A validated inductively coupled plasma mass spectrometry (ICP-MS) method for the quantification of total platinum content in plasma, plasma ultrafiltrate, urine and peritoneal fluid

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A Validated Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Method for the Quantification of Total Platinum Content in Plasma, Plasma Ultrafiltrate, Urine and Peritoneal Fluid.

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Abstract

Oxaliplatin is a platinum (Pt)[1] containing antineoplastic agent that is applied in current clinical practice for the treatment of colon and appendiceal neoplasms. A fully validated, highly sensitive, high throughput inductively coupled plasma mass spectrometry (ICP-MS) method is provided to quantify the total Pt content in plasma, plasma ultrafiltrate, urine and peritoneal fluid. In this ICP-MS approach, the only step of sample preparation is a 1000-fold dilution in 0.5% nitric acid, allowing the analysis of 17 samples per hour. Detection of Pt was achieved over a linear range of 0.01 – 100 ng/mL. The limit of quantification was 18.0 ng/mL Pt in plasma, 8.0 ng/mL in ultrafiltrate and 6.1 ng/mL in urine and peritoneal fluid. The ICP-MS method was further validated for inter-and intraday precision and accuracy (≤15%), recovery, robustness and stability. Short-term storage of the biofluids, for 14 days, can be performed at -4°C, -24°C and -80°C. As to long-term stability, up to 5 months, storage at -80°C is encouraged. Furthermore, a timeline assessing the total and unbound Pt fraction in plasma and ultrafiltrate over a period of 45 hours is provided. Following an incubation period of 5 hours at 37°C, 19 - 21% of Pt was recovered in the ultrafiltrate, emphasizing the extensive and rapid binding of oxaliplatin-derived Pt to plasma proteins. The described method can easily be implemented in a routine setting for pharmacokinetic studies in patients treated with oxaliplatin-based hyperthermic intraperitoneal perioperative chemotherapy.

Keywords

Platinum; ICP-MS; plasma; plasma ultrafiltrate; urine; peritoneal fluid

Highlights

- ICP-MS method to quantify the total Pt content in several biofluids
- Method was validated for LOD, LOQ, inter-and intraday precision and accuracy
- Method was further validated for recovery, robustness and stability
- Timeline assessing the extent of oxaliplatin-derived Pt plasma protein binding
- Pharmacokinetic case study of patient treated with oxaliplatin-based chemotherapy

[1] HIPEC, hyperthermic intraperitoneal perioperative chemotherapy; HNO_3, nitric acid; Pt, platinum; RE, relative error; Tb, terbium

1. Introduction

Oxaliplatin (oxalato-*cis*-1,2-diaminocyclohexane-platinum(II)), together with infusional 5-fluorouracil and leucovorin, is applied as chemotherapeutic agent in current clinical practice for the treatment of colon and appendiceal neoplasms[1]. It was developed to overcome side effects and resistance associated with Pt agents, cisplatin and carboplatin. After intracellular uptake, this antineoplastic agent forms intra- and interstrand Pt-DNA crosslinks on adjacent guanine bases resulting in apoptotic cell death[2-4]. Several *in vitro* and *in vivo* studies have demonstrated that oxaliplatin undergoes rapid, spontaneous and nonenzymatic biotransformation in biological fluids. Several intermediates, including complexes with chloride, water, methionine and glutathione, were identified using chromatography coupled to inductively coupled plasma mass spectrometry (ICP-MS) or to electrospray ionization mass spectrometry (MS)[5]. These intermediates are thought to bind rapidly to amino acids, proteins and other macromolecules[6-8]. When the bonds are irreversible, the complexes are considered inactive and will be secreted. At present, however, there is no consensus whether the oxaliplatin-derived intermediates contribute to the cytotoxicity of the drug. Therefore, throughout literature, various analytical methods have been published, focusing on the determination or quantification of either intact oxaliplatin, its biotransformation products or the total Pt content. These methods include high performance liquid chromatography (HPLC), MS, atomic absorption spectrophotometry (AAS) and ICP-MS.

