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RESEARCH PAPER

Low-Level (PPB) Determination of Cisplatin in Cleaning Validation (Rinse Water) Samples. I. An Atomic Absorption Spectrophotometric Method

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ABSTRACT

Suitable analytical methods are required for quantitative determination of trace levels of ingredients in samples obtained for purposes of cleaning validation. We describe below an atomic absorption method for the quantitation of cisplatin, an antineoplastic agent, in aqueous samples. Cisplatin was reacted with diethyldithiocarbamic acid (DDTC), sodium salt, to yield a platinum-DDTC (Pt-DDTC) complex. The Pt-DDTC chelate was extracted into methylene chloride, the extract was mixed with acetonitrile, and the platinum content was then determined using a Zeeman atomic absorption (AA) spectrophotometer. The extraction conditions and AA experimental conditions were set up such that the detection level could be extended to 0.5 ng/ml. Reproducible results were obtained at a quantitative working standard concentration of 5 PPB. The absorbance response was found to be a linear function of cisplatin concentration in the region between 0.5 PPB and 20 PPB, which is about 10% to 400% of the target analyte concentration of 5 PPB. The target analyte concentration was set at 5 PPB such that it was at least 10 times the detection limit of about 0.5 PPB.

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INTRODUCTION

Since pretreatment procedures to reduce nephrotoxicity associated with the intravenous injection of cisplatin [cis-dichlorodiamineplatinum (II), CDDP] have been proposed (1), CDDP has been widely used for the treatment of solid tumors of the head, neck, bladder, ovaries, testes, lungs, and bone (2–6). Many antineoplastic agents, including CDDP, are cytotoxic. In view of this, there are many recommendations for the safe handling of parenteral antineoplastic agents (7–13). In addition, in the pharmaceutical industry, cleaning the equipment in manufacturing facilities of active drugs to very low levels is a necessity to prevent cross contamination of products subsequently manufactured with that equipment. Cleaning process validation and validation of analytical methods have been emphasized in many recent publications (14–22).

Low-level quantitation of cisplatin in different matrices has been attempted; these attempts were reviewed in Refs. 23 and 24. Quantitation of cisplatin or total platinum requires either parts per million levels of cisplatin (mostly determined as total platinum) or, for low levels, multihyphenated techniques like electrothermal atomization (or volatilization) atomic absorption spectrometry (ETA-AAS) or ETV-ICP-AES (electrothermal volatilization–inductively coupled plasma–Auger electron spectroscopy) or ETV-ICP-MS (electrothermal volatilization–inductively coupled plasma–mass spectrometry) (25,26). Even with these techniques, detection levels are of the order of 50 PPB or higher, and thus simple and rapid methods for the quantitation of cisplatin at 5 PPB were lacking. Derivatization of cisplatin with diethyldithiocarbamic acid, followed by high-performance liquid chromatography (HPLC) yielded detection, and sometimes quantitation, at 100-PPB levels (27). The measurement of platinum levels by simple AA methods was found to be highly dependent on the matrix, and hence recoveries from the matrices, as well as reproducibility of the results obtained, were found to be poor (28).

The reported methods describe either derivatization of cisplatin to a platinum-DDTC (Pt-DDTC) complex for quantitation using HPLC or ultraviolet (UV) studies or methods of sample preparation of cisplatin by conversion to platinum salts for AA measurements. We judiciously combined these two procedures to derivatize the cisplatin to Pt-DDTC complex and determine the platinum content of this complex to extend the detection limit to as low as 0.5 PPB. In this article, procedures are described for the conversion of cisplatin to Pt-DDTC complex, the conditions for extraction, and the Zeeman AA conditions required to quantitate at 5-PPB levels. In addition, method validation data for reproducibility and linearity of signal as a function of concentration of cisplatin in samples containing trace levels of cisplatin are also provided.

MATERIALS AND METHODS

Reagents and Materials

All reagents were analytical grade, and aqueous solutions were prepared from deionized water. HPLC grade methylene chloride was obtained from Burdick and Jackson Laboratories (Muskegon, MI). DDTC sodium salt was obtained from Aldrich Chemical Company (Milwaukee, WI). USP CDDP reference standard or a high-purity USP cisplatin bulk drug was used for preparation of standards. Platinum AA standard (1000 PPM) was obtained from J. T. Baker, Incorporated (Phillipsburg, NJ).

