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Determination of platinum originated from antitumoral drugs in human urine by atomic absorption spectrometric methods

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ABSTRACT

Cisplatin and carboplatin are the most common platinum-based drugs used in cancer treatment. Pharmacokinetic investigations, the evaluation of the body burden during the treatment, as well as baseline levels of platinum in humans have attracted great interest. Thus, accurate analytical methods for fast and easy Pt monitoring in clinical samples become necessary. In the present study atomic absorption spectrometric methods for the determination of platinum in the forms of cisplatin and carboplatin in human urine were investigated. Platinum, in these different forms, could be determined in urine, after simple sample dilution. Regarding electrothermal atomic absorption spectrometry, the optimum parameters were defined by a central composite design optimization. Multiplicative matrix effects were overcome by using a mixture of HCl and NaCl as modifier. The limit of detection (LOD) was 0.004 mg L^{-1} of platinum in the original sample. For the analysis of more concentrated samples, high resolution continuous source flame atomic absorption spectrometry was also investigated. Flame conditions were optimized by a multivariate D-optimal design, using as response the sum of the analyte addition calibration slopes and their standard deviations. Matrix matched external calibration with $PtCl_2$ calibration solutions, was possible, and the LOD was 0.06 mg L^{-1} in the original sample. The results obtained by the proposed procedures were also in good agreement with those obtained by an independent comparative procedure.

1. Introduction

In 1977, Einhorn et al. [1] described the use of platinum-based drugs in the treatment of testicular cancer. According to this study, 80% of patients reached complete remission and 20% a partial cancer remission. In 1978, cisplatin was officially approved for clinical use by the FDA (Food and Drug Administration). Today, cisplatin is the most utilized antitumoral drug in the world, and is effective in the treatment of testicular and ovarian cancer. In addition, it also contributes in the treatment of other kinds of malignant cancers, such as oropharyngeal carcinoma, cervical carcinoma, lymphoma, osteocarcinoma, melanoma and neuroblastoma. Despite its continuous use, chemotherapy with cisplatin presents several disadvantages, including nephrotoxicity and neurotoxicity, and some tumors present a natural resistance to this drug [2]. Ever since its development, different cisplatin analogues have been synthesized and experimentally tested for verification and study of their toxicity and potential use for clinical treatment of tumor cells, originating several new and diverse substances, such as carboplatin, oxaliplatin and nedaplatin [3,4].

The accumulation, distribution and biotransformation of platinum compounds in the body are determinant factors in the physiological behavior of these drugs. Thus, accurate determination of low platinum levels in clinical samples is a prerequisite in order to understand the pharmacokinetics, pharmacodynamics and metabolism of platinum drugs, and the reliable determination of the concentration of platinum in these samples can play an important role in monitoring treatments, as well as in the development of new drugs. Even though some platinum derivatives can act as chemotherapeutic agents in the treatment of certain tumors, these and other compounds are toxic and even carcinogenic, hence the need for assessment of human exposure to these species, both occupationally and environmentally. Such studies involve the assessment and identification of biological indicators, and also the need for simple, fast and reliable analytical methods for monitoring platinum in fluids and tissues samples [5]. Platinum determination in urine has been used in several studies regarding occupational exposure, and high concentrations of this metal have been observed

