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Validated method using liquid chromatography-electrospray ionization tandem mass spectrometry for the determination of contamination of the exterior surface of vials containing platinum anticancer drugs

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ABSTRACT

Contamination of the exterior surface of vials of cytostatic drugs by the drugs themselves is a potential hazard to human health. This study developed a validated method using liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) for the determination of contamination of the exteriors of vials of cisplatin and carboplatin. Large Alpha® sampling swabs were employed to wipe the vial exterior. Cisplatin or carboplatin and gold(III) as an internal standard were derivatized by *N*,*N*-diethyldithiocarbamate (DDTC). $Pt(DDTC)_3^+$ and $Au(DDTC)_2^+$ were monitored by the respective transitions of *m*/*z* 639.3–490.9 and 493.0–345.0, respectively. Each separation was completed within 9 min using a 3 μm particle ODS-column. Calibration curves for cisplatin and carboplatin were linear over concentration ranges of 30–10,000 and 30–30,000 pg $vial^{-1}$, respectively. The accuracies and precisions were 96.1–102.5% and within 8.2% for intra-assay and 99.6–103.3% and within 7.6% for inter-assay, respectively. Their lower limit of quantification was 30 pg $vial^{-1}$. Amounts of 0.17–17.0 ng $vial^{-1}$ as cisplatin and 0.48–794 ng $vial^{-1}$ as carboplatin were detected from the exterior surface of the vials. This validated method using LC-ESI-MS/MS for the determination of platinum anticancer drugs is helpful for monitoring contamination of the exterior surface of drug vials.

1. Introduction

Exposure of healthcare workers to hazardous drugs has been a problem since the 1970s [1]. Cytostatic drugs are widely used for the treatment of cancer and non-neoplastic diseases, however, they can also be carcinogenic, mutagenic, and/or teratogenic in humans [2]. Recently, occupational exposure to cytostatic drugs has been recognized as a potential health hazard [3]. The exposure of healthcare workers results from several sources such as the preparation, treatment, and disposal of the drugs, as well as through contact with patient excrement. Some studies have described environmental contamination by cytostatic drugs via these routes in hospital settings [4–6].

Several recent studies have investigated contamination of the exterior surface of cytostatic drug vials containing cyclophosphamide, ifosfamide, 5-fluorouracil, etoposide, doxorubicin, or docetaxel as a potential health hazard [7–10]. Contamination by platinum anticancer drugs was also confirmed in several earlier studies [11–13]. Cisplatin and carboplatin induce apoptosis in tumor cells by binding to nuclear DNA, and they also possess toxicity and carcinogenicity in normal cells [14]. Cisplatin is classified into Group 2A in the International Agency for Research on Cancer (IARC) Monographs. Carboplatin, like cisplatin, is also believed to play a role in carcinogenesis. Accordingly, quantification of external vial contamination of platinum anticancer drugs is needed to evaluate any potential health hazards in hospital settings.

Several analytical methods have been reported for the determination of cisplatin and carboplatin. These methods include coupled plasma mass spectrometry (ICP-MS) [6,12,15,16], stripping voltammetry [13,17], atomic absorption spectrometry (AAS) [18], liquid chromatography-ultraviolet absorbance detection (LC-UV) [19–21], and electrospray ionization mass spectrometry (ESI-MS) [22]. ICP-MS and stripping voltammetry are the techniques as choice for the detection of traces as a result of their sensitivity. In ICP-MS, no sample pretreatment is necessary aside from desorption from compresses. However, the determination of platinum compounds using ICP-MS could be potentially interfered by monoatomic isobars such as mercury and osmium [23]. Stripping voltammetry provides access to free ions in solution or to ions bound to labile complexes and thus requires to derivatize platinum

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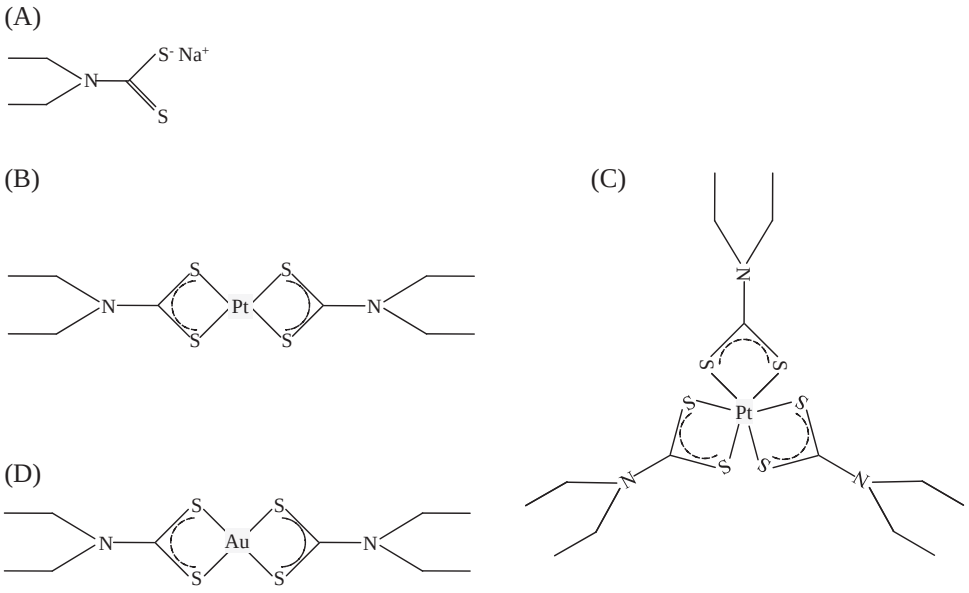

