Journal of Chromatography B, 879 (2011) 285–290

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short Communication

Quantification of 3-deazaneplanocin A, a novel epigenetic anticancer agent, in rat biosamples by hydrophilic interaction liquid chromatography–tandem mass spectrometric detection

Feng Sun [a], Jasmine Chiat-Ling Ong [a], Qiang Yu [b], Eli Chan [a,*]

[a] Department of Pharmacy, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore
[b] Cancer Biology and Pharmacology, Genome Institute of Singapore, A*STAR (Agency for Science, Technology and Research), Biopolis, Singapore

ARTICLE INFO

Article history:
Received 15 July 2010
Accepted 11 December 2010
Available online 21 December 2010

Keywords:
3-Deazaneplanocin A
LC–MS/MS
Rat biosamples
Pharmacokinetics
Tissue distribution
Excretion studies

ABSTRACT

A sensitive and selective LC–MS/MS based bioanalytical method was developed and validated for the quantification of 3-deazaneplanocin A (DZNep), a novel epigenetic anti-tumor drug candidate, in Sprague–Dawley (SD) rat biosamples (plasma, urine, feces and tissue samples). The method comprises a phenylboronic acid (PBA)-containing solid phase extraction procedure, serving for binding and clean-up of DZNep in rat biosamples spiked with tubercidin (as internal standard). The analytes were separated on an Agilent hydrophilic interaction chromatography (HILIC) column. LC–MS/MS in positive ion mode was used to perform multiple reaction monitoring at *m/z* of 263/135 and 267/135 for DZNep and tubercidin, respectively. The limit of quantification (LOQ) of DZNep in rat biosamples was 20 ng/mL. The data of intra-day and inter-day accuracy were within 15% of nominal concentration while the precision (relative standard deviation) less than 10% for all biosamples. The extraction recoveries for DZNep and tubercidin were consistent and reproducible (around 80%) and the matrix effects were negligible (around 10% suppression) in all biosamples. This method was demonstrated to be applicable for pharmacokinetic studies of DZNep in SD rats.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

3-Deazaneplanocin A (DZNep), a cyclopentanyl analogue of 3-deazaadenosine that was first synthesized by Glazer et al. [1], has broad and potent antiviral activity [2,3]. Recently, DZNep appears to be a unique chromatin remodelling (epigenetic) compound that can deplete the cellular EZH2 proteins, which are abnormally overexpressed in varied metastatic cancer [4,5] and inhibit the associated histone methylation [6–8]. In turn, DZNep can effectively reverse EZH2 and histone methylation-mediated gene silencing and induce cancer cell death but not in corresponding normal cells with normal EZH2 expression. Hence, DZNep may hopefully open the therapeutic potential via inhibiting this epigenetic regulator. By far, DZNep has not been extensively investigated in a pre-clinical study using animal models. Thus, a sensitive and reliable assay is needed for quantifying DZNep in animal biosamples.

In previous study, the limit of quantification (LOQ) of the radiolabeling DZNep in mice was in the picomolar range using in-line radiochemical flow detection [9]. However, the inherent costs and safety issues of radiolabeling technique make this assay impractical and unfeasible during the early drug discovery and optimization processes. Additionally, the pharmacokinetic and disposition properties of intact DZNep could not be distinguished only by a radioassay method. To specifically quantify non-radioactive DZNep in rat biological fluids, a sensitive liquid chromatography coupled with electrospray ionization/tandem mass spectrometry (LC-ESI–MS/MS) method was thus employed in this study. In addition, the protein precipitation (PPT) for the extraction of DZNep from plasma and tissue samples was reported [9]. Although PPT is a quick and simple technique, it fails to sufficiently remove endogenous interference in the biosamples, which could cause variability in analyte signal intensity in a mass spectrometer [10]. Here, a solid phase extraction (SPE) technique using bond elute phenylboronic acid (PBA)-SPE cartridges (Varian, Palo Alto, CA) specifically for the purification of compounds containing vicinal hydroxyl groups, was evaluated to reduce endogenous interference and to minimize the matrix effect of DZNep in rat biosamples. To achieve good retention and minimize peak tailing of DZNep, a hydrophilic interaction

Abbreviations: DZNep, 3-deazaneplanocin A; ESI, electrospray ionization; LC–MS/MS, Liquid chromatography coupled with tandem mass spectrometry; SPE, solid phase extraction; PBA, phenylboronic acid; IS, internal standard; HILIC, hydrophilic interaction chromatography; RP, reversed-phase; SD, Sprague Dawley; QC, quality control; LOQ, limit of quantification; ULOQ, upper limit of quantification; RSD, relative standard deviation; LogK_{ow}, the logarithm of the water/octanol partition coefficient; MRM, multiple reaction monitoring mode.

