Isotope Dilution Gas Chromatography/Mass Spectrometry for Platinum Determination in Urine

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The therapeutic importance of platinum (Pt) compounds, the growing accessibility of gas chromatography/mass spectrometry (GC/MS) systems in clinical laboratories, and the lack of a mass spectrometric method for the determination of Pt in biological samples motivated us to develop an isotope dilution GC/MS assay for Pt. The method is based on the use of lithium bis(trifluoroethyl)dithiocarbamate, Li(FDEDTC), as a chelating agent and enriched ^{192}Pt for isotope dilution. Conditions were optimized for the precise and accurate determination of isotope ratios of Pt by using a 10-m DB-1 fused silica capillary column and a reverse-geometry double-focusing mass spectrometer with selected ion monitoring. An overall precision of 1% was obtained by combining within-run precision and between-run precision at the 10-ng level. No appreciable memory effect was observed when samples with different isotope ratios were analyzed sequentially. The method was validated by the quantitation of Pt in National Institute of Standards and Technology freeze-dried urine sample SRM 2670. A concentration value of 125 ± 6 μg/L (n = 6) was obtained by using four different sets of isotope ratios in the molecular ion and supports the National Institute of Standards and Technology recommended value of 120 ± ? μg/L. Limits-of-quantitation, estimated at 3 μg/L, are made possible by the high sensitivity of the method and the low blank value for Pt. *(J Am Soc Mass Spectrom 1991, 2, 85–90)*

Platinum (Pt) compounds are important antineoplastic drugs. Because urine concentrations of these drugs are closely related to the non-protein-bound drug concentration in the blood [1], measurement of urine Pt concentration offers an excellent, noninvasive way to monitor drug levels. Many of the analytical methods used for determining Pt in biological samples are either too insensitive or too plagued by other problems like matrix effects to be of value for this measurement. The obvious advantages of isotope dilution are freedom from matrix effects and results that are not dependent on the quantitative recovery of the analyte.

In our laboratory we have been pursuing gas chromatography/mass spectrometry (GC/MS) determination of trace metals in biological samples [2–4]. The main factors that appear to have precluded the widespread use and acceptability of GC/MS for metals determination are (1) lack of suitable chelating agents, (2) memory problem leading to cross-contamination in the sequential analyses of samples with different isotope ratios, and (3) poor precision and accuracy in isotope ratio measurements. Isotope dilution GC/MS (IDGC/MS) methods have been reported by others for the determination of Cr [5–7] and Se [8, 9]. We have reported the development of IDGC/MS methods for the determination of Ni [3] and Cr [4] in urine samples. This study reports the extension of our techniques to the determination of isotope ratios and concentration of Pt in urine samples. Memory effects were studied by sequential analyses of samples with different isotope ratios. Precision and accuracy in isotope ratio measurements were established by replicate determinations of the natural Pt on different days. Finally, the IDGC/MS method for determining Pt concentration was validated by analysis of National Institute of Standards and Technology (NIST) urine reference material.

Experimental

Instrumentation

The mass spectrometer used was a double-focusing reverse-geometry instrument (Model 8230, Finnigan MAT, San Jose, CA) with a SpectroSystem 300 data system. The instrument was operated in the electron ionization (EI) mode using 70-eV electrons with a source temperature of 200 °C, the conversion dynode at −5000 V, and the secondary electron multiplier at 2000 V. The focusing conditions of the mass spectrometer were optimized and the mass calibration was

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established by injecting perfluorokerosene into the reference inlet. The fragmentation pattern of the metal chelate was recorded by employing the exponential scanning mode (1 s per decade) and adjusting the source and the collector slits to obtain a triangular peak shape. Isotope ratios were measured in the selected ion monitoring mode using voltage peak switching. The source and the collector slits were adjusted to obtain trapezoidal peaks with flat tops (collector slit width > source slit width) and the data were acquired at 2 Hz, yielding approximately 20 data points across the 10-s wide GC peak. A mass resolution of 1000 was used throughout. The isotope ratios were calculated by integrating the ion current for various chromatographic peaks rather than by taking the peak heights. The overall linearity of the mass spectrometer was checked by injecting methyl stearate into the GC column and measuring the isotope ratios with m/z values of 299/298, 300/298, 301/298, and 302/298; these ratios differed by four orders of magnitude. Other details of the measurement methodology were published earlier [2].

