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High-performance liquid chromatographic determination of *cis*-dichlorodiammineplatinum(II) in plasma ultrafiltrate

V. Augey [a], M. Cociglio [b], M. Galtier [a], R. Yearoo [a], V. Pinsani [c], F. Bressolle [a,d,*]

[a] *Laboratoire de Pharmacocinétique, Hôpital Carémeau, 30900 Nîmes, France*
[b] *Laboratoire de Pharmacologie Clinique, Faculté de Médecine, 34060 Montpellier Cedex 01, France*
[c] *Centre Régional de Pharmacovigilance, Centre Hospitalier Saint Charles, 34295 Montpellier Cedex 5, France*
[d] *Laboratoire de Pharmacocinétique, Faculté de Pharmacie, 34060 Montpellier Cedex 01, France*

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Abstract

A reproducible, simple and sensitive high-performance liquid chromatographic method was described for the quantitative analysis of *cis*-diamminedichloroplatinum(II) (CDDP) in ultrafiltrate plasma in the presence of nickel chloride as internal standard. CDDP and the internal standard were chelated by exchange with diethyldithiocarbamate. After derivatization, the mixture was directly injected into the column. Chromatography was performed on an Ultrasphere column and the eluent measured spectrophotometrically at 260 nm for CDDP and at 250 nm for the internal standard. The peak area ratio of CDDP to the internal standard varied linearly with concentration over the range 0.05–10 μg ml^{-1}. Precision and reproducibility were both excellent and the limit of quantification was 0.03 μg ml^{-1} using only 0.5 ml of ultrafiltrate. The present method, without extraction, should be entirely automated. This assay may be suitable for therapeutic drug monitoring in patients receiving CDDP.

Keywords: *cis*-Dichlorodiammineplatinum(II); High-performance liquid chromatography; Plasma ultrafiltrate

1. Introduction

Cisplatin, or *cis*-diamminedichloroplatinum(II) (CDDP), is one of the most potent antineoplastic agents in current use. It is widely utilized in the treatment of ovarian, lung, bladder, breast, head and neck, and testicular cancer [1].

The original studies of CDDP pharmacokinetics were accomplished by measuring total platinum in plasma or urine. Most investigators used the amount of CDDP in plasma ultrafiltrate to estimate the concentration of free CDDP [2].

The most widely used technique to measure this drug in biological fluids was graphite furnace atomic absorption spectrometry; however, the accuracy and precision of this method are highly dependent on the sample matrix [3]. Ultraviolet spectrometry and X-ray fluorescence spectrometry have also been used. However, all these methods are non-specific in that they respond only to the total concentration of metal-containing species. Selectivity was improved using high-performance liquid chromatography (HPLC), a technique now available in most laboratories. Some methods have been described to quantify CDDP by HPLC involving derivatization and organic extraction using chloroform as an extractive solvent followed by evaporation of the organic phase [2–6]. Consequently, the use

* Corresponding author.

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of this solvent requires special precautions for safe handling. Electrochemical detection in HPLC was also employed [7–9]. An HPLC method has been developed to quantify simultaneously *cis*- and *trans*-DDP [10].

CDDP is a nephrotoxic drug highly bound (90%) to serum protein with a narrow therapeutic index (0.7–2 μg ml^{-1}). However, in most cases, in therapeutic drug monitoring programmes, total platinum was quantified in plasma by atomic absorption spectrometry. In this work, we describe a rapid, reliable and sensitive reversed-phase HPLC method for measuring free CDDP in plasma ultrafiltrate from patients receiving CDDP therapy. This method was validated according to validation procedures, parameters and acceptance criteria based on USP XXIII guidelines [11] and the recommendations of Shah et al. [12]. It is routinely used in our hospital to monitor closely the parent drug concentration in human plasma ultrafiltrate in order to prevent renal toxicity.