* Corresponding author. Tel.: +65 65162932; fax: +65 67791554.
E-mail address: phaelic@nus.edu.sg (E. Chan).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.12.009

chromatography (HILIC) column, a powerful tool in bioanalysis of numerous polar compounds coupled with MS/MS detection [11], was employed. The purpose of the present work was to develop and validate a sensitive, selective and reliable LC–MS/MS method for the quantification of DZNep in rat biosamples.

2. Experimental

2.1. Chemical reagents and animals

3-Deazaneplanocin A (DZNep, purity >99%) was purchased from OKeanos Tech. Co. (Beijing, China). Tubercidin (purity >99%) was from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile was obtained from TEDIA (Fairfield, USA). Deionized water was purified using a Milli-Q system (Millipore, Milford, MA, USA). All other chemicals and solvents used were obtained from standard vendors, and were of the highest quality available. The Sprague Dawley (SD) rats were supplied by Laboratory Animal Center (National University of Singapore, Singapore), and housed in temperature controlled room (25 °C) with a 12-h light-dark cycle. The study was approved by the Animal Ethics Committee of National University of Singapore.

2.2. Instrument

A LC–MS/MS system used was composed of a series 1200 HPLC instrument (Agilent Technologies, Palo Alto, CA, USA) with a Q TrapTM 3200 hybrid triple quadrupole linear ion trap mass spectrometer (Applied Biosystems/MDS Sciex, Concord, Ontario, Canada). Data processing was performed with AnalystTM 1.4.2 software package (Applied Biosystems).

2.3. Chromatographic conditions

Chromatographic separations were performed on a Hydrophilic interaction chromatography column (2.1 mm × 50 mm, Zorbax HILIC plus, Agilent) with a Silica Security Guard Cartridge (3.0 mm × 4 mm, Phenomenex). The mobile phase was made up of solvent A (H_2O, 0.1% formic acid) and B (acetonitrile, 0.1% formic acid). For method validation and sample analysis, the chromatographic analysis was conducted under gradient elution as follows: solvent A: 9% (0–0.10 min), from 9% to 20% (0.10–0.11 min), 20% (0.11–5.00 min), from 20% to 9% (5.00–5.01 min), and 9% (5.01–12.00 min). The separation was performed at a flow rate of 0.3 mL/min. The chromatographic run time of each sample was 12 min. The temperatures of column and autosampler were both maintained at ambient temperature (25 °C). A 10 μL full loop sample injection was used.

2.4. Mass spectrometric conditions

The mass spectrometer was operated using ESI source in the positive ion detection. The optimized instrument parameters for monitoring DZNep and tubercidin were as follows: source temperature (TEM), 400 °C; turbo spray voltage (IS), 4500 V; curtain gas (CUR), 10; Nebulising gas (GS1), 30; turboionspray gas (GS3), 30; collision gas (CAD), medium; declustering potential (DP), 50 V (DZNep) and 41 V (tubercidin); entrance potential (EP), 7 V (DZNep) and 8.5 V (tubercidin); collision energy (CE): 29 eV (DZNep) and 27 eV (tubercidin); collision cell exit potential (CXP), 4 V (both). Quantification was performed using the multiple reaction monitoring (MRM) mode with the following transitions: m/z 263/135 for DZNep, and m/z 267/135 for tubercidin (IS), respectively, with a dwell time of 200 ms. Representative precursor/product ion mass spectra of these compounds are shown in Fig. 1.

2.5. Preparation of calibration standards and quality control (QC) samples

Calibration standards at final concentrations of 20, 50, 200, 800, 2000, 8000, 10,000 ng/mL of DZNep were prepared by spiking an appropriate quantity of the intermediate DZNep solution in blank plasma, urine and feces samples, while 20, 50, 100, 500, 1000, 5000 ng/mL of DZNep in blank tissue samples. Samples of limit of quantification QC, low QC, medium QC, and high QC were prepared at 20, 30, 500, 7500 ng/mL of DZNep in rat plasma, urine and feces samples, while at 20, 30, 300, 3000 ng/mL in tissue samples. In addition, dilution QC samples were prepared at 40,000 ng/mL of DZNep with fivefold dilution for plasma and tenfold dilution for urine. All standards and QC samples were spiked with 100 ng/mL of IS and then stored at 4 °C before analysis.

