a triple quadrupole mass spectrometer API 2000 (Applied Biosystems/MDS SCIEX, Concord, Canada). Data were collected and analysed using the Analyst 1.4.1 software from Applied Biosystems/MDS SCIEX. A Purospher® Star RP-18 endcapped analytical column (125 mm × 2mm, 5 μm, Merck KGaA, Darmstadt, Germany) in combination with a Purospher® Star RP-18 endcapped guard column (4 mm × 4 mm, 5 μm, Merck KGaA, Darmstadt, Germany) was used to perform the chromatography. This tetraalkoxysilane column provides a high stability up to pH 10.5 and was tempered to 30 °C. The isocratic mobile phase consisted of 50% (v/v) methanol and 50% (v/v) water modified with 2% (v/v) ammonia in aqueous solution. Hence, the resulting pH value of the mobile phase was strongly alkaline (pH 10.5). Since no splitting device was used to adjust the flow rate to 0.3 ml/min, resulting in back-pressures of 180 bar. The tandem mass spectrometer was tuned in the multiple reaction monitoring (MRM) mode to monitor the *m/z* 278.1 → 234.9 and the *m/z* 288.1 → 133.1 transitions for CDV and IS, respectively, using negative electrospray ionization. The mass spectra and the proposed patterns of CDV and IS fragmentation as well as a summary of the adjusted MS conditions and the compound-specific MS-parameters are presented in Fig. 1 and Tables 1 and 2.

2.5. Validation

A full validation based on the FDA guideline for bioanalytical method validation was required, due to the fact that this method is applied for the first time, and therefore performed [15].

2.5.1. Selectivity

The assay selectivity was assessed by analysing serum extracts from seven different paediatric cancer patients not receiving a CDV therapy. Potential interferences in these sera by either endogenous compounds or other drugs concomitantly administered to these patients were investigated. An "interfering drug" or an "interfering endogenous compound" was considered if there was a signal close to 0.3 min of the retention time of the analyte or the IS with the potential capability to cause ion