A Varian 3700 gas chromatograph equipped with a DB-1 (J. W. Scientific, Rancho Cordova, CA) poly(dimethylsiloxane) bonded-phase fused silica capillary column, 10 m × 0.32 mm, with a 0.25-μm film thickness was used. Platinum chelate samples were injected by using an on-column injector (OCI-3, Scientific Glass Engineering, Austin, TX) at an oven temperature of 100 °C followed by a 25 °C/min temperature ramp to 300 °C. High-purity helium was used as a carrier gas.

Reagents

The ^{192}Pt (50 atom%) used as a spike for isotope dilution was obtained from Oak Ridge National Laboratory (Oak Ridge, TN). Certified Atomic Absorption Standard (Pt in 10% HCl) purchased from Spex Industries Inc. (Edison, NJ) was used as the primary standard for spike calibration. Double subboiling quartz-distilled HNO_3 in Teflon bottles was obtained from the NIST (Gaithersburg, MD). Ultrex grade NH_4OH solution (30%), HCl (12 N), and ortho-H_3PO_4 (86%) were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). Formic acid (90%) and stabilized hydrogen peroxide (50%) were obtained from Fisher Scientific (Fairlawn, NJ). The standard reference material, freeze-dried urine SRM 2670, was also purchased from the NIST and prepared according to their directions. Lithium bis(trifluoroethyl)dithiocarbamate, Li(FDEDTC), was synthesized by reacting bis(trifluoroethyl)amine (PCR Inc., Gainesville, FL) and *n*-butyl-lithium (Aldrich Chemical Co., Milwaukee, WI) in an inert atmosphere at −70 °C followed by the addition of carbon disulfide (Aldrich) [2, 10].

Contamination control, always important in trace metals analysis, would not be considered a major problem when working with Pt because of the rarity of this element. Nonetheless, normal trace metals procedures to avoid contamination were followed, e.g., use of acid-leached Polypropylene/Teflon labware, deionized water (DW), powder-free gloves, and a clean hood with laminar flow.

Preparation and Standardization of Spike Solution

The ^{192}Pt spike solution was prepared by dissolving the metal in a minimum amount of aqua regia at 70 °C. This solution was then taken to the appropriate volume with dilute HCl. More dilute spike solutions were prepared from this stock solution on a weight basis for isotope dilution experiments. The isotopic composition of Pt in the spike was determined experimentally by GC/MS analysis of $Pt(FDEDTC)_2$ chelate. The spike solution was calibrated as previously reported by reverse-isotope dilution GC/MS using the natural Pt primary standard [3, 4].

Digestion of Urine Samples and Chelate Formation

A known volume (0.5 mL) of the reconstituted urine reference material was mixed with a weighed amount of ^{192}Pt spike solution in a Teflon beaker. The spiked mixture was treated with 2.5 mL of concentrated HNO_3 and 0.5 mL of concentrated H_3PO_4 (5:1, v/v) and allowed to stand overnight to partially digest the organic matter and reduce foaming during subsequent heating. The partially digested solution was then heated gently on a hot plate at 70 °C to reduce the volume, and 1 mL of 50% H_2O_2 solution was slowly added to the hot solution until no visible brown fumes of nitrogen dioxide were released. Subsequently, 0.5 mL of formic acid was added to destroy the residual HNO_3 [11]. The solution was evaporated to 0.5 mL, and 0.5 mL of 10% HCl was added. The volume of the solution was taken to 5 mL with DW and extracted with 2 mL of CH_2Cl_2, discarding the organic phase to remove any undigested lipids. The pH of the aqueous phase was adjusted to 2–3 by using about 200 μL of concentrated NH_3 solution; 1 mL of pH 3 acetate buffer was added and the Pt chelate formed by adding 200 μL of a 20-mM solution of the chelating agent Li(FDEDTC). The Pt chelate was extracted with two 500-μL aliquots of CH_2Cl_2. The organic extract containing the Pt chelate was allowed to evaporate to dryness at room temperature in the laminar flow hood and reconstituted in 20 μL of CH_2Cl_2 for analysis.

