Communications to the Editor

[Chem. Pharm. Bull. 33(10)4614—4617(1985)]

A METHOD FOR DETERMINING CIS-DICHLORODIAMMINEPLATINUM(II) IN PLASMA AND URINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH DIRECT ULTRAVIOLET DETECTION

Ryoichi Kizu,* Shin-ichi Higashi, and Motoichi Miyazaki

Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1, Takara-machi, Kanazawa 920, Japan

A method was developed for determining cis-dichlorodiammine-platinum(II) (CDDP) in plasma and urine by HPLC with direct UV detection. After one ml of plasma or urine was pretreated with Dowex 1-x8, an aliquot (100 µl) of the treated plasma or urine was subjected to HPLC using a column packed with Hitachi Gel #3013-N. A mobile phase was prepared with 10 mM sodium chloride in water/methanol (85:15) and the detection wavelength was 210 nm. There was a linear relationship between peak height and the concentration of CDDP in plasma and urine in a range from 1 µM to 500 µM. The detection limit was 0.5 µM of CDDP in plasma and urine.

CDDP in the plasma and urine of a patient receiving CDDP treatment can be analyzed by this method.

KEYWORDS ——— antitumor drug; cis-dichlorodiammineplatinum(II); high performance liquid chromatography; direct ultraviolet detection; plasma; urine

The cancer chemotherapy drug cis-dichlorodiammineplatinum(II) (CDDP) has been used clinically to treat several solid tumors.[1] However, the pharmacokinetics of CDDP, distinct from its biodegradation products, has been incompletely understood owing to the lack of an adequate method for determining CDDP in biological substances such as plasma and urine.

For determining CDDP in plasma or urine, there are three HPLC methods: one coupled with off-line flameless atomic absorption spectrophotometry (off-line AAS method),[2] reductive amperometry (LCEC method),[3] and post-column derivatization (post-column method).[4] However, none of these methods is sufficient to be widely accepted since procedures are troublesome and time-consuming or the HPLC systems are complicated. Furthermore, there is no method applicable to both plasma and urine.

This report describes an HPLC method for determining CDDP in plasma and urine by a common direct UV detection method following a simple pretreatment of the plasma and urine.

From the UV spectrum of CDDP, it was found that the absorptivity of CDDP was significantly larger below 230 nm. The molar absorption coefficient of CDDP was 2300 at 220 nm, 5300 at 210 nm and 6500 at 206 nm. Therefore, the detection wave-

length was chosen at 210 nm in view of the sensitivity and the noise level of the base line.

A column packed with Partisil 10 SAX[2a,b] or ODS[2c-4] on which hexadecyltrimethylammonium bromide was adsorbed has been used for CDDP analysis. However, the capacity factors of CDDP obtained on both columns are below 2 and these are too small for our purpose. In order to analyze CDDP in plasma and urine accurately by detection at 210 nm, CDDP should be completely separated from its biodegradation products and the constituents of plasma and urine, since many of these substances have significant absorptivity at 210 nm. Therefore, a column was sought on which CDDP was retained more strongly than on any columns known to date.

Zipax SAX, Hitachi Gel #3013-N, MCI Gel CPK08 and Hitachi Gel #3010 were slurry packed in stainless steel columns (4.6 $mm_{i.d.}$ x 15 cm) according to standard methods, and then tested. As CDDP was most strongly retained on a Hitachi Gel #3013-N column among the four columns examined, it was used for CDDP analysis in subsequent studies. A standard chromatogram of CDDP and HPLC conditions are shown in Fig. 1. The capacity factor of CDDP on the Hitachi Gel #3013-N column was 6 and this value was much larger than those of any known columns. The capacity factor and peak height of CDDP were constant through a range from 1 mM to 100 mM of sodium chloride in the HPLC mobile phase. A linear relationship was found between peak height and concentration of standard CDDP in a range from 0.1 μM to 500 μM. Therefore, the amount of CDDP can be determined from its peak height.

