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Journal of Pharmaceutical and Biomedical Analysis
25 (2001) 465–475

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Validation of an AAS method for the determination of platinum in biological fluids from patients receiving the oral platinum derivative JM216☆

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Received 31 March 2000; accepted 21 October 2000

Abstract

A flameless atomic absorption spectrometric (AAS) method has been developed and validated for the determination of platinum (Pt) in human plasma, plasma ultrafitrate and urines from cancer patients receiving the orally available platinum derivative, JM216. Sample pretreatment is minimal for urine, which is diluted with 10% HCl prior to AAS analysis. Pt analysis in plasma requires the application of the matrix modifier 5% Triton X-100 directly onto the integrated L'vov platform of the graphite furnace prior to the addition of plasma samples. For Pt in ultrafiltrates, enhanced sensitivity is achieved by pre-concentrating ultrafiltrate samples onto the platform prior to the ashing/atomisation step. The AAS program was set specifically for each considered matrix enabling to achieve limit of quantitations as low as 50, 10 and 5 ng Pt ml^{-1} for urine, plasma and plasma ultrafiltrate, respectively. The calibration was linear ($r^2 > 0.993$) over the working range 5–150 ng Pt ml^{-1}. The method has been validated according to the Recommendations on Bioanalytical Methods Validation. The stability of Pt in samples has been explored, as well as the specificity of the method. In the urine intra-assay precision of control samples at 60, 90 and 140 ng Pt ml^{-1} is always lower than 3.0, 1.3 and 4.7%, respectively, with concentrations not deviating more than -5.5 to -1.0% from their nominal values, while inter-assay precision is within 5.7–7.7% and inter-assay deviation within the -1.9 to $+4.3$% range. Intra-assay precision of plasma control samples at 20, 70 and 140 ng Pt ml^{-1} is always lower than 8% and concentrations never deviating more than 7.1% from their nominal values. Inter-assay precision of plasma control samples is always lower than 9% with inter-assay deviation from their nominal concentrations within the -3.9 to $+1.8$% range. In plasma ultrafiltrate, intra-assay CVs of control samples at 12,

☆ Presented in part at the 10th NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, 7–9 May 1998 (S. Vouillamoz-Lorenz, L.A. Decosterd, J. Bauer, T. Buclin, and F. Lejeune, Ann. Oncol. 9;(1998):130) and at the Annual Assembly of the Clinical Pharmacology Section of the Swiss Society of the Internal Medicine, 1998 (S. Vouillamoz-Lorenz, L.A. Decosterd, J. Bauer, T. Buclin, and F. Lejeune, Schweiz. Med. Wochenschr. 128 (suppl. 96);(1998):45s).

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PII: S0731-7085(00)00508-2

25 and 45 ng Pt ml^{-1} are always lower than 2.6, 1.7 and 6.8%, respectively, with concentrations not deviating more than -2.6 to -0.2% from their nominal values, while inter-assay CVs are within 5.1–9.5% and inter-assay deviation within the -1.6 to $+5.3$% range. The proposed method has, therefore, the required performance to measure Pt in biological samples and has been successfully applied to the determination of Pt in samples from cancer patients receiving JM216 in a phase I (daily administration for 14 days, dose escalation 10–50 mg m^{-2}) and a phase II (fixed dose 120 mg m^{-2} over 5 days) clinical study. In phase I study, both total and ultrafiltrable Pt accumulated upon repetitive dosings, showed long elimination half-lives ($t_{1/2}$) and were measurable 2 weeks after the end of JM216 administration.

Keywords: Platinum complexes; AAS; Pharmacokinetics; Oral administration; Assay validation

1. Introduction

JM216 (bis-acetato-ammine-dichlorocyclohexylamine platinum(IV)) [1] (Fig. 1) is a new platinum analogue currently in phase I/II clinical studies, which can be administered to cancer patients by the oral route, an approach likely to increase patients' quality of life, by promoting home and cost effective chemotherapy. Moreover, JM216 may represent an effective addition to the current platinum-based anticancer chemotherapy by circumventing the resistance of several tumor cells [2] to the platinum analogues available to date — cisplatin [3], carboplatin and oxaliplatin [4] (Fig. 1), and by possibly demonstrating in the clinics, a more favourable profile of platinum-related toxicities [5]. Indeed, in vitro studies of JM216 have shown comparable cytotoxicity to cisplatin and encouraging activity against some cisplatin-resistant tumor lines [2]. However, JM216 was ineffective in tumor models resistant or insensitive to cisplatin [1] and its in vivo antitumor activity is often comparable to that of cisplatin or carboplatin administered parenterally [2].