2.6. Sample preparation

200 μL of rat blood samples in heparinized 1.5 mL microtubes were centrifuged for 10 min at 2000 g at 10 °C to separate plasma samples. Tissue samples (heart, liver, spleen, lung, kidney, stomach, adipose, muscle, brain) harvested from sacrificed rats were rinsed with ice-cold 0.9% NaCl (saline) and then gently blotted with absorbent paper. The tested tissues and feces were firstly minced or crushed to pieces in ice bath. Then 0.2 g tissues and feces were added with 1 mL deionized water and crushed by a Diax 900 homogenizer (Heidolph, Germany). IS working solution was added to aliquots of 100 μL of biosamples (plasma, urine, and homogenized feces and tissues) to obtain the final concentration of 100 ng/mL. Then, the biosamples were mixed with 1 mL of 0.2 M ammonium acetate buffer (pH 9.0). The Bond Elute PBA-SPE cartridges (Varian, Palo Alto, CA) were preconditioned by consecutive washing with 1 mL methanol, 1 mL 0.1 M formic acid, and 1 mL 0.2 M ammonium acetate buffer (pH 9.0). The biosample mixtures were loaded into the column. Then, the columns were washed thrice with 1 mL 0.2 M ammonium acetate buffer (pH 9.0). Samples were eluted with 1.5 mL 0.1 M formic acid and freeze-dried overnight using a lyophilizer (Labconco, Kansas City, MO). Following this, the freeze-dried residues were reconstituted in 100 μL reconstitution solution (80% solvent B plus 20% solvent A), and 10 μL was injected onto column for analysis.

2.7. Method validation

The method validations for rat biological samples (plasma, urine, feces and tissue) were similarly carried out as follows.

The selectivity of the method was evaluated by comparing the chromatograms of blank samples with the corresponding spiked samples at the LOQ level. The peak areas of endogenous compounds co-eluting with DZNep should be less than 20% of the peak area of the LOQ standard.

The calibration curves were fitted by a weighted ($1/y^2$) least squares linear regression method through the measurement of the peak-area ratio of the DZNep to IS. The r^2 value (i.e., coefficient of determination) of greater than 0.99 was set for the acceptable linearity of a calibration curve. The accuracy and precision at the LOQ for each biosample should be within 20% of the nominal concentration and less than 20% RSD ($n = 5$), respectively.

Matrix effects and extraction recoveries of DZNep were evaluated at QC samples under low, medium and high concentrations. Three groups of samples ($n = 5$) were prepared: (A) DZNep spiked in the reconstitution solution; (B) DZNep spiked in extract of blank biosamples (post-extraction); (C) DZNep spiked in blank biosamples and then extracted (pre-extraction). Matrix effect was calculated as the percentage ratio of the response of B to A. Extraction recovery was calculated as the percentage ratio of the response

