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# Operational Protocol for Detection of Contamination by Actinides U, Pu and Am in Urine Using Calixarene Columns: From Mineralization to ICP-MS Measurement

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## Abstract

Individual monitoring of workers exposed to the risk of intake of actinides requires suitable methods for measuring low level of excreted activity. The current protocols used for actinides analysis in bioassay are usually complicated and highly time consuming. In this work, a protocol based on the microwave digestion of urines followed by the separation of actinides using calix[6]arene-based chromatography columns and their measurement by a quadrupole ICP-MS is developed and validated, for the first time, on urine samples containing the three actinides, U, Pu and Am. With this protocol, the total analysis time is about 2 days, including the mineralization of urine and the chromatographic separation of actinides. Detection limits of actinides in urine are determined and compared to those obtained after "dilute and shoot" ICP-MS analysis or after alpha spectrometry measurement.

## Keywords

Actinides, Urine, Calix[6]Arene, Analysis, ICP-MS

## 1. Introduction

The analysis of actinides is of great importance due to their high radiotoxicity and the severe damages their alpha radiation may induce at the cellular level. After contamination, radiotoxicological analyses of bioassay samples (e.g. urine and feces) are required to detect and quantify the contamination by the actinides and to estimate internal dose related to this actinides intake in the body. The

most widely used technique for determination of alpha emitters (U, Pu, Am) in biological samples is alpha spectrometry. Alpha particles heavily ionize matter and then quickly lose their kinetic energy. Therefore alpha particles deposit all their energies along their short paths in matter. To detect alpha particles emitted by actinides in urine or feces, it is necessary to destroy all the organic matter and to separate the actinides from mineral compounds present in bioassay samples. Furthermore, the measurement of actinides by alpha spectrometry requires the separation of actinides from each other because of their interferences in alpha spectrometry. Therefore the current methods using alpha spectrometry to detect contamination by actinides require mineralization of bioassay samples then separation of actinides on various chromatographic columns and finally long counting times to achieve very low activity level (0.5 mBq/L) [1]. Thus, these procedures are usually tedious and highly time consuming. These last years, new protocols have been developed to respond to radiological/nuclear emergency event with a requirement of high sample throughput [2] [3] [4]. However, detection limits obtained are much higher (50 mBq/L) than those for routine protocols, as reduced sample volume and shorter counting time by alpha spectrometry are used. Previous studies showed that chromatography columns based on calix[6]arene molecules exhibit a good selectivity for actinides in urine matrix. In particular, we demonstrated in previous works that carboxylic or hydroxamic calix[6]arene are excellent extractants of U, Pu, Am, and Th [5] [6] [7] [8]. Their immobilization on an inert solid support, as polymer resin for example, allows to use it as chromatography columns for actinides separation from urine and uranium analysis in drinking water [9] [10] [11]. The combination of two columns was suggested, the hydroxamic calix[6]arene column for the Pu isolation followed by the carboxylic calix[6]arene column for the U/Am separation with an elution step by EDTA. Lastly, a method based on the coupling of the hydroxamic calix[6]arene column to a quadrupole ICP-MS for on-line actinide analysis was developed [12]. In this method, the calixarene column is used for the precon- centration of actinides, whereas the ICP-MS allows the separation of actinides and their on-line detection. All these studies were performed on synthetic samples or urine samples containing a single actinide or both actinides U and Am.

The aim of this work was to optimize all steps from the mineralization of urine samples with microwave oven to the actinides separation with calixarene columns on urine samples containing the three actinides U, Pu and Am and to evaluate the robustness of the optimized protocol on various urine samples. In this paper, the ICP-MS measurement was carried out for the detection of actinides for convenient purposes, but this separation protocol has been developed to be suitable for alpha spectrometry measurement, and the advantages and the drawbacks of both techniques for actinides analysis are also discussed.

## 2. Materials and Methods

### 2.1. Reagents and Instrumentation

$^{239}$Pu and $^{243}$Am were purchased from Eckert & Ziegler (USA) and AEA Tech-

nology (Isotrak QSA Amersham, USA), respectively. Natural uranium was from SPEX Certiprep, Ind. (USA). All chemicals from Aldrich or VWR used in this study were of analytical grade and the water was purified by a Milli-Q® Synergy 185 water purification system from Millipore (Merck). Calixarene molecules were synthesized as described in the patent [13].

Digestion of urine samples were performed with a closed vessel digestion system, the Milestone EthosOne© microwave (Thermo Electron, France) and the analyses of total organic carbon with a Vario TOC cube analyser (Elementar, France).

All the measurements of $^{239}$Pu, $^{243}$Am and $^{238}$U in aqueous phases were performed by Inductively Coupled Mass Spectrometry (ICP-MS) using a quadrupole mass spectrometer "Agilent 7700" (Agilent, France).

## 2.2. Mineralization of Urine Samples

In case of suspected contamination of nuclear workers by actinides, their urines are collected on 24 hours (about 1 L) and analyzed to detect and quantify the contamination. To control the process of urine treatment (mineralization, chromatographic separation) before the actinides measurement, each urine sample is spiked with an isotope of each actinide not initially present in the urine such as, for example, $^{233}$U, $^{242}$Pu and $^{243}$Am.