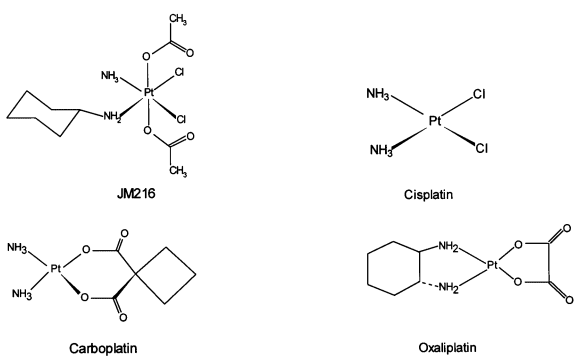

Fig. 1. Chemical structures of JM216, cisplatin, carboplatin and oxaliplatin.

The determination of platinum (Pt) in biological fluids remains an analytical challenge. Several specific methods have been reported to measure both parent Pt drugs, as well as their individual metabolites concentrations [6–12]. Off- and on-line analytical methods have indeed been reported for the specific determination of JM216 and its metabolites, either by high performance liquid chromatography (HPLC) followed by off-line atomic absorption spectrophotometry (AAS) of the eluted fractions [13–15] or by liquid chromatography coupled with electrospray ionisation mass spectrometry (LC-ESI/MS) [16,17]. Most reported analytical methods, however, applied AAS for the quantification of the Pt atom contained in several Pt derivatives notably cisplatin [18–24], or JM216 [25,26]. In addition, inductively coupled plasma-mass spectrometry (ICP-MS) of Pt has also been applied for the assay of cisplatin [27] or JM216, itself [28]. In most cases, the detailed procedures of the analytical validation of the AAS method are not reported.

We describe here the setting-up and the validation of an atomic absorption spectrometric method that enables accurate and sensitive measurements of Pt concentrations down to 5 ng Pt ml^{-1} in biological fluids for its application in pharmacokinetic studies. Total platinum (P_{tot}) was determined in plasma and urine and ultrafiltrable platinum (UPt) in plasma. Three different temperature programs were developed to take into account the specific matrix effect of the considered biological fluids (urine, plasma and plasma ultrafiltrate).

Table 1
Analytical conditions

Wavelength	265.9 nm
Slit width	0.7 nm
Slit height	Low
Hollow-cathode lamp current	15 mA
Calibration	External-standard
Standard	5–150 ng Pt ml^{-1}
Number of replicates	2
Read delay	0.0 s
Integration time	5.0 s (plasma, urine) and 7.0 s (plasma ultrafiltrate)
Signal processing	Peak area
Sample volume	50 μl (plasma, urine), 3×50 μl (plasma ultrafiltrate)
Background correction	Deuterium

The detailed assay validation reported here was performed according to the recommendations of the Conference Report on Bioanalytical Method Validation [29]. This method was successfully applied for the analysis of samples collected from cancer patients receiving JM216 in phase I and II clinical studies.

2. Experimental

2.1. Chemicals

JM216 standard reference (lot no. 2R216) used for the preparation of calibration and quality control samples was generously provided by Bristol–Myers Squibb (Pharmaceutical Research Institute, Princeton, NJ, USA) and was calculated to contain 38.78% (w/w) of Pt (BMS Certificate of Analysis). Hydrochloric acid solutions were prepared from 37% HCl (Merck, Darmstadt, Germany). Triton X-100 was purchased from Fluka (Buchs, Switzerland) and solutions were prepared with double distilled water. The blank plasma used for the preparation of calibration and control samples was obtained from outdated transfusion bags. A large volume of this plasma was also subjected in portion to ultrafiltration (see below), which yielded blank plasma ultrafiltrate used throughout the analysis for the preparation of calibration and control ultrafiltrate samples.