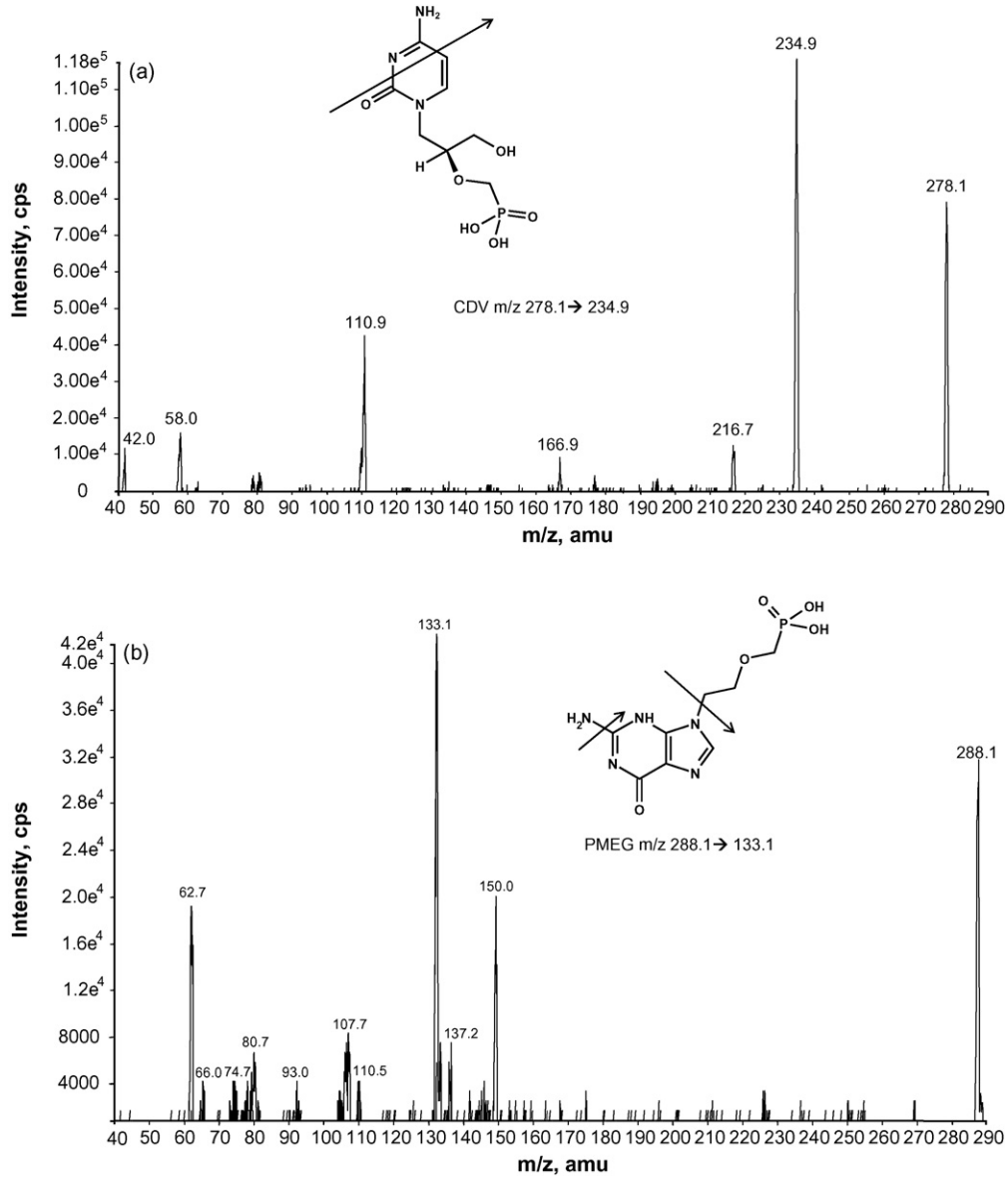

Fig. 1. Product ion mass spectra (negative electrospray ionization) and proposed patterns of fragmentation of (a) CDV and (b) PMEG (internal standard). The deprotonated molecules were used as precursor ions for MS/MS.

suppression or enhancement. In addition, possible "cross-talk" effects between the MS/MS channels used for the monitoring of CDV and the IS were assessed. This was achieved by separately injecting CDV at the highest concentration of the calibration line (10,000 ng/ml) and monitoring the response in the IS channel and by injecting a serum sample spiked only with the IS and monitoring the response in the respective channel for CDV.

2.5.2. Accuracy, precision and calibration

Accuracy and precision were assessed by determining quality control samples at the lower limit of quantification, low, mid and high concentrations (five samples from each concentration) on three subsequent validation days. The criteria used to assess the suitability of precision and accuracy was as follows: the mean percentage deviation should not exceed 15%. The precision was expressed as relative standard deviation (CV (%)) and the accuracies were expressed as percentages (%) of the nominal concentrations. These criteria are compliant with the FDA guidelines for bioanalytical method validation [15]. For the calibration, experiments were performed on three validation days with three independent calibration sets (consisting of eight calibration points) on each day. The calibration data were collected and analysed using the Analyst 1.4.1 software from Applied Biosystems/MDS SCIEX (Concord, Canada). The calibration curve was obtained by fitting the ratio of the integrated mass peak area of CDV to the integrated mass peak area of the IS (y-axis) against the range of added analyte concentration (x-axis) using $1/x$ linear regression plots.

2.5.3. Recovery

For the determination of the absolute recovery of CDV and the IS from the extraction procedure the method described by

Table 1
MS conditions for cidofovir analysis

Mass spectrometer	API 2000
Interface	Electrospray
Polarity	Negative
Scan type	MRM
Resolution	Q1-unit resolution[a]
Curtain gas (CUR)	Q3-unit resolution[a]
Collision gas (CAD)	8
IonSpray voltage (IS)	−4500
Temperature (TEM)	375 °C
Ion source gas 1 (GS 1)	30
Ion source gas 2 (GS 2)	50
Solvent split ratio	None

Compound	Mass to charge ratio (*m*/*z*)		Dwell time (ms)
	Q1 (amu)	Q3 (amu)	
Cidofovir	278.1	234.9	250
PMEG (internal standard)	288.1	133.1	250

[a] Unit: FWHM is 0.7 ± 0.1 amu.

Matuszewski et al. [16] was applied. The absolute recovery was determined by a comparison of the peak areas derived from serum samples (QC samples at three concentrations, with five samples for each concentration) spiked before extraction with the peak areas derived from serum samples spiked after the extraction procedure with the same amount of analyte. Similarly, the recovery of the IS was determined by comparing the mean peak areas of extracted QC samples to mean peak areas of IS in serum samples prepared by spiking the same amounts of IS after the extraction procedure. The absolute recovery (%) was calculated as the peak area divided by the peak area multiplied by 100.

