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# Determination of platinum originating from carboplatin in canine sebum and cerumen by inductively coupled plasma mass spectrometry

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ABSTRACT

We present highly sensitive, reliable methods for the determination of platinum originating from carboplatin in canine sebum and cerumen. The methods are based on the measurement of platinum by inductively coupled plasma mass spectrometry and allow quantification of 0.15 pg platinum per $cm^2$ body surface in canine sebum and of 7.50 pg platinum per sampled ear canal. The sample pretreatment procedure involved extraction of wipe samples followed by dilution with appropriate diluents. The performance of the methods, in terms of accuracy and precision, fulfilled the most recent FDA guidelines for bioanalytical method validation. Validated ranges of quantification were 15.0–1.00 × $10^4$ ng $L^{-1}$ for platinum in canine sebum extraction solution (corresponding to 15.0 pg per wipe sample or 0.15 pg $cm^{-2}$) and 7.50–1.00 × $10^4$ ng $L^{-1}$ for platinum in canine cerumen extraction solution (corresponding to 7.50 pg per sampled external acoustic meatus). Canine matrices may not always be obtained in sufficient quantities. Therefore, we also confirmed the legitimacy of the application of human matrix samples for the preparation of calibration standards and quality control samples as alternatives, to be used in future clinical studies. The assays are used to support human biomonitoring studies and pharmacokinetic oncology studies in pet dogs treated with carboplatin.



## 1. Introduction

Pt containing compounds are an important class of chemotherapeutics. Carboplatin (*cis*-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) is a second generation Pt containing compound (Fig. 1). Due to its less severe side effects as compared to cisplatin this compound is gaining popularity in veterinary oncology.

Several investigations have underlined the importance of skin contact as a route of exposure to hazardous substances, including antineoplastic drugs [1–3]. This has led to the development of guidelines for veterinary practices and owners, regarding the handling of pets treated with these drugs [4–6]. Questions are now arising about the justification of those guidelines and recommendations. Thus far it is not known which matrices should be of concern. Therefore, in addition to the assays we developed for the measurement of carboplatin in excretion products such as urine, faeces, and oral fluid, we now have developed assays to monitor the excretion of platinum originating from carboplatin in the less studied excretion products, sebum and cerumen, in dogs. Because contact with these excretion products is inevitable when handling animals, it is relevant to know the extent and duration of excretion of platinum originating from carboplatin in these matrices. To our knowledge, the excretion of Pt containing compounds in sebum and cerumen has never been monitored, neither in humans nor in companion animals.

As carboplatin is a hydrophilic compound, we can assume that excretion via sebum and cerumen will yield lower levels as compared to urinary and faecal excretion. Therefore, the use of an ultra sensitive analytical technique such as inductively coupled plasma mass spectrometry (ICPMS) is essential. ICPMS can be applied to a wide range of sample matrices including those of biological and environmental origin [7–12]. Moreover, sample pretreatment methods are usually straightforward and simple. For all these reasons usage of ICPMS and therefore monitoring of Pt originating from carboplatin seemed the most suitable approach.

In this article we describe the development and validation of ICPMS assays for platinum, according to most recent FDA guidelines on bioanalytical method validation [13].

The clinical applicability of the assays was demonstrated by analysis of samples obtained from a pet dog treated with carboplatin.

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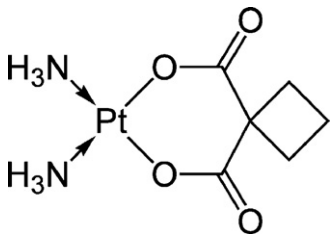