Fig. 1. Chemical structures of *N,N*-diethyldithiocarbamate (DDTC), platinum-DDTC complexes, and gold-DDTC complex. (A) DDTC, (B) $Pt(DDTC)_2$, (C) $Pt(DDTC)_3$, and (D) $Au(DDTC)_2$.

compounds [13,17]. Some hospitals have LC-MS/MS for identification and monitoring of drugs and toxic substances in blood and urine. In contrast, there were few hospitals possessing ICP-MS or stripping voltammetry due to their lower frequency of use. The LC-MS/MS is more available than ICP-MS and stripping voltammetry in hospital settings.

AAS and LC-UV are not suitable to determine vial exterior contamination due to their poor sensitivity and selectivity. An analytical method using ESI-MS employs a derivatization reagent to promote platinum ionization and consequently enables the sensitive detection of platinum anticancer drugs [22]. LC-electrospray ionization tandem mass spectrometry (ESI-MS/MS) involves LC separation, thus avoiding compounds that can interfere with analytes, and consequently possesses better sensitivity and selectivity than ESI-MS.

To date, a simple and validated wiping method for the determination of external vial contamination has not been fully established. In addition, there have only been a few reports on monitoring of external contamination in different lots of platinum anticancer drug vials delivered to a hospital pharmacy. The aim of this study was to develop a validated method using LC-ESI-MS/MS for the determination of external vial contamination of vials containing cisplatin or carboplatin.

2. Experimental

2.1. Chemicals and reagents

Cisplatin and carboplatin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Gold standard solution ($1\,mg\,mL^{-1}$) as an internal standard, HPLC-grade acetonitrile, HPLC-grade dichloromethane, HPLC-grade propan-2-ol, and AAS-grade *N,N*-diethyldithiocarbamate (DDTC) (Fig. 1A) were obtained from Wako Pure Chemicals (Osaka, Japan). All other reagents were analytical grade and commercially available.

2.2. Solution

Stock solution of cisplatin ($100\,\mu g\,mL^{-1}$) was prepared with normal saline solution. Stock solutions of carboplatin ($100\,\mu g\,mL^{-1}$) and gold ($2\,\mu g\,mL^{-1}$) were prepared with ultra-pure water purified by Aquarius® (Advantec Toyo Kaisha, Ltd., Tokyo). Standard solutions of cisplatin (0.75, 1.5, 2.5, 7.5, 15, 25, 75, 250, and $2500\,ng\,mL^{-1}$) were obtained by the dilution of stock solution with normal saline solution. Standard solutions of carboplatin (0.75, 1.5, 2.5, 7.5, 15, 25, 75, 250, 750, and $7500\,ng\,mL^{-1}$) and an internal standard solution ($200\,ng\,mL^{-1}$) were obtained by the dilution of stock solution with ultra-pure water. DDTC solutions for cisplatin and carboplatin were prepared to concentrations of 0.5 M and 1.0 M with 50 mM sodium hydroxide, respectively. For the calibration standard, 40 μL of standard solution was added into polypropylene tubes containing acetonitrile and ultra-pure water. The calibration standards were prepared at final amounts of 30, 60, 100, 300, 600, 1000, 3000, 10,000, and 100,000 pg vial^{-1} for cisplatin and 30, 60, 100, 300, 600, 1000, 3000, 10,000, 30,000, and 300,000 pg vial^{-1} for carboplatin. Quality controls for cisplatin and carboplatin were prepared at levels of 300, 1000, and 10,000 pg vial^{-1} using the same dilution procedures. The terminal solution consisted of 1% hydrochloric acid.