2.5.4. Matrix effects

It is known that the reliability of quantitative assays using the LC–MS/MS methodology may be adversely affected by matrix effects [16–19]. To analyse possible matrix effects at the point of elution the postextraction addition technique was applied according to the method described by Taylor [20]. The reasoning is that the analyte and the internal standard elute both under 1 min retention time, which is often within the matrix elution. Therefore, sample extracts with the analyte of interest added postextraction were compared with pure solutions prepared in mobile phase containing equivalent amounts of the analyte of interest. The difference of the resulting peak areas between the postextraction samples and the pure solution peak areas divided by the pure solution peak areas determines the degree of matrix effect occurring under chromatographic conditions.

Table 2
Compound-specific MS-parameters

Parameter	Cidofovir	PMEG
Focusing potential (FP)	−340	−330
Entrance potential (EP)	−7	−7.5
Cell entrance potential (CEP)	−16	−14
Collision energy (CE)	−22	−42
Cell exit potential (CXP)	−10	−8

2.5.5. Stability

Relevant data regarding the stability of CDV and IS at different conditions were not available. For this reason, stability tests were conducted in the following different matrices: stock solution, postextracted solution and serum. For the stock solution stability, peak area of each drug obtained from fresh stock solution was compared to peak area obtained after 1 month storing at 4 °C. To evaluate the stability of the postextracted solution, the analytical results from the extracted QC samples at four different concentrations (five samples on each concentration) were compared with those obtained after 24 h storing at room temperature within the autosampler. To investigate the stability in human serum, the deviation of analytical results obtained from extracted QC samples at three different concentrations (five samples on each concentration) after 1 month storing at −20 °C were compared with the nominal results. The stability in human serum after three freeze–thaw cycles was also tested.

2.6. Calculations and statistical methods

The pharmacokinetic parameters of the paediatric cancer patient were determined from the concentration–time data. The elimination half-life ($t_{1/2}$) was calculated with the non-compartmental model of TopFit 2.0: pharmacokinetic and pharmacodynamic data analysis system (Gustav Fischer Verlag, Stuttgart, Germany). The area under the concentration–time curve from time zero (AUC_0) to the last measurable concentration point t_{last} ($AUC_{0-t_{last}}$) was calculated by the linear trapezoidal method. Extrapolation to time infinity ($AUC_{0-\infty}$) was calculated as follows: $AUC_{0-\infty} = AUC_{0-t_{last}} + c_t/k_e$, where c_t is the last measurable concentration and k_e is the elimination rate constant. The maximum concentration c_{max} was defined as the first measured concentration. The calculation of the resulting parameters clearance (Cl) and volume of distribution (V_d) has been carried out using the following equations: Cl = dose/AUC and $V_d = Cl/k_e$.

2.7. Samples of a paediatric cancer patient

To apply and test the new LC–MS/MS method under realistic application conditions, we analysed blood samples of a paediatric cancer patient who intravenously received 2.5 mg per kg bodyweight CDV [21]. Blood samples were collected at 0 h (pre-treatment), 1 h, 2 h, 3 h, 4 h and 6 h after infusion. The blood collection system "S-Monovette" (Sarstedt AG, Nürnbrecht, Germany) was used to take the blood samples (serum extraction). It contains additive carriers as clotting activator but no chemical additives (such as heparin, citrate or EDTA). Analytical interferences are therefore not expected. After withdrawal of approximately 1.2 ml whole blood, the blood was centrifuged at 3220 × *g* (this is equivalent to 4000 rpm) over 20 min at 4 °C (Centrifuge Eppendorf 5810R, Eppendorf AG, Hamburg, Germany). The supernatant of the centrifuged blood (approximately 600 μl, sufficient for a duplicate analysis) was then used for further sample extraction procedure.

3. Results

3.1. Selectivity

Endogenous peaks at the retention time of the analyte or the IS were not observed in any of the seven sera evaluated and no "cross-talk" was observed. Typical ion chromatograms of blank human serum- and QC-extracts at the lower limit of quantification (LLOQ) are presented in Fig. 2.

