Journal of Chromatography B, 876 (2008) 277–282

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short communication

Determination of intact oxaliplatin in human plasma using high performance liquid chromatography-tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 13 August 2008
Accepted 30 October 2008
Available online 7 November 2008

Keywords:
Oxaliplatin
HPLC
Mass spectrometry

ABSTRACT

A HPLC-tandem mass spectrometry method was developed and validated for the quantitation of intact oxaliplatin in human plasma. Plasma ultrafiltrates were precipitated with acetonitrile and separation was performed on a 250 mm Beckman ODS reverse phase column using a gradient mobile phase. The mass spectrometer was operated in positive ionization mode using TurboionSpray and precursor–product ion combinations of m/z 391.1 → 305.1 and 371.1 → 247.0 were monitored for oxaliplatin and carboplatin, the internal standard, respectively. The lower limit of quantitation for oxaliplatin was 20 ng/ml. The linear range of the method was 20–1000 ng/ml. The between- and within-day relative standard deviations ranged from 3.1 to 7.7%, and accuracy was within 5%. This method was successfully applied in a clinical study of oxaliplatin.

1. Introduction

Oxaliplatin, one of the platinum analogues, is characterized by a 1,2-diaminocyclohexane carrier ligand, which confers important advantages in terms of anti-tumor activity and toxicity, compared with other platinum analogues, such as cisplatin and carboplatin. Randomized controlled studies have shown that oxaliplatin combined with infusional 5-fluorouracil (5-FU) plus leucovorin (FOLFOX) has significantly higher response rates and longer overall survival with acceptable toxicity than 5-fluorouracil plus leucovorin in patients with locally advanced or metastatic colorectal cancer [1–3]. The FOLFOX regimen has become a commonly accepted standard of care in these patients.

Like other platinum agents, oxaliplatin rapidly forms a variety of reactive intermediates after intravenous administration [4]. These intermediates then irreversibly bind to other macromolecules and are inactivated. Total platinum contents in plasma ultrafiltrate which represent all unbound platinum species are generally measured with atomic absorption or mass spectrometry methods [5–7]. However, these methods could not distinguish intact oxaliplatin from platinum intermediates which are not biologically active.

Recently, Ehrsson and Wallin reported a HPLC method for quantitating intact oxaliplatin in whole blood ultrafiltrates [8]. However, this method involves post-column derivatization and microwave heating. We developed a HPLC-tandem mass spectrometry method for measuring intact oxaliplatin in human plasma.

2. Experimental

2.1. Chemical and reagents

Oxaliplatin (Cat No. O09512) and Carboplatin (Internal standard) (Cat No. C2538) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Liquid nitrogen (99.999%) was purchased from Praxair Canada Inc. (Hamilton, Ont., Canada). Purified de-ionized water was prepared with the Milli-Q Academic Quantum Purification System (Millipore, Bedford, MA, USA). Amico Centrifree® Micropartition Devices were purchased from Millipore Corporation (Bedford, MA, USA). All other solvents and reagents were of HPLC or analytical grade. Human plasma used for the preparation of quality controls and calibration standards was obtained from the Canadian Blood Services (Toronto, Canada).

2.2. Chromatography conditions

The chromatography system consisted of a SIL-20-AC autoinjector and a LC-20AB pump (Shimadzu Scientific Instruments, Columbia, MD, USA). Oxaliplatin and carboplatin were sepa-

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doi:10.1016/j.jchromb.2008.10.055

rated at ambient temperature on a Beckman ODS reverse phase analytical column (250 mm × 4.6 mm, 5 μm particles, Beckman Instruments Inc. Fullerton, CA) mated with a C18 guard column (4.0 mm × 3.0 mm internal diameter)(Phenomenex, Torrance, CA, USA). The binary mobile phase consisted of mobile phase A: a mixture of 3.5/96.5 methanol/water containing 0.1% acetic acid and mobile phase B: a mixture of 90/10 methanol/water containing 0.1% acetate acid. The mobile phase was linearly increased from 10% B to 60% B from 0.0 to 1.0 min and kept constant until 6.5 min, at which time point it was linearly decreased from 60% B to 10% B in 6.7 min and kept there until 10 min. The flow rate was 0.6 ml/min.