In our study, urine samples (1000 mL) from people non-exposed to actinides contamination were collected then spiked with 0.1 µg (or 2.5 mBq) of natural uranium, 0.1 µg (or 230 Bq) of $^{239}$Pu and 0.3 ng (or 2.1 Bq) of $^{243}$Am, to control the contamination. After acidifying with 20 mL of 8 M $HNO_3$, the urine was heated under reflux on a hot plate with magnetic stirring for 2 h. In the still warm urine, ammonia water (20 wt%) was added until precipitation of alkali-earth phosphate was observed. The supernatant was discarded and the precipitate was centrifuged and digested by microwave oven before actinides separation with extraction chromatography. In this paper, the microwave digestion was optimized on urine samples spiked with natural uranium. The mineralization efficiency was checked by controlling the extraction yield of uranium from mineralized urine samples on the carboxylic calixarene column (CC column) and comparing it with those obtained after a classical mineralization of urine samples using an ashing step of the alkali-earth phosphate precipitate in a muffle furnace at 1170 K during about 14 h. The influence of nitric acid volume (10 to 50 mL of 67 wt% nitric acid) and hydrogen peroxide volume (2 to 10 mL of 30 wt% hydrogen peroxide) and the effect of the heating profile were investigated. This digested solution is called "mineralized urine".

## 2.3. Chromatographic Separation Procedure with Calixarene Columns

The resin CH used in the hydroxamic calixarene column is composed of $1.78 \times 10^{-5}$ mol of 1, 3, 5-$OCH_3$-2, 4, 6-$OCH_2CONHOH$-*p-tert*-butylcalix[6]arene dissolved in heptanol and immobilized on 1 g polystyrene divinylbenzene (126 µm

diameter), (CG-3000 Rohm & Haas) [13]. The resin CC used in the carboxylic calixarene column is composed of 3.70 × $10^{-5}$ mol of 1, 3, 5-$OCH_3$-2, 4, 6-$OCH_2COOH$-*p-tert*-butylcalix[6]arene dissolved in heptanol and immobilized on 1 g polystyrene divinylbenzene (126 µm diameter), (CG-3000 Rohm & Haas) [13]. CH and CC resins were conditioned with 20% v/v MeOH in 0.04 M $NaNO_3$ for a week before use to make sure the resin is sufficiently and homogeneously moisten to avoid a poor extraction yield on the first use. The CH and CC columns were filled with 1 g of bed of CH resin or CC resin, respectively. The column height was about 4.5 cm. The various solutions were passed through the calixarene columns without applying any pressure. Before the loading of mineralized urine for actinides extraction, CH column was preconditioned with sodium formate buffer at pH 2.7 ± 0.1 and CC column with sodium acetate buffer at pH 6.0 ± 0.1, otherwise stated.

The pH of mineralized urine is increased, using ammonia water, to about 2.7 before its loading on the CH column for Pu extraction. pH values of 3 and 3.5 were also tested for Pu extraction. The CH column was then rinsed with 10 mL of 0.04 M $NaNO_3$ solution adjusted at the same pH than the mineralized urine and the combined effluent (from the loading and the rinsing steps) was retained for uranium and americium separation (solution A). Plutonium was finally eluted with 30 mL of hydrochloric acid solution (2, 3 or 4 M) or with 30 mL of 0.1 M hydroxylammonium chloride in 2 M HCl (Pu fraction). The pH of the retained americium and uranium fraction (solution A) is adjusted at 5.2 ± 0.1 using ammonia water, before its loading on the CC column. The CC column was then rinsed with 10 mL of 0.04 M $NaNO_3$ solution adjusted at pH 6.0 ± 0.1. In this paper, two eluents, ethylene diamine tetraacetic acid (EDTA) and diethylene triamine pentaacetic acid (DTPA) were investigated for the separation of americium/uranium on the CC column. The effect of acetate buffer in these eluents was also studied. Finally, the elution of uranium is performed with 30 mL of 1 M HCl. The optimized protocol is illustrated in **Figure 1**.

All chromatographic separation steps were performed at room temperature (22˚C ± 4˚C) to ease the use of the separation protocol.

## 2.4. ICP-MS Analysis

Aliquots are sampled before and after the extraction of actinides on CH and CC columns and then analyzed by ICP-MS to determine the extraction yield for each actinide. Each elution fraction was also analyzed by ICP-MS to determine the elution yield ($Y_E$) of actinides and the chemical recovery (R) of each actinide, according to the equations (1) and (2), respectively. Each sample was prepared using a 2-fold dilution in nitric acid (2 wt%) before its ICP-MS measurement.

$$Y_E = \frac{Q_{eluted}}{Q_{extracted}} = \frac{[An]_E \times V_E}{[An]_0 \times V_{urine} - [An]_{L+R} \times V_{L+R}} \quad (1)$$

$$R = \frac{Q_{eluted}}{Q_{urine}} = \frac{[An]_E \times V_E}{[An]_0 \times V_{urine}} \quad (2)$$

