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RESEARCH PAPER

Low-Level (PPB) Determination of Cisplatin in Cleaning Validation (Rinse Water) Samples. II. A High-Performance Liquid Chromatographic Method

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ABSTRACT

A high-performance liquid chromatographic (HPLC) method is described for the determination of residual levels of cisplatin from extracts of surfaces with very low surface area; from extracts of surfaces of coupons made of Teflon® (polytetrafluoroethylene, PTFE), stainless steel, and glass; and in aqueous solution collected after rinsing equipment and parts. Initially, the method was developed to determine cisplatin at concentrations ranging from 20 to 200 ng/ml by direct injection. Retaining the same method conditions, the scope of the method was expanded by the addition of a sample preconcentration step, allowing analyses at levels ranging from 0.5 ng to 20 ng/ml. Preconcentration is necessary for the determination of cisplatin in rinse waters at a quantifiable concentration of about 2 PPB. Under these conditions, the detection limit is about 0.2 to 0.3 ng/ml. Residual cisplatin on different types of surfaces, including surfaces with very low surface area, can be determined by swabbing each test surface with a derivatizing solution. The cisplatin recovered in the swabbing solution can be analyzed by HPLC using direct injection or preconcentration, depending on the expected level of cisplatin in the sample. Initial methods were developed to quantitate at a cisplatin concentration of about 100 PPB or higher in solution extracted from surfaces. However, when surface areas are limited because of the size of the parts, solution concentration becomes very low as a result of

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the minimum volume required for extraction. To support the application of swabbing techniques to surface analysis, stainless steel, Teflon, and glass surfaces were spiked with cisplatin at 2.5 to 20 ng/cm^2. Satisfactory overall recoveries of 90% ± 10% were obtained from all surfaces. Cisplatin has no ultraviolet/visible (UV/Vis) spectral-active functional group that can be used to detect low levels of cisplatin. Hence, diethyldithiocarbamate (DDTC) was used as a derivatizing agent to increase sensitivity to UV absorption at 340 nm. Diethyldithiocarbamate forms complexes with the platinum in cisplatin to yield a platinum-DDTC (Pt-DDTC) complex with a high molar-extinction coefficient. The Pt(DDTC)^2 complex thus formed was chromatographically separated and then quantitated by comparison of its detector response to that of a similarly derivatized standard preparation. DDTC also has application as a cleaning agent for cisplatin (e.g., for production equipment cleaning, spill cleanup). Destruction of cisplatin can be effected by the reaction of cisplatin with this cleaning agent. Derivatization of cisplatin will convert active cisplatin to platinum-DDTC on surfaces or in solution. Final cleaning can be accomplished using a water-for-injection rinse. After such a cleaning process, the rinse water, when collected and analyzed, showed levels of free cisplatin less than the detection concentration of 0.2 PPB and a total platinum concentration less than 10 PPB as Pt-DDTC complex.

INTRODUCTION

The coordination complex of platinum, cisplatin [*cis*-diaminedichloroplatinum (II)], has played a major role in the therapeutic treatment of a variety of neoplasms over the past three decades (1,2). A large number of cisplatin analogues and other platinum coordination compounds have been evaluated for therapeutic effect (3–8). Synergistic effects of cisplatin with other therapeutic agents have also been studied (9,10). There is a general demand for analytical techniques that are widely applicable for platinum antitumor agents and that can be used for the quantitation of these compounds at trace levels, especially in complex matrices. Methods were developed to differentiate between various platinum compounds, as well as different platinum species, in biological and other matrices (11). In part I of this study, a number of reports were cited (12) that showed the need for validation of cleaning procedures and method requirements for the cleaning process. High-performance liquid chromatography (HPLC) with electrochemical detection (13–16), quenched phosphorescence detection (17), post- and precolumn derivatization techniques (18–24) for quantitation, as well as ultraviolet (UV) monitoring at 210 nm after HPLC separation (25), have all been utilized to quantitate cisplatin. Many multihyphenated spectrometric techniques for detection coupled with chromatographic separation have been attempted (11). Many of these techniques and methods are either too complex and inadequate to quantitate cisplatin at low levels or they had matrix interference or required expensive instrumentation. In spite of the complexity involved, the detection levels are of the order 10 to 40 PPB with quantitation limits of the order of 100 PPB or higher.

There is a need to quantitate cisplatin and other platinum compounds, which are used as antineoplastic agents at very low concentrations, at detection limits of 0.5 PPB or lower. Methods are needed to evaluate the cleanability of equipment and surfaces after manufacturing of pharmaceutical drugs containing these antineoplastic agents. In this article, an HPLC method with a column-switching technique is described to expand the detection limits to 0.2 PPB, with a limit of quantitation of about 2 PPB or higher. Procedures for preconcentration and conditions for HPLC separation and quantitation of cisplatin as Pt-DDTC complex are described.