2.3. Sample preparation and derivatization

Seventy percent propan-2-ol was used as the wiping solution for cisplatin vials and ultra-pure water was selected as the wiping solution of carboplatin vials. The head of a swab (Large Alpha® sampling swab, ITW Texwipe, Mahwah, NJ, USA) was moistened on one side with 100 μL of wiping solution. The lid, bottom, and side of the drug vials were wiped twice using the wet and then dry side of the swab. After cutting off the handle of the swab, the head paddle was dipped in a polypropylene tube containing 100 μL of ultra-pure water and 1200 μL of acetonitrile. The tubes were kept at −35 °C until derivatization. Forty μL of internal standard solution, 40 μL of dilution solution (normal saline solution for cisplatin or ultra-pure water for carboplatin), and 20 μL of DDTC solution were added to the polypropylene tubes. The mixtures were incubated at room temperature for 1 h, and then 20 μL of terminal solution was added to stop the derivatization. After the derivatization, the head paddle of the swab was removed from the tube and the residue solution was evaporated to dryness. The dried residues were dissolved with 400 μL of ultra-pure water and 1200 μL of dichloromethane, and then the mixtures were shaken at 140 rpm for 10 min. The dichloromethane phase (1000 μL) was evaporated

Table 1
Parameters of LC and MS/MS.

Mobile phase	80% acetonitrile containing 5 mM ammonium acetate
Separation column	TSKgel ODS-100V (particle size, 3 μm, 2.0 mm I.D. × 75 mm)
Guard column	TSKguardgel ODS-100V (particle size, 5 μm, 2.0 mm I.D. × 10 mm)
Flow rate	0.2 mL min^{-1}
Column temperature	40 °C
Injection volume	10 μL
Ion source	Electrospray ionization
Capillary temperature	300 °C
Scan mode	Positive ion mode
Monitor ion (*m*/*z*)	
$Pt(DDTC)_3^+$	693.0/491.0
$Au(DDTC)_2^+$	493.0/345.0
Collision energy	
$Pt(DDTC)_3^+$	−25 eV
$Au(DDTC)_2^+$	−15 eV

to dryness. The residues were reconstituted in 120 μL of the mobile phase and then injected onto the analytic column.

2.4. LC-ESI-MS/MS conditions

Table 1 shows LC and MS/MS parameters. The LC system (Agilent Technologies, Palo Alto, CA, USA) consisted of a G1312A binary pump, G1367 autosampler, and G1316A thermostatted column compartment. Separation was performed using TSKgel ODS-100V (particle size, 3 μm, 2.0 mm I.D. × 75 mm, Tosoh, Tokyo) with TSKguardgel ODS-100V (particle size, 5 μm, 2.0 mm I.D. × 10 mm, Tosoh). The mobile phase consisted of 80% acetonitrile containing 5 mM ammonium acetate. The flow rate was 0.2 mL min^{-1} and the column temperature was set at 40 °C. The injection volume was 10 μL. MS/MS analyses were performed using a Finnigan model TSQ®-7000 triple-quadrupole MS (Thermo Fisher Scientific, Waltham, MA, USA) with an ESI interface to the LC. Data were collected and analyzed using Xcalibur software (version 1.2, Thermo Fisher Scientific). The ESI source was operated with the spray voltage at 4.5 kV with 68 psi of sheath gas. The heated capillary was maintained at 300 °C in positive ion mode. $Pt(DDTC)_3^+$ and $Au(DDTC)_2^+$ were monitored by the respective transitions of *m*/*z* 639.0–491.0 and 493.0–345.0 with collision energy levels of −25 eV and −15 eV, respectively.

2.5. Optimization of derivatization conditions and wiping solutions

Derivatization of the platinum anticancer drugs and gold with DDTC was optimized under the following conditions: DDTC concentration (0.1, 0.15, 0.2, 0.25, 0.5, and 1.0 M), reaction time (0.5, 1, 2, and 4 h), and temperature (4, 20, 40, 50, and 60 °C). Four different solvents (ultra-pure water, normal saline solution, 1% hydrochloric acid, and 50 mM sodium hydroxide) were evaluated as wiping solutions for cisplatin vials with respect to recovery and reproducibility. Seven solvents (ultra-pure water, 10%, 30%, 70% propan-2-ol, 10%, 30%, and 70% methanol) were evaluated as wiping solutions for carboplatin vials. Cisplatin or carboplatin on glass plates was wiped with swabs that had been moistened with each solvent. The recovery and its reproducibility for 300, 1000, and 10,000 pg of cisplatin and carboplatin were evaluated for each optimal wiping solution.