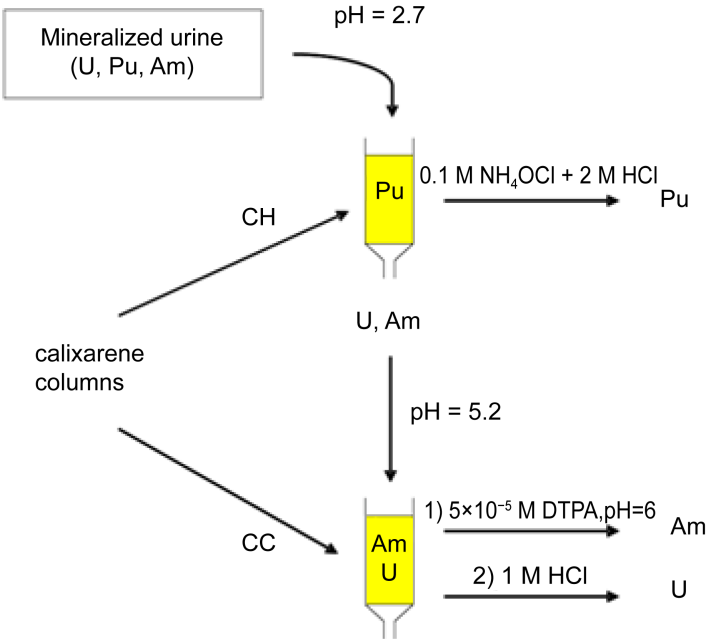


**Figure 1.** Protocol optimized for the separation of U, Pu and Am from mineralized urine using calix[6]arene columns (CH = hydroxamic calixarene column; CC = carboxylic calixarene column).

where $Q_{urine}$, $Q_{extracted}$, and $Q_{eluted}$ is the quantity of each actinide initially in the urine sample, extracted and eluted from the calixarene column CC or CH, respectively. $[An]_E$, $[An]_0$ and $[An]_{L+R}$ is the concentration of each actinide An (An = $^{238}U$, $^{239}Pu$ or $^{243}Am$) in the elution fraction, in the urine and in the combined effluent (loading + rinsing steps), respectively. $V_E$, $V_{urine}$ and $V_{L+R}$ is the volume of the elution fraction, the volume of urine, the volume of the combined effluent (loading + rinsing steps), respectively.

All analytical results in this paper are from at least three replicates, and the uncertainties are the standard deviations of the replicates expressed with k = 1. In all these experiments, the mass balance for each actinide was checked.

## 3. Results and Discussion

### 3.1. Optimization of Mineralization Step

As mentioned earlier, the mineralization of urine is essential for detection of alpha articles emitted by the actinides. The aim of this study was to optimize this mineralization step. The classical mineralization was usually performed by calcination of the calcium phosphate precipitate containing the actinides in a muffle furnace. This step takes about 14 hours. The microwave technology has been already used for mineralization of biological samples [14] [15]. In this paper, the mineralization step with microwave oven was improved in order to be adjusted to the subsequent step of actinides extraction on calixarene columns. The optimization of mineralization protocol was performed on urine samples only spiked with uranium, for radiation protection consideration, then validated on a mixture of actinides U, Pu and Am in urine.

### 3.1.1. Optimization of Nitric Acid and Peroxide Hydrogen Quantity

The required quantity of reagents, nitric acid and hydrogen peroxide, two usual reagents used for mineralization [15], was first determined. The total volume of mineralization reagents that can be used is limited by the volume of the microwave reaction vessel (50 mL). In a first set of experiments, only nitric acid was used to oxidize organic matter. The results are shown in **Figure 2(a)**.

When the quantity of nitric acid increases, a more efficient mineralization is achieved leading to a better extraction of uranium on CC column. But these results demonstrate that nitric acid cannot be used alone for a complete digestion of urine sample, as the uranium extraction yield on the CC column is still lower than those achieved after classical mineralization (79 ± 6)%.

Then a second series of experiments was performed by adding various volumes of hydrogen peroxide (2 to 10 mL of 30 wt% hydrogen peroxide) to 30 mL of nitric acid (67 wt%), as it is known that the use of hydrogen peroxide enhances the oxidation properties of nitric acid especially in the digestion of organics [15]. The results are shown in **Figure 2(b)**. As expected, when the molar ratio of hydrogen peroxide/nitric acid increases, the digestion of urine sample is better and the extraction yield of U on CC column increases. Indeed the presence of organic matter in the sample can prevent the extraction of U by calixarene molecules by complexing it. The best results for U extraction on calixarene column are obtained with a molar ratio of hydrogen peroxide/nitric acid equal to 0.11, corresponding to 5 mL of hydrogen peroxide (30 wt%) in 30 mL of nitric acid (67 wt%). Higher molar ratio of hydrogen peroxide/nitric acid gives slightly lower extraction of U on CC column. Other volumes of both reagents were tested with the same molar ratio (hydrogen peroxide/nitric acid = 0.11), and the results (data not shown) confirm that the optimal conditions of mineralization are obtained with 5 mL of hydrogen peroxide and 30 mL of nitric acid.