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[6]. Although concentrations in both urine and blood provide an evaluation of platinum exposure, the former presents some advantages, such as easy sampling and storage and requiring less effort in the pre-treatment step [6,7]. In general, pre-treatment is an important step in the determination of platinum in clinical samples. In the case of biological fluids, usually a simple sample dilution with HNO_3 1–2% (v/v) is enough, decreasing the dissolved solid concentrations to the necessary level. In some cases, however, sample digestion is reported as necessary [8,9]. Messerschmidt et al. [10] performed bodily fluids decomposition (blood, plasma and urine) with nitric, chloridric and perchloric acid in a high pressure digestor, with a gradual temperature increase until 320 °C and posterior detection by adsorptive voltammetric procedures (AV). The dry digestion of the sample, at 500 °C for 24 h, followed by residue dissolution with *aqua regia* or nitric acid was used in the determination of platinum in serum and urine by electrothermal atomic absorption spectrometry (ET AAS) [11]. Nygren et al. [12] described a method for the determination of platinum in blood. These authors used dry ashing of blood samples in a muffle furnace, and the determination of platinum was performed by AV, ET AAS and ICP-MS (inductively coupled plasma-mass spectrometry). Microwave assisted digestion has also been used to mineralize human blood samples, and platinum concentrations have been determined by inductively coupled plasma optical emission spectrometry (ICP OES) [13] and ICP-MS [14,15]. Indeed, due to their sensitivities, plasma techniques are the most used atomic spectrometric methods for platinum determination in body fluids [15–22]. Casetta et al. [23] developed a method for determining platinum in biological fluids from patients treated with cisplatin by ICP-MS, and the results obtained in this study showed agreement with those obtained by ET AAS. An inductively coupled plasma-mass spectrometer (ICP-MS) method for the determination of Pt in biological fluids (plasma, ultrafiltrate and urine) of patients treated with antitumor agents has been developed and validated by Bettinelli [24]. The limits of quantification (LOQ) in the three matrices were 1.0, 0.1, and 2.0 $\mu g\,L^{-1}$, respectively. In spite of their inherent advantages, plasma instrumentations are still expensive to purchase and to maintain, especially if only one element is to be determined at a time. This explains why atomic absorption spectrometry (AAS) has maintained its place among the spectrometric trace analysis methods, since these techniques show a good compromise between adequate limits of detection and costs, especially in monoelemental analysis. Thus, AAS is widely used for the determination of trace metals in biological samples, and has also been applied in the determination of platinum in clinical samples [25–32]. ET AAS, due to its high sensitivity and low limits of detection is particularly suitable for the analysis of clinical samples. Moreover, since part of the matrix is eliminated along the temperature program, sample pre-treatment can be simplified (usually, only a dilution step), minimizing contamination risks or losses, as can the pre-instrumental work [33]. Thus, Vouillamoz-Lorenz et al. [25] developed and validated a method for the determination of platinum in human plasma, plasma ultrafitrate and urine from cancer patients in treatment with the platinum derivative JM216, by ET AAS. Urine samples were diluted with 10% (v/v) HCl prior to the ET AAS analysis. For plasma and ultrafitrate samples, no sample pre-treatment was necessary. Warmerdan et al. [28] also developed a method for the determination of platinum in plasma, plasma ultrafiltrate, saliva and urine of patients receiving carboplatin, by ET AAS and, in this case, the pre-treatment of the samples involved a simple dilution with a solution containing 0.15 mol L^{-1} NaCl and 0.20 mol L^{-1} HCl. Thotill et al. [34] compared the results of platinum determinations in plasma, tissues and animals bones by ET AAS and ICP-MS, and concluded that both methods showed good agreement. In a variant of the AAS detection, Aucélio et al. [35] developed a graphite furnace technique for the determination of ultra-trace amounts of platinum in biological and environmental samples, based on laser-excited fluorescence spectrometry (ETA-LEAFS). A high repetition rate copper vapor laser was employed as a dye laser pump in order to probe the platinum atoms generated in the graphite furnace more efficiently. The absolute limit of detection was 50 fg, based on the fluorescence values of aqueous standard solutions. On the other hand, flame atomic absorption spectrometry (F AAS) although simple to use and relatively accessible, is not sensitive enough for the determination of low levels of platinum commonly present in these samples [25]. However, high resolution continuous source flame atomic absorption spectrometry (HR-CS F AAS) is a new instrumental concept that surpasses the usual limitations of conventional atomic absorption spectrometers with line sources, and presents several advantages, such as improved accuracy and limits of detection due to higher signal to noise ratio, the visibility of the spectral region around the analytical line and a simultaneous background correction [36,37].

Thus, the objective of the present work is to describe a development of analytical methodologies for the determination of platinum in human urine samples using atomic absorption spectrometric techniques. We aimed to use external calibration and platinum inorganic salts in the preparation of the calibration solutions, avoiding intensive sample pre-treatment, appropriate for the various platinum derivatives used in chemotherapy treatment, as well as providing adequate detection limits.

Table 1
Optimized temperature program for the determination of Pt in urine by ET AAS.

Step	Temperature (°C)	Ramp (°C s^{-1})	Hold (s)	Gas flow
Drying	90	20	10	Max
Drying	120	15	15	Max
Pyrolysis	900	100	10	Max
Pyrolysis	1600	200	10	Max
AZ	1600	0	6	Stop
Atomization	2480	1800	5	Stop
Cleanout	2600	1000	3	Max

Injected volume: 20 μL.

2. Experimental

2.1. Instrumentation

The instrumental measurements were performed in two instruments: a continuum source atomic absorption spectrometer model ContrAA 300 (Analytik Jena, Jena, Germany) with a flame atomization system, equipped with a xenon short-arc lamp (as the continuum radiation source), an Echelle double monochromator and a CCD line detector and a model ZEEnit 60 atomic absorption spectrometer (Analytik Jena, Jena, Germany), equipped with a transversally heated graphite atomizer with a transverse Zeeman-effect based background correction system and an AS-52 autosampler. For the continuum source spectrometer, the analytical wavelength line was 265.945 nm.The equipment permits a simultaneous evaluation of 200 pixels, which corresponds to a spectral environment of approximately ±0.2 nm around the central pixel. For the measurements, 5 pixels were used: the central one and the 4 closest neighbors. An air–acetylene flame was used and the reading time was of 10 s. For the line source spectrometer, the spectral band pass was fixed at 0.2 nm, the hollow cathode lamp operated at 10 mA and the wavelength fixed at 265.9 nm. Pyrolytically coated graphite tubes with pin platforms (Analytik Jena, Part no. 407-152.314) were used. All measurements were made in integrated absorbance, using 4 s for the integration time. Argon (99.99%, Linde, Rio de Janeiro, Brazil) was used as the protective and carrier gas. The graphite furnace temperature program used for the platinum determinations is shown in Table 1. The data were analyzed using the Statistica 7.0 Statsoft software. In the comparative pro-