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A full validated hydrophilic interaction liquid chromatography-tandem mass spectrometric method for the quantification of oxaliplatin in human plasma ultrafiltrates

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Abstract

Oxaliplatin is a platinum agent that is used for treatment of colorectal cancer. A sensitive and selective hydrophilic interaction liquid chromatography-tandem mass spectrometric method for the quantification of oxaliplatin was developed. Human plasma ultrafiltrates were precipitated by acetonitrile containing carboplatin as an internal standard and further diluted with acetonitrile. Chromatographic separation of oxaliplatin and the internal standard was achieved with a column modified with phosphorylcholine and an isocratic mobile phase (acetonitrile/water/acetic acid = 90:10:0.1, v/v/v) at the flow rate of 0.2 mL/min. The lower limit of quantification for oxaliplatin was 25 ng/mL. The linearity range of the method was from 25 to 5000 ng/mL. The intra-day precision and inter-day precision (RSD) ranged from 0.8 to 6.1%, and the accuracy (RE) was within ±4.5%. The extraction recoveries from human plasma ultrafiltrates were 83.6-91.6%, and ion suppression caused by matrix components was 86.7-88.5% at three different levels, respectively. This method was applied to a clinical pharmacokinetic study of oxaliplatin in a cancer patient. The maximum concentration of colorectal cancer patient administered oxaliplatin was 1650 ng/mL.

Keywords

Oxaliplatin; Hydrophilic interaction liquid chromatography-tandem mass spectrometry; Plasma ultrafiltrates

1. Introduction

Oxaliplatin (1R, 2R-diaminocyclohexane-oxalatoplatinum (II)) is a third-generation of platinum agent that is used for treatment of colorectal cancer. Oxaliplatin was developed to reduce the nephrotoxicity of cisplatin as the first-generation platinum agents and myelosuppression of carboplatin as the second-generation platinum agents. Platinum agents are activated intracellularly to form reactive platinum complexes that bind to nucleophilic groups producing both inter- and intra-strand crosslinks in DNA, thereby inhibiting DNA replication [1]. The majority of previously untreated metastatic colorectal cancer patients are treated with oxaliplatin and infusional 5-fluorouracil/leucovorin or capecitabine (FOLFOX or XELOX) [2]. It has been reported that the AUC of platinum agents correlates with the degree of adverse events such as thrombopenia [3,4]. Besides, oxaliplatin was one of major organic cation transporter (OCT) 2 substrates. OCT2 is located on the basolateral membrane of the proximal tubular epithelium in the kidney and related to uptake of cationic drugs in the kidney. Burger et al. indicated that high OCT2 expression caused to platinum toxicity [5]. Therefore, therapeutic drug monitoring of platinum agents could be an effective tool to reduce adverse effects. To establish therapeutic drug monitoring of platinum agents, it is necessary to determine the plasma target concentration and to clarify the cause of variation in pharmacokinetics.

Methods using atomic absorption spectrometry [6] and inductively coupled plasma–mass spectrometry [7] have been developed for the determination of oxaliplatin. However, the total quantity of the element platinum is measured by these methods, and the intact form cannot be distinguished from inactive platinum intermediates. For pharmacokinetic investigation, it is necessary to measure intact oxaliplatin. Ficarra et al. reported the HPLC-UV method for measuring intact oxaliplatin [8]. However, the inherent lack of favorable UV absorption properties of these agents has led to poor sensitivity. They reported that the limit of quantification of oxaliplatin was 70 μg/mL [8]. To obtain more sensitivity with UV detection, post-column derivatization techniques have been needed [9,10].

The LC/MS/MS system has been widely used for the determination of drugs because of its high sensitivity and selectivity. Luo et al. reported the LC/ESI-MS method for the determination of the intact oxaliplatin and its biotransformation products in rat plasma [11]. Recently, Zhang et al. reported the LC/MS/MS method for the determination of the intact oxaliplatin in human plasma [12]. The sensitivity of their method is comparable with that of atom absorption spectrometry. However, they used a long ODS column (250 mm) to avoid the matrix effect by endogenous impurities. Hydrophilic interaction chromatography (HILIC) using bare silica or polar bonded phase and low aqueous/high organic mobile phase has been shown to be a suitable method for the quantification of polar compounds in biological samples. HILIC is superior separation of polar compounds in biological samples with reversed retention to the traditional reversed-phase liquid chromatography [13-16]. Recently, Hemström et al. and Falta et al. reported the ICP-MS method combined with HILIC for the determination of total platinum concentrations in human plasma ultrafiltrates [17,18]. However, the method was not validated, so we considered that their method did not have higher confidence. Their method could determine total platinum concentrations, but that could not distinguish intact oxaliplatin from platinum intermediates which do not have biological activity. Moreover, the method was not applied to the actual patients, who were administered oxaliplatin.

