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Short communication

Quantification of intact carboplatin in human plasma ultrafitrates using hydrophilic interaction liquid chromatography–tandem mass spectrometry and its application to a pharmacokinetic study

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ABSTRACT

Carboplatin is a platinum agent that is used for treatment of non-small-cell lung cancer and ovarian cancer. A sensitive and selective analytical method for the quantification of carboplatin in human plasma ultrafiltrates using liquid chromatography–tandem mass spectrometry was developed. Human plasma ultrafiltrates were precipitated by acetonitrile containing carboplatin-d4 as an internal standard and were further diluted with acetonitrile. Chromatographic separation was performed on a Accucore HILIC (50 mm × 2.1 mm i.d., 2.6 μm) column using mobile phase (acetonitrile–water–acetic acid = 90:10:0.1, v/v/v) at the flow rate of 0.2 mL/min. Detection was performed on electrospray ionization triple quadrupole tandem mass spectrometer using low-energy collision induced dissociation (CID-MS/MS) analysis operating in the selected reaction monitoring (SRM) scan mode. The lower limit of quantification for carboplatin was 0.025 μg/mL. This method covered a linearity range of 0.025–50 μg/mL. The intra-day precision and inter-day precision (R.S.D.) ranged from 1.5 to 4.3%, and the accuracy (R.E.) was within ±2.9%. The present method was applied to a clinical pharmacokinetic study of carboplatin in a cancer patient.

1. Introduction

Carboplatin (cis-diammine-1,1-cyclobutanedicarboxylatoplatinum (II)) is a second-generation platinum compound that is used for treatment of non-small-cell lung cancer and ovarian cancer. Carboplatin was developed for reducing the nephrotoxicity, nausea and vomiting induced by cisplatin, a first-generation platinum agent. Carboplatin in combination with other anti-neoplastic drugs such as paclitaxel is widely used for treatment of ovarian and lung cancers [1]. Platinum agents are widely associated with adverse events such as myelosuppression. It has been reported that the area under the plasma-concentration time curve (AUC) of platinum agents correlates with the degree of adverse events such as thrombocytopenia [2,3]. At present, the dose of carboplatin is calculated on the basis of a patient's renal function as defined by the glomerular filtration rate (GFR). There are various formulas used to estimate GFR based on serum creatinine, weight, sex and age. Therefore, the differences of calculated GFR among the formulas could be existed. Collins et al. mentioned that accurate estimation of renal function is important for the optimal dosing of carboplatin [4]. Therapeutic drug monitoring of platinum agents could be an effective tool to reduce adverse effects. To establish a method for therapeutic drug monitoring of platinum agents, it is necessary to determine the plasma target concentration and to clarify the cause of variation of pharmacokinetics.

Techniques using atomic absorption spectrometry [5] and inductively coupled plasma–mass spectrometry [6] have been developed for the determination of carboplatin. 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 carboplatin. HPLC-UV method for measuring intact carboplatin was reported by Villarino et al. [7]. 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 carboplatin was 1 μg/mL [7]. To obtain more sensitivity using

Abbreviations: HILIC, hydrophilic interaction chromatography; LLOQ, lower limit of quantification; SRM, selected reaction monitoring.

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UV detection, complex post-column derivatization techniques are needed [8].

The LC/MS/MS system has been widely used for determination of concentrations of drugs because of its high sensitivity and selectivity. Carboplatin molecules are hydrophilic and are consequently poorly retained on a reversed-phase chromatography column. Recently, highly sensitive LC/MS/MS methods using SCX column for determination of intact carboplatin in human plasma ultrafiltrates were reported [9,10]. The sensitivity of these methods (the reported LLOQ was 2 ng/mL) is comparable with that of atomic absorption spectrometry. Hydrophilic interaction chromatography (HILIC) using bare silica or a polar bonded phase and a low aqueous/high organic mobile phase is superior for separation of polar compounds in biological samples with reversed retention to the traditional reversed-phase liquid chromatography [11,12]. Recently, Falta et al. reported the usefulness of the ICP-MS method combined with HILIC for determination of total platinum concentrations in human plasma ultrafiltrates [13]. However, the method was not validated, so it was considered that their method did not have higher confidence. In this study, HILIC/MS/MS method for determining intact carboplatin in human plasma ultrafiltrates was developed and the method was applied to a pharmacokinetic study in a cancer patient. The sample preparation of our present method employs protein precipitation and dilution with acetonitrile without evaporation step. It means that not only it needs simple technique but also the exposure of antineoplastic drugs could be avoidable.

