Journal of Chromatography B, 895–896 (2012) 10–16

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

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

Liquid chromatography–electrospray quadrupole linear ion trap mass spectrometry method for the quantitation of palonosetron in human plasma and urine: Application to a pharmacokinetic study

Pengfei Li, Ping Ma, Yan Wang, Weihang Tong, Jing Wang, Cheng Wu, Lihong Liu *

Pharmacy Department of the Second Artillery General Hospital PLA, Beijing 100088, PR China

ARTICLE INFO

Article history:
Received 19 July 2011
Accepted 2 March 2012
Available online 7 March 2012

Keywords:
Palonosetron
LC–MS/MS
Pharmacokinetics

ABSTRACT

The new analytical method for the determination of palonosetron in human plasma and urine has been developed based on liquid chromatography–mass spectrometry. The method utilized tramadol as the internal standard (IS). Separation was carried out on a Zorbax Eclipse TC-C_{18} column using methanol–1 mM ammonium formate in water (containing 0.1% formic acid, v/v, pH = 2.8) as mobile phase for gradient elution. Detection is carried out by multiple reaction monitoring (MRM) on 3200Qtrap™ mass spectrometry. The method has a chromatographic run time of 5.5 min and is linear within the concentration range 0.01–5.00 ng/mL for plasma and 0.10–30.00 ng/mL for urine both with a LOD of 0.003 ng/mL. Intra- and inter-day RSD of the concentration was 3.66–6.60%, 1.29–7.71% for plasma and 2.39–5.76%, 2.06–7.13% for urine. The relative error (RE) was −4.58% to 3.26% for plasma and −1.47% to 2.53% for urine. The recovery rates of palonosetron and IS both for plasma and urine were more than 90%. Palonosetron was stable under all the conditions tested. The method was successfully used to analyze palonosetron in human plasma and urine over a period of 168 h after intravenously pumping a single dose of 0.25 mg to volunteers. No significant differences were found between the pharmacokinetic parameters and urine accumulated excretory rate for male and female volunteers ($P > 0.05$). A two-compartment model was obtained after administrations. Palonosetron was eliminated at a slow rate in volunteers. The mean urine accumulated excretory rate was 25.97 ± 12.87%. Inter-individual differences could not be neglected due to the high coefficient of variety in several pharmacokinetic parameters and the urine accumulated excretion.

1. Introduction

Palonosetron is an antiemetic and antinauseant agent approved by the FDA for the prevention of acute and delayed chemotherapy-induced nausea and vomiting since 2003. It is a selective serotonin subtype 3 (5-HT3) receptor antagonist with a strong binding affinity for this receptor [1–7]. Palonosetron demonstrated potent antiemetic activity in animal models of chemotherapy induced emesis as well as in phase III clinical trials [8]. Palonosetron exists as a single stereoisomer with two chiral centers (Fig. 1), while the other 5-HT3 receptor antagonists exist as racemic mixtures.

After intravenous dosing of palonosetron in healthy subjects and cancer patients, an initial decline in plasma concentrations is followed by a slow elimination from the body [9–15]. Mean maximum plasma concentration (C_{max}) and area under the concentration–time curve ($AUC_{0-\infty}$) are generally dose proportional over the dose range of 0.3–90 μg/kg in healthy subjects and in cancer patients. Approximately 62% of palonosetron is bound to plasma proteins. Palonosetron is eliminated by multiple routes with approximately 50% metabolized to form two primary metabolites: N-oxide-palonosetron and 6-S-hydroxy-palonosetron. These metabolites each have less than 1% of the 5-HT3 receptor antagonist activity of palonosetron. In vitro metabolism studies have suggested that CYP2D6, CYP3A and CYP1A2 are involved in the metabolism of palonosetron. However, clinical pharmacokinetic parameters are not significantly different between poor and extensive metabolizers of CYP2D6 substrates. After a single intravenous dose of 10 μg/kg [^{14}C]-palonosetron, approximately 80% of the dose was recovered within 144 h in the urine with palonosetron representing approximately 40% of the administered dose [15]. In healthy subjects the total body clearance of palonosetron was 160 ± 35 mL/h/kg and renal clearance was 66.5 ± 18.2 mL/h/kg. Mean terminal elimination half-life is approximately 40 h.

Enantioseparation of palonosetron hydrochloride was selected by capillary zone electrophoresis with high-concentration beta-cyclodextrin. The baseline separation of the four stereoisomers of

* Corresponding author at: Pharmacy Department of the Second Artillery General Hospital PLA, 16 Xinwai Road, Beijing 100088, PR China. Tel.: +86 010 66343245; fax: +86 010 62076963.
E-mail address: hongllh@yahoo.com.cn (L. Liu).

