Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 2, pp. 165–170, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

Short Communication

Quantitative determination of cisplatin in body fluids by liquid chromatography with quenched phosphorescence detection

R. A. BAUMANN[1], C. GOOIJER[1]*, N. H. VELTHORST[1], R. W. FREI[1], I. KLEIN[2] and W. J. F. VAN DER VIJGH[2]

[1] *Free University, Department of General Chemistry and Analytical Chemistry, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*
[2] *Research Laboratory of Internal Medicine, Free University Hospital, De Boelelaan 1117, 1007 MB Amsterdam, The Netherlands*

Keywords: *Cisplatin; solvent generated anion exchanger; phosphorescence detection.*

Introduction

Cisplatin [CDDP, *cis*-dichlorodiammineplatinum(II)], which has a high activity towards solid tumours [1], is widely used in anticancer chemotherapy. After administration, cisplatin is rapidly bound to proteins [2] whereas the unbound, intact cisplatin is thought to be the therapeutically effective agent. Accordingly, study of its pharmacokinetic behaviour may elucidate the therapeutic and toxic effects.

Various platinum assay techniques have been applied, including X-ray fluorescence [3], flameless atomic absorption [4–6] (NFAA) and pre-column derivatization with diethyldithiocarbamate (DDTC) combined with high-performance liquid chromatography (HPLC) and on-line UV [7, 8] or off-line NFAA [8, 9] detection. These methods give information about the amount of total platinum or reactive platinum, respectively. Methods for the specific determination of CDDP, and in some cases its metabolites, also have been published. They are based on detection of the different species after HPLC separation. Use has been made of post-column reaction detection [10], chloride-assisted [11] or dual electrode [12] electrochemical detection and fraction collection followed by NFAA [13]. Furthermore, a column switching technique [14] followed by off-line NFAA has been developed.

This paper describes the determination of cisplatin in plasma and urine by HPLC with quenched phosphorescence detection — a technique that has already been applied to the

*To whom correspondence should be addressed.

determination of inorganic anions [15] (nitrite, sulphite) and some platinum(II) coordination complexes [16]. Owing to the presence of interferences in CDDP containing urine samples, as described in ref. [16], it has been necessary to look for an improved sample handling technique. The procedure developed makes use of a clean-up step, after which the samples are chromatographed on a solvent generated anion exchanger (SGAX) system as developed by Bannister *et al.* [17].

Experimental

Chemicals

All reagents used were analytical grade. Cisplatin was obtained from the research laboratory of Internal Medicine, Free University Hospital, Amsterdam. Plasma was provided by the Onze Lieve Vrouwe Gasthuis (Amsterdam, The Netherlands), Amberlite CG-400 (200 mesh particle size) was purchased from Rohm and Haas (Philadelphia, PA, USA).

The sample clean-up column was prepared as follows: the Amberlite resin, a strong anion-exchanger, was conditioned in aqueous methanol (50:50, v/v) and packed into a glass column (i.d. 4.5 mm) to a bed height of 21 mm. Before packing, a quartz wool plug was placed in the column.

Chromatographic procedure

A description of the HPLC system has been given previously [16, 18]. Separations were performed on a 250 × 4.6 mm i.d. 5 μm ODS Spherisorb column, coated with hexadecyltrimethylammonium chloride (HTACl), according to Riley *et al.* [14]. The mobile phase consisted of methanol–1 × 10^{-3}M citrate buffer (pH 5.8) (2:98, v/v) containing 5 × 10^{-3}M biacetyl and 2 × 10^{-5}M HTACl. The injection volume was 100 μl and the flow-rate 1.5 ml min^{-1}.

The Perkin–Elmer LS-2 (Perkin–Elmer, Beaconsfield, UK) filter fluorimeter used as detector was equipped with a pulsed source and a gated photomultiplier and was operated in the phosphorescence mode. The delay time was 0.01 ms and the gating time 1.00 ms. For excitation a broad band filter with maximum transmission at 400 nm was used; the emission wavelength was 518 nm.