To determine intact oxaliplatin in blood ultrafiltrate, Ehrsson *et al.* published a HPLC method with post-column derivatization in a microwave field followed by photometric detection[9]. However, an adequate validation of the proposed method is lacking. Furthermore, the method started with a large sample volume of blood (3 mL) and the limit of quantification (LOQ) was only 0.04 µg/mL. Other research groups combined HPLC, either reversed phase or hydrophilic interaction chromatography (HILIC), with tandem MS and provided a fully validated method to quantify intact oxaliplatin in plasma ultrafiltrate[10, 11]. The described methods require extensive sample preparation, have overall run times of 10-15 minutes per sample and LOQ ranges between 20 ng/mL and 25 ng/mL. To separate intact oxaliplatin and its biotransformation products, Luo *et al*. developed a reversed phase HPLC method which allowed quantification of the different complexes in rat plasma. Major drawbacks associated with this method were the extensive run time of 90 minutes per sample and the use of labeled oxaliplatin (^{3}H-oxaliplatin)[12]. Ip *et al.* and Koellensperger *et al.* report a specific HPLC-ICP-MS method to quantify intact oxaliplatin and its biotransformation product, [Pt(DACH)Cl_2] in deproteinized blood plasma[13] and urine[14], respectively. Overall run times were 20 min and 12 min, the described LOQ for oxaliplatin was 19 ng/mL and 0.17 ng/mL.

Most pharmacokinetic studies regarding oxaliplatin have been conducted using AAS and ICP-MS as analytical techniques to determine the total Pt amount[15-18]. In the past, AAS was the

method of choice but nowadays, ICP-MS is the favorable technique as lower detection limits (LOD) for most elements can be accomplished. Other major advantages associated with ICP-MS are the possibility for high throughput analysis, highly automated analysis, high specificity and the ability to obtain isotopic information. Morrison *et al.* validated a highly sensitive ICP-MS method for the quantification of total Pt in several biofluids: plasma ultrafiltrate, plasma and whole blood[19]. Using a starting sample volume of only 100 µL (plasma) and 200 µL (whole blood), the method allowed to measure 1 ng/mL as LOQ for plasma ultrafiltrate and 100 ng/mL for plasma and whole blood. Although the actual analysis time of ICP-MS is very short (seconds), the disadvantage of the described method is the time-consuming sample processing, involving digestion with nitric acid for 1h. Brouwers *et al.* developed and validated an ICP-MS method to measure total Pt amount originating from either cisplatin, carboplatin or oxaliplatin in plasma ultrafiltrate[20]. Using a simple 10-fold dilution as sample preparation, a sample volume of 150 µL, a LOQ of 0.0075 ng/mL could be achieved.

All the above-mentioned methods have the limitation of either lacking sensitivity, requiring large sample volumes or having time-consuming processing procedures and are most often reported for only a limited number of matrices. To the best of our knowledge, we are the first to provide a fully validated, highly sensitive, high throughput ICP-MS method to quantify the total Pt content in plasma ultrafiltrate, plasma, urine and peritoneal fluid. The aim of this work was to design and develop an analytical method which can be implemented in pharmacologic research and a case study regarding the intraperitoneal administration of oxaliplatin to a cancer patient is presented.

2. Materials and Methods

2.1 Safety Considerations

When working with chemotherapy, standard safety precautions were applied. These include wearing personal protective equipment (eye protection, protective gloves and protecting clothing) and using standardized handling procedures, including the use of BD PhaSeal™ closed system transfer devices (Dublin, Ireland), to minimize chemotherapy associated risks. All chemotherapy associated materials and patient samples were disposed in WIVA medical waste containers.

2.2 Materials

Oxaliplatin (Eloxatin®) (Figure 1), 5 mg/mL was purchased from Sanofi (Diegem, Belgium). Platinum standard solution, 1000 mg/L, was purchased from Merck KGaA (Darmstadt, Germany). The internal standard, terbium (Tb) 10 µg/mL in 2% nitric acid (HNO_3), was purchased from Perkin Elmer (Waltham, United States). The internal standard was chosen to

correct for potential drift of the ICP-MS instrument. HNO_3, 69.0 – 70%, was purchased from J.T. Baker (Deventer, The Netherlands). At all times, ultrapure water of 20 $M\Omega cm^{-1}$ was used in the preparations (arium®611UV, Sartorius, Gottingen, Germany). Drug-free human EDTA plasma, urine and peritoneal fluid were kindly provided by the Department of Laboratory Medicine at the Ziekenhuis Oost-Limburg (Genk, Belgium). Plasma ultrafiltrate was obtained by ultracentrifugation of plasma using the Microcon 30 kDa Centrifugal Filter Unit with Ultracel-30 Membrane (Merck Millipore Ltd., Tullagreen, Carrigtwohill Co., Cork, Ireland) at 14 000 x g for 25 minutes at room temperature. The cut-off of 30 kDa was selected because the main serum binding proteins of oxaliplatin are albumin (65-70 kDa) and gamma-globulines (1193 kDa)[8].

2.3 Calibration Standards

Pt calibration standards were prepared by serially diluting the Pt standard solution in 0.5% HNO_3, resulting in concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 50 and 100 ng/mL. Calibration standards were freshly prepared each day.