2.6. Method validation

The stabilities of platinum-DDTC and gold-DDTC complexes in mobile phase at 4 °C were evaluated by comparing the peak areas obtained from each specimen after 3 and 6 h of storage with the initial peak area. Linear regression was calculated using the ratio of the peak areas for platinum-DDTC to gold-DDTC complexes. Calibration standards for cisplatin and carboplatin were prepared with final amounts of 30–10,000 pg $vial^{-1}$ and 30–30,000 pg $vial^{-1}$, respectively. Calibration standards of 100,000 pg $vial^{-1}$ for cisplatin and 300,000 pg $vial^{-1}$ for carboplatin were used for drug vials detected as having more than 10,000 and 30,000 pg $vial^{-1}$, respectively. Accuracy was determined by evaluation of the analytical recovery of known amounts of standard specimens. Intra- and interassay precision values were also evaluated for quality controls and expressed as the coefficient of variation (CV). The quality controls for cisplatin and carboplatin were prepared at levels of 300, 1000, and 10,000 pg $vial^{-1}$. The stabilities of cisplatin and carboplatin in the swab were evaluated by comparing the peak area after 24 h of storage at room temperature with the initial peak area. Long-term stability in the swab at −35 °C was determined after one month.

2.7. Evaluation of vial external contamination

Randa® injection (10 and 50 mg, Nippon Kayaku Co., Ltd., Tokyo) for cisplatin and Paraplatin® injection (150 and 450 mg, Bristol-Myers Squibb, Tokyo) for carboplatin were selected as drug vials for the evaluation of external contamination. Five vials were sampled from each lot number from May to December 2009. Unfilled vials were donated by the respective manufacturers of the injection formulations. External contamination of the drug-filled vials and unfilled vials was determined using the present method.

2.8. Statistical analysis

Statistical analyses were performed using SPSS software (version 15.0J, SPSS Japan Inc., Tokyo). The Kruskal–Wallis test was used to determine the difference in external contamination of each standard among lot numbers. The Mann–Whitney *U* test was used to analyze the differences in external contamination of each standard between the drug vials and unfilled vials. A $P < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Derivatization of platinum anticancer drugs with DDTC

The peak area of platinum-DDTC increased in conjunction with the DDTC concentration up to 0.15 M. Concentrations above 0.5 M DDTC did not have an effect on the peak areas of platinum-DDTC. The calibration curve for carboplatin with 1.0 M DDTC exhibited linearity that was superior to that with 0.5 M DDTC at high levels of carboplatin. The DDTC concentration did not affect the peak area of gold-DDTC complex. There were no significant differences in the peak areas of platinum-DDTC and gold-DDTC complexes among the derivatization temperatures. No significant differences were observed in the peak areas of platinum-DDTC and gold-DDTC complexes among the derivatization times. The derivatization of platinum anticancer drugs and gold was optimized under the following reaction conditions: DDTC concentration, 0.5 M for cisplatin and 1.0 M for carboplatin; derivatization time, 1 h; and derivatization temperature, room temperature (approximately 20 °C).

3.2. Mass spectral analysis

$Pt(DDTC)_2^+$ of *m*/*z* 491.0 and $Pt(DDTC)_3^+$ of *m*/*z* 639.3 were detected as precursor ions of platinum anticancer drugs for MS/MS analysis (Fig. 1). Fig. 2 shows the MS/MS spectra of a fragment produced from $Pt(DDTC)_2^+$ and $Pt(DDTC)_3^+$. Platinum anticancer

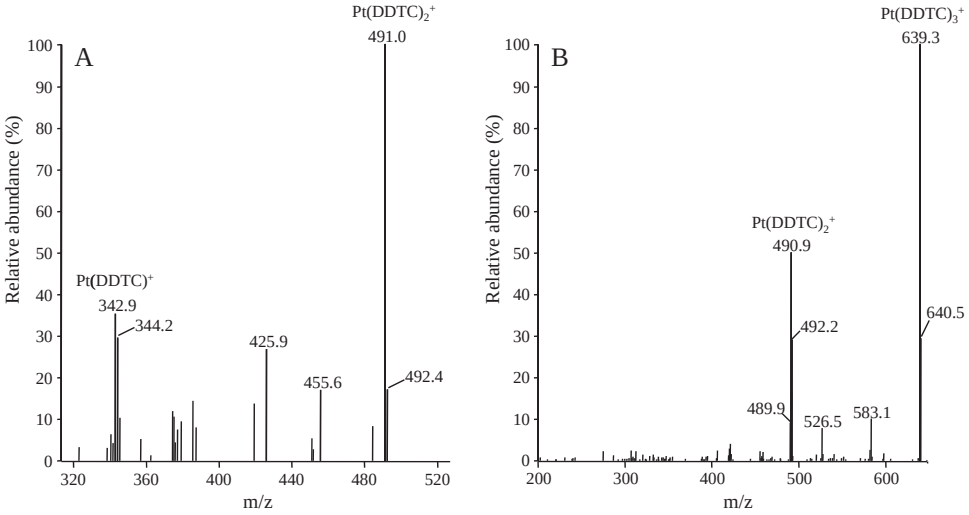

Fig. 2. MS/MS spectrum product ions and proposed fragmentation patterns of platinum-*N*,*N*-diethyldithiocarbamate (DDTC) complexes and gold-DDTC complex. (A) $Pt(DDTC)_2$, (B) $Pt(DDTC)_3$, and (C) $Au(DDTC)_2$.

drugs were identified using MS/MS and monitored for $Pt(DDTC)_3^+$ as a major product ion because product ions of $Pt(DDTC)_3^+$ were 3-fold higher than that of $Pt(DDTC)_2^+$. Product ion fragments of $Au(DDTC)_2^+$ were used for the quantification of gold.