On the basis of these results, analytical conditions for CDDP were investigated further. When plasma ultrafiltrate[5] or urine were injected directly, CDDP could not be analyzed by using various HPLC mobile phases because interfering peaks due to the constituents of plasma or urine appeared at the same retention time as CDDP. To remove these interfering peaks, plasma and urine were pretreated in various ways. When plasma and urine were pretreated with Dowex 1-x8 column[6] and chromatographed with 10 mM sodium chloride in water/methanol (85:15), no interfering peak was observed at the retention time of CDDP. This is shown in Figs. 2a and 2c which are chromatograms of blank plasma and urine of a patient just before receiving CDDP treatment. Figs. 2b and 2d are chromatograms of plasma and urine

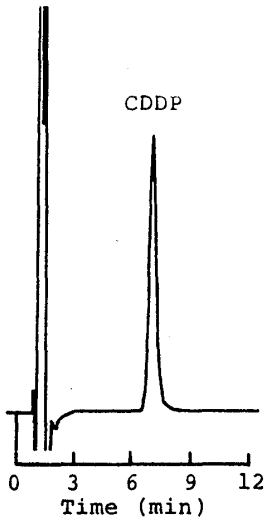

Fig. 1. High Performance Liquid Chromatogram of CDDP

HPLC conditions

Pump	: Shimadzu LC-5A
Detector	: Shimadzu SPD-2A
Injector	: Rheodyne 7125 with a 100 μl loop
Column	: Hitachi Gel #3013-N (4.6 $mm_{i.d.}$ x 15 cm)
Mobile phase	: 10 mM NaCl in H_2O/MeOH (85:15)
Flow rate	: 1 ml/min
Wavelength	: 210 nm
Column temperature	: 40°C
Injection volume	: 100 μl

freshly spiked with CDDP at 25 μM. Next, the influence of the biodegradation products of the CDDP on the analysis was examined using samples spiked with CDDP and samples from patients after receiving CDDP treatment. No peak due to biodegradation products was observed at the retention time of CDDP. Since CDDP was separated from its biodegradation products and from the constituents of plasma and urine, it was possible to determine CDDP in plasma and urine by direct UV detection.

The proposed method was applied in analyzing CDDP in the plasma and the urine of a patient receiving CDDP treatment. As shown in Fig. 3, CDDP could be analyzed and its concentration was 2.1 μM in plasma and 5.9 μM in urine.

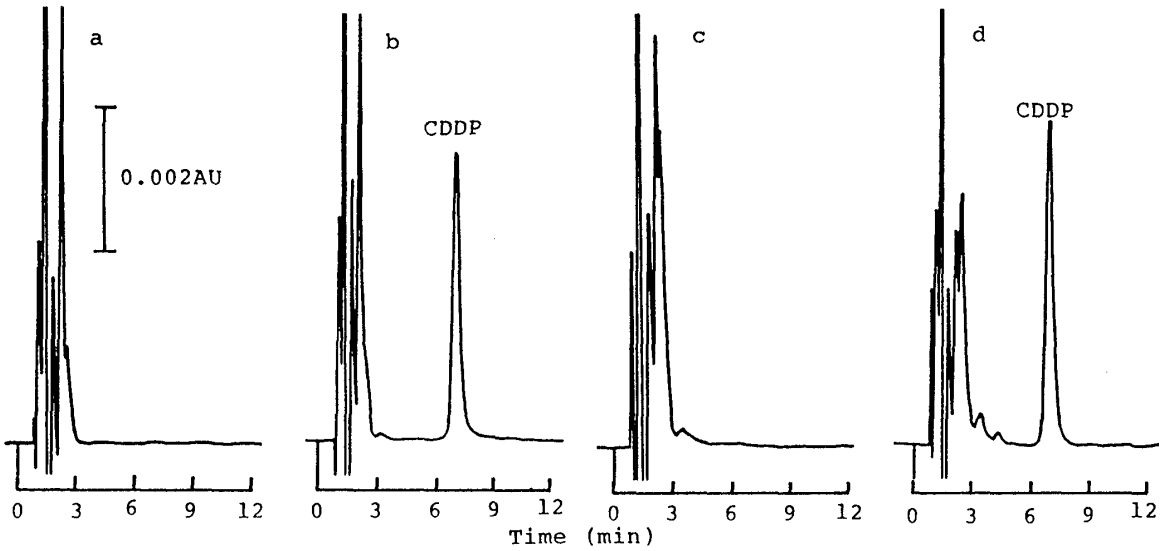

Fig. 2. Chromatograms of Blank Plasma and Urine

a: blank plasma, b: plasma freshly spiked with CDDP at 25 μM,
c: blank urine, d: urine freshly spiked with CDDP at 25 μM.
Plasma and urine were obtained from a patient with ovarian carcinoma just before receiving CDDP treatment. HPLC conditions are the same as shown in Fig. 1.

