Journal of Analytical Science and Technology

RESEARCH ARTICLE Open Access

Quantification of venadaparib, a novel PARP inhibitor, in the rat and dog plasma using liquid chromatography/tandem mass spectrometry

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Abstract

Venadaparib (VEN), a next-generation inhibitor of poly (ADP-ribose) polymerases, is under development for oral use in patients having cancers with deoxyribonucleic acid repair defects. The objective of this study was to develop and validate a sensitive and robust analytical method for quantifying VEN in a small volume of plasma samples from rats and dogs, and to assess the feasibility of the assay for application in pharmacokinetic/toxicokinetic studies. Plasma samples were subjected to deproteination, and an aliquot was injected into an LC–MS/MS system. VEN and imipramine were analyzed in the positive ion mode and quantified by monitoring the transition at *m/z* 407.2 → 70.0 for VEN and 281.2 → 86.1 for imipramine. The lower and upper limits of the assay were determined to be 1 and 1000 ng/mL, respectively, with acceptable linearity ($r^2 > 0.995$). Validation parameters, such as accuracy, precision, dilution, recovery, matrix effect, and stability, were within acceptable ranges. This method was adequately applied to the characterization of pharmacokinetics of VEN in rats and dogs at the oral dose of 30 and 0.5 mg/kg, respectively. These findings suggest that the validated assay is applicable to the kinetic studies of VEN with a small volume of plasma samples from the animals.

Keywords Method validation, Bioanalysis, Mass spectrometry, Pharmacokinetics, Poly (ADP-ribose) polymerases inhibitor, Venadaparib

Introduction

Genomic instability, a characteristic feature of cancer development, occurs due to defects in cellular responses to deoxyribonucleic acid (DNA) damage (Pilie et al. 2019). Although the damage is normally corrected by DNA repair systems, some may still remain unrepaired/misrepaired, leading to an enhanced risk of cancer development (Alhmoud et al. 2020; Hoeijmakers 2001). Poly (ADP-ribose) polymerases (PARPs), essential enzymes in the repair of damage in single-strand DNA (Sugimura and Miwa 1994), are responsible for recruiting proteins to promote DNA repair/cell survival when cells are exposed to genotoxic insults. Therefore, inhibition of PARPs can result in the accumulation of unrepaired damage in single-strand DNA and, ultimately, breaks in DNA double strands (Haber 1999; Ashworth 2008). When cells

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with genetic defects in the DNA repair system, notably in the homologous recombination pathway (e.g., breast cancer type 1 and 2 susceptibility proteins), are treated with PARP inhibitors, breaks in DNA double strands would not be adequately repaired, viz. synthetic lethal condition (Bryant et al. 2005; Farmer et al. 2005; Lord and Ashworth 2017). In this respect, PARP inhibition appears to be a pharmaco-therapeutically attractive approach for targeting tumors. Currently, four PARP inhibitors, olaparib, rucaparib, niraparib, and talazoparib, are commercially available in the USA (AstraZeneca 2022; Clovis Oncology 2021; GlaxoSmithKline 2021; Pfizer 2021).

Venadaparib (VEN, Fig. 1A), a novel derivative of phthalazinone, selectively inhibits PARPs with a balanced trapping effect (Lee et al. 2017, 2018, 2023). The compound reportedly possesses potent in vitro and in vivo antitumor effects with a wide therapeutic range, as evidenced by the fact that the inhibition of tumor growth by VEN is greater than that by other PARP inhibitors in various cancer models at a tolerable dose (Lee et al. 2017, 2018, 2023). Furthermore, VEN was found to be well tolerated in human subjects in a recently completed phase I clinical study in Korea (Identifier NCT03317743 2022; Kim et al. 2021), suggesting that the new investigational drug is a next-generation PARP inhibitor for the management of advanced cancer patients with limited therapeutic options. The compound is currently under phase Ib/IIa studies in the USA, Korea, and China (Identifier NCT04174716 2022; Identifier NCT04725994 2022; Lee et al. 2021; Im et al. 2021).

PK and toxicokinetic (TK) studies are often necessary for the further development of new drugs (e.g., drug–drug interaction and toxicology studies). For the case of PARP inhibitors, hematologic toxicities, including anemia, neutropenia, and thrombocytopenia, are the common adverse events (AstraZeneca 2022; Clovis Oncology 2021; GlaxoSmithKline 2021; Pfizer 2021). It is expected that blood samples are collected for multiple

