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Optimal sample preparation conditions for the determination of uranium in biological samples by kinetic phosphorescence analysis (KPA)

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Abstract

Kinetic phosphorescence analysis (KPA) is a proven technique for rapid, precise, and accurate determination of uranium in aqueous solutions. Uranium analysis of biological samples require dry-ashing in a muffle furnace between 400 and 600°C followed by wet-ashing with concentrated nitric acid and hydrogen peroxide to digest the organic component in the sample that interferes with uranium determination by KPA. The optimal dry-ashing temperature was determined to be 450°C. At dry-ashing temperatures greater than 450°C, uranium loss was attributed to vaporization. High temperatures also caused increased background values that were attributed to uranium leaching from the glass vials. Dry-ashing temperatures less than 450°C result in the samples needing additional wet-ashing steps. The recovery of uranium in urine samples was $99.2 \pm 4.02\%$ between spiked concentrations of 1.98–1980 ng (0.198–198 μg l^{-1}) uranium, whereas the recovery in whole blood was $89.9 \pm 7.33\%$ between the same spiked concentrations. The limit of quantification in which uranium in urine and blood could be accurately measured above the background was determined to be 0.05 and 0.6 μg l^{-1}, respectively. © 2000 Published by Elsevier Science B.V.

Keywords: Uranium analysis; Kinetic phosphorescence analysis; Depleted uranium; Blood; Urine

1. Introduction

During the Persian Gulf War many soldiers were injured and/or exposed to depleted uranium (DU). DU is made by depleting uranium of U^{234} and U^{235} during the uranium enrichment process. Because of the DU density, availability, and low relative cost, it has been incorporated into both projectiles and armor by the military of the United States. Soldiers may have inhaled airborne DU particles, ingested DU particles, and/or experienced wound contamination by DU. Many of

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the toxicological properties of uranium have been previously reported [1,2]. The exposure of soldiers to DU has resulted in renewed interest in toxicology studies of uranium. Initial studies on uranium production of free radical mechanisms and cell transformation have been reported [3–5]. With increased interest in uranium toxicology, the need to measure uranium fast, accurately, and inexpensively in biological samples has gained great interest.

Determination of uranium in environmental, geological, and biological fields are currently being accomplished by several methods. These methods for uranium analysis include inductively coupled plasma-mass spectroscopy (ICP-MS), inductively coupled plasma emission spectroscopy (ICP-AES), α-spectroscopy, spectrophotometry, fluorometry, and kinetic phosphorescence analysis (KPA) [6–15]. All these methods require complex sample preparation procedures to achieve accurate results. Furthermore, biological samples require extensive sample preparation procedures to eliminate organic matrixes that interfere with measurements and affect the accuracy of uranium analysis.

KPA is an inexpensive, sensitive, fast, and accurate method for the determination of uranium with a detection limit of 0.005 μg l^{-1} [14]. The KPA technique has been utilized for uranium determination in biological tissues and fluids [15]. Good reproducibility of the KPA has been shown by the fact that uranium standards measured have a coefficient of variation (CV) from 0.25 to 2.1% [7]. However, extensive sample preparation procedures are required to digest the organic matrix present in biological samples. The organic and inorganic components in biological samples can alter the luminescence of the uranyl ion. These components can prevent and quench the luminescent property associated with the uranyl ion affecting the accuracy of the KPA technique.

The primary objective of this study was to develop a sample preparation method for biological samples that minimized uranium loss and quenching for the KPA technique. It is necessary and common practice in various laboratories to digest the biological matrix by heat, also referred to as dry-ashing. Samples can be processed faster by increasing the temperature during dry ashing; however, uranium loss will occur as the temperature is increased due to vaporization. This can affect the accuracy of the technique by lowering the uranium content in the samples. The procedures reported in this work have been fully validated by applying the analysis methods to biological fluids containing known amounts of uranium.