In this paper, we describe a HILIC/MS/MS method for the determination of oxaliplatin in human plasma ultrafiltrates and results of application of the method to a pharmacokinetic study in a cancer patient. The sample preparation of our established method employs protein precipitation and dilution with acetonitrile without evaporation step, meaning that not only it needs simple technique but also the exposure of antineoplastic drugs could be avoidable.

2. Materials and methods

2.1. Materials

Oxaliplatin, carboplatin, and acetic acid were purchased from Wako (Osaka, Japan). Purity of oxaliplatin standard was 97.0+%. All other solvents and regents were of HPLC grade. Human

plasma with sodium heparin as the anticoagulant was obtained from Terumo (Tokyo, Japan).

2.2. Sample preparation

2.2.1. Preparation of stock and working solution

A stock solution of oxaliplatin was prepared in methanol/water (50:50, v/v) at a concentration of 100 μg/mL and was stored at -80°C. As an internal standard solution, 10 μg/mL carboplatin in acetonitrile was stored in the dark at -80°C.

2.2.2. Preparation of calibration standards and validation samples

We obtained human plasma ultrafiltrates using Centrifree® Uitrafiltration Devices purchased from Millipore (Tokyo, Japan). Calibration standards of oxaliplatin in human plasma ultrafiltrates were prepared by diluting a stock solution at a concentration of 5000 ng/mL and serially diluting a plasma working solution at concentrations of 25, 50, 100, 200, 500, 1000 and 2000 ng/mL. Quality Control samples (QC) of oxaliplatin in human plasma ultrafiltrates were prepared by diluting human plasma ultrafiltrates working solutions at concentrations of 25, 50, 500 and 5000 ng/mL. All solutions were stored at -80°C.

2.3. Sample preparation

Human plasma ultrafiltrates were precipitated by acetonitrile containing carboplatin as the internal standard. To 40-μL sample volumes, 60 μL internal standard in acetonitrile containing 10000 ng/mL of internal standard was added. The samples were then centrifuged for 10 min at 10,000×*g*. The supernatant was diluted twenty-fold with acetonitrile. Ten μL of solution was injected into the HPLC column.

2.4. Chromatographic and mass spectrometric conditions

The HPLC system consisted of a fully equipped Prominence20A (Shimadzu, Kyoto, Japan).

For the analytical column, we used PC HILIC (50 × 2.0 mm i.d., 3 μm, Shiseido, Tokyo, Japan). The column temperature was maintained at 40°C. Mobile phase A consisted of acetonitrile/water/acetic acid (90:10:0.1, v/v/v), and mobile phase B consisted of acetonitrile/water (50:50, v/v). The initial mobile phase composition was 100% mobile phase A, pumped at a flow rate of 0.2 mL/min for 5.0 min. From 5.0 to 5.5 min, mobile phase B was increased linearly from 0 to 100%. For removal of insoluble salts and matrix components, this setting was held for 4.5 min. From 10.0 to 10.5 min, mobile phase B was decreased to 0% and kept at that rate until 15.0 min, after which the next sample was injected. From 0 to 5 min, the flow was introduced into a mass spectrometer using a switching valve. The overall run time was 15.0 min.

Mass spectrometry was carried out in an API3200 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). Positive ionization electrospray mass spectrometry was performed. For the determination oxaliplatin, the ionspray voltage was set at 5000 V. The turbospray gas (N_2) probe was heated at 500°C. Nitrogen was used as curtain gas, gas 1 and gas 2, and their flows were set to 50, 60 and 80 units, respectively. Unit mass resolution was set in both mass resolving quadrupole Q1 and Q3. The declustering potential (DP) and collision energy (CE) for oxaliplatin were 32 V and 19 eV, and these for internal standard were 46 V and 27 eV, respectively. The transitions of *m/z* 398 → 306 and *m/z* 372 → 294 on selected reaction monitoring (SRM) were used for monitoring oxaliplatin and carboplatin, respectively. The dwell time was 500 msec. Data were collected and processed using Analyst 1.4.2 data collection and integration software (Applied Biosystems).