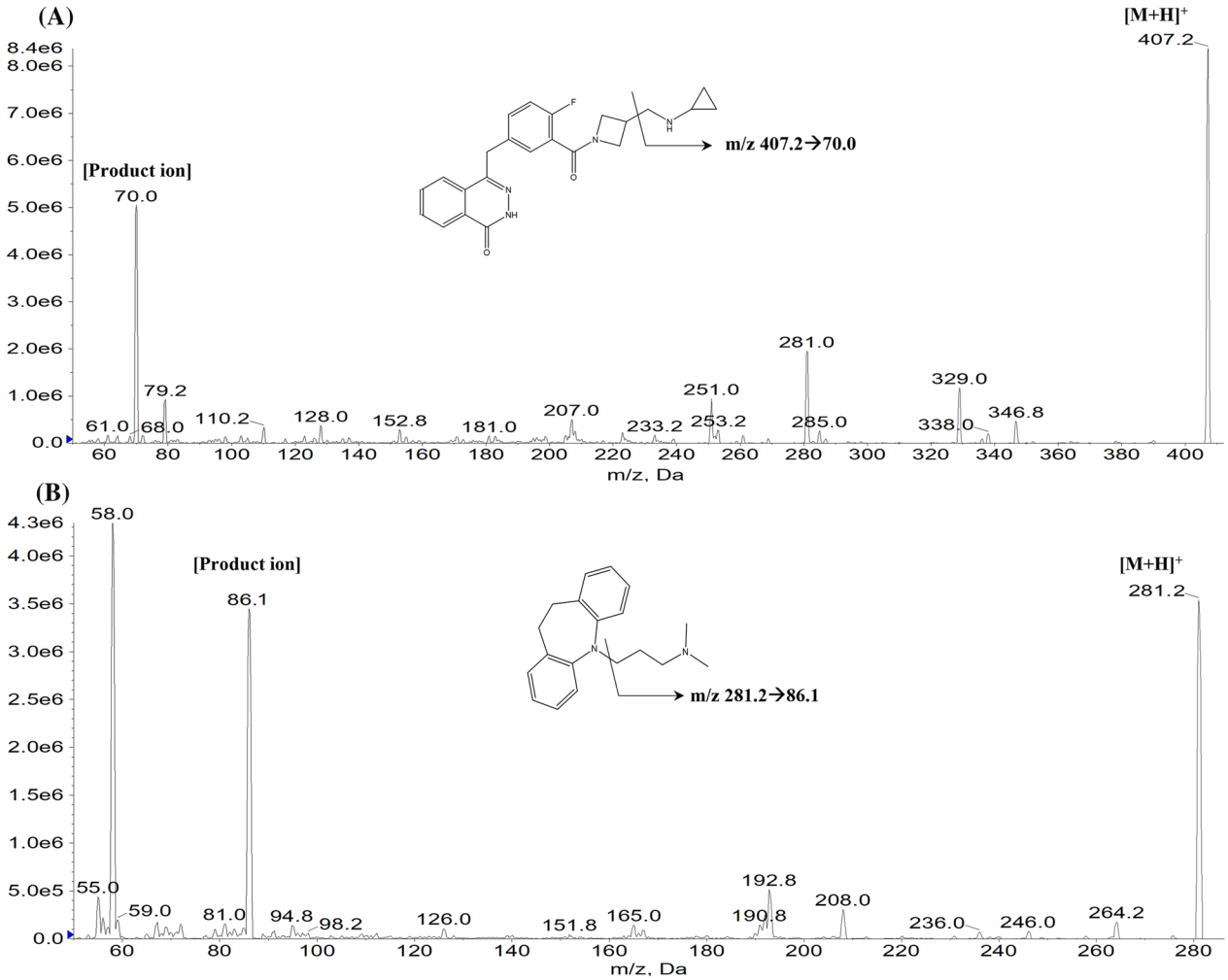

Fig. 1 Structures and product ion scan spectra of **A** VEN and **B** IS (i.e., imipramine)

purposes (e.g., TK, hematology, and clinical chemistry) in repeated dose toxicity studies for VEN. Therefore, it is important to keep the amount of blood sample to the minimum in those studies: If the blood removal is above 7.5% of the total blood volume, more than 1 week recovery period is necessary (Diehl et al. 2001) for the animal. In addition, reducing the blood sampling below 3% of the total blood volume in a week allowed to reduce pain and impact on hematological parameters (Yokoyama et al. 2020; Wang and Li 2021).

Considering these, we were interested in developing a sensitive bioanalytical assay that would involve minimal volumes of blood samples. The objective of this study was to develop and validate a sensitive and robust analytical methodology, consistent with the guidelines for assay validation recommended by the US Food and Drug Administration and the European Medicines Agency (Food and Drug Administration 2018; European Medicines Agency 2011), for quantifying VEN in plasma samples from typical preclinical animal species, namely rats and dogs. Accordingly, the desired sensitivity of the assay was set at a lower limit of quantification (LLOQ) of 1 ng/mL using 20 µL of plasma samples for PK/TK studies. The findings represented herein indicate that the validated analytical method is successfully developed with a limited volume of plasma samples from the animal models and that the assay is applied to PK/TK studies in the animals.

Methods/experimental

Chemicals and reagents

VEN (100.3% purity, hydrochloride salt form) was provided by Ildong Pharmaceutical (Seoul, Korea), and imipramine (IS) was purchased from Cerilliant Corporation [Dorset, UK]. LC–MS grade acetonitrile, methanol, formic acid, and analytical reagent grade dimethyl sulfoxide were obtained from VWR International (Poole, UK) and used without further purification. Deionized water was purified using the Sartorius Arium Comfort system (Sartorius AG, Göttingen, Germany). Blank plasma and blood samples were obtained from Covance Laboratories Ltd. (Huntingdon, UK).

Liquid chromatographic conditions

An Acquity LC system (Waters Corporation, Milford, Massachusetts, USA), consisting of a binary solvent manager with an in-built degasser, a sample manager with a sample organizer, and a column manager, was used in this study. Chromatographic separation was achieved with an InfinityLab Poroshell 120-EC C18 column (3.0 mm × 50 mm, with a particle size of 2.7 µm, Agilent Technologies, Santa Clara, California, USA; temperature maintained at 40 °C). The mobile phase was composed of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol) and was delivered at a flow rate of 0.6 mL/min. Compounds were separated by a gradient elution [i.e., (the percent of solvent composition in v/v%) 0–0.2 min, 25% B; 0.2–2.5 min, 25–40% B; 2.5–3.0 min, 40–80% B; 3.0–4.0 min, 80% B; 4.0–4.1 min, 80–98% B; 4.1–4.5 min, 98% B; 4.5–4.6 min, 98–25% B; 4.6–5.5 min, 25% B]. In addition, the injection volume was set at 3 µL, and the temperature of the sample manager was maintained at 10 °C.

Mass spectrometry conditions

Mass spectrometric detection was performed using an API 5000 triple quadrupole mass spectrometer (Sciex, Framingham, Massachusetts, USA) equipped with an electrospray ionization source operating in the positive ion mode by optimization. The ions were simultaneously monitored in the multiple reaction monitoring (MRM) mode at ion transitions of m/z 407.2 → 70.0 for VEN and m/z 281.2 → 86.1 for the IS. The first and second quadruples were set at unit mass resolution while maintaining the dwell time at 70 ms. The optimized source temperature was 600 °C, and the ion spray voltage was 5,500 V. The conditions for curtain gas, nebulizer gas, heater gas, and collision gas were 30, 50, 50, and 5 psi, respectively. The analytical parameters for mass spectrometry for the VEN and IS are summarized in Table 1. All data collection from the mass spectrometric detector, processing, and storage was performed using Analyst software (Version 1.6; Sciex, Framingham, Massachusetts, USA) running on a computer.

Preparation of standard and quality control solutions

The primary stock solution of VEN was prepared in dimethyl sulfoxide at a concentration of 1 mg/mL. The VEN standard working solutions and quality control (QC) solutions were then prepared by successive dilution of the stock solution with methanol–water–formic acid (25:75:0.1, v/v/v). The primary standard solution of IS was obtained as a certified solution of concentration 1 mg/mL. The IS working solution was obtained by diluting the standard solution with methanol–water (50:50, v/v). An aliquot of the VEN standard solution was added to rats and dogs blank plasma, resulting in eight nonzero

Table 1 Analytical condition of mass spectrometry for VEN and IS

Analyte	SRM transition	Declustering potential (V)	Collision energy (eV)	Cell exit potential (V)
VEN	407.2 → 70.0	90	38	11
IS	281.2 → 86.1	90	23	14

calibration standards, with VEN at concentrations of 1, 2, 10, 50, 100, 500, 900, and 1,000 ng/mL. In our preliminary studies, the addition of a small volume of organic solvent to plasma samples had no appreciable effect on the outcome of the assay. Using a similar method, the LLOQ, low QC (LQC), mid-QC (MQC), and high QC (HQC) samples were prepared to obtain plasma with VEN at a concentration of 1, 3, 300, or 800 ng/mL of plasma, respectively. All calibration and QC samples were prepared immediately and separately before analysis, except for the samples used in stability studies.