2. Materials and methods

Nitric acid (TraceMetal grade) was obtained from Fisher Scientific Co. (Fair Lawn, NJ). Hydrogen peroxide (semiconductor grade) and uranium atomic absorption standard solution were obtained from Aldrich Chemical Co. (Milwaukee, WI). Uraplex was obtained from Chemchek Instruments (Richland, WA). Deionized water from a Sybron Barnstead NANO pure system was used for all solutions. A kinetic phosphorescence analyzer model KPA-11 manufactured by Chemchek Instruments with a Gilson 222 Sample Changer and 401 Dilutor autosampler was utilized in this study. Dry-ashing was carried out in an Isotemp Programmable muffle furnace obtained from Fisher Scientific Co.

2.1. Principle of KPA

The principle of KPA has been described previously in detail by others [6]. Briefly, the KPA is a technique that measures the emission of phosphorescence from the uranyl ion as a function of time. The uranyl ion is excited at 420 nm with a nitrogen dye laser (3 ns pulse duration, 20 pulses per s). The emission of luminescence is filtered and measured at 90° to the laser pulse using a photomultiplier tube. In this study, 1000 laser pulses were used for each measurement. The emission intensity is measured every 13 μs for 1.651 ms. To eliminate fluorescence via luminescence leaving only the phosphorescence component, the first 65 μs are removed from the decay profile. The data from 65 to 585 μs is analyzed using first order-decay kinetics. The phosphorescence intensity at time zero is determined from the y-intercept of

the plot log phosphorescence decay versus time. The initial phosphorescence intensity versus uranium concentration is used to construct a calibration curve.

2.2. Sample preparation

The biological samples were processed in 20-ml glass scintillation vials. Samples were initially dried in an oven at 120°C for 24 h. Blood samples were further dried in an oven at 200°C for an additional 24 h. Samples were then dry-ashed in a muffle furnace at 300°C for 24 h, then at 450, 500 or 600° C for 4 h. After dry-ashing, samples were wet-ashed with 2-ml concentrated nitric acid and 0.5 ml of 30% hydrogen peroxide. The mixture was heated to just below boiling until complete evaporation. The samples were cooled and wet-ashed three more times in the same manner. The samples were further dry-ashed at 450, 500 or 600°C for 4 h. The samples were then wet-ashed four more times with 2-ml concentrated nitric acid and 0.5-ml hydrogen peroxide as described above. If the samples were not completely ashed by visual inspection at this point, they were dry-ashed at 450, 500 or 600°C for an additional 4 h and wet-ashed two more times. At completion, the samples consisted of a white residue that was dissolved in 5 ml of 1 M nitric acid for KPA analysis.

2.3. Sample analysis

Uranium standard solution traceable to a NIST uranium standard was diluted to 0.099, 0.99, 9.9, 99, and 990 μg l^{-1} in 1 M nitric acid by serial dilutions. The KPA instrument has a low and high calibration range to cover a wide range of uranium concentrations. The low series of standards used were 0.099, 0.198, 0.495, 1.98, 4.95, 9.90, and 19.8-ng uranium in 5-ml 1 M nitric acid while the high series of standards were 19.8, 49.5, 99.0, 198, 495, 990, and 1980-ng uranium in 5-ml 1 M nitric acid.

A Gilson 222 Sample Changer and 401 Dilutor autosampler was used for calibration and sample analysis. The autosampler mixes 1 ml of standard or sample solution to 1 ml of Uraplex and injects 1.8 ml into the KPA's flow cell. A sample of 1 M nitric acid was used as a blank to determine the background and reagent uranium concentration. The blank's phosphorescence intensity was subtracted from all KPA uranium measurements. The calibration curves for the low and high ranges were constructed correlating the initial phosphorescence intensity and uranium concentration.

3. Results and discussion

The KPA technique has been used previously for uranium determination in biological samples. The instrumentation has been proven to be sound [4]. However, this is the first extensive report on sample preparation and luminescence lifetimes associated with the KPA technique in biological samples. Although the sample preparation requires a few days, a large number of samples can be prepared simultaneously. Between 50 and 100 samples are typically analyzed each day.