Gas Chromatography / Mass Spectrometry

The Pt isotope ratios were measured in duplicate by injecting 1 μL of the chelate solution and monitoring the cluster of peaks corresponding to the molecular ion. Because the measurements were performed without using a lock mass, it was necessary to determine the m/z value of the peak maximum experimentally

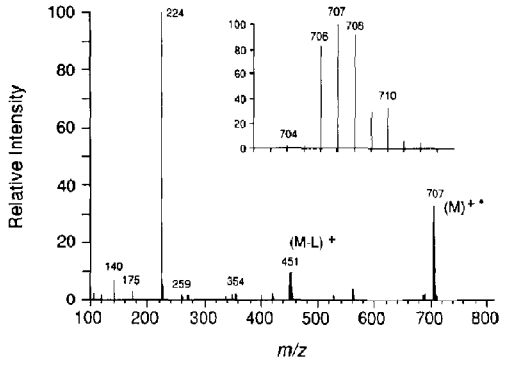

Figure 1. Electron ionization mass spectrum of $Pt(FDEDTC)_2$ (the sample contained 10 ng of Pt).

by obtaining a histogram of the ion current adjacent to and including the calculated m/z value of a given ion. Details of the methodology were published earlier [2]. Concentrations of Pt were then calculated by the usual method of isotope dilution [12].

Results and Discussion

The EI mass spectrum of $Pt(FDEDTC)_2$, shown in Figure 1, exhibits two groups of ions containing Pt isotopic information. These ions are the molecular ion $Pt(FDEDTC)_2^{+\cdot}$ and a fragment ion $Pt(FDEDTC)^+$, designated by $M^{+\cdot}$ and $[M - L]^+$, respectively. The ion at m/z 224, corresponding to $(CF_3CH_2)_2NCS^+$, is the base peak but is not useful for Pt analysis. The most abundant isotopic group, the $M^{+\cdot}$ ion, was used throughout these experiments to achieve highest possible sensitivity. This ion group consists of nominal m/z values of 702, 704, 706, 707, 708, and 710, corresponding respectively to ^{190}Pt, ^{192}Pt, ^{194}Pt, ^{195}Pt, ^{196}Pt, and ^{198}Pt isotopes. Further, the presence of the molecular ion as well as the observation of symmetrical and sharp chromatographic peaks indicated the adequate thermal stability and volatility of the Pt chelate at nanogram levels under the conditions used in these experiments.

Tables 1 and 2 show the results obtained for isotope ratio measurements of natural Pt and enriched ^{192}Pt, respectively. For these measurements, 1 μL of the chelate solution containing about 10 ng of Pt was used for analysis. The atom percent abundances shown in Table 1 for the different isotopes in natural Pt are the recommended values based on the values measured experimentally by different laboratories [13]. The atom percent abundances shown in Table 2 for the different Pt isotopes in enriched ^{192}Pt are the values provided by the Oak Ridge National Laboratory. The calculated values for the abundances of the molecular ion of the chelate, also given in Tables 1 and 2, were obtained by calculating the contributions of the isotopes of Pt, carbon, nitrogen, and sulfur in the molecular ion [14, 15]. As shown in Tables 1 and 2, there is good agreement between the calculated and measured abundances of the molecular ion in natural Pt and in the ^{192}Pt spike. No correction has been applied to the experimental data shown in Tables 1 and 2 for any mass discrimination among the different isotopes. Moreover, any mass discrimination factor would be canceled in isotope dilution experiments because the spike calibration is also done in the same experiment.