3.2. Accuracy, precision and calibration

The intra- and inter-day precisions (relative standard deviation (%)) for CDV were less than 7.8% and the accuracies (% of deviation from nominal level) within ±12.1%. The complete results of the validation are listed in Table 3. The mean linear regression equation for the calibration curve was $y = 0.000234(\pm 0.00002) + 0.00659(\pm 0.00125)$ and the resulting correlation coefficients (r) were greater than 0.996 for the calibration curve determinations during the method validation. The results of the calibration are shown in Table 4.

3.3. Recovery

The extent of recovery of the analyte and the IS was equivalent. The mean absolute recovery for CDV determined at 78 ng/ml, 1250 ng/ml and 10,000 ng/ml (five samples for each concentration) were 62.69%, 51.38% and 52.45%, respectively. The mean absolute recovery for IS at 10,000 ng/ml ($n = 15$) was 60.94%.

3.4. Matrix effect

The mean deviation of the peak areas derived from the post-extraction samples compared with the peak areas of the pure solutions prepared in mobile phase at 78 ng/ml, 1250 ng/ml and 10,000 ng/ml for CDV (five samples for each concentration) range from −72.94% to −72.14% and −74.96%, respectively. Similarly, the matrix effect for the IS was investigated at the concentration used for the sample extraction (10,000 ng/ml, $n = 15$), the mean deviation amounted to −69.68%. These data clearly indicate a pronounced ion-suppressive matrix effect. The extent of the matrix effect on the ionisation of the IS is consistent with the matrix effect of the analyte. Therefore, the technical neces-

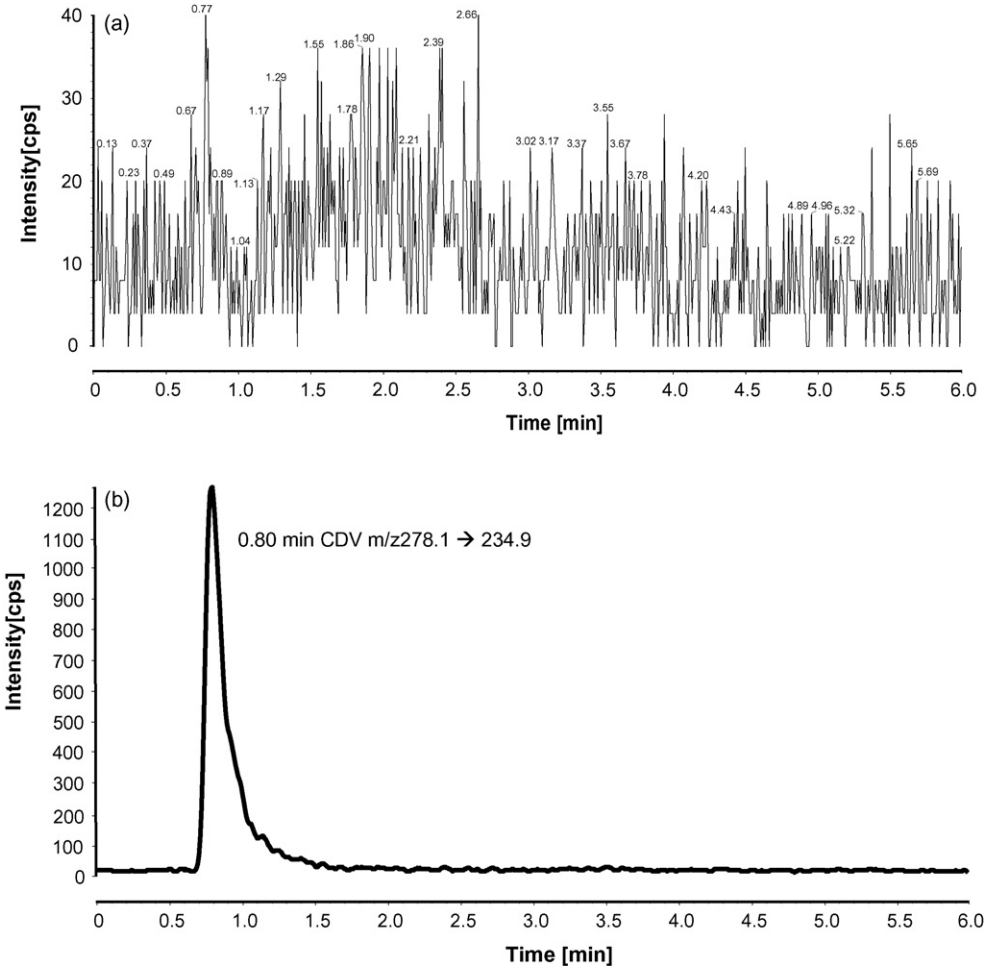

Fig. 2. (a) Typical ion chromatogram of blank human serum extract. (b) Typical ion chromatogram of LLOQ-human serum extract (spiked with 78.125 ng/ml CDV) for CDV.

