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Liquid chromatography–tandem mass spectrometry method for simultaneous determination of seven commonly used anticancer drugs in human plasma

Jingya Zhou, Shouhong Gao, Feng Zhang, Bo Jiang, Qin Zhan, Fei Cai, Jingxian Li, Wansheng Chen*

Department of Pharmacy, Changzheng Hospital, Second Military Medical University, Shanghai 200003, PR China

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ABSTRACT

This paper describes the development and validation of a novel, general liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the simultaneous determination of cyclophosphamide, ifosfamide, irinotecan, etoposide, gemcitabine, carboplatin and pemetrexed concentrations in human plasma. Samples were prepared by two kinds of extraction method and analyzed using a gradient separation over an Atlantis T3-C18 column (2.1 mm × 100 mm, 3 μm, Waters). Positive electrospray ionization was employed as the ionization source. The mobile phase consisted of acetonitrile–water (0.1% formic acid and 10 mM ammonium acetate) at a flow rate of 0.25 mL/min. Linear coefficients of correlation were >0.992 for all analytes. The intra- and inter-day relative standard deviation across three validation runs over the entire concentration range was less than 9.2%, while the accuracy was within ±10.5%. The mean recovery of all the analytes ranged from 50.0 to 81.0%. This method was successfully applied to clinical samples from cancer patients.

1. Introduction

Cytotoxic drugs are still widely used today in the treatment of cancers [1]. However, due to the narrow therapeutic index (NTI) of these drugs, small changes in doses may cause poor anti-tumor effects or unacceptable toxicity and limit the anticancer efficacy [2]. Even worse, it could damage not only cancer cells but also normal and healthy tissues [3]. So it is a great challenge to develop agents that combine efficacy, safety and convenience. All of these lead to a call for more enlightened in vivo establishment and optimization of therapeutic drug monitoring (TDM), which entails the measurement and interpretation of drug concentrations in biological fluids and the individualization of drug dosages to maximize therapeutic effects, minimize toxicities, or both, for commonly used anticancer agents [4].

With the increased incidence of smoking and the aggravation of environmental pollution, lung cancer incidence and mortality have been increasing in recent years (since the early 1980s, over the past 10 years) in China [5]. Chemicals used as therapeutics including alkylating agents (e.g., cyclophosphamide and ifosfamide), topoisomerase inhibitors (e.g., irinotecan and etoposide), metal complexes (e.g., carboplatin) and antimetabolites (e.g., pemetrexed and gemcitabine) (Fig. 1). But the different types of adverse reactions to these drugs have been reported in clinical [6–12]. Now the combination regimens are commonly used as adjuvant treatment of lung cancer (e.g., pemetrexed plus carboplatin, gemcitabine plus carboplatin, etoposide plus carboplatin and ifosfamide plus etoposide). When applying those drug combinations, adverse reactions are still serious [13]. So a plasma concentration method is urgently needed, which could offer ease of sample preparation, high sample throughput with a small sample size requirement, and high sensitivity and selectivity. HPLC methods combined with ultraviolet detection, fluorescence detection, or mass spectrometry for quantification of cyclophosphamide, ifosfamide, irinotecan, etoposide, gemcitabine, carboplatin or pemetrexed have been developed in the last five years [14–20]. In addition, a colorimetric method for the determination of cyclophosphamide and ifosphamide in pure and in dosage forms was suggested [21]. To date, only few methods were reported to simultaneously detect anticancer drugs including one or two of the anticancer agents mentioned above. James M. Rideout et al. determined etoposide and teniposide in serum by HPLC with electrochemical detection [22]. Krogh-Madsen et al. used an HPLC system with ultra-violet and fluorescence detection to simultaneously detect cytosine arabinoside, daunorubicin and etoposide in human plasma [23]. Llewellyn et al. developed a method for the determination of the cytotoxic drugs cyclophosphamide and ifosfamide in sewage effluent by LC–MS/MS [24]. However, no method is yet available which can simultaneously determine these drugs in biological samples, and can be time-saving, labor-saving and widely applicable.

* Corresponding author at: Department of Pharmacy, Changzheng Hospital, Second Military Medical University, No. 415, Fengyang Road, Shanghai 200003, PR China. Tel.: +86 21 81886181; fax: +86 21 33100038.
E-mail address: chenwanshengsmmu@yahoo.com.cn (W. Chen).

