Talanta 82 (2010) 1647–1653

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

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

Anilton Coelho da Costa Júnior[a], Mariana Antunes Vieira[b,*], Aderval Severino Luna[c], Reinaldo Calixto de Campos[a,d]

[a] Pontifícia Universidade Católica do Rio de Janeiro (PUC-Rio), Departamento de Química, Rio de Janeiro, RJ, Brazil
[b] Universidade Federal de Pelotas(UFPEL), Departamento de Química Analítica e Inorgânica, Capão do Leão, RS, Brazil
[c] Universidade do Estado do Rio de Janeiro (UERJ) – Instituto de Química, Rio de Janeiro, RJ, Brazil
[d] Instituto Nacional de Ciência e Tecnologia de Bioanalítica/CNPq, Brazil

ARTICLE INFO

Article history:
Received 19 April 2010
Received in revised form 12 July 2010
Accepted 15 July 2010
Available online 21 July 2010

Keywords:
Human urine
Platinum
Cisplatinum
Carboplatinum
HR-CS F AAS
ET AAS

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

* Corresponding author. Fax: +55 21 3114 1309.
E-mail address: maryanavieira@hotmail.com (M.A. Vieira).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.07.029

[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 pretreatment 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].

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.

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.

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-

cedure, microwave assisted digestion was carried out in a closed system (Provecto Analítica, São Paulo, Brazil).

2.2. Materials, reagents, solutions

All reagents were of analytical reagent grade. Ultrapure water, obtained from a Gehaka Master System apparatus (Gehaka, São Paulo, Brazil) was used throughout the experiments. Analytical grade HNO_3 (65%, m/v, specific mass 1.39 g/cm^3) and HCl (36.5%, m/v, specific mass 1.19 g/cm^3), both from Vetec (Rio de Janeiro, Brazil) were purified by sub-boiling distillation using a Teflon® still (Hans Kürner Analysentechnik, Rosenheim, Germany). The 1000 μg mL^{-1} Pt stock solution was prepared by the adequate dilution of a Titrisol concentrate (Merck, Darmstadt, Germany) with 0.2% (v/v) HNO_3. Aqueous calibration solutions were prepared by further dilution of the Pt stock solution with 0.2% (v/v) HNO_3. Pharmaceutical stock solutions of cisplatin (500 mg L^{-1}) and carboplatin (10,000 mg L^{-1}) both from Chiral (Juiz de Fora, Brazil) were also used, as well as Triton X-100 (Merck, Darmstadt, Germany). All plastic and glassware was washed with tap water, immersed in Extran (48 h), rinsed with tap and deionized water, and immersed in 20% (v/v) HNO_3 for at least 24 h. Before use these materials were thoroughly rinsed with ultrapure water.

2.3. Samples

Samples from healthy individuals not subjected to treatment with platinum drugs were collected in polypropylene tubes (50 mL) and stored at 4 °C until analysis. The time between collection and analysis was of up to 7 days. These samples were collected from individuals of different ages and sex, aiming to characterize the different variations to be found in the matrices in the case of a toxicological study. Urine samples from a patient submitted to the chemotherapeutic treatment with cisplatin were collected in sterile plastic bottles and stored at −4 °C, since a longer storage time was necessary. They were collected within 48 h after the drug intake.

2.4. Comparative procedure

The comparative procedure consisted in a microwave assisted digestion of 2 mL of urine samples, in closed Teflon® tubes with 1 mL of 63% (v/v) HNO_3 and 1 mL of 80% (v/v) H_2O_2. The heating program was as follows: 300 W (5 min); 500 W (10 min); 600 W (5 min); and 0 W (10 min, cooling). The volume was made up to 5 mL with 0.2% (v/v) HCl solution. The platinum content was determined by ET AAS according to the temperature program shown in Table 1, but with a pyrolysis temperature of 1300 °C and atomization temperature, ramp and hold of 2300 °C, 1400 °C s^{-1} and 8 s, respectively. Since in this comparative procedure the samples were previously digested, a lower pyrolysis temperature was sufficient for an efficient pyrolysis step. External calibration was conducted with calibration solutions prepared with the inorganic platinum salt ($PtCl_2$).