Table 3
Intra- and inter-assay performance data for Cidofovir in human serum at four concentration levels on three subsequent validation days (five samples on each concentration)

Validation day	Nominal concentration (ng/ml)	Measured concentration (ng/ml)	Accuracy (%)	Precision (%)
1	76.1	74.6	98.0	4.6
2	76.1	66.9	87.9	2.6
3	76.1	69.1	90.9	2.5
Inter-assay (1–3)	76.1	70.2	92.3	5.7
1	152.2	164.6	108.2	4.6
2	152.2	142.4	93.6	5.2
3	152.2	145.2	95.4	1.5
Inter-assay (1–3)	152.2	150.7	99.0	7.8
1	1217	1342.4	110.3	1.3
2	1217	1252.0	102.8	3.6
3	1217	1142.0	93.8	4.0
Inter-assay (1–3)	1217	1245.5	102.3	7.4
1	4870	5257.4	108.0	1.7
2	4870	5038.0	103.5	4.2
3	4870	4766.0	97.9	3.4
Inter–assay (1–3)	4870	5020.5	103.1	5.1

sity for the reliable quantification may not be adversely affected by this matrix effect.

3.5. Stability

The deviation of the peak area derived from the freshly prepared stock solutions in comparison with the peak area for the stock solutions after storage at 4 °C for 1 month was −1.91% and 0.14% for CDV and IS, respectively, indicating that the stock solutions of CDV and the IS are stable under these conditions. The results for the postextraction solution stability test demonstrate that the postextraction solution is stable at room temperature for at least 24 h. The mean percentage deviation of the analytical results from extracted QC samples compared with the results of those samples after 24 h at room temperature ranged from 11.51% (for the lowest concentration) to 0.92% (for the highest concentration). The observed deviations from the nominal results for the stability tests in human serum

Table 4
Calibration results of three independent sets for Cidofovir in human serum

Set	Expected concentration (ng/ml)	Number of values	Mean concentration	S.D.	CV (%)	Mean accuracy (%)
1	78.125	3	77.4	5.215	6.7	99.1
1	156.25	3	160.9	13.288	8.3	103.2
1	312.5	3	279.6	24.504	8.8	89.3
1	625	3	654.2	79.233	12.1	104.7
1	1250	3	1283.4	23.220	1.8	102.7
1	2500	3	2458.9	106.906	4.3	98.4
1	5000	3	5263.1	55.372	1.1	105.3
1	10,000	3	9744.6	366.148	3.8	97.4
2	78.125	3	76.4	2.641	3.5	97.8
2	156.25	3	134.4	4.423	3.3	86.2
2	312.5	3	288.8	42.219	14.6	92.3
2	625	3	711.2	36.135	5.1	113.8
2	1250	3	1258.5	115.376	9.2	100.7
2	2500	3	2815.6	90.116	3.2	112.6
2	5000	3	5031.1	424.062	8.4	100.6
2	10,000	3	9606.2	532.497	5.5	96.1
3	78.125	3	62.6	4.685	7.5	80.2
3	156.25	3	169.4	16.819	9.9	108.6
3	312.5	3	324.5	15.266	4.7	103.7
3	625	3	657.5	33.973	5.2	105.2
3	1250	3	1258.4	160.04	12.7	100.7
3	2500	3	2568.3	138.029	5.4	102.7
3	5000	3	5024.3	90.401	1.8	100.5
3	10,000	3	9857.4	661.391	6.7	98.6

Table 5
Stability of cidofovir in human serum

Sample concentration (ng/ml; $n=5$)	Mean concentration (ng/ml)	Accuracy (%)	Precision (%)
Three freeze–thaw cycles			
156	166	106.15	3.38
1250	1398	111.84	5.93
5000	5518	110.36	7.21
Stability for 1 month at −20 °C			
156	147	94.36	5.20
1250	1234	98.72	5.08
5000	4936	98.72	2.31