MATERIALS AND METHODS

Reagents and Materials

All reagents were analytical grade, and aqueous solutions were prepared from deionized water. HPLC grade methylene chloride, obtained from Burdick and Jackson Laboratories (Muskegon, MI), was used for extraction studies. USP *cis*-diaminedichloroplatinum (II) reference standard or high-purity USP bulk drug cisplatin was used for the preparation of cisplatin standards. Platinum (II)

chloride and diethyldithiocarbamic acid, sodium salt, were obtained from Aldrich Chemical Company (Milwaukee, WI). Sodium nitrate was obtained from Mallinckrodt (St. Louis, MO). For purposes of swabbing, polyurethane swabs (Texswab™) from Texwipe Company (Upper Saddle River, NJ) were used. Pyrex® glass, 316 stainless steel, and Teflon® plates were prepared by in-house machinists for typical swab surfaces. Glass scintillation vials, 20 ml with screw caps, were obtained from Kimble (Vineland, NJ).

All glassware was thoroughly cleaned with 10% nitric acid, then water, 50:50 acetonitrile/methanol mixture, and finally rinsed with water and dried before use. For cisplatin solutions, low actinic glassware was used.

Sodium diethyldithiocarbamate (DDTC) (0.5%) solution was prepared fresh daily. Saturated sodium nitrate was prepared by dissolving 40 g of sodium nitrate in a sufficient amount of water in a 50-ml volumetric flask by heating. The solution was diluted to volume with water and allowed to cool to room temperature to form crystals at the bottom of the flask; the solution can be used for up to a month when stored at room temperature.

Standard cisplatin solutions were prepared by accurately weighing about 100 mg of USP cisplatin reference standard or high-purity cisplatin bulk drug and dissolving it in 100 ml of 0.9% sodium chloride solution. This then was serially diluted to a concentration of 100, 50, 20, 5, or 2 PPB in cisplatin as required using 0.09% sodium chloride solution as diluent. Stock standard at a concentration of 1 PPM was prepared, and the rest of the solutions were prepared fresh daily for use.

Material contact parts, surfaces, or appropriate coupons were swabbed with the swabbing solution. In a 40-ml centrifuge, 8 ml of 0.09% sodium chloride, 1.0 ml of 0.5% sodium DDTC solution (derivatizing solution), and 1.0 ml of saturated sodium nitrate solution were mixed. The same number of centrifuge tubes as the number of plates, surfaces, or coupons to be analyzed were prepared.

To aqueous wash samples, as soon as they were sampled, an appropriately weighed amount of solid sodium chloride was added such that the concentration of sodium chloride was about 0.09%.

High-Performance Liquid Chromatography Equipment

The HPLC system employed consisted of SP Thermo Separation Products (San Jose, CA), Spectra System pump P-4000, UV 2000 detector (or Applied Biosystem ABS 785A programmable UV/Vis wavelength detector, Foster City, CA) set to monitor at 340 nm, AS 3000, variable volume injector (or Perkin-Elmer Advanced LC Sample Processor model ISS 200, Norwalk, CT). A Waters 501 HPLC pump (Milford, MA) was used as the secondary pump when the column-switching technique was required. For column switching, columns connected through a VICI (Valco Instruments Co., Houston, TX), auto/manual switching model E36, was used. A 2-ml loop was connected through a Rheodyne valve system (Rohnet Port, CA) when required (see Fig. 1 for description). Chromatograms were processed using Fison's (Beverly, MA) Chromatography X Chrom, VG Data data systems package.

High-Performance Liquid Chromatography Method

The choice of analytical procedure depends on the level of cisplatin to be quantitated. Table 1 summarizes the applicability of this assay. The detection limits attainable by this method under different conditions are presented. At a cisplatin standard concentration of 20 PPB or less, 2 ml of a solution are injected into a preconcentration column using a binary pump with water as the mobile phase; cisplatin was concentrated at the head of a guard column used as the concentrating column. This column was then connected in series to an analytical column by column switching, and the mobile phase was allowed to pass through the guard column, connected now in series with the analytical column. The details of the chromatographic conditions are listed in Table 2, and that of the procedure for preconcentration are listed in Table 3.

To establish that surfaces exposed to cisplatin at the dose level of 1.0 mg/ml can be cleaned of cisplatin, a laboratory procedure with many different parts was de-

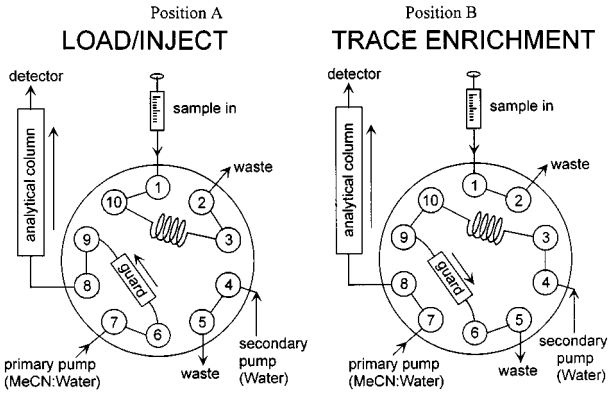


Figure 1. Connections to 10-port valve.

Table 1

Applicability of the Method for Different Sources of Samples

Assay	Detection Limit	Sample Solution	Injection Mode
Final wash water samples	0.2 ng/ml	—	Preconcentration
Surfaces with low surface area	0.03 ng/cm^2	Surface extracts	Preconcentration
Surface coupons	0.3 ng/cm^3	Surface extracts	Direct

signed. The laboratory procedure adopted is graphically illustrated in Fig. 2. The swabbing procedure for the parts is given in Table 4.