2.5. Method validation

2.5.1. Linearity and lower limit of quantification (LLOQ)

For validation, oxaliplatin standards (eight non-zero standards of the analyte, 25, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL for oxaliplatin) were prepared in control human plasma ultrafiltrates and analyzed. Linear regression of ratio of the areas of the analyte and internal standard peaks

versus the concentration were weighted by 1/x (reciprocal of the concentration). LLOQ was defined as the concentration with a signal-to-noise ratio of at least 10 and acceptable precision and accuracy data (RSD and RE less than 20%).

2.5.2. Precision and accuracy

Intra-day (n=6) and inter-day (n=6) precision and accuracy were investigated at four different levels, 50, 500 and 5000 ng/mL, for oxaliplatin. The precision (RSD) was determined on the basis of the coefficient of variation (RSD (%)), and the accuracy was calculated as ((found concentration–theoretical concentration)/theoretical concentration) × 100 (RE (%)).

2.5.3. Specificity and selectivity

To investigate whether endogenous matrix constituents interfered with the assay, control drug-free plasma ultrafiltrate samples containing neither analyte nor internal standard (double blank) and plasma ultrafiltrate samples containing LLOQ (25 ng/mL) of oxaliplatin and internal standard were prepared. Human plasma ultrafiltrates were prepared from six different donors.

2.5.4. Stability

The stability of oxaliplatin in human plasma ultrafiltrates was examined by analyzing three concentrations (50, 500 and 5000 ng/mL) in triplicate. These samples were stored at -80°C for 4 weeks and at 4°C for 4 hours to evaluate long-term and short-term stability, respectively. Freeze-thaw stability was tested following three freeze-thaw cycles (-80°C to 4°C). Stability of the processed samples was assessed by reinjecting the samples after 4 hours in an autosampler (4°C).

2.5.5. Extraction recovery from human plasma ultrafiltrates and matrix effect

Extraction recovery of oxaliplatin from human plasma ultrafiltrates was evaluated at three concentrations by comparing peak areas obtained from samples spiked with oxaliplatin before

extraction with those obtained from samples to which oxaliplatin was added after extraction.

The matrix effect of components from human plasma ultrafiltrates on oxaliplatin ionization was also evaluated at three concentrations by comparing peak areas obtained from plasma ultrafiltrate samples after extraction with those obtained from samples without matrix components.

For infusion experiments, reference standard oxaliplatin (50 μg/mL, 0.1 mL/min) was continually infused and then the corresponding matrix samples with (processed samples) or without dilution (only protein precipitation samples) with acetonitrile were injected.

2.6. Application to clinical pharmacokinetic study

The method was applied to the determination of plasma concentrations of oxaliplatin in a colorectal cancer patient hospitalized in Hokkaido University Hospital. Before treatment, the patient gave written informed consent as approved by the institutional review board. We applied our method to samples obtained from a cancer patient receiving 85 mg/m^2 of oxaliplatin i.v. over 2 hours. The blood samples were collected into sodium heparin-containing tubes at 0, 0.5, 1 and 1.5 h post-dosing. Samples were immediately stored at 4 °C, and plasma ultrafiltrates were obtained. The resultant ultrafiltrates were stored at -80°C until use.

3. Results and discussion

3.1. Mass spectrometry

^{194}Pt, ^{195}Pt, ^{196}Pt and ^{198}Pt are four major natural isotopes of platinum, and their natural abundances are of 32.9, 33.9, 25.3 and 7.2%, respectively. Positive ion full-scan mass spectra (Q1) of oxaliplatin indicated the presence of protonated molecular ions $[M+H]^+$ with *m/z* of 397, 398, 399 and 401, corresponding to Pt isotope abundance ratio. A protonated molecular ion with *m/z* of 398 was most strongly generated and was used for the precursor ion. The product ion mass spectrum of $[M+H]^+$ at *m/z* of 306 is shown in Fig. 1A. The product ion at *m/z* of 306 was the most strongly produced and was used for quantitative SRM of oxaliplatin. The proposed fragmentation pattern for oxaliplatin is

presented in Fig. 1A.

Positive ion full-scan mass spectra (Q1) of carboplatin as an internal standard indicated the presence of protonated molecular ions $[M+H]^+$ with *m/z* of 371, 372, 373 and 375, corresponding to Pt isotope abundance ratio. A protonated molecular ion with *m/z* of 372 was most strongly generated, and it was used for the precursor ion. The product ion at *m/z* of 294 was the most strongly produced (Fig. 1B) and was used for quantitative SRM of the internal standard.