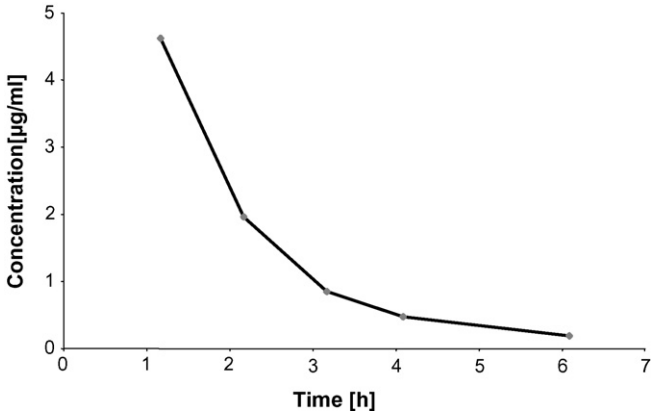

Fig. 3. Concentration–time profile of a paediatric cancer patient who intravenously received 2.5 mg/kg CDV.

after three freeze–thaw cycles and after storage for 1 month at −20 °C for three concentrations (five samples on each concentration) show that CDV is stable under these conditions (Table 5). Overall, the conducted stability tests indicate reliable stability properties for CDV and the IS under experimental conditions.

3.6. Samples of a paediatric cancer patient

The pharmacokinetic properties of the paediatric cancer patient derived from the analysis of the concentration–time profile (Fig. 3) in comparison to published adult pharmacokinetic properties [6] are presented in Table 6. Fig. 4 shows an ion chromatogram of a quality control extract compared with one of the paediatric patient sample extract.

Table 6
Comparison of paediatric and adult pharmacokinetic properties

Parameter	Cundy et al.[a] [6]	Paediatric patient
Dose (mg/kg)	3	2.5
$t_{1/2}$ (h)	2.2 (0.6)	1.51
c (max) (μg/ml)	8.08 (1.39)	4.62
$AUC_{0-\infty}$ (μg h/ml)	19.87(2.85)	9.07
Cl (ml/h kg)	154 (23)	257
V_d (ml/kg)	488 (171)	560

[a] Values are means of five subjects (S.D.).

4. Discussion

The developed LC–MS/MS method shall be applied for the realisation of a pharmacokinetic study in paediatric patients, which is a fundamental prerequisite for the development of a paediatric dosing schedule that incorporate potential age-dependent pharmacokinetic properties of CDV. However, the adequate treatment of paediatric cancer patients often requires polymedication which is related to potential analytical interference. Therefore, analytical methods for the determination of CDV in human serum necessitate a selective detection. For this reason, we implemented the application of tandem mass spectrometry for the development of this new method as modern approach to provide high selectivity, because only a fragment ion derived from the $[M-H]^-$ ion of the analyte was monitored in the multiple reaction monitoring mode. Nevertheless, the reliability of a quantitative LC–MS/MS method is susceptible to be affected by matrix effects. In the presented method, the conducted tests to investigate these effects clearly indicate a pronounced ion-suppressive matrix effect. But this effect was consistent for both the analyte and the internal standard. Therefore, the technical necessity for the reliable quantification may not be adversely affected by this matrix effect. In addition, the observed validation data for accuracy and precision indicate that the LC–MS/MS methodology can be applied for the measurement of CDV concentrations in human serum. The expected concentrations in adult patients who intravenously received 3.0 mg/kg CDV range from 8 μg/ml for the peak concentration (c_{max}) to approximately 400 ng/ml after 8 h [6]. With a linear signal response in the concentration range from 78.125 ng/ml to 10,000 ng/ml, the developed method covers the expected concentrations in patients at least up to four half-lives. Since multiple blood sampling from paediatric patients is restricted, the required blood volume for the analysis of a pharmacokinetic profile is of particular interest. The presented method requires 300 μl serum to determine a single data point. Therefore, for the determination of a concentration–time profile consisting of six data points and a duplicate analysis a total of 7.2 ml whole blood is required. This volume is far below the Blood Draw Guidelines of the Institutional Review Boards of the University of Michigan Medical School (IRBMED) [22]. To test the method, the concentration–time profile of a paediatric cancer patient who intravenously received CDV was investigated. The