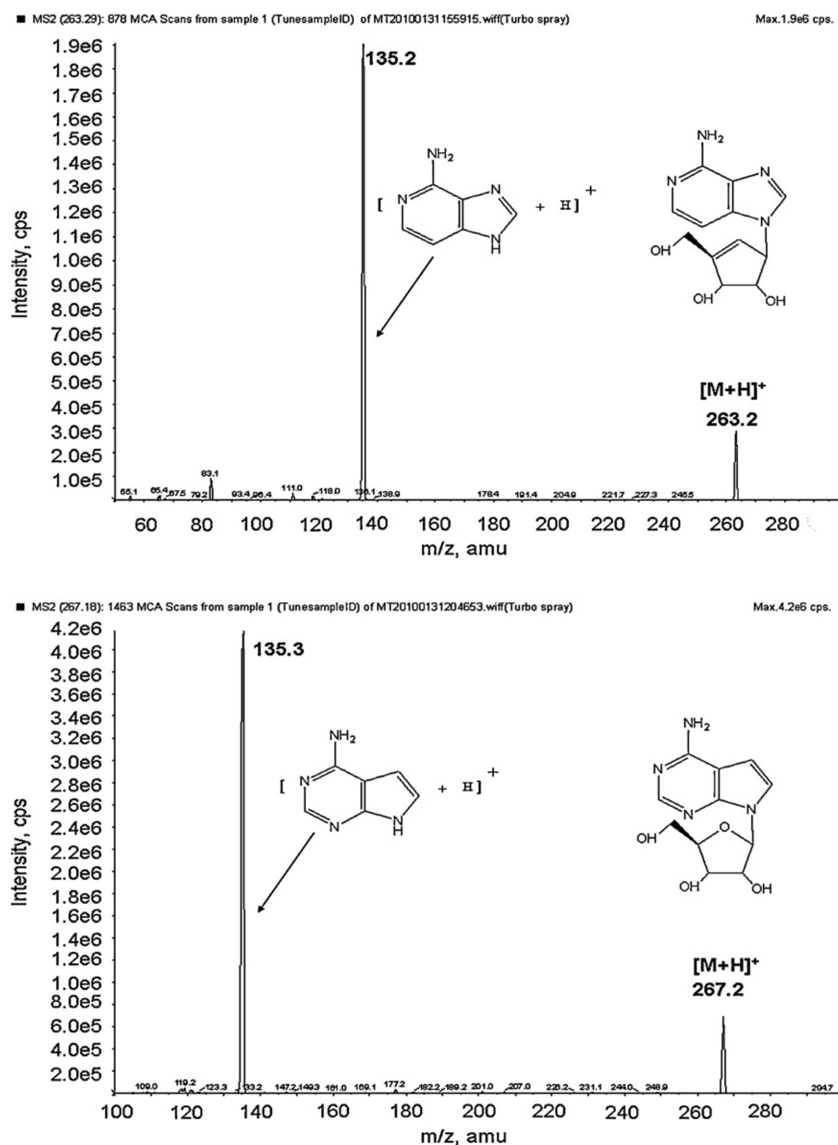

Fig. 1. Representative precursor/product ion mass spectra and chemical structure of DZNep (A) and tubercidin (B), respectively. For mass spectrometer conditions, see Section 2.4.

of C to B. Also, the evaluation of matrix effect and extraction recovery was performed for 100 ng/mL of IS in each biosample.

Intra-day accuracy and precision of five replicates of QC samples were analyzed at each concentration level within the same day. Inter-day accuracy and precision were evaluated at the same QC samples on three consecutive days. Accuracy was calculated as the percentage ratio of the mean of the measured concentration to the spiked concentration. Precision was expressed by the relative standard deviation (RSD) of the measured concentration. The accuracy and precision should be within 15% of the nominal concentration and less than 15% RSD, respectively. Five replicates of plasma and urine dilution QC samples were measured after a fivefold dilution with blank plasma and tenfold dilution with blank urine, respectively, to assess the dilution integrity. An extracted blank sample was placed after the upper limit of quantification (ULOQ) standard to determine the carry-over of the LC–MS/MS system.

The stability of DZNep in rat biosample ($n = 5$) was evaluated at three concentration levels (low, medium and high). The analytes were considered stable when the accuracy was within 15% of the spiked concentration and the precision was below 15% RSD. For freeze–thaw stability, QC samples were stored at −20 °C for 24 h and thawed unassisted at room temperature. When completely thawed, the samples were refrozen at −20 °C for over 24 h. The freeze–thaw cycles were repeated two more times in 4 weeks, and then QC samples were extracted and analyzed on the third cycle. Short-term temperature stability was assessed by analyzing QC samples, which were kept at ambient temperature (25 °C) for 12 h before sample preparation. The post-preparative stability of QC samples was analyzed by re-injecting QC sample prepared under autosampler condition (25 °C) 5 times in 24 h.

3. Results and discussion

3.1. Optimization of the mass spectrometric conditions

Mass spectrometric analysis was set up in the multiple reaction monitoring (MRM) mode in positive polarity. The MRM transition of *m/z* 263/135 and *m/z* 267/135 were selected for DZNep and IS, respectively. Fig. 1 shows the MS/MS spectra of both DZNep and IS. The other MS parameters optimized to enhance the sensitivity of two compounds are summarised in Section 2.4.

Table 1
The linearity of calibration curves for DZNep in rat biosamples and the precision and accuracy of LOQ samples ($n = 5$).