2.3. Standard solutions

Two independent stock solutions of oxaliplatin were prepared by dissolving 1.5 mg oxaliplatin in 3.0 ml of methanol:water (50:50) to obtain a concentration of 500 μg/ml, and stored at −20 °C. The internal standard (IS), carboplatin, was also prepared as a stock solution of 500 μg/ml by dissolving 1.5 mg of carboplatin in 3.0 ml of methanol:water (50:50), and stored at −20 °C.

One of the oxaliplatin stock solutions was serially diluted with methanol:water (50:50) to prepare calibration standard working solutions. Calibration standards were prepared by diluting calibration standard working solutions further with pooled blank human plasma ultrafiltrate each day to obtain oxaliplatin concentrations of 20, 40, 200, 750, 1000, 2000, and 2500 ng/ml. The other stock solution of oxaliplatin was serially diluted with methanol:water (50:50) to prepare the standard working solutions for quality control samples. Pools of quality control (QC) samples were prepared by mixing appropriate amounts of QC standard working solutions and pooled blank plasma ultrafiltrate to obtain oxaliplatin concentrations of 50, 500, and 2500 ng/ml, and stored in batch at −20 °C for the duration of validation procedure. The IS was serially diluted with methanol:water (50:50) to yield a working solution of 4000 ng/ml.

2.4. Mass spectrometry conditions

The HPLC system was interfaced with a API 3200 mass spectrometer (MDS Sciex, Concord, Ontario, Canada) with TurboIonSpray (TISP) probe. The TISP probe was maintained at 600 °C. Curtain gas, nebulizer gas and turbo gas pressures (nitrogen) were set at 25, 60 and 55 psi, respectively. Sampling orifice potential and ion spray voltage were set at 46 and 5000 V, respectively. The dwell time was 150 ms, and the temperature of the interface heater was set at 100 °C. Mass analyzers Q1 and Q3 were operated at unit resolution. The mass spectrometer was programmed to admit the protonated molecules (M+H) at m/z 397.1 for oxaliplatin and m/z 371.1 for IS via the first quadrupole filter (Q1). Collision induced fragmentation at Q2 yielded the product ions at Q3 of m/z of 305.1 and 247.0 for oxaliplatin and IS, respectively. Peak area ratios obtained from multiple reaction monitoring of the analytes (m/z 397.1 → 305.1)/(371.1 → 247.0) were utilized for the construction of calibration lines, using weighted ($1/\chi^2$) linear least-square regression of the plasma concentrations and measured peak area ratios. Data collection, peak integration, and calculation were performed using MDS Analyst 1.4.2 software.

2.5. Patient sample preparation

Blood samples were taken at various times after the completion of a 2-h oxaliplatin infusion at a dose of 85 mg/m^2 from patients with metastatic colorectal cancer and treated with FOLFOX. Venous blood was drawn into BD Vacutainer® tubes containing sodium heparin, kept on ice and centrifuged at 1500 × g. Plasma was separated and divided into two 0.5 ml aliquots. Each aliquot was then loaded into an Amico Centrifree® Micropartition Devices with a molecular weight cutoff of 30,000 and centrifuged at 1900 × g for 30 min at 4 °C. The plasma ultrafiltrates were frozen and stored at −20 °C.

Plasma ultrafiltrates were thawed at ambient temperature. A 50 μl volume of plasma ultrafiltrate was pipetted into a polypropylene tube (100 mm × 14 mm), and 25 μl of the working IS solution was added and they were mixed for 30 seconds on a vortex-mixer. Subsequently, 3.0 ml acetonitrile was added to the mixture and they were vigorously shaken for 1 min on a vortex-mixer. Samples were centrifuged at 1500 × g for 30 min, and the supernatants were transferred to 1.5 ml vials containing 200 μl of ammonium acetate (10 mM, pH 7.4) and evaporated to near dryness using the Universal Vacuum System (Thermo Electron Corp., Milford, MA, USA). The residues were reconstituted with 200 μl of water, and 20 μl was injected onto the HPLC-MS/MS system for analysis.