Procedure for the Preparation and Analysis of Samples

There are three different types of cleaning sample solutions. These are (*a*) solutions swabbed from surfaces of glass, Teflon, or stainless steel coupons; (*b*) solutions obtained after swabbing surface parts that were exposed to cisplatin and were subsequently cleaned as described, with these parts having very low surface area; or (*c*) aqueous rinse samples, which were to be analyzed for both residual cisplatin and Pt-DDTC complex. The solutions prepared for analysis are described below. In all cases, a blank preparation was necessary.

Blank Solution Test Preparation

For blank solution test preparation, 8.0 ml of 0.09% sodium chloride solution was pipetted into a 40-ml centrifuge tube, and 1.0 ml of derivatizing reagent solution and 1.0 ml of saturated sodium nitrate solution was added to the centrifuge tube. The solution was heated in a water bath maintained at 60°C (±2°C) for 60 (±5) min. After heating under derivatization conditions, the centrifuge tube was cooled under tap water to bring the solution rapidly to room temperature. A sample of the solution was transferred to an appropriate vial for analysis.

If surface analysis was being carried out, two polyurethane swabs were first added to the vial described above, and the vial was sonicated for 5 min. The solutions were collected from the swabs by pressing the swabs against the sides of the centrifuge tube and then removing them. Finally, the solution was heated as specified above. (Note that derivatization of blank solution test preparation, all standard solution test preparations, and sample solution test preparations were carried out simultaneously under the same conditions of temperature and time.)

Table 2

Chromatographic Conditions for the Quantitation of Cisplatin

Parameter	Condition
Column	Waters Novapak C_{18} 4-μ column
Temperature	Ambient
Flow rate	1.0 ml per min
Injection volume	200 μl (2 ml for preconcentration)
Detector wavelength	340 nm
Mobile phase	50:50 acetonitrile/water mixture
Approximate retention time of cisplatin	16 min
Run time	45 min

Standard and Control Solution Test Preparations

For direct injection, 50-PPB standard and 20-PPB control standard were prepared; 5-PPB and 2-PPB standard and control standard, respectively, were prepared for preconcentration injection. Of the standard preparation and control solution preparation, 8 ml were pipetted into separate 40-ml centrifuge tubes. To each centrifuge tube, 1.0 ml of derivatizing agent solution and 1.0 ml of saturated sodium nitrate solution were added. The solution was derivatized as given above in the preparation of blank solution test preparation for chromatographic separation and quantitation.

Aqueous Sample Test Preparation

Underivatized Sample Test Preparation

For the underivatized sample test preparation, 8.0 ml of sample was pipetted into a 40-ml centrifuge tube, and 1.0 ml of 0.09% sodium chloride solution and 1.0 ml of saturated sodium nitrate solution were added. (This solution was not heated; if not analyzed within 4 hr, it was

Table 3

Procedure for Preconcentration of Derivatized Sample

Step	Procedure
A.	Connect the two pumps, guard column, analytical column, 2-ml sample loop, and waste lines as shown in Fig. 1. Begin with the valve in position A. Allow the mobile phase to flow through the system until minimal detector fluctuation is observed.
B.	Keep the valve in position A. Fill the 2-ml loop with the derivatized solution by flushing at least 3.0 ml of solution through the loop. (After each injection of the sample, rinse the loop with acetonitrile, purge with air, rinse with water, and again purge the air before the next sample is injected.)
C.	Change the valve to position B. Let deaerated water, previously filtered through a 0.4-μ filter, flow through the trace enrichment column for 4 min.
D.	At the end of 4 min, change the position of the valve back to position A and start the integrator or data system. Monitor the signal for about 45 min. The baseline noise should be very low between 12 and 20 min of run time.

Note: In step B, the loop is filled with the solution to be analyzed. In step C, the analyte is concentrated in the enrichment column, while the interferants in the matrix are eluted to waste. In step D, the analyte is moved from the trace-enrichment column to the analytical column and finally to the detector.

refrigerated or used as a refrigerated autosampler at 0°C–5°C.)

Derivatized Sample Test Preparation

To determine total cisplatin, 8.0 ml of sample was pipetted into a 40-ml centrifuge tube. To this was added 1.0 ml of derivatizing agent solution and 1.0 ml of saturated sodium nitrate solution. This solution was derivatized (heated) as given above and analyzed immediately or stored in the refrigerator.

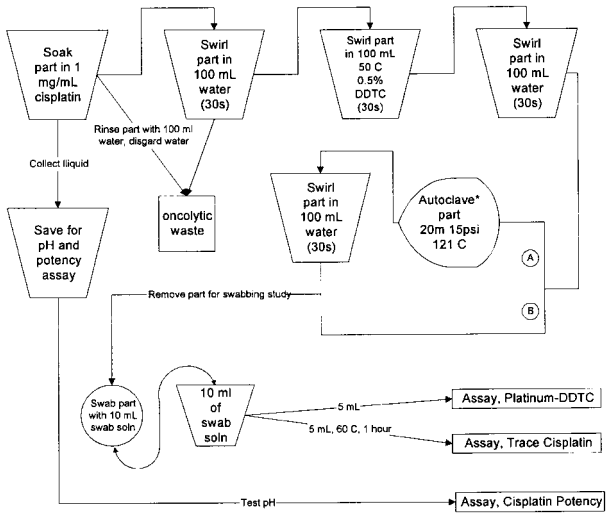


Figure 2. Procedure for cleaning contact surface parts.

