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Journal of Pharmaceutical and Biomedical Analysis
24 (2000) 1–10

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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Validation of a highly sensitive ICP-MS method for the determination of platinum in biofluids: application to clinical pharmacokinetic studies with oxaliplatin

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Received 7 February 2000; received in revised form 18 May 2000; accepted 27 May 2000

Abstract

ELOXATIN® (Oxaliplatin) is a novel platinum containing anti-cancer agent with a diaminocyclohexane carrier ligand which has been approved in several major European countries. Clinical studies have demonstrated that the compound exhibits marked activity against colorectal cancers in combination with 5-fluorouracil (5-FU). The aim of this work was to develop and validate a highly sensitive inductively coupled plasma mass spectrometry assay for the determination of oxaliplatin-derived platinum in plasma ultrafiltrate, plasma and whole blood and to apply this technique to clinical pharmacokinetic studies with oxaliplatin. Ultratrace detection of platinum in plasma ultrafiltrate was achieved using ultrasonic nebulisation combined with ICP-MS. This technique allows detection of platinum at the 0.001 μg Pt/ml level in only 100 μl of matrix. Assays in blood and plasma utilised a standard Meinhardt nebuliser and spray chamber, achieving detection limits of 0.1 μg Pt/ml in 100 and 200 μl of matrix, respectively. The assays were validated (accuracy and precision within ±15%) over the concentration ranges: 0.001–0.250 μg Pt/ml in plasma ultrafiltrate and 0.1–10 μg Pt/ml for plasma and whole blood. The effect of sample digestion, dilution, long term frozen storage and quantitation in the presence of 5-FU were also investigated and validated. The method was used to monitor platinum exposure following oxaliplatin administration (130 mg/m^2) to cancer patients. Following a 2 h i.v. infusion, peak platinum levels declined in a triphasic manner in all blood compartments. Free platinum was detected in plasma ultrafiltrate at low levels (0.001–0.010 μg Pt/ml) for up to 3 weeks. In conclusion, a highly sensitive and specific assay has been developed for the determination of platinum in biofluids. This method enabled characterisation of the long term exposure to platinum in patients following oxaliplatin treatment.

Keywords: Inductively coupled plasma mass spectrometry; Ultrasonic nebulisation; Assay validation; Platinum; Oxaliplatin; Pharmacokinetics

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0731-7085/00/$ - see front matter
PII: S0731-7085(00)00377-0

1. Introduction

Platinum anticancer drugs have been in clinical use for almost 30 years following the discovery of cisplatin [*cis*-diamminedichloroplatinum (II)]. However the use of cisplatin is associated with dose limiting toxicities including nephrotoxicity and neurotoxicities. Research has focused on developing new agents which are less toxic with a broader spectrum of anti-tumour activity. Until now, carboplatin is the only agent which is generally accepted to offer any real advantage over cisplatin in terms of safety, although it exhibits a broadly similar pattern of anti-tumour activity [1]. Oxaliplatin is a new platinum agent with a diaminocyclohexane carrier ligand and carboxylic acid leaving group (Fig. 1), which has recently been approved in several major European countries for first line treatment of colorectal cancer in combination with 5-fluorouracil.

The ability to measure total platinum exposure to platinum-containing anticancer agents in patients is a prerequisite to an understanding of their pharmacokinetics, pharmacodynamics and toxicity. This is of particular significance with platinum agents, as free plasma ultrafilterable drug is thought to represent the pharmacological and toxicologically significant fraction in blood, the remaining material is biological inactive being irreversibly bound to plasma proteins, blood cells and tissues. However, the low levels of free drug related products in the ultrafilterable plasma compartment challenge the detection limits of most analytical instrumentation.

The analytical techniques employed for measuring platinum in biofluids have been well documented and reviewed [2].

Until recently, atomic absorbance spectrometry (AAS) has been the method of choice for platinum analysis. Because of availability and convenience it is still used today to characterise platinum exposure. However, the major limitation of this method is a lack of adequate sensitivity. This becomes important when trying to assess the long term exposure of free circulating products. Recent clinical pharmacokinetic studies with oxaliplatin have shown that the limit of quantitation (LOQ) of AAS is not adequate (LOQ = 0.03 μg/ml) to fully characterise the terminal elimination of 'free' platinum in plasma ultrafiltrate beyond 24 h [3].

