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Short communication

Rapid and sensitive determination of vinorelbine in human plasma by liquid chromatography–tandem mass spectrometry and its pharmacokinetic application

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ABSTRACT

Vinorelbine is a semi-synthetic vinca alkaloid with demonstrated high activities against various types of advanced cancer. To support a clinical pharmacokinetic study, a simple, rapid and sensitive method to determine vinorelbine in human plasma was developed using reversed phase liquid chromatography (LC) coupled with electrospray ionization mass spectrometry/mass spectrometry (ESI-MS/MS). Vinorelbine and vinblastine (the internal standard) were extracted from human plasma by one-step liquid–liquid extraction (LLE) with methyl-t-butyl ether. The chromatographic separation was achieved on a Spursil polar-modified C_{18} column (50 mm × 2.1 mm, 3 μm, Dikma Technologies) with an isocratic mobile phase of a 75:25 (v/v) acetonitrile–4 mmol/L ammonium formate (pH 3.0) mixture at a flow-rate of 0.4 mL/min. The MS/MS detection was performed in the positive ion multiple reaction monitoring (MRM) mode by monitoring the precursor → product ion transitions at *m/z* 779.4 → 122.0 and *m/z* 811.3 → 224.2 for vinorelbine and the internal standard, respectively. The assay was validated in the range 0.1–200 ng/mL ($r > 0.997$), the lowest level of this range being the lower limit of quantification (LLOQ) based on 50 μL of plasma. The intra- and inter-day precisions were within 6.0%, while the accuracy was within ±4.7% of nominal values. Detection and quantification of both analytes within 2 min make this method suitable for high-throughput analyses. The method was successfully applied to evaluate the systemic pharmacokinetics of vinorelbine after a 20-min intravenous infusion of 25 mg/m^2 of vinorelbine to patients with metastatic breast cancer.

1. Introduction

Vinorelbine (5′-nor-anhydro-vinblastine), a semi-synthetic vinca alkaloid, has shown activity in various tumors, especially metastatic breast cancer [1,2] and non-small-cell lung cancer [3,4]. The mechanism of action of vinorelbine mainly involves the disruption of microtubules by the reversible binding to tubulin leading to mitotic spindle dissolution and metaphase arrest in dividing cells [5]. Vinorelbine chemically differs from other vincas by a modification of the catharanthine moiety of the molecule, which renders it less neurotoxic than other marketed vinca alkaloids [6]. Although in most clinical studies vinorelbine has been well tolerated, the dose-limiting myelotoxicity has limited the delivered dose to 20–30 mg/m^2 when given on a weekly schedule [7,8]. Early population pharmacokinetic studies using the limited-sampling strategy have demonstrated marked inter-individual pharmacokinetic variations in patients with various types of advanced cancer [8,9]. Since vinorelbine has been commonly included in the combination therapy used as the first-line treatment for metastatic breast cancer, a clinical study in our hospital was carried out to characterize vinorelbine pharmacokinetics and determine the dose–response relationships. In support of the pharmacokinetic study, a simple, rapid and sensitive LC–MS/MS assay method was developed and validated for the quantification of vinorelbine concentrations in human plasma.

Previous methods for determination of vinorelbine in biological samples applied high-performance liquid chromatography with UV [10–12], fluorescence [13–15] and electrochemical [16,17] detection, which often involved complex sample preparation and long runtime. Several LC–MS/MS methods for vinorelbine quantification in biological matrices have been reported over the past decade [18–24]. One of these methods [20] selected Sphersorb cyano column with an isocratic elution by a mixture of 40 mmol/L ammonium acetate buffer (pH 3) and acetonitrile (55:45, v/v) as the mobile phase. However, a large plasma volume of 500 μL was needed to reach an LLOQ of 0.25 ng/mL and the analysis time was longer than 9 min. Very recently, Damen et al. [22] reported a method for the determination of vinorelbine by LC–MS/MS with

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much smaller plasma sample (50 μL) to achieve the LLOQ of 0.1 ng/mL. This method used a high pH mobile phase (pH 10.5) and applied heated ESI source to improve the sensitivity.

The current study described a rapid, sensitive and practical LC–MS/MS method for the determination of vinorelbine in human plasma. A polar-modified Spursil C_{18} column markedly improved peak shape and retention of vinorelbine with an acidic isocratic system. The assay showed a short analytical time (2.0 min per sample), a high sensitivity (an LLOQ of 0.1 ng/mL, sufficiently sensitive for this clinical study), and the sample preparation procedure involved a one-step liquid–liquid extraction. This method, providing a good alternative over previously reported, was successfully applied to a clinical pharmacokinetic study.