1570-0232/$ – see front matter.
doi:10.1016/j.jchromb.2012.03.001

palonosetron in solution was achieved within 35 min, which was not suitable for bio-sample test [16]. For pharmacokinetic study, LC–MS/MS method has been developed for the determination of palonosetron in plasma. However, the method suffers from disadvantages such as low sensitivity (0.021 ng/mL), extensive sample preparation and larger biosamples (500 μL), and has not been applied to the determination of palonosetron in human urine in healthy subjects [17]. In this paper, we describe an LC–MS/MS method for the determination of palonosetron in human plasma and urine after one-step protein precipitation, and its application to a clinical pharmacokinetic study in healthy volunteers given a 0.25 mg dose of palonosetron.

2. Experimental

2.1. Materials and reagents

Palonosetron hydrochloride (99.5%) and tramadol (99.0%) (Fig. 1) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Acetonitrile was HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). All other chemicals were of analytical grade and used without further purification. Blank (drug free) human plasma and urine was obtained from the Second Artillery General Hospital PLA (Beijing, PR China). Distilled, demineralized water was produced by a Milli-Q Reagent Water System (Millipore, MA, USA).

2.2. Instrumentation

The LC–MS/MS system consisted of an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA) coupled to an Applied Biosystems Sciex 3200Qtrap™ mass spectrometer (Applied Biosystems Sciex, Ontario, Canada). Applied Biosystems/MDS SCIEX Analyst software was used for data acquisition and processing.

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

Stock solutions of palonosetron (0.40 mg/mL) were prepared by dissolving 11.21 mg palonosetron hydrochloride (containing 10.00 mg palonosetron) in a 25-mL volumetric flask and filling the flask to the volume with acetonitrile. These solutions were stored at 4 °C. Plasma and urine standards of palonosetron (100.00 ng/mL) were prepared freshly by spiking the appropriate stock solutions into the blank plasma and urine, respectively. Calibration curves for plasma were prepared by spiking the appropriate plasma standards into the blank plasma at concentrations of 0.01, 0.02, 0.04, 0.10, 0.30, 1.00, 2.50 and 5.00 ng/mL. Low, medium and high QC

