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A sensitive method for the determination of uranium in biological samples utilizing kinetic phosphorescence analysis (KPA)

Mohsen A. Hedaya *, Harry P. Birkenfeld, Ronald L. Kathren

Department of Pharmaceutical Sciences and United States Transuranium and Uranium Registries, College of Pharmacy, Wegner Hall, Room 309, Washington State University, Pullman, WA 99164-6510, USA

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Abstract

Kinetic phosphorescence analysis is a technique that provides rapid, precise and accurate determination of uranium concentration in aqueous solutions. This technique utilizes a laser source to excite an aqueous solution of uranium, and measures the emission luminescence intensity over time to determine the luminescence decay profile. The lifetime of the luminescence decay profile and the linearity of the log luminescence intensity versus time profile are indications of the specificity of the technique for uranium determination. The luminescence intensity at the onset of decay (the initial luminescence intensity), which is the luminescence intensity at time zero after termination of the laser pulse used for excitation, is proportional to the uranium concentration in the sample. Calibration standards of known uranium concentrations are used to construct the calibration curve between the initial luminescence intensity and uranium concentration. This calibration curve is used to determine the uranium concentration of unknown samples from their initial luminescence intensity. We developed the sample preparation method that allows the determination of uranium concentrations in urine, plasma, kidney, liver, bone spleen and soft tissue samples. Tissue samples are subjected to dry-ashing in a muffle furnace at 600°C and wet-ashing with concentrated nitric acid and hydrogen peroxide twice to destroy the organic component in the sample that may interfere with uranium determination by KPA. Samples are then solubilized in 0.82 M nitric acid prior to analysis by KPA. The assay calibration curves are linear and cover the range of uranium concentrations between 0.05 μg l^{-1} and 1000 μg l^{-1} (0.05–1000 ppb). The developed sample preparation procedures coupled with the KPA technique provide a specific, sensitive, precise and accurate method for the determination of uranium concentration in tissue samples. This method was used to quantify uranium in different tissue samples obtained over a period of 90 days following a single intraperitoneal uranium dose of 0.1 mg kg^{-1} in rats. © 1997 Elsevier Science B.V.

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1. Introduction

Determination of uranium in biological and environmental samples is necessary to assess occu-

* Corresponding author. Tel.: + 1 509 3555622; fax + 1 509 3350162; e-mail: Hedaya@mail.wsu.edu

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pational exposure, environmental contamination and natural levels of uranium in soil, air, water and other environmental media. The need for a sensitive, accurate, precise and reproducible method for uranium determination in real-world samples is felt in the biological, environmental and geological fields. The methods currently employed in the analysis of uranium include inductively coupled plasma-mass spectroscopy (ICP-MS) which has been used in the determination of uranium in environmental samples with a sensitivity as low as 0.1 μg l^{-1} [1]. Inductively coupled plasma emission spectroscopy (ICP-AES) has also been utilized for uranium analysis, but this method is subject to interference and has a relatively high detection limit, typically above 2 μg l^{-1} [2]. Other common techniques used for uranium analysis include α-spectroscopy, spectroscopy and fluorometry [3–9]. Although fluorometry has historically been the method of choice, particularly for analysis of uranium in urine, this technique has a relatively high detection limit, typically in the order of 1 μg l^{-1} [9]. Moreover, the use of these methods in routine sample analysis is limited because of the extensive chemical separation and sample preparation procedures that are required [5–9].

Kinetic phosphorescence analysis (KPA) has been utilized for uranium determination in urine and water samples [4,6,9–11]. The KPA technique is rapid and accurate with an easily achievable detection limit of 0.005 μg l^{-1} for uranium [4,6,11]. However, the use of KPA for analysis of uranium in biological samples has been limited because of interference from endogenous substances present in biological samples. The organic components of biological samples cause quenching of the luminescence associated with the uranyl ion. Quenching can compete with phosphorescence by shortening the excited-state lifetime and reducing the luminescence intensity, thus affecting the specificity, sensitivity and accuracy of the technique. The KPA technique has been successfully utilized for uranium determination in urine samples because interference from endogenous compounds in urine can be eliminated by simple sample preparation. However, this technique has never been used for uranium analysis in complex biological matrices.

The primary objective of this study was to develop a sensitive, precise and accurate method for the determination of uranium concentration in biological samples utilizing the KPA technique. This analytical method has been fully validated and has been applied to the analysis of tissue samples obtained after administration of uranium to rodents.