3.1. Ashing effects on standards

The wet-ashing and dry-ashing effects were studied using five replicates of uranium standards over the entire calibration range (Table 1). Standards were wet-ashed eight times as biological samples would be during normal preparation. Because all samples and standards were dissolved in 5 ml of 1 M nitric acid, the total amount of uranium (0.099–1980 ng) added per sample was used in the analysis results. Minimal effects were observed from wet-ashing by the fact that the average recovery was $101 \pm 4.26\%$ for uranium concentrations between 1.98–1980 ng (Table 1). Another five sets of standards were wet-ashed eight times and dry-ashed three times at 500°C as normal sample preparation. Dry-ashing at 500°C caused a significant loss of uranium. The dry-ashed standards containing 1.98–1980 ng of uranium are shown in Table 1 and have a recovery of $75.7 \pm 24.4\%$. Since the results in Table 1 show that wet-ashing alone does not produce any loss of uranium, it can be concluded that the observed loss occurs during the dry-ashing procedure and is

Table 1
Recovery of uranium standards

Uranium added (ng)	Wet-ashed			Wet and dry-ashed		
	Uranium[a,b] measured (mean ± S.D.)	Recovery (%)	CV (%)	Uranium[a,b] measured (mean ± S.D.)	Recovery (%)	CV (%)
0.00[c]	0.20 ± 0.11			0.19 ± 0.12		
0.099	0.08 ± 0.05	84.4	59.1	0.12 ± 0.08	120	67.2
0.198	0.23 ± 0.06	117	25.4	0.25 ± 0.08	124	32.4
0.495	0.53 ± 0.07	106	12.8	0.29 ± 0.10	59.1	32.7
1.98	2.10 ± 0.23	106	11.0	1.59 ± 0.13	80.3	8.15
4.95	4.89 ± 0.16	98.9	3.35	3.38 ± 0.87	68.3	25.8
9.90	10.3 ± 1.16	104	11.3	6.53 ± 2.42	66.0	37.0
19.8	19.6 ± 0.15	98.8	0.77	15.9 ± 2.29	80.3	14.4
49.5	47.1 ± 1.25	95.1	2.66	2.95 ± 10.4	59.5	35.3
99.0	98.8 ± 2.63	99.3	2.67	76.7 ± 13.8	77.5	18.0
198.0	202 ± 5.10	102	2.52	166 ± 25.7	83.9	15.5
495.0	501 ± 15.2	101	3.03	384 ± 98.9	77.5	25.8
990.0	1010 ± 24.2	102	2.40	696 ± 407	70.3	58.4
1980.0	1990 ± 59.0	101	2.96	1850 ± 105	93.6	5.64
Low[d]		103	32.4		101	44.1
Low[e]		102	6.59		73.7	21.3
High[f]		100	2.43		77.5	24.7
Low[e] and High[f]		101	4.26		75.7	24.4

[a] Uranium content minus background (added uranium-background).
[b] $n = 5$.
[c] The background value is 0.00.
[d] Averages of low calibration range between 0.0198 and 0.099 μg l^{-1} (0.099, 0.198, and 0.495 ng uranium).
[e] Averages of low calibration range between 0.396 and 3.96 μg l^{-1} (1.98, 4.95, 9.9, and 19.8 ng uranium).
[f] Averages of high calibration range between 3.96 and 396 μg l^{-1} (19.8, 49.5, 99.0, 198, 495, 990, and 1980 ng uranium).

possibly due to vaporization of uranium in the sample. This result is expected by the fact that all metals vaporize with increasing temperatures. This is the basic principle for atomic spectroscopy. In order to investigate the effect of temperature on uranium vaporization in the processing procedure, two sets of standards in triplicate were studied over a range in temperature between 200 and 600°C (Fig. 1). The first set of standards were dry-ashed and the second set of standards were initially wet-ashed four times and then dry-ashed at temperatures between 200 and 600°C. At temperatures greater than 400°C, uranium loss was observed in both sets (Fig. 1). Initial wet-ashing did increase the recovery of uranium from 89 to 93% and 51 to 79% at 500 and 600°C, respectively. The concentrated nitric acid and hydrogen peroxide added during wet-