### 3.1.2. Optimization of Heating Profile

Various heating profiles were tested with one or two-step temperature ramp as

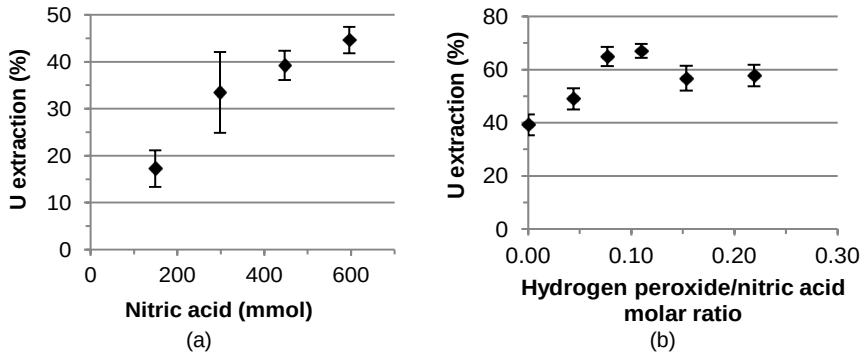


**Figure 2.** Influence of nitric acid quantity (a) or molar ratio of hydrogen peroxide/nitric acid (b) used for the microwave digestion of urine samples on the U extraction yield by the carboxylic calixarene column at pH 5.2. (a) Only nitric acid (10 to 50 mL of 67 wt% nitric acid) was used for the step of microwave digestion. (b) 30 mL of nitric acid (67 wt%) was used in mixture with various volumes of hydrogen peroxide (2 to 10 mL of 30 wt% hydrogen peroxide).

described in Table 1. The total mineralization time includes one hour of cool-down time to come back to temperature below 40˚C, for a safe handling of samples after the mineralization step. The uranium extraction yields on CC column from urine samples after microwave mineralization in these different conditions are shown in Table 1. As we can see there is no significant difference between these heating profiles, U extraction yields being very similar, except a higher discrepancy of results for the heating profiles P1 and P3. The program P4 could be recommended as it is the shortest.

To complete this study, the analyses of total organic carbon were performed on urine samples before and after mineralization with the optimized procedure using the microwave program P4. The results show that more than 99.5% of organic carbon amount is destroyed thanks to this mineralization procedure.

Finally this mineralization procedure was also validated on the actinides Pu and Am in mixture in urine and their extraction was then performed on CH and CC column, respectively. The extraction yields were (78 ± 15)% for Pu and (92 ± 2)% for Am, respectively. All these results allow validating the mineralization protocol of urines by microwave digestion for actinides (U, Pu and Am) analysis, with a total recovery of actinides in mineralized urine.

In conclusion, the goal of this study on mineralization step was achieved: it was demonstrated that the protocol of mineralization with microwave furnace exhibits excellent digestion performance. With the judicious choice of reagents and heating profile allowing total sample decomposition, shorter mineralization time (about 1.5 h) is achieved compared to classical calcination in muffle furnace (14 h) with a high sample throughput, since up to 10 urine samples can be mineralized per run.

### 3.2. Separation of Pu from U and Am with CH Column

The previous studies on hydroxamic calix[6]arene and carboxylic calix[6]

Table 1. U extraction yield (%) at pH 5.2 on the carboxylic calixarene column after microwave mineralization of urines using 5 mL of hydrogen peroxide (30 wt%) and 30 mL of nitric acid (67 wt%) with different heating profiles.

| Program (total mineralization time) | Heating profile | | U extraction (%) |
|---|---|---|---|
| | Time (min) | Temperature (˚C) | |
| P1 (2h05) | 0 → 5<br>5 → 10<br>10 → 20<br>20 → 35 | 0 → 100<br>100<br>100 → 200<br>200 | 53 ± 16 |
| P2 (1h35) | 0 → 10<br>10 → 25<br>25 → 35<br>35 → 65 | 0 → 100<br>100<br>100 → 200<br>200 | 65 ± 7 |
| P3 (1h40) | 0 → 5<br>5 → 25 | 0 → 200<br>200 | 63 ± 13 |
| P4 (1h25) | 0 → 10<br>10 → 40 | 0 → 200<br>200 | 62 ± 7 |

arene extractants showed that the extraction of actinides by these molecules is controlled by the deprotonation of their hydroxamic or carboxylic functions and thus is pH dependent [5] [6] [7]. From these results, it was recommended using two different calixarene columns to separate plutonium from uranium and americium [10]. The first column, CH column, based on hydroxamic calixarene molecules exhibits a good affinity for Pu(IV) thanks to its hydroxamic functions and a possible separation of Pu(IV) from U(VI) and Am(III) can also be achieved if the Pu extraction is performed at pH of about 2 on this first column. Then a second column, CC column, based on carboxylic calixarene molecules can be used to separate uranium from americium. These previous results were obtained on urine samples spiked with one single actinide.

#### 3.2.1. Optimization of Extraction pH of Pu on CH Column

In this paper, the influence of the extraction pH on the separation of Pu from U and Am was investigated with CH column. These experiments were carried out on urine samples containing the mixture of actinides, U, Pu and Am. Three pH values were studied: 2.7, 3 and 3.5, because previous results showed that this pH range can be used to separate Pu from U and Am. The results are presented in Table 2.

As we can see, the increase of extraction pH improves the extraction of plutonium on CH column, as it promotes the deprotonation of hydroxamic functions of calix[6]arene molecules and a better repeatability is achieved on the Pu extraction yield. But this increase of extraction pH leads also to a possible co-extraction of U and Am, and thus a lower selectivity of CH column.

In conclusion if the separation of actinides is critical (for example in case of alpha spectrometry measurement), it is recommended to perform the first extraction step on CH column at pH 2.7 to achieve the best separation of Pu from U and Am. Otherwise, it may be judicious to carry out the plutonium extraction at pH 3 to improve its extraction on CH column.