2.4 Validation Standards

A stock solution of oxaliplatin in water was available at a concentration of 5 mg/mL, equivalent to 2.454 mg/mL Pt, and was stored at room temperature in the dark. Working solutions containing 1.25, 125 and 375 µg/mL Pt were prepared by diluting the stock solution of oxaliplatin in ultrapure water. Quality control samples of Pt in matrix were prepared by 5-fold dilution of the working solutions in drug-free plasma, urine and peritoneal fluid to reach final Pt concentrations of 0.25, 25 and 75 µg/mL. Quality control samples of Pt in ultrafiltrate were prepared by ultracentrifugation of the spiked plasma samples. Working solutions and quality control samples were freshly prepared each day.

2.5 Sample Preparation

Plasma, urine, peritoneal fluid samples (100 µL) and ultrafiltrate samples (50 µL) were diluted 1000-fold with 0.5% HNO_3 and mixed thoroughly on a vortex-mixer prior to ICP-MS analysis. Automatic dilution of the internal standard resulted in a final concentration of 20 ng/mL Tb for each sample.

2.6 ICP-MS Analysis

The ICP-MS system is housed in a thermostatically regulated room (22°C) and consisted of a Perkin Elmer NexION 350S system equipped with the Syngistix software version 1.1. and an ESI PrepFast sample delivery system (500 µL sample volume) controlled by the ESI SC software version 2.9.0.202. The analytes, isotopes of Pt and Tb were monitored at *m/z* Pt 194

(Pt 193.963), Pt 195 (Pt 194.963) and Tb 159 (Tb 158.925). More operating details are summarized in Table 1.

2.7 Method Validation

The described ICP-MS method was validated for the following parameters according to the general accepted guidelines[21]:

2.7.1 Linearity

Linearity was assessed using the previously described calibration standards. The ratio of counts per second (cps) for the Pt isotopes and the internal standard isotope was plotted against the analyte concentration and fitted in a linear manner.

2.7.2 Limit of Detection

Limit of detection was defined as three times the standard deviation (SD) of ten consecutive measurements of drug-free samples of plasma, ultrafiltrate, urine and peritoneal fluid.

2.7.3 Limit of Quantification

LOQ was defined as ten times the concentration defined as LOD that can be measured with an acceptable precision and accuracy. Precision was expressed as the relative standard deviation (RSD%); calculated as ((SD/mean) x 100). The accuracy was expressed as the relative error (RE%); calculated as ((found concentration – theoretical concentration)/theoretical concentration) x 100). Acceptable precision and accuracy (n=10) was defined as RSD and RE $\leq$15% or $\leq$ 20% at the lowest level, i.e. the LOQ[19].

2.7.4 Precision and Accuracy

Interday (n=10) and intraday (n=10) precision and accuracy were evaluated at three different validation levels; 0.25, 25 and 75 µg/mL (before dilution). Acceptable precision and accuracy was defined as RSD and RE $\leq$15%.

2.7.5 Recovery

Recovery of Pt in plasma, ultrafiltrate, urine and peritoneal fluid was determined at three different validation levels by comparing the absolute cps of the Pt isotopes in the respective biofluids with the absolute cps of Pt isotopes in the corresponding Pt calibration standards. Working solutions containing 0.5, 50 and 500 µg/mL Pt were prepared by diluting the stock solution of oxaliplatin in ultrapure water. Quality control samples of Pt in matrix were prepared by 5-fold dilution of the working solutions in drug-free plasma, urine and peritoneal fluid to reach Pt concentrations of 0.1, 10 and 100 µg/mL Pt before 1000-fold dilution. For each concentration, three independent samples were analyzed.

2.7.6 Stability

The influence of storage conditions on the recovery of Pt in the different biofluids as compared to freshly prepared samples was assessed at three different validation levels, 0.25, 25 and 75 µg/mL Pt (before 1000-fold dilution). Short-term and long-term stability were evaluated by preserving the different samples at 4°C, -24°C and -80°C for 14 days and 5 months respectively. For each concentration, three independent samples were analyzed.

2.7.7 Robustness

Matrix effect was evaluated for the ICP-MS analysis of plasma. Working solutions containing 0.61 and 18.27 µg/mL (for 61-fold dilution), 5.02 and 150 µg/mL (for 500-fold dilution), 9.96 and 300 µg/mL (for 1000-fold dilution) Pt were prepared by diluting the stock solution of oxaliplatin in ultrapure water. Quality control samples of Pt were prepared by 5-fold dilution of the working solutions in drug-free plasma. During sample preparation, plasma (100 µL) was diluted 61-, 500- and 1000-fold respectively with 0.5% HNO_3, to reach final Pt concentrations of 2.0 and 60.0 ng/mL, and mixed thoroughly on a vortex-mixer prior to ICP-MS analysis. For each concentration and dilution factor, three independent samples were analyzed and accuracy and precision were determined.