2. Materials and methods

2.1. Materials

Carboplatin was purchased from Wako (Osaka, Japan). Purity of carboplatin standard was 98.0+%. Carboplatin-d4 was purchased from Toronto Research Chemicals (Toronto, Canada). All other solvents and regents were of HPLC grade. Human plasma with sodium heparin as an anticoagulant was obtained from Terumo (Tokyo, Japan).

2.2. Sample preparation

2.2.1. Preparation of stock and working solutions

A stock solution of carboplatin was prepared in methanol–water (50:50, v/v) at a concentration of 500 μg/mL and was stored at 4 °C. As the internal standard solution, 1 μg/mL carboplatin-d4 in acetonitrile was used for the determination of carboplatin and was stored in the dark at −80 °C.

2.2.2. Preparation of calibration standards and validation samples

Human plasma ultrafiltrates were obtained using Centrifree(R) Uitrafiltration Devices from Millipore (Tokyo, Japan). Calibration standards of carboplatin in human plasma ultrafiltrates were prepared by diluting a stock solution at concentrations of 0.025, 0.05, 0.25, 0.5, 2.5, 5, 25 and 50 μg/mL. Quality control samples (QC) of carboplatin in human plasma ultrafiltrates were prepared by diluting a plasma working solution at concentrations of 0.1, 1 and 10 μg/mL. All solutions were stored at −80 °C.

2.3. Sample pretreatment

Human plasma ultrafiltrates were precipitated by acetonitrile containing internal standard. To each 20 μL sample volume, 80 μL of acetonitrile containing 1 μg/mL of internal standard was added. The samples were then centrifuged for 10 min at 10,000 × *g*. The supernatant was diluted twenty times with acetonitrile. Five μL of solution was injected onto the HPLC column.

2.4. Chromatographic and mass spectrometric conditions

The HPLC system was a fully equipped Prominence 20A (Shimadzu, Kyoto, Japan) with the Accucore HILIC column (50 mm × 2.1 mm i.d., 2.6 μm, Thermo, Waltham, MA). 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, 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 other materials, this setting was held for 2.5 min. From 7.5 to 8 min, mobile phase B was decreased to 0% and kept at that rate until 12.0 min, after which the next sample was injected. From 0 to 5 min, the flow was introduced into mass spectrometer using a switching valve. The overall run time was 12.0 min.

Mass spectrometry was carried out on an API 3200 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) using low-energy collision induced dissociation (CID-MS/MS) analysis operating in the selected reaction monitoring (SRM) scan mode. Positive ionization electrospray mass spectrometry was performed. For the determination of carboplatin, the ionspray voltage was set at 5000 V. The turbospray gas (N_2) probe was heated at 600 °C. Nitrogen was used as curtain gas, gas 1 and gas 2, and their flows were set to 50, 40, and 70 units, respectively. Unit mass resolution was set in both mass resolving quadrupole Q1 and Q3. The declustering potential (DP) and collision energy (CE) for carboplatin were 32 V and 19 V, respectively, and those for the internal standard were 32 V and 19 V, respectively. The transitions of *m/z* 372 → 294 and *m/z* 376 → 298 on selected reaction monitoring (SRM) were used for monitoring carboplatin and carboplatin-d4, respectively. The dwell time was 500 msec. Data were collected and processed using Analyst 1.4.2 data collection and integration software.

2.5. Method validation

2.5.1. Linearity and lower limit of quantification (LLOQ)

For validation, carboplatin standards (eight non-zero standards of the analyte; 0.025, 0.05, 0.25, 0.5, 2.5, 5, 25 and 50 μg/mL for carboplatin) were prepared in blank human plasma ultrafiltrates and analyzed. Linear regression of the 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 (R.S.D. and R.E. less than 20%).