2.6. Method validation

This method was validated for selectivity, accuracy, precision, recovery, matrix effect and stability according to guidelines issued by the Food and Drug Administration [9]. Validation was performed on five consecutive days, and included calibration curve and QC samples at low, medium and high concentration.

The specificity of the method was evaluated using human plasma ultrafiltrates prepared from six different donors' plasma. Chromatograms were visually inspected for the presence of endogenous interfering peaks. To determine oxaliplatin recovery, 5 QC samples at each concentration were processed as described above. The recovery was determined by comparing areas obtained from QC samples with those spiked solutions of same concentrations, and expressed as a percentage.

The matrix effect was assessed by duplicate analyses of oxaliplatin (500 ng/ml) in six lots of different human plasma ultrafiltrates prepared from six different donor plasma and comparing concentrations with theoretical values, expressed as a percentage. The stability of oxaliplatin in human plasma ultrafiltrates was evaluated using QC samples following three full freeze–thaw cycles. The stability of sample solution as prepared above was evaluated at 0, 15, and 24 h under normal laboratory conditions. The stabilities of oxaliplatin standard solutions were evaluated after 1 day and one week under −20 °C. The stability of oxaliplatin in acetonitrile was evaluated at 4, 6 and 24 h after mixing.

3. Results and discussion

3.1. Mass spectra

There are four major natural isotopes of platinum: ^{194}Pt, ^{195}Pt, ^{196}Pt and ^{198}Pt with natural abundances of 32.9, 33.9, 25.3 and 7.2, respectively. As oxaliplatin and the internal standard, carboplatin are platinum-containing metal organic compounds, their molecular spectra are distinguishable by a group m/z values resulting from the presence of these isotopes. ESI positive mass spectra of oxaliplatin and internal standard carboplatin from the selected m/z regions due to oxaliplatin (397.1 g/mol) and carboplatin (371.3 g/mol) indicated that each molecular m/z group was actually comprised of several mass values corresponding to the four platinum isotopes, with three peaks roughly the same intensity and a much smaller 4th peak (Fig. 1). The product ion mass spectra of oxaliplatin and internal standard protonated molecules indicated the presence of intense product ions at m/z 305.1 and 247.0 for oxaliplatin and internal standard (Fig. 2), respectively. Based on their product ion full scan positive ion mass spectra, precursor-