Biosamples	r^2	LOQ (20 ng/mL)	
		Accuracy (%)	Precision (%)
Plasma	0.9996	97.7–118.2	7.4
Urine	0.9981	82.1–105.7	9.8
Feces	0.9982	81.7–116.3	11.8
Heart	0.9991	91.5–114.7	13.4
Liver	0.9999	89.7–114.5	16.1
Spleen	0.9986	89.2–114.9	11.8
Lung	0.9986	81.3–111.1	14.2
Kidney	0.9997	81.1–113.0	19.4
Stomach	0.9992	94.8–111.5	13.8
Adipose	0.9994	80.3–113.8	15.1
Muscle	0.9996	86.9–115.4	15.2
Brain	0.9983	83.4–106.2	10.6

3.2. Optimization of the chromatographic conditions

Reversed-phase (RP) columns were preliminarily screened and found improper separation tools for a highly hydrophilic compound like DZNep with a low partition constant ($\text{Log}K_{\text{ow}} = -0.71$, pH 7.4). Thus, a Zorbax HILIC column, which is typically used for the retention and separation of small, polar analytes, was tested. Tubercidin, a nucleoside analogue structurally similar to DZNep, was chosen as the internal standard (IS) because it not only works well in tracking DZNep in the solid phase extraction but also effectively diminishes the intensity variation caused by the gradient elution. Using a gradient elution as specified in Section 2.3 after optimizing the mobile phase compositions, appropriate peak shape and minimal matrix effects for DZNep and IS were achieved on the HILIC column.

3.3. Optimization of sample preparation conditions

In the case of DZNep and tubercidin, their highly hydrophilic nature renders liquid–liquid extraction not a viable option. Additionally, the insufficient cleanliness of the biosamples using PPT resulted in significant matrix suppression and unsymmetrical peaks of DZNep in the chromatograms. The purification, using Bond Elut PBA SPE columns, was based on the principle that the molecules with vicinal hydroxyl (diol) groups, such as DZNep and tubercidin, specifically bind to PBA in a neutral or alkaline medium [12–14]. Once these compounds were retained, other components could be washed off. Then, the covalent bond was broken when the medium pH was lowered followed by the elution of these compounds. In fact, the extraction and purification of DZNep from rat biosamples, using this SPE column, were significantly efficient because the extraction recoveries were around 80% and the matrix effects were around 90%.

3.4. Method evaluation

3.4.1. Selectivity and calibration model

Injection of blank rat biosamples from 6 SD rats to evaluate the selectivity, showed no interference signals at the location of DZNep and IS peaks (Fig. 2A and B). Furthermore, as shown in Fig. 2C, the elution time for DZNep and IS in rat biosamples was found 6.1 ± 0.3 min and 4.5 ± 0.3 min, respectively. The analytical run time of 12 min allowed for mobile phase gradient re-equilibration between sample injections. Five replicates of a series of 6 or 7 DZNep concentrations in the biosamples were analyzed to create calibration curves (concentration range seen in Section 2.5). As shown in Table 1, the acceptable linearity was achieved as the r^2 values were found greater than 0.99 in all biological samples. The back-calculation results for all calibration standards except for LOQ sample showed less than 10% RSD as well as within 15% of the nominal concentration (data not shown). As for 20 ng/mL DZNep samples, the RSD was lower than 20% (7.4–19.4%) and the accuracy was within 20% of the nominal value (Table 1), which were within the acceptance criteria. Measurements of the lower concentration at 15 ng/mL resulted more often in outliers. Therefore, the LOQ of DZNep in this method was set at 20 ng/mL.

3.4.2. Matrix effect and recovery

The matrix effect is caused by ionization competition between the analytes and existing co-eluents when using LC–MS/MS for analysis [15]. Evaluation of the mean peak areas of DZNep in the reconstitution solution compared to those in the biosample extracts showed matrix effects in all the biosamples ranging within 84.8–94.7% at various QC biosamples (Table 2). For the 100 ng/mL of IS, similar matrix effects were found ranging from 85.4 to 93.0% (Table 2). These results indicate that minimal matrix effects (around 10% suppression) across different biosamples and spiked concentrations for both DZNep and IS, did not compromise the performance of the assay.

The extraction recovery of DZNep ranged from 71.6 to 85.8%, while that of IS ranged from 76.2 to 84.8% at 100 ng/mL, at various QC samples of tested biofluids (Table 2). In addition, these recoveries were consistent, precise and reproducible in the same biosamples under different concentrations. Collectively, the pre-column SPE-solid phase extraction procedure used is effectively minimizing the matrix effects by sufficiently cleaning up all the rat biosamples and achieving the acceptable extraction recovery.

