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Determination of platinum derived from cisplatin in human tissues using electrospray ionization mass spectrometry

Kayoko Minakata*, Hideki Nozawa, Naoko Okamoto, Osamu Suzuki

*Department of Legal Medicine, Hamamatsu University School of Medicine,
1-20-1, Handayama, Hamamatsu 431-3192, Japan*

Abstract

Determination of platinum (Pt) derived from cisplatin in tissues was performed by electrospray ionization mass spectrometry (ESI-MS) using silver (Ag) as the internal standard. Pt and Ag reacted with diethyldithiocarbamate (DDC), and were extracted using isoamylalcohol and acidified with oxalic acid. The compounds were termed Pt(DDC)_3^+ and Ag(DDC)_2^+ , based on their m/z values exhibiting the highest peaks at m/z 639 and m/z 405, respectively. The limit of detection was 30 pg and the quantitation range was from 100 to 10000 pg using 5 mg tissue. The present method allowed the determination of Pt in wet-ashed tissue in 10 minutes.

Key words: Platinum; Electrospray ionization; Mass spectrometry
Diethyldithiocarbamate; Silver

*Corresponding author: Tel/fax: +81-53-435-2233

E-mail address: kminakat@hama-med.ac.jp

1. Introduction

Platinum (Pt) compounds such as cisplatin, *cis*-diamminedichloro platinum(II), and carboplatin are well-known antitumor agents [1]. Diethyldithiocarbamate (DDC, $(\text{C}_2\text{H}_5)_2\text{NCSS}^-$) is known to form a complex with Pt replacing other ligands previously bonded [2,3]. We observed that the Pt-DDC complex could be extracted thoroughly with isoamylalcohol (IAA) at pH 3 – 7. The extraction of the complex with IAA results in not only its concentration but also in the elimination of most substances contained in biological materials, enabling injection of the sample in the direct mode without staining the inside of the capillary in electrospray ionization (ESI)-mass spectrometry (MS). In addition, we found that acidification of the Pt-DDC complex, i.e., the formation of a ternary complex, enhanced the signal of $\text{Pt}(\text{DDC})_3^+$ in ESI-MS. MS is a powerful technique for the determination of Pt. Mass dependent determinations of Pt in several biological samples were performed using inductively coupled plasma (ICP) MS [4,5,6]. However, ICP-MS, requires rather large sample sizes that are not applicable to small animal tissue as well as human biopsy tissue. Previous studies have shown that the absolute amounts of Pt required at the limit of detection were 10 ng in 0.1 ml human plasma [4], 60 pg in 300 mg fish liver [5] and 1 ng in 1 ml human plasma [6], respectively. In the present ESI-MS study, the limit of detection was 30 pg of Pt in 5 mg tissue where this amount enabled the measurement several times, since only 1 μl out of 10 μl IAA dissolving 30 pg Pt was injected in each measurement. Furthermore, monoatomic isobars such as Hg and Os could potentially interfere with the determination of Pt by ICP-MS in addition to interference by polyatomic isobars such as WO, YbO, HfO, LuO and TaO [5]. Among these elements, interference from Hg and W should be noted since concentrations of Hg and W in tissues are occasionally elevated due to strong

binding to thiol proteins following absorption. The present ESI-MS study confirmed that 10 ng of Hg, W (and Hf) did not interfere with the determination of Pt. Ag was used as the internal standard (IS) in the quantitation, since interfering signals from biological substances are not observed in the $\text{Ag}(\text{DDC})_2^+$ signal and the signal intensity of Ag per atom is comparable to that of Pt under the present treatment.

2. Materials and Methods

2.1. Chemicals

HNO_3 , Pt^{4+} , Ag^+ and other metal ion standard solutions of atomic absorption grade, cisplatin and other chemicals of analytical grade were obtained from Wako Pure Chemical Ltd., Japan. IAA suitable for nucleic acid purification was obtained from Sigma-Aldrich Co., USA. Pt^{4+} and Ag^+ standard solutions at 1 $\mu\text{g}/\mu\text{l}$, respectively, were used as stock solutions. Cisplatin was dissolved in 1 M HCl at 0.5 $\mu\text{g Pt}^{2+}/\mu\text{l}$ and was used as a stock solution. Pure water, having a specific resistance of 18 M Ωcm , was used. All glassware or plastics were soaked in conc. or 0.3 M HNO_3 , respectively, overnight and rinsed at least 10 times with pure water.

2.2. Materials

Ethical approval was obtained for the removal of tissue from one patient and three reference subjects. A 68-year-old male with malignant lymphoma received an accidental overdose of 165 mg cisplatin/24 h, administered as an intravenous infusion for almost 3 days, until the patient noted the onset of hearing loss. The total cisplatin dose received was 426 mg and the patient's body weight was 56.3 kg. Intravenous hydration and administration of diuretics were initiated immediately. The patient received 13 sessions of plasmapheresis,

7 sessions of hemodialysis and 3 sessions of bilirubin adsorption totally. Death of the patient occurred on day 44, following the accidental overdose of cisplatin. A forensic autopsy was performed and cerebrum, cerebellum, thymus, heart, adrenal, testis, pancreas, lung, spleen, kidney and liver were collected and Pt levels were examined. Corresponding tissues from a healthy 60-year-old male and two healthy females (32 years and 64 years old) subjected to forensic autopsy, were obtained and subsequently used as the reference material for validation of the assay.