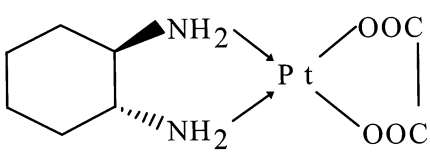

Fig. 1. Chemical structure of Oxaliplatin.

ICP-MS is a relatively new technique for the determination of platinum in biological matrices. The high sensitivity of ICP-MS enables ultratrace determinations of free platinum in plasma ultrafiltrate, with the specificity synonymous with mass spectrometric detection. The obvious benefits of ICP-MS have led to it's development and use in the study of platinum drug interactions with DNA [4] and other biomolecules [5] and more recently for pharmacokinetic studies with platinum drugs [6,7]. There is still however, only limited data on the accuracy and precision of ICP-MS methods. In addition, possible effects of short and long term frozen storage of samples has not been fully examined. For an accurate assessment of platinum exposure in patients it is important to consider the effects of blood processing on free and bound platinum. Studies using cisplatin and oxaliplatin have shown that it is necessary to process blood within a fixed time period [8,9].

In general, current ICP-MS methods utilise relatively large sample volumes (up to 300 μl of ultrafiltrate) which for clinical pharmacokinetic studies are not always easily obtainable. Small clinical samples, typically 100 μl, and a need to measure long term exposure to oxaliplatin highlights the need for robust and sensitive analytical techniques.

We describe, for the first time the use of ultrasonic nebulisation coupled with ICP-MS for low level detection of platinum in biomatrices. Ultrasonic nebulisers are sample introduction systems for ICP-MS. The aqueous sample is introduced to a quartz faceplate of a piezo-electric transducer where it is dispersed into a fine uniform aerosol, and then desolvated to produce a dense

Table 1
ICP-MS operating conditions

ICP-MS	
Instrument	Finnigan MAT SOLA ICP
Platinum isotope mass	195
Iridium internal standard isotope mass	193
Detector mode	Multiplier (pulse count)
Acquisition modes	Scanning (whole blood and plasma) peak jumping (plasma ultrafiltrate only)
Dwell time	8 ms
Channels/amu	8
Passes/scan	60
No. scans/sample	3
Nebuliser type	Concentric and ultrasonic (ultrafiltrate only)
Sample uptake flow rate	10 and 16 (ultrafiltrate only) (Arbitrary units)
Parameter	Intensity ratio
Wash solution	8% aqua Regis
Ultrasonic nebuliser	
Model	U-5000AT+
Carrier gas	1.0 l/min
Desolvation temperature	140°C
Desolvation cooling temperature	3°C
Ultrasonic frequency	1.4 MHz

dry aerosol. Ultrasonic nebulisation is significantly more efficient than pneumatic nebulisers producing marked increases in sensitivity for ICP-MS analysis [5].

Cisplatin, carboplatin and oxaliplatin all undergo rapid biotransformations to aquated derivatives and conjugates. The reactivity and transient nature of the biotransformation products makes monitoring of individual components extremely difficult both in terms of sample handling and analysis. Therefore, the generally accepted approach for monitoring these agents is to determine total platinum products, as 'free' platinum in plasma ultrafiltrate or the bound component as 'total platinum' in plasma and whole blood or blood cells.

The aim of this work was to develop a highly sensitive ICP-MS method for the determination of platinum in biological fluids in order to fully characterise the exposure to platinum following administration of oxaliplatin to cancer patients.