#### 3.2.2. Optimization of Pu Elution on CH Column

To develop a protocol that could be suitable also for alpha spectrometry measurement, hydrochloric acid and hydroxylammonium chloride were investigated as eluent, since these reagents are commonly used in protocols for plutonium determination [16]. Three different concentrations (2, 3 and 4 M) of hydrochloric acid and 0.1 M hydroxylammonium chloride in 2 M HCl were studied for Pu elution. The results are summarized in Table 3.

**Table 2.** Influence of extraction pH on the separation of Pu from Am and U from mineralized urine sample by using the hydroxamic calixarene column.

| Extraction pH | 2.7 | 3 | 3.5 |
|---|---|---|---|
| Pu extraction (%) | 81 ± 9 | 97 ± 1 | 91 ± 2 |
| U extraction (%) | 3 ± 4 | 10 ± 4 | 25 ± 1 |
| Am extraction (%) | 3 ± 1 | 0 ± 0 | 11 ± 3 |

**Table 3.** Pu elution yield (%) from hydroxamic calixarene column with different eluents, after Pu extraction at pH 2.7 from mineralized urines.

| Eluent | 2 M HCl | 3 M HCl | 4 M HCl | 0.1 M hydroxylammonium chloride in 2 M HCl |
|---|---|---|---|---|
| Pu elution (%) | 62 ± 9 | 62 ± 7 | 76 ± 6 | 78 ± 8 |

The best Pu elution is achieved with 4 M HCl and 0.1 M hydroxylammonium chloride in 2 M HCl. This last reagent was chosen since it is already used in other protocols for Pu elution.

## 3.3. U/Am Separation with CC Column

### 3.3.1. Influence of EDTA Solution pH on U/Am Separation

A previous study demonstrated that the use of 0.1 mM EDTA solution at pH 3.2 enhances the U/Am separation after their co-extraction at pH 5.2 on carboxylic calixarene column [10]. These preliminary results have already shown that the pH of EDTA solution used as eluent was a key parameter on the efficiency of U/Am separation.

In a first time, the repeatability of U/Am separation from carboxylic calixarene column was investigated with 0.1 mM EDTA solution at pH 3.2, as suggested in our previous work, on four urine samples. The results exhibit that the U/Am separation is not repeatable from sample to sample in these conditions (data not shown).

As the extraction of actinides by the calixarene columns is pH-dependent [5] [6] [10] [11], new experiments (n = 6) were performed to understand the influence of pH during the elution of Am with EDTA. After the extraction of U and Am at pH 5.2 on CC column, the elution step with 0.1 mM EDTA solution at pH 3.2 was performed and pH of EDTA solution was measured at the bottom of the column in aliquots of 5 to 10 mL. The pH variation of the EDTA solution as a function of its volume passed through the carboxylic calixarene column is presented in **Figure 3**.

pH value starts at 5.2, that is the pH used for the extraction and rinsing steps, and then it increases quickly until about 6. This pH increase goes along with the beginning of the americium elution. The pH is relatively stable until that 25 mL of EDTA solution is percolated through the column and then decreases to achieve the final pH of 3.2. This decrease of pH is more or less rapid and the uranium elution starts to be significant as the pH is below 5. The correlation between the pH of EDTA solution measured at the bottom of the column and the elution yields of U and Am is illustrated in **Figure 4**.

The actinides elution from the carboxylic column is controlled by two reactions:

- The reprotonation of the carboxylic functions of calixarene molecules when the pH of mobile phase decreases in the calixarene column
- The action of chelating agent, as EDTA (ethylene diamine tetraacetic acid), that can complex the actinides and then lead to their elution from the calixarene column.

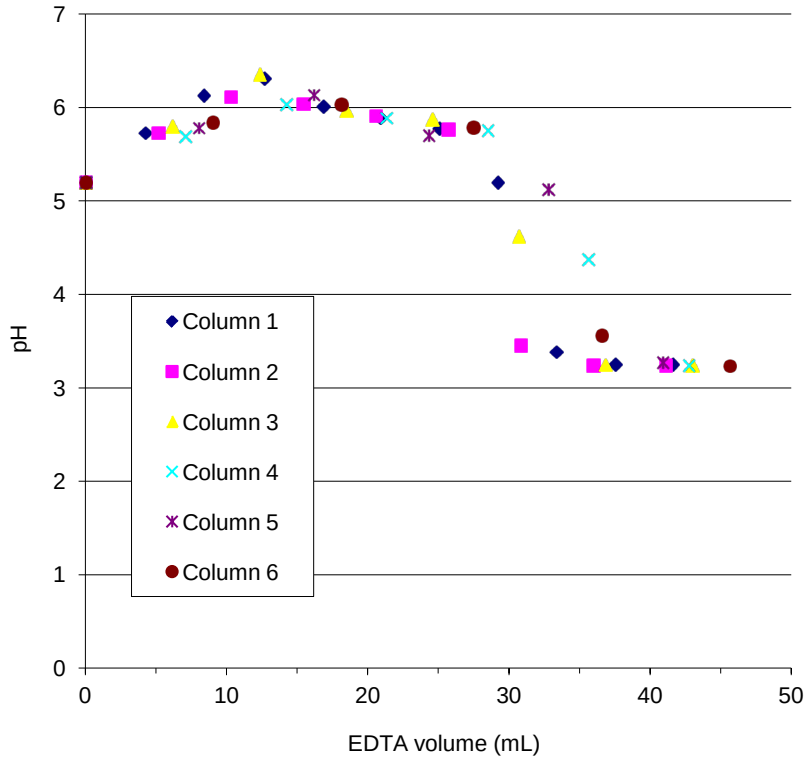


**Figure 3.** pH of EDTA eluent (0.1 mM) measured after its loading in carboxylic calixarene (CC) column as a function of its percolated volume, after U and Am extraction from mineralized urines on CC column at pH 5.2.