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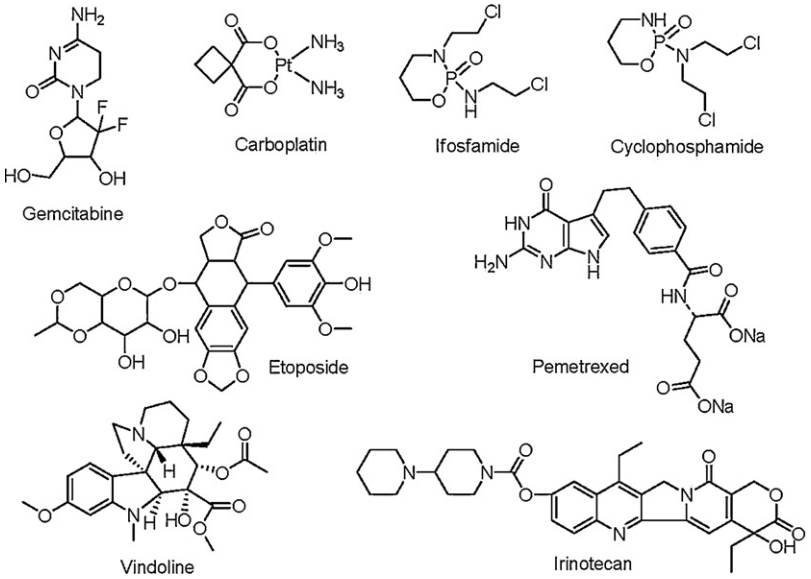

Fig. 1. Chemical structures of cyclophosphamide, ifosfamide, irinotecan, etoposide, gemcitabine, carboplatin, pemetrexed and internal standard (IS, vindoline).

LC–MS/MS technology allows liquid chromatography with high separation ability and mass spectrometry with high sensitivity and selectivity in one method, and has become the most powerful tool for rapid quantification of complex drugs within various biological fluids [25]. In this paper, a specific LC–MS/MS method for the simultaneous determination of these seven drugs in human plasma was developed and validated for TDM.

2. Materials and methods

2.1. Chemicals and reagents

Cyclophosphamide, ifosfamide, irinotecan, etoposide, gemcitabine, carboplatin, pemetrexed and vindoline (IS) were purchased from Sigma (St. Louis, USA). HPLC grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Analytical grade formic acid, ethyl acetate and ammonium acetate were purchased from Tedia (Tedia, USA). Ultrapure water (0.22 μm) was deionized and further purified by means of a Milli-Q Plus water purification system (Millipore, USA). All other reagents were of commercially available analytical grade. Human blank plasma (plasma from subjects who were not receiving the drug) was obtained from the Shanghai Red Cross Blood Center (Shanghai, China).

2.2. Chromatographic conditions

An Atlantis T3-C18 analytical column (2.1 mm × 100 mm, 3 μm, Waters) was used with a linear gradient mobile phase consisting of acetonitrile–water containing 10 mM ammonium acetate and 0.1% formic acid at a flow rate of 0.25 mL/min. The column temperature was held at 30 °C and the sample compartment was at ambient temperature. Mobile phase-A was water containing 0.1% formic acid and 10 mM ammonium acetate, and mobile phase-B was acetonitrile. The gradient profile started with 100% mobile phase-A and held for 1 min, was then switched to 100% mobile phase-B at 1.01 min and lasting until 9 min, and finally switched back to 100% mobile phase-A at 9.01 and lasting until 18 min, after which the system was returned to the initial condition. Under these conditions, the analytes co-eluted with the internal standard within 9 min.

2.3. Mass spectrometry

An Agilent 6410 triple-quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Agilent, USA) operating in the positive ion mode was used as a detector. For quantification, multiple reaction monitoring (MRM) chromatograms were acquired with MassHunter Analysis software version B.01.04 (Agilent, USA). The analytes were quantified by the MRM transitions (Table 1). The optimized mass spectrometric parameters were as follows:interface temperature of 350 °C, cone gas flow of 10 L/min, nebulizer pressure of 40 psi, rough vacuum of 2.05 Torr, high vacuum of 3.03×10^{-5} Torr, and source temperature of 105 °C.

2.4. Preparation of standards and quality control (QC) samples in human plasma

The standard stock solutions of cyclophosphamide, ifosfamide, irinotecan, etoposide, gemcitabine, carboplatin and pemetrexed were prepared from two independent weighs to a final concentration of 1 mg/mL for each analyte in methanol, which were further diluted to seven or nine concentrations with control human plasma for working solutions in the range of 1–1000 ng/mL, 1–1000 ng/mL, 10–10,000 ng/mL, 5–5000 ng/mL, 50–5000 ng/mL, 50–5000 ng/mL and 100–10,000 ng/mL, respectively. The internal standard working solution (100 ng/mL) was prepared by diluting the stock solution (1 mg/mL) with 50% methanol. The working solution A is the mixture of the four analytes including irinotecan, etoposide, ifosfamide and cyclophosphamide, and the working solution B is the mixture of the remaining analytes including pemetrexed disodium, carboplatin and gemcitabine. All stock and working solutions were stored at nominally −20 °C until use.