3. Results and discussion

3.1. Determination of platinum in urine by HR-CS F AAS

Urine samples from patients submitted to chemotherapy with platinum based drugs may present platinum concentration at the mg L^{-1} level [38]. Thus, this part of the present study explores the possibility of determining platinum in these samples by HR-CS F AAS, which provides appropriate detection limits for this concentration range.

3.1.1. Dilution ratio

A preliminary study has shown the need of sample dilution to prevent the formation of solids in the slot of the burner head. No solid was formed after only a 1 + 1 dilution of the urine samples with a 1% (v/v) HCl solution. Thus, aiming at minimizing the dilution in order to avoid the excessive impoverishment of the detection limit, this dilution ratio was adopted.

3.1.2. Diluent nature and concentration

It is known that the diluent nature and concentration may influence the AAS response. At first, the use of 1% (v/v) HCl was investigated. Analyte addition curves in 1 + 1 diluted samples ($n = 5$) as well as an external calibration curve in the same medium, using $PtCl_2$, were performed and compared. The results obtained in this step demonstrated that the sensitivity observed in the aqueous solution was significantly lower than that observed in urine. Furthermore, the sensitivities observed for the analyte addition curves were different for each of the specific urine samples, which would lead to the conclusion that calibration by analyte addition should be used. However, with the increase of the HCl concentration to 2% (v/v), not only was the sensitivity increased in the urine analyte addition curves, but also the curve slopes were closer to each other. Thus, all further tests were performed using 2% (v/v) HCl as diluent, at a 1 + 1 dilution ratio.

3.1.3. Flame atomization conditions – multivariate optimization

In the present case, the variables taken into consideration were the flame stoichiometry (controlled by acetylene flow) and the local flame composition (defined by the observation height). Three levels of each variable were selected, and a factorial experiment with two replicates was performed. The planning matrix for the first experiment round is shown in Table 2, where the real values and coded levels are displayed. A first-order model was initially assumed as an adequate approximation of the relationship between the investigated response and the factors to be optimized. This is truer in the small region nearby the starting values, and the method of the steepest ascent can be used for moving sequentially in the direction of the maximum increase of the response [39]. A D-optimal analysis was performed using Design Expert 7.0 software. ANOVA was performed for this set of experiments, considering a multiple response: The sum of the slopes of the analytical curves as well as the relative standard deviation between the slopes. This was made in order to find the conditions for maximum sensitivity (maximum sum of the slopes) and minimum matrix effects (minimum relative standard deviation of the slopes). The approach for

Table 2
Studied variables with their respective levels in the first and second rounds of the D-optimal design in the determination of Pt by HR-CS F AAS.

Variable code	Description	1st round			2nd round		
		Levels			Levels		
		−1	0	+1	−1	0	+1
A	Acetylene flow (L h^{-1})	65	70	75	70	75	80
B	Observation height (mm)	12	13	14	13	14	15

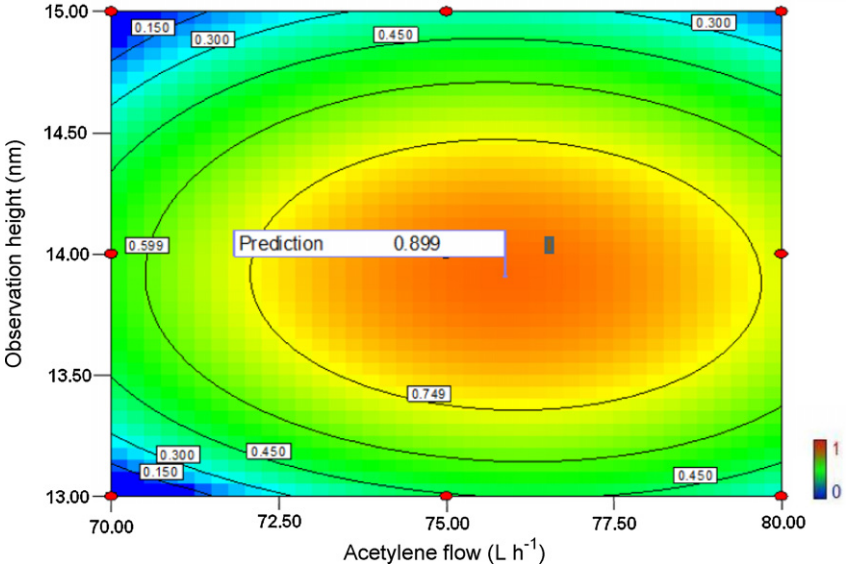

Fig. 1. Desirability function graphic for the second round for D-optimal planning experiments.