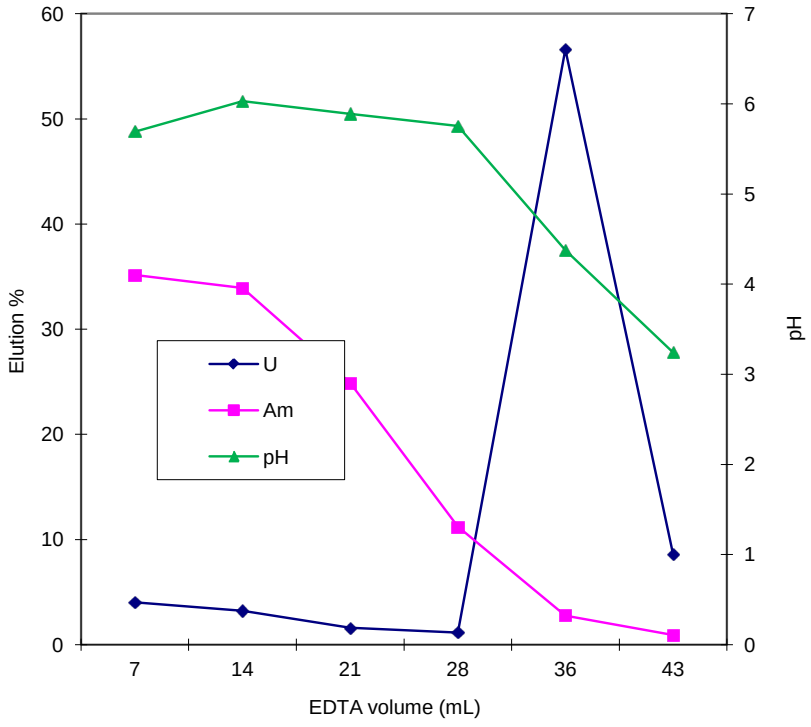


**Figure 4.** Correlation between the pH of EDTA solution (0.1 mM) measured at the bottom of the carboxylic calixarene (CC) column and the elution yields (%) of U and Am as a function of EDTA volume (mL) percolated through the column, after U and Am extraction from mineralized urines on CC column at pH 5.

The complexation constant of americium by EDTA is much higher than the one between uranium and EDTA, as shown in **Table 4**. Thus the americium elution is essentially governed by the EDTA complexation whereas the U elution is mainly controlled by the protonation of the carboxylic functions of calixarene molecules.

These new results on various urine samples show that the pH of EDTA solution has to be controlled to achieve a reproducible separation of U/Am. The use of buffering agent in EDTA solution was then investigated.

### 3.3.2. Influence of Acetate Buffer in 0.1 mM EDTA Solution pH on U/Am Separation

New experiments were carried out on urine samples containing a mixture of U and Am. For U/Am separation, 0.1 mM EDTA solution with 0.03 M sodium acetate as buffering agent was used to control the pH during the elution step. pH of 4, 5.2 and 6 were investigated as buffer pH values. The elution yields for U and Am were determined and the pH of EDTA eluent was measured at the bottom of the column in aliquots of 10 mL. The results for pH 4 or 5.2 are not presented, because no separation of U/Am was achieved in these conditions, the co-elution of both actinides started with the beginning of the loading of the mixture of EDTA solution with sodium acetate buffer into the CC column. The results obtained for EDTA solution with sodium acetate buffer at pH 6 are shown in **Figure 5**.

The results demonstrate that the use of sodium acetate buffer in EDTA solution leads to a better control of pH during the elution step and then prevents the co-elution of uranium with americium. However, it is noticed that the Am elution yield is slightly lower compared with EDTA eluent without buffering agent (about 70% compared to 100%). This could be explained by a salt effect due to the increase of ionic strength, along with the addition of sodium acetate buffer in the EDTA eluent [18].

### 3.3.3. Improvement of Americium Elution

To improve the americium elution yield, the influence of EDTA concentration in presence of sodium acetate buffer (pH 6) on the separation of U/Am from the CC column was investigated on urine samples after mineralization. The results are summarized in **Table 5**.

When the EDTA concentration increases, the americium elution yield increases but the co-elution of uranium also increases, driving by the actinides complexation by EDTA. Thus this parameter cannot be used to improve the elution of americium, because EDTA is not a chelating agent selective enough for the U/Am separation.