QC samples were prepared by the same operation listed above (2, 10, 100 ng/mL for cyclophosphamide and ifosfamide; 20, 100, 1000 ng/mL for irinotecan; 10, 50, 500 ng/mL for etoposide; 100, 500, 2500 ng/mL for gemcitabine and carboplatin; 200, 1000, 5000 ng/mL for pemetrexed). The QC samples were stored at −20 °C and brought to room temperature (25 °C) before being processed together with the clinical samples.

Table 1
Optimized MRM parameters for the analytes and IS.

Analyte	Precursor ion	Fragmentor energy (V)	Collision energy (eV)	Product ion
Cyclophosphamide	261.0	80	14	140.1
Ifosfamide	260.7	80	20	154.0
Irinotecan	587.1	200	40	167.1
Pemetrexed	428.3	80	10	281.2
Gemcitabine	264.1	80	10	112.0
Carboplatin	372.2	80	10	294.1
Etoposide	589.0	150	10	229.1
Vindoline (IS)	457.2	150	20	188.0

2.5. Collection and storage of plasma samples

Of the 51 patients (24 women and 27 men), the means (ranges) of age and body weight were 42.5 (18–67) years and 62.5 (49–76) kg, respectively. Venous blood samples were collected from Shanghai Changzheng Hospital (Shanghai, China), which were gently placed in an ice bath until centrifugation. Some samples were obtained from patients at 0 h and 24 h after intravenous infusion at 300 mg/m^2 and 1000 mg/m^2 dosage for irinotecan and cyclophosphamide. The others came from patients at 0 h, 3 h and 24 h after intravenous infusion at 1200 mg/m^2, 80 mg/m^2, 2200 mg/m^2, 330 mg/m^2 and 800 mg/m^2 dosage for ifosfamide, etoposide, gemcitabine, carboplatin and pemetrexed. After centrifugal separation (2000 × *g*, 10 min, 4 °C), plasma was transferred to polypropylene tubes and immediately frozen (−20 °C) until analysis. All samples were processed within an hour. The experimental method was reviewed and approved by the Ethics Committee of Changzheng Hospital, and performed in Changzheng Hospital, Shanghai, China.

2.6. Sample preparation

For working solution A, 200 μL aliquot of the plasma sample, 20 μL of IS working solution (100 ng/mL) and 3 mL ethyl acetate were added successively. Extraction was performed by vortex mixing the tube vigorously for 3 min and then centrifugation for 10 min at 3500 × *g* at room temperature. The upper organic layer was collected and transferred into a 5 mL glass centrifuge tube, followed by evaporating at 45 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μL of the mobile phase. After vortex mixing, the reconstituted solvent was transferred into 1.5 mL eppendorf tubes and then centrifuged for 10 min at 13,000 × *g*. The clean supernatants were transferred to glass auto-sampler vials with inserts for analysis. A 10 μL aliquot of the resulting solution was injected into the chromatographic system for LC–MS/MS.

For working solution B, 100 μL aliquot of the plasma sample was treated with 300 μL methanol containing the IS (100 ng/mL). The mixture was vortex mixed for 3 min and centrifuged for 10 min at 13,000 × *g*. The upper organic layer was evaporated at 45 °C under a gentle stream of nitrogen. The residue was reconstituted in 100 μL of the mobile phase, and then vortex mixed (1 min), centrifuged (13,000 × *g*, 10 min), and injected for LC–MS/MS.

2.7. Method validation

A full validation procedure was performed including selectivity, linearity, lower limit of quantification (LLOQ), intra-day and inter-day precision and accuracy, recovery of analytes and stability after sample preparation [26].

Six pre-dose plasma samples from different humans were processed with and without analytes and IS in order to ensure the absence of interfering peaks, which was used to evaluate the selectivity.

Five calibration curves of the recovered standards were prepared to establish linearity and reproducibility of the LC–MS/MS system. Graphs were constructed correlating the peak area ratio of each analyte with the internal standard versus each analyte concentration, using a weighting factor of $1/\chi^2$.

The lower limit of quantification (LLOQ) is defined as the lowest concentration of the standard curve, giving a signal-to-noise ratio of 10:1, which can be measured with an acceptable level of precision (%RSD < 20%) and accuracy (%RE ± 20%).

Five replicates of QC samples at three levels were included in each run to determine the intra-day and inter-day precision and accuracy of the assay. Precision was evaluated as the % relative standard deviation (%RSD) of peak area ratios for each QC sample. Accuracy was expressed by the % relative error (%RE) of the measurement.