2.5.2. Precision and accuracy

Intra-day ($n = 6$) and inter-day ($n = 6$) precision and accuracy were investigated at three different levels, 0.1, 1 and 10 μg/mL, for carboplatin. The precision was determined on the basis of the coefficient of variation (R.S.D. (%)), and the accuracy was calculated as (found concentration–theoretical concentration)/theoretical concentration) × 100 (R.E. (%)).

2.5.3. Specificity and selectivity

To investigate whether endogenous matrix constituents interfered with the assay, blank plasma ultrafiltrates containing neither the analyte nor internal standard (double blank) and plasma ultrafiltrates containing LLOQ (0.025 μg/mL) of carboplatin and carboplatin-d4 were prepared. Human plasma ultrafiltrates were prepared from six different donors.

2.5.4. Extraction recovery from human plasma ultrafiltrates and matrix effect

Extraction recovery of carboplatin from human plasma ultrafiltrates was evaluated at three concentrations (0.1, 1 and 10 μg/mL) by comparing peak areas obtained from samples spiked with carboplatin before extraction with those obtained from samples to which carboplatin was added after extraction.

The matrix effect of components from human plasma ultrafiltrates on carboplatin ionization was also evaluated using a post-column infusion system with a syringe pump (10 μg/mL, 0.01 mL/min). After an equilibrium state has been reached, drug-free plasma ultrafiltrate samples with (processed samples) or without dilution (only protein precipitation samples) with acetonitrile were injected. Effluent from the HPLC column combined with the infused analytes entered into the detector.

2.5.5. Stability

The stability of carboplatin in human plasma ultrafiltrates was examined by analyzing three concentrations (0.1, 1 and 10 μg/mL) in triplicate. These samples were stored at −80 °C for 4 weeks and at 4 °C for 4 h to evaluate long-term stability 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 h in an autosampler (4 °C).

2.6. Application to clinical pharmacokinetic study

The method was applied to samples obtained from a cancer patient receiving 350 mg/body of carboplatin i.v. over 2 h. Before treatment, the patient gave written informed consent as approved by the institutional review board. The blood samples were collected into sodium heparin-containing tubes at 0, 0.5, 1, 2, 3, 6, 12.75 and 24 h post-dosing. Samples were immediately stored at 4 °C and plasma ultrafiltrates were obtained. The resultant ultrafiltrates were stored at −80 °C until measurement.

3. Results and discussion

3.1. Method development

^{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. The ESI-MS full-scan mass spectra (Q1) of carboplatin recorded in the positive ion mode afforded the protonated molecular ions $[M+H]^+$ at *m/z* of 371, 372, 373 and 375, with the expected ratio corresponding to Pt isotope abundance (Fig. 1A). The CID-MS/MS analysis of the protonated molecular ion at *m/z* of 372 afforded the major product ion at *m/z* of 294 which was used for the quantitative SRM analysis of carboplatin (Fig. 2A).

The ESI-MS full-scan mass spectra (Q1) of carboplatin-d4 as an internal standard recorded in the positive ion mode afforded the protonated molecular ions $[M+H]^+$ at *m/z* of 375, 376, 377 and 379, with the expected ratio corresponding to Pt isotope abundance (Fig. 1B). The product ion scan of $[M+H]^+$ at *m/z* of 376 afforded the major product ion at *m/z* of 298, which used for quantitative SRM of the internal standard (Fig. 2B).

We have already developed the quantification of oxaliplatin using HILIC mode with PC HILIC (Shiseido, Tokyo, Japan) column [14]. There was no problem that carboplatin was used as the internal standard as far as we analyzed the quantification of oxaliplatin. However, profound matrix effects were observed for carboplatin in human plasma ultrafiltrates samples. Therefore, we utilized another column for the quantification of carboplatin. The present method employed HILIC with an Accucore HILIC column.