2. Materials and methods

2.1. Chemical and reagents

Vinorelbine ditartrate ($C_{45}H_{54}N_4O_8{\cdot}2C_4H_6O_6$, potency 72.0%) and vinblastine sulphate ($C_{46}H_{58}N_4O_9{\cdot}H_2SO_4$, potency 86.0%) were supplied by Jiangsu Hanson Pharmaceutical Co. Ltd. (Jiangsu, China). HPLC grade acetonitrile were purchased from Merck (Darmstadt, Germany). Ammonium acetate and methyl-t-butyl ether (MTBE) were purchased from Tedia (Fairfield, OH, USA). Water was distilled and purified using a Millipore Milli-Q Plus system (Bedford, MA, USA). Heparinized blank (drug-free) human plasma was obtained from Shanghai Blood Center (Shanghai, China).

2.2. Liquid chromatography/mass spectrometry

The HPLC was performed using a LC-20AD Prominence binary solvent delivery system with a column oven, a DGU-20A3 on-line degasser and a SiL-20ACHT autosampler (Shimadzu, Kyoto, Japan). The chromatographic separation was performed on a Dikma Spursil C_{18} column (50 mm × 2.1 mm, 3 μm, Dikma Technologies) at 40 °C. A mixture of acetonitrile–4 mmol/L ammonium formate pH 3.0 (75/25, v/v) was used as mobile phase for isocratic elution at a flow rate of 0.4 mL/min.

The HPLC system was coupled with an API 3200QTrap (Applied Biosystem/MDS SCIEX, Foster City, CA, USA) fitted with a TurboIonSpray ionization (ESI) source in positive mode. Multiple reaction monitoring (MRM) of the precursor–product ion transitions *m/z* 779.4 → 122.0 for vinorelbine and *m/z* 811.3 → 224.2 for vinblastine (internal standard, IS) was used for quantification. The optimal instrument conditions were as follows: curtain gas of 30 psi, nebulizer gas of 50 psi, TurboIonSpray gas of 45 psi, entrance potential (EP) of 10 V, TurboIonSpray voltage of 5000 V, and temperature of 500 °C. Declustering potential (DP) was set at 47, 54 V, the collision energy (CE) at 40, 60 V and the collision cell exit potential (CXP) at 3, 7 V for vinorelbine and IS, respectively. Scan time was 0.15 s per transition. All the parameters of LC and MS were controlled by Analyst 1.5 software (Applied Biosystem/MDS SCIEX).

2.3. Preparation of standards and quality control samples

Stock solutions of vinorelbine and IS were prepared separately in methanol at a target concentration of 200 μg/mL as free base. Working solutions of vinorelbine (0.01–20.0 μg/mL) and IS (0.25 μg/mL) were prepared by independent diluting stock solutions with 50% aqueous methanol. Stock solutions of both compounds were stable for at least 6 months and working solutions for at least 2 weeks when stored at −20 °C.

On each day of analysis, calibration standards were freshly prepared in duplicate at 0.1, 0.2, 0.5, 2.0, 5.0, 20, 80, and 200 ng/mL, by spiking 5 μL of the appropriate vinorelbine working solution to 45 μL of blank human plasma.

A second stock solution of vinorelbine was prepared to obtain quality control (QC) samples in blank human plasma at four levels including: 0.1 ng/mL, the lower limit of quantification (LLOQ); 0.2 ng/mL, the low QC (LQC); 5.0 ng/mL, the medium QC (MQC); and 160 ng/mL, the high QC (HQC). The prepared QCs were stored at −20 °C. An additional QC for dilution test was prepared at 1,600 ng/mL of vinorelbine and diluted 1:10 (v/v) in blank human plasma for quantitation.

2.4. Plasma sample preparation

Liquid–liquid extraction was chosen for the sample preparation. All frozen human plasma samples were thawed at ambient temperature. A 5 μL aliquot of IS (0.25 μg/mL) standard solution were added to 50 μL of each plasma sample in a 1.5 mL polypropylene microfuge tube and then vortex-mixed. The mixture was extracted with 0.5 mL MTBE, vortex-mixed for 3 min, and centrifuged at 14,000 rpm for 10 min at 4 °C. The organic layer was transferred to another tube and evaporated to dryness under a gentle stream of nitrogen gas at 30 °C. The residue was reconstituted with 50 μL methanol–water (50:50, v/v), vortex-mixed for 30 s, and centrifuged at 14,000 rpm for 5 min. The supernatant was pipetted to an autosampler vial. The injection volume was 5 μL and, to avoid carryover, the autosampler was rinsed with methanol before and after sample injection.