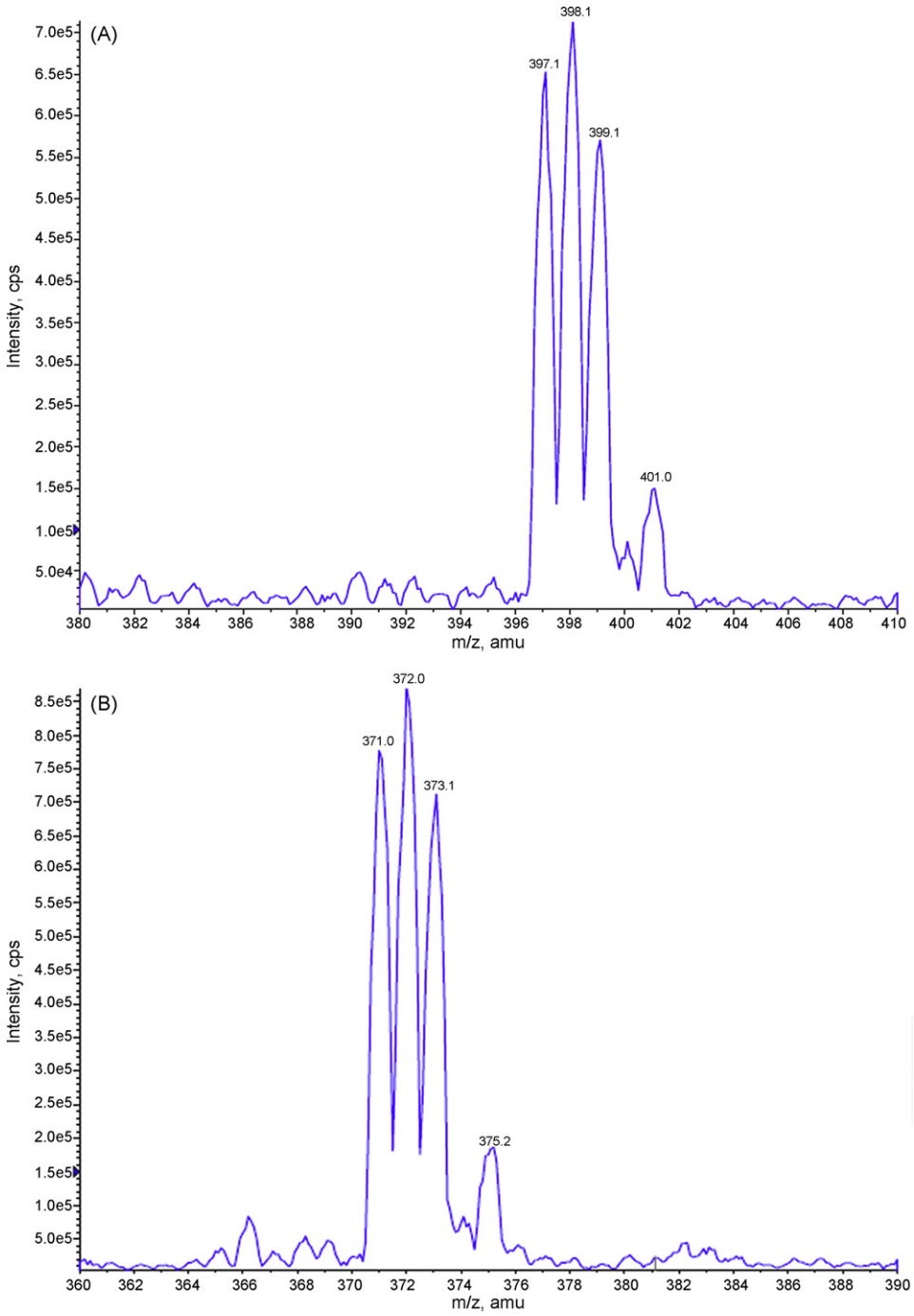

Fig. 1. ESI Positive mass spectra of oxaliplatin (A) and carboplatin (B).

product ion combinations of m/z 397.1 → 305.1 and 371.1 → 247.0 were selected for oxaliplatin and carboplatin, respectively, by multiple reaction monitoring mode.

3.2. Chromatography

In this study, carboplatin, rather than isotope-labeled oxaliplatin, was used as the internal standard. The matrix effect was assessed under different column conditions. When a short column was used, oxaliplatin and carboplatin were co-eluated with endogenous impurities. As a result, the ionization efficiency of oxaliplatin and carboplatin were suppressed by undetected co-eluting endogenous impurities. By using a 250 mm long Beckman ODS reserve phase column, the matrix effect was significantly reduced and the assay sensitivity improved. The measured concentrations of 500 ng/ml samples in six lots of different human plasma ultrafiltrates all gave values close to their theoretical concentrations, with accuracy ranging from 89.1 to 101.8%, indicating that the method was robust with respect to matrix suppression and plasma batch variability.