the optimization of the multiple responses was that of Derringer and Suich that makes use of the desirability functions. In short, each response y_i is first converted into an individual desirability function d_i that varies over the range $0 \leq d_i \leq 1$, where the response y_i is at its goal or target, then $d_i = 1$, and if the response is outside an acceptable region, $d_i = 0$. Then the design variables are chosen to maximize the overall desirability $D = (d_1 \times d_2 \times \ldots \times d_m)^{1/m}$ where there are m responses [39]. The linear regression model, in terms of the real variables levels, obtained for the first round was Absorbance = $-0.024 + 0.0003A + 0.0021B$, where A is the acetylene flow rate and B the observation height. Results showed that the linear model was well adjusted ($p < 0.05$) and it did not show lack of fit.

The desirability function graph (not shown) indicated that greater acetylene flow rate and observation height lead to the desired situation. Thus, a second round of experiments was carried out in accordance to the matrix also shown in Table 2, which displays the real values and codified levels of studied variables. Taking into consideration the same criteria as the first round, the best adjusted model at this time was a quadratic one ($p < 0.05$). ANOVA was performed for this new set of experiments using the same software aforementioned. The point of greater desirability is located at an acetylene flow rate of 75 L h^{-1} and the observation height of 14 mm as can be seen in Fig. 1.

3.1.4. Calibration test

Ten different urine samples collected from individuals of different ages and sex were taken for investigation. These were spiked with known increasing amounts of different forms of platinum ($PtCl_2$, cisplatin and carboplatin). Their slopes were significantly different from those observed for $PtCl_2$ in an aqueous (1%, v/v HCl) medium, indicating that external calibration in this medium with inorganic platinum salt is not possible because of the matrix effect. However, a parallelism test of these analyte addition curves performed by the GraphPad Prism 5.0 software, found out that, in the optimal conditions, no statistical difference ($p < 0.05$) between their slopes was observed. Thus, external calibration in matrix matched medium using an inorganic salt of platinum ($PtCl_2$) can be used for analysis.

3.2. Platinum determination in urine by ET AAS

In this study, for the determination of Pt by ET AAS, the temperature program (atomization and pyrolysis temperature and atomization ramp), sample volume and diluent nature and concentration should be defined. A 2^k factorial design can be used to identify the factors or variables that have large effects. The factors identified as important are then investigated more thoroughly in subsequent experiments. However, in this design, as the number (k) of variables or factors increases, the number of runs required may prohibitively grow. If the experimenter can reasonably assume that certain high-order interactions are negligible, information on the main effects and low-order interactions may be obtained by running only a fraction of the complete factorial experiment [39].

Thus, for the first round, a screening experiment was performed by a 2^{7-2} fractional factorial design using urine samples spiked with 200 μg L^{-1} of $PtCl_2$. The response was the maximum integrated absorbance. The real values and codified levels of the studied variables in this first round are presented in Table 3. Statistica 7.0 Statsoft software estimated the individual effects and interactions: factor C (atomization temperature) and the interactions AB and BC have shown to be statistically significant ($p < 0.05$). These results were also confirmed by the analysis of variance (ANOVA).

A second round of experiments was performed eliminating three of the experimental variables and planning a full factorial design with four variables, at two levels each (factorial 2^4). The variables acid nature, dilution factor and sample volume were fixed as HNO_3, 1 + 1, and 20 μL, respectively. The com-

Table 3
Studied variables with their respective levels of factorial planning 2^{7-2} in the optimization of Pt determination in urine by ET AAS.

Variable code	Description	Levels	
		−1	+1
A	Diluent solution	HCl	HNO_3
B	Diluent concentration (%, v/v)	0.4	2
C	Atomization temperature (°C)	2300	2500
D	Pyrolysis temperature (°C)	1200	1600
E	Atomization ramp (°C s^{-1})	1600	2000
F	Dilution factor	0	1:1
G	Sample volume injected (μL)	10	20

Table 4
Studied variables with their respective levels of factorial planning 2^{4-0} in the second and third round of experiments for optimization of Pt determination in urine by ET AAS.