All glassware was washed thoroughly with 10% nitric acid, then with water, and then finally rinsed with 0.2% nitric acid. For cisplatin solutions, low actinic glassware was used. (*Caution*: The solvent in which the sample was prepared for AA analysis was a 1:1 mixture of methylene chloride and acetonitrile. Therefore, only Teflon or glass cups should be used for assay of solutions.)

For purposes of system suitability, a solution of 1000 PPM platinum AA reference standard was serially diluted to a concentration of about 80 PPB in water. A blank was prepared using 50 ml of 0.09% sodium chloride. Cisplatin USP reference standard was used to prepare a 0.5 mg/ml solution of cisplatin in 0.

9% sodium chloride. This solution was then serially diluted to obtain a final concentration of 5 PPB using 0.09% (note, 0.09%) sodium chloride solution as the diluent.

To 50 ml of cisplatin reference standard solution (5 PPB) in 0.09% sodium chloride, add 2 ml of 0.5% sodium diethyldithiocarbamate (DDTC) in water. The solution was heated for about 30 min in a water bath maintained at 60°C (±5°C), then cooled to room temperature and extracted with 5 ml of methylene chloride. Then, 2 ml of methylene chloride extract was combined with 2 ml of acetonitrile. Each extracted solution was kept in a stoppered vial for analysis. The above procedure was repeated for 50 ml of 0.09% sodium chloride (blank preparation) and for 50 ml of each sample being analyzed. (Cisplatin in rinse samples was quantitated using a cisplatin standard.)

Table 1

Graphite Furnace Method Conditions

Step	Furnace Temperature (°C)	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (ml/min.)
1	90	10	60	300
2	1300	10	20	300
3[a]	2500	0	3	0
4	2700	1	5	300
5	20	1	5	300

Steps 1 and 2 are repeated for multiple injections of samples.
[a] Gas for Internal gas flow is Argon and the absorbance signal is measured in this step.

Equipment and Equipment Conditions

The instrument used for AA measurements was a Perkin-Elmer Zeeman (Norwalk, CT) graphite furnace AA spectrophotometer (model 3030) equipped with an autosampler and pyrolytically coated graphite tube. Platinum line at 265.9 nm with a slit width of 0.7 nm was monitored. The lamp current was 15 mA. The sample injection volume was 50 μL. The graphite furnace method conditions are given in Table 1, and the cleaning conditions are given in Table 2.

Procedure

First, the following instrumental conditions were used for the pyrocoated tube to remove any impurities adhering to its surface that might interfere in the determination of sample absorbance.

Analysis was done using the diluted 80-PPB AA standard prepared by making a single injection and using the instrumental conditions provided in Table 1. For optimal performance of the instrument, the Zeeman corrected peak area should not be less than 0.06 units. (Note that the platinum AA standard is used only for system suitability.) Then, a similar analysis was performed with five injections of the derivatized blank solution. The Zeeman corrected peak area should be less than 0.02 absorbance units. If these two conditions were met, the standard and sample solutions that were derivatized under method conditions were analyzed using five injections for each. Using the ratio of the sample absorbance to that of the standard absorbance and the concentration of the standard solution (~5 PPB), the concentration of cisplatin in the sample could be calculated. If the sample concentration was greater than 20 PPB, the derivatized solution could be diluted with acetonitrile. Note that 5 injections of 5-PPB cisplatin standard solution is equivalent to a single injection of 80-PPB platinum AA standard; 5 PPB × (50/5) (the concentration by extraction) × (1/2) (dilution with acetonitrile) × 5 (the number of injections is increased to five in the case of cisplatin) × (190.5/300.1) (the conversion factor for platinum content in cisplatin).

Table 2

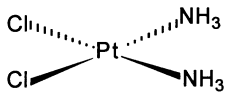
Graphite Furnace Cleaning Conditions

Step	Furnace Temperature (°C)	Ramp Time (sec)	Hold Time (sec)	Internal Gas Flow (ml/min.)
1	2650	60	2	300
2	20	1	20	300
3	2650	10	10	300
4	20	1	20	300
5	2650	10	10	300
6	20	1	20	300
7	2650	10	10	300