2.5. Method validation

During pre-study validation, three validation runs were conducted on 3 separate days. Each validation run consisted of two sets of calibration standards, and at least five replicates of regular QC samples at each level (0.2, 5.0 and 160 ng/mL). One of the batches included five replicates of QC 1,600 ng/mL after a 10-fold dilution with blank plasma. In one of the batches, the LLOQ standards (0.1 ng/mL) in quintuplicate were analyzed to assess the precision and accuracy of at the LLOQ. Blanks and QC samples were interspersed among calibration standards. Carry-over effects were routinely controlled by injecting blanks immediately after the highest calibration standard (200 ng/mL) or HQC (160 ng/mL).

2.5.1. Selectivity and matrix effect

Selectivity was investigated by comparing the chromatograms of six different batches of blank human plasma with the corresponding plasma samples spiked with vinorelbine and IS.

To evaluate the matrix effect in the experiment, chromatographic peak areas of each analyte from the spike-after-extraction samples, at low, medium and high concentration levels, were compared to those for the neat standard solutions at the same concentrations.

2.5.2. Linearity

Linearity was determined in the range of 0.10–200 ng/mL by plotting the peak area ratio of vinorelbine to IS versus the nominal concentration of vinorelbine in plasma. The calibration curves were constructed by weighted ($1/x^2$) least squares linear regression. There should be no co-eluting peaks with areas of more than 20% of the analyte peak area at the LLOQ.

2.5.3. Accuracy and precision

Accuracy and precision were assessed by determination of QC samples using five replicate preparations of plasma samples at concentration levels (0.2, 5.0 and 160 ng/mL) on three validation days. Sample concentrations were determined using calibration standards prepared on the same day. Accuracy was expressed as the relative error (RE), i.e. (measured concentration − nominal concentration)/(nominal concentration) × 100%, while the precision was

given by the inter- and intra-day relative standard deviations (RSD). The lower limit of quantification (LLOQ) was determined as the lowest concentration for which vinorelbine spiked in six different sources of plasma resulted in measurements with acceptable precision and accuracy (less than 20% for each criterion).

2.5.4. Effect of dilution

To enable the analysis of clinical samples for which plasma concentrations were found to be above the upper limit of quantification (ULOQ), sample dilution was validated. Plasma control samples spiked with 1,600 ng/mL (5 μL) were diluted 10-fold with human blank plasma (45 μL) in quintuplicate. Resulting concentration values were multiplied by the dilution factor, and accuracy and precision were calculated using these data. A bias of less than 10% between the measured undiluted concentration and nominal undiluted concentration was accepted.

2.5.5. Extraction recovery

The extraction recoveries for vinorelbine at three QC concentrations were determined by comparing the peak area of extracted samples spiked with known amount of the analytes with that of spike-after-extraction at corresponding concentrations.

2.5.6. Stability

Vinorelbine stability in human plasma was assessed by analyzing five replicates of QC samples at concentrations of 0.2, 5.0 and 160 ng/mL, respectively, exposed to different conditions of time and temperature. The short term bench-top stability of 3 h was based on the actual exposure time for each sample during analysis at room temperature of about 22 °C and long-term stability was examined after storage of the standard spiked plasma samples at −80 °C for 6 months. The freeze/thaw stability was performed by three complete freeze/thaw cycles between a −80 °C freezer and room temperature on consecutive days. The stability of vinorelbine in the final reconstituted solution was assessed by placing QC samples in the HPLC autosampler at 4 °C for 12 h. The stability of stock solutions was also tested for 6 months upon refrigeration (−20 °C).

2.6. Pharmacokinetic application

The LC/MS/MS procedure developed here was used to investigate the plasma profiles of vinorelbine administered as a 20-min intravenous infusion at a dose of 25 mg/m^2 to Chinese patients treated for metastatic breast cancer. The pharmacokinetic analysis is a part of a study approved by the Fudan University Cancer Hospital Ethics Committee for clinical investigation, and the patient provided written informed consent.

These samples were collected into heparinized evacuated glass tubes before infusion, and after 10, 20, 40 min, and 1, 3, 6, 12, 24, 48, 72, 96 and 144 h after its commencement. Plasma was separated by centrifugation of treated blood at 2000 × *g* for 10 min and stored at −80 °C until subsequent analysis.