2.8 Extent of Plasma Protein Binding

A timeline was created assessing the extent of oxaliplatin-derived Pt plasma protein binding at 37°C over a period of 45 hours. Working solutions containing 6.22 and 186.59 µg/mL Pt were prepared by diluting the stock solution of oxaliplatin in ultrapure water. Quality control samples of Pt were prepared in plasma by 51-fold dilution of the working solutions in plasma resulting in final Pt concentrations of 122 ng/mL and 3.66 µg/mL. At intervals, two aliquots plasma, 100 µL and 50 µL respectively, were sampled. Ultrafiltrate was obtained by plasma (100 µL) ultracentrifugation at 14 000 x g for 25 minutes at room temperature. During sample preparation, plasma (50 µL) and ultrafiltrate (50 µL) were diluted 61-fold (see Results and Discussion section Robustness) with 0.5% HNO_3, to reach final Pt concentrations of 1.99 and 60.0 ng/mL Pt. Sample were mixed thoroughly on a vortex-mixer prior to ICP-MS analysis. For each concentration, two independent samples were analyzed.

2.9 Case Study

The described ICP-MS method was implemented in a pharmacokinetic case study as part of a prospective randomized, non-blinded phase III clinical study, called the COBOX trial (NCT03028155). A patient diagnosed with peritoneal carcinomatosis from colorectal origin was treated with the cytoreductive surgery and hyperthermic intraperitoneal perioperative chemotherapy (HIPEC) combination therapy. During the 30-minute HIPEC, oxaliplatin was administered at a dose of 460 mg/m^2 in 0.9% saline carrier solution (2 L/m^2) at a target

temperature of 42°C. At each 5-minute time interval, plasma, peritoneal fluid and urine were sampled and processed as previously described. Plasma samples were ultracentrifugated within 45 minutes after sampling. Samples were stored at -80°C until day of analysis. The participant provided written informed consent. The study complies with the Declaration of Helsinki and the study protocol was approved by the local committee on human research.

3. Results and Discussion

All the obtained ICP-MS results were comparable for both the Pt isotopes, *m/z* Pt 194 and *m/z* Pt 195. Therefore, the results are presented for the most abundant Pt isotope, Pt 195.

3.1 Linearity

During optimization of the proposed ICP-MS method, the potential difference between calibration standards prepared with oxaliplatin or with the Pt standard was evaluated. ICP-MS analysis was performed for two Pt concentrations, 3.07 and 30.68 ng/mL and expressed in terms of recovery. With respect to the Pt calibration standards, the recoveries were 101 $\pm$ 1% for both concentrations. Regarding safety measures, minimizing exposure of laboratory staff to the chemotherapeutic agent, we therefore chose to proceed with the Pt calibration standards for the calibration curve throughout the validation. Linearity of the calibration curve was also compared between calibration standards (with the Pt standard) prepared in 0.5% HNO_3 or in the different biofluids, i.e. plasma, ultrafiltrate, urine and peritoneal fluid. We report that there is no difference between calibration standards prepared in 0.5% HNO_3 or in the biofluids, denoted by the same slope of the calibration curves, i.e. 0.011 ($y = 0.011x + 0.000$) with high correlation coefficients ($r^2>0.999$). We therefore proceeded with the use of calibration standards prepared with the Pt standard in 0.5% HNO_3 for the calibration curve. The described ICP-MS method was linear over a range of 0.01 – 100 ng/mL Pt. A typical calibration curve in 0.5% HNO_3 was $y = 0.002x + 0.000$ with a correlation coefficient (r^2) of 0.999973.

3.2 LOD

The LOD was 1.76 ng/mL Pt in plasma, 0.39 ng/mL Pt in ultrafiltrate, 0.29 ng/mL in urine and 0.30 ng/mL in peritoneal fluid.

3.3 LOQ

According to the theoretical definition of the LOQ, ten times the concentration defined as LOD, we evaluated the following Pt concentrations: 18 ng/mL in plasma, 4 ng/mL in ultrafiltrate, and 3 ng/mL in urine and peritoneal fluid for accuracy and precision. However, the RSD and RE for the ultrafiltrate, urine and peritoneal fluid analysis were not within the predefined 20% deviation of their expected values. Therefore, the theoretical LOQ concentrations were elevated with a factor 2 and evaluated for accuracy and precision. LOQ was 18.0 ng/mL Pt in