Low-Level Surface Extract (Swabbing) Sample Test Preparation

All swabbed samples were sonicated with the swabs in place. The solutions were collected from the swabs by pressing the swabs against the sides of the centrifuge tube and then removing them; 3 ml of this solution was removed and designated as the underivatized sample test preparation. (This solution was not heated; if not analyzed within 4 hr, it was refrigerated or used as a refrigerated autosampler at 0°C–5°C.)

The remaining 6–7 ml was derivatized (heated) as given above and designated the derivatized sample test preparation.

Procedure for Analysis

1. For analysis, 200 μl of the derivatized solution of the 50-PPB standard was injected, and the chromatogram was monitored with the detector wavelength set at 340 nm for 45 min. Alternatively, 2 ml of derivatized solution of 5-PPB standard for samples that require the preconcentration was analyzed. This solution was chromatographed using the procedure for preconcentration of derivatized solutions listed in Table 3. In either case, there should have been a peak eluting around 15 min (12 to 20 min), with two other peaks, one on each side of this peak (retention time of about 10 and 25 min, respectively). The middle peak is the

Table 4

Swabbing Procedure

Preparation of Swabs for Swabbing

Place about 100 polyurethane swabs upside down in a beaker. Add enough water to cover the heads of the swabs. Sonicate for 20 min. Drain the water. Repeat the procedure with methanol as the wash solvent. Finally, wash with water again. Dry the swabs at 60°C under vacuum overnight.

Swabbing Procedure

Identify an area equivalent to about 10 cm × 10 cm on the surface being analyzed if it is a Teflon, glass, or stainless steel coupon. Otherwise, measure the total surface area of the contact surface if it is less than 100 cm^2; if total suface area available is huge, cut parts into 12 cm × 12 cm coupons or coupons of greater surface area such that total surface area available for swabbing is greater than a 100 cm^2 area.

Add about 5 to 10 drops of the swabbing solution to the surface using the prepared swab. Spread the solution over the entire surface area. Swab the surface by moving along the horizontal direction for about 10 strokes and for about 10 strokes in the vertical direction. If the part is a curved surface, pass the swab along the surface back and forth for about 20 times.

Immerse the swab in the swabbing solution contained in the centrifuge tube. Reswab as given above with the moistened swab. Remoisten at least two times from the same centrifuge tube.

Place the swab in the same centrifuge tube and then take a fresh swab and swab the surface to near dryness. Place the second swab in the same centrifuge tube.

Repeat with fresh dry swabs, if necessary. Place all swabs in the centrifuge tube.

Refrigerate the sample if it cannot be analyzed immediately.

chromatographic peak of the pt-DDTC complex (see Fig. 3).

2. The derivatized blank solution (200 µl) (or 2 ml for solution requiring preconcentration) was injected, and the chromatogram was monitored for 45 min. There should have been no peak in the region between 12 and 20 min of run time. (Sometimes, in the quantitation of analytes with cisplatin concentration below 5 PPB, there is a potential for carryover from previous use of the instruments, especially if the injector has been used for the quantitation of cisplatin in cisplatin solutions. This step ensures that any potential carryover problems are eliminated before the analysis of the samples.) If interfering peaks due to carryover were observed, mobile phase solution was injected, as necessary, until there were no interfering peaks in the region of interest.
3. Next, 200 µl of the 20-PPB (or 2 ml of 2-PPB control for solutions requiring preconcentration) derivatized control solution were injected, and a peak considerably higher than three times the noise level appeared with the same retention time as that observed for derivatized standard solution in step 1 above.
4. The chromatographic system was considered suitable for analysis if all three conditions were met. Then, standard and sample test preparations for analysis were injected. For standards, the average peak area *As* was not to vary by more than 7.0% from either of two consecutive injections of the derivatized standard preparation. If the peak area corresponding to cisplatin in any sample test preparation was less than that of the control solution test preparation (2 or 20 PPB), a single injection was adequate. Otherwise, at least two injections of the sample test preparation were made, and then peak areas were averaged *Au* for calculation.

Calculations

For Direct Injection, Solution Analysis

$$\frac{Au}{As} \times \frac{\text{Std. Wt. (mg)}}{100\ \text{ml}} \times 0.00050 \times \frac{1000\mu\text{g}}{\text{mg}} \times \frac{1000\ \text{ng}}{\mu\text{g}} = \text{Cisplatin (ng/ml)}$$

where 0.00050 is the standard dilution factor.