3.2. Chromatography

Oxaliplatin and the internal standard are hydrophilic molecules and are consequently poorly retained on reversed-phase chromatography columns such as Inertsil ODS-3 (GL Science, Tokyo, Japan), CACELL PAK MGII (Shiseido, Tokyo, Japan), and TSKgel ODS 100V (TOSOH, Tokyo, Japan). The present method employed HILIC using a column modified with phosphorylcholine. HILIC mode is suitable for polar compounds such as oxaliplatin.

Using the mobile phase described in section 2.4, oxaliplatin and the internal standard were base-line separated. The retention time of oxaliplatin was 2.4 min and that of the internal standard was 4.1 min. We avoided reduction of the signal by ion suppression from impurities in plasma samples by washing the analytical column using mobile phase B.

3.3. Validation

3.3.1. Linearity and lower limit of quantification (LLOQ)

The present method covered a linearity range of 25 to 5000 ng/mL for the concentration of oxaliplatin in plasma ultrafiltrates with a weighting by 1/x. The correlation coefficient (r) was >0.999 for oxaliplatin determination. A typical standard curve was y = 0.646x - 0.00802. The LLOQ for oxaliplatin was 25 ng/mL. This value is comparable with the method by Zhang et al. [12].

3.3.2. Precision and accuracy

Intra-day (n=6) and inter-day (n=6) precision and accuracy were investigated at four different levels, 25, 50, 500 and 5000 ng/mL, for oxaliplatin. The results are summarized in Table 1. For the determination of oxaliplatin, the intra- and inter-day precisions were less than 6.1% for all concentrations. The accuracies were within ±4.5% for all concentrations. These results suggest that intact oxaliplatin in human plasma can be measured accurately and reproducibly by the present method.

3.3.3. Specificity and selectivity

We evaluated the specificity and selectivity of the method. A representative chromatogram of blank human plasma and chromatograms of LLOQ of oxaliplatin spiked in human plasma are shown in Fig. 2A, 2B. There is no interference from an endogenous substance.

3.3.4. Stability

The stability of oxaliplatin in human plasma ultrafiltrates is shown in Table 2. We confirmed that oxaliplatin in human plasma ultrafiltrates is stable for 4 weeks at a temperature below -80°C, 4 hours at a temperature below 4°C, and after processing. In addition, freeze-thaw stability also had good value. Therefore, we decided to preserve oxaliplatin in human plasma ultrafiltrates at -80°C and thaw them just before measurement.

3.3.5. Extraction recovery from human plasma ultrafiltrates and matrix effect

The extraction recoveries from human plasma ultrafiltrates were 83.6-91.6% (Table 3). Ion suppression caused by the matrix components is shown in Fig. 3. Because of the matrix components, ionization of oxaliplatin was suppressed to 50% (Fig. 3 (B)); however, by diluting plasma with acetonitrile, ionization of oxaliplatin recovered to nearly 90% (Fig. 3 (C)). The dilution of matrix components with acetonitrile was possible to remove the effect of matrix components and improve the ionization of the oxaliplatin. However, we confirmed that more than twenty times dilution with

acetonitrile caused to decline of sensitivity due to high dilution ratio. Thus, we thought that this sample preparation was best condition for our method.

3.4. Application to a clinical pharmacokinetic study

The plasma intact oxaliplatin concentration versus time curve in a colorectal cancer patient after infusion for 2 hours is shown in Fig. 4. Plasma concentration of oxaliplatin at 2 hours after infusion was less than the LLOQ. The chromatogram of oxaliplatin in plasma ultrafiltrates taken from a patient at the end of oxaliplatin fusion is shown in Fig. 2D. The maximum concentration was 1650 ng/mL. This result agrees with past report [12], indicating the applicability of this method to pharmacokinetic study of oxaliplatin.

4. Conclusion

We have developed a sensitive and selective hydrophilic interaction liquid chromatography-tandem mass spectrometric method for the quantification of oxaliplatin in human plasma. Using samples diluted with acetonitrile, we succeeded in reducing ion suppression caused by the matrix effect. Our method does not need an evaporation step for sample preparation, which makes a brief sample preparation and a minimal occupational exposure. Validation demonstrated that this method was high levels of sensitivity and selectivity and sufficient precision and accuracy. The method was applied to a clinical pharmacokinetic study of oxaliplatin in a cancer patient.