plasma (RSD: 4.24%, RE: -0.97%), 8.0 ng/mL Pt in ultrafiltrate (RSD: 7.03%, RE: -11.34%), 6.1 ng/mL Pt in urine (RSD: 9.96 %, RE: -3.56 %) and 6.1 ng/mL Pt in peritoneal fluid (RSD: 5.09%, RE: -1.19%). The LOQ for the reported ICP-MS method in plasma is significantly lower than the one achieved in the previously described ICP-MS method published by Morrison *et al*., e.g. 100 ng/mL. The LOQ reported in plasma UF by Morisson and colleagues is 1 ng/mL, which is lower than described by our ICP-MS method. This can potentially be contributed to the fact that they employed an 18-fold dilution in internal standard solution as sample preparation combined with the use of ultrasonic nebulization [19]. Brouwers *et al.* reported a lower LOQ of 0.0075 ng/mL in plasma UF for their ICP-MS method by using a 10-fold dilution with 1% HNO_3 [20]. Both publications demonstrate a lower LOQ for the ICP-MS analysis of Pt in plasma UF. However, we demonstrated in a case study (section 3.9) that our validated ICP-MS method is adequately sensitive to be used in a routine setting of total Pt quantification in pharmacologic research regarding the intraperitoneal administration of oxaliplatin to cancer patients.

3.4 Precision and Accuracy

The inter- and intraday precision and accuracy are summarized in Table 2. In summary, the inter- and intraday precision and accuracy were investigated at three different Pt levels, 0.25, 25 and 75 µg/mL (before 1000-fold dilution), in the biofluids. Both the RSD and RE are less than 5.46% for the ICP-MS analysis of plasma, urine and peritoneal fluid. The reported RSD and RE for the ultrafiltrate analysis are less than 3.70% and 14.36% respectively. The described RE value in ultrafiltrate is higher than the ones achieved for the other biofluids. This is because Pt, derived from oxaliplatin and its intermediates, binds rapidly and irreversible to plasma proteins such as albumin and gamma-globulins (see section 2.8)[22]. These Pt complexes are eliminated through ultracentrifugation, accounting for the loss of Pt in the ultrafiltrate and hence the higher RE. However, the RE is still within the predefined limit of 15%. Therefore, our results confirm that Pt can be determined accurate and precise in all biofluids.

3.5 Recovery

The recovery from plasma, ultrafiltrate, urine and peritoneal fluid is summarized in Table 3. In summary, the recovery was assessed at three different Pt levels; 0.1, 10 and 100 µg/mL (before 1000-fold dilution). The reported recoveries range from 85 – 103%. We can conclude that a 1000-fold dilution in 0.5% HNO_3 as sample preparation results in a limited to no loss of sample. Moreover, at this level of dilution, potential signal suppression due to the matrix, is eliminated.

3.6 Stability

The short-term and long-term stability at 4°C, -24°C and -80°C of Pt in plasma, ultrafiltrate, urine and peritoneal fluid is summarized in Table 4. Stability was assessed at three different Pt levels; 0.25, 25 and 75 µg/mL (before 1000-fold dilution), and expressed in terms of recovery. For all biofluids, Pt remains stable after 14 days of storage at all defined storage conditions, with a recovery ranging from 86 – 114%. As to long-term stability; when stored at 4°C, Pt stability is less predictable, denoted by the high variability of the mean recovery values and the reported SD of the analysis. Storage at -24°C results in a recovery ranging from 83 – 100% for all the biofluids except for peritoneal fluid, 57 – 89%. Long-term storage of the lowest validation level of peritoneal fluid at -80°C results in a recovery of 83%, which exceeds the acceptable 15% variation. Therefore, we confirm that long-term storage, up to 5 months, should be conducted at -80°C with extra caution for the peritoneal fluid samples that should be stored for a shorter time period. Freeze-thaw stability was not investigated as part of this validation study. In a pharmacologic/clinical setting, regarding the intraperitoneal administration of oxaliplatin to cancer patients, the biological samples are only thawed once before analysis and discarded afterwards. Therefore, freeze-thaw stability was not relevant for the particular application [23]. The described ICP-MS method is a high-throughput method, allowing the quantification of total Pt in about 170 samples per day. To avoid very long runs and long storage times at room temperature in the autosampler, samples were prepared and analyzed in batches of about 60, including calibration standards, quality controls and on average 49 samples. Analysis time for each sample is on average 106 seconds, resulting in a total batch analysis time of 1 hour and 50 minutes.

3.7 Robustness

Potential signal suppression due to matrix effect was evaluated for the ICP-MS analysis of plasma using a 61-, 500- and 1000-fold dilution in 0.5% HNO_3 as sample preparation (Table 5). In summary, matrix effect was assessed at two Pt levels, 1.99 ng/mL and 60.0 ng/mL, after dilution. Results confirm that the described dilution factors, employed during sample preparation, have no influence on both the RSD (≤1.97%) and RE (≤2.91%) of the Pt quantification in plasma.