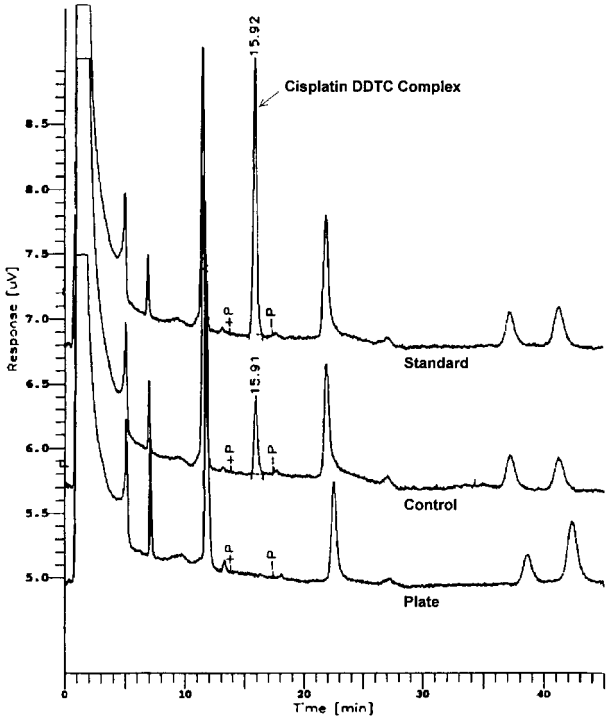


Figure 3. Chromatograms of derivatized solutions of blank, a standard at 100 PPB, and a control at 20 PPB.

For Platinum-DDTC Complex

$$\frac{Au}{As} \times \frac{\text{Std. Wt. (mg)}}{100\ \text{ml}} \times 0.00050 \times \frac{491.6}{300.1} = \text{Cisplatin } (\mu g/ml)$$

where 0.00050 is the standard dilution factor, and 491.6/300.1 is the molecular weight conversion factor.

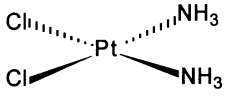
For Direct Injection, Surface Analysis

$$\frac{Au}{As} \times \frac{\text{Std. Wt. (mg)}}{100\ \text{ml}} \times 0.00050 \times \frac{8}{10} \times 0.1 = \text{Cisplatin } (\mu g/cm^2)$$

where 0.00050 is the standard dilution factor, and 0.1 is the surface to volume ratio factor to calculate the cisplatin concentration on surfaces.

For All Samples Involving Preconcentration

For all samples involving preconcentration, the results were calculated as given above using a dilution factor of 0.000050.



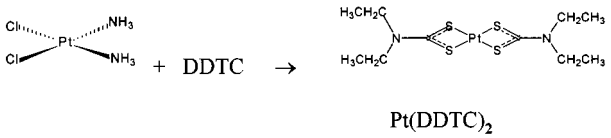
Structure 1. Cisplatin

RESULTS AND DISCUSSION

Cisplatin [*cis*-diaminedichloroplatinum (II)] is a square planar complex of platinum. The oxidation state of platinum is +2. The positive charge of the metal ion in cisplatin is neutralized by the negative charges of the two chloride ions in the inner coordination sphere of platinum, resulting in a neutral coordination complex. The structure of cisplatin is given in Structure 1.

Because of the inclusion of the chloride ions in the inner coordination sphere of the platinum metal, the chloride in cisplatin solutions do not ionize to yield chloride ions in solution as in ionic metal salts. But, under the aqueous conditions of a solution, the chloride ion in the complex may become labile enough to yield aquated and hydroxylated complexes of platinum and free chloride ions in equilibrium with these complexes. To avoid potential hydrolysis and loss of platinum by adsorption or other processes, therefore making it unavailable for derivatization, an excess chloride ion concentration was always maintained in solution to drive the equilibrium to cisplatin by diluting the sample in 0.09% sodium chloride solution. There are no chromophores that are amenable to direct spectrophotometric detection, particularly for low-level quantitation. It is well known that cisplatin reacts with DDTC to yield a *cis*-di-DDTC complex, [$Pt(DDTC)_2$]. The reaction is given in Scheme 1 (26).

The validation procedure adopted consisted of several stages. First, HPLC methods were developed and validated to determine analyte concentrations at appropriately targeted levels of the source of the sample. Once the methods were developed, linear response behavior at appropriate concentration levels and reproducibility under specified conditions were established for the extraction of surfaces. Then, a procedure to mimic cleanability of low surface area materials was designed. The effec-



Scheme 1. Reaction of cisplatin with diethyldithiocarbamate.

tiveness of cleaning was validated by monitoring low levels of cisplatin on these surfaces. Aqueous samples collected after rinsing parts were assayed for low levels of cisplatin and Pt-DDTC complex. These validation data are presented. In addition, examples of results showing that surfaces are effectively cleaned are also presented.

Reproducibility of the Derivatization Procedure

The reproducibility of the derivatization was established by mixing cisplatin solution at 100 PPB with sodium DDTC and sodium nitrate in six independent centrifuge tubes. The resulting mixture was derivatized under recommended method conditions. Each was analyzed chromatographically and was quantitated against a similarly derivatized cisplatin standard. The peak area of each sample thus derivatized was compared with that of the standard preparation. The result averaged 103% ± 10%, with the lowest value at 90% and the highest at 116%.