Precision in the determination of various isotope ratios was evaluated by performing measurements of chelated natural Pt on four different days. Three to six replicate analyses of 10 ng of Pt were made on each of the four days and the data are summarized in Table 3. The mean values for each day were used to calculate the mean of means and its standard deviation, referred to as between-run precision in Table 3. The within-run precision was obtained by considering the standard deviation values obtained on the individual days. Overall precision was calculated by combining the within-run and between-run precision values to evaluate the effects of any variations in the mass spectrometer operating parameters that may affect the isotope ratio data from one day to another. No significant difference was observed among the isotope ratios measured on different days. Overall precision values of approximately 1% were obtained for all the isotope ratios except the m/z 704/707 ratio, which

Table 1. Calculated and measured abundances of different ions in natural $Pt(FDEDTC)_2$

		Molecular ion ($M^{+\cdot}$)		
Isotope	Atom% abundance	Ion (m/z)	Calculated abundance[a] (%)	Measured abundance (%)
^{190}Pt	0.01	702	0.01	—[b]
^{192}Pt	0.79	704	0.65	0.76
^{194}Pt	32.90	706	26.95	26.85
^{195}Pt	33.80	707	31.67	31.07
^{196}Pt	25.30	708	29.90	29.43
^{198}Pt	7.20	710	10.84	11.89

[a] Including the contributions of the isotopes of Pt, carbon, nitrogen, and sulfur in the ion $Pt(FDEDTC)_2^{+\cdot}$.
[b] Intensity below detection limits.

Table 2. Calculated and measured abundances of different ions in $^{192}Pt(FDEDTC)_2$

Isotope	Atom% abundance[a]	Molecular ion ($M^{+\cdot}$) Ion (m/z)	Calculated abundance[b] (%)	Measured abundance (%)
^{190}Pt	< 0.05	702	< 0.04	0.09
^{192}Pt	50.03	704	40.20	37.84
^{194}Pt	24.25	706	27.06	26.74
^{195}Pt	16.23	707	17.05	17.72
^{196}Pt	8.00	708	12.63	13.70
^{198}Pt	1.49	710	3.01	3.90

[a]Values given by Oak Ridge National Laboratory.
[b]Including the contributions of the isotopes of Pt, carbon, nitrogen, and sulfur in the ion $Pt(FDEDTC)_2^{+\cdot}$.

gave a precision of 5% due to the low natural abundance of ^{192}Pt.

Evaluation of the Memory Effect

Memory effect refers to cross-contamination in the GC/MS system during the sequential analyses of samples with different isotope ratios. This cross-contamination can adversely affect the accuracy of the measurement of altered isotope ratios. In these studies, the memory effect was evaluated in two different ways.

The first approach used to investigate the memory effect was an evaluation of the accuracy of determining isotope ratios different from those of natural Pt. For this experiment, three synthetic mixtures differing in the m/z 704/707 ratio by a factor of about 16 (range 0.05–0.81) were prepared by mixing weighed aliquots of the primary standard solution and the enriched ^{192}Pt solution in differing proportions but containing almost equal amounts of total Pt. This was done to observe the extent of memory in samples that are most commonly encountered in isotope dilution experiments. Three analyses, each with 10 ng of Pt, were made for each mixture, and the isotope ratios m/z 704/706, 704/707, and 704/708 were determined. The mixtures were analyzed in the sequence of increasing isotope ratios and then in the reverse sequence. Figure 2 shows the m/z 704/707 isotope ratios determined in these mixtures. Only a slight memory effect is observed in analysis number 13, which immediately followed samples with dramatically different isotope ratios.

The second approach involved the sequential analyses of a solution of natural Pt and a solution of enriched ^{192}Pt. The ratio m/z 704/707 was measured because it corresponds to the maximum abundant isotopes, ^{192}Pt in the spike and ^{195}Pt in the natural Pt. The analyses were carried out in the following sequence: five injections of natural Pt, five injections of ^{192}Pt spike, followed by seven injections of natural Pt. The results are presented in Figure 3. Again, only a slight memory effect is observed in analysis number 11, which immediately followed samples with isotope ratios differing by a factor of about 80. Hence a small memory effect is seen for only the first new sample after a large change in ratio. The slight extent of memory effects is not a problem.