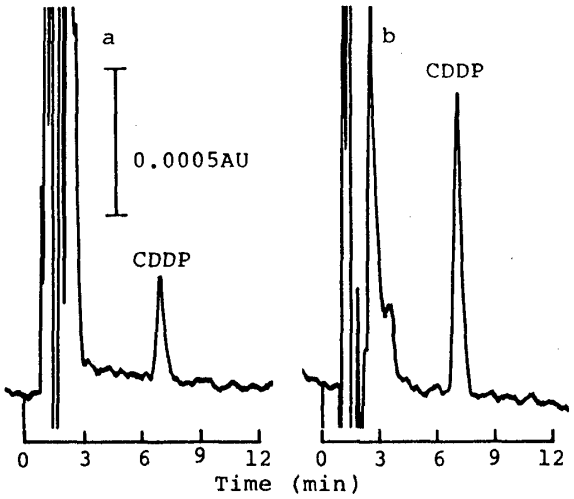

Fig. 3. Chromatograms of Plasma and Urine of the Patient 3 h after Starting CDDP Treatment

a: plasma, b: urine.

The recommended procedures are as follows. Blood samples are immediately centrifuged at 1000 x g for 3 min to obtain plasma. One ml of plasma or urine is immediately placed on a Dowex 1-x8 column[6] and is eluted with water at the flow rate of 1.2 ml/min. The initial 8 ml portion of the effluent is discarded and the subsequent 10 ml portion is collected. An aliquot (100 µl) of the effluent collected is immediately subjected to HPLC. All of these pretreatment procedures are carried out at 5 ± 2°C. The HPLC conditions are described in Fig. 1.

The calibration curves for CDDP showed good linearity in a range from 1 µM to 500 µM of CDDP in plasma and urine. The detection limit of CDDP was 0.5 µM in plasma and urine (S/N = 2.5). Recovery of CDDP in pretreatment was 84.1 ± 3.3% in blood, 83.5 ± 2.9% in plasma and 91.8 ± 2.2% in urine for six determinations.

An HPLC method for determining CDDP in plasma and urine by direct UV detection is presented in this study. The features of the proposed method are i) CDDP in plasma and urine can be directly analyzed by UV absorption of CDDP with a common HPLC system, ii) It is applicable to both plasma and urine with the same analytical conditions, iii) The sensitivity is comparable to those of the LCEC method[3] and the post-column method,[4] iv) The procedures of the pretreatment are quite simple. The proposed method appears to be useful for the metabolic study of CDDP.

ACKNOWLEDGEMENT The authors are grateful to Dr. E. Hara of Saitama Cancer Research Institute and Dr. K. Shimizu of Tohma Hospital for providing us with the plasma and urine of a patient receiving CDDP treatment. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.

REFERENCES AND NOTES

1) A.W. Prestayko, S.T. Crooke, and S.K. Carter (ed.), "Cisplatin ; Current Status and New Development", Academic Press, Inc., New York, 1980.

2) a) Y. Chang, L.A. Sternson, and A.J. Repta, Anal. Lett., B11, 449 (1978); b) A.J. Repta, and D.F. Long, "Cisplatin; Current Status and New Development," ed. by A.W. Prestayko, S.T. Crooke, and S.K. Carter, Academic Press, Inc., New York, 1980, p.287; c) C.M. Riley, L.A. Sternson, A.J. Repta, and R.W. Siegler, J. Chromatogr., 229, 373 (1982); d) C.M. Riley, L.A. Sternson, and A.J. Repta, J. Pharm. Sci., 72, 351 (1983).

3) a) S.J. Bannister, L.A. Sternson, and A.J. Repta, J. Chromatogr., 273, 301 (1983); b) I.S. Krull, X-D. Ding, S. Braverman, C. Selavka, F. Hochberg, and L.A. Sternson, J. Chromatogr. Sci., 21, 166 (1983).

4) a) L.A. Sternson, K.C. Marsh, S.J. Bannister, and A.J. Repta, Anal. Proc., 20, 366, (1983); b) K.C. Marsh, L.A. Sternson, and A.J. Repta, Anal. Chem., 56, 491 (1984).

5) Plasma ultrafiltrate was prepared by centrifuging 0.6 ml of plasma in Amicon MPS-1 starter kit fitted with Amicon YMT membrane (10000 M.W. cut off) at 1000 x g for 15 min at 4°C.

6) Dowex 1-x8 is a strong poly(styrene-divinylbenzene) based anion exchanger, (Bio-Rad Laboratories). The Dowex 1-x8 column was prepared by packing Dowex 1-x8 (Cl form, 200-400 mesh) in a glass column (5 $mm_{i.d.}$ x 6 cm). The exchanger was washed with 1 M sodium hydroxide, 1 M hydrogen chloride and water before use.

(Received May 27, 1985)