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References

[1] Z.H. Siddik, Cisplatin: mode of cytotoxic action and molecular basis of resistance, Oncogene. 22

(2003) 7265-7279.

[2] S. Welch, K. Spithoff, R.B. Rumble, J. Maroun, Bevacizumab combined with chemotherapy for patients with advanced colorectal cancer: a systematic review, Ann. Oncol. 21 (2010) 1152-1162.

[3] M.J. Egorin, D.A. Van Echo, S.J. Tipping, E.A. Olman, M.Y. Whitacre, B.W. Thompson, J. Aisner, Pharmacokinetics and dosage reduction of cis-diammine (1,1-cyclobutanedicarboxylato) platinum in patients with impaired renal function, Cancer Res. 44 (1984) 5432-5438.

[4] K. Hanada, M. Suda, N. Kanai, H. Ogata, Pharmacokinetics and toxicodynamics of oxaliplatin in rats: application of a toxicity factor to explain differences in the nephrotoxicity and myelosuppression induced by oxaliplatin and the other platinum antitumor derivatives, Pharm. Res. 27 (2010) 1893-1899.

[5] H. Burger, W.J. Loos, K Eechoute, J. Verweij, R.H. Mathijssen, E.A. Wiemer, Drug transporters of platinum-based anticancer agents and their clinical significance, Drug Resist. Updat. 14 (2011) 22-34.

[6] C. Kloft, H. Appelius, W. Siegert, W. Schunack, U. Jaehde, C. Kloft, H. Appelius, W. Siegert, W. Schunack, U. Jaehde, Determination of platinum complexes in clinical samples by a rapid flameless atomic absorption spectrometry assay, Ther. Drug Monit. 21 (1999) 631-637.

[7] J.G. Morrison, P. White, S. McDougall, J.W. Firth, S.G. Woolfrey, M.A. Graham, D. Greenslade, Validation of a highly sensitive ICP-MS method for the determination of platinum in biofluids: application to clinical pharmacokinetic studies with oxaliplatin, J. Pharm. Biomed. Anal. 24 (2000) 1-10.

[8] R. Ficarra, M.L. Calabro, P. Cutroneo, S. Tommasini, S. Melardi, M. Semreen, S. Furlanetto, P. Ficarra, G. Altavilla, Validation of a LC method for the analysis of oxaliplatin in a pharmaceutical formulation using an experimental design, J. Pharm. Biomed. Anal. 29 (2002) 1097-1103.

[9] R. Kizu, T. Yamamoto, T. Yokoyama, M. Tanaka, M. Miyazaki, A sensitive postcolumn derivatization/UV detection system for HPLC determination of antitumor divalent and

quadrivalent platinum complexes, Chem. Pharm. Bull. 43 (1995) 108-114.

[10] H. Ehrsson, I. Wallin, Liquid chromatographic determination of oxaliplatin in blood using post-column derivatization in a microwave field followed by photometric detection, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 795 (2003) 291-294.

[11] F.R. Luo, T.Y. Yen, S.D. Wyrick, S.G. Chaney, High-performance liquid chromatographic separation of the biotransformation products of oxaliplatin, J. Chromatogr. B Biomed. Sci. Appl. 724 (1999) 345-56.

[12] W. Zhang, L. Seymour, E.X. Chen, Determination of intact oxaliplatin in human plasma using high performance liquid chromatography-tandem mass spectrometry, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 876 (2008) 277-282.

[13] W. Naidong, Bioanalytical liquid chromatography tandem mass spectrometry methods on underivatized silica columns with aqueous/organic mobile phases, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 796 (2003) 209-224.

[14] P. Hemstrom, K. Irgum, Hydrophilic interaction chromatography, J. Sep. Sci. 29 (2006) 1784-1821.

[15] D.V. McCalley, Is hydrophilic interaction chromatography with silica columns a viable alternative to reversed-phase liquid chromatography for the analysis of ionisable compounds?, J. Chromatogr. A 1171 (2007) 46-55.

[16] B. Dejaegher, D. Mangelings, Y.V. Heyden, Method development for HILIC assays, J. Sep. Sci. 31 (2008) 1438-1448.

[17] P. Hemström, Y. Nygren, E. Björn, K. Irgum, Alternative organic solvents for HILIC separation of cisplatin species with on-line ICP-MS detection, J. Sep. Sci. 31 (2008) 599-603.