2. Experimental

2.1. Materials

Oxaliplatin analytical standard (Lot No. L093T021) (Fig. 1) was obtained from Tanaka Kikinzoku Kogyo K.K., Japan, and dissolved in water to give a working stock of approximately 1 mg Pt/ml. Platinum standard solution was supplied by Sigma as a 1 mg/ml solution (atomic absorption spectrometric grade). Iridium was chosen as internal standard for the assay as it was the closest element to platinum in atomic mass. Iridium internal standard (9.9 mg/ml) was obtained from Aldrich (Lot 01425MY, ICP/DCP grade) with further dilutions being made in 1% (plasma ultrafiltrate) or 7% (plasma and whole blood) nitric acid to achieve the desired working concentration. Nitric acid and hydrochloric acid were obtained from Fisher Scientific and were of trace metal grade purity. Aqua regis (3:1 hydrochloric acid: nitric acid) was prepared as an 8% solution in water. In all cases, water was purified by deionization with a minimum conductivity value of 15 mΩ/cm. Whole blood was obtained from healthy volunteers into lithium heparin draw tubes. Plasma ultrafiltrate was prepared from fresh plasma using Amicon® Centrifree Filters with a molecular cut-off of 30 000 Da (Centrifugation at $3000 \times g$ for 0.5 h, maintained at 4°C).

2.2. ICP-MS

The ICP-MS system comprised a Finnigan MAT SOLA ICP-MS with a Gilson model 222 autosampler. Sample nebulisation was performed using a concentric nebuliser for plasma and whole blood and an ultrasonic nebuliser (Cetac, UK) for plasma ultrafiltrate. The major isotopes of platinum and iridium were monitored at m/z, 195 and 193, respectively. The detection modes for both isotopes were 'scanning' (plasma whole blood assays) or 'peak jumping' (plasma ultrafiltrate only). Details of the ICP-MS operating conditions are given in Table 1. Quantitation was based on the

mean ($n = 3$) intensity ratios for platinum and iridium against a calibration curve using linear regression analysis.

2.3. Sample processing

Ultrafiltrate samples (100 μl) were diluted using a Gilson ASPEC XLi programmed to deliver 1.8 ml of iridium internal standard (0.005 μg/ml, in 1% nitric acid). Samples were then mixed thoroughly prior to ICP-MS analysis.

In plasma (100 μl) and whole blood (200 μl), following addition of iridium internal standard (100 μl, 5 μg/ml), samples were digested in 1 ml of concentrated nitric acid (70%) for 1 h at 90–100°C, allowed to cool to room temperature and diluted to 10 ml with de-ionised water.

2.4. Preparation of standards

Matrix matched calibration samples were prepared in plasma ultrafiltrate (100 μl) plasma (100 μl) and whole blood (200 μl) by spiking with an equal volume of an appropriate platinum solution to give final platinum concentrations in matrix of 0.250, 0.200, 0.125, 0.05, 0.006, 0.003 and 0.001 μg Pt/ml for plasma ultrafiltrate and 10.0, 7.50, 5.00, 0.300 and 0.100 μg Pt/ml in plasma and whole blood. Replicate standards ($n = 3$) were prepared at the upper and lower levels.

Validation samples were prepared by spiking matrix with oxaliplatin standard solutions. Plasma ultrafiltrate samples (100 μl) were spiked with appropriate oxaliplatin stock solution to give final concentrations of platinum in matrix of 0.250, 0.200, 0.125, 0.003 and 0.001 μg Pt/ml. Similarly, validation samples were prepared in plasma (100 μl) and whole blood (200 μl) by spiking with an equivalent volume of an appropriate oxaliplatin stock solution to give nominal platinum concentrations in matrix of 10.0, 7.50, 5.00, 0.300 and 0.100 μg Pt/ml. Blank samples were prepared by substituting water for the oxaliplatin standard.

2.5. Validation studies

The assay was validated by assessing within and between run accuracy and precision for each matrix. For the former, the accuracy (Eq. 1) and precision (Eq. 2) of the assay was examined at five validation levels ($n = 6$) in one run. In the case of the between-run assessment, one sample from each of the five validation levels was assessed in six separate runs.

The stability of samples (plasma ultrafiltrate: 0.200 and 0.003 μg Pt/ml, plasma or whole blood: 7.5 and 0.300 μg Pt/ml) under various storage conditions was also investigated.