**Table 4.** Stability constants of complexes between EDTA or DTPA and Am or U [17].

| Log $K_1$ (I = 0) | $UO_2^{2+}$ | $Am^{3+}$ |
|---|---|---|
| EDTA | 13.7 | 19.7 |
| DTPA | 11.0 | 26.2 |

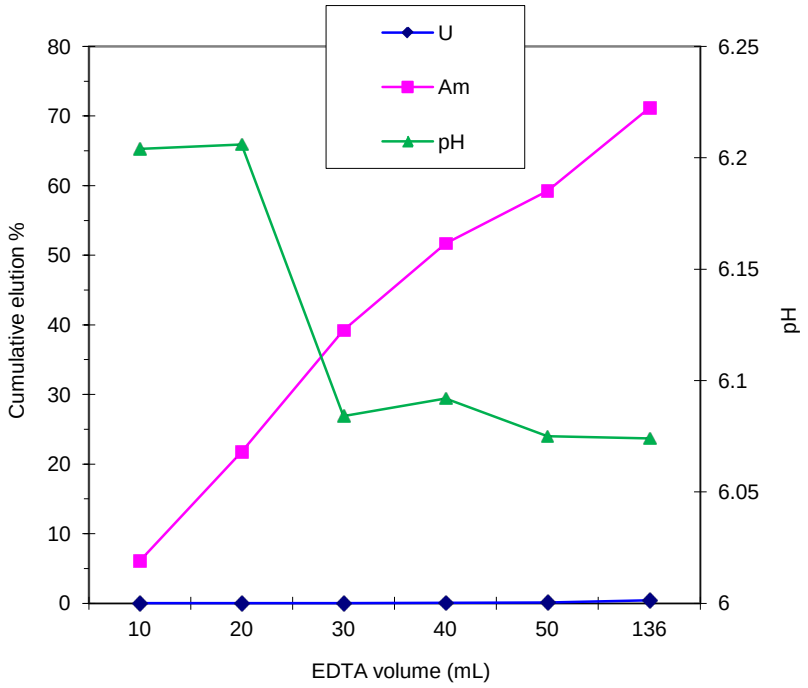


**Figure 5.** Cumulative elution yield (%) of U and Am from the carboxylic calixarene (CC) column and pH variation of EDTA solution (0.1 mM EDTA + 0.03 M sodium acetate buffer at pH 6) as a function of EDTA volume passed through the column. U and Am extraction from mineralized urines was previously performed at pH 5.2 on CC column.

**Table 5.** Comparison of elution yield (%) of Am and U on mineralized urines from the carboxylic calixarene column with two eluents, EDTA and DTPA, at various concentrations. Sodium acetate buffer is added in eluents to control the pH at 6 in the column.

| | EDTA | | | DTPA | |
|---|---|---|---|---|---|
| | 0.1 mM | 0.5 mM | 1 mM | 0.01 mM | 0.05 mM |
| Eluted Am (%) | 72 ± 4 | 71 ± 7 | 95 ± 6 | 70 ± 7 | 81 ± 8 |
| Eluted U (%) | 2 ± 2 | 25 ± 3 | 49 ± 5 | 1 ± 1 | 3 ± 2 |

To optimize the americium elution, the use of DTPA (diethylene triamine pentaacetic acid) as eluent instead of EDTA was studied. Indeed, according to the complexation constants of EDTA and DTPA for uranium and americium given in Table 4, the affinity of DTPA for Am is higher than those between EDTA and Am, whereas the affinity of DTPA for U is lower than those between EDTA and U. DTPA should be a better candidate for the separation of U and Am.

Two concentrations of DTPA were examined: 0.01 mM and 0.05 mM. The pH of DTPA solution was controlled with 0.03 M sodium acetate buffer at pH 6. The results are presented in Table 5. As it could be expected according to the complexation constants, DTPA is a better eluent than EDTA, since a better americium elution yield is obtained with lower concentrations of DTPA than those of EDTA. Furthermore, DTPA is a better ligand for the separation U/Am since an efficient separation U/Am can be achieved with the use of 0.05 mM

DTPA at pH 6.

### 3.4. Separation of U, Pu, Am: Repeatability Study on 10 Urine Samples

To validate this protocol, a set of experiments were carried out on 10 various urines samples (1000 mL) spiked with a mixture of actinides U, Pu and Am according to the optimized protocol using calixarene columns illustrated in Figure 1. The extraction yields of U, Pu and Am for both calixarene columns and the recovery for each actinide in each elution fraction were determined. The results are presented in Table 6.

These results show the robustness of this protocol for actinides separation. Very good extraction yields of plutonium on the hydroxamic calixarene column and of uranium and americium on the carboxylic calixarene column are achieved with a good repeatability that confirms the good affinity of these extractants for actinides from urine samples. The first calixarene column allows an efficient separation of Pu from U and Am. By this way, the possible polyatomic interference between $^{1}H^{238}U$ and $^{239}Pu$ would not be a concern for ICP-MS measurement with a quadrupole mass spectrometer. Furthermore the measurement of both isotopes $^{239}Pu$ and $^{240}Pu$ is possible by ICP-MS, whereas they can't be distinguished by alpha spectrometry measurement due to their alpha energy that are very close (<15 keV). However, the use of quadrupole ICP-MS to detect a possible internal contamination of $^{238}Pu$ in urine is still impossible due to the short half-life of this isotope and the isobaric interference with $^{238}U$ that is naturally present in all chemical reagents. The extraction of Pu on the second calixarene column is also very low leading to a very low Pu contamination in the americium fraction and in the uranium fraction. Concerning the separation of U from Am with the carboxylic calixarene column, the first elution step with DTPA solution in sodium acetate buffer allows a good recovery of americium (65%) with less than 10% of uranium. Then the last elution step with 1 M HCl allows the recovery of 55% of uranium with about 10% of americium. This poor separation of U/Am could be a concern for alpha spectrometry measurement of actinides due to possible

Table 6. Repeatability study of separation protocol of actinides using calixarene columns (as described in Figure 1) on 10 urines samples spiked with a mixture of actinides U, Pu and Am.