3. Results and discussion

3.1. Optimization of LC–MS/MS

Vinorelbine contains four nitrogen atoms (as shown in Fig. 1), which make it a weakly basic alkaloid. Vinorelbine ditartrate possesses good hydrophilicity and the measured Log *P* is 1.32 [25]. In the present study, method development began with the optimization of chromatographic conditions including mobile phase composition and column type based on the chromatographic behaviors and ionization responses of vinorelbine and IS. Chromatographic analysis of the analytes and IS was initiated under isocratic conditions with an aim to develop a simple separation process with a short run time. Acetonitrile was selected as the organic phase instead of methanol because of its higher eluting ability. When developing a reversed phase method for basic compounds, better ionization and reproducibility usually come with acidic mobile phases. Thus, an ammonium formate buffer acidified with formic acid (pH 3.0) was optimal for both chromatographic separation and MS sensitivity.

As column packing materials strongly influence the chromatographic separation and sensitivity, reversed-phase C_{18} silica columns, including Shimadzu Shim-pack XR-ODS C_{18} column, Phenomenex Kinetex C_{18} column, Agilent Zorbax C_{18} column and Agela Venusil MP C_{18} column, were tested. However, broad, tailing peaks were observed since basic compounds interact strongly with the silanols on the bare silica surface and are not eluted thoroughly by the mobile phase. On the other hand, as a sort of polar compound, vinorelbine showed poor retention on silica-based stationary phases and required low content of acetonitril in mobile phase, which resulted in poor ionization efficiency in MS. When a Spursil C_{18} column (50 mm × 2.1 mm, 3 μm, Dikma Technologies) was employed, the retention and peak shape of vinorelbine were improved significantly. The polar column, which was developed to retain polar, especially basic compounds, as well as reduce silanol interactions by the inclusion of polar-modified stationary phase, exhibited satisfactory chromatographic performance in terms of peak shape and selectivity. The IS vinblastine, an analogue of vinorelbine, performed in the similar way.

With the enhancement of retention to the polar-modified stationary phase, the acetonitrile portion could be increased so as to improve chromatographic and MS performance. Satisfactory results were obtained with an isocratic mobile phase of a 75:25 (v/v) acetonitrile and 4 mmol/L ammonium formate (pH 3.0) mixture at a flow rate of 0.4 mL/min. Vinorelbine and IS could be separated with symmetric peak shapes on the Spursil C_{18} column along with significantly improved sensitivity and good reproducibility. The retention time of vinorelbine and IS were 1.2 and 1.0 min, respectively and the total runtime was 2.0 min, considerably shorter than that of previous methods [10–20], which made it possible to analyse a large number of samples in a relatively short period of time.

For evaluation of sensitivity of vinorelbine to ESI, the signal intensity obtained in the positive mode was much higher than that in the negative mode. The ESI mass spectrum showed that the protonated molecular ion $[M+H]^+$ of vinorelbine was at *m/z* 779.4. The major fragment found at *m/z* 122.0, corresponding to the opening of the octatomic ring at the catharanthine moiety of the molecule, was present in the highest abundance and hence selected for subsequent monitoring in the third quadrupole. The mass spectrum of IS, vinblastine, showed a $[M+H]^+$ at *m/z* 811.3, and the high collision energy (60 V) gave a major product ion at *m/z* 224.2. The fragment was formed by breaking the carbon–carbon bond which bridges catharanthine ring with vindoline part, and by opening the nine-atomic ring at the catharanthine part of the molecule. The product ion mass spectra and the possible structures of these fragments were shown in Fig. 1.

Vinblastine was selected as IS due to its similar ionization condition, chromatographic, extraction recovery and MS behaviors compared to vinorelbine. Vinblastine was shown to be stable under the described experimental conditions, and it did not interfere with the analysis of vinorelbine.