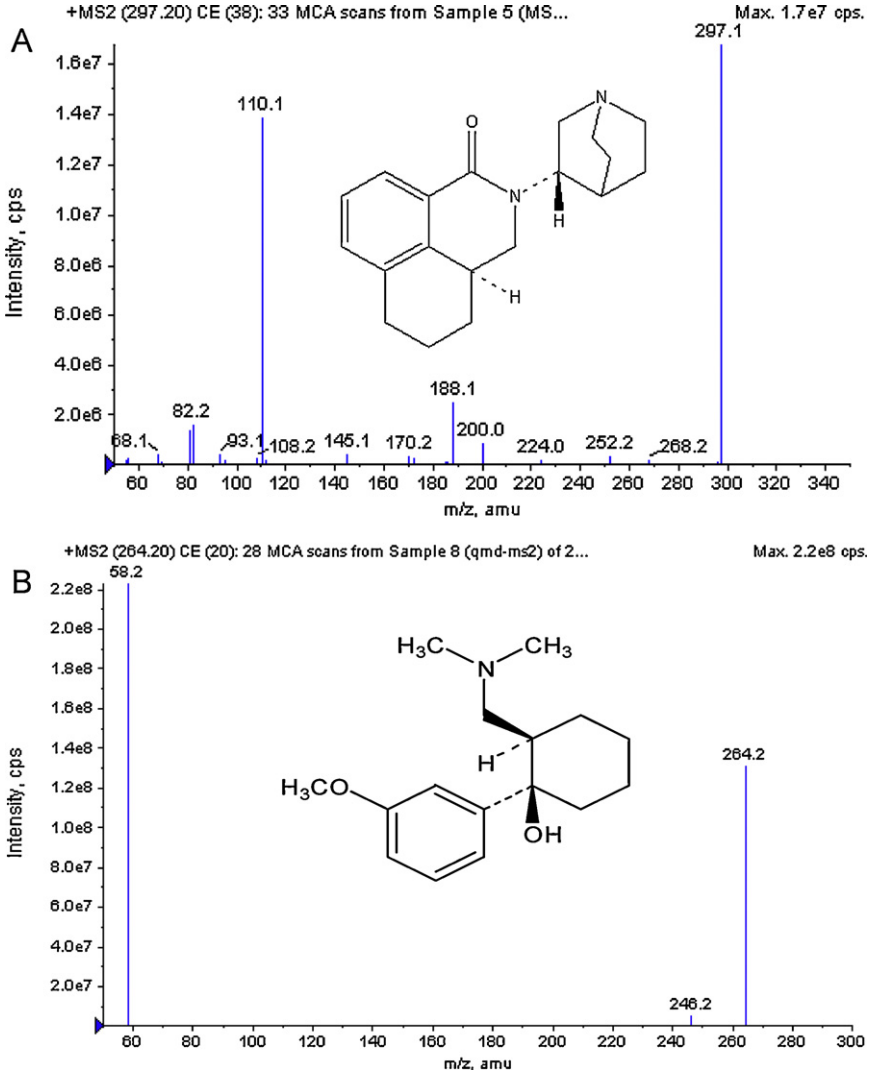

Fig. 1. Full-scan product ion spectra of $[M+H]^+$ for (A) palonosetron and (B) tramadol.

samples for plasma (0.02, 0.30, 4.00 ng/mL) were also prepared. Calibration curves for urine were prepared by spiking the appropriate urine standards into the blank urine at concentrations of 0.10, 0.30, 1.00, 3.00, 10.00 and 30.0 ng/mL. Low, medium and high QC samples for urine (0.30, 3.00, 24.00 ng/mL) were also prepared. The stock solution of IS (0.10 mg/mL) was prepared by dissolving 10.00 mg tramadol in a 100-mL volumetric flask and diluted to 10.00 ng/mL working solution with acetonitrile. Care was taken to protect palonosetron solutions and QC samples from direct sunlight. In each analytical run, calibration standards, QC samples and unknowns were extracted together.

2.4. Sample preparation

Human plasma samples were collected from blood (1 mL) by centrifugation at 5000 × *g* for 5 min, and stored at −20 °C prior to analysis. An aliquot of 200 μL plasma was transferred into a 1.5 mL eppendorf tube, together with 100 μL of IS working solution. 300 μL acetonitrile was added to precipitate plasma proteins, the mixture was vortexed for 1 min and centrifuged at 12,000 × *g* for 10 min. The supernatant was transferred into a 200 μL autosampler vial and 20 μL was injected into the instrument for analysis by LC–MS/MS.

An aliquot of 100 μL human urine sample was transferred into a 1.5 mL eppendorf tube, together with 100 μL of IS working solution. 600 μL acetonitrile was added to dilute urine. The mixture was vortexed for 1 min and centrifuged at 12,000 × *g* for 10 min. The supernatant was transferred into a 200 μL autosampler vial and 5 μL was injected into the instrument for analysis by LC–MS/MS.

2.5. Chromatographic conditions

Gradient elution chromatography (as shown in Table 1) was carried out on a 150 mm × 4.6 mm, 5 μm Zorbax Eclipse TC-C_{18} column (Agilent Technologies, Palo Alto, CA, USA) maintained at 35 °C using a mobile phase of methanol–1 mM ammonium formate in water (containing 0.1% formic acid, v/v, pH = 2.8) at a flow-rate of 1.2 mL/min. The column effluent was split so that approximately 0.6 mL/min entered the mass spectrometer. Under these conditions, retention times were typically 2.94 min for palonosetron and 2.86 min for tramadol.

2.6. Mass spectrometer conditions

The electrospray ion (ESI) source was used in positive ion mode for all experiments. The LC–MS/MS detector was operated at low resolution in the MRM mode using the mass transition ion-pairs *m*/*z* 297.1 → *m*/*z* 110.1 for palonosetron and *m*/*z* 264.2 → *m*/*z* 58.2 for tramadol. In order to optimize MS parameters, a standard solution of analyte and IS was infused into the mass spectrometer using a syringe pump. Optimized parameters were as follows: curtain gas, gas 1 and gas 2 (nitrogen) 15, 50 and 60 units, respectively; dwell time 200 ms; source temperature 450 °C; ionspray voltage 2500 V. Declustering potential (DP) and collision energy (CE) were, respectively, 53 V and 36 eV for palonosetron and 25 V and 40 eV for tramadol. The collision gas was set to high mode and the interface heater to on mode.

Hydrophilic impurities were diverted to waste for 2 min after an injection using a ten-way switching valve. Data acquisition was carried out by Analysis 1.4.2 software on a *DELL* computer.

Table 1
The conditions of gradient elution.