Carboplatin and the internal standard were separated by difference of molecular weight. The retention time of carboplatin and the internal standard was 2.3 min. We avoided reduction of signal by ion suppression from impurities in plasma samples by washing the analytical column using mobile phase B. Our method does not need an evaporation step for sample preparation, enabling quick sample preparation and minimal occupational exposure.

3.2. Validation

3.2.1. Linearity and lower limit of quantification (LLOQ)

The calibration curve of concentration for carboplatin in human plasma was linear in the range from 0.025 to 50 μg/mL with a weighting by $1/x$. The correlation coefficient (r) was >0.999 for carboplatin determination. A typical standard curve was $y = 0.175x + 0.00307$. The LLOQ for carboplatin was 0.025 μg/mL.

3.2.2. Precision and accuracy

Intra-day ($n = 6$) and inter-day ($n = 6$) precision and accuracy were tested at three different concentrations (0.1, 1 and 10 μg/mL for carboplatin). The results are summarized in Table 1. For the determination of carboplatin, the intra- and inter-day precisions ranged from 1.5% to 4.3%. The accuracies were at most within ±2.9% for all concentrations. These results suggest that intact carboplatin in human plasma ultrafiltrates can be measured accurately and reproducibly by the present method.

3.2.3. Specificity and selectivity

The specificity and selectivity of the method were evaluated. A representative chromatogram of blank human plasma and chromatograms of LLOQ of carboplatin spiked in human plasma are shown in Fig. 3. There is no interference from an endogenous substance.

3.2.4. Extraction recovery from human plasma ultrafiltrates and matrix effect

The extraction recoveries from human plasma ultrafiltrates were 88.4, 85.1 and 96.1% at the concentrations of 0.1, 1 and 10 μg/mL, respectively. The effect of matrix components is shown in Fig. 4. Because of the matrix components, ionization of carboplatin was suppressed to 50% (Fig. 4B). Therefore, plasma extract was diluted with acetonitrile to reduce the effect of matrix components and improve the ionization of carboplatin. With the plasma extract being diluted five times and ten times with acetonitrile, ionization of carboplatin was recovered to 70% and 120%, respectively (Fig. 4C and D). By diluting plasma twenty times with acetonitrile which was adopted in this method, ionization of carboplatin recovered up to almost 100% (Fig. 4E). In addition, more than twenty times dilution with acetonitrile caused a decline in sensitivity due to the high dilution ratio. Thus, this sample preparation was the best condition for our method.

3.2.5. Stability

The stability of carboplatin in human plasma ultrafiltrates is shown in Table 2. Carboplatin in human plasma ultrafiltrates is stable for four weeks at −80 °C.

3.3. Application to clinical pharmacokinetic study

The plasma intact carboplatin concentration versus time curve in a patient after 2-h carboplatin infusion is shown in Fig. 5. Plasma concentration of carboplatin at 24 h after infusion was less than the LLOQ. The maximum concentration was 3.51 μg/mL. This result indicated the applicability of this method to pharmacokinetic study of carboplatin.