3.2. Optimization of sample preparation

Sample extraction and cleanup are vital aspects for the quantitative bioanalysis. Preliminarily, a protein precipitation method was tried to extract vinorelbine from human plasma, but strong ion suppression from the residual plasma matrix components were

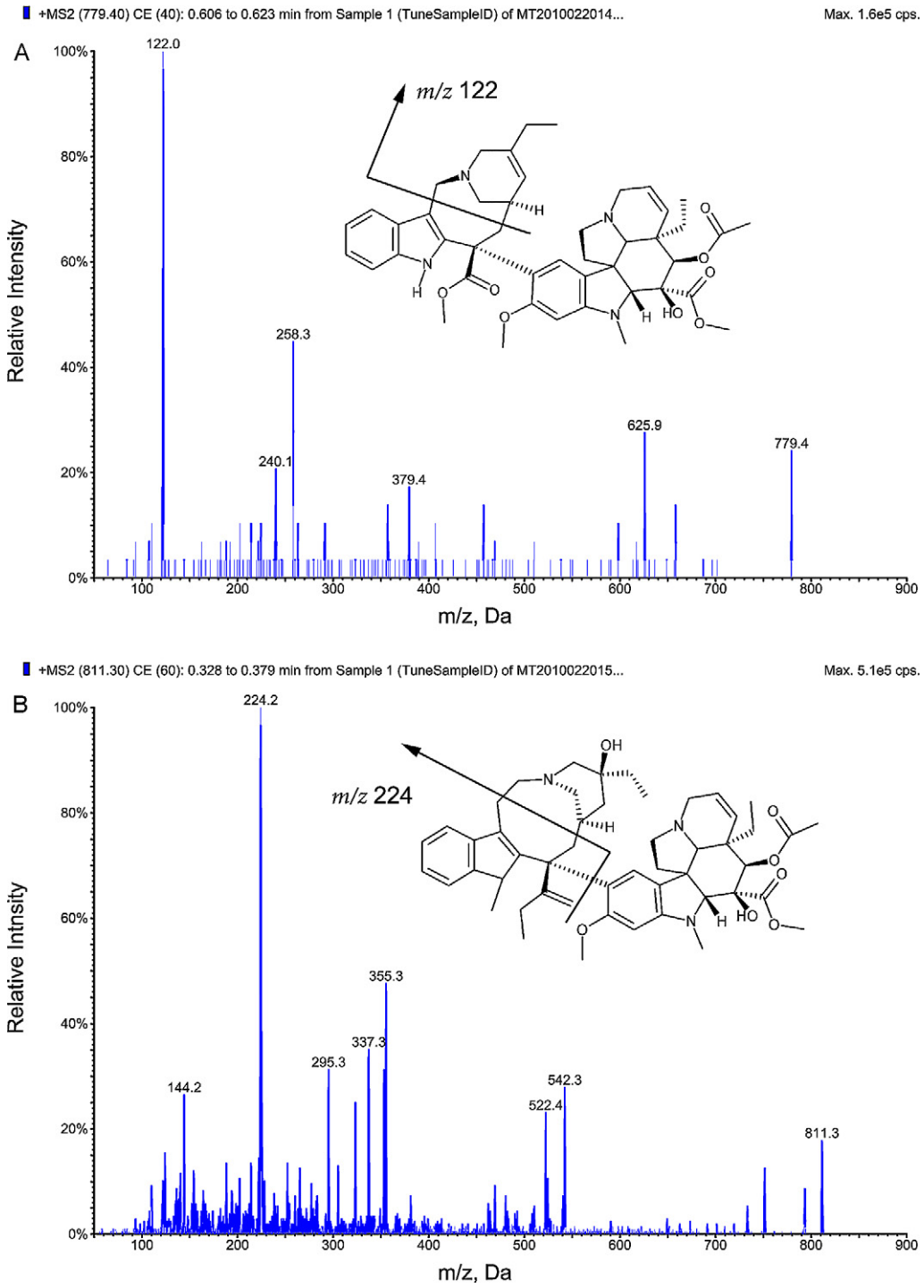


Fig. 1. Product ion mass spectra of $[M+H]^+$ ions of vinorelbine (A) and vinblastine (B) in positive electrospray ionization mode.

observed, suggesting those co-eluting components interfered with the ionization of the analytes and made the precision of the method unacceptable. Gradient elution could be used to eliminate the matrix effect but at the cost of longer analytical times, which was unfavorable for pharmacokinetic studies.

Compared with protein precipitation (PPT), liquid–liquid extraction (LLE) could produce a relatively clean sample and reduce the possibility of introducing highly polar materials into the column and MS system. Then plasma samples were subjected to a LLE procedure with different solvents, such as ethyl acetate, methyl-t-butyl ether, diethyl ether, hexane and dichloromethane. By evaluating for recovery and sample clean up, the best extraction was obtained using MTBE, but not diethyl ether which was reported in previous studies [13,17] as a suitable extraction solvent. In fact, MTBE yielded an extraction recovery 20% higher than diethyl ether with one step LLE.