[18] T. Falta, G. Koellensperger, A. Standler, W. Buchberger, R.M. Mader, S. Hann, Quantification of cisplatin, carboplatin and oxaliplatin in spiked human plasma samples by ICP-SFMS and hydrophilic interaction liquid chromatography (HILIC) combined with ICP-MS detection, J. Anal. At. Spectrom. 24 (2009) 1336-1342.

Figure legends

Figure 1 Product ion mass spectra of oxaliplatin (A) and internal standard (B) and proposed fragmentation patterns.

Figure 2 Representative chromatograms of blank plasma (A) and LLOQ of oxaliplatin (25 ng/mL) (B) and an internal standard (C) spiked into human plasma ultrafiltrates and the chromatogram of oxaliplatin in plasma ultrafiltrates taken from a patient at the end of oxaliplatin infusion (D).

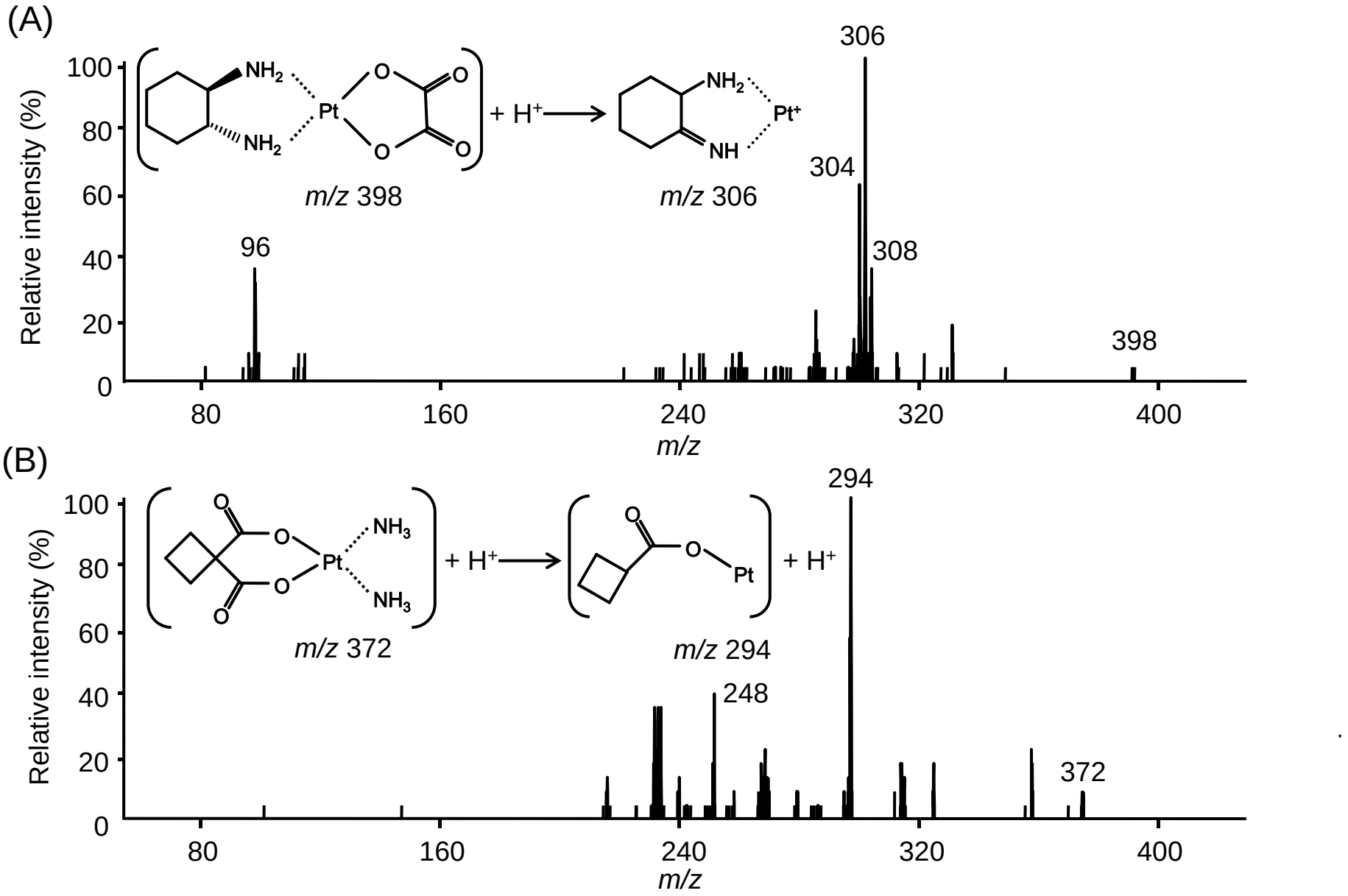
Figure 3 Matrix effect of plasma matrix components on oxaliplatin ionization. Arrow indicates the retention time of oxaliplatin.
Reference standard oxaliplatin (50 μg/mL, 0.1 mL/min) (black line), injection of plasma extract without diluting with acetonitrile (blue line), and injection of plasma extract diluted with acetonitrile (red line).