Time (min)	Flow rate (μL/min)	Methanol (%)	1 mM ammonium formate water, 0.1% formic acid, pH = 2.8 (%)
0.00	1200	15	85
0.70	1200	80	20
1.70	1200	85	15
2.30	1200	95	5
2.31	1200	15	85
5.50	1200	15	85

2.7. Assay validation

Calibration standards and QC samples ($n = 6$) were analyzed on three separate days. Linearity of calibration curves based on peak areas was assessed by weighted ($1/x^2$) least-squares analysis. Intra- and inter-day precision was calculated as coefficient of variation (CV) and accuracy as relative error. The limit of quantitation (LOQ) was determined as the concentration below which the inter-day CV exceeded 20%. The LOD was determined as the concentration with signal-to-noise ratio of 3. The absolute recovery rates of palonosetron and tramadol were evaluated by comparing peak areas of extracted QC samples with those of reference QC solutions reconstituted in blank plasma or urine extracts, respectively. Matrix effects were evaluated by comparing peak areas of QC solutions and internal standard solutions reconstituted in blank plasma extracts with that of the same solutions injected directly into the LC–MS system. Both the absolute recovery rates and matrix effects tests contain three samples in each concentration.

Stability of palonosetron in plasma and urine was assessed at −20 °C for 20 days and three freeze–thaw cycles. After extracting and reconstitution, stability was also investigated in the autosampler at room temperature for 20 h. The samples for stability tests were quantified using freshly prepared calibration standards.

2.8. Pharmacokinetic study

The proposed analytical method was used in a pharmacokinetic (PK) study. The study protocol was approved by the State Food and Drug Administration, PR China (SFDA). This study was carried out on a group of five male and five female healthy volunteers. A single dose of 0.25 mg (0.25 mg/5 mL per ampule) of palonosetron was intravenously pumped to volunteers in 5 min. Blood samples were collected before (0 h) and at 0.083, 0.167, 0.333, 0.50, 1.0, 2.0, 4.0, 8.0, 12.0, 24.0, 48.0, 72.0, 96.0, 120.0, 144.0 and 168 h after administration. The plasma was obtained by centrifugation of whole blood at 5000 × *g* for 5 min and kept frozen at −20 °C until analysis. Urine samples were collected before (0 h) and 0–2, 2–4, 4–8, 8–12, 12–24, 24–48, 48–72, 72–96, 96–120, 120–144 and 144–168 h after administration. The urine samples were kept frozen at −20 °C until analysis after the volume was measured.

The descriptive statistics of PK parameters were computed using Drug and Statistics Program (DAS) version 2.0 (Anhui Provincial Center for Drug Clinical Evaluation, China) using the batch processing method. The pharmacokinetic parameters were calculated by noncompartmental methods. All data were expressed as mean ± standard deviation (SD). Statistical differences of the data were determined by means of analysis of variance (ANOVA).

The following PK parameters were determined for the period of 0–168 h: the area under the plasma concentration–time curve from time zero to the last measurable palonosetron sample time (AUC_{0-t}), the maximum plasma concentration (C_{max}), and the time to reach C_{max} (T_{max}). The area under the curve from time zero to infinity ($AUC_{0-\infty}$) and the area under the first moment of the drug concentration curve from time zero to infinity ($AUMC_{0-\infty}$) were calculated as $AUC_{0-t} + C_t/K_e$ and $AUMC_{0-t} + t \times C_t/K_e + C_t/K_e$, respectively, where C_t is the last detectable plasma concentration and t is the time at which this concentration occurred. The elimination rate (K_e) was as the slope of the linear regression of the log-transformed concentration–time curve data in the terminal phase. The half-life ($t_{1/2}$) was calculated by dividing ln 2 by K_e. The total systemic

clearance (CL) of palonosetron was calculated as dose/$AUC_{0-\infty}$. The apparent volume of distribution (V_d) was calculated as CL/K_e. The mean residence time from time zero to last sampling time (MRT_{0-t}) was calculated from the ratio of $AUMC_{0-t}$ to AUC_{0-t}. Accumulated excretory rates were calculated and the accumulated excretory rate–time curves of palonosetron were drawn after the concentrations of the urine samples were quantitated by the LC–MS/MS analytical method.

3. Results and discussion

3.1. Mass spectrometry

According to the chemical structure of palonosetron, ESI in positive mode is expected to be the best source for LC–MS/MS. Full-scan positive mode spectra of palonosetron and tramadol contained predominant protonated molecules at m/z 297.1 and 264.1, respectively. Product ion spectra of $[M+H]^+$ showed fragment ions at m/z 110.1 and 188.1 for palonosetron and at m/z 58.0 for tramadol (Fig. 1). The fragment ions at m/z 110.1 and 58.0 were present in highest abundance and were chosen for multiple reaction monitoring (MRM) acquisition of palonosetron and tramadol, respectively.