3.3. LC separation

Fig. 3 shows the MS/MS chromatograms of platinum-DDTC and gold-DDTC complexes in the swabs. The MS/MS chromatograms were completed within 9 min. Platinum-DDTC and gold-DDTC complexes were eluted at 6.5 and 3.7 min, respectively. No peaks interfering with platinum-DDTC and gold-DDTC complexes were observed.

3.4. Calibration curve and sensitivity

The calibration curve was linear over a concentration range of 30–10,000 pg vial^{-1} for cisplatin and its correlation coefficient was 0.999. The lower limit of quantification (LLOQ), defined as the concentration of drug giving a signal-to-noise ratio of 10, was 30 pg vial^{-1} and its precision and accuracy were 7.7% and 102.7%. The carboplatin calibration curve was linear over a concentration range of 30–30,000 pg vial^{-1} and its correlation coefficient was 0.999. The LLOQ was 30 pg vial^{-1} and the precision and accuracy were 7.2% and 103.0%.

3.5. Assay accuracy and precision

Tables 2 and 3 show the single results of the intra- and inter-assay investigations, respectively. The accuracy and precision values for cisplatin were 1.6–8.2% and 99.5–102.5% for intra-assay and 0.2–4.4% and 100.1–101.4% for inter-assay, respectively (Table 4). The corresponding values for carboplatin were 3.0–6.3% and 96.1–100.4%, and 1.4–7.6% and 99.6–103.3%.

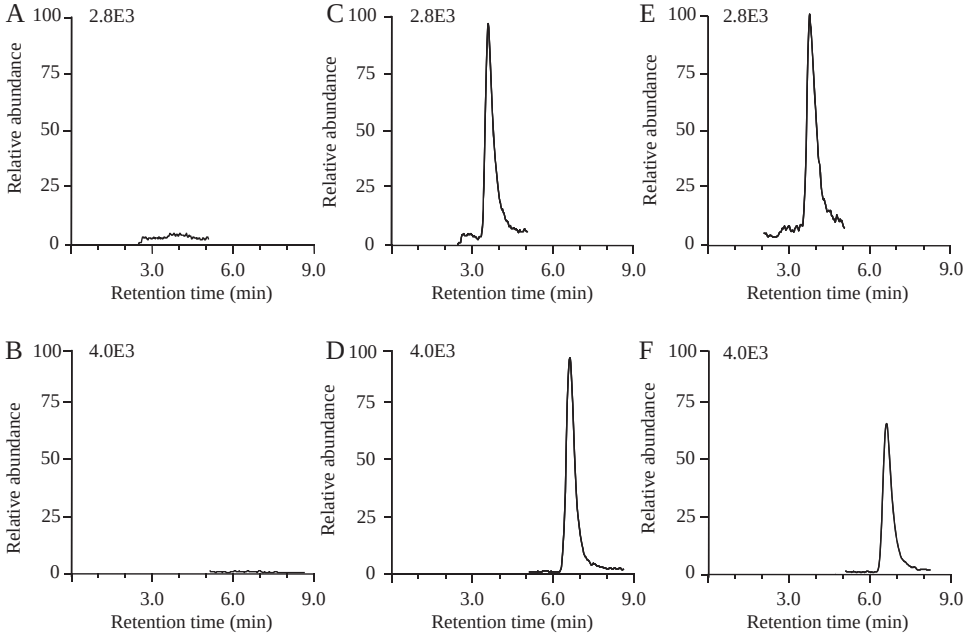

Fig. 3. MS/MS chromatograms of platinum-*N*,*N*-diethyldithiocarbamate (DDTC) complex and gold-DDTC complex. Background [(A) $Pt(DDTC)_3$, (B) $Au(DDTC)_2$], standard solution [(C) $Pt(DDTC)_3$, (D) $Au(DDTC)_2$], and external vial contamination [(E) $Pt(DDTC)_3$, (F) $Au(DDTC)_2$].

Table 2
Intra-assay accuracies and precisions of cisplatin and carboplatin.