2.2. Equipment

A Perkin-Elmer 1100B atomic absorption spectrophotometer (Ueberlingen, Germany) equipped with a HGA-700 graphite furnace and an AS-70 autosampler was used. The tubes were coated with pyrolytic graphite and equipped with an integrated L'vov platform. A Pt hollow cathode lamp was operated at 15 mA with a 0.7 nm slit. The wavelength was set at 265.9 nm. The analytical conditions are given in Table 1. Argon was used as purge gas delivered at a flow rate of 300 ml min^{-1} (stop-flow during atomisation). Deuterium background correction was applied.

2.3. Standard solutions

A stock solution of JM216 (MW = 500.29, corresponding to 195.08 g Pt $mole^{-1}$ of JM216) was used to prepare the Pt calibration standards in pooled blank urines at 50, 75, 100, 125 and 150 ng Pt ml^{-1}, in plasmas at 10, 30, 60, 100 and 150 ng Pt ml^{-1} and in plasma ultrafiltrates at 5, 10, 20, 30 and 50 ng Pt ml^{-1}. Control samples in urine at 60, 90 and 140 ng Pt ml^{-1}, in plasma at 20, 70 and 140 ng Pt ml^{-1} and in plasma ultrafiltrate at 12, 25 and 45 ng Pt ml^{-1} were similarly prepared. All samples were stored in polypropylene Eppendorf tubes at -24°C and thawed individually on the day of analysis. Calibration solutions were used to establish the calibration curve and control samples were added in each analytical run every four samples.

2.4. Sample processing

According to a protocol approved by the local Ethics Committee of the University Hospital (see infra), blood samples were collected from cancer patients on EDTA-K (Monovette, Sarstedt, Nümbrecht, Germany), centrifuged during 10 min at $2000 \times g$ at 4°C. An aliquot of the plasma was transferred into polypropylene tube, flash-frozen in dry ice and stored at -80°C for plasma P_{tot}

determination. Plasma ultrafiltrate sample was prepared immediately with the remaining plasma, which was introduced in an Amicon Centrifree Micropartition filters (30 000 MW cut-off; Amicon Division, W.R. Grace Co, Beverly, MA, USA). The filters were centrifuged at $3000 \times g$ for 45 min at 4°C with a fixed-angle rotator (Beckmann, J2–21 model, rotor angle JA–20). The ultrafiltrates were collected in polypropylene tubes, immediately flash-frozen and stored at −80°C until UPt analysis.

Urine was collected by spontaneous voiding up to 24 h. An aliquot of the two considered urine collection periods (0–8 h and 8–24 h) was immediately frozen for P_{tot} determination. Due to the variability of the volume of patients' voidings, some exploratory P_{tot} analyses were necessary to assess the correct dilution to carry out with 10% HCl to reach Pt absorption values encompassed by the 50–150 ng ml^{-1} calibration range.

A matrix modifying procedure with Triton X-100 was applied to plasma samples to control at best the matrix interferences, to circumvent the variability of sample drying procedure, and to minimise the unavoidable loss of P_{tot} during the charring/atomisation step. Best conditions were found by applying 15 μl of 5% Triton X-100 solution directly onto the integrated L'vov platform of the tubes, immediately followed by a 35 μl aliquot of the plasma sample prior to the start of the programmed drying/ashing/atomisation procedure. An automatic recalibration using the calibration program featured in the Perkin–Elmer 1100B AAS was performed every 10 samples with a plasma standard containing 130 ng Pt ml^{-1}.

Since Pt was present at the low ng ml^{-1} range of concentrations in the plasma ultrafiltrates, it was necessary to enhance the sensitivity of the assay by pre-concentrating large volume of sample into the graphite tube. This was achieved by applying a total volume of 150 μl of plasma ultrafiltrate onto the tube platform by three successive additions of 50 μl separated by drying steps and by prolonging the atomisation time.

Furnace conditions were experimentally established to provide reproducible results with acceptable sensitivity while adequately removing the biological matrix. Optimal drying, charring and atomisation temperatures were determined specifically for urine, plasma and plasma ultrafiltrate samples (see Table 2). Atomisation temperatures were comprised between 2200 and 2400°C, for urine and plasma, respectively.

2.5. Validation of the method

Throughout the analysis of biological samples, the control samples at three concentrations levels (see Tables 3–5) were assayed every four samples. Duplicate analysis was performed on each individual sample. The complete validation of this assay was carried out according to the guidelines recommended by the Conference Report on Bioanalytical Method Validation [29].