2.3. Wet-ashing

Tissues from reference subjects were spiked with cisplatin (either 0, 6, 20, 100, 200 or 2000 pg Pt^{2+}) and Ag^+ (either 0 or 1000 pg)/mg wet weight. Patient tissues were spiked with Ag^+ at 1000 pg/mg wet weight. Five mg of wet tissue was mixed with 5 μl of conc. HNO_3 and wet-ashed at 85°C for 8 h [7]. The final volume was adjusted to 10 μl . The amounts of samples and reagents in wet-ashing could be increased proportionally. The wet-ashed solutions could be used for one month at room temperature.

2.4. Sample preparation for examining recoveries and matrix effects

Pt^{2+} (at 200 and 2000 pg/mg) and Ag^+ (at 1000 pg/mg) were spiked to reference tissues before and after the wet-ashing to examine the recoveries of Pt and Ag in wet-ashing. Similarly, Pt^{2+} (at 100 and 1000 pg/ μl) and Ag^+ (at 500 pg/ μl) were spiked to either wet-ashed solutions of the reference tissues or 7M NaNO_3 aqueous solution to examine the matrix effect of wet-ashed tissue solution on ionization.

2.5 Sample preparation for calibration and quality control

Calibration standard solutions and quality control solutions were prepared by spiking Pt^{2+} at either 0, 3, 10, 50, 100 or 1000 pg/ μl to wet-ashed solutions of reference tissues containing Ag^+ at 500 pg/ μl .

2.6. Analytical procedure

The pH of wet-ashed tissue solution (10 μl) was adjusted to 3 - 7 with either 10 M NaOH or 7 M HNO_3 . (Small differences of aqueous volumes due to pH adjustment did not influence greatly on the volume of IAA added in the subsequent step, since the solubility of IAA in the pH adjusted tissue solution was quite low.) 1 μl of 1 M DDC was then added to the solution. After 3 min, 10 μl of IAA was added and mixed for 30 sec, and separated by centrifugation. The IAA layer was mixed with 10 μl of 1 M oxalic acid for 10 sec and centrifuged. A 1- μl aliquot of IAA layer was subjected to ESI-MS. The peaks of both $\text{Pt}(\text{DDC})_3^+$ and $\text{Ag}(\text{DDC})_2^+$ appeared 1 min after the injection.

2.7. ESI-MS operating conditions

ESI-MS was performed by using a TSQ 7000 LC/MS/MS quadrupole mass spectrometer (Thermo Quest, Japan) in the positive ion mode. One μl of the IAA layer was injected manually in the direct mode. The mobile phase consisted of methanol at a flow rate of 200 $\mu\text{l}/\text{min}$. The spray voltage was set at +4.5 kV, and the fused silica capillary temperature was set at 280 $^\circ\text{C}$ since the peaks of both $\text{Pt}(\text{DDC})_3^+$ and $\text{Ag}(\text{DDC})_2^+$ increased following an increase in temperature from 170 to 280 $^\circ\text{C}$. Nitrogen was used as the sheath gas (68 p.s.i.) and the auxiliary gas (8 units) to assist with nebulization. The electron multiplier was set at 1.3 kV and the scan time, 1.8 sec between m/z 100 and 1000.

The quantitation of Pt was conducted by simultaneously detecting two molecular ions at m/z 639 for $\text{Pt}(\text{DDC})_3^+$ and m/z 405 for $\text{Ag}(\text{DDC})_2^+$ as IS in selected ion monitoring (SIM) mode. Eight different types of ions can be monitored simultaneously by TSQ 7000.

3. Results and discussion

3.1 ESI-MS

Figure 1 shows an ESI-MS of 1 μl of IAA containing 1 ng Pt and 1 ng Ag. A cluster of peaks at around m/z 405 were the signal of $\text{Ag}(\text{DDC})_2^+$; those at around m/z 491, the signal of $\text{Pt}(\text{DDC})_2^+$; and those at around m/z 639, the signal of $\text{Pt}(\text{DDC})_3^+$, respectively (Fig.1(a)). The highest peak at m/z 639 corresponds to $^{195}\text{Pt}(^{12}\text{C}_5^1\text{H}_{10}^{14}\text{N}^{32}\text{S}_2)_3$ and other peaks, to the mixture of isotopes with natural abundances as Pt of ^{192}Pt (0.8%), ^{194}Pt (32.9%), ^{195}Pt (33.8%), ^{196}Pt (25.3%), ^{198}Pt (7.2%), S of ^{32}S (95.0%), ^{33}S (0.8%), ^{34}S (4.2%) and C of ^{12}C (98.9%), ^{13}C (1.1%), as shown in the expanded spectrum (Fig. 1(b)). Ag consists of two isotopes ^{107}Ag (51.8%) and ^{109}Ag (48.2%). Molecular ions showed clusters of peaks with their isotopes, and the shapes of the clusters proved useful for identification purposes. Figure 2(a) shows the mass spectrum of 10 pg Pt and 50 pg Ag. However, in the negative ion mode, Pt and Ag did not show any peaks corresponding to their compounds under the same treatment as that in the positive ion mode. Figure 3 shows the mass spectra of flow injections monitored at m/z 639 for $\text{Pt}(\text{DDC})_3^+$.