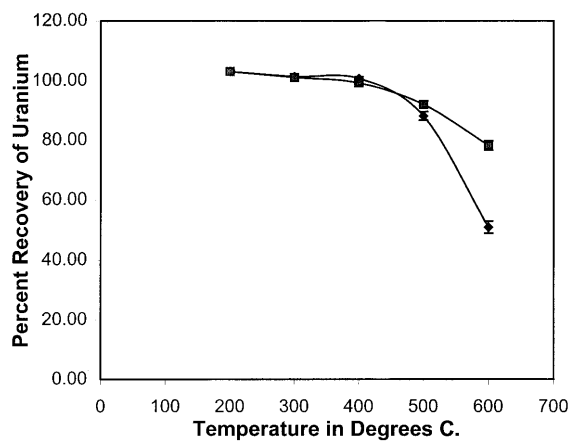

Fig. 1. The percent recovery of uranium vs. temperature from standards (◆) dry-ashed and (■) wet and dry-ashed. Bars represent mean ± S.E.M. ($n = 3$).

ashing acted as matrix modifiers, which prevented analyte loss during the ashing step by converting the analyte to a less volatile form.

3.2. Recovery of uranium from urine

The temperature effect of dry-ashing urine samples was studied at 450, 500 and 600°C (Table 2). The uranium recovery experiments were calculated from five replicates of spiked urine samples. Samples were prepared by adding 10 ml of urine to vials in which uranium standards had been dried. Background samples were 10 ml of urine with no uranium added to the vial. The samples were ashed as described in the methods and analyzed as unknown samples. The assay accuracy was determined by comparing the measured uranium content of the samples minus the background with the nominal amount of uranium added to each sample. The observed accuracy for the urine samples was $>95\%$ recovery for uranium concentrations >0.495 ng (Table 2). The limit of quantification was determined to be greater than 0.5 ng based on the high coefficients of variation $>20\%$ below this point. Since the sample size was 10-ml urine, the detection limit for urine was 0.05 ng ml^{-1} or 0.05 μg l^{-1}. The standards were categorized into four sets based on concentrations. The first three standards in the low calibration range were averaged because of the high CV of 34.5–733%. The remaining four standards in the low calibration range, the high calibration range, and all the standards above the determined detection limit of 0.5-ng uranium were averaged at each dry-ashing temperature (Table 2). A recovery of $99.2 \pm 4.02\%$ was obtained at 450°C. The increased dry-ashing temperature of 500 and 600°C lowered the recoveries to 95.6 ± 7.62 and $96.0 \pm 2.52\%$ (Table 2). Interestingly, the recovery of the standards at 495, 990, and 1980-ng uranium at 600°C decreased from the average of $96.0 \pm 2.52\%$ to a range between 90.8 and 93.6% (Table 2). Once again the uranium loss from the samples was attributed to vaporization at the high temperatures. The urine samples at 500°C had a 19.9% higher recovery than the uranium standards in Section 3.1 from 75.7 ± 24.4 to $95.6 \pm 7.62\%$. The inorganic material in the urine acts as an additional modifier stabilizing the uranium and preventing its vaporization at high temperatures. The measured background values increased from 0.35 ± 0.40 ng uranium to 4.59 ± 0.31 ng uranium as the temperature was raised from 450 to 600°C (Table 2). There are two possibilities for this observed increase in uranium background concentration measured at high temperatures above 450°C. The first possibility is the deposition into all samples of uranium evaporated from the standards containing the higher uranium concentrations. Since this deposition would be evenly distributed, it would mainly affect the lower concentration standards. The second possibility would be the leaching of uranium from the glass vials at high temperatures (>450°C). To address the first possibility of vaporization, the samples were processed separately. The low concentration standards were done separately from the high concentration standards. These measurements produced the same results of increased background uranium values. This leaves only the second possibility of the uranium leaching out of the glass vials (>450°C) thus yielding the higher background measurement. The increased uranium in the background was attributed to leaching from the glass.