Chromatograms of a blank plasma ultrafiltrate sample, a human plasma ultrafiltrate sample spiked with oxaliplatin at the lower limit of quantitation (LLOQ) and a patient sample were shown in Fig. 3. The retention times for oxaliplatin and the internal

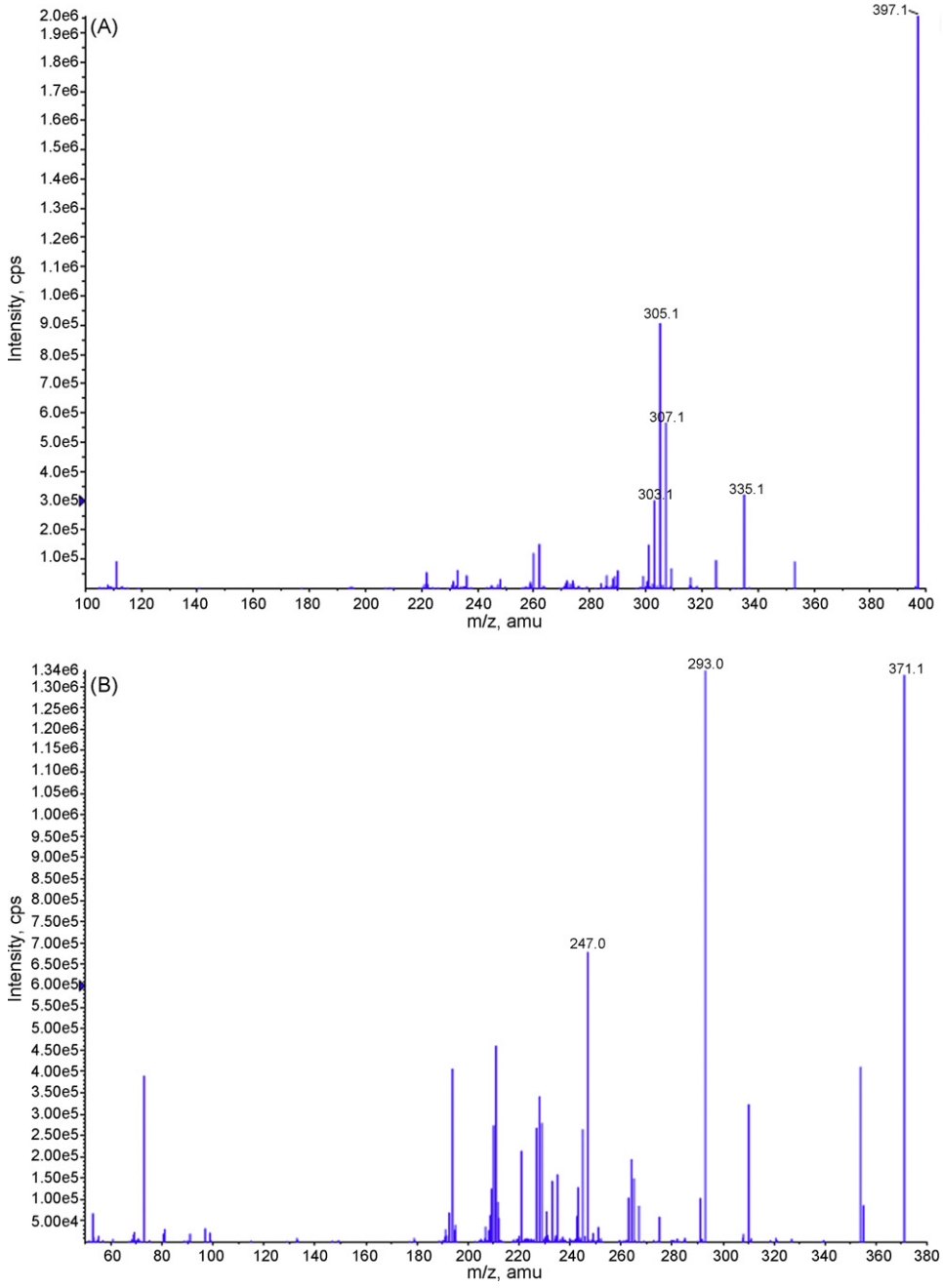

Fig. 2. Product ion mass spectra of oxaliplatin (A) and carboplatin (B).