**Fig. 1.** Molecular formula of carboplatin ($M_w$ 371.249 g mol$^{-1}$).

## 2. Experimental

### 2.1. Chemicals

Carboplatin reference standard, used for preparation of calibration solution and quality control (QC) samples, was obtained from Calbiochem (San Diego, CA, USA). Iridium Chloride ICP standard, containing 1000 mg L$^{-1}$ iridium in 7% $HNO_3$, used for internal standardisation, was purchased from Merck (Darmstadt, Germany). Nitric acid ($HNO_3$) 70% and HCl 35% Ultrex II ultrapure reagent were obtained from Mallinckrodt Baker (Philipsburg, NJ, USA). Water used for the ICPMS analysis was sterile water for irrigation (Aqua B. Braun Medical, Melsungen, Germany). A multi-element solution containing 10.0 mg L$^{-1}$ of Ba, Be, Ce, Co, In, Mg, Pb, Th, Tl (VAR-TS-MS) in 5% $HNO_3$ was purchased from Inorganic Ventures/IV Labs (Lakewood, NJ, USA). Drug-free human sebum from healthy volunteers was used. Drug-free canine sebum and cerumen from healthy dogs was used. Van Linde Gas Benelux (Schiedam, The Netherlands) provided argon gas (4.6) of 99.996% purity.

### 2.2. Instrumentation

Analyses were performed on an ICP-quadrupole-mass spectrometer (Varian 810-MS) equipped with a 90° reflecting ion mirror (Varian, Mulgrave, Victoria, Australia). The sample introduction system consisted of a Micromist glass low flow nebuliser (sample uptake 0.12 mL min$^{-1}$), a peltier-cooled (4 °C) double pass glass spray chamber, a quartz torch, and a nickel sampler and skimmer cone (Varian). The spray chamber was cooled to reduce the vapour loading on the plasma, increasing the available energy for atomisation and ionisation of the elements of interest. Sample transport from the SPS-3 autosampler (Varian) to the nebuliser was perfomed using a peristaltic pump. The instrument was cooled by using a Kühlmobil 142 VD (Van der Heijden, Dörentrup, Germany). Data were acquired and processed using the ICPMS Expert Software version 1.1 b49 (Varian). Further data handling was performed using Excel 2003 (Microsoft, Redmond, WA, USA). All measurements were carried out in a dedicated temperature-controlled, positively pressurised environment in order to maintain optimum instrument performance and minimise contamination. All solutions were prepared using plastic pipettes (VWR International B.V., Amsterdam, The Netherlands) and 10 mL (Plastiques-Gosselin, Hazebrouck Cedex, France) and 30 mL (Sarstedt AG&Co., Nümbrecht, Germany) polypropylene tubes. Prior to method development, all sample pretreatment devices were checked thoroughly for Pt contamination and appeared to be suitable for Pt analyses.

### 2.3. Determination of Pt by ICPMS

To optimise the ICPMS signal for the mid range masses and to reduce the formation of oxides and doubly charged ions, a solution containing 5000 ng L$^{-1}$ of Th, In, Ce, Ba, and Pt was used. Typically this 5000 ng L$^{-1}$ solution gave readings of $^{115}$In: $2.40 \times 10^6$ counts per second (cps); $^{232}$Th: $5.80 \times 10^5$ cps and $^{194}$Pt: $1.60 \times 10^5$ cps. The production of $CeO^+$ was less than 1% of the total $Ce^+$ counts. The formation of doubly charged $Ba^{2+}$ was less than 2%. Instrument settings are summarised in Table 1. The performance was checked daily. Other than a daily torch alignment, there was no need to tune any of the other instrumental parameters. The conditions as depicted in Table 1 were kept constant and only replacement of consumable parts such as torch, nebuliser and cones required additional tuning of the instrument settings. Thus, the signals never deviated more than 15% of the values for In, Th, Pt, doubly charged, and oxides as mentioned above.

**Table 1**
ICPMS instrument settings.

| | |
|---|---|
| **Flow parameters** (L min$^{-1}$) | |
| Plasma flow | 18.0 |
| Auxiliary flow | 1.65 |
| Sheath gas | 0.25 |
| Nebuliser flow | 1.05 |
| **Torch alignment** (mm) | |
| Sampling depth | 5.00 |
| **Ion optics** (volts) | |
| First extraction lens | −12.0 |
| Second extraction lens | −220 |
| Third extraction lens | −230 |
| Corner lens | −240 |
| Mirror lens left | 37.0 |
| Mirror lens right | 35.0 |
| Mirror lens bottom | 20.0 |
| Entrance lens | 5.00 |
| Fringe bias | −3.00 |
| Entrance plate | −50.0 |
| Detector focus | −500 |
| Pole bias | 0.00 |
| **Other** | |
| RF power (kW) | 1.30 |
| Pump rate (mL min$^{-1}$) | 0.28 |
| Stabilization delay (s) | 40.0 |