Sample preparation

One ml urine or plasma sample was added to 4 ml 0.03 M potassium chloride in methanol for protein precipitation. After vortexing for 15 s, the mixture was centrifuged at 2000 **g** for 10 min. The sample clean-up column was flushed with 3 ml 0.15 M potassium chloride (pH 2.2) in water and 2 ml 0.03 M potassium chloride in methanol, respectively. Subsequently, 4.5 ml (urine) or 3.8 ml (plasma) of the supernatant was transferred to the column. After washing the Amberlite resin again with 2 ml of the potassium chloride solution in methanol, cisplatin was eluted with the 0.15 M potassium chloride (pH 2.2) solution in water. Under these conditions it was found that cisplatin eluted completely between 1.0 and 5.0 ml. The first ml of the eluate was always discarded. For recovery measurements the fraction 1.0–5.0 ml was collected and after deoxygenation an aliquot was directly injected onto the analytical column. For calibration purposes and limit of detection measurements the fraction with the highest CDDP concentration, 2.0–3.0 ml, was collected and analyzed. This fraction contained about 50% m/m of the total amount of cisplatin from a sample.

Results and Discussion

In an earlier paper [16] we described the analysis of CDDP and *cis*-diammine-1,1-cyclobutanedicarboxylate platinum(II) (CBDCA) by HPLC with quenched phosphorescence detection. This detection method is based on the measurement of the phosphorescence of biacetyl (2,3-butanedione) present as a solute in the mobile phase. Eluting compounds which are able to quench the phosphorescence signal give rise to a decrease of the original intensity I_o to I. Under dynamic quenching conditions the relation between I_o/I and the analyte concentration $[A]$ is given by the Stern–Volmer equation

$$I_o/I = 1 + k_A \cdot \tau^{B_o} [A], \quad (1)$$

where k_A is the bimolecular rate constant of the quenching process in $M^{-1}\ s^{-1}$ and τ^{B_o} is the phosphorescence lifetime of biacetyl in absence of quenchers in s.

The sensitivity of the detection method is determined by τ^{B_o} and k_A. A high τ^{B_o} is reached by using analytical grade chemicals, thorough cleaning of the whole HPLC system and effective removal of oxygen from the system, requirements that can be met easily [18]. For CDDP and CBDCA we reported [16] k_A values of 7×10^8 and $4 \times 10^8 M^{-1}\ s^{-1}$, respectively (measured in 0.15 M aqueous sodium chloride) which gave rise to detection limits for standard solutions in the low ng range. However, measurements in diluted urine samples showed interfering compounds co-eluting with the platinum species. This prompted us to look for an efficient sample clean-up procedure, details of which are now presented.

In Fig. 1 chromatograms obtained from a blank urine sample and from a sample spiked with CDDP (5×10^{-6}M) are shown. The samples were prepared as described in the experimental. It can be seen from Fig. 1a that two small peaks with retention times of 4.9 and 5.5 min interfere with the CDDP peak. Nevertheless, after correction for the blank it is possible to determine CDDP in urine down to a concentration of 3×10^{-7}M. The part

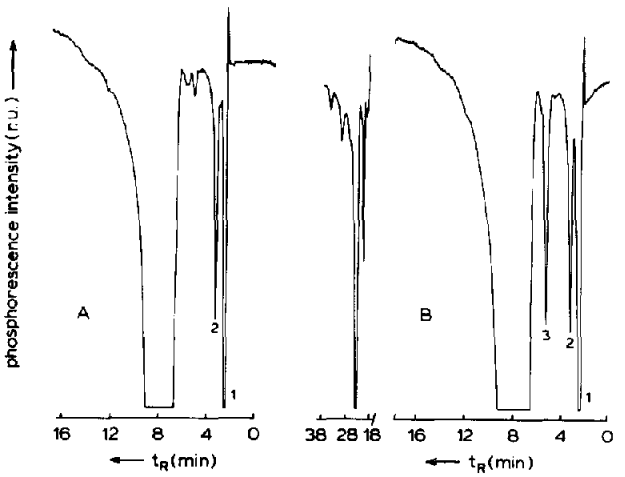

Figure 1
Chromatogram of a blank urine sample (A), and of a urine sample spiked with 5×10^{-6}M CDDP (B). Identification of peaks: (1) chloride, (2) unknown and (3) CDDP. The other peaks were not identified.