Quantitative Nature of the Derivatization Process

Platinum chloride was dissolved in dimethyl sulfoxide (DMSO); platinum ethylenediamine complex was dissolved in water. These were suitably diluted such that the platinum content in each was about 80 PPB. Cisplatin was prepared in 0.9% sodium chloride, suitably diluted in 0.09% sodium chloride solution. Platinum ethylenediamine complex and cisplatin were then derivatized using the derivatization procedure and extracted in methylene chloride, and the platinum content was determined using an atomic absorption method for the determination of platinum content (2). The data (Table 5) clearly show that the conversion of cisplatin to the complex was quantitative, and the derivatization was complete in 60 min when derivatized at 60°C.

Standard/Addition Recovery Data from Stainless Steel, Teflon, and Glass Coupons

The stainless steel, Teflon, and glass coupons were utilized to show that, if these surfaces were contaminated with cisplatin, they can be cleaned. In addition, if a cisplatin spill occurs, an acceptable procedure can be used to show that the surfaces are adequately cleaned. Figure 3 shows the typical chromatograms of a derivatized blank solution, control solution at 20 PPB, and standard solution at 100 PPB. The chromatogram of the blank solution corresponds to that of a blank derivatizing solution used under normal coupon swabbing conditions, that is, exposure to swabs of about 100 to 200 cm^2 area of coupon. The resulting solution was treated suitably under derivatization conditions. The chromatograms show a number of peaks in the blank solution, which are attributable to trace metal ions present on the surface, in solution, or in the swabs. The concentration of DDTC was chosen high enough such that complexation of DDTC with these other metals did not affect the quantitative conversion of cisplatin to Pt-DDTC complex. A comparison of the chromatograms of the blank solution with either that of the 20-PPB control solution or that of the standard solution

Table 5

Cisplatin Quantitation: Calculated Percentage Conversion

Compound	Platinum Content Found (PPB)	Platinum Calculated (PPB)	Conversion (%)
Platinum chloride	84.4	80.2	Used as is; not applicable
Platinum ethylenediamine complex	Used as standard	80 (PPB)	
Cisplatin			
1	74.3	81.4	91.3
2	81.6	81.4	100.2
3	80.7	81.4	99.1

Note that when cisplatin is present at levels as low as 0.5 PPB, linear response of absorbance under atomic absorption method conditions was observed. The concentration of cisplatin was about 125 PPB, corresponding to about 80 PPB of platinum.

shows that cisplatin is clearly separated from the rest of potential interferants.

Standard addition/recovery studies were carried out on three different surfaces: Teflon, stainless steel, and glass. These data (Table 6) show overall recoveries of 90% ± 9%. Starting at about 20 ng/cm^2, spike levels were decreased to about 2.5 ng/cm^2. In all cases, the recoveries were acceptable. These recoveries are considered excellent in terms of all the surfaces involved, the low levels at which cisplatin was spiked to these surfaces, and the number of steps required for the processing and analysis of the samples. In addition to these recovery studies, Teflon surfaces were spiked with cisplatin at 50 times the highest spike level of 20 ng/cm^2 (i.e., 50 × 20 ng/cm^2 = 1 μg/cm^2), swabbed per the swabbing procedure described in Table 4, and cleaned with water. The resulting plate was then swabbed for detection of residual levels of cisplatin in the cleaned coupons. No detectable levels were found in the plate. This swabbing procedure leaves no residual cisplatin; therefore, an appropriate procedure can be recommended for spill cleanup, as well as for decontamination of surfaces irrespective of the surface that needs cleaning.

During the initial studies, the analyses were usually performed within 4 hr of derivatization. The data presented in Table 6 correspond to the validation data generated within 4 hr after derivatization. During these initial studies, the derivatization was carried by mixing the target analyte with 0.5% sodium DDTC. Trace metals from surfaces were found to suppress the reaction or accelerate the degradation process. Addition of 1 ml of saturated sodium nitrate (26) was found to increase the stability of the derivatized sample solutions for at least 16 hr and considerably more if the sample was refrigerated. Thus, to establish that this modification does not affect the recovery data, the standard addition recovery was repeated under these conditions for Teflon surfaces. Reproducible results were obtained (Table 7). At the 95% confidence interval, these two means are the same for the population in each set of data.

Table 6

Percentage Recovery of Cisplatin Added to Different Surfaces

Added (ng/cm^2)	Recovered (ng/cm^2)	Recovery (%)
Teflon		
20.06	16.25	81
15.04	13.51	90
	13.85	92
10.03	9.21	92
	8.01	80
5.02	4.37	87
	4.28	85
2.51	1.93	77
	1.94	77
Mean		85
Relative standard deviation		±7%
Stainless steel		
20.06	18.21	91
	17.09	85
15.04	13.71	91
	13.16	88
10.03	9.02	90
	11.68	116
5.02	4.99	99
	5.27	105
2.51	1.98	79
	2.18	87
Mean		93
Relative standard deviation		±12%
Glass		
15.08	13.60	90
	14.73	98
10.05	9.48	94
5.03	4.87	97
	5.06	101
Mean		96
Relative standard deviation		±4%
Overall average recovery		90
Overall standard deviation		±9
Overall relative standard deviation		±10%

Surface Parts with Low Surface Area

According to the procedure outlined in Fig. 2, each part was rinsed for 30 sec using a cleaning solution containing DDTC. Then, the part was rinsed with water for 30 sec. The two-step rinsing process was designed to remove *most* of the active cisplatin from the surface of each part. The conversion of cisplatin to Pt-DDTC was not an instantaneous reaction; complete conversion of surface-bound cisplatin to Pt-DDTC was not effected during cleaning. Therefore, after rinsing, each part was subjected to autoclaving. Under these conditions, the DDTC remaining on the surface converted cisplatin to the adduct. Each part retained some residual cisplatin and Pt-DDTC complex. Then, the contact part was swabbed, and the swab solution was analyzed to determine the surface concentration of the two species.