For bioanalytical LC–MS/MS assays, the matrix effects are generally due to the influence of co-eluting, undetected matrix components reducing or enhancing the ion intensity of the analytes, which affected the reproducibility and accuracy of the assay. By comparing neat standards peak areas ratios (analyte/IS) to the peak areas (analyte/IS) obtained with blank plasma samples spiked after the extraction, the matrix effect of ionization was evaluated. The recovery was assessed by comparing the peak area ratios (analyte/IS) obtained from spiked plasma samples to the peak area ratios (analyte/IS) spiked in deproteinized plasma samples.

The stability of analytes in spiked human plasma samples after three freeze/thaw cycles from nominally −20 °C to ambient temperatures was investigated in five replicates by comparing QC samples that had been frozen and thawed three times with the initial concentrations. The short-term stability was examined in human plasma by comparing the concentration of QC samples stored at room temperature for 12 h after spiking and mixing, before being frozen (nominal −20 °C), prior to analysis. The long-term stability was studied by analyzing QC samples which were stored at −20 °C for a whole month.

3. Results and discussion

3.1. Optimization of chromatographic and MS/MS conditions

In order to develop a faster and more sensitive analytical system, several HPLC columns: Agilent Zorbax SB-C18 column (2.1 mm × 100 mm, 3.5 μm), Agilent Zorbax SB-C8 column (2.1 mm × 100 mm, 3.5 μm), Atlantis T3-C18 column (2.1 mm × 100 mm, 3 μm) were tested. Elution of pemetrexed, carboplatin and gemcitabine needed high percentage (at least 80%) of organic solvent in the mobile phase. However, by using a high proportion of organic solvent (85% or so), the analytes could not be totally separated from the endogenous plasma components on the SB-C18 and C8 column. Fortunately, the T3-C18 column provided sufficient retention and suitable separation. Based on unique ultra-pure silica with specific modifications which was achieved by using a trifunctional C18 alkyl bonded phase and proprietary end-sealing technology, the T3-C18 column was used for further method development. Several mobile phase had been tested: 0.05% formic acid, 0.1% formic acid, 0.05% acetic acid, 0.1% acetic acid,