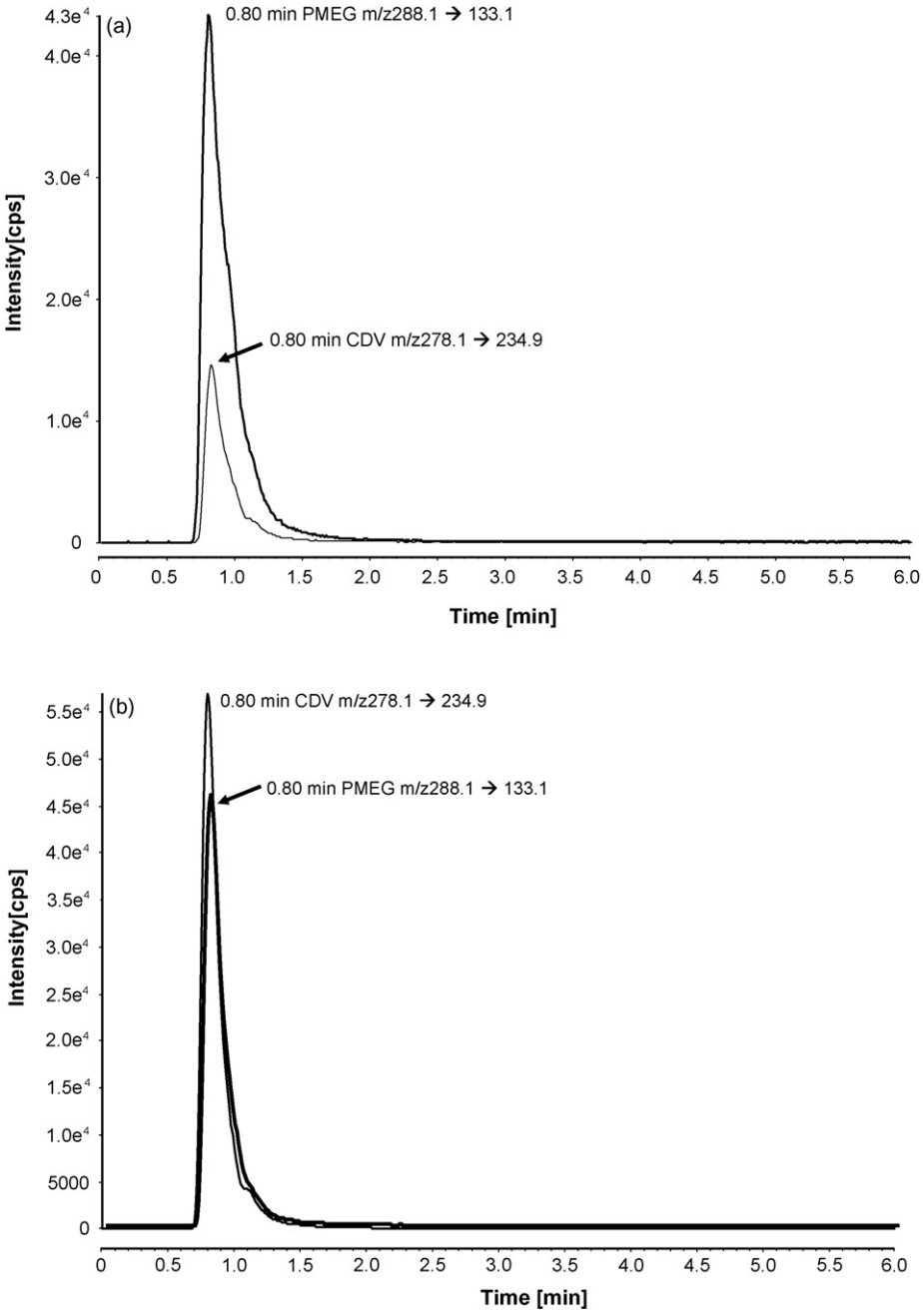

Fig. 4. (a) Ion chromatogram of a quality control extract (spiked with 1250 ng/ml CDV) for CDV and the internal standard PMEG. (b) Ion chromatogram of a paediatric patient sample extract (blood withdrawal 1 h after infusion of 2.5 mg/kg CDV, the measured CDV concentration amounted to 5270 ng/ml) for CDV and the internal standard PMEG.

resulting pharmacokinetic properties indicate a higher body-weight normalised clearance in paediatric patients compared to the clearance in adults. This finding supports our hypothesis that it is crucial to determine the individual concentrations and the resulting drug exposure of an antiviral compound in paediatric patients. In this case, a dose adaption seems to be required for the paediatric patient to obtain the same drug exposure as adults have. This can be reasoned by the fact that the observed clearance difference amounts for 60%. If the observed individual deviation in the clearance is an age-specific difference remains to be verified. For this purpose, the analysis of more serum concentration–time profiles of paediatric patients within the framework of a pharmacokinetic study is required.