Figure 4 Intact oxaliplatin concentration in plasma ultrafiltrates versus time curve in a colorectal cancer patient after infusion for 2 hours.

Table 1 Precision and accuracy of the quantification of oxaliplatin in human plasma ultrafiltrates

Analyte	Concentration (ng/mL)	Intra-day (n=6)			Inter-day (n=6)		
		Found (ng/mL)	RSD (%)	RE (%)	Found (ng/mL)	RSD (%)	RE (%)
Oxaliplatin	25	24.5±1.3	5.40	-1.93	25.1±1.5	6.07	0.27
	50	49.7±2.8	5.54	-0.70	50.2±1.5	2.95	0.40
	500	493±14	2.76	-1.50	501±18.7	3.73	0.17
	5000	4770±147	3.08	-4.53	5000±378	0.75	0.07

Figure 1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40
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NH2
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Pt+
NH
m/z 398
m/z 306
306
304
308
96
398
80
160
240
320
400
m/z
(B)
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m/z 294
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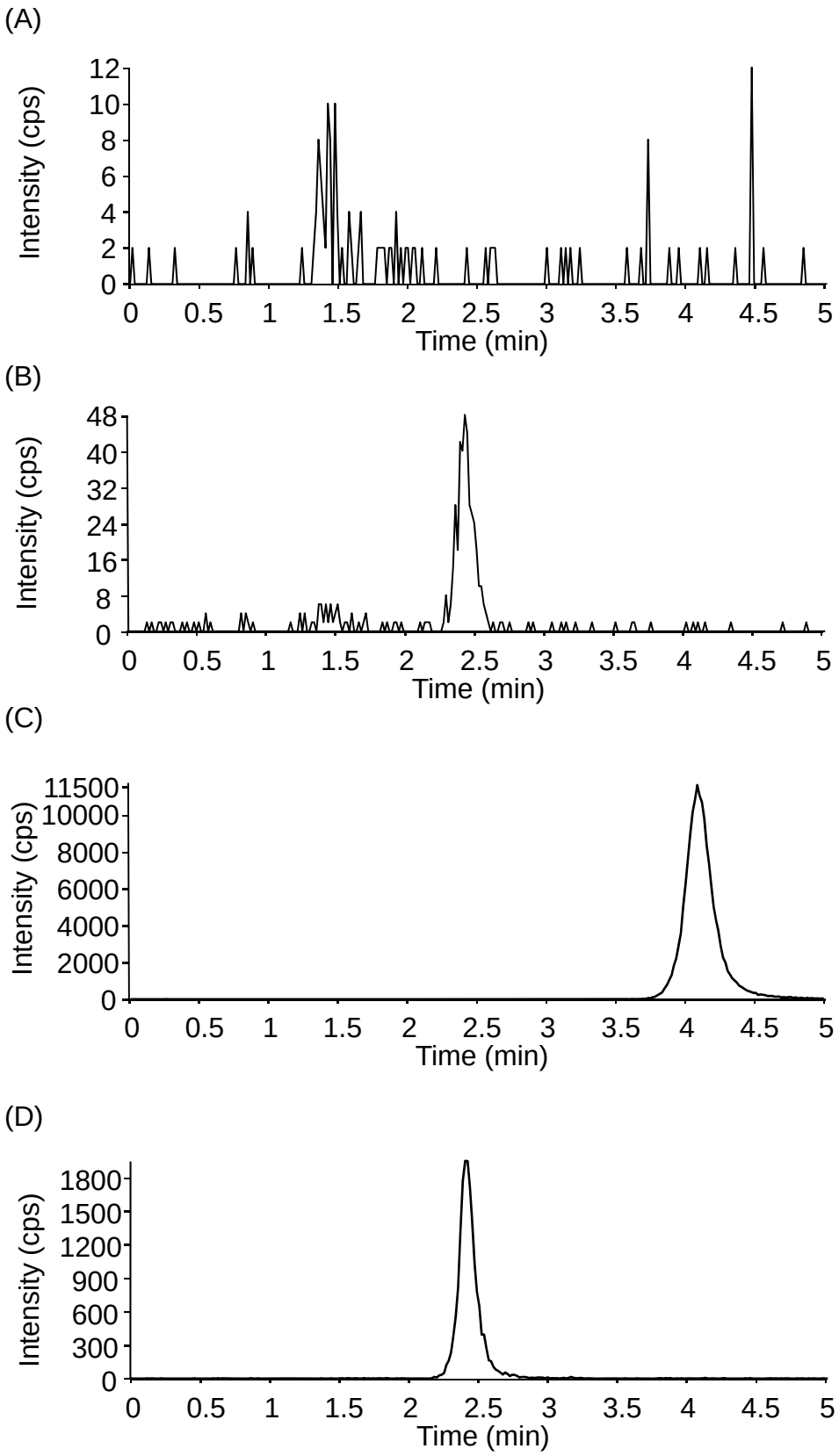
m/z