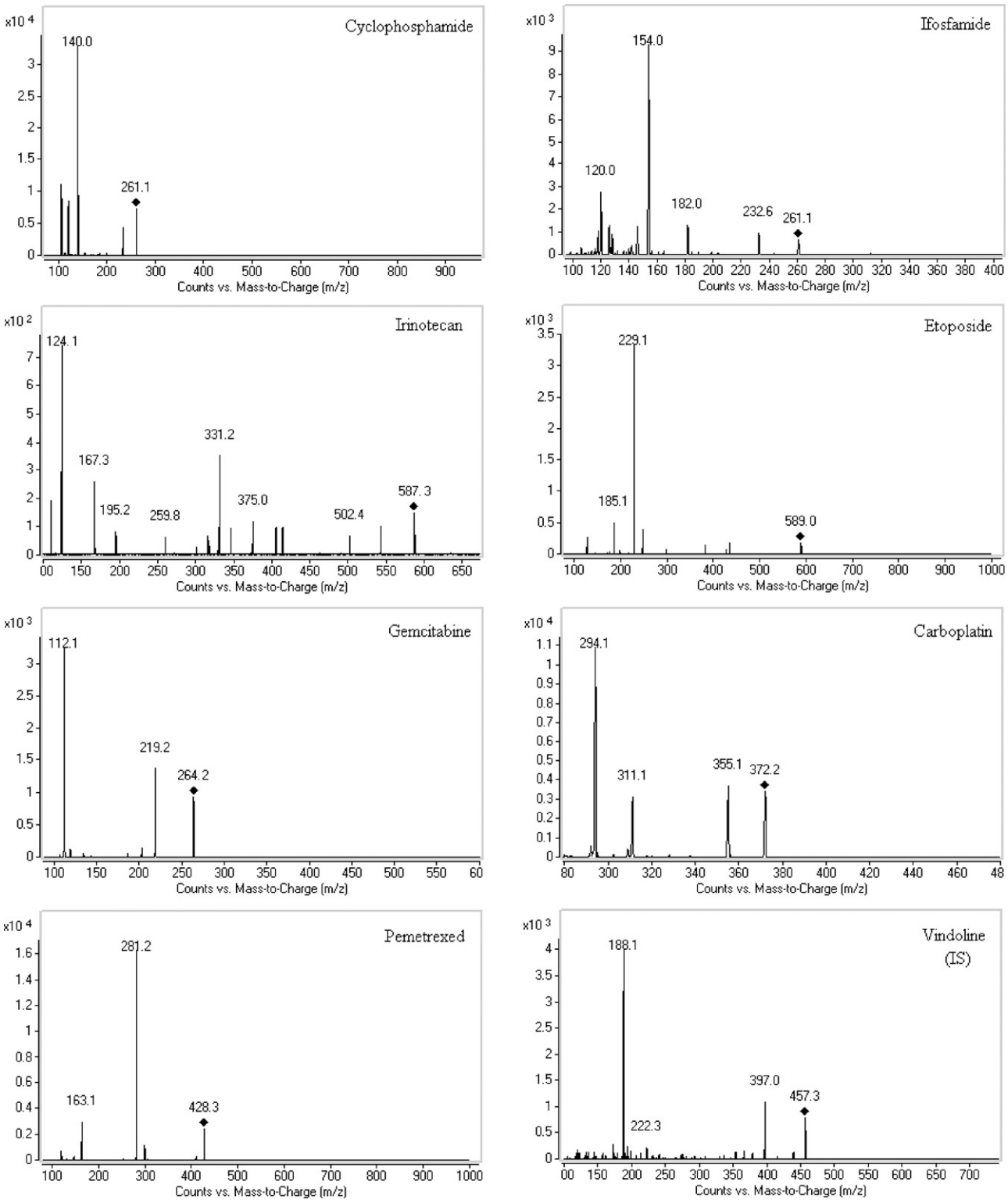

Fig. 2. Full scan product ion of precursor ions of cyclophosphamide, ifosfamide, irinotecan, etoposide, gemcitabine, carboplatin, pemetrexed and IS.

5 mM ammonium acetate, 10 mM ammonium acetate in combination with either methanol or acetonitrile. With methanol as organic solvent moderate tailing was observed, while with acetonitrile split peaks were obtained. As the temperature is raised, the resolution improved as indicated by the narrower the peak width at 10% of the height, HPLC column temperatures from 20 to 45 °C were tested. In conclusion, the most appropriate eluent was acetonitrile–10 mM ammonium acetate containing 0.1% formic acid in water pumped at a flow rate of 0.25 mL/min at 30 °C.

Since ESI source was suitable to analyze the polar compounds, while atmospheric pressure chemical ionization (APCI) source was good for the non-polar compounds, ESI source was chosen. Both positive and negative ionization had been tested. The results revealed that all the compounds were more sensitive in positive