When different pH values of the extraction system were tested, the pH in the range of 7.0–9.0 was found to give similar, consistent and reproducible recoveries (>80%) of analytes from plasma. In view of the convenience of sample preparation, no buffer solution was added into the sample since human plasma normally presents an alkalescence pH of 7.35–7.45. Thus a simple and efficient extrac-

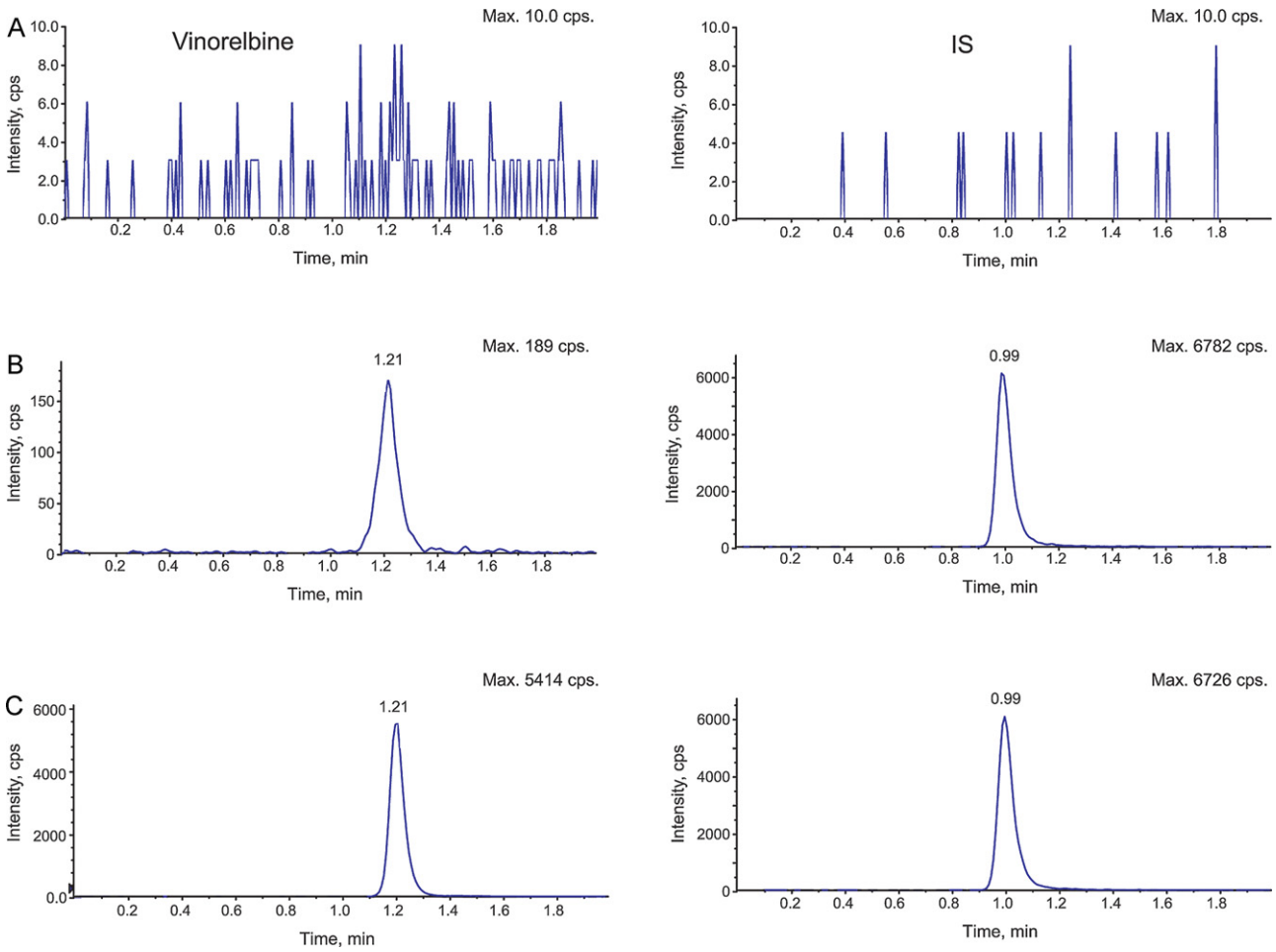


Fig. 2. Representative MRM chromatograms of vinorelbine (*m/z* 779.4 → 122.0) and internal standard (*m/z* 811.3 → 224.2) for (A) blank plasma; (B) blank plasma spiked with vinorelbine at the LLOQ of 0.1 ng/mL and IS (25.0 ng/mL); (C) a plasma sample 3 h after intravenous administration of vinorelbine at a dose of 25 mg/m^2 in cancer patients. The retention times for vinorelbine and IS were 1.2 and 1.0 min, respectively.

tion method with MTBE was utilized to extract vinorelbine and IS from plasma samples. This simple LLE procedure demonstrated to have advantages in rapidity, column maintenance and sensitivity improvement.