RESULTS AND DISCUSSION

Platinum at low levels can be determined using AA spectrometric techniques. The lowest absolute mass of platinum that can be detected is 115 pg for an absorbance of 0.0044 (characteristic mass). When the sample contains nitric acid, the response is decreased, and hence the detection limit increases to about 400 pg. Theoretically, using the characteristic mass information, a calculation of the absolute mass of platinum needed to attain a sensitivity of 0.1 A_u yields a value of 2.6 ng. Under method conditions, 4.0 ng of cisplatin were required to attain this sensitivity. In addition, it was observed that, to destroy the toxic effects of cisplatin, the cisplatin itself needs to be destroyed and converted into innocuous inorganic platinum salts. During the conversion of cisplatin to these salts using a concentrated nitric and sulfuric acid mixture, reproducibility of the method was found to be poor. However, in the derivatization procedure used to convert to Pt-DDTC adduct, the conversion was not only quantitative, but the reproducibility was excellent. To extend the limit of detection, we decided that a judicious combination of derivatization with DDTC and the extraction of the DDTC complex in methylene chloride might yield an increased concentration of platinum. This increased concentration could be used for the determination of platinum (and consequently, of cisplatin) by the AA method.

Cisplatin, *cis*-diaminedichloroplatinum (II), is a square planar complex of platinum. The oxidation state of platinum is +2. The positive charges of the divalent metal ion are neutralized by 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.



Structure 1. Cisplatin.

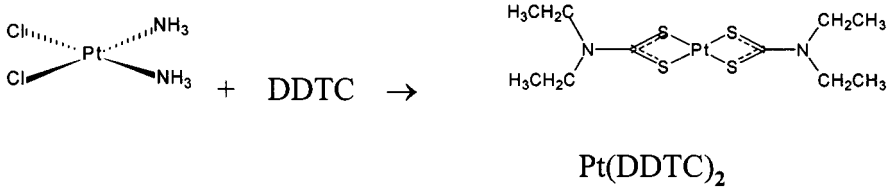
Because of the inclusion of the chloride ions in the inner coordination sphere of the platinum metal, the chloride ion in cisplatin solutions does not ionize to yield chloride ions in solution as in ionic metal salts. However, under the aqueous conditions of a solution, the chloride ion in the complex 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 concentration was always maintained in solution to drive the equilibrium to cisplatin by diluting the sample in 0.09% sodium chloride at low levels of cisplatin. In addition, it is recommended that solid sodium chloride (900 mg/L) be added to the rinse water samples immediately after sampling to prevent absorption of platinum to the surfaces of the glass container.

Cisplatin reacts with DDTC to yield a *cis*-di-DDTC complex [$Pt(DDTC)_2$]. The reaction is given in Scheme 1.

The complex is more soluble in methylene chloride than in water. Therefore, a 10-fold increase in concentration of the solution is effected by just extracting the complex in a smaller volume of organic solvent (10:1 ratio). In addition, by increasing the number of injections to five during analysis, the AA absorbance measurement is made to correspond to about 80 PPB in platinum content in solution. It was noted that platinum DDTC adduct degrades when stored under aqueous conditions. However, after extraction into the methylene chloride, even if there is degradation of the adduct, there is no loss in the total platinum extracted. Therefore, reproducible results for absorbance were obtained.

To validate the method we

1. Showed that there was no interference from any source when the entire procedure was carried out with a blank solution containing no cisplatin.
2. Established that, during dilution, there was no loss of cisplatin by adsorption to the surfaces of the glassware at these low levels.



Scheme 1. Reaction of cisplatin with diethyldithiocarbamate.

Table 3

Measured Absorbances of 5 PPB Solutions of Cisplatin (Instrumental Conditions per Method)

Preparation	Analyst 1	Average	Analyst 2	Average
Procedure 1	0.112		0.076	
	0.107	0.122	0.086	0.081
	0.147			
Procedure 2	0.105		0.085	
	0.092	0.101	0.098	0.099
	0.106		0.113	
Overall average		0.112 ± 0.019		0.096 ± 0.014
Overall average for all data				0.102 ± 0.019

3. Established that the response (peak area-sec, area under the curve) was a linear function of concentration in the range between 0.5 and 20 PPB.

Blank absorbances were normally low and were usually around 0.005 absorbance units. However, values as high as 0.015 were also observed occasionally. To show that, at low levels, there is no loss of cisplatin due to adsorption, at first (procedure 1) 2.5 ml of a 100-PPB solution was added to a mixture containing 50 ml of 0.09% and 2 ml of DDTC and derivatized immediately. In the second procedure (procedure 2), cisplatin was serially diluted to 5 PPB, thus exposing the same absolute amount of cisplatin in solution to about 40 times more surface area than in procedure 1. These data (Table 3) show that the absorbances are nearly identical within the limits of the experimental error.