Sample analytes	Theoretical value (pg vial^{-1})	Platinum contamination (ng vial^{-1})							
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
Cisplatin	300	290	313	322	301	335	326	260	284
	1000	1048	1009	1077	1071	1008	943	1030	1012
	10,000	9907	9986	9671	9759	10,080	10,065	10,152	9964
Carboplatin	300	286	305	266	278	311	287	308	265
	1000	967	980	969	967	1080	1020	1057	989
	10,000	9978	10,644	10,038	9866	9729	10,077	9656	10,024

Table 3
Inter-assay accuracies and precisions of cisplatin and carboplatin.

Sample analytes	Theoretical value (pg vial^{-1})	Platinum contamination (ng vial^{-1})							
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8
Cisplatin	300	316	289	314	293	290	290	320	296
	1000	1011	1036	1009	987	997	1048	1052	974
	10,000	10,018	10,018	10,022	9990	9998	9986	10,029	9980
Carboplatin	300	309	325	313	327	294	284	286	342
	1000	1087	962	1099	998	992	977	967	1177
	10,000	10,101	10,022	9703	9784	9983	9984	9978	10,085

Table 4
Summary of intra- and inter-assay accuracies and precisions of cisplatin and carboplatin.

Sample analytes	Theoretical value (pg vial^{-1})	Intra-assay (n = 8)			Inter-assay (n = 8)		
		Mean ± SD	Accuracy (%)	CV (%)	Mean ± SD	Accuracy (%)	CV (%)
Cisplatin	300	304 ± 25	101.3	8.2	301 ± 13	100.4	4.4
	1000	1025 ± 43	102.5	4.2	1014 ± 29	101.4	2.8
	10,000	9948 ± 164	99.5	1.6	10,005 ± 19	100.1	0.2
Carboplatin	300	288 ± 18	96.1	6.3	310 ± 21	103.3	6.7
	1000	1004 ± 44	100.4	4.4	1032 ± 79	103.2	7.6
	10,000	10,002 ± 301	100.0	3.0	9955 ± 140	99.6	1.4

SD, standard deviation and CV, coefficient of variation.

Table 5
Wiping recovery and its reproducibility of cisplatin and carboplatin.

Sample analytes	Theoretical value (pg vial^{-1})	Wiping recovery and its reproducibility (%)									
		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	Mean	SD	CV
Cisplatin	300	66.0	49.2	64.7	55.7	53.1	52.0	65.4	58.0	7.1	12.3
	1000	64.4	58.5	69.0	65.0	61.1	60.9	68.6	63.9	4.0	6.2
	10,000	68.8	75.4	67.2	55.6	65.9	66.8	55.1	65.0	7.3	11.2
Carboplatin	300	43.9	42.8	41.2	37.0	39.4	39.4	37.2	40.1	2.6	6.6
	1000	41.7	44.2	44.2	39.2	39.4	42.5	39.7	41.6	2.2	5.2
	10,000	38.2	43.6	37.8	42.5	35.2	34.7	37.2	38.5	3.4	8.9

SD, standard deviation and CV, coefficient of variation.

3.6. Stability of platinum anticancer drugs and platinum-DDTC complex

The stock solutions of cisplatin and carboplatin were stable at 4 °C for at least 3 months. The cisplatin and carboplatin in the swabs were stable at room temperature for at least 24 h and at −35 °C for at least one month. Platinum-DDTC and gold-DDTC complexes were stable in mobile phase at 4 °C for at least 6 h.

3.7. Wiping recovery and its reproducibility

The highest peak areas of platinum-DDTC complex were obtained from the 70% propan-2-ol wiping solution for cisplatin and ultra-pure water for carboplatin. The wiping recovery and its reproducibility were 58.0–65.0% and 6.2–12.3% for cisplatin and 38.5–41.6% and 5.2–8.9% for carboplatin (Table 5). There were no significant differences in the wiping recovery and its reproducibility among the different amounts of cisplatin and carboplatin.

3.8. Analysis of external vial contamination

Amounts of 0.17–3.12 ng (median, 0.58 ng) and 0.50–17.0 ng (median, 1.59 ng) as cisplatin were detected from 10 and 50 mg Randa® injection, respectively (Fig. 4). Significant differences were observed in the external platinum contamination of 10 mg (P = 0.03) and 50 mg (P < 0.01) Randa® injection between lot numbers. There were significant differences in the external platinum contamination of 10 mg (P = 0.01) and 50 mg (P < 0.01) Randa® injection between the drug vial and its unfilled vial. With respect to Paraplatin® injection, 0.48–44.5 ng (median, 2.09 ng) and 0.89–794 ng (median, 7.93 ng) as carboplatin were found from the sampled 150 mg and 450 mg vials (Fig. 5). Significant differences were observed in the external platinum contamination of 150 mg (P = 0.05) and 450 mg (P = 0.04) Paraplatin® injection between lot numbers. There were significant differences in the external platinum contamination in 150 mg (P < 0.01) and 450 mg (P < 0.01) Paraplatin® injection between the drug vial and its unfilled vial.