Variable code	Description	2nd round		3rd round	
		Levels		Levels	
		−1	+1	−1	+1
B	HNO_3 (%, v/v)	0.3	0.6	0.20	0.30
C	Atomization temperature (°C)	2300	2400	2350	2450
D	Pyrolysis temperature (°C)	1400	1600	1600	1660
E	Atomization ramp (°C s^{-1})	1700	1800	1800	1813

binations between the coded factors and their normal levels are shown in Table 4. In the present case, the effects *B* (diluent concentration), *C* (atomization temperature), *D* (pyrolysis temperature) and *E* (atomization ramp) and the *CD* interaction were statistically significant and confirmed by analysis of variance. The regression model obtained was: Integrated absorbance = $0.0140 - 0.0039B + 0.0159C + 0.0047D + 0.0021E - 0.0029CD$ in codified values, where the coefficient of determination was equal to 0.8355, meaning that 83.55% of the variability was explained by the model.

In order to refine the optimum conditions for the analysis, the response surface methodology (RSM) was used. The atomization temperature was chosen to establish the path of maximum slope, since it is associated to largest coefficient. Thus, the displacement of the other factors was determined and a third round of experiment was established. Once again a full factorial design with four factors at two levels was proposed (Table 4). The experiments were carried out in triplicate. In this case, factors B (HNO_3), C (atomization temperature), *E* (atomization ramp) and the *BD* interaction were statistically significant. As the pyrolysis temperature was not significant in the studied range in this experiment, one can conclude that 1600 °C is the optimal value for this factor.

To verify the existence of quadratic terms in the regression model, a central composite design with three variables, *B*, *C* and *E* was proposed: 2^3 full factorial design, plus 6 axial points with $\alpha = \pm 1.68$ and triplicate on the central point. The whole experiment was carried out in triplicate. The variables with their respective values for each level are shown in Table 5, and Statistica 7.0 Statsoft software was used for data analysis. The statistically significant effects were *B* (linear term), *B* (quadratic term), *C* (linear term) and *C* (quadratic term) which it was confirmed by analysis of variance ($p < 0.05$). The regression equation according this model was: Integrated absorbance = $0.0901 + 0.0041B - 0.0025B^2 + 0.0037C - 0.0017C^2$. Fig. 2 shows the relation between observed and predicted values for this model. The dispersion of points indicates that the model has a limited capacity to explain the variability of the data ($R^2 = 0.63$). The resulting optimized program is presented in Table 1.

3.2.1. Calibration tests

Using the optimized conditions, the adequate calibration procedure was assessed. Analyte addition curves using 10 different urine samples collected from individuals of different ages and sex and three Pt forms were investigated. Their respective correlation and angular coefficients were obtained by the method of least squares. It was found that, even under optimal conditions, the sensitivity in aqueous solution was significantly higher than in urine, and was also different for each urine sample. However, sensitivity was non-dependent on the urine form (Pt^{2+}, cisplatin and carboplatin). Thus, in these circumstances, analyte addition calibration should be used, using any of the studied Pt forms. However, since calibration by the analyte addition technique implies in larger analysis time and expanded errors in the final results, further experiments were performed looking for a more adequate calibration procedure.

3.2.2. Modification studies

Aiming at overcoming the multiplicative matrix effects observed, that led to the analyte addition calibration, the use of modifiers was investigated. In the present case, due to the already high pyrolysis temperature used, the modifier action should necessarily not aim for its increase. Thus, at first, 5 μL of 0.5% (v/v) Triton X-100 were added to the sample, but no change was observed. Then, 10 μL of a mixture containing 0.30 mol L^{-1} NaCl and 0.40 mol L^{-1} HCl was added to the sample in the graphite furnace, in accordance to Warmerdam et al. [28]. In this case, no statistical evidence permitted the rejection of the hypothesis that the slopes of the analyte addition curves using different urine samples or the platinum forms under investigation were equal ($p = 0.65$, GraphPad Prism 5.0 software), at a significance level of 0.05. Moreover, the same was true for calibration curves performed in the same medium as the blank, meaning that external calibration in this medium is permitted.