3.8 Extent Plasma Protein Binding

Knowledge about the extent of Pt binding, derived from oxaliplatin and its intermediates, to plasma proteins is paramount in the process of sampling, subsequent sample processing and storage. Two timelines on two separate days were created to assess the extent of Pt binding to plasma proteins (Figure 2). Accounting for extreme Pt loss, a 61-fold dilution in 0.5% HNO_3 was employed during sample preparation instead of the earlier described 1000-fold dilution, to

ensure accurate and precise quantification of Pt. In a first timeline, Pt plasma protein binding was evaluated over a period of 6 hours and thereafter at 24 hours. In a second timeline, we evaluated Pt plasma protein binding in the first hour and subsequently over a period of 14 – 45 hours of incubation. In the common time periods, both timelines demonstrate similar recoveries for both the analysis of the total Pt content in plasma, as well the unbound Pt fraction in ultrafiltrate. This again emphasizes the quality and reproducibility of our validated ICP-MS method. The total Pt content in plasma remained stable over a period of 45 hours for the two Pt concentrations 122 ng/mL and 3.66 µg/mL, with an average recovery of 102 ± 4%. When oxaliplatin was incubated in plasma at 37°C for approximately 5 hours of incubation, 19 – 21% of total Pt was recovered in ultrafiltrate, which is in line with findings of Pendyala *et al.*[24] (12 – 15%). These results demonstrate the extensive and rapid binding of oxaliplatin-derived Pt to plasma proteins. From 5 hours incubation onwards, the recovery remained stable over a period of 17 hours. Furthermore, the extent of protein binding was independent on the Pt concentration (122 ng/mL and 3.66 µg/mL). Therefore, we can confirm that stabilization of the recovery is not due to saturable binding over the concentration range 122 ng/mL - 3.66 µg/mL Pt in plasma. In the last 23 hours of the timeline, a slight increase is observed in the recovery of Pt in ultrafiltrate. However, this increase is also observed in the analysis of plasma, and hence the total Pt content. A possible explanation could be the loss of plasma volume due to an incubation period of approximately 22 hours in a thermostatically regulated water bath at 37°C. This could account for the higher total Pt concentration found and therefore a higher recovery. These results confirm, that rapid ultracentrifugation of plasma after sampling is warranted to achieve accurate results, especially when conducting pharmacologic research where time-dependent samples are collected.

3.9 Case Study

The concentration versus time curve of the total Pt content in plasma, plasma ultrafiltrate, peritoneal fluid and urine is depicted in Figure 3. The total Pt content was quantified in the respective biofluids sampled at each 5-minute time interval during the HIPEC procedure. The peritoneal fluid concentration curve demonstrates the high initial concentration of Pt due to intraperitoneal administration of the chemotherapeutic agent and the subsequent decrease in Pt concentration due to uptake in the systemic circulation and surrounding tissues. The concentration in peritoneal fluid ranged from 45.53 – 85.79 µg/mL. The plasma concentration, 1 min after initial administration of oxaliplatin, was 0.61 µg/mL and increased to 4.32 µg/mL after the 30-minute HIPEC procedure. The plasma ultrafiltrate concentration ranged from 0.51 – 3.60 µg/mL. The plasma and ultrafiltrate levels of the drug represent the toxicity of the HIPEC treatment. Pt concentration in urine, representing the major excretion route, ranged from 0.01

– 41.10 µg/mL. These results confirm the applicability of the described ICP-MS method for the pharmacokinetic study with oxaliplatin-based HIPEC.

4. Conclusion

We have developed a fully validated, highly sensitive, high throughput ICP-MS method to quantify the total Pt content in plasma ultrafiltrate, plasma, urine and peritoneal fluid. Our ICP-MS method does not require expensive and time-consuming sample preparation methods. Sample preparation is confined to a 1000-fold dilution in 0.5% HNO_3. This implies that the ICP-MS analysis has become the rate-limiting step (17 samples per hour). The strong dilution also reduces the fouling of the ICP-MS instrument by the biological background and the need for maintenance interventions. Furthermore, we implemented a timeline assessing the total and unbound Pt fraction in plasma and ultrafiltrate over a period of 45 hours. In this way, an imperative estimation can be made of the percentage of unbound Pt that has been lost due to protein binding during the process from plasma sampling to ultracentrifugation and storage. Today, this ICP-MS method is applied successfully in both a preclinical and a clinical pharmacokinetic study of patients treated with oxaliplatin-based hyperthermic intraperitoneal perioperative chemotherapy, HIPEC (COBOX trial: NCT03028155). The outcome of these studies will be communicated on another occasion.

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6. Conflict of Interest

Conflicts of interest: none.