3.3. Method validation

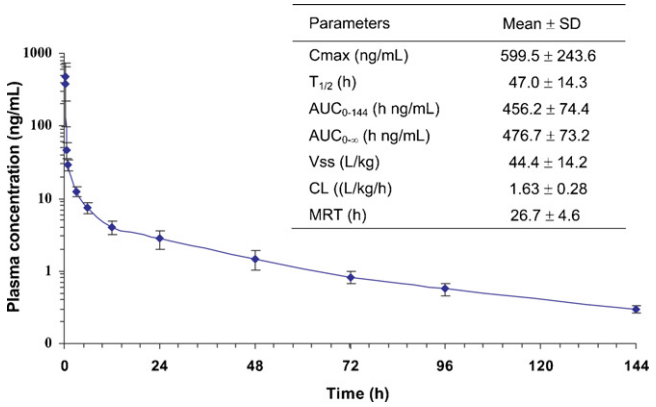
3.3.1. Selectivity and matrix effect

Fig. 2 shows the typical chromatograms for a blank plasma, a spiked plasma sample with vinorelbine (0.1 ng/mL) and IS (25.0 ng/mL), and a plasma sample from a patient 3 h after an intravenous administration of 25 mg/m^2 vinorelbine. There were no significant interferences from endogenous substances observed at the retention times of the analyte and IS. Carry-over was less than 20% of LLOQ, indicating there was no carry-over.

The post-extraction addition technique was used to determine the degree of matrix effect relative to the response of vinorelbine. The matrix effects on recovery of blank plasma samples spiked after the sample preparation with 0.2, 5.0 and 160 ng/mL of vinorelbine were found to be 106.7%, 95.8% and 103.0% respectively. The same evaluation was performed on IS, and no significant peak area differences were observed.

3.3.2. Linearity and LLOQ

Calibration curves were linear over the concentration range of 0.1–200 ng/mL with correlation coefficients always better than 0.997. For each point on the calibration curves for vinorelbine, the concentration back-calculated from the equation of the regression analysis showed a deviation ranging from −2.1 to 5.3%. The mean linear regression equation of the calibration curves generated during the validation was: $y = 0.0056(\pm 0.0015) + 0.1773(\pm 0.009)x$, where y represented the peak-area ratios of vinorelbine to IS, and x represented the plasma concentration of vinorelbine, expressed as ng/mL.



Parameters	Mean ± SD
Cmax (ng/mL)	599.5 ± 243.6
$T_{1/2}$ (h)	47.0 ± 14.3
$AUC_{0\text{-}144}$ (h ng/mL)	456.2 ± 74.4
$AUC_{0\text{-}\infty}$ (h ng/mL)	476.7 ± 73.2
Vss (L/kg)	44.4 ± 14.2
CL ((L/kg/h)	1.63 ± 0.28
MRT (h)	26.7 ± 4.6

Fig. 3. Mean plasma concentration-time profile of vinorelbine after an intravenous administration of 25 mg/m^2 to cancer patients (n = 12, mean ± SD). Pharmacokinetic parameters obtained were summarized inset table.

Table 1
Accuracy, intra- and inter-day precision and recovery of vinorelbine in human plasma.

Nominal concentration (ng/mL)	Intra-day (n = 5)		Inter-day (n = 15)		Extraction recovery (n = 5)	
	RE (%)	RSD (%)	RE (%)	RSD (%)	Mean (%)	RSD (%)
0.10 (LLOQ)	3.8	6.5	7.2	8.1	–	–
0.20	2.5	4.4	3.4	5.6	84.5	4.0
5.00	−2.1	6.0	−2.4	3.7	84.0	3.8
160	−3.4	3.6	−4.7	3.9	81.8	4.9

"–" no experimentation was performed.

Based on 50 μL of plasma, the LLOQ for vinorelbine was established 0.1 ng/mL with a signal-to-noise ratio greater than 10:1 and was set as the lowest standard of the calibration curve.

3.3.3. Accuracy and precision

Accuracy and precision were analyzed at LLOQ, low, medium and high concentrations. Accuracy was found to be always better than 7.2%, and intra-day and inter-day precision were constantly better than 6.5% and 8.1%, respectively (Table 1), which promised its reliable application to the quantification of samples from clinical trails.