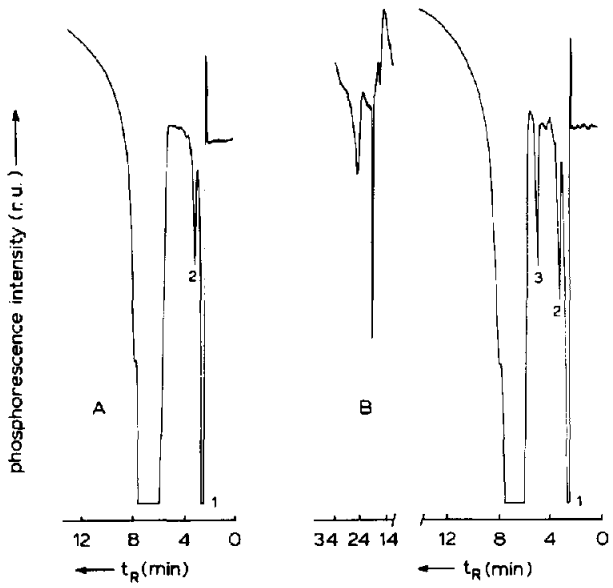

Figure 2
Chromatogram of a blank plasma sample (A), and of a plasma sample spiked with 5×10^{-6}M CDDP (B). Identification of peaks: (1) chloride, (2) unknown (same as in urine?) and (3) CDDP. The other peaks were not identified.

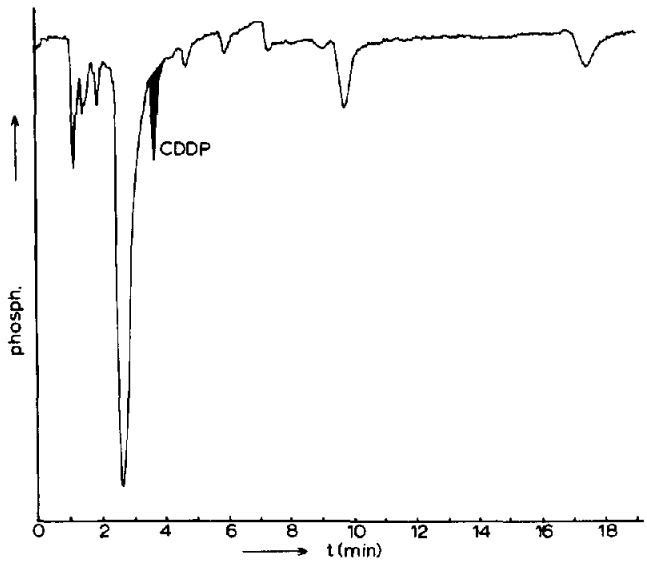

Figure 3
Chromatogram of a urine sample spiked with 7×10^{-5}M CDDP and diluted 1:7 with mobile phase. Chromatographic conditions: mobile phase 100% water, 1×10^{-2}M citrate buffer pH 5.0, 1×10^{-2}M biacetyl and 2×10^{-5}M hexadecyltrimethyl ammonium bromide (HTAB); column 10 cm × 4.6 mm i.d. 5 μm ODS Hypersil coated with HTAB; flow rate 1.1 ml min^{-1}.

of the chromatogram from 18–38 min is observed for both the blank and the spiked sample.

Figure 2 shows chromatograms obtained from a blank plasma sample and of a plasma sample spiked with CDDP (5×10^{-6}M). It can be seen that there are no interfering peaks and that the limit of detection in plasma calculated with reference to the signal-to-noise ratio is 1.5×10^{-7}M. The part of the chromatogram from 14 to 34 min is caused by the plasma matrix. To demonstrate the effectiveness of the clean-up step a chromatogram from a diluted urine sample, injected without any further pretreatment, containing CDDP (7×10^{-5}M) is shown in Fig. 3. With the recommended sample clean-up the sensitivity is improved by a factor of at least 20.

The recovery and reproducibility data for the analytical procedure are summarized in Table 1. It is obvious that the recovery of CDDP from plasma is complete for the whole concentration range studied. The recovery of cisplatin from urine is complete for concentrations exceeding 2.5×10^{-6}M, but at lower concentrations the recovery tends to decrease with decreasing CDDP concentration. This could be caused by partial degradation of cisplatin in urine during the sample clean-up. Calibration curves for urine as well as plasma samples are linear from the limit of detection up to a concentration of at least 1×10^{-4}M, which is approximately the maximum concentration found in body fluids obtained from patients undergoing chemotherapy [2]. Linear regression data (least-squares method) for calibration curves were $Y = (1.00 \pm 0.01) \times 10^4 X + (1.003 \pm 0.002)$ ($r = 0.9998$) for urine and $Y = (0.87 \pm 0.01) \times 10^4 X + (1.000 \pm 0.002)$ ($r = 0.9996$) for plasma samples, where Y is the ratio I_0/I and X the molar concentration of cisplatin according to equation (1).