2. Material and methods

Nitric acid, hydrogen peroxide, uranium octoxide (U_3O_8), sodium phosphate monobasic, and potassium phosphate monobasic were obtained from J.T. Baker (Phillipsburg, NJ). Human plasma was supplied by Biological Speciality (Colmar, PA). All chemicals were reagent grade. The water used for reagent preparation, and glassware cleaning and rinsing was double distilled demineralized water. A kinetic phosphorescence analyzer model KPA-11 manufactured by Chemchek Instruments (Richland, WA) was utilized in this study. Uraplex™, was also supplied by Chemchek Instruments. The KPA-11 is equipped with a nitrogen laser source manufactured by Laser Science (Newton, MA) and the stilbene-420 dye was obtained from Exciton (Dayton, OH). A muffle furnace was supplied by Barnstead/Thermolyne (Dubuque, IA). Male Wistar rats weighing 100-150 g were purchased from Simonsen Laboratories (Gilroy, CA), and maintained on a 12 h light/dark cycle with Purina chow pellets and water ad libitum for at least 7 days before use in experiments. Rats were kept 3 per cage in a temperature and humidity controlled environment.

Since naturally occurring uranium can leach from glass, all glassware was boiled in 4 M nitric acid for 48 h to remove any leachable uranium. The glassware was then rinsed with deionized water and dried. Plasticware was boiled in deionized water for 48 h and then rinsed and dried to remove potential interference from leachable plasticizers.

2.1. Principle of KPA

The principle of KPA has been described previously in detail by Bushaw [4] as well as others [6,10–12]. Briefly, the KPA is a computer-controlled, luminescence analyzer that utilizes a pulsed nitrogen laser (3 ns pulse duration, 20 pulses s^{-1}) coupled with a stilbene 420 dye. This provides an excitation wavelength of 420 nm which is close to the uranyl ion absorption maximum of 415 nm. This laser source is used to excite aqueous solutions of uranium in reference and sample cells. The complexing agent Uraplex™ is added to each sample to minimize quenching by solvent molecules, and, thus, increasing the intensity of the longer lifetime phosphoresce. Following termination of each laser pulse, the luminescence intensity is measured every 13 μs. Luminescence intensities measured at each time point after each laser pulse are accumulated over the number of laser pulses used in the measurement to obtain the luminescence decay curve. In the current study, 1000 laser pulses were used for each measurement, for total sample analysis time of approximately 50 s.

The kinetic analysis of the luminescence intensity measurements depends on the fact that the luminescence decay of the excited uranyl complex follows first-order kinetics [10]. This means that the intensity of the uranyl luminescence emission declines exponentially after excitation with each laser pulse and log luminescence intensity is a linear function of time. Log luminescence intensities measured after each laser pulse are fitted by least squares regression to determine the decay profile curve. The first four measurements (taken over 52 μs) after termination of each laser pulse are not used in the calculations to eliminate the effect of emission from short-lived luminescence sources on the phosphorescence kinetic analysis. The luminescence intensity at time zero after termination of the laser pulse (the initial luminescence intensity), is proportional to the uranium concentration in the sample. The initial luminescence intensity is determined from the y-intercept of the luminescence decay profile. The calibration curve is constructed between the initial luminescence intensities of different standard solutions, and uranium concentrations. The initial luminescence intensity obtained during the analysis of unknown sample is used to estimate its uranium concentration from the calibration curve.

2.2. Uranium calibration curves

Uranium octoxide was dissolved in 0.82 M nitric acid to prepare the uranium standard solutions used to construct the calibration curves. Two different series of calibration standards were prepared to cover a wide range of uranium concentrations. Uranium concentrations in the first series of standards were 0.05, 0.1, 0.5, 1, 5, 10 and 20 μg l^{-1}, while that of the second series were 20, 200, 500 and 1000 μg l^{-1}. These two sets of standards were used to construct the calibration curves for the low and high ranges of uranium concentrations.

2.3. Sample preparation

Standard 20 ml glass liquid scintillation vials with screw caps were used to contain the samples during processing. Tissue samples were initially dried in a muffle furnace at 100°C for 24 h. Samples were then dried at 300°C for another 24 h, then at 600°C for an additional 48 h. This gradual increase in the drying temperature was necessary to avoid sample loss due to foaming during the drying process. Samples were wet-ashed with 10 ml concentrated nitric acid and 200 μl 30% hydrogen peroxide. The acid–sample mixture was heated to just below boiling to avoid liquid splashing, until complete evaporation. Urine and plasma samples did not require the initial dry-ashing step, and they were wet-ashed directly with 5 ml nitric acid and 200 μl 30% hydrogen peroxide. After wet-ashing, urine, plasma, and tissue samples were dry-ashed at 600°C for 8–12 h and then wet-ashed with nitric acid and hydrogen peroxide for a second time. After the second wet-ashing, the residue in each vial was dissolved in 0.82 M nitric acid and was analyzed by KPA.