For the detection of Pt, three isotopes $^{194}$Pt (abundance 33%), $^{195}$Pt (33.8%) and $^{196}$Pt (25.2%) were monitored [14]. All three monitored Pt isotopes can be subject to the interference of hafnium(Hf)-oxides [15]. However, because of low oxide formation and low observed Hf counts in all the analysed samples, these oxides were insignificant and no corrections were necessary. The interference of Hg on $^{196}$Pt was corrected on-line by monitoring $^{202}$Hg. In order to monitor unanticipated isobaric interferences, the $^{194}$Pt/$^{195}$Pt and $^{196}$Pt/$^{195}$Pt ratios were measured for all samples. When ratios were similar to those reported for natural Pt, it proved that the isotopic signals reflected the Pt content of the sample with no other spectral interference. The Pt isotope used for calculation of the validation parameters was $^{194}$Pt. The detection mode for all isotopes was based on peak jumping with peak dwell times of 968 ms, 25 scans per replicate and three replicates per sample. The total measurement time for one sample during validation procedures was 3.3 min.

Iridium (Ir) was used as internal standard. It is expected that, because of its similar mass and ionisation potential, the behaviour of Ir will accurately reflect that of Pt in a way that it will respond similar to matrix effects and possible plasma fluctuations. Internal standardisation was performed on each replicate using $^{191}$Ir. Quantification was based on the mean concentration of three replicates analysed against a calibration curve using weighted linear regression analysis. By using weight factors the calibration points with higher deviations will not have a major influence on the calibration curve function.

### 2.4. Assay development

#### 2.4.1. Wipe material and sampling method

Sebum sampling was performed by wiping a body surface area of 10 cm × 10 cm on both the coat and the skin of the sampled dog. All wipe samples were collected using a uniform sampling procedure by wiping the predefined body surface thrice. Cerumen sampling was performed by wiping the accessible part of the ear canal. We used Kimtech Science precision wipes (Kimberley-Clark Professional, Irving, TX, USA). Wipe samples were stored in 50 mL disposable polypropylene flasks (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA) at −20 °C until further processing.

#### 2.4.2. Extraction procedure

One percent HCl (v/v) was used as extraction solvent. Prior to analysis, 10 mL of extraction solvent was added to the sample and vessels were kept in an ultrasonic bath at 40 °C for 60 min. Then, samples were filtered (Sartorius minisart, Sarstedt, 0.2 μm) in order to remove particles which could block the nebuliser, or could interfere with the analysis. Afterwards Ir was added as internal standard (100 ng $L^{-1}$, 10 μL per mL sample). Two millilitres of sample were introduced directly into the ICPMS.

### 2.5. Validation

For canine sebum and cerumen, a partial validation was carried out. According to the FDA guidelines, a partial validation is sufficient to test the method, when a change in matrix with the same analyte is concerned or when a change in species with the same matrix and the same analyte is concerned. We have developed and validated a method earlier for the determination of carboplatin in surface samples by ICPMS [16]. A partial validation was performed for canine sebum and cerumen.

#### 2.5.1. Matrix effect and effectiveness of internal standardisation

In order to test the matrix effect of canine sebum and cerumen extraction solutions on the detector response and to validate the use of Ir as internal standard, the extraction solutions were diluted with 1% HCl to 0, 1, 10, 20, 50 and 100% extraction solution. These were then spiked with carboplatin in order to obtain solutions containing 50.0 ng $L^{-1}$ Pt. The signals at ($m/z$) of 194 were monitored. Pt concentrations were calculated against a calibration curve in 1% HCl using Ir as internal standard.

#### 2.5.2. Limit of quantification

The LLOQ was defined as the concentration at which the analyte response was at least five times the response of a blank wipe sample [13]. Furthermore, the LLOQ, when spiked on blank wipes, had to be determined with a precision less than 20% and the mean value was not allowed to deviate more than 20% of the actual value. The LLOQ was determined using six samples from individual batches of drug-free canine sebum and cerumen.

#### 2.5.3. Carry-over

To evaluate and minimise the effect of carry-over, we studied the signals of blank readings following the analysis of the ULOQ calibration sample ($1.00 \times 10^4$ ng $L^{-1}$ Pt in the canine sebum and cerumen extraction solutions) and we optimised the rinse-time.