The acceptance criteria was based on industry standards and accepted practices [10]. Accuracy was defined as the percentage difference between the observed concentration and the expected value. Precision was expressed as the coefficient of variation expressed as a percentage. Acceptance criteria for the validation was as follows: the mean %D and CV% could not exceed $\pm 15\%$ (or $\pm 20\%$ at the lowest level); 75% of all samples had to be within $\pm 15\%$ of expected concentrations (or $\pm 20\%$ at the lowest level); no more than two of six samples within a given concentration could exceed $\pm 15\%$ of their expected values (or $\pm 20\%$ at the lowest level).

2.6. In vitro blood distribution studies

Two separate incubations were designed to examine the blood distribution of oxaliplatin-derived platinum when samples were left at room temperature. The first incubation (10 ml) was prepared from fresh plasma containing oxaliplatin (0.3 and 7.5 μg/ml) stored at room temperature for up to 24 h. The second, prepared from fresh whole blood (3 ml) containing oxaliplatin (0.3 and 7.5 μg/ml), left over the same time period. Following the addition of oxaliplatin to the incubation mixtures, aliquots (1 ml) were then removed at 0, 1, 2, 3, 6 and 24 h and placed on ice. The samples from the plasma incubates were processed by membrane filtration (Amicon filters, $2000 \times g$ for 30 min) to obtain plasma ultrafiltrate, the whole blood being centrifuged ($2000 \times g$ for 10 min) to obtain plasma. The resultant fractions were then analysed by ICP-MS for platinum content.

2.7. Assay interference studies

As oxaliplatin is administered clinically in combination with 5-flurouracil, drug interference stud-

ies were conducted to examine any effects on the accurate measurement of platinum. A nominal concentration of 5-fluorouracil (0.3 μg/ml, Sigma) was chosen which mimicked steady state plasma concentrations reported in the literature following continuous intravenous infusion (2.25 g/m^2 per day $\times$ 5) [11].

3. Results

3.1. Linearity of response

Platinum calibration curves in plasma ultrafiltrate, plasma and whole blood were linear across the concentration ranges studied. A regression