Figure 2


(A)
Intensity (cps)
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10
8
6
4
2
0
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5
Time (min)
(B)
Intensity (cps)
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40
32
24
16
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0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5
Time (min)
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0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5
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Time (min)

Figure 3

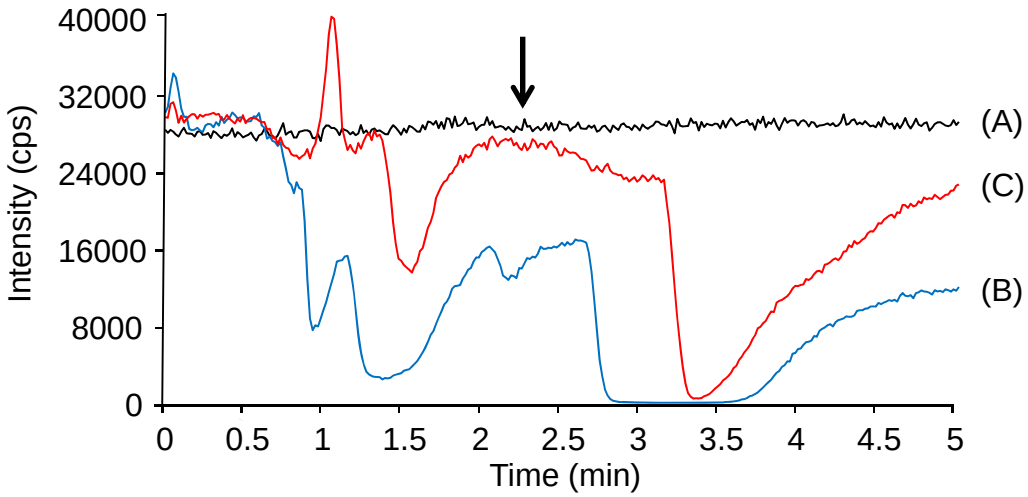
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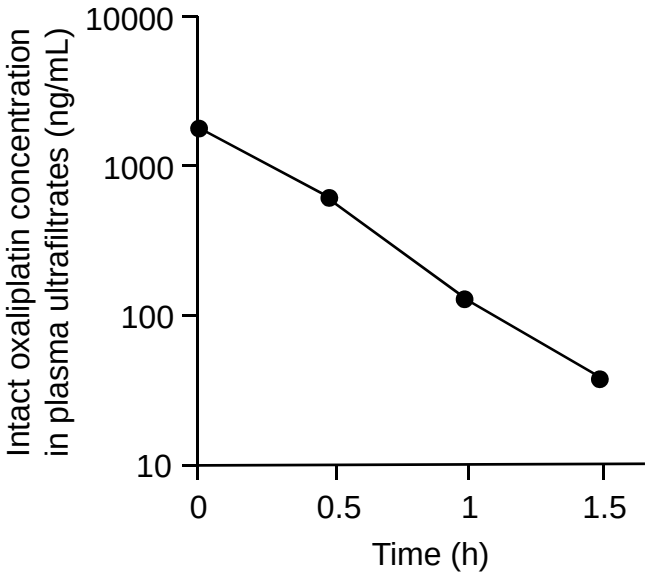
Figure 4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1000
100
10
0
0.5
1
1.5
Time (h)