3.4.3. Precision and accuracy of the method

The intra-day and inter-day precision values, expressed as RSD, were less than 10% at various concentrations of QC biosamples. In addition, the data of the intra-day accuracy were all within 96.8–108.7%, while the inter-day within 93.9–103.2%. These results demonstrate that the precision and accuracy of this assay are within the acceptable range in rat biosamples. The dilution integrity was determined by measuring dilution QC samples (only plasma and urine) after a 5-fold dilution with blank plasma and 10-fold dilution with blank urine. Both intra-day and inter-day accuracy values in rat plasma and urine dilution QC samples ranged from 94.9 to 104.4%, while the precision from 5.4 to 8.5%. These results demonstrate that the concentrations in plasma and urine samples, which are higher than ULOQ, can be determined by dilution with blank plasma and blank urine. No carry-over of the LC–MS/MS system was observed in the chromatograph of the blank biosamples after running ULOQ standards.

3.4.4. Stability of DZNep in rat biosamples

The stability tests were designed to cover the anticipated conditions that preclinical samples may experience. Three freeze–thaw cycles and ambient temperature storage of the QC samples, appeared to have little effect on the stability, with accuracy ranging from 87.9 to 95.3%. Of the spiked concentrations, the accuracy ranged from 95.5 to 105.8% for short-term stability, while that ranged from 96.1 to 102.9% for post-preparative stability. Collectively, no apparent stability issues were encountered in all these experiments.

3.5. Applications

This method was successfully applied to the determination of DZNep in rat biosamples. The concentrations of DZNep were measured in plasma and kidney samples obtained at 1 h after an intravenous injection of 5 mg/kg DZNep to a SD rat. Two chromatograms obtained from the LC–MS/MS analysis of the plasma and kidney samples are demonstrated in Fig. 2D.