#### 2.5.4. Linearity

A carboplatin stock solution containing 400 mg $L^{-1}$ Pt in water was prepared to obtain working solutions with concentrations ranging from 50.0 to $5.00 \times 10^3$ ng $L^{-1}$ Pt in water. For sebum samples, working solutions were diluted with 1% HCl (v/v) to obtain calibration standards ranging from 1.50 to 100 ng $L^{-1}$ Pt. For cerumen samples, working solutions were diluted with 1% HCl (v/v) to obtain calibration standards ranging from 0.75 to 100 ng $L^{-1}$ Pt. Calibration standards were injected directly into the ICPMS. An internal standard solution of $1.00 \times 10^4$ ng $L^{-1}$ Ir was prepared from an Ir reference solution of 1000 mg $L^{-1}$. Before analysis, 20 μL of Ir internal standard solution was added to 2 mL of each calibration standard (final internal standard concentration 100 ng $L^{-1}$). The seven non-zero calibration standards were processed and analysed in one analytical run. The calibrations were back-calculated from the responses. Deviations from the nominal concentration were evaluated.

The FDA guidelines require deviations of within ±20% for the LLOQ and ±15% for other concentrations [13].

#### 2.5.5. Precision

Quality control (QC) samples were prepared to obtain information on the recovery and precision of the extraction method and Pt analysis. Therefore, another carboplatin stock solution, prepared from a separate weighing, was diluted with water in order to obtain working solutions with Pt concentrations ranging from 500 to $5.00 \times 10^6$ ng $L^{-1}$. For drug-free human and canine sebum, the working solutions were further diluted to obtain spiking solutions with concentrations ranging from 30.0 to $2.00 \times 10^3$ ng $L^{-1}$. Tissues with drug-free human and canine sebum were spiked with these solutions serving as QC samples at the following concentration levels: $1.50 \times 10^{-2}$, $2.50 \times 10^{-2}$, 0.10, and 1.00 ng Pt on the tissues, corresponding to 1.50, 2.50, 10.0, and 100 ng $L^{-1}$ Pt in the final solution after extraction with 10 mL 1% HCl. For drug-free human sebum and canine cerumen, the working solutions were further diluted to obtain spiking solutions with concentrations ranging from 15.0 to $2.00 \times 10^3$ ng $L^{-1}$. Tissues with drug-free human sebum and canine cerumen were spiked with these solutions serving as QC samples at the following concentration levels: $0.75 \times 10^{-2}$, $2.50 \times 10^{-2}$, 0.10, and 1.00 ng Pt on the tissues, corresponding to 0.75, 2.50, 10.0, and 100 ng $L^{-1}$ Pt in the final solution after extraction with 10 mL 1% HCl. These tissues were processed as described earlier. To 2 mL diluted QC sample, 20 μL of internal standard solution was added (final internal standard concentration 100 ng $L^{-1}$). Five replicates of each sample were analysed in one analytical run. Accuracy was expressed as a percentage of the nominal concentration and it had to be within 80–120% for the LLOQ and within 85–115% for the other concentrations. Within-run precisions were calculated by analysis of variances (ANOVA) for each test concentration using the analytical run as the grouping variable. The precision should not exceed ±20% for the LLOQ and ±15% for the other concentrations [13].

#### 2.5.6. Inter-exchangeability of human and canine matrices

Canine sebum and cerumen cannot be collected in large quantities. On the other hand, human sebum can be more easily obtained. For future clinical studies, it would be more convenient to prepare quality control samples using the human alternative. Therefore, we assessed the legitimacy of using human sebum for the preparation of QC samples as a substitute for canine sebum. The collection of human cerumen samples is subjected to the same limitations as the collection of canine cerumen samples. As sebum and cerumen share most of their constituents [17–20], we evaluated the inter-exchangeability of canine cerumen QC samples with human sebum QC samples instead. Calibration standards were prepared using 1% HCl. Both human and canine QC samples were measured using this calibration curve.