3.2 Difference between cisplatin and Pt^{4+}

The height of two peaks at m/z 639 and m/z 491, in addition to the

peak-height ratio between them, 5 : 1, derived from cisplatin, were the same as those derived from Pt^{4+} , respectively, in either 7M NaNO_3 aqueous solution or wet-ashed tissue solution where the pH was adjusted between 3 - 7, indicating that cisplatin reacted similarly as Pt^{4+} in approximately 7M NO_3^- solution. When the pH of the solution was below 3, the peak at m/z 639 relatively decreased, and the peak at m/z 491 increased in both cisplatin and Pt^{4+} . When the concentration of Cl^- is decreased, the hydrolysis of cisplatin occurs [8]. The majority of metal ions are oxidized to ions with higher valence states in NO_3^- solution, and these ions also favor higher valence states when the pH of the solution is elevated. These properties may explain why a tiny amount of cisplatin (Pt^{2+}) behaved similarly to Pt^{4+} in wet-ashed tissue solution, i.e., approximately 7M NaNO_3 solution.

3.3 Effects of solvents and acids

The ionization efficiencies of both $\text{Pt}(\text{DDC})_3^+$ and $\text{Ag}(\text{DDC})_2^+$ were relatively low without acid treatment following IAA extraction from 7M NaNO_3 solution. The ionization efficiencies of $\text{Pt}(\text{DDC})_3^+$ after several acid treatments were compared using IAA as an extractor, and were expressed as a % assuming the efficiency of 1 M oxalic acid to be 100 %. The efficiencies were 100 % by either 0.2 – 2 M oxalic acid or 1 M HCl , 60 % by either 1 M citric acid or 0.5 M H_2SO_4 and 40 % by 1 M HNO_3 , respectively. The ionization efficiencies of $\text{Ag}(\text{DDC})_2^+$ after acid treatment were 100 % by 1 M oxalic acid and 50 % by 1 M HCl , respectively. Using 1 M oxalic acid as an acidifier, the extraction efficiencies of several solvents were compared. The extraction efficiencies of IAA, cyclohexanol,

octanol and chloroform were 100, 100, 100 and 20 %, respectively, for Pt and those of IAA, cyclohexanol and octanol were 100, 70 and 60 %, respectively, for Ag. Since IAA and 1 M oxalic acid gave the best result for Pt and Ag, IAA and 1 M oxalic acid were used throughout the measurements, and hereafter IAA was defined as the IAA acidified by 1 M oxalic acid.

3.4 Recoveries and effects of matrix

The peak areas of $\text{Pt}(\text{DDC})_3^+$ and $\text{Ag}(\text{DDC})_2^+$ in IAA, extracted from two types of wet-ashed solutions, were compared. The solution where Pt^{2+} and Ag^+ were spiked to tissues before wet-ashing and the solution where Pt^{2+} and Ag^+ were spiked after wet-ashing were used. They were the same within a difference of 5 % in all eleven kinds of tissues spiked with 200 and 2000 pg Pt^{2+}/mg and 1000 pg Ag^+/mg wet weight. These high recoveries may be due to stable and non-volatile properties of Pt^{2+} and Ag^+ . Owing to these high recoveries, the calibration standard and the quality control can be prepared by dissolving Pt^{2+} and Ag^+ to wet-ashed solutions of reference tissues.

To examine the effects of wet-ashed tissue solution on ionization, the peaks of $\text{Pt}(\text{DDC})_3^+$ and $\text{Ag}(\text{DDC})_2^+$ in IAA extracted from wet-ashed tissue solutions were compared with those extracted from the 7M NaNO_3 aqueous solution. Since both peaks of $\text{Pt}(\text{DDC})_3^+$ and $\text{Ag}(\text{DDC})_2^+$ in IAA extracted from wet-ashed tissue solutions showed 30 % lower than those extracted from the 7M NaNO_3 aqueous solution, calibration standards as well as quality controls should be prepared using wet-ashed tissue solutions. In ESI-MS, the possibility of ionization suppression of analytes by a matrix must be considered

especially for methods involving direct injection and LC methods with a short run time [9,10,11]. The suppression is only 30 % in the present direct injection analysis, even though the procedure is reasonably simple and requires only 10 min in total for the extraction and detection of analytes. We confirmed that, in addition to common salts and amino acids, transition metals such as Fe, Zn, Mn, Mo and Cr were also eliminated mostly in the extraction of analytes with IAA from wet-ashed tissue solution at pH 7. When the IAA was mixed with 1 M oxalic acid to form ternary complexes of analytes, most impurities still contained in the IAA were removed using 1 M oxalic acid. In the present study, matrix effects were also relieved since the peak of analyte and that of IS were observed at the same time.