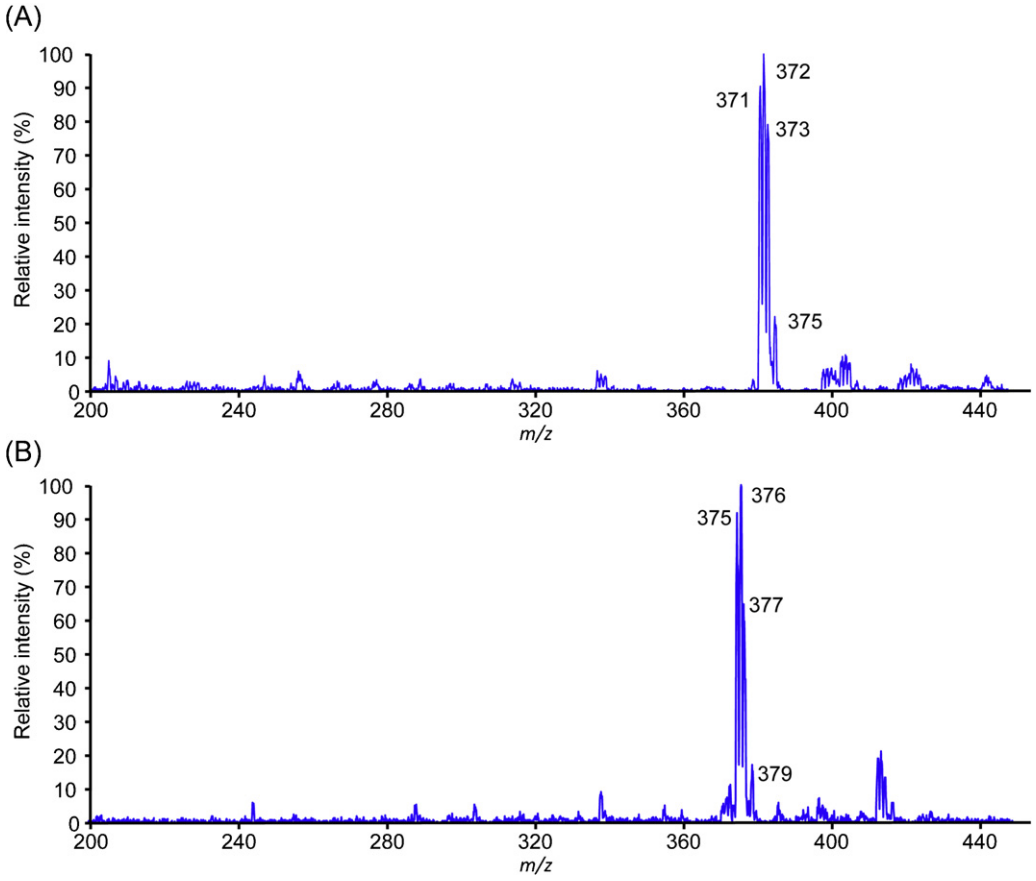

Fig. 1. The ESI-MS of the carboplatin (A) and carboplatin-d4 (B).