2. Experimental

2.1. Materials and reagents

CDDP, diethyldithiocarbamic acid (sodium salt, DDTC) and nickel chloride hexahydrate (internal standard) were obtained from Sigma (St. Quentin Fallavier, France). Methanol was of Chromasol grade (SDS, Peypin, France) and used without further purification. Purified water was obtained from Flandre Laboratories (Ludres, France). Sodium hydroxide (Merck, Nogent-sur-Marne, France) was of analytical grade.

DDTC (10%) was extemporaneously prepared by dilution in 0.1 M sodium hydroxide aqueous solution.

A stock solution of CDDP (1 mg ml^{-1}) in 0.9% sodium chloride (Macopharma, Turcoing, France) was diluted 10- and 200-fold with 0.9% sodium chloride when appropriate. Solution of nickel chloride, internal standard (0.1 mg ml^{-1}), was achieved in 0.9% sodium chloride.

Pooled plasma samples from healthy volunteers were used for validation of the method.

2.2. Separation of ultrafiltrate

Separation of ultrafiltrate was attained by convective filtration through an anisotropic, hydrophilic YMT-1 ultrafiltration membrane (diameter 14 mm; Amicon, Epernon, France). Ultrafiltration was performed from 1-ml plasma; the driving force for filtration was provided by centrifugation at 2000*g* for 45 min at ambient temperature (20 °C), with a fixed angle rotor providing polarization control to minimize the potential for non-ideal protein–protein interactions.

Ultrafiltrate was immediately frozen and stored at −20 °C to await analysis.

CDDP adsorption onto the membrane was studied by performing replicate analyses ($n = 4$) of spiked samples in ultrafiltrate at concentrations of 0.1, 1 and 10 μg ml^{-1}. Comparison of drug concentrations, determined against a calibration curve, was performed before and after the ultrafiltration procedure.

2.3. Instrumentation

The isocratic system consisted of the following components: a model P4000 quaternary gradient pump from Thermo Separation Products (Orsay, France) with a Rheodyne loading valve (model 7010) fitted with a 100-μl sample loop, an automatic sample injection system (model 231; Gilson Medical electronic, Villiers le Bel, France), a guard column (20 × 4.6 mm i.d.; SFCC, Neuilly Plaisance, France) packed with Hypersil ODS C18, and a stainless-steel column (250 × 4.6 mm i.d., Beckman, Gagny, France) packed with Ultrasphere (5 μm).

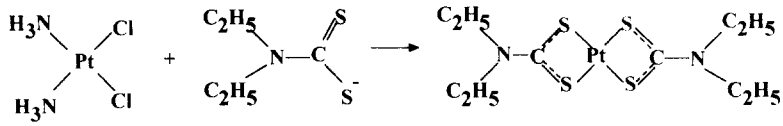
The column effluent was monitored with a Spectra Focus spectrophotometric detector (Thermo Separation Products, Orsay, France). The HPLC system was interfaced with an IBM compatible-DX computer/data station and controlled through Thermo product PC 1000 software, which allowed post-data analysis whilst allowing further on-line acquisition of data.

2.4. Chromatographic conditions

The mobile phase, methanol–water (75:25, v/v), was degassed ultrasonically before use. Methanol and water were filtered through a membrane filter (0.45 μm; Millipore, Molsheim, France). The oven temperature was 30 °C, and the flow rate was 1.5 ml min^{-1}. The detector was set at 260 nm for the first 6.5 min and at 250 nm from 6.5 to 10 min.

2.5. Extraction procedure

Ultrafiltrate samples (0.5 ml) were pipetted into a 5 ml glass centrifuge tube. Internal



1 2 3

Fig. 1. Formula showing the formation of chelate (3) from CDDP (1) and DDTC (2). (2 moles of DDTC react with 1 mole of CDDP [3]).

standard solution (30 µl) and 50 µl of a 10% solution of DDTC in sodium hydroxide were added in turn to each sample. The tubes were capped and incubated at 37 °C for 1 h, and then chilled. A 300 µl aliquot of the solution was transferred in vials protected from light and 100 µl was injected into the column.