Table 3. Precision of isotope ratio determination of natural Pt as $Pt(FDEDTC)_2$

	Isotope ratio[a] (mean ± rsd) 704/707	706/707	708/707	710/707
Day 1 (n = 6)	0.0256 ± 3.2%	0.8593 ± 1.1%	0.9488 ± 0.4%	0.3849 ± 1.2%
Day 2 (n = 6)	0.0250 ± 3.9%	0.8677 ± 0.7%	0.9422 ± 0.7%	0.3790 ± 2.3%
Day 3 (n = 5)	0.0250 ± 6.5%	0.8620 ± 0.9%	0.9489 ± 0.8%	0.3832 ± 1.2%
Day 4 (n = 3)	0.0230 ± 3.3%	0.8677 ± 0.1%	0.9483 ± 0.5%	0.3832 ± 1.2%
Mean of means	0.0246	0.8642	0.9471	0.3826
Within-run precision,[b] %	2.2	0.4	0.3	0.8
Between-run precision, %	4.6	0.5	0.3	0.7
Overall precision,[c] %	5.1	0.6	0.4	1.1

[a]Not corrected for any mass discrimination factor.
[b]Calculated by using the formula $S_i = (\sum_{j=1}^{n} S_j^2)^{1/2}/n$, where s_j represents the standard deviation obtained on each individual day and n is the number of days.
[c]Overall precision S_t was calculated by combining within-run precision (S_i) and between-run precision (S_e) according to the formula $S_t = (S_i^2 + S_e^2)^{1/2}$.

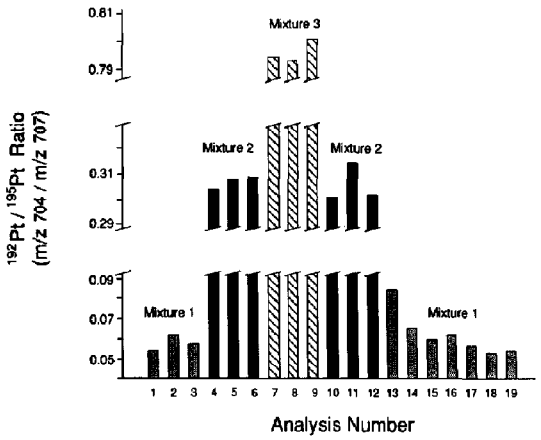

Figure 2. Evaluation of cross-contamination in consecutive analyses of samples with slightly altered isotopic composition.

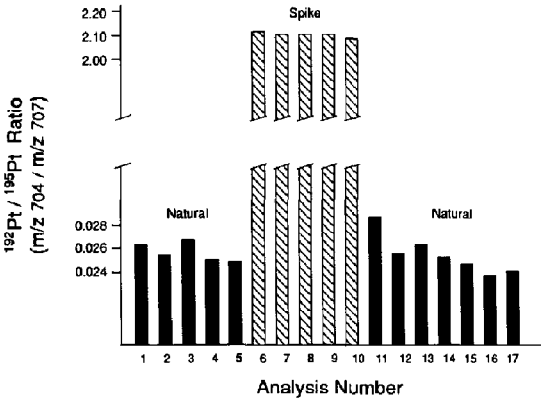

Figure 3. Evaluation of cross-contamination of samples with isotopic composition differing by a factor of 80. Analyses 1–5 and 11–17 are of a natural Pt sample and analyses 6–10 are of enriched ^{192}Pt sample.