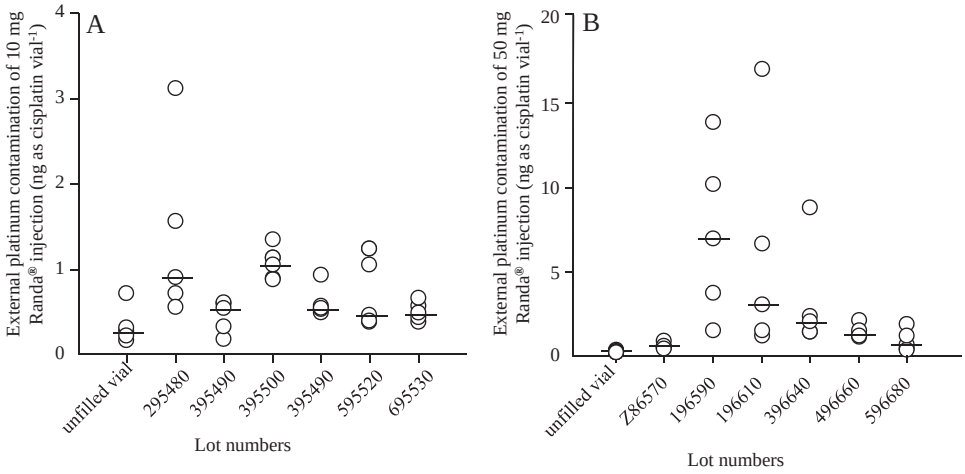

Fig. 4. External vial contamination of cisplatin in 10 mg (A) and 50 mg (B) of Randa® injection.

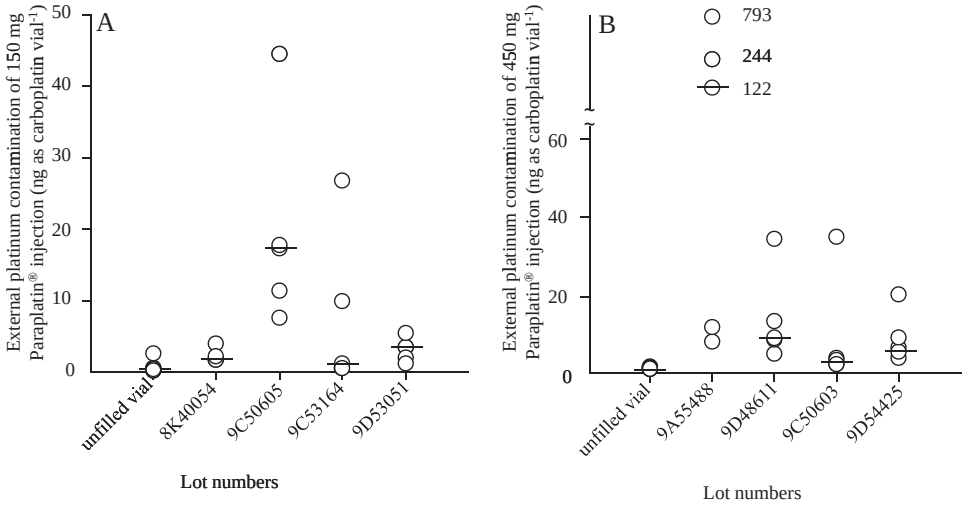

Fig. 5. External vial contamination of carboplatin in 150 mg (A) and 450 mg (B) of Paraplatin® injection.

4. Discussion

Contamination of the external surface of cytostatic drug vials is a problem since it is a potential human health hazard. A sensitive and rapid method for monitoring contamination of the external surface of vials and that does not require specialized equipment is needed hospital settings. In the present study, we developed a validated method using LC-ESI-MS/MS for the determination of contamination of vial exteriors by cisplatin and carboplatin. To the best of our knowledge, this is the first report on the determination of platinum anticancer drugs using LC-ESI-MS/MS. The suitability of the method was confirmed using drug vials delivered to our hospital pharmacy.