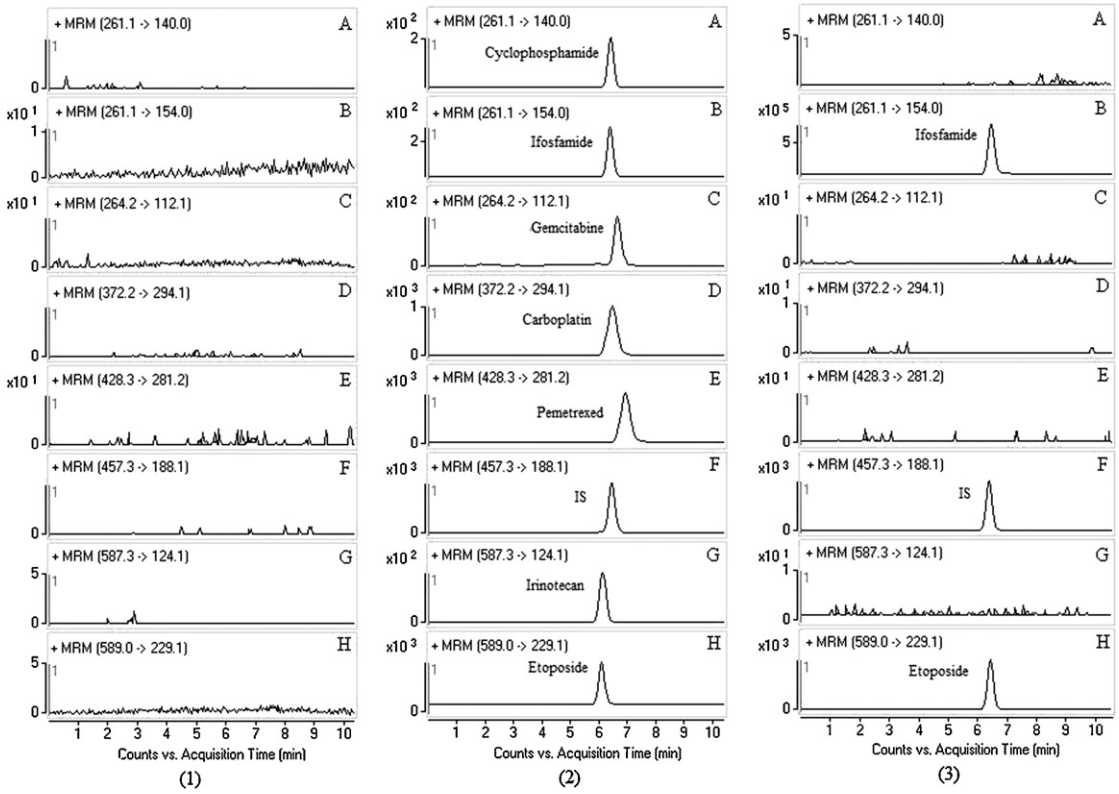

Fig. 3. Representative MRM chromatograms of cyclophosphamide (A), ifosfamide (B), gemcitabine (C), carboplatin (D), pemetrexed (E), vindoline (F, IS), irinotecan (G) and etoposide (H): (1) blank plasma sample, (2) blank plasma sample spiked with seven analytes at LLOQ and IS, and (3) plasma sample collected from a patient at 3 h after combined administration of etoposide and ifosfamide at the usual dose.

ionization mode. The advantages of ESI were demonstrated in our experiment by much less matrix influence and better sensitivity. These product ions were extracted for quantification as shown in Fig. 2.

3.2. Sample pre-treatment

Once the chromatographic conditions were properly adjusted, the extraction procedure was explored. Due to the complex nature of plasma, a sample pretreatment is often needed to remove protein and potential interferences prior to LC–MS/MS analysis. Different methods of sample pretreatment were investigated. Biological matrices were extracted on Oasis HLB cartridges pre-conditioned with methanol and acetate buffer, which was developed for gemcitabine by Vainchtein et al. [27], but none of these solvents were able to efficiently extract the analytes. As protein precipitation (PPT) was by far the easier and faster way of sample pretreatment, different type of solvents and reconstituted solutions were tested to extract the analytes [28]. The total recovery of the compounds by using methanol was higher and more reproducible than with acetonitrile for carboplatin, pemetrexed and gemcitabine. As LLE was widely described by several authors [29–31], tert-butyl ether, diethyl ether, ethyl acetate, dichloromethane and mixture in various proportions were investigated. By evaluating recovery and sample clean up, ethyl acetate proved to be the best extraction solvent and a miniaturized extraction procedure was tested for cyclophosphamide, ifosfamide, irinotecan and etoposide.

3.3. Method validation

3.3.1. Selectivity

Selectivity was verified by examining any interference with the peaks for analytes and IS at the same mass transitions and retention times from using six extracted individual blank human plasma samples compared to six replicates of plasma spiked to contain analytes at the LLOQ prior to extraction. Selectivity in spiked samples was within the acceptable criteria and no endogenous peaks interfering were observed in the MRM chromatograms of blank human plasma

Table 2
Linearity parameters of the analytes determined ($n = 5$).

Analyte	Linearity range (ng/mL)	$A = ac + b$		R^2	LLOQ (ng/mL)
		a	b		
Cyclophosphamide	1–1000	4.4292	0.0056	0.9940	1
Ifosfamide	1–1000	8.0562	0.0024	0.9929	1
Irinotecan	10–10,000	3.2214	−0.3354	0.9942	10
Etoposide	5–5000	0.1760	−0.0053	0.9924	5
Gemcitabine	50–5000	0.8479	1.7650	0.9940	50
Carboplatin	50–5000	0.0332	−0.0053	0.9982	50
Pemetrexed	100–10,000	0.3433	−0.0531	0.9959	100

Table 3
Intra- and inter-day precision and accuracy for the analytes in human plasma ($n = 15$, 5 replicates per day for 3 days).