3.3. Accuracy studies

Since no certified reference material for Pt in urine was available, accuracy was assessed by comparing the results obtained in the analysis of different urine samples with independent methods, as well as by recovery tests. Eight urine samples were prepared by mixing different proportions of one obtained from a patient submitted to chemotherapy with cisplatin with those from health individuals of different ages and sex, in order to simulate different matrices. All determinations were performed using optimized conditions for each sample. The results are shown in Table 6. No statistically significant difference among the results (ANOVA, $p = 0.65$) was observed. By the analysis of variance (ANOVA) demonstrated that the results obtained by HR-CS FAAS were not statistically different ($p = 0.65$) from those obtained by an independent comparative procedure (ET AAS). Recoveries of 98 ± 4% were observed for the different concentration levels studied. This successful comparison with a method that makes use of sample mineralization has also shown that the present methods are independent of the

Table 5
Studied variables with their respective levels of central composite design optimization of Pt determination in urine by ET AAS.

Variable code	Description	Level				
		−1.68	−1	0	+1	+1.68
B	HNO_3 (% v/v)	0.14	0.20	0.30	0.40	0.46
C	Atomization temperature (°C)	2400	2420	2450	2480	2500
E	Atomization ramp (°C s^{-1})	1790	1800	1815	1830	1840

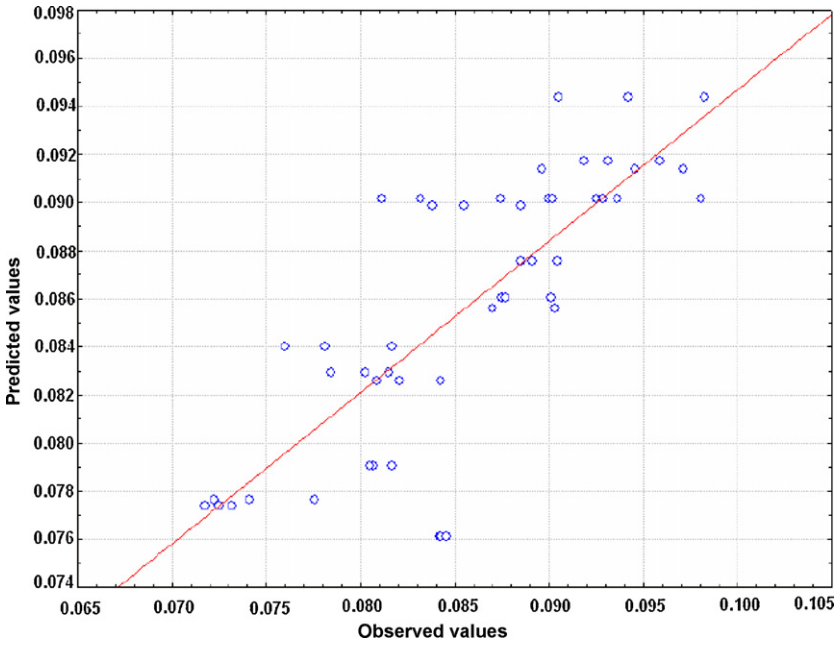

Fig. 2. Observed versus predicted values according to the mathematical model obtained in the multivariate optimization of the determination of Pt in urine by ET AAS.

Table 6
Concentrations ($mg\,L^{-1} \pm SD$) of total Pt obtained by HR- CS FAAS, ET AAS and by the comparative procedure in urine samples of different individuals spiked with a sample of a patient submitted to chemotherapy with cisplatin ($n = 5$).

	Proposed procedures		Comparative procedure
	HR-CS F AAS	ET AAS	
Urine 1	3.67 ± 0.08	3.54 ± 0.16	3.68 ± 0.04
Urine 2	5.62 ± 0.07	5.87 ± 0.36	5.58 ± 0.05
Urine 3	5.85 ± 0.07	6.03 ± 0.39	5.80 ± 0.15
Urine 4	5.63 ± 0.07	5.80 ± 0.47	5.48 ± 0.10
Urine 5	0.72 ± 0.09	0.66 ± 0.06	0.72 ± 0.02
Urine 6	1.74 ± 0.09	1.62 ± 0.08	1.73 ± 0.05
Urine 7	1.39 ± 0.09	1.42 ± 0.04	1.49 ± 0.03
Urine 8	1.44 ± 0.09	1.41 ± 0.11	1.54 ± 0.05

presence of unknown Pt metabolites that exist in urine of patients submitted to treatment with Pt based drugs [40,41].