Figure 4 shows the chromatograms of derivatized solutions of a blank test preparation, a control test prepara-

Table 7

Percent Recovery of Cisplatin Added to Teflon Surfaces Repeated Under Conditions Containing Sodium Nitrate

Added (ng/cm^2)	Recovered (ng/cm^2)	Recovery (%)
15.2	10.4	69
	12.1	79
10.1	9.0	90
	7.9	79
5.1	3.6	71
	3.3	65
Mean		76
Standard deviation		±9
Relative standard deviation		±12

tion, the swab sample solution (from the surface of a part) at the limit of detection, and a 20-PPB standard solution of cisplatin similarly derivatized and chromatographed under the HPLC conditions. The limit of detection for the solution under the conditions of the method was about 0.3 ng/cm^2.

The results obtained after cleaning some of these parts with low surface areas are presented in Table 8. For proprietary reasons, the parts are identified only by part numbers; however, typical parts include silicone rubber triclamp gaskets, rubber O rings, Kynar bushings, and the like. Each part was swabbed thoroughly to collect both residual cisplatin and Pt-DDTC. The swabbing solution was then divided into two portions and analyzed. The first portion was analyzed by HPLC without pretreatment. The result obtained in the first portion yields the surface concentration of Pt-DDTC only (column 2 in Table 8), while the second portion, which was analyzed after heating, gives a measure of the total Pt-DDTC concentration. The difference between the two results is a measure of active cisplatin (column 3 in Table 8). Except for parts 3, 36, 60, and 66 (Table 8), all other parts were thoroughly cleaned below a surface concentration of 0.5 ng/cm^2.

Assay for Aqueous Samples

To establish that manufacturing equipment and surfaces in a typical manufacturing area after a cleaning process show only very low levels of cisplatin and Pt-DDTC complex, a final wash was carried out on selected parts with water for injection. The sample of water for injection collected after rinsing the parts, under these conditions, will contain the Pt-DDTC complex that was formed

Table 8

Cleanability Study Results

Part Number	Platinum-DDTC (ng/cm^2)	Cisplatin (ng/cm^2)
Steam applied during cleaning process (autoclave)		
2	0.23	Not detected
8	0.04	0.15
16	1.53	0.05
20	0.05	Not detected
20	Not detected	Not detected
21	Not detected	0.12
65	Not detected	0.12
65	0.05	0.22
66	0.24	0.75
67	Not detected	0.18
Steam not applied during cleaning process[a]		
1	—	0.19
2	—	1.42[b]
3	—	0.76
36	—	1.45
58	—	0.14
60	—	0.85

[a] For the parts that were not steamed, the result obtained is the sum of active cisplatin and platinum-DDTC.

[b] The levels of platinum-DDTC and cisplatin for this part were measured before and after steaming. Thus, both platinum-DDTC and cisplating can be quantitated to very low levels.

as a result of reaction with DDTC during cleaning and free unreacted cisplatin. The concentration of Pt-DDTC can be ascertained by injecting the underivatized solution as is or immediately after mixing with the reagents. Thus, the rinse water sample will show a peak characteristic of the Pt-DDTC complex only. But, when another portion of the same solution was heated with DDTC (i.e., when derivatized) and then analyzed, an increase in Pt-DDTC peak was be observed since any cisplatin unreacted during the cleaning process was derivatized in the second derivatization process. Thus, this can be ascertained from an examination of the chromatograms of the rinse water sample (Fig. 5) before and after derivatization. The peak height is approximately at the level of detection of about 0.1 PPB for the underivatized sample. Other typical chromatograms of a rinse water sample, a 2-PPB control solution, and a 5-PPB standard are shown in Fig. 5.

Linear Response Behavior

The linearity of detector response experiments was designed to show that the detector response will be a linear

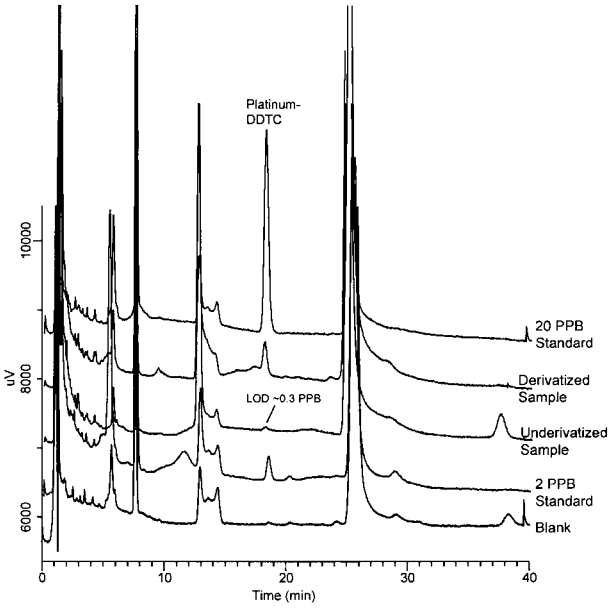


Figure 4. Chromatograms of derivatized solutions (description of each chromatogram in the figure).