Both signals of Pt(DDC)_3^+ and Ag(DDC)_2^+ in IAA extracted from wet-ashed tissue solution remained constant for 3 h, and decreased to approximately 80 % after 24 h. However, both signals in IAA extracted from 7M NaNO_3 aqueous solution remained constant for 48 h. Although Cl^- easily precipitates Ag^+ , interference from Cl^- was not observed in wet-ashed tissue solution. Possibly, large amounts of NO_3^- contained in the wet-ashed solution may prevent the precipitation of AgCl . However, when Cl^- was added to wet-ashed tissue solution, the signal of Ag(DDC)_2^+ decreased, but that of Pt(DDC)_3^+ remained constant.

3.5 Accuracy, precision, limit of detection, limit of quantitation and linearity

In IAA extracted from 7M NaNO_3 aqueous solution, the limit of detection and the limit of quantitation were 3 and 10 pg, since $\text{S/N} = 3$ and 10, respectively (Fig. 3(a)). These concentrations corresponded

to 6 and 20 pg Pt/mg wet weight, respectively, in tissues. In the case of blank kidney tissue (Fig. 3(b)), blank tissues did not exhibit any peaks at m/z 639 and the limit of detection in tissue was 6 pg/mg wet weight in all tissues. Intra-day and inter-day accuracy and precision of the method were examined and expressed as recovery and co-efficient of variation (C.V.) of the determination for Pt spiked at 20, 100, 200 and 2000 pg/mg wet weight (Table 1). The deviation from the nominal value and the C.V. were lower than 10 % in all samples in both intra-day and inter-day variations at 200 and 2000 pg/mg wet weight, respectively. Therefore, the values for inter-day variation at 200 and 2000 pg/mg wet weight were not listed. The limit of quantitation was 20 pg/mg wet weight since the recoveries were in the range of 78 – 118 %, and the C.V. was less than 21 %, respectively, at 20 pg/mg in both intra-day and inter-day variations. The integrated areas of mass spectra monitored at m/z of $\text{Pt}(\text{DDC})_3^+$ relative to the area of $\text{Ag}(\text{DDC})_2^+$, (y in pg), were proportional to the amounts of Pt, (x in pg), in the concentration ranges studied. Specifically, the calibration equations calculated on 12 points (four concentrations such as 20, 100, 200 and 2000 pg/mg wet weight, three determinations per each concentration) were exceptional for all tissues (Table 2) .

3.6 Patient samples

Fig. 2(b) demonstrates the mass spectrum of 1 μl of IAA extracted

from 0.5 mg patient kidney tissue containing 500 pg Ag as IS. Although the tissue contained several metals such as Fe and Cu reacting with DDC [7] at high concentrations, the peaks of $\text{Pt}(\text{DDC})_3^+$ and $\text{Ag}(\text{DDC})_2^+$ were not interfered with, e.g. the kidney. This fact was also confirmed by the observation that metal ions such as Hg^{2+} , W^{6+} , Hf^{4+} , Fe^{3+} , Zn^{2+} , Cu^{2+} , Mn^{2+} , Ti^{4+} , V^{5+} , Cr^{6+} , Cd^{2+} and Zr^{4+} at 10^{-4} M, i.e., at approximately 10 ng, did not show peaks at the peaks of $\text{Pt}(\text{DDC})_3^+$ and $\text{Ag}(\text{DDC})_2^+$, respectively, under the same treatment.

The mass spectra of flow injections monitored at m/z 639 are shown in Fig. 3(b). One μl of IAA extracted from 0.5 mg of the reference samples and patient samples were injected in duplicate. These samples were those of blank kidney, the kidney standard spiked with Pt at 1000 pg/mg wet weight and patient samples such as cerebrum, cerebellum, thymus, heart, adrenal, testis, pancreas, lung, spleen, left kidney, right kidney and liver, respectively, from left to right. Pt levels in tissues obtained from the patient were calculated based on the kidney standard (Fig. 3(b)), and were listed as value 1 in Table 3. Following measurement in triplicate for each tissue, patient Pt levels were also calculated based on the respective calibration curve in Table 2, and are listed as value 2 in Table 3. In previous reports using healthy animals, the Pt level of the kidney was 10-fold greater than that of the liver after two hours [12] and 3-fold greater after 8 days [3], respectively. The Pt level of the kidney was lower than that of the liver in our patient in the

present study, whose death occurred 44 days after receiving an accidental overdose of cisplatin. We believe that the duration of time may be one of the contributing factors, which affected the distribution of Pt.

Conclusion

Herein, we propose a method for the rapid and decisive determination of platinum by ESI-MS. Various metals in tissues did not interfere with the assay. The method was employed for the quantitation of platinum in several tissues obtained from a patient with cancer who died 44 days after receiving accidental overdose of cisplatin.

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References

- [1] G. Chu, R. Mantin, Y.M. Shen, G. Baskett, H. Sussman, *Cancer* 72 (1993) 3707.
- [2] S.J. Bannister, L.A. Sternson, A.J. Repta, *J. Chromatogr.* 173 (1979) 333.
- [3] R.S. DeWoskin, J.E. Riviere, *Toxicol. Appl. Pharmacol.* 112 (1992) 182.
- [4] J.G. Morrison, P. White, S. McDougall, J.W. Firth, S.G. Woolfrey, M.A. Graham, D. Greenslade, *J. Pharm. Biomed. Anal.* 24 (2000) 1.
- [5] S. Zimmermann, C.M. Menzel, Z. Berner, J.D. Eckhardt, D. Stüben, F. Alt, J. Messerschmidt, H. Taraschewski, B. Sures, *Anal. Chim. Acta* 25 (2001) 203.