3.3. Analysis of whole blood

Uranium recovery experiments from whole blood were calculated from five replicates of spiked whole blood samples (Table 3). All samples were prepared from the same unit of blood. Samples were prepared by adding 5 ml of whole blood to vials in which uranium standard solutions were dried. The samples were then prepared as described in the methods with dry-ashing done at 500°C. The whole blood samples were treated as unknowns and analyzed. The assay accuracy was determined by comparing the measured uranium content of the spiked blood samples minus the background with the nominal amount of uranium added to each sample.

The developed method was only successful in the determination of uranium content in whole blood after the samples had been diluted with 1 M nitric acid. The manufacturer reports that iron

Table 2
Temperature effect on recovery of uranium in urine samples[a]

Uranium added (ng)	Temperature dried-ashed								
	450°C			500°C			600°C		
	Uranium[b,c] measured (mean ± S.D.)	Recovery (%)	CV (%)	Uranium[b,c] measured (mean ± S.D.)	Recovery (%)	CV (%)	Uranium[b,c] measured (mean ± S.D.)	Recovery (%)	CV (%)
0.00[d]	0.35 ± 0.40			0.57 ± 0.22			4.59 ± 0.31		
0.0990	0.13 ± 0.20	129	154	0.14 ± 0.06	139	41.2	0.02 ± 0.46	23.8	1950
0.198	0.40 ± 0.20	202	50.9	0.33 ± 0.16	164	64.2	0.29 ± 0.27	148	93.4
0.495	0.71 ± 0.21	142	29.1	0.58 ± 0.08	116	14.2	0.40 ± 0.64	81.6	157
1.98	2.00 ± 0.25	101	12.4	1.95 ± 0.30	98.3	15.6	1.92 ± 0.08	96.9	4.37
4.95	4.75 ± 0.27	96.0	5.77	4.77 ± 0.50	96.3	10.4	4.92 ± 0.18	99.4	3.75
9.90	9.88 ± 0.30	99.8	2.99	9.46 ± 0.73	95.5	7.68	9.60 ± 0.16	97.0	1.71
19.8	19.7 ± 1.01	99.3	5.15	18.6 ± 0.64	94.2	3.44	20.4 ± 0.70	103	3.42
49.5	50.0 ± 1.87	101	3.74	46.1 ± 2.57	93.1	5.57	47.7 ± 1.42	96.3	2.97
99.0	99.3 ± 1.21	100	1.21	93.7 ± 4.43	94.7	4.73	95.1 ± 0.80	96.1	1.85
198.0	197 ± 3.27	99.5	1.66	189 ± 13.1	95.6	6.92	187 ± 2.07	94.4	1.11
495.0	489 ± 12.4	98.7	2.54	466 ± 39.4	94.2	8.46	463 ± 3.90	93.6	1.84
990.0	976 ± 25.6	98.6	2.65	960 ± 55.8	96.9	5.82	899 ± 16.4	90.8	1.83
1980.0	1940 ± 38.8	98.0	2.00	1920 ± 145	96.9	7.54	1820 ± 43.2	92.0	2.37
Low[e]		158	78.0		140	34.5		84.7	733
Low[f]		99.0	6.60		96.1	9.28		99.1	3.31
High[g]		99.3	2.71		95.1	6.07		95.2	2.20
Low[f] and High[g]		99.2	4.02		95.6	7.62		96.0	2.52

[a] Volume of urine samples, 10 ml.
[b] Uranium content minus background (uranium measured − background measured).
[c] $n = 5$.
[d] The background value is 0.00.
[e] Average of low calibration range between 0.0198 and 0.099 μg l^{-1} (0.099, 0.198, and 0.495 ng uranium).
[f] Average of low calibration range between 0.396 and 3.96 μg l^{-1} (1.98, 4.95, 9.9, and 19.8 ng uranium).
[g] Average of high calibration range between 3.96 and 396 μg l^{-1} (19.8, 49.5, 99.0, 198, 495, 990, and 190 ng uranium).