Most methods for determining external contamination of vials for platinum anticancer drugs employ ICP-MS [6,12,15,16]. However, ICP-MS is not commonly available in hospital settings. Several methods using LC-UV employed derivatization reagents because platinum anticancer drugs possess structural instability and no specific UV absorption [19–21]. Minakata et al. used ESI-MS with DDTC as a chelating agent to assist with the ionization of platinum [22]. DDTC is known to form a complex with platinum replacing other ligands previously bonded (Fig. 1) and the platinum-DDTC complex is stable and can be quantitatively concentrated into chloroform [24,25]. The method enabled the detection of platinum anticancer drugs at a picogram level [22]. In the present study, derivatized platinum was detected by LC-ESI-MS/MS, which possesses better sensitivity and selectivity than UV and ESI-MS. The sensitivity of LC-ESI-MS/MS is inferior to that of ICP-MS, however, the LLOQ of our method is sufficient to determine external contamination of vials.

Analytical methods using graphite furnace AAS and LC-UV detected platinum anticancer drugs as $Pt(DDTC)_2$ [19,26,27]. In the present method, $Pt(DDTC)_3^+$ as a major precursor ion was monitored using MS/MS analysis because the peak area of the product ion made from $Pt(DDTC)_3^+$ was 3-fold higher than that from $Pt(DDTC)_2^+$. An analytical method using ESI-MS also detected $Pt(DDTC)_3^+$ as a monitor ion [22]. $Pt(DDTC)_3$ and $Pt(DDTC)_2$ were eluted at same retention time using LC separation. The earlier LC-UV methods using DDTC as a chelating agent may detect the platinum anticancer drugs as the sum of the peak areas of $Pt(DDTC)_2$ and $Pt(DDTC)_3$ [19,27].

The derivatization of platinum anticancer drugs with DDTC was optimized in this method. Cisplatin and carboplatin were derivatized at room temperature because the reaction temperature did not affect the peak areas of the platinum-DDTC and gold-DDTC complexes. Andersson et al. reported that the derivatization of cisplatin with DDTC proceeded with a maximum yield of 90% after 1 h at 70 °C in methanol [28]. The derivatization with DDTC in acetonitrile was completed at room temperature. Minakata et al.

employed 3-methyl-1-butanol as a reaction solvent for derivatization with DDTC [22]. 3-Methyl-1-butanol is difficult to evaporate after derivatization due to its higher boiling point. The DDTC concentration affected the peak area of platinum-DDTC complex, but did not affect the peak area of gold-DDTC complex. In addition, the concentrations of platinum anticancer drugs and gold did not affect each peak area of the complexes.

The MS/MS chromatograms were completed within 9 min. If injection time is not included, the present method enables the measurement of 40 samples in 6 h due to the fast LC separation using a 3 μm particle ODS-column. In addition, more than 500 chromatographic runs could be performed with one ODS column without any deterioration of the separation performance. With respect to method validation, this method provides acceptable precision and accuracy in accordance with FDA guidance for industry bioanalytical method validation [29]. This validated method can be utilized to evaluate the contamination of the exterior of platinum anticancer drug vials.

Monitoring of contamination of vial exteriors by hazardous drugs has been performed using various sampling techniques [8]. Many methods employed sampling techniques using a swab, while others used an immersion sampling technique. The present method used Large Alpha® sampling swabs, which have been validated for wiped tests. Chappuy et al. also used Large Alpha® sampling swabs to monitor environmental contamination by cytostatic drugs in hospital settings [26]. Our wiping method using Large Alpha® sampling swabs was optimized with respect to the wiping solution, recovery rate, and reproducibility, and consequently is suitable for assessing contamination of vial exteriors.

The present method was used to determine platinum contamination on the exterior surface of Randa® injection and Paraplatin® injection supplied to our hospital pharmacy. Amounts of 0.17–17.0 ng vial^{-1} as cisplatin and 0.48–794 ng vial^{-1} as carboplatin were detected from the vials tested. Mason et al. reported external contamination levels of less than 9 ng and 7–251 ng in 13% of cisplatin vials and 100% of carboplatin vials, respectively [11], while Connor et al. found levels of less than 256 ng in all cisplatin vials they tested [12]. Nygren et al. reported detecting 0.2–99 ng on the outside of vials and 0.6–21 ng in the cap cover of all cisplatin vials from 3 different manufacturers [13]. The contamination levels on the exterior surface of vials of platinum anticancer drugs in earlier reports were comparable to our results. Significant differences between lots were observed for both Randa® injection and Paraplatin® injection, indicating that contamination of the external surface of all vials occurred during the manufacturing process. In addition, there were significant differences in contamination between the drug-filled vials and unfilled vials for Randa® injection and Paraplatin® injection. This result indicates that the platinum compounds detected from drug vials were due to contamination by cisplatin and carboplatin.

5. Conclusion

This study developed a validated method using LC-ESI-MS/MS for the determination of contamination of the exterior surface of vials by cisplatin and carboplatin. The suitability of the method for determining the contamination was confirmed in drug vials supplied to our hospital pharmacy. This validated method for the determination of platinum anticancer drugs is helpful for monitoring contamination of the exterior surface of vials.

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