3.2. Chromatography

Various combinations of acetonitrile, methanol, acetic acid and formic acid were investigated to optimize the mobile phase for sensitivity, speed and peak shape. The inclusion of 1 mM ammonium acetate instead of pure water reduced matrix effects without decreasing response. Peak shape was improved by using 0.1% formic acid. Further improvement in peak shape with reduced cycle time was achieved by splitting the column effluent and increasing the flow rate. After a number of C_{18} columns (Nova-Pak, Nucleosil, Zorbax Eclipse XDB and Zorbax Eclipse TC) were evaluated, Zorbax Eclipse TC-C_{18} gave the best chromatogram using gradient elution. With a flow rate of 1.2 mL/min, the cycle time was 5.5 min allowing a sample throughput of 120–150 samples per day. Under optimized HPLC conditions, palonosetron and tramadol were detected at retention times of 2.93 and 2.85 min, respectively.

3.3. Sample preparation

In this study, one-step protein precipitation, which is economical and convenient, was adopted to simplify sample preparation. Comparing with methanol and trifluoroacetic acid, acetonitrile was selected to be the protein precipitant due to excellent precipitation and fewer matrix effects. In the experiment, we tested different volumes of protein precipitant such as 200 μL, 300 μL, 600 μL and 900 μL. It was found that 300 μL for plasma and 600 μL for urine was the best choice for both ion suppression and precipitation efficiency. The recovery rate was high and the analytes were stable under these conditions.

3.4. Selection of IS

It is necessary to use an IS to obtain good accuracy and precision when a mass spectrometer is used as the HPLC detector. Tramadol was adopted as IS because of the similarity of its retention time with that of the analyte, and it also ionizes well in the positive ionization mode.

3.5. Assay validation

3.5.1. Selectivity

Selectivity was assessed by comparing the chromatograms for six different blank human plasma or urine with those for the corresponding standard spiked samples. Typical chromatograms are shown in Fig. 2 for plasma and urine. There was no significant interference from endogenous substances observed at the retention times of the analytes and the IS. The results suggested that no considerable endogenous contribution from human plasma and urine interferes with the measurement of the analytes, demonstrating the selectivity and specificity of the MRM technique.

3.5.2. Linearity and sensitivity

The assay was linear over the concentration range 0.01–5.00 ng/mL for plasma and 0.10–30.00 ng/mL for urine both with an LOD of 0.003 ng/mL. Typical linear regression equations of the calibration curves were as follows:

$$\text{Plasma}: \quad y = 0.082x + 0.000355 \quad r = 0.9970$$

$$\text{Urine}: \quad y = 0.101x + 0.0798 \quad r = 0.9963$$

where y represents the ratio of analyte peak area to that of the IS, and x represents the concentration of the analyte. Good linearity was shown in the stated concentration ranges.

The LOQ were determined to be 0.01 ng/mL for plasma and 0.10 ng/mL for urine, which were sufficient for clinical PK studies.

3.5.3. Precision and accuracy

The precision was calculated by using the relative standard deviation (RSD) and the accuracy was evaluated using the relative error (RE). In this assay, intra- and inter-day RSD of the concentration was 3.66–6.60%, 1.29–7.71% for plasma and 2.39–5.76%, 2.06–7.13% for urine. Meanwhile, RE was −4.58% to 3.26% for plasma and −1.47% to 2.53% for urine (Table 2). The above values were within the acceptable range, and the method was thus judged to be suitably accurate and precise.

3.5.4. Recovery, matrix effect, stability

The absolute recovery rates of palonosetron in the protein precipitation with acetonitrile for plasma were 92.8 ± 5.4%, 91.5 ± 4.4%, 95.8 ± 2.3% at 0.02, 0.30 and 5.00 ng/mL, respectively. While, the absolute recovery rates of palonosetron for urine were 98.5 ± 2.0%, 97.1 ± 2.1%, 101.0 ± 1.9% at 0.30, 3.00 and 24.00 ng/mL, respectively. The absolute recovery rates of the internal standard tramadol for plasma and urine were 90.3 ± 2.7% and 99.0 ± 1.6%, respectively.

Table 2
Precision and accuracy for the determination of palonosetron in human plasma and urine (6 samples of different concentrations each 3 days).