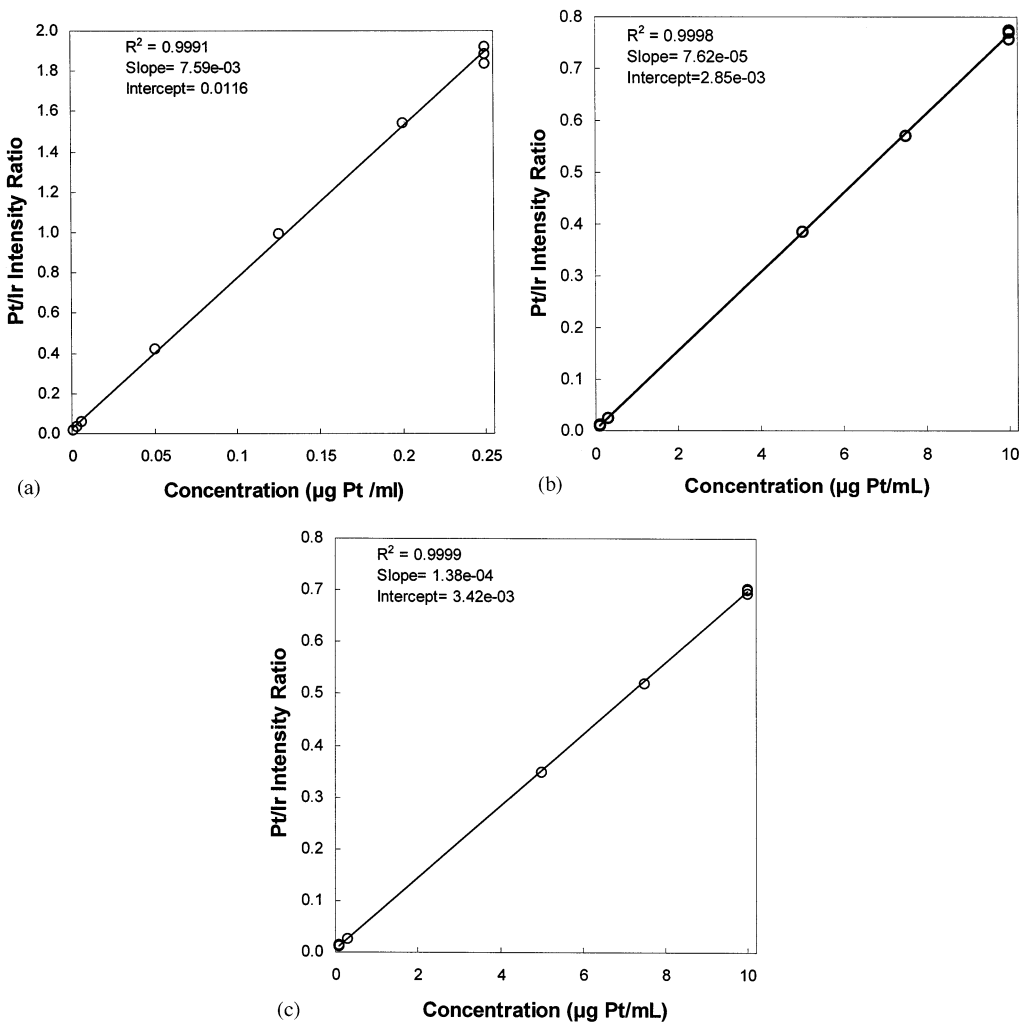

Fig. 2. Typical calibration curve for platinum in (a) plasma ultrafiltrate, (b) plasma and (c) whole blood.

Table 2
Summary of within and between-day validation results for platinum in plasma ultrafiltrate, plasma and whole blood

	Type	Parameters ($n = 6$/level)	Observed concentration (μg Pt/ml)				
	Actual conc.		**0.256**	**0.205**	**0.128**	**0.00307**	**0.00102**
Plasma ultrafiltrate	Within-day	Mean	0.249	0.199	0.116	0.00314	0.00110
		Accuracy (%)	−2.6	−2.9	−9.2	2.4	9.6
		Precision (%)	1.8	1.2	1.1	3.2	4.8
	Between-day	Mean	0.248	0.203	0.127	0.00272	0.0010
		Accuracy (%)	−3.0	−0.8	−0.4	−12	2.1
		Precision (%)	3.9	2.8	7.0	15	14
	Actual conc		**10.2**	**7.58**	**5.08**	**0.305**	**0.101**
Plasma	Within-day	Mean	10.2	7.40	4.98	0.271	0.086
		Accuracy (%)	0.2	−2.3	−1.9	−11	−16
		Precision (%)	0.6	0.3	0.9	4.2	17
	Between-day	Mean	9.65	6.96	4.54	0.277	0.114
		Accuracy (%)	−4.1	−7.7	−9.7	−8.2	12
		Precision (%)	3.0	3.5	5.9	10	14
	Actual conc		**10.3**	**7.52**	**5.08**	**0.310**	**0.103**
Whole blood	Within-day	Mean	10.3	7.35	5.00	0.305	0.095
		Accuracy (%)	−0.2	−2.3	−1.5	−1.7	−8.2
		Precision (%)	1.1	1.3	0.4	1.7	4.3
	Between-day	Mean	10.4	7.38	5.01	0.314	0.111
		Accuracy (%)	0.08	−1.9	−1.2	1.1	7.4
		Precision (%)	2.9	2.5	2.8	4.0	9.2

line with $1/X$ weighting best fitted the data (Fig. 2).

3.2. Accuracy and precision of assay

The within and between-day accuracy and precision of the assay are summarised in Table 2. In all matrices, the observed values were within the acceptance criteria outlined above. Overall, the within day accuracy and precision for the assay was in the range −16–9.6 and 0.3–17%, respectively. The corresponding values for the between day runs were similar giving an accuracy of −12–12% and precision of 2.5–15%. The response of the blank samples were below that observed at the LOQ of the assays. It was also found that 70 samples could be conveniently analysed in an overnight run, without any significant change in instrument response.

3.3. Freeze/thaw effects

Similarly, freeze/thawing of samples did not affect the accurate quantitation of platinum in plasma ultrafiltrate (0.200 and 0.003 μg Pt/ml), plasma or whole blood (7.5 and 0.300 μg Pt/ml) with no loss of platinum observed following three freeze/thaw cycles (Fig. 3a and b).