- [6] M. Bettinelli, *Microchem. J.* 79 (2005) 357.
- [7] K. Minakata, O. Suzuki, *Anal. Chem.* 74 (2002) 6111.
- [8] K. Lenz, S. Hann, G. Koellensperger, Z. Stefanka, G. Stingeder, N. Weissenbacher, S.N. Mahnik, M. Fuerhacker, *Sci. Total Environ.* 345 (2005) 141.
- [9] R. Bonfiglio, R.C. King, T.V. Olah, K. Merkle, *Rapid Commun. Mass Spectrom.* 13 (1999) 1175.
- [10] Y. Hsieh, M. Chintala, H. Mei, J. Agans, J-M Brisson, K. Ng, W.A. Korfmacher, *Rapid Commun. Mass Spectrom.* 15 (2001) 2481.
- [11] C. Müller, P. Schäfer, M. Srörtzel, S. Vogt, W. Weinmann, *J Chromatogr. B* 773 (2002) 47.
- [12] K. Hanada, N. Nagai, H. Ogata, *J. Chromatogr. B* 663 (1995) 181.

Figure captions

Figure 1. Mass spectra of 1 µl of IAA containing 1 ng Pt and 1 ng Ag were recorded from m/z 400 – 650 (a) and m/z 636 – 646 (b), respectively.

Figure 2. (a): Mass spectrum of 1 µl of IAA containing 10 pg Pt and 50 pg Ag. (b): Mass spectrum of 1 µl of IAA, extracted from 0.5 mg kidney tissue from the patient, containing 500 pg Ag as IS.

Figure 3. (a): Mass spectra of flow injections monitored at m/z 639.

One μl of IAA containing either 0, 3, 10 or 100 pg Pt was injected in triplicate for each amount, indicating that the limit of detection was 3 pg Pt per injection; (b): Mass spectra of flow injections monitored at m/z 639. One μl of IAA extracted from 0.5 mg of blank kidney, the kidney standard spiked with 1000 pg Pt/mg, and patient samples such as cerebrum, cerebellum, thymus, heart, adrenal gland, testis, pancreas, lung, spleen, left kidney, right kidney and liver, respectively, from left to right in duplicate.

Table 1

Recovery and coefficient of variation of determination for Pt spiked at 20 to 2000

pg/mg wet tissue. Inter-day values spiked at 200 and 2000 pg, see the text.

Pt spiked(pg)	Intra-day (3 times)				Inter-day (3 days)	
	Recovery % (C.V. %)				Recovery % (C.V. %)	
	20	100	200	2000	20	100
cerebrum	115 (21.0)	116 (13.0)	103 (8.0)	100 (4.0)	87 (16.5)	103 (12.3)
cerebellum	118 (20.5)	114 (7.9)	99 (4.5)	97 (7.1)	105 (13.5)	103 (6.0)
thymus	97 (9.4)	95 (9.6)	94 (4.2)	105 (5.0)	84 (11.8)	94 (5.6)
heart	107 (17.7)	100 (3.5)	98 (9.4)	97 (6.8)	87 (10.8)	102 (2.1)
adrenal	115 (13.1)	113 (6.6)	102 (5.2)	97 (3.6)	109 (13.3)	100 (8.2)
testis	114 (11.9)	102 (11.1)	99 (1.6)	95 (1.9)	109 (15.2)	95 (4.1)
pancreas	103 (15.4)	106 (15.2)	100 (2.6)	98 (0.4)	99 (10.7)	101 (4.5)
lung	78 (12.7)	81 (9.1)	98 (5.1)	97 (5.6)	94 (20.2)	93 (10.3)
spleen	96 (7.1)	82 (10.3)	105 (4.8)	96 (3.8)	102 (14.4)	95 (10.3)
kidney	103 (9.4)	108 (11.4)	104 (2.5)	100 (5.5)	87 (13.1)	98 (2.3)
liver	83 (12.5)	86 (9.5)	95 (6.1)	99 (2.5)	91 (15.2)	102 (8.0)

Table 2

Calibration curve and correlation coefficient for Pt spiked at 20 – 2000 pg/mg wet tissue.

Sample	Calibration equation $y = a x + b$ (y, x : pg)	S.D. a	S.D. b (pg)	Correlation coefficient
cerebrum	$y = 0.988 x + 4.6$	0.015	7.4	0.999
cerebellum	$y = 0.967 x + 4.2$	0.028	14.0	0.996
thymus	$y = 1.053 x - 5.6$	0.020	10.0	0.998
heart	$y = 0.969 x + 1.1$	0.026	13.3	0.996
adrenal	$y = 0.966 x + 5.2$	0.014	7.2	0.999
testis	$y = 0.952 x + 3.0$	0.008	3.8	0.999
pancreas	$y = 0.983 x + 2.1$	0.006	3.0	0.999
lung	$y = 0.974 x - 3.4$	0.022	11.1	0.997
spleen	$y = 0.960 x + 0.5$	0.016	7.8	0.998
kidney	$y = 0.997 x + 3.0$	0.021	10.8	0.998
liver	$y = 0.993 x - 4.1$	0.010	5.1	0.999

Table 3

Pt level listed as “Value 1” was calculated based on the comparison between the peak in each tissue ($n=2$) and that in standard kidney shown in Fig. 3 (b), and that listed as “Value 2” was calculated from another peak in each tissue ($n=3$) using the respective calibration curve listed in table 2.