	Nominal conc. (ng/mL)	Calculated conc. (ng/mL)	Intra-day RSD (%)	Inter-day day RSD (%)	RE (%)
Plasma	0.04	0.04	6.60	7.71	−4.58
	0.30	0.30	4.12	1.29	0.30
	4.00	4.13	3.66	6.90	3.26
Urine	0.30	0.31	5.76	2.06	2.53
	3.00	2.96	3.65	7.13	−1.47
	24.00	23.96	2.39	6.80	−0.16

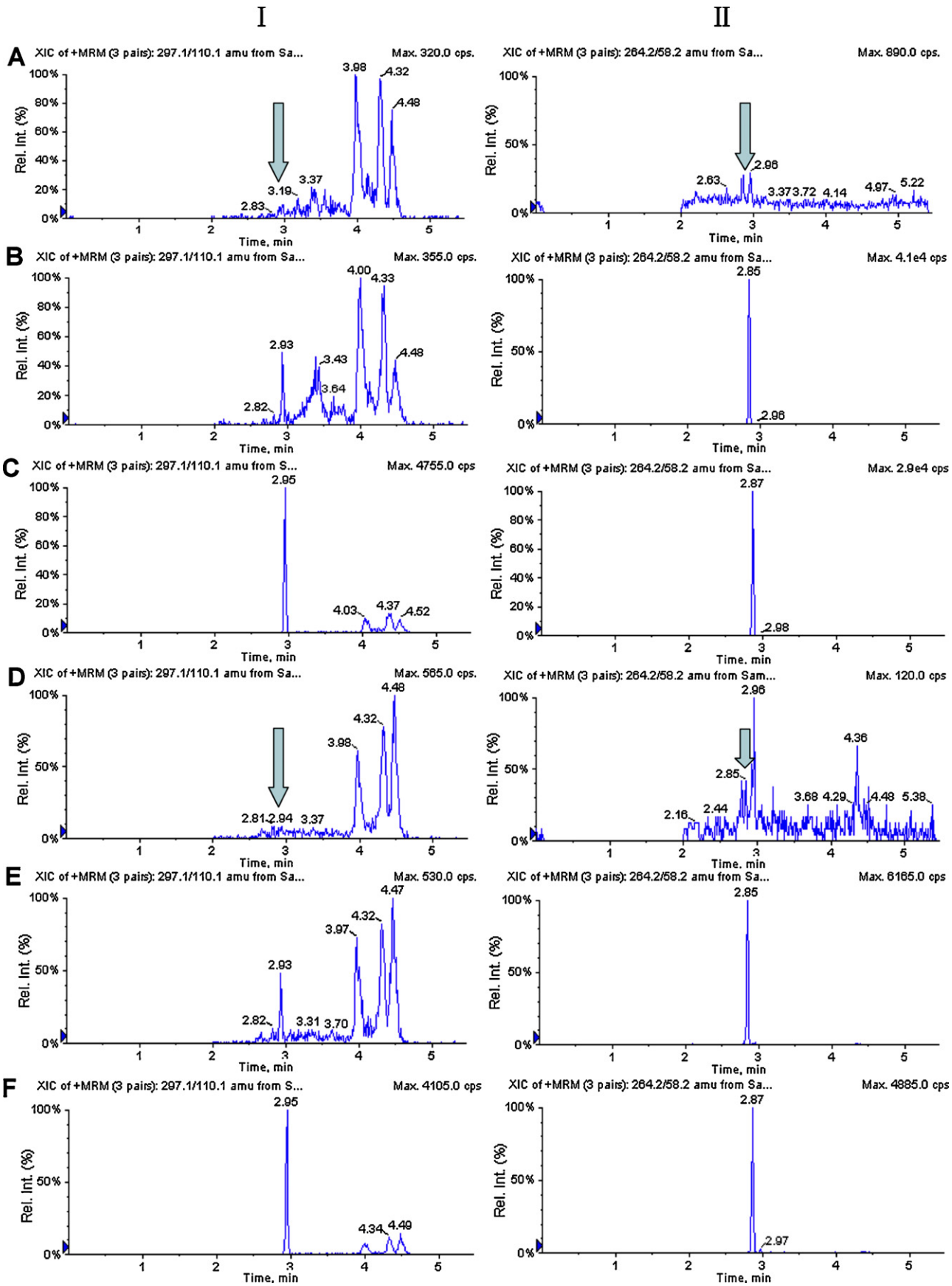

Fig. 2. Representative single reaction monitoring chromatograms of (A) blank plasma, (B) blank plasma spiked with palonosetron at the limit of quantitation (0.01 ng/mL) and tramadol, (C) a plasma sample 5 min after injected 0.25 mg palonosetron to healthy volunteers, (D) blank urine, (E) blank urine spiked with palonosetron at the limit of quantitation (0.1 ng/mL) and tramadol and (F) a urine sample 0–2 h after injected 0.25 mg palonosetron to healthy volunteers. (I) Palonosetron and (II) tramadol.

Table 3
Stability data of palonosetron during the routine analyses (3 samples of different concentrations each tests).