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Tables

Table 1 ICP-MS operating details

ICP-MS operating conditions	
Instrument	Perkin Elmer Nexion 350 S
Software	Synergistix version 1.1
Platinum isotope masses	193.963
	194.965
Terbium isotope mass	158.925
Sample delivery system	ESI PrepFast
Software	ESI SC version 2.9.0.202
Nebulizer	PFA ST Microflow
Type	Concentric
Spray Chamber	Quartz Cyclonic (Peltier cooled)
Injector	1.8 mm Sapphire
Plasma gas flow (L/min)	17.0
Auxiliary gas flow (L/min)	1.20
Nebulizer gas flow (L/min)	1.02
Collision cell	Standard mode
Detector mode	Multiplier (pulse count)
Acquisition mode	Peak hopping
Dwell time (ms)	100
Sweeps per sample	20

Measurement time (sec)	18
Read delay (sec)	40
Wash time (sec)	30
Wash solution	0.5% HNO_3
Replicates per sample	3
Parameter	Intensity ratio

496

497 **Table 2** Interday and intraday precision (RSD%) and accuracy (RE%) in plasma, ultrafiltrate, urine and
498 peritoneal fluid after dilution

	n=10	Validation parameter	Theoretical concentration (ng/mL Pt)		
			0.25	25	75
Plasma	Interday	Mean observed concentration (ng/mL Pt) ± SD	0.25 ± 0.01	24.25 ± 1.10	70.93 ± 1.88
		RSD (%)	5.34	4.54	2.65
		RE (%)	1.12	-3.16	-5.46
	Intraday	Mean observed concentration (ng/mL Pt) ± SD	0.25 ± 0.01	24.09 ± 0.46	71.88 ± 0.66
		RSD (%)	2.70	1.92	0.92
		RE (%)	-0.27	-3.79	-4.20
Ultrafiltrate	Interday	Mean observed concentration (ng/mL Pt) ± SD	0.23 ± 0.01	22.64 ± 0.55	67.37 ± 2.49
		RSD (%)	2.43	2.45	3.70
		RE (%)	-8.52	-9.60	-10.22
	Intraday	Mean observed concentration (ng/mL Pt) ± SD	0.22 ± 0.01	21.49 ± 0.22	64.26 ± 0.78
		RSD (%)	1.03	1.04	1.21
		RE (%)	-13.17	-14.20	-14.36
Urine	Interday	Mean observed concentration (ng/mL Pt) ± SD	0.25 ± 0.01	24.96 ± 0.33	72.78 ± 1.42
		RSD (%)	2.85	1.33	1.95
		RE (%)	1.47	-0.31	-3.00
	Intraday	Mean observed concentration (ng/mL Pt) ± SD	0.25 ± 0.01	24.77 ± 0.38	73.18 ± 1.11
		RSD (%)	1.73	1.55	1.52
		RE (%)	-0.80	-1.10	-2.47
Peritoneal fluid	Interday	Mean observed concentration (ng/mL Pt) ± SD	0.25 ± 0.01	25.05 ± 0.42	72.47 ± 2.23
		RSD (%)	2.91	1.70	3.08
		RE (%)	1.01	0.02	-3.41
	Intraday	Mean observed concentration (ng/mL Pt) ± SD	0.26 ± 0.01	24.72 ± 0.21	74.23 ± 0.66
		RSD (%)	2.52	0.84	0.89
		RE (%)	4.63	-1.30	-1.07

499

500 **Table 3** Recovery from plasma, ultrafiltrate, urine and peritoneal fluid after dilution[a]

	Theoretical concentration (ng/mL Pt)	Recovery (%) ± SD
Plasma	0.1	100.0 ± 1.3

	10	91.33 ± 1.92
	100	91.14 ± 1.94
Ultrafiltrate	0.1	86.12 ± 1.10
	10	84.53 ± 0.59
	100	86.16 ± 1.39
Urine	0.1	103.4 ± 0.5
	10	95.53 ± 1.08
	100	95.82 ± 2.06
Peritoneal Fluid	0.1	101.7 ± 1.2
	10	91.96 ± 0.96
	100	90.53 ± 3.88

[a]For each concentration, three independent samples were analyzed (n=3).