The chelating effect of DDTC was rapid at 37 °C, for both CDDP and the internal standard in plasma ultrafiltrate. 78 and 93% of maximum chelation occurred at 60 min for CDDP and the internal standard, respectively [4]. The reaction between CDDP and DDTC is shown in Fig. 1. A similar chelate has been shown to form between DDTC and nickel.

2.6. Instrument calibration

Calibration standards for control ultrafiltrate were prepared using concentrations of 0.05, 0.1, 0.3, 0.5, 1.0, 5.0, and 10.0 µg ml $^{-1}$. The standard samples were prepared by adding appropriate volumes of CDDP solutions to ultrafiltrate. The volume added was always smaller than 2% of the total volume of the sample, so that the integrity of the ultrafiltrate was maintained. These concentrations were arbitrarily selected to cover the concentrations anticipated in clinical samples.

2.7. Data analysis

The peak-area ratios of CDDP to internal standard were used to construct the standard curves. Unweighted least-squares linear regression of the peak-area ratios as a function of the theoretical concentrations was applied to each standard curve.

The linearity of the method was confirmed using the classical statistical tests, i.e. comparison of intercept with zero and correlation coefficients.

2.8. Specificity

To evaluate the specificity of the method, 0.5 ml of drug-free ultrafiltrate was used for the assay procedure, and the retention time of endogenous compounds were compared with those of CDDP and internal standard.

The interference from a variety of metal ions (iron, calcium, copper, phosphorus, lead, cobalt) suitable for forming similar chelates with DDTC was studied.

The interference from other drugs that could be co-administered was also studied. The following drugs were checked: 5-fluorouracil, etoposide, vinorelbine, cyclophosphamide, and mitomycin.

2.9. Precision and accuracy

The between-day and within-day repeatabilities of the assay were assessed by performing replicate analyses of spiked samples at high, middle, and low concentrations (0.075, 3, and 7 µg ml $^{-1}$) against a calibration curve. The procedure was repeated for different days on the same spiked standards to determine between-day repeatability. The within-day repeatability was determined by treating spiked samples in replicate on the same day. The accuracy, expressed as percentage deviation of observed concentration from theoretical concentration, with the relative error, was evaluated.

2.10. Determination of the limit of quantification (LOQ)

The LOQ was determined from the peak and the standard deviation of the noise level (S_N). The LOQ was defined as the sample concentration of CDDP resulting in a peak height of 10 times S_N. An estimate of S_N was obtained by extrapolation to zero. To determine the analytical error in the LOQ, spiked ultrafiltrate was used.

2.11. Stability study

CDDP is known to be very unstable in both aqueous solution and biological samples [13,14], so its stability during both sample treatment and storage was investigated.

The stability of the analytes (CDDP and internal standard) in plasma ultrafiltrate after derivatization was also investigated. Spiked samples (0.075, 3 and 7 μg ml^{-1}) were treated as mentioned above and left on the autosampler at ambient temperature prior to HPLC analysis. Periodic analyses, every 40 min, over a span of 12 h were then performed.

3. Results

3.1. CDDP adsorption onto the membrane

In the concentration range studied, CDDP adsorption onto the membrane was considered negligible. The mean recoveries at concentrations of 0.1, 5, and 10 μg ml^{-1} were 96.2 ± 5.54, 98.3 ± 3.5, and 98.2 ± 3.9%, respectively.

3.2. Retention times

The observed retention times were 5.7 and 7.4 min for CDDP and the internal standard, respectively. The capacity factors k' were 2.8 for CDDP and 3.9 for the internal standard. The resolution between these two compounds was 8.4 and the selectivity was 1.41. Representative chromatograms are shown in Fig. 2.

3.3. Specificity

There were no significant interfering peaks in the control ultrafiltrate (Fig. 2) at the retention time of the respective analytes.

No interference was noted with a number of metals likely to form complexes with DDTC or drugs that could be co-administered with CDDP.