The control samples were used for the determination of the precision and accuracy of the method, precision being calculated as the R.S.D. (%) within a single run (intra-assay) and between different assays (inter-assay), and accuracy as the percentage of deviation between nominal and measured concentrations with the established calibration curves.

Precision and accuracy of the lower limit of quantitation (LLQ) were also examined in samples containing Pt concentration corresponding to the LLQ.

The stability of Pt in urine, plasma and ultrafiltrate plasma was determined as follows;

1. by storing biological samples containing Pt (at 50 and 150 ng ml^{-1}, 20, 70 and 140 ng Pt ml^{-1}, and 8, 25 and 45 ng Pt ml^{-1}, for urine, plasma and plasma ultrafiltrate, respectively) at room temperature up to 8 h.
2. by subjecting aliquots of urine (50 and 150 ng Pt ml^{-1}) and plasma at 20 and 140 ng Pt ml^{-1} to two freeze-thaw cycles; frozen duplicate samples were allowed to thaw at ambient temperature for 2 h and were subsequently refrozen. Their Pt concentration was compared with aliquots that had not been subjected to the freeze-thaw cycles.

The specificity of the method was determined by analysing six different Pt-free urine, plasma and plasma ultrafiltrate samples in order to evaluate the non-specific absorption response. The limit of detection (LOD) was set at three standard

deviations (S.D.) of the mean background absorbance of blank (i.e. unspiked) samples.

2.6. Application of the analytical method for pharmacokinetic studies

The present method was applied to determine the pharmacokinetics of total and ultrafiltrable Pt after the oral administration of JM216 to 19 cancer patients included in Lausanne, according to two multicentric research protocols approved by the Ethics Committee of the Lausanne University Hospital. As a part of a multicentric phase I clinical study [28], 14 patients with various advanced cancers were included and received daily administration of JM216 for 14 days (dose escala-

Table 2
Standardised furnace conditions for urine, plasma and plasma ultrafiltrate samples

Step	T (°C)	Ramp-time (s)	Hold-time (s)	Argon flow (ml min^{-1})
Urine				
Dry	140	10	80	300
Char I	800	15	20	300
Char II	1700	10	40	300
Atomisation	2200	0	5	0
Clean	2600	1	5	300
Plasma				
Dry I	110	5	40	300
Dry II	140	5	60	300
Dry III	450	15	20	300
Char I	600	5	20	300
Char II	1750	10	20	300
Atomisation	2400	0	5	0
Clean	2600	1	5	300
Plasma ultrafiltrate				
Dry I	140	2	90	300
Dry II	140	2	90	300
Dry III	140	2	90	300
Char I	800	30	20	300
Char II	1700	10	50	300
Atomisation	2300	0	7	0
Clean	2600	1	5	300

Table 3
Precision and accuracy of the assay of platinum in urine samples

Nominal concentration (ng ml^{-1})	Concentration found (ng ml^{-1})	Precision (R.S.D.%)	Accuracy[a] (S.D.%)
Intra-assay ($n = 5$)			
60.0	59.4 ± 1.8	3.0	−1.0
90.0	89.0 ± 1.1	1.3	−1.1
140.0	132.3 ± 6.2	4.7	−5.5
Inter-assay ($n = 14$)			
60.0	58.9 ± 3.4	5.7	−1.9
90.0	91.6 ± 5.4	5.9	1.8
140.0	146.0 ± 11.2	7.7	4.3

[a] Found − nominal/nominal × 100.

Table 4
Precision and accuracy of the assay of platinum in plasma sample

Nominal concentration (ng ml^{-1})	Concentration found (ng ml^{-1})	Precision (R.S.D.%)	Accuracy[a] (S.D.%)
Intra-assay (n = 5)			
20.0	20.6 ± 1.7	8.0	2.7
70.0	75.0 ± 3.0	4.0	7.1
140.0	147.2 ± 1.8	1.2	5.1
Inter-assay (n = 21)			
20.0	20.4 ± 1.8	9.0	1.8
70.0	68.9 ± 4.1	5.9	−1.6
140.0	134.5 ± 7.7	5.7	−3.9

[a] Found − nominal/nominal × 100.