#### 2.5.7. Selectivity

From six individual batches of drug-free canine sebum wipe tissues, samples containing neither analyte nor internal standard (blank), and samples containing 1.50 ng $L^{-1}$ Pt and 100 ng $L^{-1}$ internal standard were prepared and injected directly into the ICPMS. The same procedure was followed for drug-free canine cerumen

wipe tissue. These were spiked with carboplatin in order to obtain concentrations of 0.75 ng $L^{-1}$ Pt and were injected directly into the ICPMS. These samples were prepared to determine whether endogenous compounds interfered at the masses selected for Pt or internal standard. All samples were analysed in one analytical run. The signal of any interfering peak at $m/z$ 194 in blank solutions was not allowed to exceed 20% of the response of the LLOQ standard. The response of any interfering peak at $m/z$ 191 in the blank solution should not exceed 5% of the response of 100 ng $L^{-1}$ internal standard. Accuracies of the samples spiked with Pt at the LLOQ standard level had to be within 80–120% of the nominal value [13].

#### 2.5.8. Cross analyte/internal standard interference test

Interference of the internal standard solution on the $m/z$ 194 and interference of the carboplatin solution on $m/z$ 191 had to be assessed. Drug-free canine sebum and cerumen wipe tissues were spiked with carboplatin at ULOQ standard level and the Ir signal at $m/z$ 191 was monitored. The response of the interfering peak at $m/z$ 191 should be less than 5% of the response of 100 ng $L^{-1}$ internal standard [13].

Drug-free canine sebum and cerumen wipe samples were prepared and spiked with Ir in order to obtain a concentration of 100 ng $L^{-1}$. The response of the interfering peak at $m/z$ 194 should be less than 20% of the response of the LLOQ standard [13].

### 2.6. Application of ICPMS assay

The described analytical methods are used to support clinical pharmacokinetic studies to monitor Pt originating from carboplatin in canine sebum and cerumen. An example of the analysis of sebum wipe samples from the coat and skin, and cerumen wipe samples of a pet dog treated with carboplatin, 300 mg/$m^2$ as a 30 min intravenous infusion, is given here. The samples were collected on the day of administration until day 3 postinfusion and processed according to the methods described above.

## 3. Results and discussion

### 3.1. Validation

#### 3.1.1. Matrix effect and effectiveness of internal standardisation

Some signal suppression occurs with increasing canine sebum extraction solution concentration (see Fig. 2). Compared to 1% HCl, an extract of 10 mL of canine sebum caused an ion suppression of 10%. The internal standard corrected well for this matrix effect.

Increasing canine cerumen extraction solution caused a minor signal enhancement (see Fig. 2). Compared to 1% HCl, an extract of 10 mL of canine cerumen caused a signal enhancement of 1.5%. Again, the internal standard corrected well for this matrix effect.

#### 3.1.2. Limit of quantification

For canine sebum, the LLOQ of the assay was set at a Pt concentration of 1.50 ng $L^{-1}$ in 1% HCl, corresponding to 15.0 pg per wipe sample or 0.15 pg $cm^{-2}$ taking into account a body surface of 10 cm × 10 cm. For canine cerumen, the LLOQ of the assay was set at a Pt concentration of 0.75 ng $L^{-1}$ in 1% HCl, corresponding to 7.50 pg per wipe sample or per sampled acoustic meatus.

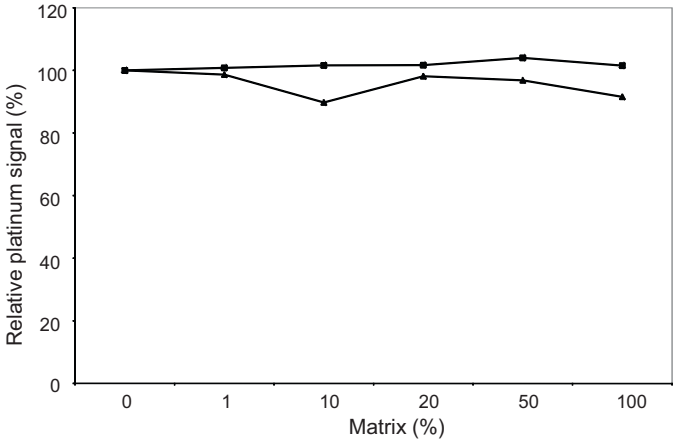


**Fig. 2.** Matrix effect of canine sebum (▲) and cerumen (■) extraction solution. The left $y$-axis depicts $^{194}$Pt signals relative to the signal of Pt in 1% HCl.