3.4. Linearity

In ultrafiltrate plasma, the peak-area ratio of CDDP to the internal standard varied linearly with concentration over the range used (0.05–10 μg ml^{-1}). The correlation coefficients (r) for calibration curves were equal to or better than 0.9996. Intra-assay reproducibility was determined for calibration curves prepared the same day in replicate ($n = 6$) using the same stock solutions. The intraday average slope of the fitted straight lines was 0.118 ± 6.06 × 10^{-3} (relative standard deviation, RSD = 5.14%), the correlation coefficient was 0.9998 ± 8.17 × 10^{-5} (RSD = 8.17 × 10^{-3}%) and the mean intercept was 5.56 × 10^{-4} ± 1.77 × 10^{-3}. For calibration curves prepared on different days ($n = 11$), the mean results were as follows: slope = 0.113 ± 4.6 × 10^{-3} (RDS = 4.11%), r = 0.9998 ± 1.25 × 10^{-4} (RDS = 0.0125%) and intercept = −1.69 × 10^{-4} ± 1.73 × 10^{-3}.

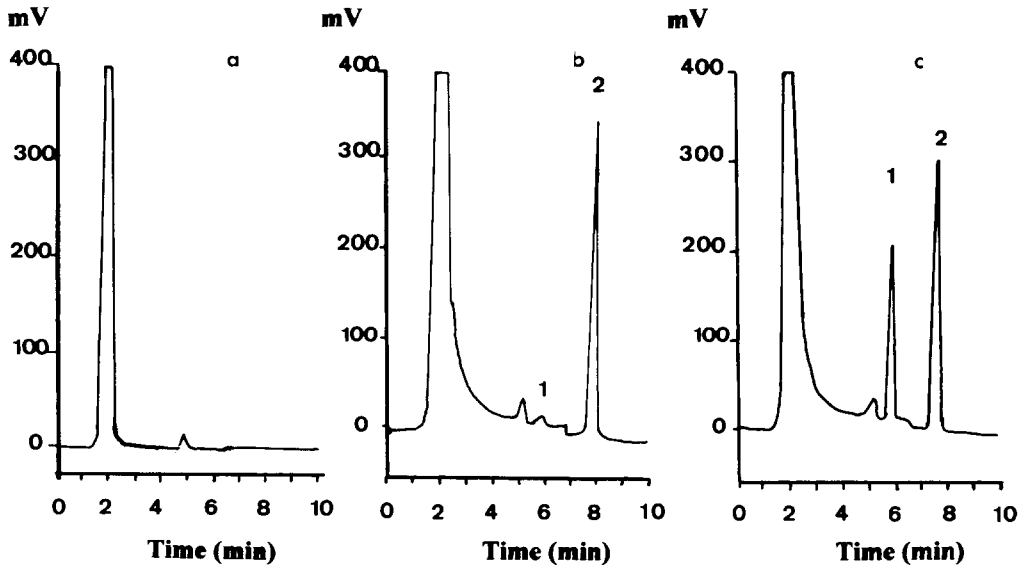


Fig. 2. (a) HPLC chromatogram of a blank plasma ultrafiltrate. HPLC chromatogram of ultrafiltrate plasma spicked with CDDP at concentrations of (b) 0.1 μg ml^{-1} and (c) 5 μg ml^{-1}. Peaks: (1) CDDP, (2) internal standard. For chromatographic conditions, see text.

For each point of the calibration standards, the concentrations were recalculated from the equation of the linear regression curves (experimental concentrations) and the percentage relative standard deviations (RSDs) were computed. Inter-day and intra-day variabilities at the concentration of calibration standards are presented in Table 1.

The linearity of this method was statistically confirmed. For each calibration curve, the intercept was not statistically different from zero. Moreover, the RSDs of the response factors (peak-area/concentration ratio) of each point of the calibration standards was 8.02% for calibration curves performed on the same day and 7.96% for calibration curves performed on different days. In addition, the mean values of these response factors were always very close to the slopes of the linear calibration curves.

