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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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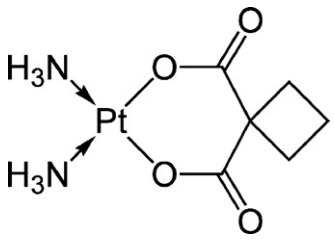

Fig. 1. Molecular formula of carboplatin (M_w 371.249 g mol^{-1}).

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: 2.40×10^6 counts per second (cps); ^{232}Th: 5.80×10^5 cps and ^{194}Pt: 1.60×10^5 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.