3.4. Figures of merit

The HR-CS F AAS calibration curve showed a linear behavior up to 80 $mg\,L^{-1}$. The limits of detection (LODs) were calculated from 10 successive measurements of the blank solutions ($k = 3$) as recommended by IUPAC. The limit of detection was determined considering the "blank", the signal of the urine sample with the lowest response among those analyzed. The respective LOD and quantification (LOQ) were 0.06 and 0.18 $mg\,L^{-1}$ in the original sample, by HR-CS F AAS. Using the GF AAS, the calibration curve was linear up to 2.7 $mg\,L^{-1}$ and the LOD and LOQ for the method were 0.004 and 0.012 $mg\,L^{-1}$, respectively, in the original sample.

4. Conclusions

Platinum, in the forms of cisplatin and carboplatin, could be determined in urine, after simple 1 + 1 sample dilution, by flame HR-CS AAS, as well as by ET AAS, covering a large concentration range with minimal sample handling. In both procedures, external calibration with an inorganic platinum salt ($PtCl_2$) could be use: For the flame procedure matrix matched calibration solutions should be used while, in the ET AAS procedure, using NaCl + HCl as modifier, calibration could be performed with calibration solutions prepared in the same medium as the blank. Accuracy was confirmed by the good agreement between the results obtained by the proposed procedures and those obtained by an independent method, in which the sample was mineralized. This also confirms the adequacy of the method in relation to the presence of unknown Pt metabolites that exist in urine of patients submitted to treatment. The limits of detection and dynamic ranges were adequate for the follow-up of patients submitted to chemotherapy with these Pt drugs.

References

[1] L.H. Einhorn, J.P. Donahue, J. Urol. 117 (1977) 65.
[2] B. Rosenberg, C.L. Van, J.E. Trosko, V.H. Mansour, Nature 222 (1969) 385.
[3] R.D. Wong, C.M. Giandomenico, Chem. Rev. 99 (1999) 2451.
[4] P. Kopf-Maier, H. Koph, Chem. Rev. 87 (1987) 1137.
[5] M. Zeiner, M. Ovari, G. Zaray, I. Steffan, Microchem. J. 93 (2009) 22.
[6] W.A.J. de Waal, F.J.M.J. Maessen, J.C. Kraak, J. Pharm. Biomed. Anal. 8 (1990) 1.
[7] B. Bocca, A. Alimonti, A. Cristaudo, E. Cristallini, F. Petrucci, S. Caroli, Anal. Chim. Acta 512 (2004) 19.
[8] K.S. Subramanian, Biol. Trace Elem. Res. 49 (1995) 187.
[9] K.E. Levine, J.D. Batchelor, C.B. Rhoades, B.T. Jones, J. Anal. At. Spectrom. 14 (1999) 49.
[10] J. Messerschmidt, J. Alt, G. Tölg, J. Angerer, K.H. Schaller, Fresenius J. Anal. Chem. 343 (1992) 391.
[11] M. Mcgahan, K. Tyczkowska, Spectrochim. Acta Part B At. Spectrosc. 42 (1987) 665.
[12] O. Nygren, G.T. Vaughan, T.M. Florence, G.M.P. Morrison, I.M. Warner, L.S. Dale, Anal. Chem. 62 (1990) 1637.
[13] V. Dinoto, D. Ni, L.D. Via, F. Scomazzon, M. Vidali, Analyst 120 (1995) 1669.
[14] J. Begerow, L. Dunemann, J. Anal. At. Spectrom. 11 (1996) 303.
[15] F. Morazzoni, C. Canevali, I. Moschetti, R. Todeschini, S. Caroli, A. Alimonti, F. Petrucci, G. Ravasi, A.V. Bedini, F. Milani, M. Palazzi, S. Villa, G. Giudice, Cancer Chemother. Pharmacol. 35 (1995) 529.
[16] C. Sessa, C. Minoia, A. Ronchi, M. Zucchetti, J. Bauer, M. Borner, J. Jong, O. Pagani, J. Renard, C. Weil, M. D'Incalci, Ann. Oncol. 9 (1998) 1315.
[17] C. Sessa, G. Capri, L. Gianni, F. Peccatori, G. Grasselli, J. Bauer, M. Zucchetti, L. Viganò, A. Gatti, C. Minoia, P. Liati, S. Van den Bosch, A. Bernareggi, G.S. Camboni, Ann. Oncol. 11 (2000) 977.
[18] J.G. Morrison, D. Bissett, I.F.D. Stephens, K. Mckay, R. Brown, M.A. Graham, A.M. Fichtinger-Schepman, D.J. Kerr, Int. J. Oncol. 2 (1993) 33.
[19] E. Gamelin, P. Allain, P. Maillart, A. Turcant, R. Delva, A. Lortholary, F. Larra, Cancer Chemother. Pharmacol. 37 (1995) 97.
[20] S. Hann, G. Koellensperger, Zs. Stefánka, G. Stingeder, M. Fürhacker, W. Buchberger, R.M. Mader, J. Anal. At. Spectrom. 18 (2003) 1391.
[21] M. Óvári, G. Muránszky, M. Zeiner, I. Virág, I. Steffan, V.G. Mihucz, E. Tatár, S. Caroli, G. Záray, Microchem. J. 87 (2007) 159.
[22] J.E. Thorsten, M. Galanski, B.K. Keppler, J. Anal. At. Spectrom. 11 (1996) 747.
[23] B. Casetta, M. Roncadin, G. Montanari, M. Furlanut, At. Spectrosc. 12 (1991) 81.