Analyte	Nominal concentration (ng/mL)	Intra-day ($n = 5$)			Inter-day ($n = 5$)		
		Measured concentration (mean ± S.D., ng/mL)	Precision (%RSD)	Accuracy (%RE)	Measured concentration (mean ± S.D., ng/mL)	Precision (%RSD)	Accuracy (%RE)
Cyclophosphamide	2	1.99 ± 0.18	9.0	0.2	2.09 ± 0.15	7.2	−4.3
	10	9.79 ± 0.34	3.5	2.1	9.81 ± 0.48	4.9	1.9
	100	96.11 ± 2.98	3.1	3.9	98.81 ± 2.55	2.6	1.2
Ifosfamide	2	2.10 ± 0.08	3.9	−5.0	2.06 ± 0.07	3.4	−3.1
	10	10.14 ± 0.54	5.4	−1.4	9.75 ± 0.51	5.2	2.5
	100	98.00 ± 3.59	3.6	1.0	99.88 ± 3.68	3.7	0.1
Irinotecan	20	18.21 ± 0.26	1.4	9.0	17.97 ± 0.19	1.1	10.1
	100	94.78 ± 1.95	2.1	5.2	93.49 ± 6.63	7.1	6.5
	1000	935.94 ± 34.89	3.7	6.4	924.88 ± 42.47	4.6	7.5
Etoposide	10	9.54 ± 0.64	6.7	4.6	9.37 ± 0.86	9.2	6.3
	50	44.84 ± 1.28	2.8	10.3	47.10 ± 1.90	4.0	5.8
	500	459.55 ± 17.63	3.8	8.1	464.08 ± 7.99	1.7	7.2
Gemcitabine	100	102.87 ± 6.47	6.3	−2.9	106.42 ± 6.93	6.5	−6.4
	500	503.98 ± 42.92	8.5	−0.8	511.38 ± 27.72	5.4	−2.3
	2500	2647.73 ± 129.92	4.9	−5.9	2629.66 ± 159.83	6.1	−5.2
Carboplatin	100	109.59 ± 4.72	4.3	−9.6	109.61 ± 4.37	4.0	−9.6
	500	531.50 ± 34.46	6.5	−6.3	551.33 ± 21.61	3.9	−10.3
	2500	2711.20 ± 140.81	5.2	−8.4	2593.96 ± 188.96	7.3	−3.8
Pemetrexed	200	206.32 ± 15.10	7.3	−3.2	213.85 ± 16.56	7.7	−6.9
	1000	1103.17 ± 35.11	3.2	−10.3	1104.96 ± 44.94	4.1	−10.5
	5000	5397.36 ± 448.56	8.3	−7.9	5080.36 ± 334.63	6.6	−1.6

samples. Under the conditions set forth, the retention time was 6.44 min for cyclophosphamide, 6.39 min for ifosfamide, 5.99 min for gemcitabine, 1.79 min for carboplatin, 6.25 min for pemetrexed, 6.11 min for irinotecan, 6.41 min for etoposide and 6.42 min for IS. Fig. 3 showed the representative chromatograms of the blank plasma, blank plasma spiked with seven analytes and IS, and plasma obtained 3 h after combined administration of etoposide and ifosfamide.

3.3.2. *Linearity of calibration curves and LLOQ*

The linearity of the method was evaluated by analyzing seven calibration standards in duplicate over the nominal concentration, and it complied with the predefined acceptance criteria in Table 2. Graphs were constructed correlating the peak area ratio of each analyte with IS versus each analyte concentration. A weighting factor $1/\chi^2$ was used. Five of equation curves exhibited an excellent relationship with a mean ± SD.

Table 4
Recovery data for the analytes in human plasma ($n = 5$).

Analyte	Nominal concentration (ng/mL)	Extraction recovery		Matrix effect	
		Mean (%)	RSD (%)	Mean (%)	RSD (%)
Cyclophosphamide	2	65.6	9.3	85.6	8.8
	10	58.6	10.6	81.7	6.4
	100	61.6	7.2	85.0	7.1
Ifosfamide	2	65.1	7.9	71.0	6.4
	10	56.6	5.4	72.5	3.1
	100	62.4	7.1	78.8	3.5
Irinotecan	20	73.9	6.0	89.9	6.6
	100	59.6	5.4	86.7	5.9
	1000	66.8	5.9	79.8	6.0
Etoposide	10	67.2	11.3	77.1	11.2
	50	61.0	9.8	78.3	10.5
	500	72.8	8.6	78.4	2.0
Gemcitabine	100	81.0	7.1	75.3	13.1
	500	80.5	3.8	64.3	4.7
	2500	73.6	6.8	66.6	4.3
Carboplatin	100	61.7	5.5	86.9	10.0
	500	50.0	2.9	78.1	8.4
	2500	56.5	4.9	82.7	4.2
Pemetrexed	200	72.0	9.0	81.7	9.9
	1000	64.7	8.1	74.3	7.1
	5000	60.2	7.4	77.9	4.4
IS	100	98.0	2.8	99.6	3.1

Table 5
Stability of analytes in human plasma ($n = 5$).

Analyte	Nominal concentration (ng/mL)	Three freeze–thaw (%RE[a])	Short-term (12 h at 25 °C) (%RE)	Long-term (1 m at −20 °C) (%RE)
Cyclophosphamide	2	−1.9	1.0	−3.9
	10	−2.2	2.2	1.1
	100	−8.6	−6.0	1.4
Ifosfamide	2	5.3	−1.4	−0.9
	10	−9.0	−0.1	−0.2
	100	−9.7	−2.4	−3.4
Irinotecan	20	9.2	10.6	12.3
	100	7.0	6.6	5.3
	1000	−9.6	−2.3	7.9
Etoposide	10	4.9	2.5	7.3
	50	7.9	4.7	5.4
	500	−9.1	−6.2	3.0
Gemcitabine	100	−2.2	−3.2	−2.5
	500	−2.3	0.2	−0.7
	2500	−4.8	−4.9	−8.1
Carboplatin	100	−4.4	−6.4	−5.5
	500	−5.8	−7.2	−5.7
	2500	−2.0	−10.2	−3.4
Pemetrexed	200	−1.5	−3.2	−0.3
	1000	−9.4	−7.5	−8.1
	5000	−3.7	−9.3	0.3

[a] RE is expressed as (measured concentration/freshly prepared concentration − 1) × 100%.