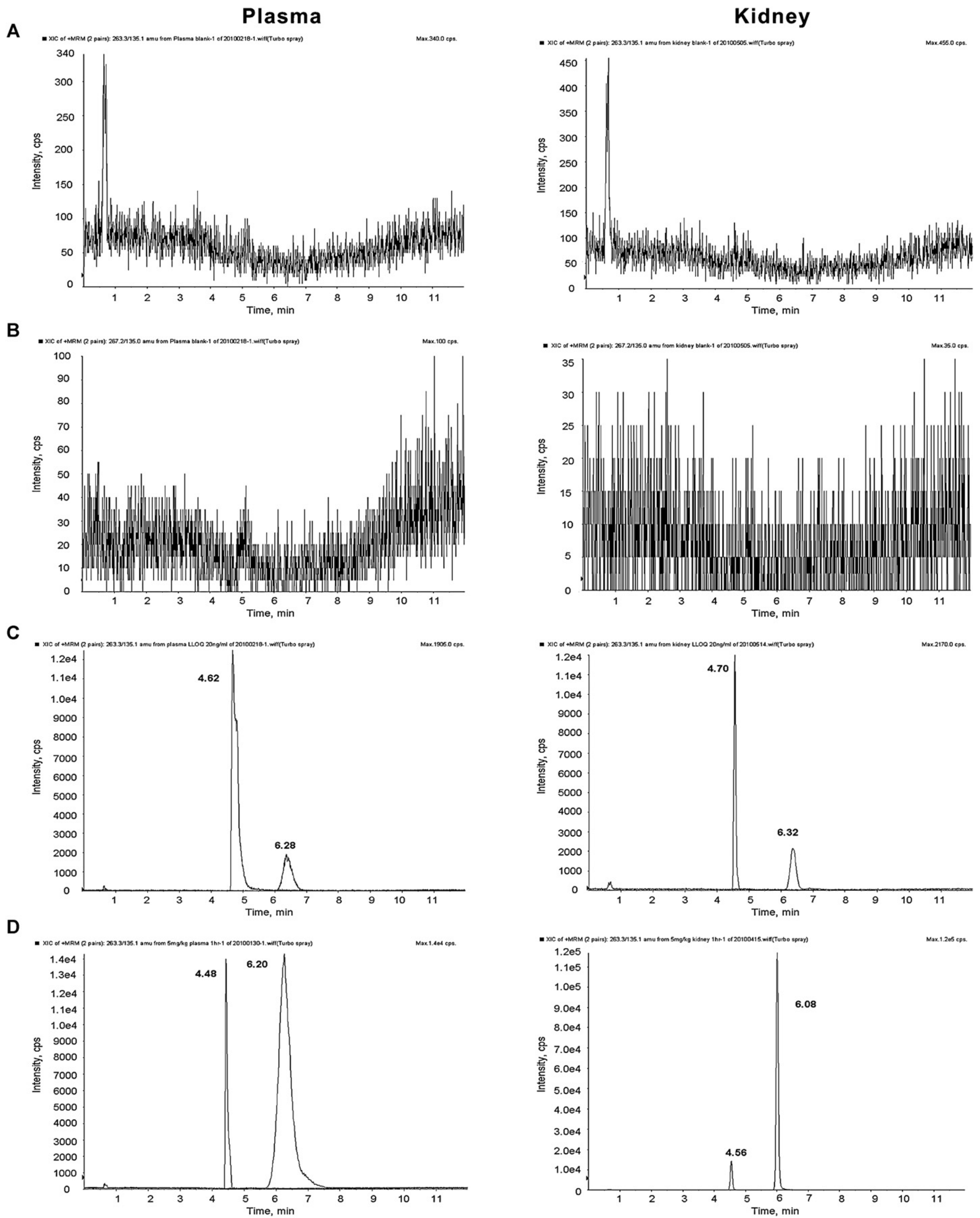

Fig. 2. Mass chromatograms of DZNep and tubercidin in plasma (left column) and kidney (right column) samples under MRM mode: (A) mass chromatograms of DZNep in blank samples (*m/z*, 263/135); (B) mass chromatograms of IS in blank samples (*m/z*, 267/135); (C) LOQ plasma and kidney samples spiked with DZNep at 20 ng/mL and IS at 100 ng/mL; (D) a plasma sample and a kidney sample obtained at 1 h after an intravenous dose of 5 mg/kg DZNep to a rat.

Table 2
Matrix effect and extraction recovery of DZNep and IS in rat biosamples ($n = 5$).

Biosample	Drug	Drug conc. (ng/mL)	Recovery (%)	Matrix effect (%)
Plasma	DZNep	30	79.9 ± 4.7	91.3 ± 8.0
		500	83.6 ± 5.8	86.0 ± 5.9
		7500	79.7 ± 8.2	91.1 ± 4.6
	Tubercidin	100	82.0 ± 7.7	88.5 ± 4.9
Urine	DZNep	30	78.2 ± 4.3	93.3 ± 3.9
		500	76.3 ± 5.5	94.7 ± 4.6
		7500	83.7 ± 4.1	92.1 ± 3.3
	Tubercidin	100	80.2 ± 4.0	86.1 ± 2.8
Feces	DZNep	30	85.8 ± 5.2	89.9 ± 5.4
		500	81.3 ± 7.1	90.9 ± 5.2
		7500	81.8 ± 7.4	88.3 ± 5.2
	Tubercidin	100	80.5 ± 7.7	85.4 ± 6.3
Heart	DZNep	30	79.9 ± 5.7	90.4 ± 5.3
		300	74.1 ± 8.4	86.8 ± 5.7
		3000	71.6 ± 8.3	86.1 ± 6.9
	Tubercidin	100	79.4 ± 4.5	90.4 ± 4.7
Liver	DZNep	30	79.3 ± 6.8	93.2 ± 5.6
		300	81.9 ± 5.8	90.6 ± 6.6
		3000	78.6 ± 7.2	91.0 ± 7.1
	Tubercidin	100	81.7 ± 7.1	88.0 ± 7.2
Spleen	DZNep	30	77.3 ± 7.8	93.0 ± 7.1
		300	81.8 ± 7.0	91.8 ± 4.7
		3000	79.2 ± 4.7	91.1 ± 5.6
	Tubercidin	100	76.6 ± 5.4	89.4 ± 5.5
Lung	DZNep	30	81.7 ± 8.9	91.0 ± 6.3
		300	76.1 ± 7.0	91.5 ± 5.7
		3000	80.0 ± 7.7	90.6 ± 7.2
	Tubercidin	100	79.2 ± 6.1	89.6 ± 5.5
Kidney	DZNep	30	74.9 ± 8.9	88.7 ± 8.0
		300	75.5 ± 10.0	88.2 ± 7.8
		3000	78.7 ± 8.2	90.1 ± 7.5
	Tubercidin	100	83.9 ± 7.6	90.7 ± 7.9
Stomach	DZNep	30	82.2 ± 9.4	91.2 ± 7.0
		300	78.0 ± 8.4	86.6 ± 6.8
		3000	77.0 ± 8.2	90.3 ± 6.7
	Tubercidin	100	76.4 ± 8.5	89.3 ± 6.5
Adipose	DZNep	30	79.6 ± 9.5	90.3 ± 7.9
		300	73.1 ± 5.6	93.1 ± 5.9
		3000	78.2 ± 10.5	88.7 ± 7.3
	Tubercidin	100	76.2 ± 7.7	93.0 ± 6.5
Muscle	DZNep	30	81.2 ± 4.6	92.7 ± 8.0
		300	82.7 ± 8.6	90.9 ± 6.6
		3000	85.7 ± 8.1	86.3 ± 7.5
	Tubercidin	100	82.6 ± 8.4	87.6 ± 5.5
Brain	DZNep	30	84.1 ± 7.3	90.9 ± 4.5
		300	82.5 ± 9.0	92.2 ± 5.9
		3000	82.9 ± 8.3	84.8 ± 7.5
	Tubercidin	100	84.8 ± 9.6	89.9 ± 5.8