standard were approximately 5.6 and 5.8 min, respectively. The total runtime is 10 min. There are no interferences from endogenous substances. In human plasma ultrafiltrates, calibration curves of oxaliplatin were linear over the range of 20–2500 ng/ml. The mean slope of calibration curves and Y-intercept were 2.88 ± 0.25 (SD) and 0.0025 ± 0.0033 respectively, with the mean r^2 being 0.996 ± 0.0059. The LLOQ of oxliplatin was determined to be 20 ng/ml, which was improved compared to that reported by Ehrsson and Wallin [8]. Accuracy and precision were 87.5% and 4.43% at LLOQ, and −0.77 and 6.93% at upper limit of quantitation of 2500 ng/ml. The between- and within-day precision for QC sample at three different concentrations ranged from 3.14 to 7.74%. The accuracy of QC samples for oxaliplatin ranged from 98.9 to 103.8% (Table 1).

Table 1
Validation characteristics of intact oxaliplatin in human plasma ultrafiltrates.

	Concentration (ng/ml)		
	50	500	2500
Accuracy (%)	98.9	103.8	99.2
Precision (%)			
Within	3.1 ($n = 5$)	3.8 ($n = 5$)	4.5 ($n = 5$)
Between	5.0 ($n = 13$)	7.7 ($n = 13$)	6.9 ($n = 13$)
Recovery (%)	92.5	92.3	88.1