Storage conditions	Drug	Concentration (ng/mL)		RSD (%)	RE (%)
		Nominal	Calculated		
Freezing for 20 days at −20 °C	Plasma	0.04	0.04	2.50	7.50
		0.30	0.32	4.17	6.78
		4.00	4.26	0.86	6.39
	Urine	0.30	0.29	2.62	−3.33
		3.00	3.15	4.39	5.00
		24.00	24.22	1.85	0.92
Three freeze–thaw cycles	Plasma	0.04	0.04	2.50	7.50
		0.30	0.32	7.00	6.33
		4.00	3.91	3.19	−2.14
	Urine	0.30	0.32	1.9	6.67
		3.00	2.88	4.12	−4.00
		24.00	24.01	3.33	0.04
Stability at room temperature for 20 h (after extracting and reconstitution)	Plasma	0.04	0.04	2.89	3.33
		0.30	0.31	1.45	2.33
		4.00	4.13	2.09	3.28
	Urine	0.30	0.31	5.77	3.33
		3.00	3.22	7.52	7.33
		24.00	23.85	3.14	−0.62

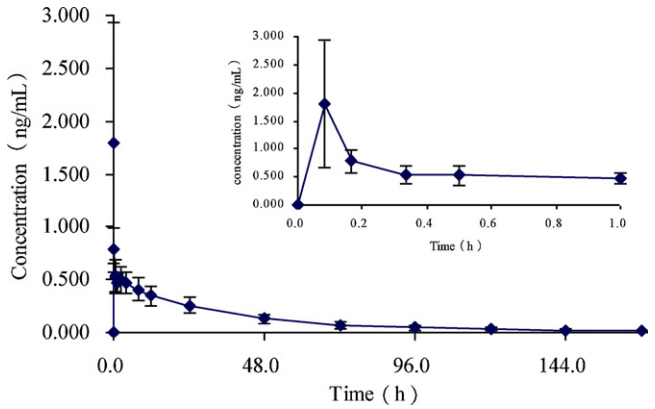

Fig. 3. Average plasma concentration–time profile for palonosetron with a single dose of 0.25 mg intravenously pumping to volunteers in 5 min ($n = 10$).

In relation to matrix effects, the relative errors based on mean peak areas for both palonosetron and internal standard tramadol were lower than 10% at all the concentration levels. The results indicate that no co-eluting endogenous substances significantly influenced the ionization of palonosetron and internal standard. Palonosetron was stable under all the conditions evaluated with RE of −4.00% to 7.50% in Table 3 ($n = 18$).

Table 4
Pharmacokinetic parameters of palonosetron with a single dose of 0.25 mg intravenously pumping to volunteers in 5 min ($n = 10$).

Parameter	Estimate (mean ± SD)
C_{max} (ng/mL)	1.810 ± 1.124
T_{max} (h)	0.0917 ± 0.0264
$AUC_{0-168\,h}$ ((ng h)/mL)	19.16 ± 5.65
$AUC_{0-\infty}$ ((ng h)/mL)	19.93 ± 5.91
K_e (1/h)	0.0199 ± 0.0031
$t_{1/2}$ (h)	35.62 ± 5.50
CL (L/h)	14.07 ± 4.03
V_d (L)	724.13 ± 250.90
MRT_{0-t} (h)	35.41 ± 5.93

3.6. Pharmacokinetic study

The method described above was successfully used to analyze palonosetron in human plasma and urine. The procedure developed was sensitive enough to quantitate palonosetron in plasma with acceptable accuracy and precision over a period of 168 h after intravenously pumping a single dose of 0.25 mg to volunteers in 5 min. The plasma concentration–time profile and urine accumulated excretory rate–time profile for palonosetron after intravenously pumping are shown in Figs. 3 and 4. All the pharmacokinetic parameters are listed in Table 4. No significant differences were found between the pharmacokinetic parameters and urine accumulated excretory rate for male and female volunteers ($P > 0.05$). In this