2.4. Sample analysis

For each of the calibration standards, 1 ml standard solution and 1.5 ml Uraplex™ were added to the sample cell. The luminescence decay profile was determined for all the standard solutions. The calibration curve was constructed between the initial luminescence intensity and uranium concentration. Two-calibration curves were constructed to cover the low and high ranges of uranium concentrations. A blank sample was used to determine the background and reagent uranium concentration, and this was subtracted from all KPA measurements. Unknown samples were analyzed by adding 1 ml sample residue solution in nitric acid to 1.5 ml Uraplex™ and determination of the luminescence decay profile. The initial intensity of the luminescence decay profile of the unknown sample was used to estimate the uranium concentration in the sample using the appropriate calibration curve depending on uranium concentration in the sample. This assay was validated by determining the specificity, reproducibility, sensitivity, precision and accuracy of the method in the analysis of uranium concentration in different tissue samples.

2.5. Application of the assay

This assay was used to study uranium biodistribution in the rat after intraperitoneal (i.p.) uranium administration. In this experiment, 36 male Wistar rats weighing 100–150 g were used. Each rat received a single i.p. dose of 0.1 mg kg^{-1} uranium contained in Sorenson's buffered saline (pH 6.0). A group of six rats were sacrificed on day 16. These control rats were used to determine the background uranium tissue concentrations. Rats were sacrificed with an overdose of i.p. pentobarbital. Samples collected from each animal included liver, kidney, spleen, femur, and sternum. Samples were weighed and were kept at −20°C until analysis.

3. Results and discussion

The KPA technique has been used previously for uranium determination in water, urine, milk and soil samples [6,9–12]. However, this is the first report of the application of this technique in the analysis of uranium in more complex biological matrices. Although the sample preparation procedure takes a few days because of the repeated wet-ashing and dry-ashing steps, large numbers of samples can be analyzed simultaneously. This is because only small tissue samples are needed for the analysis due to the high sensitivity of the KPA technique. Typically we analyzed 80 samples during each analysis run.

3.1. Assay characteristics

3.1.1. Specificity

The specificity of the assay for uranium analysis was determined by examining the lifetime and the linearity of the luminescence decay curve for each sample. Interfering substances in the sample cause quenching which can shorten the lifetime of the excited uranyl ion and decrease its luminescence intensity. The lifetime of the luminescence decay curve of uranium samples treated with Uraplex™ are typically more than 200 μs [10,11]. All calibration standards and tissue samples analyzed utilizing the current method exhibited lifetimes well above 200 μs. The other criterion used for the determination of the specificity of the assay was the linearity of the log luminescence intensity versus time plot. Interference with the uranium assay results in loss of the linearity of this plot. The analysis was accepted only if the correlation coefficient of the log intensity versus time plot was $>$ 0.98 which is a good indication for the linearity of the decay profile. These two criteria were met in the analysis of all calibration standards and tissue samples, which indicates that the sample preparation procedures utilized in this assay can minimize interference due to the matrix of the sample.

3.1.2. Reproducibility

The reproducibility of the KPA instrument was determined by analyzing one set of standards in

Table 1
The analytical precision of uranium assay calibration

Uranium concentration ($\mu g\ l^{-1}$)	Within-run ($n = 4$)[a]		Run-to-run ($n = 5$)[b]	
	Initial luminescence intensity[c] ($\times 10^{-3}$) Mean ± S.D.	CV (%)	Initial luminescence intensity[c] ($\times 10^{-3}$) Mean ± S.D.	CV (%)
0.05	1.3 ± 0.082	6.3	1.37 ± 0.089	6.5
0.1	2.6 ± 0.125	4.8	2.65 ± 0.106	4.0
0.5	12.4 ± 0.312	2.5	12.7 ± 0.246	1.9
1.0	24.6 ± 0.565	2.3	25.2 ± 0.479	1.9
5.0	121 ± 1.73	1.4	123 ± 3.8	3.1
10.0	237 ± 2.58	1.1	242 ± 7.4	3.1
20.0	464 ± 6.5	1.4	469 ± 12.8	2.7
Slope	23.3 ± 0.3	1.3	23.5 ± 0.671	2.9
20	5.46 ± 0.12	2.1	5.41 ± 0.174	3.2
200	54.8 ± 1.66	3.0	54.2 ± 1.24	2.3
500	137 ± 3.2	2.3	134 ± 3.36	2.5
1000	267 ± 4.7	1.7	261 ± 6.61	2.5
Slope	0.267 ± 0.005	1.7	0.261 ± 0.007	2.6