[24] M. Bettinelli, Microchem. J. 79 (2005) 357.
[25] S. Vouillamoz-Lorenz, J. Bauer, F. Lejeune, L.A. Decosted, J. Pharm. Biomed. Anal. 25 (2001) 465.
[26] J. Smeyers-Verbeke, M.R. Detaevernier, L. Denis, D.L. Massart, Clin. Chim. Acta 113 (1981) 329.
[27] M.C. McGahan, K. Tyczkowska, Spectrochim. Acta Part B At. Spectrosc. 42 (1987) 665.
[28] L.J.C. Warmerdam, O. Tellingen, R.A.Q. Maes, J.H. Beijnen, Fresenius J. Anal. Chem. 351 (1995) 777.
[29] J. Begerow, M. Turfeid, L. Dunemann, Anal. Chim. Acta 340 (1997) 277.
[30] C. Kloft, H. Appelius, W. Siegert, W. Schunack, U. Jaehde, Ther. Drug. Monit. 21 (1999) 631.
[31] M. Chappuy, E. Caudron, A. Bellanger, D. Pradeau, J. Hazard. Mater. 176 (2010) 207.
[32] N.M. Najafi, S. Shahparvizi, H. Rafati, E. Ghasemi, R. Alizadeh, J. Pharm. Biomed. Anal. 53 (2010) 58.
[33] F.W. Sunderman, J. Sporn, S.M. Hopfer, K.R. Sweeney, N.G. Chakraborty, B. Greenberg, Ann. Clin. Lab. Sci. 20 (1990) 379.
[34] P. Tothill, L.M. Matheson, J.F. Smyth, K. Mckay, J. Anal. At. Spectrom. 5 (1990) 619.
[35] R.Q. Aucélio, V.N. Rubin, B.W. Smith, J.D. Winefordner, J. Anal. At. Spectrom. 13 (1998) 49.
[36] B. Welz, H. Becker-Ross, S. Florek, U. Heitmann, High-resolution Continuum Source AAS—The Better Way to do Atomic Absorption Spectrometry, 1st Ed., Wiley-VCH, Weinheim, 2005, p. 295.
[37] B. Welz, D.L.G. Borges, F.G. Lepri, M.G.R. Vale, U. Heitmann, Spectrochim. Acta Part B 62 (2007) 873.
[38] M. Balcerzak, Analyst 122 (1997) R67.
[39] D.C. Montgomery, Design and Analysis of Experiments, 6th Ed., John, Wiley & Sons, Inc., USA, 2005.
[40] G. Koellensperger, Zs. Stefanka, K. Meelich, M. Galanski, B.K. Keppler, G. Stingeder, S. Han, J. Anal. At. Spectrom. 23 (2008) 29.
[41] S. Hann, Zs. Stefanka, K. Lenz, G. Stingeder, Anal. Bioanal. Chem. 381 (2005) 405.