Table 3
The recovery of uranium in blood[a]

Uranium added (ng)	No dilution				1/6th Dilution				1/11th Dilution			
	Uranium[b,c] measured (mean ± S.D.)	Recovery (%)	CV (%)	Lt[d] (μs)	Uranium[b,c] measured (mean ± S.D.)	Recovery (%)	CV (%)	Lt[d] (μs)	Uranium[b,c] measured (Mean ± S.D.)	Recovery (%)	CV (%)	Lt[d] (μs)
0.00[e]	0.25 ± 0.07				0.37 ± 0.17				0.22 ± 0.18			
0.099	−0.03 ± 0.10	−32.8	298	94.4	−0.07 ± 0.27	−73.8	371	219	−0.09 ± 0.09	−90.5	101	315
0.198	0.05 ± 0.14	26.5	273	104	0.06 ± 0.24	30.3	415	227	0.10 ± 0.26	50.4	256	265
4.95	0.13 ± 0.08	27.0	58.2	121	0.34 ± 0.26	67.7	76.2	233	0.27 ± 0.34	54.2	126	285
1.98	0.52 ± 0.09	26.5	16.3	142	1.70 ± 0.27	85.7	16.0	223	1.72 ± 0.39	86.7	22.5	244
4.95	1.72 ± 0.64	34.8	37.1	140	4.41 ± 0.47	89.1	10.6	220	4.48 ± 0.32	90.5	7.05	251
9.90	3.38 ± 0.31	34.1	9.20	149	9.51 ± 1.04	96.0	10.9	205	9.57 ± 1.00	96.7	10.5	253
19.8	6.11 ± 0.40	30.9	6.48	144	17.9 ± 1.01	90.5	5.66	217	18.6 ± 0.67	94.1	3.62	256
49.5	14.4 ± 0.48	29.2	3.31	146	41.2 ± 2.14	83.3	5.18	210	42.3 ± 2.08	85.5	4.93	255
99.0	29.2 ± 2.15	29.5	7.35	147	83.1 ± 6.58	84.0	7.92	213	86.4 ± 4.93	87.2	5.71	252
198	61.4 ± 2.43	31.0	3.97	147	174 ± 7.58	87.8	4.36	217	168 ± 6.53	84.8	3.89	253
495	154 ± 8.16	31.1	5.30	150	440 ± 17.5	88.9	3.97	217	438 ± 27.6	88.6	6.30	261
990	318 ± 8.36	32.1	2.63	151	915 ± 48.5	92.4	5.30	220	927 ± 32.0	93.6	3.45	259
1980	622 ± 31.9	31.4	5.13	150	1780 ± 97.6	90.0	5.48	220	1810 ± 96.0	91.2	5.31	259
Low[f]		6.90	210	106		8.07	287	226		4.70	161	288
Low[g]		31.6	17.3	144		90.3	10.8	216		92.0	10.9	251
High[h]		30.7	4.88	148		88.1	5.41	216		89.3	4.74	256
Low[g] and high[h]		31.1	9.68	147		88.8	7.54	216		89.9	7.33	254

[a] Volume of blood samples, 5 ml.
[b] Uranium content minus background (added uranium − background).
[c] $n = 5$.
[d] Lifetime of luminescence decay curve.
[e] The background value is 0.00.
[f] Average of low calibration range between 0.0198 and 0.099 μg l^{-1} (0.099, 0.198, and 0.495 ng uranium).
[g] Average of low calibration range between 0.396 and 3.96 μg l^{-1} (1.98, 4.95, 9.9, and 19.8 ng uranium).
[h] Average of high calibration range between 3.96 and 396 μg l^{-1} (19.8, 49.5, 99.0, 198, 495, 990, and 1980 ng uranium).