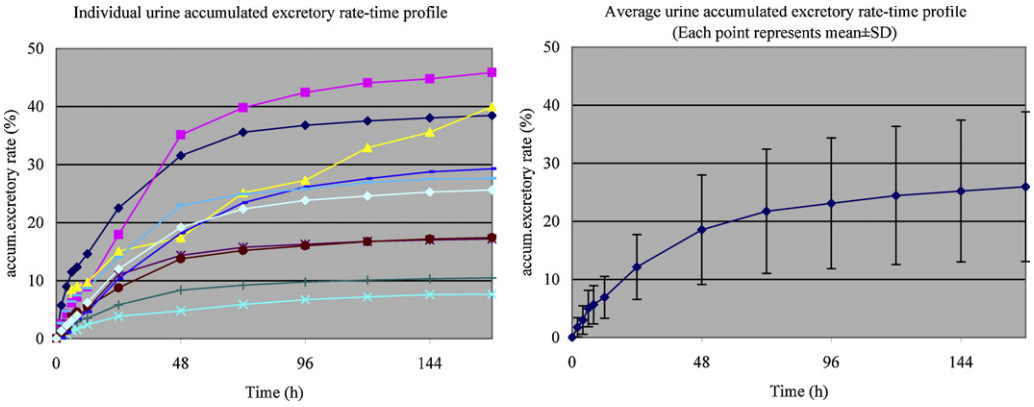

Fig. 4. Urine accumulated excretory rate–time profile for palonosetron with a single dose of 0.25 mg intravenously pumping to volunteers in 5 min ($n = 10$).

study a two-compartment model was obtained after single intravenously pumping administrations. The V_d markedly exceeded the volume of total body water of human, suggesting that a certain portion of the dose distributed into tissues. The pharmacokinetic parameters of $t_{1/2}$ and CL used to evaluate excretion suggested palonosetron was eliminated at a slow rate in volunteers, which was similar to the urine accumulated excretion study (mean urine accumulated excretory rate was $25.97 \pm 12.87\%$).

In addition, in the present pharmacokinetic study of palonosetron, inter-individual differences could not be neglected due to the high coefficient of variety (>30% in several pharmacokinetic parameters). Likewise, high coefficient of variety existed in the urine accumulated excretion study.

4. Conclusion

A LC–MS/MS method using an ESI interface for determination of palonosetron in human plasma and urine was developed and validated in this study. The method only needed a one-step protein precipitation procedure, which reduced the preparation time and allowed quantitation of palonosetron for the concentration range 0.01–5.00 ng/mL for plasma and 0.10–30.00 ng/mL for urine both with a LOD of 0.003 ng/mL. The precision, sensitivity and selectivity of the method were sufficient to determine the drug in human plasma and urine, and it is also suitable for pharmacokinetic studies.

References

[1] R.M. Navari, Expert Opin. Drug Metab. Toxicol. 5 (2009) 1577.
[2] C. Ruhlmann, J. Herrstedt, Expert Rev. Anticancer Ther. 10 (2010) 137.
[3] M. Saito, M. Tsukuda, Expert Opin. Pharmacother. 11 (2010) 1003.
[4] L.P. Yang, L.J. Scott, Drugs 69 (2009) 2257.
[5] C. Rojas, A.G. Thomas, J. Alt, M. Stathis, J. Zhang, E.B. Rubenstein, S. Sebastiani, S. Cantoreggi, B.S. Slusher, Eur. J. Pharmacol. 626 (2010) 193.
[6] C. Rojas, M. Stathis, A.G. Thomas, E.B. Massuda, J. Alt, J. Zhang, E. Rubenstein, S. Sebastiani, S. Cantoreggi, S.H. Snyder, B. Slusher, Anesth. Analg. 107 (2008) 469.
[7] G. Tonini, B. Vincenzi, D. Santini, Expert Opin. Drug Metab. Toxicol. 1 (2005) 143.
[8] M. Di Maio, C. Gallo, F. Perrone, Tumori 94 (2008) 14.
[9] R. Stoltz, J.C. Cyong, A. Shah, S. Parisi, J. Clin. Pharmacol. 44 (2004) 520.
[10] P. Eisenberg, F.R. MacKintosh, P. Ritch, P.A. Cornett, A. Macciocchi, Ann. Oncol. 15 (2004) 330.
[11] T.L. Hunt, S.C. Gallagher, M.T. Cullen Jr., A.K. Shah, J. Clin. Pharmacol. 45 (2005) 589.
[12] A.K. Shah, T.L. Hunt, S.C. Gallagher, M.T. Cullen Jr., Curr. Med. Res. Opin. 21 (2005) 595.
[13] A. Shah, T. DeGroot, G. Apseloff, J. Clin. Pharmacol. 46 (2006) 1139.
[14] A.A. Genazzani, R.A. Billington, Tumori 94 (2008) 6.
[15] R. Stoltz, S. Parisi, A. Shah, A. Macciocchi, Biopharm. Drug Dispos. 25 (2004) 329.
[16] M. Wang, X. Ding, H. Chen, X. Chen, Anal. Sci. 25 (2009) 1217.
[17] L. Ding, Y. Chen, L. Yang, A. Wen, J. Pharm. Biomed. Anal. 44 (2007) 575.