[a] Annalyzed on the same day.
[b] Analyzed over a period of 60 days.
[c] The initial luminescence intensity (The y-intercept of the luminescence decay curve).

the range 0.05–1000 $\mu g\ l^{-1}$ four times. The variability in the measured initial luminescence intensity within each concentration during the four analyses was used to determine the reproducibility of the instrument. The coefficient of variation (CV) for all concentrations ranged from 0.25–2.1%, indicating very good reproducibility for the KPA instrument.

3.1.3. Sensitivity

The assay sensitivity criteria were calculated for each of five different calibration curves prepared over a period of 45 days, using the method described by Oppenheimer et al. [13]. The critical limit is the assay response above which an observed response is reliably recognized as detectable, and it is defined as the upper 95% confidence interval for the predicted value of a blank sample. The type I error rate, at the critical limit, for predicting the presence of uranium when the sample is actually blank will be 5%. The estimated critical limit was $0.012 \pm 0.002\ \mu g\ l^{-1}$. The detection limit which is the actual assay response which may a priori be expected to lead to detection was $0.024 \pm 0.004\ \mu g\ l^{-1}$. The detection limit is a more conservative estimate than the critical limit because it protects against both type I error which concludes the presence of uranium when there is none and type II error which concludes the absence of uranium when there is some. The determination level which is the concentration which can be measured with CV of 10% was $0.046 \pm 0.011\ \mu g\ l^{-1}$. The reported values are the mean ± S.D. of the assay limits calculated from five different calibration curves.

3.1.4. Linearity and precision

The calibration curves obtained during the analysis of uranium were linear in the range of concentrations they cover. Within-run precision of the assay calibration was determined by analysis of four different sets of standard solutions on the same day. Run-to-run precision of the assay calibration was determined from the calibration curves prepared on each of five different days during 45 days. The precision of the calibration was determine from the variability in the measured initial luminescence intensity at each con-

centration (Table 1). The CV for within-run precision ranged from 1.1–6.3% and the CV for run-to-run precision ranged from 1.9–6.5% which indicates very good assay precision.

The precision of the analysis of uranium in various tissues was determined by spiking blank human urine (5 ml), human plasma (2 ml), ground beef (1 g), beef bone (1 g), and beef liver (0.5 g) with 0, 5, 10, 50, 100 and 200 ng of uranium. Within-run precision for the analysis of uranium in different tissue matrices was determined by running four different sets of each uranium concentration in each matrix simultaneously in the same run. The run-to-run precision for the analysis of uranium in different tissue matrices was determined by preparing five different sets of tissue matrices spiked with uranium on five different days, and analyzing them on five different runs over a period of 60 days. The precision of the assay was determined from the variability of the measured amounts of uranium in each sample which is the sum of the background uranium in tissue and the added uranium (Table 2). The CV for the precision for the analysis of uranium in tissue samples was different in various tissues. The highest variability was observed in the tissues that have a high uranium background level such as bone, when spiked with low uranium concentration.

3.1.5. Accuracy

The accuracy of the assay was determined from the analysis of uranium quality control samples. Quality control samples were prepared by spiking different rat tissue in triplicate with 1, 5, 10 and 50 ng of uranium. These samples were stored at −20°C until analysis. Quality control samples were treated as unknown samples and were analyzed over a period of 90 days. The assay accuracy was determined by comparing the measured uranium content of the quality control samples with the nominal amount of uranium added to each sample (Table 3).

The assay precision was determined for the analysis of the calibration standards, and for the analysis of tissue samples spiked with known amounts of uranium. Because the size of the samples was different for different tissue, we used the total amount of uranium per sample in our analysis to facilitate the comparison between different tissue. The observed accuracy and precision during the analysis of tissue samples suggest that the sample preparation utilized in this analysis was successful in minimizing the matrix effect on uranium determination in different tissue samples using the KPA technique. This also validates the use of aqueous uranium solutions as calibration standards to determine the uranium concentrations in unknown tissue samples.