Sample preparation

An aliquot (20 µL) of plasma was transferred to a well and mixed with methanol–water (20 µL, 50:50, v/v) containing the IS (15 ng/mL). The resulting mixture was vortexed for 2 min at 1,000 rpm, and then acetonitrile (100 µL) was added. The mixture was vortexed for 5 min at 1,000 rpm and centrifuged at 3,000 × g for 10 min at 4 °C. A 50 µL of the supernatant was gently mixed with 200 µL of solvent A and transferred to an analytical 96-well plate for LC–MS/MS analysis.

Method validation

The current analytical method was validated according to the guidelines for the validation of bioanalytical methods from the US Food and Drug Administration and the European Medicines Agency (Food and Drug Administration 2018; European Medicines Agency 2011).

Selectivity and specificity

The selectivity of the assay was studied by analyzing blank plasma samples from six independent sources, for the presence of interfering peaks for VEN and the IS. The interfering peaks were considered absent when the response at the retention time of VEN in the plasma was less than or equal to 20% of the response from the LLOQ samples, or when the response of the IS in the control was less than or equal to 5% of the response from the LLOQ samples.

Linearity

Calibration curves were constructed based on the results of the analysis of the eight concentrations of VEN. Duplicate calibration samples in the plasma at VEN concentrations of 1, 2, 10, 50, 100, 500, 900, and 1,000 ng/mL were analyzed. One set of standards was analyzed at the start of the batch, and one set of standards was analyzed at the end of the batch. Fresh calibration standards were prepared for each analytical batch during method validation.

Carryover

The degree of carryover, that is, transfer of VEN from the preceding sample in a batch sequence, was assessed by injecting at least two blank matrix extracts sequentially analyzed immediately after the upper limit of quantification (ULOQ) sample. The level of carryover was considered acceptable if the response in the blank matrix extract was less than or equal to 20% of the mean response from the LLOQ sample in the batch.

Accuracy, precision, and dilution integrity

Accuracy (relative error, RE) and within-run precision (coefficient of variation, CV) were assessed using six QC samples consisting of four concentrations of VEN (i.e., 1, 3, 300, and 800 ng/mL) in replicates ($n=6$), together with a set of calibration standards. Between-run precision was also determined by analyzing the four levels of QC samples on three separate occasions. The RE was determined at each concentration for both within and between (overall) batches, and the accuracy was considered acceptable if the value was within ± 15% (± 20% for LLOQ) for both within and between batches. The CV was determined at each concentration and the precision was considered adequate if the value was within ≤ 15% (≤ 20% for LLOQ) for both within and between batches.

The effect of dilution of the test samples was also studied: The samples were prepared at concentrations greater than the ULOQ (i.e., 16,000 ng/mL). A 20 µL of the sample was diluted 20-fold with blank matrix, in replicates ($n=6$). The concentration of VEN was determined in the diluted sample and the original concentration was calculated and compared with the nominal VEN concentration in the original sample.

Recovery and matrix effect

The absolute recovery of samples was determined for analytes, that is, VEN and IS, by comparing the response from the injection of the extract obtained with plasma added with the analytes to that from the injection of a mixture of the analytes added with the extract of blank plasma. Thus, blank plasma was first extracted as described in Sect. "Sample preparation," and VEN was added to obtain LQC, MQC, and HQC concentration in replicates ($n=6$) in the presence of a fixed concentration of the IS. Separately, blank plasma samples were added to VEN to obtain LQC, MQC, and HQC concentrations in replicates ($n=6$) at one fixed IS concentration. The samples were extracted as described in Sect. "Sample preparation." The mean responses of the two sets of samples were then compared.

The matrix factor was assessed using six independent sources of blank plasma from normal blood and two

sources of blank plasma from hemolyzed blood. Thus, the blank matrix sample was first extracted in replicates, as described in Sect. "Sample preparation," and then VEN was added. For this study, VEN was set at LQC and HQC concentrations, with one fixed concentration of the IS (i.e., post-extraction spiked sample). When necessary, the reference solution was prepared at LQC and HQC concentrations of VEN with one fixed concentration of the IS. In this study, the matrix factor was obtained by comparing the response of the post-extraction spiked sample with that of the reference solution at the corresponding concentrations.

Stability

The stability of the analytes was assessed under various storage and handling conditions:

Standard solutions were prepared to obtain VEN at concentrations ranging from 0.2 to 1.0 mg/mL and stored at 4 °C or allowed to stand at room temperature for 24 h. Thereafter, the response of the stored samples was compared with that of the freshly prepared standard solutions.

In whole blood sample stability, the stability of VEN was assessed at LQC and HQC concentrations (i.e., 3 and 800 ng/mL), representing 0 min, and after at least 60 min. The blood samples were prepared by dissolving VEN in pre-warmed (37 °C) whole blood. The 0 min plasma samples were then obtained by the centrifugation of the blood samples at 2,500 × g for 10 min at 4 °C. The remaining blood samples were stored for 60 min on wet ice. The samples were then subjected to the centrifugation at 2,500 × g for 10 min at 4 °C to obtain plasma. The stability of VEN in whole blood was considered adequate if the mean concentration of samples at 60 min remained within ± 15% of that of samples at time 0 min. The adequacy was also determined if CV was less than or equal to 15% for two concentrations of two time points.

In plasma sample stability, the stability of VEN was evaluated at LQC and HQG concentrations (i.e., 3 and 800 ng/mL) under typical sample-handling conditions. For the evaluation of short-term room temperature stability, aliquots of QC samples were stored at room temperature (i.e., 22 °C) over 20 h and a portion of the sample was analyzed in replicates ($n = 6$ for each concentration). When necessary, freeze–thaw stability was studied by repeating the freeze-and-thaw cycle. The QC samples were stored at − 70 °C for at least 12 h and thawed or allowed to stand for at least 2 h at room temperature. The samples were then transferred back to the freezer and maintained for at least 12 h. The cycle of thawing and freezing was repeated, and six replicates at each concentration were analyzed. In addition, long-term stability was evaluated after storing the samples for at least 3 months at − 70 °C. Aliquots of the QC samples after the storage period were analyzed in six replicates. The stability was considered adequate if the concentration of the samples was within ± 15% of the nominal concentration.

When necessary, the stability of the processed sample in the sample manager (10 °C) was studied using samples at LQC and HQC concentrations (3 and 800 ng/mL; $n = 3$ for each concentration). The QC samples were injected twice, immediately after sample preparation and again at least 24 h after the first injection. The storage in the sample manager was considered adequate when CV and RE were ≤ 15% (≤ 20% for the LLOQ) and within ± 15% (± 20% for the LLOQ) for all QC concentrations, respectively.