For a 10-PPB solution, absorbance values of 0.190 ± 0.02 absorbance units were obtained.

The data generated are acceptable for use of this method as a limit test to determine if surfaces can be cleaned such that residual levels of cisplatin in solutions collected after rinsing the surfaces are less than the target analyte concentration of 5 PPB. A typical absorption signal observed for a 5-PPB cisplatin solution carried through the procedure and that of a blank solution are shown in Fig. 1.

A series of solutions of cisplatin in the concentration range of 0.5 to 20 PPB (10% to 400% of the target analyte concentration of 5 PPB) was taken through the procedure in duplicate, and their measured absorbances were plotted as a function of concentration. This procedure was repeated on a different day to establish that reasonably reproducible linear behavior was observed even at the extremely low concentration of 0.5 PPB. Plots of data from both days are shown in Figs. 2 and 3. The data show that the results are repeatable. Since the response is a

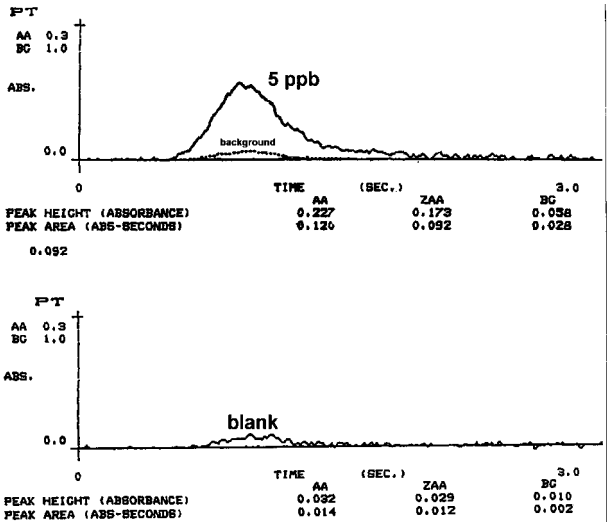


Figure 1. Typical atomization signal for a 5-PPB cisplatin standard and that for a blank solution.

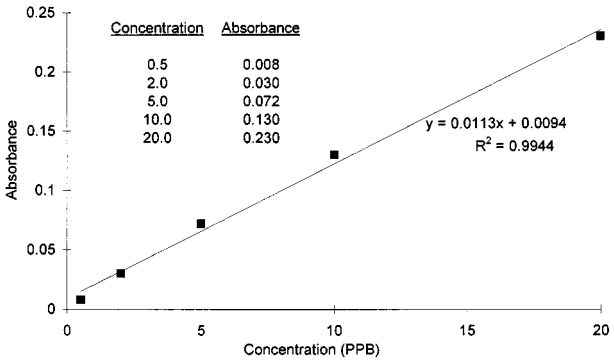


Figure 2. Plot of atomization signal strength (absorbance) versus cisplatin concentration.

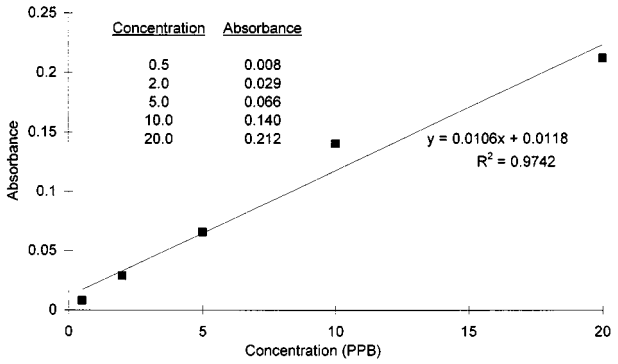


Figure 3. Plot of atomization signal strength (absorbance) versus cisplatin concentration (duplicate measurement).

linear function of concentration, a single standard preparation is adequate to quantitate cisplatin in rinse samples. To avoid potential loss of cisplatin by adsorption to the container, it was recommended that, for each 100 ml of sample collected in vials, about 1 g of solid sodium chloride be added and mixed. The method can be automated using appropriate robotics for sample preparation, derivatization, separation, and injection.

In conclusion, the procedure described in this article is simple, uses no unusual reagents or procedures, and does not require elaborate multihyphenated techniques to measure extremely low levels of this cytotoxic compound.

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