3.4. Drug interference studies

There was no affect on the accurate quantitation of platinum in any matrix following co-incubation of 5-fluorouracil with oxaliplatin in plasma ultrafiltrate (0.20 and 0.003 μg Pt/ml) plasma or whole blood (7.5 and 0.300 μg Pt/ml). Overall, accuracy and precision was within ± 10 and $< 13\%$, respectively (Fig. 3a and b).

3.5. Storage effects

There were no effects on the accurate quantitation of platinum under any of the storage conditions tested. Observed platinum concentrations did not deviate from the expected value following storage of processed plasma ultrafiltrate, plasma or whole blood samples for 3 h at room temperature or frozen storage of samples for up to 1 month. (Fig. 3a and b).

3.6. Matrix dilution

Dilution studies demonstrated that plasma ultrafiltrate samples could be diluted five fold with control matrix with no effect on the accurate quantitation of platinum. Following a fivefold dilution of ultrafiltrate containing oxaliplatin (3 μg Pt/ml) the accuracy and precision was 5.3 and 11%, respectively.

3.7. Blood distribution

The change in the distribution of oxaliplatin derived platinum (7.5 and 0.3 μg Pt/ml) following storage of whole blood and plasma at room temperature was examined in two separate incubations. In plasma incubates, free platinum levels fell markedly (~ 80% loss of initial level) over a 24 h period (Fig. 4a). More critically, the rapid decrease in platinum levels over the initial 5 h highlights the importance of processing samples rapidly within a fixed time frame. In whole blood incubates there was evidence of a concentration effect with a more rapid distribution at 7.5 μg Pt/ml than at 0.3 μg Pt/ml (Fig. 4b). The overall

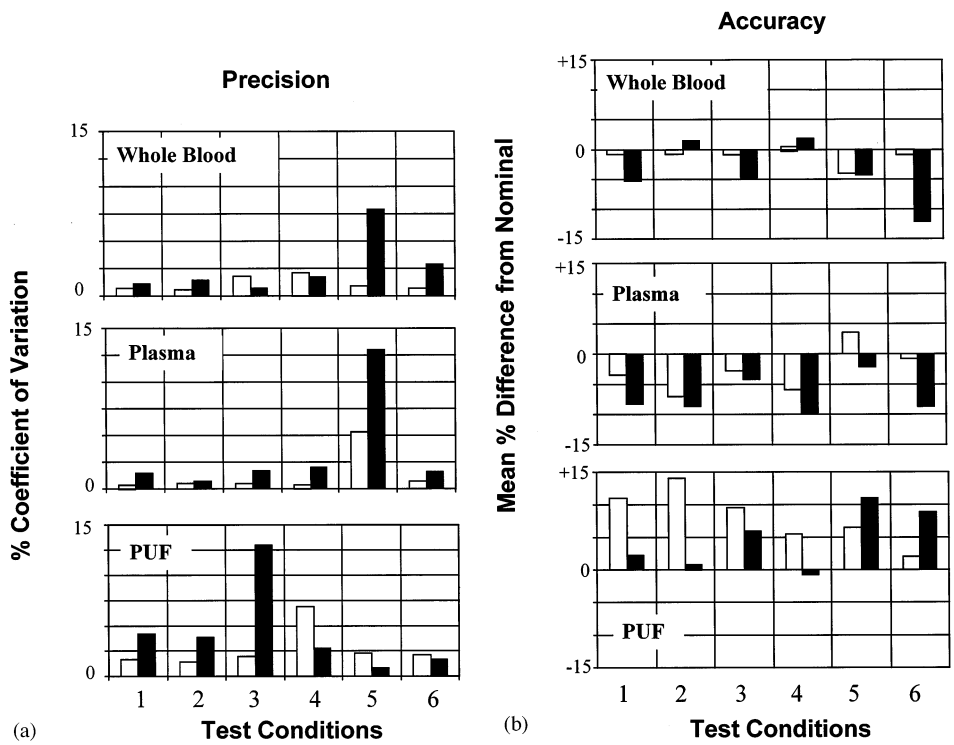

Fig. 3. Accuracy (a) and precision (b) data obtained from the analysis of oxaliplatin-derived platinum in whole blood, plasma and plasma ultrafiltrate under the following test conditions: (1) freshly prepared in control matrix; (2) after three freeze/thaw cycles; (3) co-incubation with 5-fluorouracil; (4) effects of digesting samples and the effects of (5) 1 week and (6) 1 months frozen storage. Studies were conducted at platinum concentrations equivalent to 3 × LOQ (unfilled bars) and 75% of the top calibration level (filled bars).