3.3.4. Influence of dilution

Influence of dilution was validated by diluting plasma samples at 1,600 ng/mL by a dilution factor of 10. Accuracy and precision were 98.3% and 4.5%, respectively.

3.3.5. Extraction recovery

The mean recoveries and standard deviation of vinorelbine at low, medium, and high concentrations were 84.5 ± 4.0%, 84.0 ± 3.8% and 81.8 ± 4.9%, respectively (mean ± SD, n = 5). Mean recovery for IS was 82.1% ± 5.0%.

3.3.6. Stability studies

Vinorelbine was stable at room temperature up to 3 h with less than 7.0% deviation from initial concentration. No significant degradation of vinorelbine was found in QC samples stored in a freezer at −80 °C for 6 months. The biases for freeze–thaw stability were no more than 6.5%. Other results met the criterion set up for stability (Table 2). In addition, the stock solutions of vinorelbine and IS stored for 6 months at −20 °C were comparable to the freshly made ones.

3.4. Pharmacokinetic application

This overall analytical procedure was applied to quantify vinorelbine in plasma from 12 Chinese cancer patients receiving a dose of 25 mg/m^2 by 20 min intravenous infusion. Pharmacokinetic analysis was performed using a non-compartmental method. The mean plasma concentration-time profile and major pharmacokinetic parameters of vinorelbine following administration are presented in Fig. 3. The current study confirmed the high plasma clearance (CL), the large volume of distribution at steady state (V_{ss}) and long elimination half-life described in those researches [26–29] with 25 or 35 mg/m^2 short infusion or bolus injection.

When dose was adjusted to be comparably equivalent, the calculated pharmacokinetic parameters in our study were consistent with those from a previous study by Schilling et al. [29], such as C_{max} plasma 676.6 ± 220.2 ng/mL, AUC_{inf} plasma 623.3 ± 220.1 h ng/mL, CL plasma 1.74 ± 0.97 L/kg/h, V_{ss} plasma 34.3 ± 11.2 L/kg, where six patients were administered vinorelbine by bolus injection of 35 mg/m^2 and the plasma concentrations were determined by HPLC-UV. However, the above mentioned values from plasma samples were apparently lower than those reported in Marty's study with 20 min intravenous infusion of vinorelbine at 25 mg/m^2 [26]: for C_{max} blood 761.8 ± 185.4 ng/mL and AUC_{inf} blood 1042 ± 392 h ng/mL. Such discrepancy also appeared in other clinical trials [27,28] and it is noteworthy that these pharmacokinetic values were based upon blood concentration measurements. Since *in vitro* experiments showed that platelets are the main carrier in blood for vinorelbine and several clinical studies [9,13,29] reported that vinorelbine concentrations were about 1.9 times higher in blood than in plasma, parameter comparisons had to take into account the ratio of AUC_{blood}/AUC_{plasma} which averaged 1.8. This could be the leading cause for the differences of pharmacokinetic parameters among the clinical studies on vinorelbine administered intravenously.

Table 2
Stability of vinorelbine in plasma samples (n = 5).

Storage conditions	Nominal concentration (ng/mL)	Measured concentration (ng/mL)	RSD (%)	RE (%)
Short-term (3 h, 22 °C)	0.20	0.186	6.7	−7.0
	5.00	5.184	4.6	3.7
	160	154.6	3.9	−3.4
Long-term (−80 °C, 6 months)	0.20	0.209	7.2	4.5
	5.00	4.915	1.3	−1.7
	160	168.4	4.9	5.3
Three freeze/thaw cycles	0.20	0.213	5.0	6.5
	5.00	5.102	1.5	2.0
	160	157.5	3.7	−1.6
Autosampler (4 °C, 12 h)	0.20	0.189	8.1	−5.5
	5.00	4.904	3.1	−1.9
	160	153.0	4.0	−4.4

4. Conclusions

An LC–MS/MS assay for determination of vinorelbine in human plasma was developed and validated with respect to sensitivity, accuracy, precision and reproducibility. The method is based on the use of a polar-modified Spursil C_{18} column, which significantly improved the retention and peak shape of vinorelbine with an acidic isocratic system, and therefore enhanced the sensitivity with the increase of acetonitrile portion in mobile phase. The efficiency of liquid–liquid extraction and an analytical runtime of 2.0 min per sample make this method suitable for high-throughput clinical applications.

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