Table 5
Precision and accuracy of the assay of platinum in plasma ultrafiltrate sample

Nominal concentration (ng ml^{-1})	Concentration found (ng ml^{-1})	Precision (R.S.D.%)	Accuracy[a] (S.D.%)
Intra-assay (n = 5)			
12.0	12.0 ± 0.3	2.6	−0.2
25.0	24.4 ± 0.4	1.7	−2.6
45.0	44.0 ± 3.0	6.8	−2.3
Inter-assay (n = 11)			
12.0	12.2 ± 1.2	9.5	1.6
25.0	26.3 ± 2.0	7.6	5.3
45.0	46.8 ± 2.4	5.1	3.9

[a] Found − nominal/nominal × 100.

tion from 10 to 50 mg m^{-2} per day). A phase II trial included five earlier untreated small cell lung cancer (SCLC) patients receiving daily administration of JM216 at a fixed dose (120 mg m^{-2} per day) for 5 days. An antiemetic prophylaxis with 5-HT_3 antagonists was started at 35 mg m^{-2} in the phase I study and metoclopramide and dexamethasone were given to every patient included in the phase II. JM216 capsules were given to fasten patients in a standardised way (liquid intake and patients posture controlled). In the phase I study, blood samples were taken at 0, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after the dose of day 1 and 14 of the first chemotherapy cycle. Additional blood samples were taken on day 7 and up to 2 weeks (day 28) after the end of the chemotherapy. In the phase II study, blood samples were taken at 0, 0.5, 1, 2, 3, 4, 6, 8 12 and 24 h after the JM216 dose on day 1, and additional samples were taken on days 3 and 5 of the 5-day chemotherapy course. The detailed pharmacokinetic analysis of these two studies will be submitted elsewhere.

On days 1 and 14 of the phase I study, urine were collected by spontaneous voiding and stored as two fractions (0–8 and 8–24 h). Their volumes were measured and an aliquot was taken and stored at −80°C prior to P_{tot} analysis.

3. Results and discussion

3.1. Setting-up and validation of the analytical method

The biological sample pretreatment and AAS instrument conditions were extensively explored to optimise the signal of Pt atomic absorption response yielding the highest assay sensitivity, linearity and reproducibility. The optimised condi-

tions for the analysis of Pt in urine, plasma and plasma ultrafiltrate are presented in Table 2.

For the assay of Pt in plasma, initial attempts were made by injecting untreated plasma samples directly onto the tube, but were of no avail. The tubes deteriorated very quickly with the occurrence of a solid charcoal-like residue on the platform and an unacceptable decrease of its analytical performance. Subsequent attempts implied the addition of plasma samples onto the platform after the application of solution of the matrix modifier Triton X-100 at various concentrations (from 1 to 10%) and using different plasma/Triton X-100 volume ratios. Best Pt atomic absorption responses were obtained using 7 vol. of plasma with 3 vol. of 5% Triton X-100. Deviating from this ratio resulted in a definite lost of the linearity and reproducibility of the Pt absorption signal. Importantly enough, the Triton X-100 solution and the plasma were introduced separately into the graphite tubes just before the start of the sample drying program. This successive addition of plasma sample and detergent solution resulted in an extended life span of the graphite tubes. The drying of the sample in the tube could be best achieved using a step-wise temperature program: (i.e. 110°C for 40 s, 140°C for 60 s, and 450°C for 20 s, see Table 2b). This approach produced a satisfactory drying of the sample, preventing its splashing all over the tube wall. Ashing temperatures at 600 and 1750°C yielded the best absorbance with satisfactory reproducibility and acceptable background signal. Fig. 2 shows the variation of the P_{tot} absorption signal in a plasma sample spiked with 200 ng Pt ml^{-1} when the atomisation temperature varied up to 2650°C, with a fixed ashing temperature set at 1750°C. The P_{tot} absorption signal was maximal at 2550°C, but was associated with a background signal, which was found unacceptable at temperature exceeding 2400°C. Thus, the chosen atomisation temperature was set at 2400°C for 5 s and was a compromise between the optimal signal response and acceptable background signal.