PK studies with VEN in rats and dogs

To determine the applicability of the current assay in PK studies of VEN, VEN was dissolved in deionized water, and the aqueous solution was administered orally at a dose of 30 mg/kg (as a free base; administration volume of 10 mL/kg) to Wistar Han [Crl:WI(Han)] rats (Charles River UK Ltd., Kent, UK), weighing 183–253 g (i.e., 7–8 weeks of age). In addition, VEN was administered orally in a form of a gelatin capsule at the dose of 0.5 mg/kg (as free base) to Beagle dogs (Marshall BioResources, East Yorkshire, UK), weighing 6.4–9.5 kg (i.e., 5–6 months of age). The study was conducted in accordance with the applicable sections of the UK Animals (Scientific Procedures) Act 1986 and Amendment Regulations 2012 (the Act). Blood samples (approximately 100 µL) were collected in K_2EDTA tubes (Covance, Huntingdon, UK) at pre-dosing, 0.167, 0.33, 0.5, 1, 4, 8, and 12 h post-dose either via the jugular vein in rats or pre-dosing, 0.25, 0.5 1, 4, 8, and 12 h via the jugular vein in dogs. The blood samples were centrifuged at 2,500 × g at 4 °C for 10 min, and plasma samples were collected and stored at − 70 °C prior to analysis. When necessary, systemic PK parameters, e.g., area under the curve from time zero to measurable concentration ($AUC_{0\text{-}t}$), were calculated using the linear trapezoidal method and the standard area extrapolation method (Gibaldi and Perrier 1982). The maximum concentration (C_{max}) and the time point at a maximum concentration (T_{max}) were read directly from the concentration–time profile of VEN in the plasma. In this study, the terminal phase half-life ($T_{1/2}$) was calculated by dividing 0.693 by the slope of the terminal log-linear portion of the concentration–time profile. PK parameters were calculated using Phoenix WinNonlin (version 8.1; Pharsight Corp. Mountain View, California, USA) from either the mean (rats) or individual concentration–time profiles (dogs).

Results

Mass spectrometry and chromatography

The full scan mass spectra of VEN and the internal standard (IS) indicate the presence of a prominent protonated molecule, $[M+H]^+$, at m/z 407.2 and 281.2, respectively. Major fragment ions, formed by the cleavage of amide bond, were readily evident at m/z 70.0 for VEN and 86.1 for the IS. Thus, the ion transitions of m/z $407.2 \rightarrow 70.0$, and $281.2 \rightarrow 86.1$ were used for the subsequent monitoring of VEN and the IS, respectively (Fig. 1A and B). To achieve adequate chromatographic separation of VEN/IS peaks from interfering peaks (1.82, 3.26, and 3.35 min) in the rat plasma, the gradient with 0.1% formic acid in methanol and 0.1% formic acid was used. For the case of the dog plasma, no apparent interfering peaks were evident with the elution condition. Under these chromatographic conditions, the retention time was 1.75 min for VEN and 3.45 min for the IS, and the analytical run was completed within 5.5 min per sample (Fig. 2). These analytical conditions were used in subsequent experiments.

Selectivity and specificity

Representative LC–MS/MS chromatograms of the LLOQ at 1 ng/mL are shown in Fig. 2. The chromatogram of six different lots of blank analyses indicates that the analyte peaks are adequately separated from interfering peaks, suggesting that the selectivity of the LC–MS/MS assay is adequate for the quantification of VEN in rat and dog plasma samples.

Linearity

The calibration curve was linear over the concentration ranges of 1–1,000 ng/mL for VEN in plasma samples from rats and dogs. A $1/x^2$-weighted least squares quadratic regression analysis of the data was used to calculate the slope, intercept, and coefficient of determination (r^2) for samples from the two species. The typical equation of VEN calibration curve was $y = 0.000000521x^2 + 0.00691x - 0.000260$ ($r^2 = 0.998$) and $y = 0.00623x - 0.000106$ ($r^2 = 0.998$) for rats and dogs, respectively. Where y represents the ratio of the peak area of the VEN to that of IS, and x represents the plasma concentration of VEN. The mean coefficient of determination for the calibration curves for rat and dog plasma samples was 0.996 and 0.995, respectively. The LLOQ for VEN was determined to be at 1 ng/mL with acceptable an accuracy and precision (Sect. "Accuracy, precision, and dilution integrity"), and the signal-to-noise ratio was greater than 5 for the two species studied.

Accuracy, precision, and dilution integrity

The accuracy, precision, and dilution integrity of the assays are summarized in Table 2. The QC samples at the LLOQ, LQC, MQC, and HQC were analyzed at three separate occasions, in six replicates for each occasion, together with a set of calibration standards. The within-run RE for rat and dog samples was 0.3–7.0%, and 1.7–6.0%, respectively; the CV for rat and dog samples was 1.4–7.3%, and 1.7–4.2%, respectively. The between-run RE for rat and dog samples was 4.2–9.0%, and − 2.0 to 2.0% with the CV for rat and dog samples of − 1.7 to 5.0%, and 7.4–9.0%, respectively. These results suggest that the assay is accurate and precise for the determination of VEN concentrations ranging from 1 to 1000 ng/mL in rat and dog plasma samples. To determine the adequacy of sample dilution for estimating the concentration in samples exceeding the ULOQ, a set of plasma samples containing VEN at 16,000 ng/mL was prepared and then diluted 20-fold with the corresponding blank plasma. The RE for the analyte in the plasma after the 20-fold dilution with blank rat and dog samples was 0.0% and − 0.6% with at a CV of 3.3% and 2.4% in rat and dog plasma samples, respectively. These observations indicate that samples at concentrations exceeding the ULOQ can be diluted with the corresponding blank plasma to determine accurately and precisely the original concentration using the current assay.

Recovery and matrix effect

The mean recovery of VEN in plasma samples for rats and dogs was within the range of 88.7–98.7% and 88.7–101%, respectively, with six replicates at the LQC, MQC, and HQC levels. In addition, the mean recovery for IS ranged from 88.1 to 96.2% and from 96.1 to 102% for the rat and dog samples, respectively, at a fixed IS concentration of 15 ng/mL in the presence of VEN at LQC, MQC, and HQC concentrations in the plasma. Collectively, these observations indicate that the recovery of both VEN and IS was consistent and reproducible in the concentration range. The matrix effect and IS-normalized matrix effects were also studied with six independent sources of matrix and two sources of matrix obtained from hemolyzed blood. The matrix factor of VEN and the IS was in the range of 0.95–1.05 and 0.91–1.00, respectively. The CV of the matrix factor was less than 15% regardless of the concentration or origin of the matrix. In addition, it was readily evident that the IS-normalized matrix effect was consistent (Table 3): As a result, the matrix effect of VEN and IS appeared negligible in the current assay. Taken together, these findings indicate that the current assay provides virtually complete recovery of the analytes with