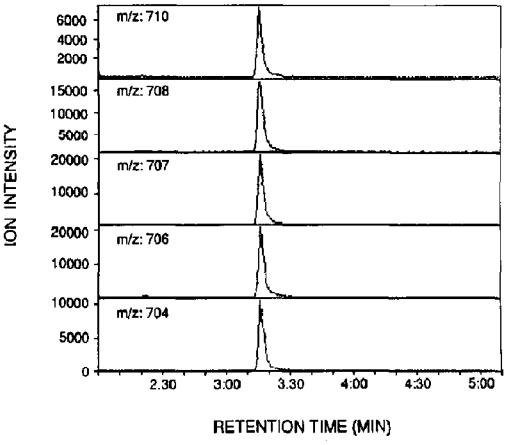

Figure 4. Reconstructed ion chromatogram for the determination of Pt in the urine sample SRM 2670 (the sample injected contained 10 ng of Pt).

Calibration of ^{192}Pt Spike Solution

The ^{192}Pt spike solution was calibrated by reverse-isotope dilution using a primary standard of natural Pt. For this standardization, five samples were prepared by mixing weighed aliquots of primary standard and ^{192}Pt spike solutions to achieve an optimum isotope ratio m/z 704/707 in the spiked mixtures. The results obtained for Pt concentration in the spike solution are given in Table 4. The values calculated from the different isotope ratios are all in good agreement. The relative standard deviations observed for these concentration values are smaller than those from synthetic mixtures because the optimum sample-to-spike ratio was measured during the reverse-isotope dilution experiment for spike calibration, whereas the synthetic mixtures comprised a range of isotope ratios.

Results on Urine Samples

The calibrated ^{192}Pt spike solution was used to quantify Pt in the NIST freeze-dried urine reference material SRM 2670. A high signal-to-noise ratio, shown in

Table 4. Determination of Pt in ^{192}Pt spike solution by reverse-isotope dilution GC/MS

	Concentration of Pt (μg of Pt /g of solution)		
		Synthetic mixtures[a]	
Ratio	Optimum spiking (mean ± rsd)[b]	A (mean ± rsd)[c]	B (mean ± rsd)[c]
704 /706	49.09 ± 1.2%	49.74 ± 2.7%	50.86 ± 3.1%
704 /707	48.29 ± 1.4%	48.81 ± 3.6%	49.69 ± 4.4%
704 /708	47.86 ± 1.0%	48.65 ± 3.4%	49.06 ± 4.3%
704 /710	47.85 ± 0.9%	—	—

[a]A and B represent the sequence of GC/MS analyses of synthetic mixtures; A denotes the analysis sequence with increasing isotope ratio and B denotes the analysis sequence in the reverse order.
[b]n = 5.
[c]n = 3.

Table 5. Determination of Pt in urine (SRM 2670)

Sample no.	Concentration of Pt (μg /L) using various ion ratios			
	704 /706	704 /707	704 /708	704 /710
1	127	126	123	117
2	122	126	123	114
3	126	126	117	109
4	131	125	129	114
5	136	129	133	124
6	132	131	134	121
Mean ± rsd	129 ± 5	127 ± 2	127 ± 7	117 ± 5

Note: Concentration of Pt recommended by NIST = 120 ± ? μg/L.

the reconstructed ion chromatogram in Figure 4, indicates potential limits of detection below the parts per billion level. Limits-of-quantitation are estimated to be better than 3 μg/L. The results obtained for Pt concentration in the urine sample SRM 2670 are shown in Table 5. Since the recommended Pt concentration values in the urine are provided in the units of μg/L by NIST, the urine samples were taken on a volume basis instead of a weight basis. The concentration values calculated by using the different isotope ratios are in very good agreement with one another. The value of 125 ± 6 μg/L calculated from these data supports the revised (April 12, 1989, NIST Certificate of Analysis) recommended value of 120 μg/L for Pt rather than the earlier (December 3, 1985) recommended value of 110 μg/L. It should be noted that the NIST recommended value does not have the standard deviation provided.

Conclusions

Stable IDGC/MS using Li(FDEDTC) as a chelating agent is a sensitive method for Pt determination in urine. There is no appreciable memory effect and the method gives good precision and accuracy for Pt isotope ratio measurements.

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