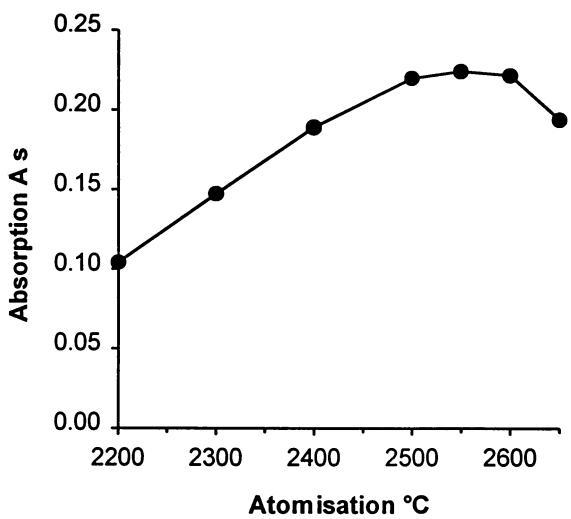

Fig. 2. Variation of platinum absorption in a plasma sample (spiked with 200 ng Pt ml^{-1}) when atomisation temperature varies from 2200 to 2650°C (ashing temperature fixed at 1750°C).

The low ng ml^{-1} ranges of Pt concentrations encountered in the ultrafiltrate samples collected in our clinical study required an enhanced sensitivity. A 150 μl total volume of ultrafiltrate sample was, therefore, injected in three successive applications on the platform tube separated by drying steps at 140°C for 90 s (see Table 2c). With such a procedure, the LLQ of Pt in plasma ultrafiltrate could be improved reaching values as low as 5 ng ml^{-1}, which was, therefore, chosen as the lowest concentration of the standard calibration curve. Ashing temperatures of 800 and 1700°C, atomisation temperature at 2300°C and the atomisation duration prolonged to 7 s were chosen for optimal concentration-absorbance linearity with minimal background signal. The Fig. 3 shows the variation of the UPt absorption signal in a ultrafiltrate sample (spiked with 200 ng Pt ml^{-1}) upon the application of various ashing and atomisation temperatures conditions.

Under these conditions, the shape of the signals were acceptable and appeared as single peaks, indicating minimal interferences of the matrix effect in the assay. The calibration curves established with Pt calibration solutions were linear over the 50–150 ng Pt ml^{-1} range in urine, 10–150 ng Pt ml^{-1} in plasma and 5–50 ng Pt ml^{-1} in plasma ultrafiltrate samples. The regression coefficients (r^2) were always higher than 0.993, 0.995 and 0.994 in urine, plasma and

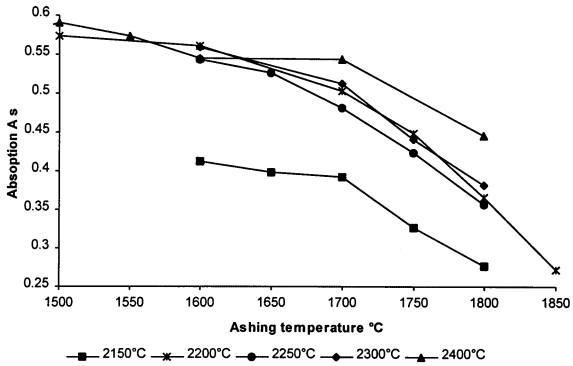

Fig. 3. Variation of platinum absorption in an ultrafiltrate sample (spiked with 200 ng Pt ml^{-1}) when ashing temperature varies from 1500 to 1850°C and atomisation temperature from 2150 to 2400°C (atomisation duration set at 7 s).

plasma ultrafiltrate, respectively. Concentrations higher than the upper limit of linearity should be appropriately diluted before the analysis to avoid saturation of the signal intensity and unacceptable memory effect observed in subsequent atomisations.

The precision and accuracy determined during the validation procedure are given in Tables 3–5. At low, medium and high concentrations in urine, the overall intra-assay precision was 3.0, 1.3, 4.7%, respectively. At the same dose levels, the precision was 8.0, 4.0, 1.2% in plasma and 2.6, 1.7, 6.8% in plasma ultrafiltrate, respectively. The same quality control samples were also analysed on different days to assess inter-assay precision, which was found lower than 5.7, 5.9, 7.7% in urine, 9.0, 5.9, 5.7% in plasma and 9.5, 7.6, 5.1% in plasma ultrafiltrate, respectively.

Precision and accuracy at the LLQ were also determined using matrix samples obtained from seven independent sources. Each sample was spiked to contain the LLQ concentration of the analyte and assayed. The deviation from the nominal LLQ concentrations ranged between -10.6% in plasma and $+7.7\%$ in plasma ultrafiltrate samples while the precision values were comprised between 2.9% in urine and 8.2% in plasma samples. This indicated that these values were, therefore, well below the $\pm 20\%$ recommended in the validation of bioanalytical methods [29].