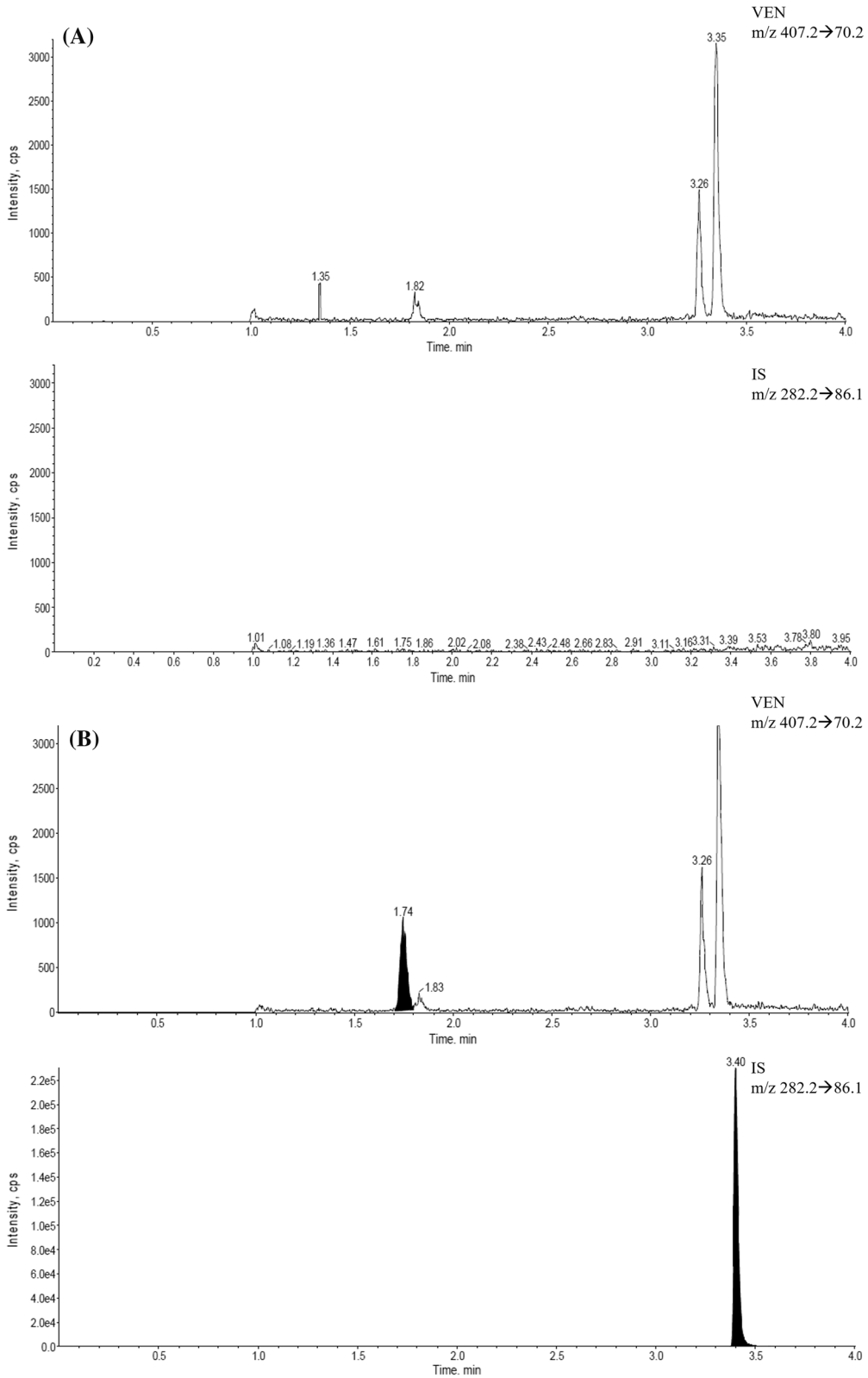

Fig. 2 MRM chromatograms for samples in the rat plasma. **A** Double blank; **B** VEN at LLOQ with IS; **C** VEN at the ULOQ with IS. MRM chromatograms for samples in the dog plasma. **D** Double blank; **E** VEN at LLOQ with IS; **F** VEN at the ULOQ with IS