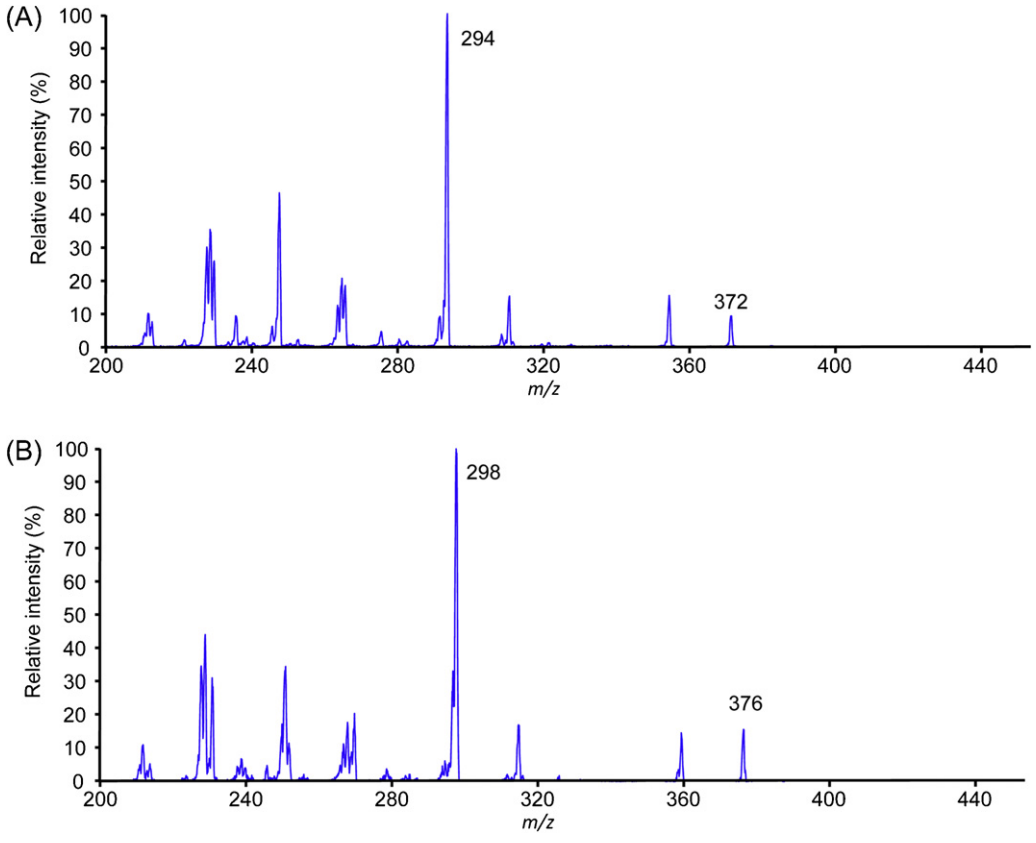

Fig. 2. The CID-MS/MS of the precursor $[M+H]^+$ ions at *m/z* 372 (A) and 376 (B).

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

Analyte	Concentration (μg/mL)	Intra-day (n = 6)			Inter-day (n = 6)		
		Found (μg/mL)	R.S.D. (%)	R.E. (%)	Found (μg/mL)	R.S.D. (%)	R.E. (%)
Carboplatin	0.1	0.10 ± 0.00	4.3	0.7	0.10 ± 0.00	3.9	2.3
	1	1.03 ± 0.02	2.3	2.9	1.01 ± 0.03	2.6	1.0
	10	10.1 ± 0.2	1.5	1.2	10.1 ± 0.4	3.7	0.7

Table 2
Stability of carboplatin in human plasma ultrafiltrates.

Concentration (μg/mL)	Remaining (%)			
	Freeze-thaw stability	Short-term stability (4 h, 4 °C)	Long-term stability (4 week, −80 °C)	Processed sample stability (4 h, 4 °C)
0.1	103.9 ± 7.9	102.9 ± 6.8	95.4 ± 5.7	101.0 ± 13.0
1	105.9 ± 1.9	105.0 ± 0.5	105.9 ± 3.2	105.9 ± 2.0
10	103.0 ± 1.7	103.0 ± 3.4	104.0 ± 3.1	104.0 ± 2.9