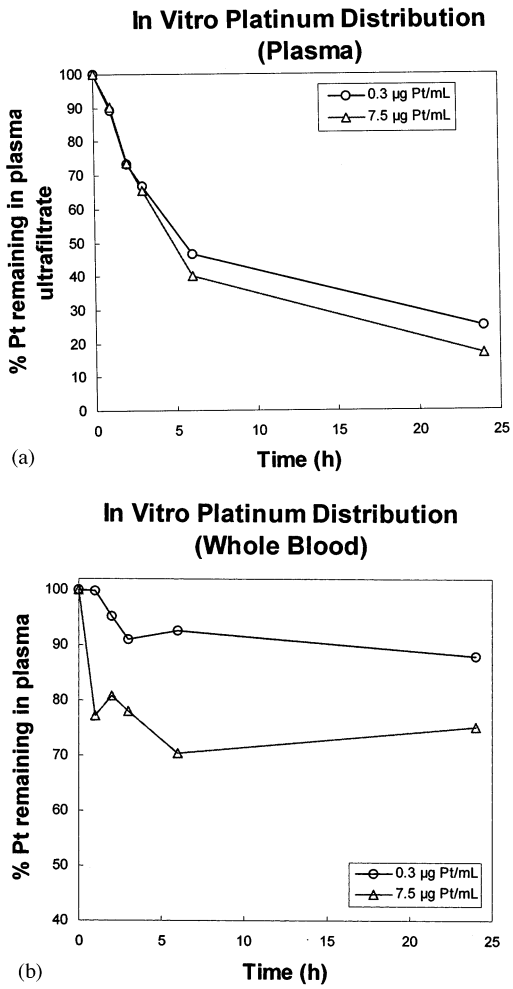

Fig. 4. In vitro blood distribution of platinum following 24 h storage of plasma and whole blood samples containing oxaliplatin (0.3 and 7.5 μg Pt/ml) at room temperature. The graphs represent (a) the percentage of free platinum remaining in plasma ultrafiltrate following incubation of oxaliplatin in plasma and (b) the percentage of platinum associated with plasma from whole blood incubates.

loss of platinum from plasma into cells was approximately 17–25% over the 24 h period.

3.8. Pharmacokinetics

Following a single 2 h intravenous infusion of oxaliplatin (130 mg/m^2) to colorectal cancer patients ($n = 15$), platinum concentrations could be detected in all whole blood, plasma and plasma ultrafiltrate samples up to 3 weeks post-dose (Fig. 5). Peak platinum levels (attained at the end of infusion) were followed by a triphasic decay in all blood compartments. Free platinum pharmacokinetics were characterised by a rapid distribution phase ($t_{1/2}\alpha = 0.2$ h), a long intermediate phase ($t_{1/2}\beta = 12.7$ h) and a protracted elimination phase ($t_{1/2}\gamma = 237$ h). The distribution ratio of platinum between blood cells, plasma, and the free fraction was approximately 3.1:3.7:1.0. at the end of infusion (C_{max}).

4. Discussion

The current assay was validated successfully for the determination of oxaliplatin-derived platinum in plasma ultrafiltrate, plasma and whole blood. The platinum concentration ranges selected reflect the exposure levels anticipated in humans following intravenous dosing with oxaliplatin; 0.001–0.250 μg Pt/ml (with an upper limit of 1.25 μg Pt/ml, using matrix dilution) in ultrafiltrate and 0.1–10 μg Pt/ml for plasma and whole blood. In addition, there was no affect on the accurate quantitation of platinum in plasma ultrafiltrate, plasma or whole blood after the following conditions: co-incubation with 5-fluorouracil, frozen storage −20°C for up to 1 month, and storage of nitric acid digests for up to 3 h at room temperature.