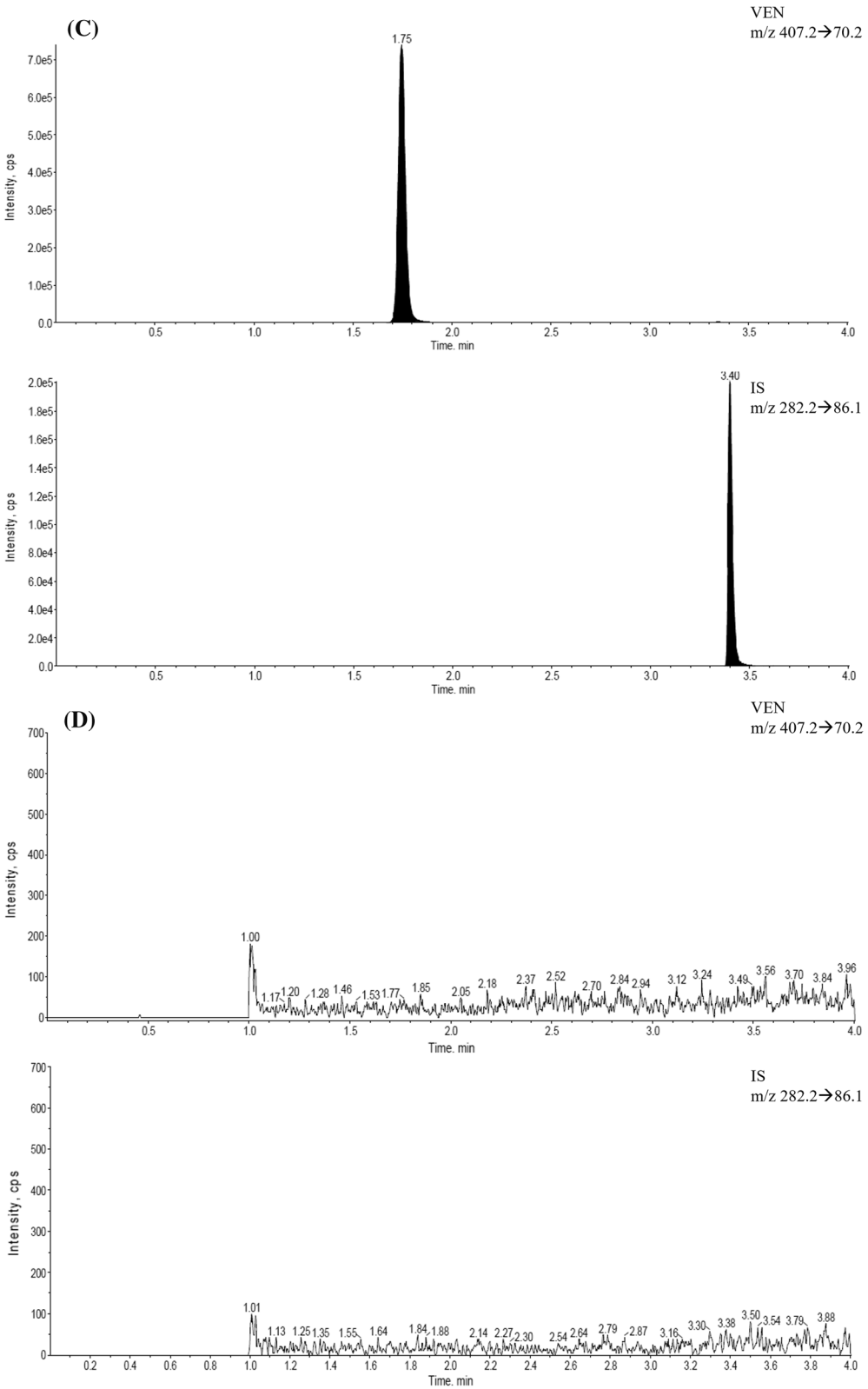

Fig. 2 continued

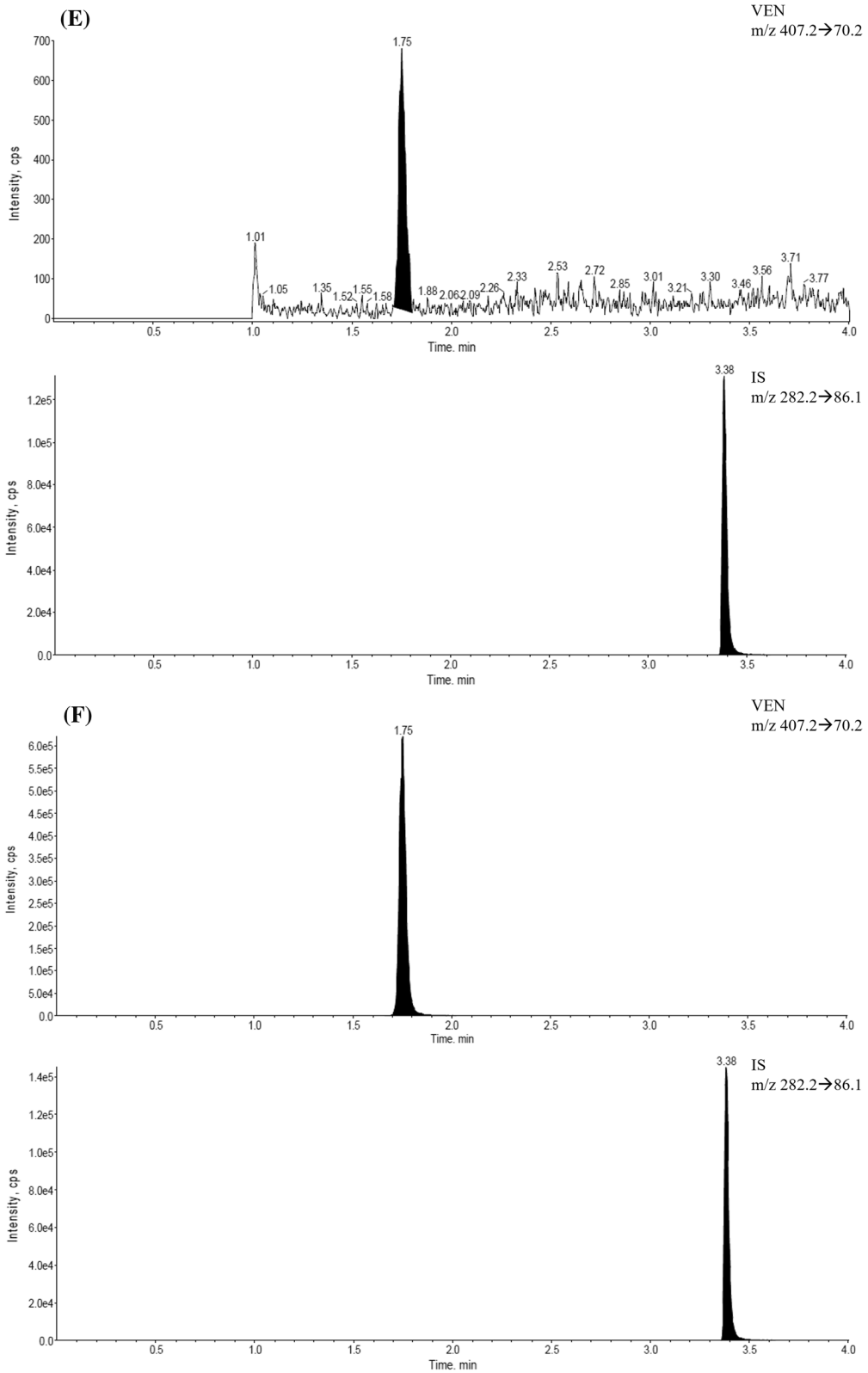

Fig. 2 continued

Table 2 Accuracy and precision of the assay for VEN in plasma samples from the rat and dog

Batch	Theoretical concentration (ng/mL)				
	1	3	300	800	800[3]
Rat plasma					
(A) Within-run accuracy and precision					
Mean estimated concentration	1.07	3.01	301	802	800
RE, %[1]	7.0	0.3	0.3	0.3	0.0
CV, %[2]	7.3	2.4	1.4	2.9	3.3
(B) Between-run accuracy and precision					
Mean estimated concentration	1.05	3.09	295	802	
RE, %	6.9	9.0	4.2	5.9	
CV, %	5.0	3.0	− 1.7	0.3	
Dog plasma					
(A) Within-run accuracy and precision					
Mean estimated concentration	1.06	3.05	308	828	795
RE, %	6.0	1.7	2.7	3.5	− 0.6
CV, %	3.6	3.3	1.7	4.2	2.4
(B) Between-run accuracy and precision					
Mean estimated concentration	1.02	2.98	296	784	
RE, %	2.0	− 0.7	− 1.3	− 2.0	
CV, %	7.6	9.0	7.4	7.8	

[1] Accuracy (RE, %) = (calculated concentration − theoretical concentration)/theoretical concentration × 100

[2] Precision (CV, %) = standard deviation of the concentration/mean concentration × 100

[3] Analyzed after a 20-fold dilution with blank plasma (viz. 16,000 → 800 ng/mL)