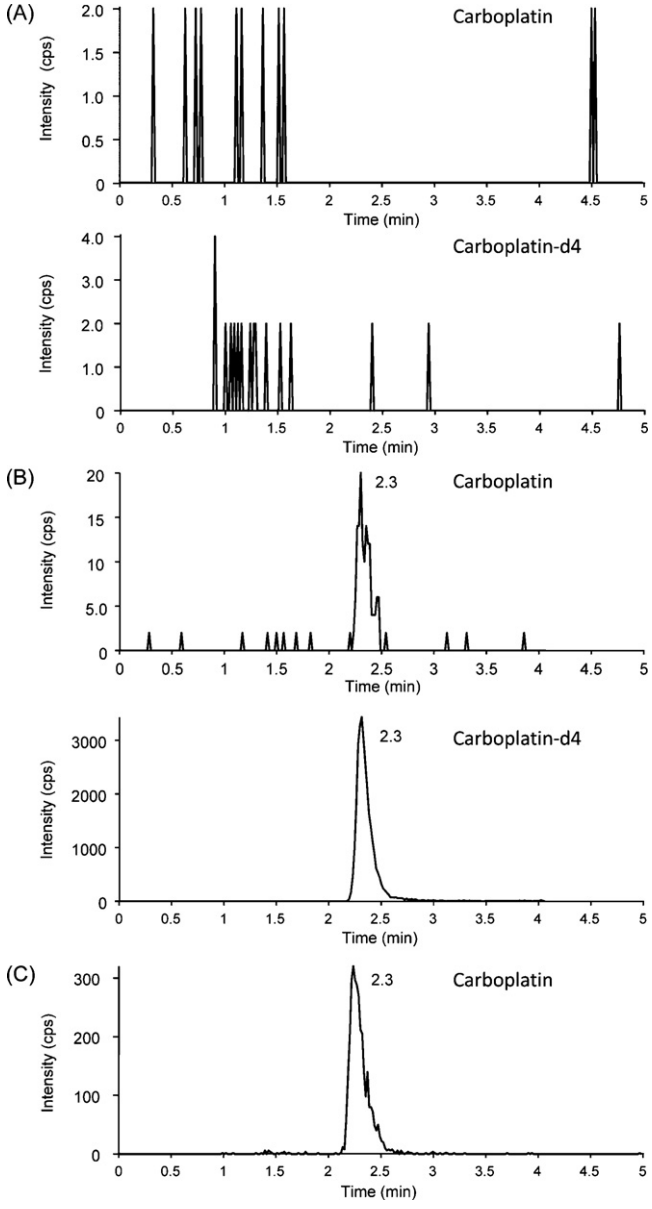

Fig. 3. Representative chromatograms of human blank plasma ultrafitrates (A), LLOQ of carboplatin (0.025 μg/mL) and internal standard (B), and plasma sample obtained from a patient at the end of 2-h carboplatin infusion at a dose of 350 mg/body (C).

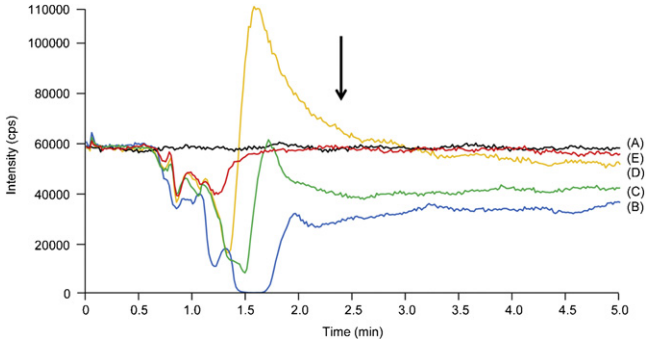

Fig. 4. Matrix effect of plasma matrix components on carboplatin ionization. Arrow indicates the retention time of carboplatin.
Reference standard carboplatin (10 μg/mL, 10 μL/min) (A), injection of plasma extract without dilution with acetonitrile (B), injection of plasma extract diluted five times with acetonitrile (C), injection of plasma extract diluted ten times with acetonitrile (D), and injection of plasma extract diluted twenty times with acetonitrlie (E).

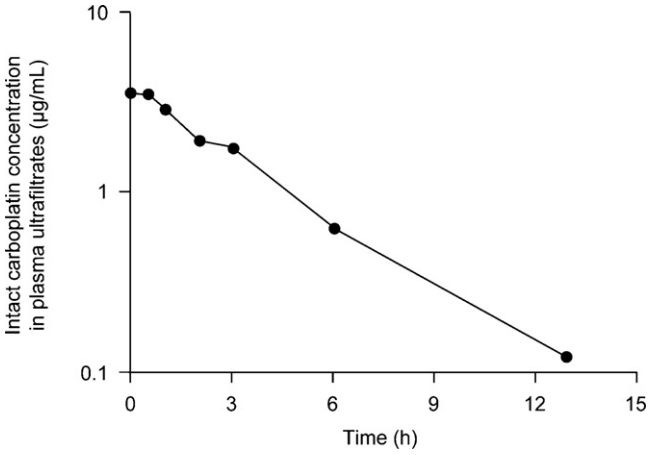

Fig. 5. Intact carboplatin concentration in plasma ultrafiltrates versus time curve in patient after 2-h infusion of carboplatin.

4. Conclusion

A method for quantification of intact carboplatin in human plasma ultrafiltrates using sensitive and selective hydrophilic interaction liquid chromatography–tandem mass spectrometry has been developed. Using samples diluted with acetonitrile, we succeeded in decreasing ion suppression caused by the matrix effect. Our established method does not need an evaporation step, which

makes a brief sample preparation and a minimal occupational exposure. Validation demonstrated that this method has adequate sensitivity and selectivity and sufficient precision and accuracy. The method was applied to a clinical pharmacokinetic study of carboplatin in a cancer patient. Further studies are needed to determine the relationship between pharmacokinetics and side effects.

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