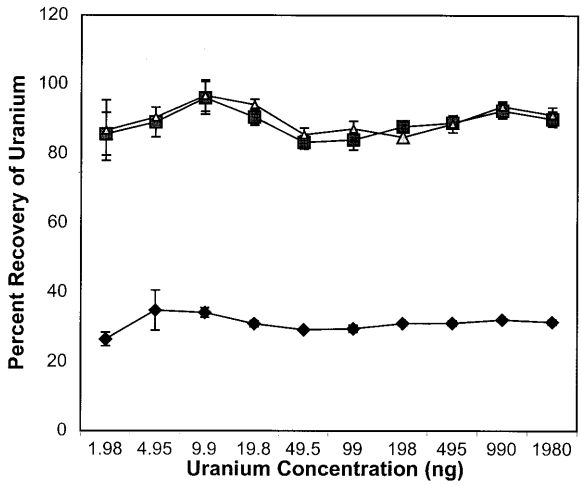

Fig. 2. The percent recovery of uranium from 5-ml blood samples that have (◆) no dilution; (■) 1/6th dilution; and (▲) 1/11th dilution. Bars represent mean ± S.E.M. ($n = 5$).

can interfere with the KPA analysis at concentrations above 0.4–0.5 mM (KPA Manual). The concentration of iron in whole blood was reported to be 7–10 mM [16]. Therefore, by diluting the sample, the iron concentration was reduced between 0.6 and 0.7 mM and acceptable luminescence decay lifetime's limits were achieved (Fig. 2 and Table 3). The recovery of uranium in whole blood was 37% for undiluted samples. The recovery increased however to 88.8 and 89.9% when the samples were diluted with 1 M nitric acid in ratios of 1:6 and 1:11, respectively (Table 3). Along with the increase in recoveries from diluting the samples, the average luminescence lifetimes increased from 147 μs when the samples were not diluted to 254 μs with a dilution ratio of 1:11 (Table 3). Again, the limit of quantification was determined to be > 0.5 ng uranium based on the high coefficients of variation of $> 20\%$ below this point. Since 5 ml of blood was analyzed and the samples requiring a 1:6 dilution, the detection limit for blood was 0.6 ng ml^{-1} or 0.6 μg l^{-1}. The 6% lower recovery in blood versus urine samples is believed to be from unavoidable foaming in the vials during the preparation steps. The blood samples are more difficult to ash due to the fact the samples tend to sputter during dry-ashing.

4. Conclusions

The described sample preparation procedure for KPA analysis was investigated in detail for accuracy. The temperature of the dry-ashing step was the most critical element of the procedure for obtaining the highest recoveries of uranium in a biological matrix. The best dry-ashing temperature was determined to be 450°C based on the fact that significant amount of uranium vaporized above this temperature and caused lower recoveries in the samples. Secondly, significant amounts of uranium leached from the glass vials above 450°C causing uranium measurements in the background and low calibration range to increase significantly.

The current method can be used with any biological tissues or fluids including whole blood as long as the lifetime of luminescent decay curve is greater than 200 μs. If the luminescent decay curve is less than 200 μs, the sample can simply be diluted until an acceptable lifetime is achieved. Starting with smaller sample sizes of 0.5 g or less will result in the ashing procedure to be quicker and prevent the need to dilute the sample to achieve acceptable luminescent lifetimes. Even though the instruments detection limit was 0.005 μg l^{-1} uranium, the limit of quantification in which uranium in urine and blood could be accurately measured above the background was determined to be 0.05 and 0.6 μg l^{-1}, respectively. The higher detection limit is believed to be from increased luminescence originating from the biological matrix. In addition, the higher detection limit for blood is due to the sample size that must be smaller than 1 g or samples larger than 1 g require dilution for acceptable luminescent lifetimes as in this study. Processing larger quantities of sample does not lower the limit of quantification because acceptable luminescent lifetimes are not achieved and the sample once again must be diluted for acceptable luminescent lifetimes.

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