Table 3 Matrix effects for VEN and IS in plasma samples of the rat and dog

	Matrix factor[1]		IS-normalized
	VEN	IS	Matrix effect[2]
Rat plasma			
LQC (3 ng/mL)			
A	0.77	0.83	0.92
B	1.06	1.00	1.06
C	0.97	1.03	0.95
D	0.99	1.07	0.92
E	0.99	0.99	1.00
F	0.91	0.98	0.93
A (Hemolyzed)	1.00	1.02	0.97
B (Hemolyzed)	1.05	0.99	1.06
Mean ± SD	0.97 ± 0.09	0.99 ± 0.07	0.98 ± 0.06
CV (%)[3]	9.5	7.0	5.8
HQC (800 ng/mL)			
A	0.96	0.94	1.02
B	1.00	1.00	1.00
C	1.01	1.00	1.01
D	1.00	0.96	1.03
E	0.98	0.99	0.99
F	1.04	1.00	1.04
A (Hemolyzed)	1.01	1.02	0.99
B (Hemolyzed)	1.03	1.01	1.02
Mean ± SD	1.00 ± 0.02	0.99 ± 0.03	1.01 ± 0.02
CV (%)	2.5	2.7	2.0
Dog plasma			
LQC (3 ng/mL)			
A	0.82	0.79	1.05
B	0.96	0.97	0.99
C	0.88	0.93	0.94
D	1.14	0.92	1.24
E	1.09	0.98	1.11
F	0.93	0.90	1.04
A (Hemolyzed)	0.80	0.80	0.99
B (Hemolyzed)	0.97	0.96	1.01
Mean ± SD	0.95 ± 0.12	0.91 ± 0.07	1.05 ± 0.09
CV (%)	12.8	8.3	8.8
HQC (800 ng/mL)			
A	1.03	0.93	1.11
B	1.03	0.97	1.06
C	1.06	1.01	1.05
D	1.11	1.03	1.08
E	1.07	1.02	1.05
F	1.04	1.00	1.04
A (Hemolyzed)	1.03	1.01	1.05
B (Hemolyzed)	1.04	1.01	1.03
Mean ± SD	1.05 ± 0.03	1.00 ± 0.03	1.05 ± 0.03
CV (%)	2.7	3.3	2.6

[1] Matrix factor = [peak area of analyte added post-extraction]/[peak area of analyte standards]

no appreciable matrix effect in plasma samples of the two species.

Stability

The stability of VEN was evaluated under various storage and handling conditions. In general, the compound was relatively stable and the assay satisfied the requirements set by the guidelines for assay validation under the conditions studied, regardless of the concentration and origin of the matrix. For example, the concentration of the compound and IS in a stock solution that had been stored for 24 h at room temperature was 97.3% and 98.6% of the initial concentration, respectively. In addition, the concentration after 1 month of storage under refrigerated conditions was 99.8% and 94.2% of the initial concentration, respectively. Furthermore, the analyte in the rat and dog plasma was found to be stable in various conditions [i.e., in room temperature for 22 and 24 h; after freeze–thaw cycles, and at − 70 °C over 3 months (Table 4)]. In this study, degradation was not apparent in the sample manager of the LC–MS/MS system (i.e., operating at 10 °C) for 8 days (Table 4). VEN was stable in whole blood on ice for up to 1 h. Collectively, these observations

Table 3 (continued)

[2] Normalized Matrix Factor = Matrix Factor for Analyte/Matrix Factor for IS

[3] CV (%) = standard deviation/mean × 100

Table 4 Summary of stability studies for VEN in QC samples

Batch	Theoretical concentration (ng/mL)			
	Rat		Dog	
	3	800	3	800
(A) Short-term stability at room temperature (22 °C, 22 h for rats, 24 h for dogs, n = 6)				
Mean estimated concentration	2.62	725	3.15	688
RE (%)[1]	−12.7	−9.4	5.0	−14.0
CV (%)[2]	5.7	5.6	7.5	3.3
(B) Freeze–thaw stability (3 cycles for rats, 4 cycles for dogs, n = 6)				
Mean estimated concentration	2.59	710	2.90	735
RE (%)	−13.7	−11.3	−3.5	−8.1
CV (%)	5.9	7.6	8.1	2.9
(C) Long-term stability (107 days for rats, 87 days for dogs, n = 6)				
Mean estimated concentration	3.42	907	29.7	693
RE (%)	14.0	13.4	−1.0	−13.4
CV (%)	12.1	5.0	4.4	4.0
(D) Processed sample stability (at 10 °C for 8 days, n = 3)				
Mean estimated concentration	3.00	773	3.08	785
RE (%)	0.0	−3.4	2.7	−1.9
CV (%)	2.4	4.1	0.7	1.4

[1] RE (%) = (calculated concentration − theoretical concentration)/theoretical concentration × 100

[2] CV (%) = standard deviation of the concentration/mean concentration × 100

suggest that VEN is stable under typical storage and handling conditions.

Applicability of the assay to pharmacokinetic studies

One of the objectives of this study was to determine whether the current assay would be applicable to PK studies of VEN in rats and dogs. The temporal profiles of the plasma concentration of the PARP inhibitor after oral administration to the rat and dog at a dose of 30 mg/kg and 0.5 mg/kg, respectively, are depicted in Fig. 3. In general, the concentration was readily detected up to 12 h after administration, suggesting that the sensitivity was adequate for the quantification of plasma concentrations of the drug in PK studies with rats and dogs at the doses tested. PK parameters of the drug in rats and dogs, calculated using standard moment analysis, are listed in Table 5. Interestingly, a gender difference in VEN PK was noted in rats (i.e., compared to male rats, the C_{max} and $AUC_{0\text{-}t}$ in female rats were 3.68- and 2.43-fold higher, respectively). However, in dogs, the gender difference was reduced for the key PK parameters (i.e., compared to male dogs, female dogs showed 1.30- and 1.49-fold higher values for C_{max} and $AUC_{0\text{-}t}$, respectively.).

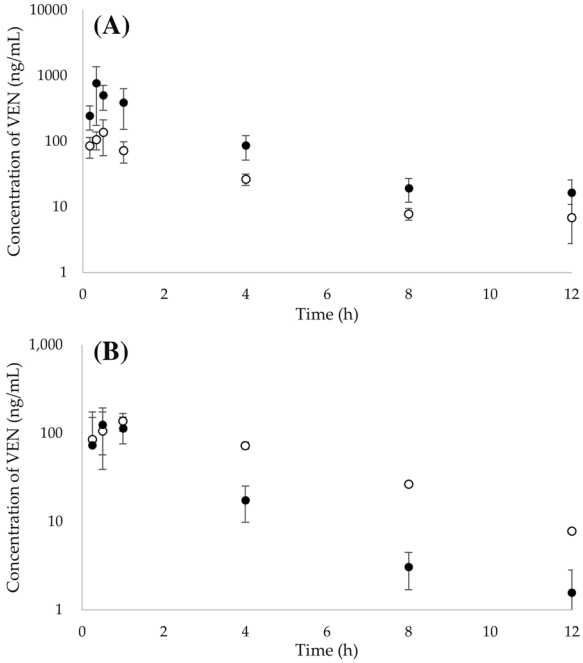

Fig. 3 Mean plasma concentration–time curves of VEN in **A** rats and **B** dogs that had received an oral administration of 30 mg/kg and 0.5 mg/kg of VEN, respectively (key: opened circles, male; closed circles, female). Data were represented as mean ± standard deviation of *n* = 3 (for rats) or *n* = 6 (for dogs)