The developed method was successful in the determination of uranium content in all tissues except whole blood samples. Even after extensive dilution of samples, neither the luminescence decay lifetime nor linearity of the decay profile were within the acceptable range (200–300 μs for lifetime and correlation coefficient of >0.98 for linearity). It has been reported that iron at concentration of 25 mg l^{-1} or above can interfere with the KPA analysis [11]. This suggests that the high iron content in whole blood samples is probably responsible for the difficulties we experienced with the analysis of whole blood samples for their uranium content. Alternatively when plasma samples were analyzed, the luminescence decay lifetime and linearity were well within the acceptable limits. Analysis of large liver samples in the range of 5 g also resulted in unacceptable luminescence decay lifetime and linearity. This problem was eliminated by analyzing smaller samples of liver (0.5 g) and calculating the total liver burden by multiplying by the total liver weight assuming homogenous distribution of uranium in the liver. Analysis of four different samples of 0.5 g liver taken from different lopes of the liver from a control rat and a rat after 1 day of i.p. uranium administration showed coefficient of variation of less than 10%, indicating homogenous distribution of uranium in the liver.

The tissue used for the determination of the assay precision were beef tissue. We did not use rat tissue to avoid the use of large numbers of animals just to obtain the tissue necessary for the assay validation. The highest variability in the accuracy and precision of this assay was observed in the tissue that contained high background uranium levels such as bone. The background ura-

Table 2
Analytical precision of uranium determination in biological samples

Uranium added (ng)	Within-run ($n=4$)[a]		Run-to-run ($n=5$)[b]	
	Measured uranium[c] (ng) mean ± S.D.	CV (%)	Measured uranium[c] (ng) mean ± S.D.	CV (%)
Urine (5 ml)				
0.00	4.8 ± 0.21		3.15 ± 1.70	
5.0	9.84 ± 0.596	6.1	8.21 ± 1.55	18.9
10.0	15.3 ± 0.338	2.2	13.3 ± 1.87	14.1
50.0	58.3 ± 0.23	0.4	52.9 ± 5.31	10.0
100.0	110 ± 0.411	0.4	103 ± 8.18	7.9
200.0	211 ± 2.22	1.1	199 ± 16.7	8.4
Plasma (2 ml)				
0.00	3.16 ± 0.373		2.21 ± 1.58	
5.0	8.62 ± 0.603	7.0	7.48 ± 2.00	26.8
10.0	13.8 ± 0.059	0.4	11.7 ± 2.7	23.1
50.0	57.1 ± 0.648	1.1	56.0 ± 6.49	11.6
100.0	107 ± 1.65	1.5	104 ± 14.5	14.0
200.0	202 ± 2.4	1.2	208 ± 23.5	11.3
Soft tissues (1 g)				
0.00	1.41 ± 0.297		1.00 ± 0.500	
5.0	6.20 ± 0.221	3.6	6.36 ± 0.718	11.3
10.0	11.5 ± 0.075	0.7	11.8 ± 1.04	8.9
50.0	52.4 ± 0.728	1.4	53.5 ± 1.90	3.6
100.0	103 ± 1.27	1.2	105 ± 1.95	1.9
200.0	200 ± 2.28	1.1	205 ± 4.2	2.0
Bone (1 g)				
0.00	13.0 ± 0.863		11.1 ± 5.8	
10.0	21.2 ± 1.42	6.7	19.7 ± 7.50	38
50.0	62.6 ± 1.62	2.6	62.8 ± 6.30	10.0
100.0	111 ± 2.89	2.6	113 ± 5.01	4.4
200.0	203 ± 2.48	1.2	208 ± 5.83	5.8
Liver (0.5 g)				
0.00	0.791 ± 0.12		1.53 ± 0.75	
5.0	5.84 ± 0.263	4.5	6.20 ± 0.560	9.0
10.0	10.8 ± 0.326	3.0	11.5 ± 0.403	3.5
50.0	53.8 ± 0.878	1.6	54.6 ± 1.58	2.9
100.0	106 ± 2.45	2.3	1.5 ± 3.72	3.5
200.0	198 ± 2.23	1.1	197 ± 11.9	6.1

[a] Analyzed on the same day.
[b] Analyzed over a period of 60 days.
[c] Total uranium content (background + added uranium).

nium content of beef bone used in the assay validation was approximately 13.0 ± 0.86 ng g^{-1}. This high background level resulted in high variability in the analysis of bone samples spiked with small amounts of uranium. Also, it has been reported that uranium is initially distributed into all surfaces of the bone, then it diffuses very slowly into the bone volume [14,19,20], resulting in heterogeneous distribution of uranium in bone. It is possible that the background uranium level in the beef bone samples used in the assay validation was variable due to the heterogeneous uranium distribution in bones. This may have been the reason for the relatively larger variability in the