Sample	Value 1 (pg/mg wet tissue)	Value 2 (pg/mg wet tissue)
cerebrum	50	48 \pm 8
cerebellum	40	36 \pm 3
thymus	40	60 \pm 12
heart	300	267 \pm 19
adrenal	460	365 \pm 61
testis	700	653 \pm 41
pancreas	460	413 \pm 57
lung	500	487 \pm 40
spleen	280	290 \pm 6
kidney	1200	1280 \pm 118
liver	1600	1680 \pm 150

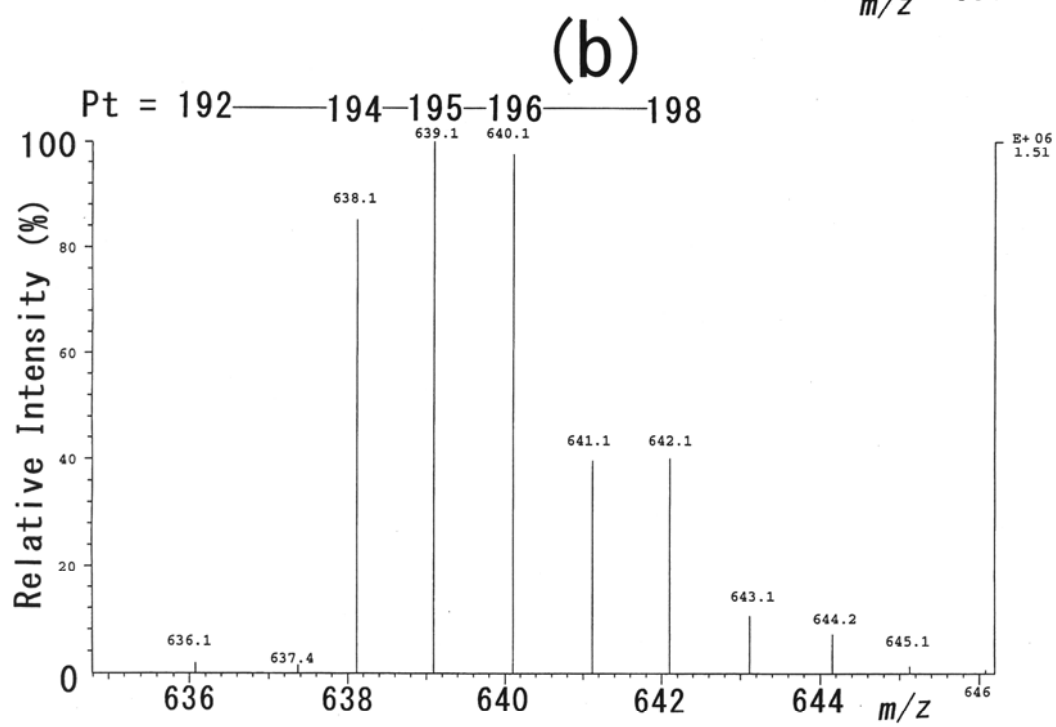
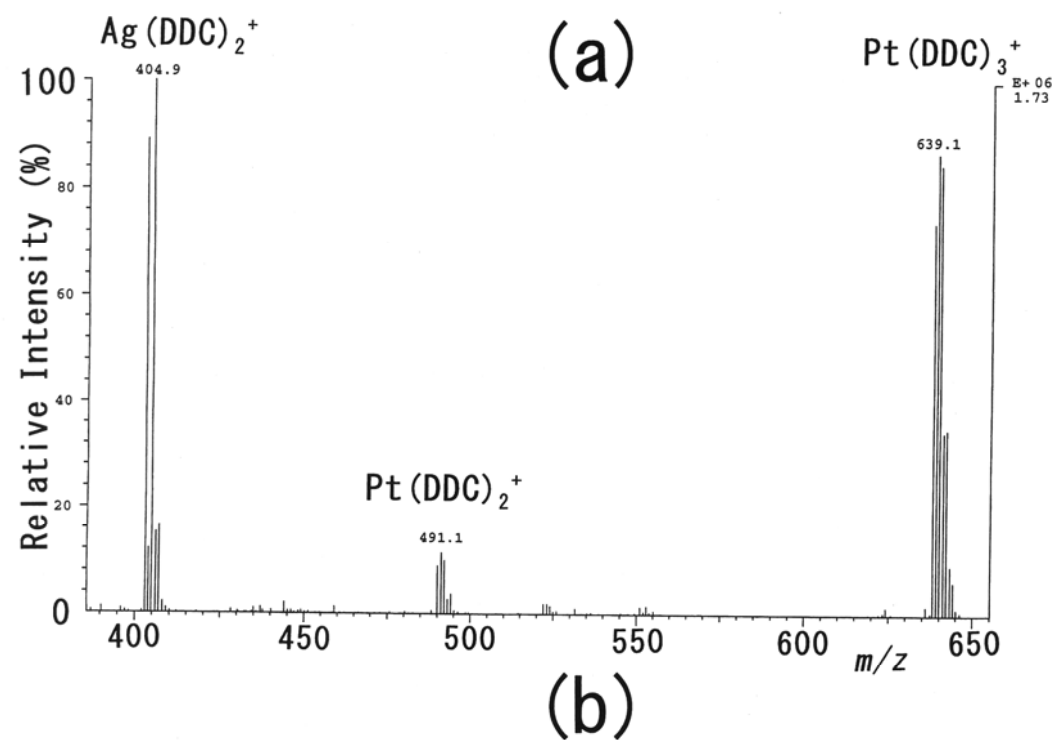