5. Conclusion

The presented HPLC–MS/MS method allows the accurate and reproducible quantification of CDV in human serum, according to the generally accepted FDA validation criteria. With the required serum volume of 300 μl for one single analysis, this method is suitable to be integrated into a pharmacokinetic study with paediatric patients.

Acknowledgements

The authors would like to thank Martha L. Vazquez from Gilead Sciences, Inc. (Foster City, USA) for her efforts to facilitate the material transfer agreement for cidofovir reference standard and the internal standard.

References

[1] M.A. Jacobson, N. Engl. J. Med. 337 (1997) 105.
[2] W.H. Kruger, J. Bohlius, O.A. Cornely, H. Einsele, H. Hebart, G. Massenkeil, S. Schuttrumpf, G. Silling, A.J. Ullmann, D.T. Waldschmidt, H.H. Wolf, Ann. Oncol. 16 (2005) 1381.
[3] P. Ljungman, P. Reusser, R. de la Camara, H. Einsele, D. Engelhard, P. Ribaud, K. Ward, Bone Marrow Transplant. 33 (2004) 1075.
[4] F.R. Schuster, A. Simon, H.J. Laws, K. Beutel, A.H. Groll, G. Jager, V. Schuster, Klin. Padiatr. 217 (Suppl. 1) (2005) S67.
[5] K.C. Cundy, Clin. Pharmacokinet. 36 (1999) 127.
[6] K.C. Cundy, B.G. Petty, J. Flaherty, P.E. Fisher, M.A. Polis, M. Wachsman, P.S. Lietman, J.P. Lalezari, M.J. Hitchcock, H.S. Jaffe, Antimicrob. Agents Chemother. 39 (1995) 1247.
[7] S. Eksborg, Herpes 10 (2003) 66.
[8] D.L. Wolf, C.A. Rodriguez, M. Mucci, A. Ingrosso, B.A. Duncan, D.J. Nickens, J. Clin. Pharmacol. 43 (2003) 43.
[9] S.R. Brody, M.H. Humphreys, J.G. Gambertoglio, P. Schoenfeld, K.C. Cundy, F.T. Aweeka, Clin. Pharmacol. Ther. 65 (1999) 21.
[10] S. Laer, J.P. Elshoff, B. Meibohm, J. Weil, T.S. Mir, W. Zhang, M. Hulpke-Wette, J. Am. Coll. Cardiol. 46 (2005) 1322.
[11] E.J. Eisenberg, K.C. Cundy, J. Chromatogr. B: Biomed. Appl. 679 (1996) 119.
[12] B.B. Ba, M.C. Saux, J. Chromatogr. B: Biomed. Sci. Appl. 764 (2001) 349.
[13] L.C. Yuan, G.J. Samuels, G.C. Visor, Am. J. Health Syst. Pharm. 53 (1996) 1939.
[14] A.N. Naiman, G. Roger, M.C. Gagnieu, J. Bordenave, S. Mathaut, S. Ayari, R. Nicollas, J.B. Bour, N. Garabedian, P. Froehlich, Laryngoscope 114 (2004) 1151.
[15] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), 2001. http://www.fda.gov/cder/guidance/4252fnl.htm.
[16] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[17] J.L. Little, M.F. Wempe, C.M. Buchanan, J. Chromatogr. B: Analyt. Technol. Biomed. Life Sci. 833 (2006) 219.
[18] T.M. Annesley, Clin. Chem. 49 (2003) 1041.
[19] R. Bonfiglio, R.C. King, T.V. Olah, K. Merkle, Rapid Commun. Mass Spectrom. 13 (1999) 1175.
[20] P.J. Taylor, Clin. Biochem. 38 (2005) 328.
[21] A. Breddemann, S. Laer, K.G. Schmidt, M. Harjes, R. Adam, A. Ludwig, T. Niehues, D.T. Schneider, Herpes 14 (2007) 41.
[22] University of Michigan, Institutional Review Boards of the University of Michigan Medical School (IRBMED), 2007. http://www.med.umich.edu/irbmed/guidance/blood_draw.htm.