The influence of the variability of urine matrix (obtained from six different healthy subjects) on the performance of the analysis of Pt in urine at its LLQ (50 ng Pt ml^{-1}) was also studied. In all cases, the deviation never departs more than $\pm$ 20% of the LLQ value, indicating that the inter-individual variability of the urine matrix does not seem significantly to influence the accuracy of the analysis of this low concentrations of Pt in urine samples.

The stability of the biological matrices at the various steps of the analysis (throughout the procedure, from sampling at the patient bedside to the processing in the laboratory) was explored to assess its possible influence on the AAS assay of Pt. Urine samples at 50 and 150 ng Pt ml^{-1}, plasma samples at 70 and 140 ng ml^{-1} and spiked plasma ultrafiltrate samples at 45 ng ml^{-1} were allowed to stand at room temperature up to 8 h before the analysis. Our results indicated no significant variations ($< 2.8\%$) of the level of the initial Pt concentration in these samples. This is in contrast with plasma samples at 20 ng ml^{-1} and plasma ultrafiltrate samples at 8 and 25 ng ml^{-1}, which showed an apparent increase in their starting nominal Pt concentration ($+14.3$, $+12.5$ and $+9.6\%$, respectively). This can be explained by the evaporation presumably occurring on the rack at room temperature, which may concentrate these low volumes of samples before their injection. Therefore, the time during which samples are stored on the autosampler rack should be minimised; they should ideally be placed in temperature-controlled autosampler just prior to the moment of the analysis.

Spiked urine (50 and 150 ng ml^{-1}) and plasma samples (20 and 140 ng ml^{-1}) subjected to two freeze-thaw cycles showed an acceptable change from their nominal starting concentration ($<$ 7.6%) after the first cycle. Therefore, urine and plasma samples should be immediately processed and frozen, stored at -80°C and thawed just prior to the analysis. However, in vitro data indicates that they should not be subjected to an additional freeze-thaw cycle.

The specificity of the assay was determined using six different Pt-free urine, plasma and plasma ultrafiltrate samples and the calculated

limits of detection (LOD) were 6.3, 5.8 and 1.9 ng Pt ml^{-1}, respectively.

3.2. Pharmacokinetics

The concentration-time curves of total (P_{tot}) and ultrafiltrable (UPt) platinum of the 14 patients enrolled in a phase I study of JM216 (dose escalating from 10 to 50 mg m^{-2} per day for 14 days) are shown in Fig. 4. The concentration profiles of P_{tot} and UPt of the five patients included in the phase II are presented in Fig. 5. JM216 is rapidly absorbed, with detectable concentrations in plasma already observed 30 min after dose administration. In the phase I, the P_{tot}/UPt ratio increased upon the administration of repetitive JM216 doses and maximal P_{tot} concentrations were 5 and 14 times higher than the corresponding UPt concentrations measured on days 1 and 14, respectively. For comparison, in the phase II, the P_{tot} at the maximal concentration measured on day 1 was also 5 times higher than the maximal UPt concentrations. This indicates, therefore, an accumulation of P_{tot} and UPt after JM216 repetitive doses. The elimination was variable and, as expected, slower for P_{tot} than for UPt with a median $t_{1/2}$ in the phase I of 9 days (range, 6.6–12.4) and 3.5 days (range, 0.4–10.8) for P_{tot} and UPt, respectively. It is not excluded that a profound compartment from which low levels of P_{tot} are slowly released may be present, but could not be observed with the analytical limitations of our AAS method, which can accurately measure concentrations down to 5 ng ml^{-1}. A more sensitive analytical method, such as ICP-MS, may enable to detect lower Pt concentration (down to 0.001 ng Pt ml^{-1}), resulting accordingly in the observance of prolonged elimination $t_{1/2}$. Similar discrepancies between the $t_{1/2}$ obtained with both techniques have been earlier described and discussed with oxaliplatin [30]. In the phase II, the elimination determined on day 1 is more rapid with median $t_{1/2}$ values of 1.6 days (range, 0.8–2.1) and of 0.4 days (range, 0.2–0.9) for P_{tot} and UPt, respectively. In the phase I study, P_{tot} was still detectable 2 weeks after the end of the first chemotherapy cycle, whereas UPt concentrations rapidly achieved the limit of quantitation of the method. The mean percentage of drug eliminated in urine during 24 h after the first administration is 6 and 3% of the dose in the phase I and II, respectively. The observed differences in Pt urinary excretion is not known, but may be explained by a saturation of renal tubular secretion mechanisms of Pt due presumably to the higher total dose administered daily in the phase II study.