Signal-to-noise (S/N) ratios were dependent on the batches of the drug-free matrices used. S/N ratios at the LLOQ level exceeded 5 during all experiments, which was in accordance with the requirement [13]. The acceptance criteria were easily met.

The lower S/N ratio observed in cerumen wipe samples is probably due to the environment created by the acoustic meatus that shields the ear canal from external influences and thereby, Pt contamination.

#### 3.1.3. Carry-over

A 55-s rinse time with 1% $HNO_3$ between two samples was required to avoid a memory effect from the preceding high concentration sample and to achieve a blank signal <20% of the LLOQ standard signal [13].

#### 3.1.4. Linearity

In Table 2, deviations for 1% HCl are presented. Deviations from the nominal concentration were between −3.48 and 4.14% for all concentration levels, which all met the requirements of within ±20% for the LLOQ and ±15% for other concentrations [13]. Correlation coefficients were higher than >0.9998.

#### 3.1.5. Accuracy and precision

Accuracy and precision data are summarised in Table 3. It can be seen from Table 3 that, at all QC concentration levels, the data were within the limits for bioanalytical method validation [13]. The data summarised in Table 3 shows that the canine and human QC samples are all inter-exchangeable. In future clinical studies, human

**Table 2**
Deviation from theoretical concentration (DEV %) for carboplatin standards in 1% HCl.

| Matrix | Nominal Pt concentration (ng $L^{-1}$) | Pt concentration in final matrix after 1:100 dilution (ng $L^{-1}$) | Mean Pt concentration back calculated (ng $L^{-1}$) | DEV (%) from nominal concentration |
|---|---|---|---|---|
| 1% HCl (used in the validation of canine sebum and cerumen) | 150 | 1.50 | 1.51 | 0.87 |
| | 500 | 5.00 | 5.21 | 4.14 |
| | $1.00 \times 10^3$ | 10.0 | 9.65 | −3.48 |
| | $2.50 \times 10^3$ | 25.0 | 24.3 | −2.75 |
| | $5.00 \times 10^3$ | 50.0 | 48.6 | −2.91 |
| | $7.50 \times 10^3$ | 75.0 | 73.2 | −2.46 |
| | $1.00 \times 10^4$ | 100 | 99.6 | −0.36 |

A partial validation was performed for sebum and cerumen. Therefore no relative standard deviations for carboplatin standards was calculated.

**Table 3**
Mean, within run precision and accuracy for each quality control sample in human and canine biological matrix ($n = 5$).

| Matrix | Nominal Pt concentration in matrix (ng $L^{-1}$) | Mean Pt concentration in canine matrix | Mean Pt concentration in human matrix | Mean canine vs human matrix[b] (%) | Within run precision canine matrix[c] (%) | Within run precision human matrix[c] (%) | Accuracy canine matrix (%) | Accuracy human matrix (%) |
|---|---|---|---|---|---|---|---|---|
| Sebum | 30.0 | 31.7 | 29.74 | 6.73 | 10.9 | 5.21 | 106 | 99.2 |
| | 50.0 | 51.6 | 50.10 | 2.96 | 2.10 | 6.69 | 103 | 100 |
| | 200 | 208 | 189 | 9.08 | 11.1 | 1.81 | 104 | 94.6 |
| | $2.00 \times 10^3$ | $1.91 \times 10^3$ | $1.87 \times 10^3$ | 2.01 | 1.71 | 0.43 | 95.5 | 93.5 |
| Cerumen[a] | 15.0 | 16.3 | 17.4 | 6.38 | 2.77 | 8.34 | 109 | 116 |
| | 50.0 | 54.5 | 55.4 | 1.86 | 4.36 | 8.35 | 109 | 111 |
| | 200 | 212 | 192 | 9.47 | 2.37 | 3.84 | 106 | 96.1 |
| | $2.00 \times 10^3$ | $2.15 \times 10^3$ | $1.9 \times 10^3$ | 11.7 | 1.18 | 2.12 | 108 | 95.1 |

A partial validation was performed for canine sebum and cerumen. Therefore no between-run precision was calculated.
[a] Human sebum quality control samples were used.
[b] Means were compared using the following formula: $(x - y)/y \times 100$, with $x$ = mean human matrix and $y$ = mean canine matrix.
[c] Within run precisions were calculated using: SD/mean × 100.

sebum can be used to prepare QC samples as an alternative for canine sebum and cerumen.