Table 5 Summary of systemic PK parameters following oral administration of VEN to rats and dogs

Pharmacokinetic parameters	Rat[1]		Dog[2]	
	Male	Female	Male	Female
	Mean		Mean ± SD	
Dose (mg/kg)	30.0		0.50	
T_{max} (h)	0.333	1.00	0.583 ± 0.342	0.583 ± 0.342
C_{max} (ng/mL)	1,130	4160	120 ± 61	156 ± 54
$T_{1/2}$ (h)	1.92	1.88	1.43 ± 0.64	1.72 ± 0.29
$AUC_{0\rightarrow t}$ (ng·h/mL)	5,310	12,900	243 ± 111	361 ± 88
$AUC_{0\rightarrow\infty}$ (ng·h/mL)[3]	5,430	13,100	267 ± 169	369 ± 98

[1] Three replicates: Each rat covered different regions in the time profile and, as results, the calculation of the standard deviation (SD) was not possible

[2] Six replicates

[3] AUC from time of dosing extrapolated to infinity

Discussion

Inhibition of PARP results in severe hematologic toxicities that form neutropenia in laboratory animals at low dose levels in chronic studies. The fact that toxic doses could be as low as one-tenth of the effective dose for

PARP inhibitors is now well established (AstraZeneca 2022; Clovis Oncology 2021; GlaxoSmithKline 2021), suggesting that an adequate dose of VEN may be lower than the efficacy or PK study in certain toxicity studies. In addition, exploratory repeated dose PK studies may be conducted in animal models (e.g., the identification of drug–drug interaction mechanisms). Frequent blood sampling could exacerbate hematologic toxicity of PARP inhibitors, a small sampling volume would be ideal for PARP inhibitors. Thus, a bioanalytical method which enables the quantification of analyte using a small volume of plasma samples could be helpful in PK/TK studies of VEN. In this study, we attempted to develop and validate a sensitive and accurate LC–MS/MS method for the quantification of VEN in the rat and dog plasma samples, and quantified VEN at concentrations as low as 1 ng/mL using 20 µL plasma samples. Assuming linear PK for VEN in the animal models, the LLOQ, 1 ng/mL, represented less than 5% of the expected C_{max} after oral administration of VEN at 1 and 0.1 mg/kg in rats and dogs, respectively. Taken together with the possibility of low-dose studies of VEN in animals, this observation suggests that the current assay can be readily applied to typical TK/PK studies of the inhibitor in two animal models.

Using gradient elution, interfering peaks originating from the plasma were reasonably separated from the analytes, while limiting a run-time of approximately 5.5 min. The deuterated form of VEN is not commercially available; hence, a series of compounds had to be screened for an IS. In the preliminary screening study, imipramine was selected as the IS, considering its recovery and matrix effects with VEN and IS (Table 3). We found that the analytes were almost completely extracted in the current assay as the recoveries for the rats and dogs, 88.7–98.7% and 88.7–101%, respectively, for VEN; 88.1–96.2% and 96.1–102%, respectively, for IS. In addition, the recovery values were consistent over the concentration range used in this study. The experimental data for selectivity, linearity, precision, accuracy, and stability were found acceptable according to the guidelines for bioanalytical method validation from the US Food and Drug Administration and the European Medicines Agency.

In this study, we found that the assay was applicable to PK studies for VEN in rats and dogs at oral doses of 30 mg/kg and 0.5 mg/kg, respectively. Based on the LLOQ of the current assay, we were able to monitor the plasma concentration–time profile up to 12 h after oral administration, which accounted for over six times the $T_{1/2}$ for VEN ($T_{1/2} \leq 2$ h for VEN, Table 5). It was noted that gender differences in PK were found for VEN in the animal models: C_{max} at 30 mg/kg was 1130 and 4160 ng/mL for male and female rats, respectively; C_{max} at 0.5 mg/kg was 120 and 156 ng/mL for male and female dogs, respectively. In this study, we did not directly study the mechanisms for the kinetic difference of VEN by gender. However, olaparib, a structural analog of VEN, was reported to have up to 14-fold higher exposure in female rats than in male rats, while the difference was markedly reduced (approximately twofold) in dogs (Application Number:206162Orig1s000 2014), suggesting a common mechanism for the gender difference in the PK for the PARP inhibitors. This aspect of PARP inhibitors warrants additional studies.

Conclusions

A sensitive and robust LC–MS/MS assay was developed and validated for the quantification of VEN plasma samples from rats and dogs in terms of accuracy, precision, dilution, recovery, matrix effect, and stability, which were within acceptable ranges. The blood sampling can be reduced below 3% of the total blood volume in PK/TK studies in rats using this method. This method can be applied to the chronic study of VEN to minimize the hematological effect of blood sampling. Collectively, these observations indicate that the current assay can be reliably applied to PK and TK studies of VEN in rats and dogs using the limited sample volume.

Abbreviations

$AUC_{0\rightarrow t}$	Area under the curve from time zero to measurable concentration
$AUC_{0\rightarrow \infty}$	Area under the curve from time zero to infinity
$AUMC_{0\rightarrow \infty}$	Area under the first moment curve from time zero to infinity
C_{max}	Maximum concentration
CV	Coefficient of variation
HQC	High quality control
IS	Internal standard
LC–MS/MS	Liquid chromatography–tandem mass spectrometry
LLOQ	Lower limit of quantification
LQC	Low quality control
MQC	Mid-quality control
MRM	Multiple reaction monitoring
MRT	Mean residence time
PARP	Poly (ADP-ribose) polymerase)
PK	Pharmacokinetic
QC	Quality control
RE	Relative error
SD	Standard deviation
$T_{1/2}$	Half-life
T_{max}	Time point at maximum concentration
TK	Toxicokinetic
ULOQ	Upper limit of quantification
UK	United Kingdom
US	United States
VEN	Venadaparib

Acknowledgements

Not applicable.

Author contributions

ML, EJ, and JL contributed to conceptualization; ML and EJ contributed to methodology; ML performed formal analysis, data curation, writing—original draft preparation, and visualization; ML, J-HL, and S-JC contributed to writing—review and editing; SC, WSL, and S-JC supervised the study; NSB and SL

administered the project; Y-WP performed funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding
This study was conducted with the National-OncoVenture supported by the National Cancer Center, designated by the Ministry of Health and Welfare Korea (HI17C2196).

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

Received: 16 November 2022 Accepted: 18 January 2023
Published online: 01 February 2023

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