Table 3
Accuracy in the analysis of uranium quality control samples

Uranium added (ng)	Recovered uranium (ng) mean ± S.D. ($n = 3$)[a]	Nominal amount (%)	CV (%)
Urine (5 ml)			
1.0	1.34 ± 0.02	134	1.5
5.0	5.49 ± 0.58	109	10.5
10.0	10.2 ± 0.15	102	1.5
50.0	53.2 ± 1.05	106	2.0
Plasma (2 ml)			
1.0	1.07 ± 0.15	107	13.8
5.0	5.33 ± 0.66	106	12.4
10.0	9.02 ± 0.33	90	3.7
50.0	48.9 ± 0.35	97	0.7
Spleen[b]			
1.0	1.01 ± 0.18	101	18.1
5.0	4.92 ± 0.22	98	4.6
10.0	10.4 ± 0.15	104	1.4
50.0	53.9 ± 1.91	107	3.5
Liver (0.5 g)			
1.0	1.06 ± 0.18	106	17.2
5.0	4.45 ± 0.23	89	5.3
10.0	10.2 ± 0.22	100	3.3
50.0	54.1 ± 0.65	108	1.2
Femur[b]			
1.0	1.00 ± 0.07	100	7.5
5.0	4.43 ± 0.26	89	6.0
10.0	9.40 ± 0.35	94	3.7
50.0	51.6 ± 1.3	103	1.4

[a] Analyzed over a period of 90 days.
[b] The entire rat tissue/organ was used in the analysis.

analysis of bone samples spiked with smaller amounts of uranium. The low variability in the analysis of the quality control samples indicated that the developed assay can be used for the determination of uranium bone content with very good accuracy.

3.2. Application of the uranium assay

The developed assay was utilized to measure uranium concentrations in tissue samples obtained after i.p. administration of uranium to rats. The method was sensitive enough to measure background or endogenous uranium concentrations in all tissue in the control animals. These values were used as uranium concentrations in tissue at time zero. After i.p. uranium administration, uranium concentrations increased gradually in all tissue. Uranium concentrations expressed as ng g^{-1} tissue weight were highest in the spleen > kidney > liver > femur > sternum. Uranium tissue concentrations did not decrease significantly during the 90 days after uranium administration (Fig. 1). These observations are not fully consistent with earlier studies of the uptake and distribution of uranium in various mammals, including, rats, rabbits, dogs and humans [14–18]. However, the data in these earlier studies were obtained with less sensitive and generally less accurate and precise methods. The results of our study show that the KPA technique coupled with the described sample preparation method provides a sensitive,

accurate and precise assay for the determination of uranium in all biological samples. The analysis of the pharmacokinetics and tissue distribution of uranium will be discussed in detail in a forthcoming paper.

4. Conclusion

In summary, the described sample preparation procedures coupled to the KPA technique represent a specific, precise and accurate method for the determination of uranium in biological samples. We were not able to compare the performance of the developed method with other techniques such as ICP-MS or ICP-AES because none of these methods have been validated for uranium determination in tissue samples. The current method uses a small sample size which allows simultaneous analysis of large numbers of samples. The limit of sensitivity of this analytical method is well below the background uranium levels in most tissue, which makes this procedure suitable for the determination of uranium content in virtually any biological sample.

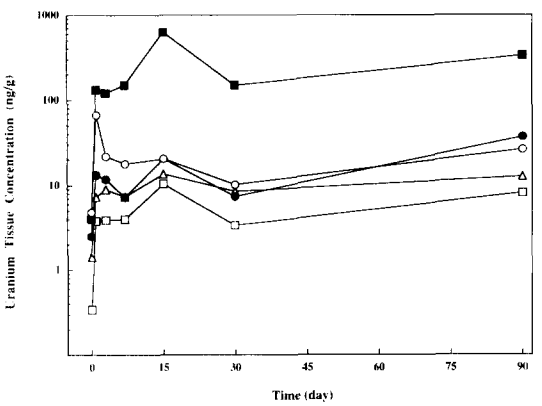

Fig. 1. Uranium concentration time profile in the (●) liver, (○) kidney, (□) femur, (■) spleen, and (◁) sternum of the rats after administration of a single intraperitoneal dose of 0.1 mg kg $^{-1}$ uranium in rats. Each point is the mean concentration in six rats.

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