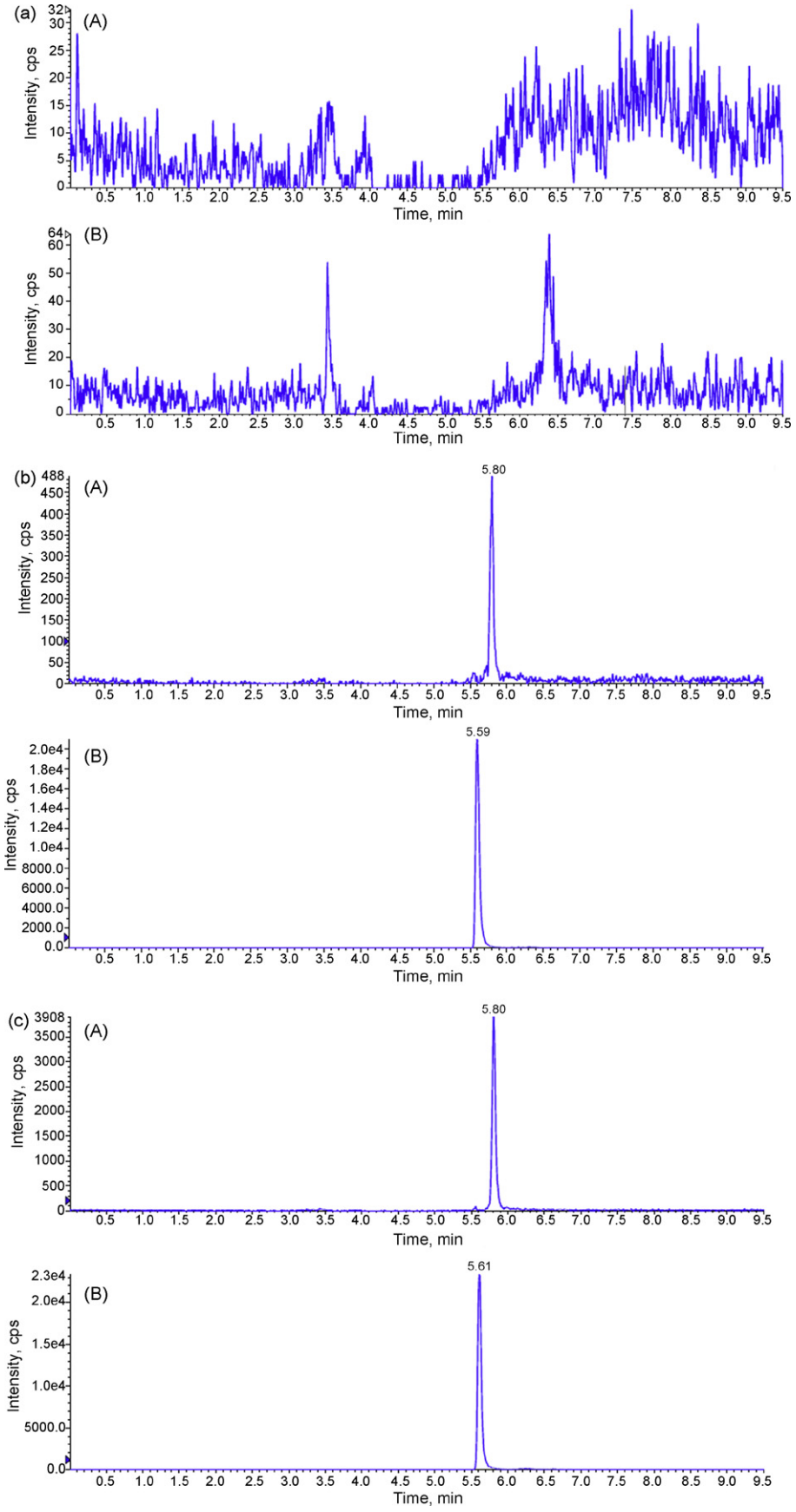

Fig. 3. Chromatograms of oxaliplatin (top) and carboplatin (bottom). (A) blank human plasma ultrafiltrate; (B) Blank human plasma ultrafiltrate spiked with 20 ng/ml of oxaliplatin and 2000 ng/ml of carboplatin; (C) a patient plasma ultrafiltrate including 2000 ng/ml of carboplatin.

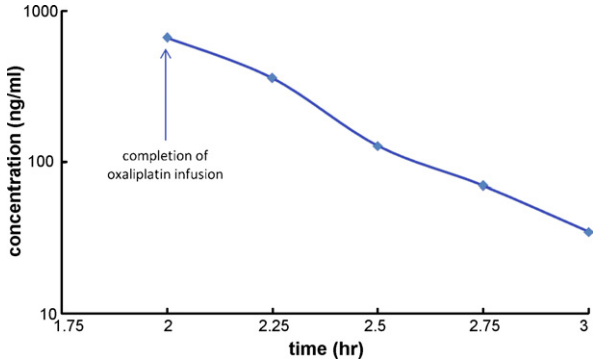

Fig. 4. Plasma intact oxaliplatin concentration versus time curve in one patient after a 2-h infusion.

By comparing ratios of peak areas of oxaliplatin from QC samples with those from spiked solutions of the same concentrations, the extraction recovery was determined to be 88.1–92.5% (Table 1). There was no significant change for oxaliplatin in human plasma ultrafiltrates through one full cycle of freeze–thaw and for oxaliplatin in sample solutions after 15 h under normal laboratory conditions. However, oxaliplatin was degraded by approximately 9% in sample solutions after 24 h under normal laboratory conditions. Oxaliplatin standard solutions were degraded after one-week storage at 4°, therefore the standard solution was prepared daily. Oxaliplatin was stable in acetonitrile for up to 6 h.

3.3. *Oxaliplatin concentrations in patients*

The plasma intact oxaliplatin concentrations versus time curve in a typical patient was shown in Fig. 4. Oxaliplatin concentrations declined rapidly after completion of intravenous infusion with a half-life of approximately 10 min. Our results were compatible with those reported by Ehrsson and Wallin who also measured intact oxaliplatin [10]. Previously, the elimination half-life of oxaliplatin was reported to be more than 30 h, likely as a result of slow release of platinum conjugates after the degradation of cellular macromolecule [4].

4. Conclusion

In conclusion, we report a robust, specific, selective and sensitive HPLC-tandem mass spectrometry method for quantifying free intact oxaliplatin concentrations in human plasma. This method determines intact oxaliplatin concentration which is biologically active, not the total concentrations of platinum complexes as determined by AAS and ICP-MS. The LLOQ of the current method is similar to that of atom absorption spectrometry. This method would be of value in studies of oxaliplatin pharmacokinetics.

Acknowledgements

We wish to thank patients who participated in this study and their families for their courage and determination; and the National Cancer Institute of Canada Clinical Trials Group for financial support.

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