4. Conclusion

A sensitive, selective and reliable LC–MS/MS method using sample preparation by Bond Elute PBA-SPE cartridges and sample separation on HILIC column was developed and validated for the quantification of DZNep in rat biosamples (plasma, urine, feces and tissue samples). To our knowledge, this is the first full validation of a LC–MS/MS procedure capable of determining the concentrations of DZNep in animal biosamples. The assay has been successfully used in the routine analysis of varied rat biosamples in the preclinical studies.

Acknowledgement

This research was supported by the National University of Singapore Academic Research Fund, R-148-000-134-112.

References

[1] R.I. Glazer, K.D. Hartman, M.C. Knode, M.M. Richard, P.K. Chiang, C.K. Tseng, V.E. Marquez, Biochem. Biophys. Res. Commun. 135 (1986) 688.
[2] E. De Clercq, M. Cools, J. Balzarini, V.E. Marquez, D.R. Borcherding, R.T. Borchardt, J.C. Drach, S. Kitaoka, T. Konno, Antimicrob. Agents Chemother. 33 (1989) 1291.
[3] M. Bray, J. Driscoll, J.W. Huggins, Antiviral. Res. 45 (2000) 135.
[4] W.R. Sellers, M. Loda, Cancer Cell 2 (2002) 349.
[5] S. Varambally, S.M. Dhanasekaran, M. Zhou, T.R. Barrette, C. Kumar-Sinha, M.G. Sanda, D. Ghosh, K.J. Pienta, R.G. Sewalt, A.P. Otte, M.A. Rubin, A.M. Chinnaiyan, Nature 419 (2002) 624.
[6] J. Tan, X. Yang, L. Zhuang, X. Jiang, W. Chen, P.L. Lee, R.K. Karuturi, P.B. Tan, E.T. Liu, Q. Yu, Genes Dev. 21 (2007) 1050.
[7] X. Jiang, J. Tan, J. Li, S. Kivimae, X. Yang, L. Zhuang, P.L. Lee, M.T. Chan, L.W. Stanton, E.T. Liu, B.N. Cheyette, Q. Yu, Cancer Cell 13 (2008) 529.
[8] F. Sun, E. Chan, Z. Wu, X. Yang, V.E. Marquez, Q. Yu, Mol. Cancer Ther. 8 (2009) 3191.
[9] R.A. Coulombe Jr., R.P. Sharma, J.W. Huggins, Eur, J. Drug Metab. Pharmacokinet. 20 (1995) 197.
[10] E. Chambers, D.M. Wagrowski-Diehl, Z. Lu, J.R. Mazzeo, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 852 (2007) 22.
[11] R.N. Xu, L. Fan, M.J. Rieser, T.A. El-Shourbagy, J. Pharm. Biomed. Anal. 44 (2007) 342.
[12] J.O. Svensson, A. Bruchfeld, R. Schvarcz, L. Stahle, Ther. Drug Monit. 22 (2000) 215.
[13] T.H. Huang, J. Wall, P. Kabra, J. Chromatogr. 452 (1988) 409.
[14] S.H. Kirsch, J.P. Knapp, J. Geisel, W. Herrmann, R. Obeid, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 3865.
[15] A. Van Eeckhaut, K. Lanckmans, S. Sarre, I. Smolders, Y. Michotte, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 877 (2009) 2198.