| | Hydroxamic calixarene column (CH) | | | Carboxylic calixarene column (CC) | | |
|---|---|---|---|---|---|---|
| | Pu | U | Am | Pu | U | Am |
| Extraction (%) | 87 ± 11 | 3 ± 2 | 2 ± 2 | 3 ± 3 | 92 ± 4 | 97 ± 4 |
| Recovery (%) in Pu fraction | 80 ± 14 | <4 | <4 | - | - | - |
| Recovery (%) in Am fraction | - | - | - | <2 | 8 ± 6 | 65 ± 11 |
| Recovery (%) in U fraction | - | - | - | <3 | 55 ± 17 | 11 ± 8 |

interferences between $^{232}$U and $^{241}$Am or $^{243}$Am isotopes, when the resolution of alpha spectrometry measurement is not sufficient (>30 keV). But for ICP-MS measurement, this separation could be sufficient since there is no possible interference between uranium and americium. Furthermore the low recovery for uranium can be compensated by the high sensitivity of ICP-MS measurement for this element.

### 3.5. Figures of Merits

With this protocol, the total analysis time is about 2 days including the mineralization and separation steps and ICP-MS measurement, and is about 5 days if alpha spectrometry measurement is used. By considering the chemical recoveries of actinides with this separation protocol, detection limits that can be achieved with ICP-MS can be calculated from nitric acid blank measurement. These detections limits are summarized in Table 7 and compared to detection limits obtained by direct ICP-MS measurement of urine sample after a 20-fold dilution ("dilute and shoot" ICP-MS analysis).

These results show that this separation protocol based on calixarene columns allows to improve detection limits by a factor 150 for U isotopes and $^{239}$Pu and by a factor 65 for $^{241}$Am, as compared with the "dilute and shoot" ICP-MS analysis of urine. It should be mentioned that the detection limits obtained for U isotopes are probably slightly overestimated because of the presence of natural uranium in all chemical reagents. Thus "blank" experiments (with all steps including mineralization, separation on calixarene columns) should be carried out for a better estimation of detection limits for U isotopes. Nevertheless, ICP-MS is the suitable technique for U measurements in urine and is recommended instead of alpha spectrometry [19] [20].

For $^{239}$Pu and $^{240}$Pu, detection limits in urine achieved by ICP-MS measurement after actinides separation on calixarene columns are close to those obtained by alpha spectrometry measurement with "emergency protocol" [3] [4], with the possibility to distinguish these both isotopes. In all cases, ICP-MS measurement time is always shorter (few minutes) than alpha spectrometry measurement (few hours to few days). For $^{241}$Am, detection limits achieved by ICP-MS measurement is still higher than those obtained by alpha spectrometry measurement, even after the preconcentration of americium on calixarene column. The coupling between the calixarene column and the ICP-MS for on-line detection could be a way to improve detection limits [12]. With new developments in

Table 7. Detection limits (DL) for actinides U, Pu, Am by quadrupole ICP-MS for direct measurement after a 20-fold dilution of urine or after mineralization of urine (1 L) and separation of actinides on calixarene columns according to the protocol represented in Figure 1.

| DL ($mBq \cdot L^{-1}$) | $^{234}$U | $^{235}$U | $^{238}$U | $^{239}$Pu | $^{240}$Pu | $^{241}$Am |
|---|---|---|---|---|---|---|
| "Dilute and shoot" ICP-MS analysis | 300 | 0.15 | 0.05 | 2500 | 10000 | 150000 |
| ICP-MS after chemical separation | 2 | $8 \times 10^{-4}$ | $3 \times 10^{-4}$ | 20 | 60 | 2300 |

ICP-MS instrument, more efficient sample introduction system or the use of a sector field ICP-MS instead of a quadrupole one, a better sensitivity and a lower background signal can be achieved implying lower detection limits [21] [22] [23].

## 4. Conclusion

Rapid and reliable bioassays methods are in great demand for occupational radiation exposure monitoring of nuclear energy workers. Current protocols used for actinides determination in urine require lengthy sample preparation and long counting time, whereas protocols dedicated to radiological/nuclear emergency often exhibit higher detection limits. In this work, a protocol using a microwave digestion of urine samples followed by the separation of actinides using calix[6]arene-based chromatography columns and ICP-MS measurement is optimized then validated on urine samples containing the three actinides, U, Pu and Am. With this protocol, the total analysis time is about 2 days including the mineralization and separation steps and ICP-MS measurement. It offers simplicity of use as only two calixarene columns are used and a simple step of pH adjustment of sample is required between both columns. The selected elution media are well suited for ICP-MS but also for alpha spectrometry measurement, without the need of any additional evaporation until dryness of sample. The preconcentration step of actinides on calixarene columns allows improving detection limits by a factor from 65 to 150 depending on the actinides as compared to "dilute and shoot" ICP-MS analysis. With this protocol, ICP-MS measurement can be competitive to alpha spectrometry measurement for U isotopes or $^{239}$Pu and $^{240}$Pu determination in urine and offers a shorter analysis time. For short-age isotopes, like $^{241}$Am or $^{238}$Pu, measurement with a quadrupole ICP-MS cannot replace alpha spectrometry measurement due to higher detection limits. For implementation in radiotoxicological laboratories, this separation protocol should be applied on certified urine samples and validated according to several key parameters like accuracy, linearity and detection limits.

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