3.3.3. Precision and accuracy

Intra-day precision and accuracy were determined by extracting plasma supplemented with all of the analytes at three QC concentrations ($n = 5$) as described in the experiment section. Intra-day precision ranged between 1.4 and 9.0%, and accuracy ranged between −10.3 and 10.3%. Inter-day precision ranged between 1.1 and 9.2%, and accuracy ranged between −10.5 and 10.1%. Precision and accuracy data of the method are presented in Table 3, which were acceptable at all QC levels for quantification in human plasma.

3.3.4. Recovery and matrix effect

When analyzing the supernatant of cyclophosphamide, ifosfamide, irinotecan and etoposide from plasma sample using PPT, salts and endogenous materials were present that could cause ion suppression or enhancement, which was greater than that of LLE and SPE. Using the mixed extracting mode, including LLE and PPT, the results of recovery from plasma and the matrix effect on ionization were presented in Table 4. The absolute matrix effects were expressed as the ratios of the mean peak areas of analytes spiked post-extraction to that of the neat standards at corresponding concentrations. The value of 100% indicates no absolute matrix effect was observed, and the value of <100% illustrates ionization suppression while the value of >100% indicates ionization enhancement. Absolute matrix effect of the analytes at high, middle and low concentrations ranged between 64.3 and 89.9%, which showed that matrix had caused strong ion suppression. The recovery of the analytes and IS was determined by calculating the ratios of the mean peak areas of regularly prepared QC samples to that of post-extraction spiked samples. Then the foregone conclusion is that the extraction recovery was less than 100%. But matrix effect of QC samples at low, middle and high concentrations were of good consistency, displaying no change by the concentration change, and CV was quite acceptable. So the accuracy and reproducibility of our method were not being affected.

3.3.5. Analyte stability

The stability of all the analytes was assessed under various conditions. Resuspended plasma extracts of all the analytes were found to be stable for 12 h in the autosampler at room temperature, and also resisted the effect of up to three freezing and thawing cycles.

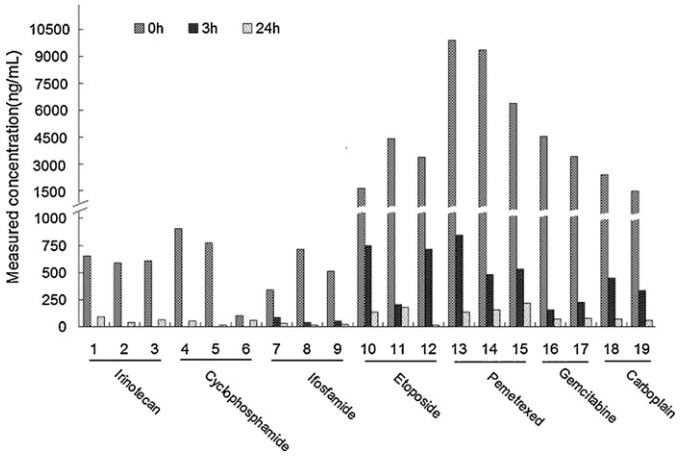

Fig. 4. The determination results of each plasma concentration.

The analytes in plasma stored at −20 °C up to 1 month also showed no significant degradation. All of the results were shown in Table 5.

3.4. Application to clinical plasma samples of cancer patients

The suitability of the present analytical method for human clinical samples was demonstrated by the determination of cyclophosphamide, ifosfamide, irinotecan, etoposide, gemcitabine, carboplatin and pemetrexed in clinical samples. Plasma samples from 51 cancer patients were assayed by the proposed method. The LC–MS/MS determining results are shown in Fig. 4.

4. Conclusion

Based on the use of a polar-modified T3-C18 column, the retention time and peak shape of polar material were significantly improved with an acidic gradient system. Using a combination of PPT and LLE, we were able to achieved a high efficiency of extraction. In a word, the LC–MS/MS method we described here provides a rapid, sensitive, reproducible, and accurate technique to

simultaneously quantitate cyclophosphamide, ifosfamide, irinotecan, etoposide, gemcitabine, carboplatin and pemetrexed, which has been successfully applied to analyze these drugs in human plasma samples from cancer patients, indicating that it appears to be reliable and clinically useful approach for TDM.

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