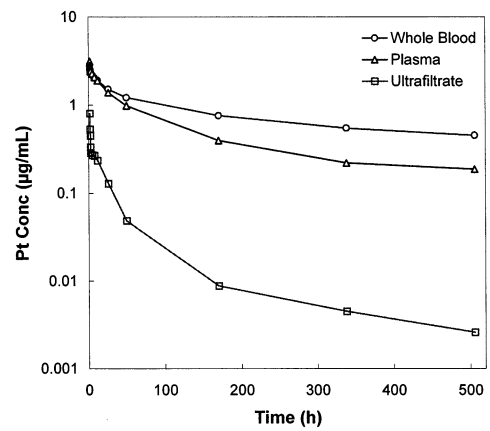

Fig. 5. Mean platinum concentration versus time profile of platinum in whole blood, plasma and plasma ultrafiltrate following a single 2 h i.v. infusion of oxaliplatin (130 mg/m^2) to cancer patients ($n = 15$).

ICP-MS within and between-day assay variability has been reported to be as low as 2% with accuracies of within 10% [6,12]. However, assay performance in these studies was estimated by repeat analysis of a single standard platinum solution and not assessed in biological matrices. In this study, the overall assay performance was good, with within day assay precision of $< 5\%$ in all matrices, except at the low level in plasma (17%).

The limits of detection reported for platinum by ICP-MS varies significantly [5,6,12,13], probably reflecting the differences in instrument efficiency, the amount of sample matrix used, and more importantly, the nebuliser efficiencies. The common Meinhardt type nebulisers produce only 2% of the fine droplet aerosol required for ICP-MS analysis. However, sensitivity can be gained by exploiting more efficient nebuliser types. In our laboratory, we observed a 25-fold increase in sensitivity using ultrasonic nebulisation.

In terms of absolute sensitivity for platinum in solution, the levels of sensitivity are in the fg/ml range for most ICP-MS instruments. However, realistically this level of sensitivity is difficult to achieve in biological matrices due to the inherent variability in the natural background level of platinum. Furthermore, we found the background level of platinum is higher in whole blood than plasma or plasma ultrafiltrate, probably reflecting the natural distribution of the element. Therefore, matrix matched calibrants are essential for ultratrace quantitative analysis.

Distribution studies highlight the importance of preparing samples rapidly following collection from patients in the clinic. It is therefore recommended that whole blood and plasma samples be placed on ice following collection and processed within an hour to minimise loss in the plasma matrix due to uptake into RBC's and/or loss of free drug as a result of protein binding.

Oxaliplatin derived platinum follows a triphasic pattern of elimination, however the terminal phase of the ultrafiltrable drug has generally been poorly defined, probably reflecting sparse sampling at later time points coupled with a poor level of assay sensitivity [3,14]. In this limited study we monitored platinum in plasma ultrafiltrate up to 3 weeks post-dose and sample collection was conducted under well controlled sample handling conditions to ensure sample integrity. In addition, platinum drug measurements were conducted using a fully validated assay.

The elimination half-life of free platinum following oxaliplatin dosing has been reported to be as low as 27 h [3]. However, we observed a long terminal elimination phase ($t_{1/2}$, 237 h approx.) possibly reflecting differences in assay sensitivity. The long retention of ultrafiltrable platinum probably represents drug covalently bound to low molecular weight protein fractions or amino acids which are able to pass through membrane filters.

In conclusion, a highly sensitive and specific assay has been developed and validated for the determination of platinum in plasma ultrafiltrate, plasma and whole blood using ultrasonic nebulisation and ICP-MS. In these studies, we demonstrated assay integrity across a working dynamic range for the determination of platinum in biofluids. This method enabled full characterisation of the long term phamacokinetic behaviour of platinum in colorectal patients following oxaliplatin treatment.

Acknowledgements

The authors wish to thank Dr David Churchman and Dr Fadi Abou-Shakra for their expert knowledge and assistance in ICP-MS and Vicki Forster for preparing this manuscript.

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