Table 4 Short- and long-term stability of Pt analysis in plasma, ultrafiltrate, urine and peritoneal fluid at 4°C, -24°C and -80°C after dilution[a]

		Temperature (°C)	Theoretical concentration (ng/mL Pt)		
			0.25	25	75
			Recovery (%) ± SD		
Plasma	Short-term (14 days)	4	85.91 ± 3.76	95.15 ± 4.31	85.65 ± 2.59
		-24	90.13 ± 2.44	95.55 ± 3.26	86.59 ± 2.29
		-80	85.73 ± 3.78	95.24 ± 0.42	87.24 ± 1.14
	Long-term (5 months)	4	102.6 ± 20.3	93.24 ± 7.72	97.69 ± 26.49
		-24	100.5 ± 7.9	88.88 ± 0.83	82.97 ± 1.60
		-80	99.16 ± 0.55	86.72 ± 2.00	92.52 ± 1.50
Ultrafiltrate	Short-term (14 days)	4	89.28 ± 0.72	98.46 ± 1.67	98.00 ± 1.10
		-24	106.4 ± 5.6	102.9 ± 0.3	103.1 ± 1.2
		-80	112.0 ± 1.7	104.3 ± 1.8	102.1 ± 0.5
	Long-term (5 months)	4	77.66 ± 1.10	88.70 ± 1.14	89.28 ± 0.79
		-24	89.41 ± 3.86	92.60 ± 1.00	89.93 ± 0.77
		-80	96.14 ± 3.30	97.80 ± 0.64	99.21 ± 3.22
Urine	Short-term (14 days)	4	114.0 ± 5.7	104.8 ± 5.8	99.09 ± 3.98
		-24	101.4 ± 1.2	102.5 ± 1.6	92.67 ± 4.73
		-80	111.2 ± 1.5	105.5 ± 2.3	102.3 ± 1.9
	Long-term (5 months)	4	169.9 ± 27.0	126.2 ± 6.4	122.7 ± 45.7
		-24	93.76 ± 2.05	89.24 ± 0.54	91.21 ± 0.11
		-80	96.16 ± 3.01	92.73 ± 1.21	94.33 ± 1.51
	Short-term (14 days)	4	99.34 ± 4.79	103.9 ± 3.4	110.5 ± 2.8

Peritoneal fluid	Long-term (5 months)		-24	97.92 ± 3.17	95.21 ± 5.51	101.8 ± 4.5
			-80	103.4 ± 2.4	107.3 ± 3.5	109 .1± 1.5
			4	48.57± 4.88	105.0 ± 26.2	111.5 ± 22.1
			-24	57.29 ± 0.92	83.90 ± 0.67	89.48 ± 0.27
			-80	83.45 ± 0.53	96.84 ± 0.74	97.60 ± 3.17

[a]For each concentration, three independent samples were analyzed (n=3).

Table 5 Matrix effect for the plasma ICP-MS analysis after 61-, 500- and 1000-fold dilution[a]

Theoretical concentration (ng/mL Pt)	n=3	Dilution factor in 0.5% HNO_3		
		61	500	1000
1.99	Mean observed concentration (ng/mL Pt) ± SD	1.99 ± 0.04	2.05 ± 0.03	2.05 ± 0.01
	RSD (%)	1.91	1.51	0.26
	RE (%)	-0.22	2.21	2.91
60.0	Mean observed concentration (ng/mL Pt) ± SD	58.50 ± 1.15	60.60 ± 0.07	60.47 ± 0.52
	RSD (%)	1.97	0.11	0.86
	RE (%)	-2.35	1.15	0.93

[a]For each concentration and dilution factor, three independent samples were analyzed (n=3).

Figure Captions

Fig 1 Chemical structure oxaliplatin

Fig 2 Recovery of the total Pt content in plasma and plasma ultrafiltrate. The graph represents the extent of oxaliplatin-derived Pt binding to plasma proteins at 37°C over a period of 45 hours. Pt is plasma represents the total Pt content, whereas Pt in ultrafiltrate represent the unbound Pt fraction. A: Recovery of Pt in plasma (purple: 2 ng/mL; green: 60 ng/mL) and ultrafiltrate (dark blue: 2 ng/mL; red: 60 ng/mL) during 6 hours of incubation and thereafter at 24 hours. B: Recovery of Pt in plasma (light blue: 2 ng/mL; orange: 60 ng/mL) and ultrafiltrate (grey: 2 ng/mL; yellow: 60 ng/mL) during 1 hour of incubation and subsequently over a period of 14 - 45 hours. Error bars represent standard deviations for two independent samples for each concentration (n=2).

Fig 3 Concentration versus time graph of intraperitoneal oxaliplatin during a 30-minute hyperthermic intraperitoneal perioperative chemotherapy procedure. At each 5-minute time interval, plasma (blue), plasma ultrafiltrate (orange), peritoneal fluid (grey) and urine (yellow)

were sampled. Concentration of Pt 195 ranged from 0.61 – 4.32 µg/mL in plasma; 0.51 – 3.60 µg/mL in plasma ultrafiltrate; 45.53 – 85.79 µg/mL in peritoneal fluid and 0.01 – 41.10 µg/mL in urine.