Fig. 1

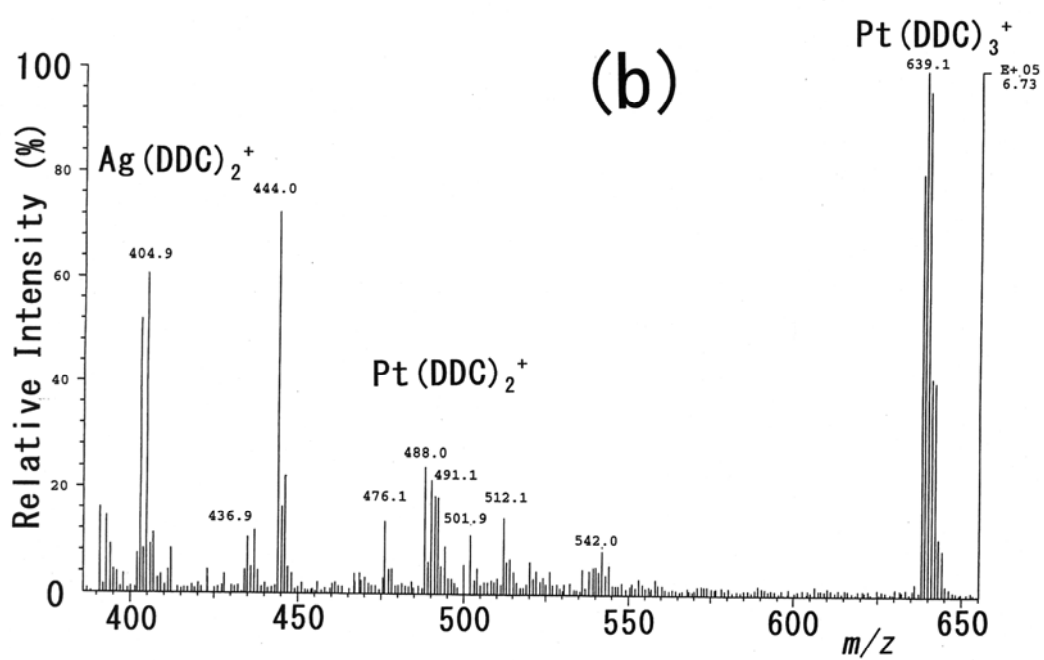
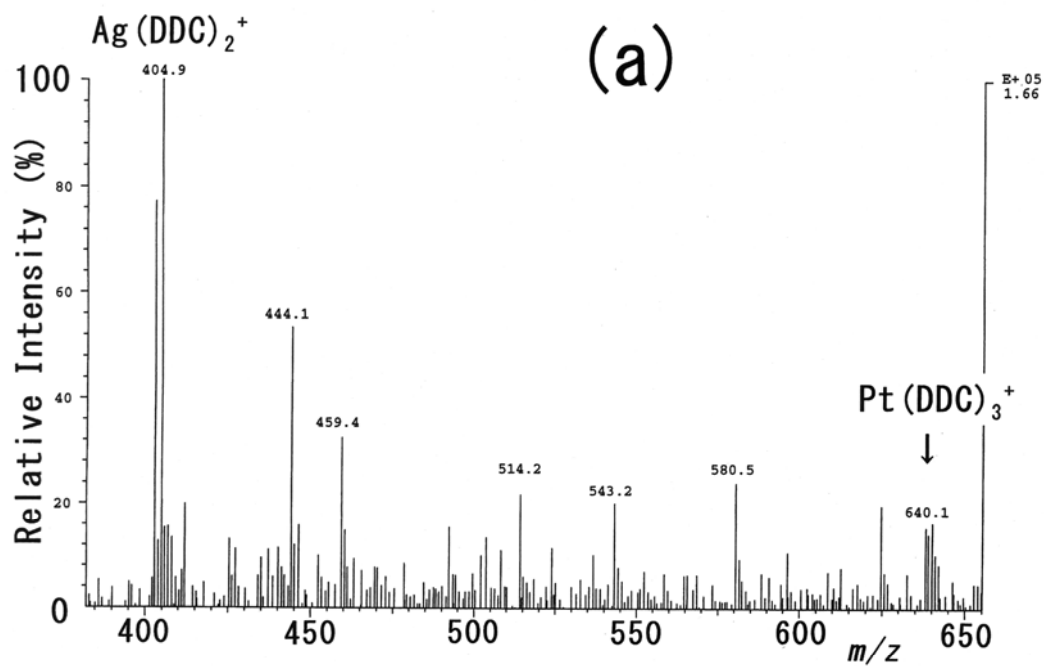