3.5. Precision and accuracy

For concentrations of calibration standards ranging from 0.1 to 10 μg ml^{-1}, the precision around the mean value did not exceed 15% RSD. This precision was 20% for concentration of 0.05 μg ml^{-1} (Table 1).

The within-run and between-run precision of the method were assessed by analysing quality control samples prepared in ultrafiltrate at different concentrations in replicate on the same day and on different days. The results for accuracy, and within-day and between-day precision are presented in Table 2.

3.6. Limit of quantification and limit of detection

The limit of quantification was 0.030 μg ml^{-1}. At this level, the average analytical error was 25%. The limit of detection representing a signal noise of 3:1 was about 0.010 μg ml^{-1}.

3.7. Stability

CDDP was found to be stable when ultrafiltrate plasma was immediately (within 30 s) thawed in hot water (80 °C), but showed a

Table 1
Intra- and inter-assay reproducibilities of the HPLC analysis

Theoretical concentration (μg ml^{-1})	Intra-assay reproducibility ($n = 6$)		Inter-assay reproducibility ($n = 11$)	
	Experimental concentration (μg ml^{-1}) (mean ± SD)	RSD (%)	Experimental concentration (μg ml^{-1}) (mean ± SD)	RSD (%)
0.050	0.0508 ± 0.0102	20.1	0.0501 ± 0.0102	20.3
0.1	0.103 ± 0.0153	14.9	0.0973 ± 0.0150	15.4
0.3	0.293 ± 0.0093	3.17	0.311 ± 0.0123	3.96
0.5	0.485 ± 0.0131	2.70	0.494 ± 0.014	2.83
1	0.939 ± 0.0373	3.97	0.94 ± 0.0271	2.88
5	5.14 ± 0.0484	0.942	5.10 ± 0.124	2.43
10	9.95 ± 0.0344	0.346	9.96 ± 0.0588	0.591

Table 2
Within-run and between-run precision of the HPLC method

Theoretical concentration (μg ml^{-1})	n	Experimental concentration (μg ml^{-1}) (mean ± SD)	RSD (%)	Mean recovery	Relative error (%)
Within-run precision					
0.075	13	0.0691 ± 0.00074	10.7	92.1	7.9
3	13	3.15 ± 0.181	5.74	105.0	5.0
7	14	7.05 ± 0.309	4.38	100.7	0.7
Between-run precision					
0.075	13	0.0776 ± 0.0120	15.5	103.5	3.5
3	7	3.12 ± 0.089	2.86	104.0	4.0
7	7	6.97 ± 0.207	2.96	99.6	0.4

significant reduction (about 5%) when samples were thawed at room temperature for 20 min. At −20 °C, ultrafiltrate samples were stable for 5 days.

The stability of CDDP and the internal standard after derivatization indicated that no statistical significant degradation occurred over a span of 6 h for the highest concentrations tested (3.0 and 7.0 μg ml^{-1}); the average percentage recovery was $101.3 \pm 2.70\%$ for 3.0 μg ml^{-1} ($n = 8$) and $97.9 \pm 2.72\%$ for 7.0 μg ml^{-1} ($n = 8$). 14 min later, a 10% reduction in concentration was observed. At low concentration (0.075 μg ml^{-1}, $n = 7$), the mixture was stable for 5 h, the percentage recovery was $98.8 \pm 8.63\%$, and a 10% decrease in concentration was observed 40 min later.

4. Discussion

All previously published methods for the quantification of CDDP by HPLC, after chelation by exchange with DDTC, involve chloroform extraction and evaporation to dryness of the organic phase. Our procedure, in contrast, does not require any extraction by an organic solvent. In the present paper we developed a simple derivatization method to quantify CDDP that is adequately sensitive and specific for the quantification of this drug in ultrafiltrate; this assay is rapid, easy to perform, and safe to analysts and the environment. Moreover, the present method requiring minimal sample preparation should be entirely automated for processing large numbers of samples. This method provides the necessary sensitivity and selectivity for chromatographic monitoring of unbound CDDP for at least 8 h after drug administration of 100 mg m^{-2} [2].

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