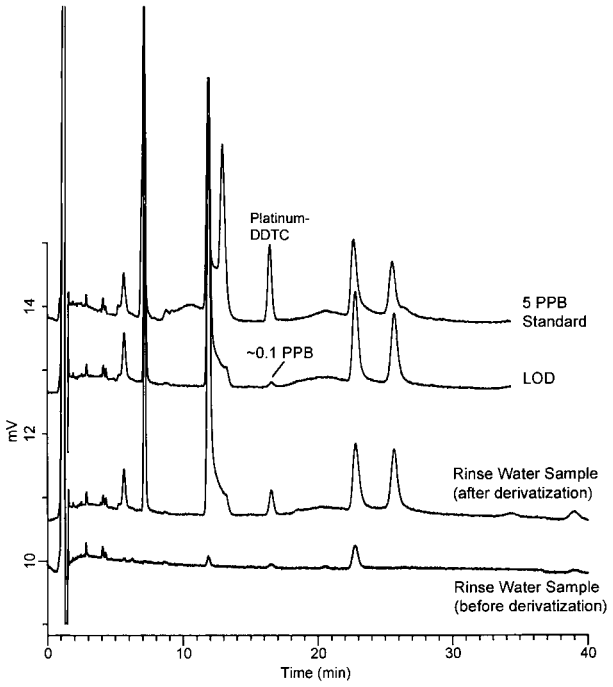


Figure 5. Chromatogram obtained under rinse water assay conditions.

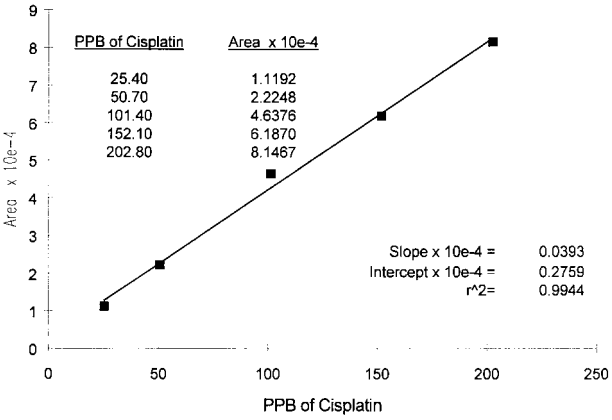


Figure 6. Plot of cisplatin concentration versus peak area.

function of concentration in the concentration range of interest. Cisplatin solutions at the required concentrations were prepared and derivatized. These derivatized solutions were then chromatographed under specified experimental conditions, and the detector response was plotted as a function of cisplatin concentration. Figure 6 shows the linear response between 25-PPB and 200-PPB levels of cisplatin in solution, which correspond to 2.5 ng/cm^2 and 20.0 ng of cisplatin/cm^2, respectively, of the coupon surface. The linear correlation coefficient was greater than 0.99, and the magnitude of the intercept was about 6%. The linear response of detector signal at low concentrations under preconcentration conditions is shown in Fig. 7. For low levels of cisplatin concentration, a preconcentration method has to be adopted. The detector responses were linear, and the intercepts pass very close to the origin under conditions involving preconcentration and direct injection. These data suggest that a single con-

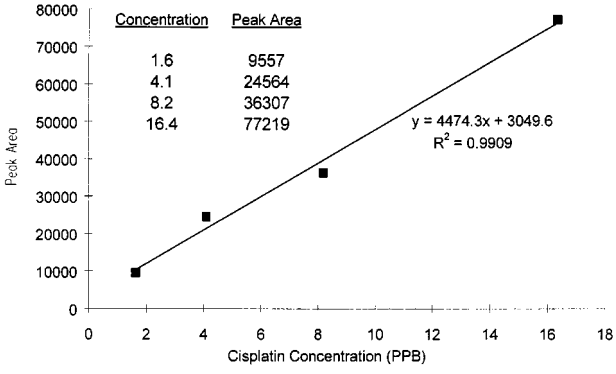


Figure 7. Plot of peak area versus concentration for low-level detection of cisplatin.

centration of the standard is adequate in each case to quantitate low levels of cisplatin under specified conditions. Since many other platinum compounds can be derivatized to yield Pt-DDTC complex, this method can be widely applied to other platinum antitumor agents like carboplatin.

CONCLUSIONS

The methods described in this article are simple and very rapid. Validation data generated demonstrate that cisplatin can be extracted from suitable surfaces and can be quantitated. In addition, excellent reproducibility can be obtained at low levels. Using the appropriate experimental conditions suggested, the samples collected from different sources, like wash water, cleaning validation coupons, and other surfaces, can be quantitated with ease and without the need for expensive and sophisticated instrumentation. The ability to detect and quantitate the adduct at low levels enhances the utility of the method since it can be applied to a number of other new platinum antitumor agents being developed.

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