Fig. 2

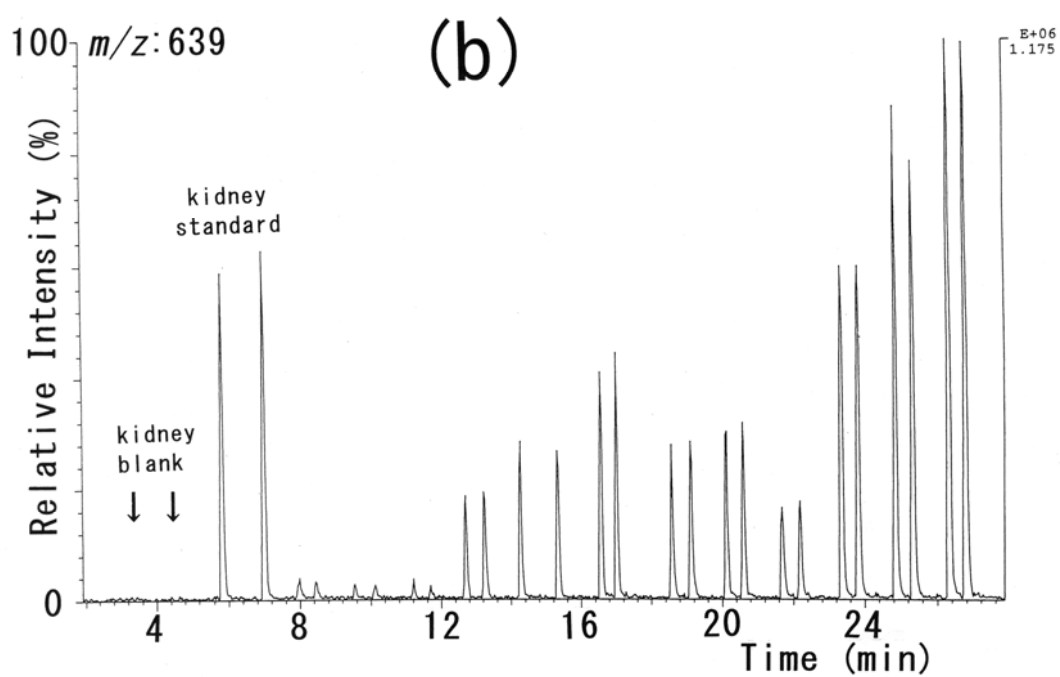
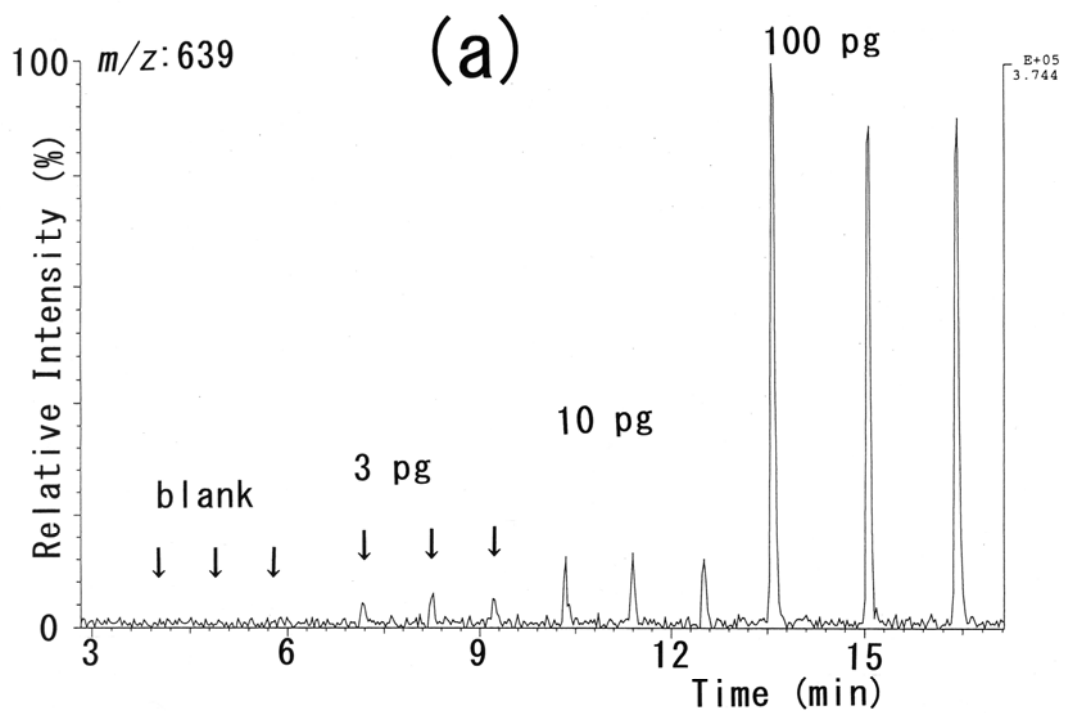


Fig. 3