Only one technique for the separate determination of CDDP in body fluids is known to be more sensitive, i.e. the elaborate HPLC fraction collection method combined with off-line flameless atomic absorption detection described by Daley–Yates and McBrien [13]. These authors claim a limit of detection for CDDP in plasma of 3.3×10^{-8}M. Some other methods [8, 10, 12] have about the same sensitivity, but the present method has the potential that in principle all Pt(II) species, regardless of their structure, can be determined with approximately the same sensitivity as cisplatin [16], which is important in metabolite studies. However, the analysis of CDDP metabolites is only possible by using an extended sample clean-up procedure.

Table 1
Recovery and reproducibility data of the determination of CDDP in urine and plasma

[CDDP] molar concentration	Recovery ± r.s.d. (%) ($n = 5$) Urine	Plasma
5.0×10^{-5}	98.7 ± 0.9	99.5 ± 0.9
2.5	100.5 ± 1.3	97.9 ± 0.3
1.0	99.2 ± 0.6	98.9 ± 0.7
5.0×10^{-6}	100.0 ± 2.2	101.2 ± 5.6
2.5	88.9 ± 3.1	100.0 ± 7.3
1.0	77.0 ± 11.2	101.6 ± 14.7
5.0×10^{-7}	*	94.8 ± 16.0

*Not measured.

Conclusions

The present work indicates that the HPLC/quenched phosphorescence method in combination with a clean-up procedure can be applied successfully to the quantitative determination of CDDP in urine and plasma. The sensitivity of the method is sufficient for the monitoring of therapeutic CDDP levels in clinical samples.

References

[1] L. H. Einhorn and S. D. Williams, *New Engl. J. Med.* **300**, 289–302 (1979).
[2] J. B. Vermorken, W. J. F. van der Vijgh, I. Klein, H. E. Gall, C. J. van Groeningen, G. A. M. Hart and H. M. Pinedo, *Clin. Pharmacol. Ther.* **39**, 136 (1986).
[3] S. J. Bannister, L. A. Sternson, A. J. Repta and G. W. James, *Clin. Chem.* **23**, 2258–2262 (1977).
[4] S. J. Bannister, Y. Chang, L. A. Sternson and A. J. Repta, *Clin. Chem.* **24**, 877–880 (1978).
[5] J. Smeyers-Verbeke, M. R. Detaevernier, L. Denis and D. L. Massant, *Clin. Chim. Acta* **113**, 329–333 (1981).
[6] M. L. Denniston, L. A. Sternson and A. J. Repta, *Anal. Lett* **14**, 451–462 (1981).
[7] S. J. Bannister, L. A. Sternson and A. J. Repta, *J. Chromatogr.* **173**, 333–342 (1979).
[8] O. H. Drummer, A. Proudfoot, L. Howes and W. J. Louis, *Clin. Chim. Acta* **136**, 65–74 (1984).
[9] P. A. Reece, J. T. McCall, G. Powis and R. L. Richardson, *J. Chromatogr.* **306**, 417–423 (1984).
[10] K. C. Marsh, L. A. Sternson and A. J. Repta, *Anal. Chem.* **56**, 491–497 (1984).
[11] W. N. Richmond and R. P. Baldwin, *Anal. Chim. Acta* **154**, 133–142 (1983).
[12] X. D. Ding and I. S. Krull, *J. Liquid Chromatogr.* **6**, 2173–2194 (1983).
[13] P. T. Daley-Yates and D. C. H. McBrien, *Biochem. Pharmacol.* **33**, 3063–3070 (1984).
[14] C. M. Riley, L. A. Sternson, A. J. Repta and R. W. Siegler, *J. Chromatogr.* **229**, 373–386 (1982).
[15] C. Gooijer, P. R. Markies, J. J. Donkerbroek, N. H. Velthorst and R. W. Frei, *J. Chromatogr.* **289**, 347–354 (1984).
[16] C. Gooijer, A. C. Veltkamp, R. A. Baumann, N. H. Velthorst and R. W. Frei, *J. Chromatogr.* **312**, 337–344 (1984).
[17] S. J. Bannister, L. A. Sternson and A. J. Repta, *J. Chromatogr.* **273**, 301–318 (1983).
[18] C. Gooijer, N. H. Velthorst and R. W. Frei, *Trends Anal. Chem.* **3**, 259–265 (1984).

[*Manuscript received 18 June 1986; revised version received 14 August 1986*]