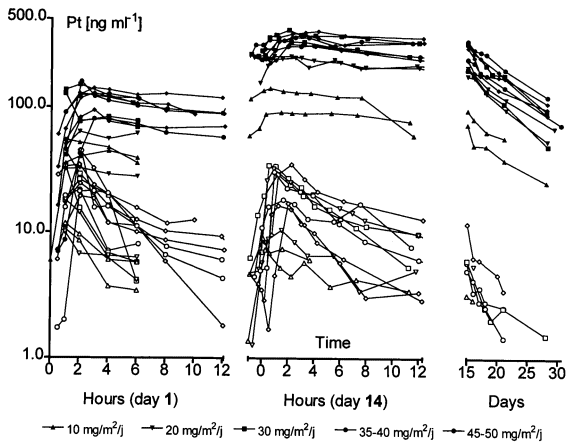

Fig. 4. Plasma levels of total platinum (P_{tot}) and ultrafiltrable platinum (UPt) in 14 patients treated with JM216 over 14 days (dose escalation: 10–50 mg m^{-2} d^{-1}).

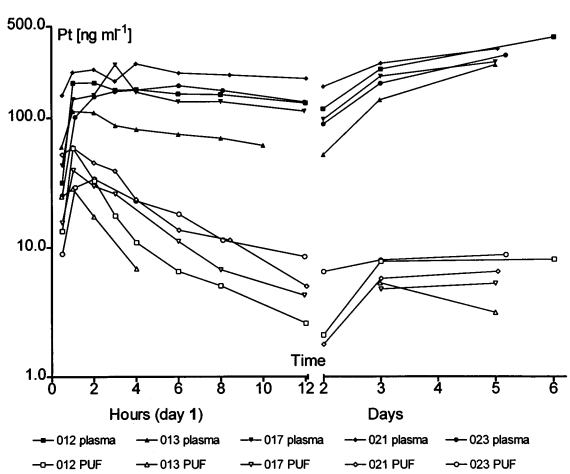

Fig. 5. Plasma levels of total platinum (P_{tot}) and ultrafiltrable platinum (UPt) in five patients treated with JM216 over 5 days (dose: 120 mg m^{-2} d^{-1}).

A detailed pharmacokinetic evaluation of JM216 in this phase I, as well in the phase II study will be presented elsewhere.

4. Conclusion

A reliable quantitative determination of the total and ultrafiltrable platinum in urine, plasma and plasma ultrafiltrate has been developed and validated using atomic absorption spectrophotometry. The method has been set-up specifically for each considered matrix, is rapid and has the required accuracy and precision to be applied for the analysis of Pt in clinical studies of Pt-containing anticancer agents, notably JM216. Interestingly enough, P_{tot} and UPt concentrations after JM216 administration were measurable 2 weeks after the end of the first chemotherapy cycle. Preliminary pharmacokinetic study indicates a long elimination $t_{1/2}$, which was particularly prolonged for P_{tot}. Moreover, both P_{tot} and UPt accumulate upon repetitive administration of daily doses of JM216.

Whereas it is not expected that a phase I demonstrates any anti-cancer activity, a subjective improvement with tumor-related pain relief and stable disease was observed in two patients with the oral administration of JM216 over a 14 day period regimen [28]. The daily doses of 40–45 mg m^{-2} for 14 days schedule is currently the recommended regime for further phase II evaluation of JM216 as a single agent [28].

While it has been reported that first-line JM216 therapy was ineffective in non-small-cell lung cancer (NSCLC) [31], the clinical evaluation of the results of our phase II study is under completion. Moreover, the potential clinical benefit of JM216 remains to be evaluated in further combination studies.

Acknowledgements

Bristol-Myers Squibbs and the MEDIC Foundation provided support for this research. We are indebted to Professor Bernard Rossier and Professor Jacques Diezy, Institute of Pharmacology and Toxicology, Faculty of Medicine, University of Lausanne, for providing laboratory space and the AAS equipment required for the setting-up of the assay.

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