### 3.1.6. Selectivity

Blank samples from six individual batches did not show interferences from endogenous material at the $m/z$ selected for Pt with a response >20% of the LLOQ standard signal. The response of interfering peaks at $m/z$ 191 did not exceed 5% of the response of 100 ng $L^{-1}$ internal standard. Deviations from the nominal concentrations at the LLOQ level were between 8.15 and 11.3% (data not shown).

### 3.1.7. Cross analyte/internal standard interference test

The response of the interfering peak at $m/z$ 194 was less than the maximum allowed 20% of the response of the LLOQ standard.

### 3.2. Application of ICPMS assay

The clinical applicability of the described assays was demonstrated by analysis of canine sebum and cerumen wipe samples obtained from a pet dog treated with carboplatin. A Pt versus time profile for Pt in canine sebum and cerumen is presented in Fig. 3.

The results show that Pt can be found in canine sebum and cerumen at concentrations above the LLOQ. However, these results are only preliminarily and should be confirmed in future studies.

The presence of Pt in sebum samples might partly be explained by external contamination of the skin and the coat of the pet dog via its surroundings or via contact with its urine and saliva. However, this cannot explain the presence of Pt in cerumen. We may assume that the ear canal shields the cerumen from external contamination. As cerumen samples also contained measurable Pt levels, the excretion of Pt in cerumen and, probably, in sebum seems plausible. Either way, Pt at concentrations above the LLOQ of the described methods can be found in sebum and cerumen of a client-owned pet dog treated with carboplatin. Therefore, handling and caressing the animal may lead to exposure of veterinary personnel, owners and others in close contact with treated animals.

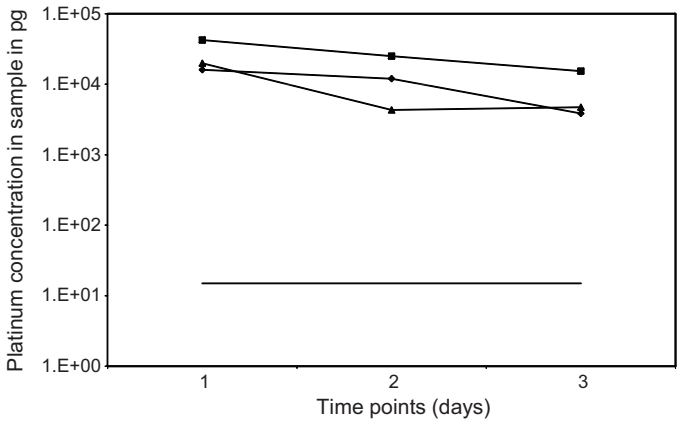


**Fig. 3.** A Pt concentration versus time plot in canine sebum (of the coat: ♦; of the skin: ■) and cerumen (▲) of a pet dog treated with carboplatin (300 mg/$m^2$). The LLOQ of the sebum assay (0.15 pg $cm^{-2}$ Pt body surface) is delineated in the figure (–).

Pt concentrations in cerumen and sebum were substantially lower than Pt concentrations in urine, faeces and saliva of the same pet dog (data not shown). Whether these amounts represent a health risk for people remains a question to be answered.

## 4. Conclusion

Highly sensitive ICPMS assays for the reliable and fast determination of Pt originating from carboplatin in canine sebum and cerumen, were developed. Subsequently, the assays were validated according to current FDA guidelines. The validated range was from 15.0 to $1.00 \times 10^4$ ng $L^{-1}$ in canine sebum extraction solution and 7.50 to $1.00 \times 10^4$ ng $L^{-1}$ Pt in canine cerumen extraction solution, corresponding to 0.15 pg $cm^{-2}$ Pt body surface and 7.50 pg Pt peracoustic meatus, respectively. The assay is now successfully applied to support pharmacokinetic studies of patients treated with carboplatin.

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