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High Throughput Determination of Ultra-trace Actinides in Urine by In-line Extraction Chromatography Combined with Quadrupole Inductively Coupled Plasma Mass Spectrometry (EC-ICP-MS)

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Abstract

Determining actinides in urine is vital for occupational exposure monitoring and radiological emergency response because of actinides' toxicity and radiological dose effects on human health. Traditional radiochemistry analytical methods used to determine actinide concentrations in urine are time-consuming (sample analysis takes several days) and are hindered by a variety of technical and instrumentation-related obstacles. A high-throughput, fully automated, precise, and accurate in-line method was developed for determining five actinides (^{241}Am, ^{239}Pu, ^{237}Np, ^{232}Th, and ^{238}U) at ng/L levels in urine using Extraction Chromatography combined with Quadrupole Inductively Coupled Plasma Mass Spectrometry (EC-ICP-MS). In this method, the five actinides are successfully separated with the required sensitivity, peak shape, and resolution using a simplified, single, Eichrom TRU column with a Dionex™ ICS-5000 system. The separated actinides are subsequently injected into an in-line PerkinElmer (PE) NexION® 300D ICP-MS for quantitative determination. Sample to sample run time is 23 minutes for automatic chemical separation and quantification using only 0.5 mL of urine. The limit of detection (LOD) obtained using this method is 0.015 ng/L, 0.022 ng/L, 0.039 ng/L, 4.5 ng/L, and 2.4 ng/L for ^{241}Am, ^{239}Pu, ^{237}Np, ^{232}Th, and ^{238}U, respectively. The method routinely has a chemical yield > 84%, as well as a linearity (R^2) coefficient of ≥ 0.999 for the calibrators. The method proved to be rapid, reliable, and effective for actinide quantification in urine and therefore is appropriate for radiological emergency response incidents.

Graphical Abstract

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Author Contributions

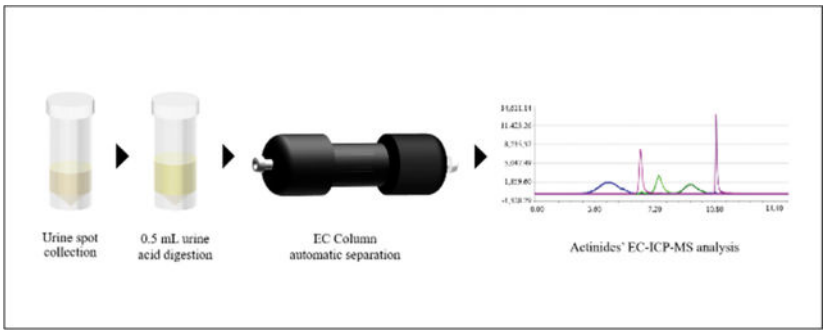
The manuscript was written through contributions of all authors.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Additional experimental details, results for sample carryover, and instrumental conditions in Figures S1–S5 and Tables S1–S5 (DOC)



INTRODUCTION

Actinides are of public health concern due to their long half-lives, alpha particle emission, and chemical toxicity. They are among several priority radionuclides that could be present in a nuclear or radiological incident, a “dirty bomb,” or other radiological dispersal device, or a nuclear power plant incident. Widespread use of nuclear power, threats of radiological incidents, growing public concern about potential environmental or incidental exposure to these radionuclides and their toxicity, and potential adverse human health outcomes all motivate development of a rapid, accurate, and precise non-invasive screening method for actinides in people. The primary exposure pathways of actinides in humans are inhalation, ingestion, and contact through wounds. Once in the body, actinides accumulate in various “target” organs and pose significant health hazards in the long term, such as increased risk of developing cancer and birth defects, even in small concentrations.[1]

Development of a rapid actinide screening method to monitor potential internal radiological contamination is within Centers for Disease Control and Prevention’s (CDC) public health mission. Quantitative analysis of actinides excreted in urine is considered a useful, noninvasive method to assess levels of contamination in humans. Rapid identification and quantification of long-lived actinides for civilian populations is desirable for several reasons. The analysis can help to monitor possible internal contamination. It can also help in calculating exposure dose (health risk) by providing information about the contaminant, including the amount of the contaminant. Therefore, the data can help model the population contaminated, if a large enough group of exposed individuals were measured. Most importantly, a timely actinide urine measurement can provide information for medical management.

Detection of actinides has traditionally been performed using a variety of analytical techniques, such as alpha spectrometry,[2–4] Thermal Ionization Mass Spectrometry (TIMS),[5,6] Accelerator Mass Spectrometry (AMS),[7,8] and Fission-Track Analysis (FTA).[9] The use of TIMS, AMS, and FTA is limited due to the scarcity and/or expense of the instruments, even though these techniques have exceptional sensitivity and limits of detection. Among the available analytical techniques, ICP-MS is the most widely used for metal analysis with great potential for actinides analysis at low concentrations. ICP-MS has low detection limits for long-lived radionuclides, short analytical time, and multi-element analytical capability. This technique is hindered by both isobaric and polyatomic

interferences from various actinide isotopes. Chemical separation of individual actinides prior to the sample measurement by ICP-MS eliminates or reduces these interferences. Although many existing methods using ICP-MS for actinide analysis have been described in the literature, they either lack individual actinide separation[10–12] or full separation for major individual actinides.[13–16]

Actinide separation involves both separation of actinides from the urine matrix and separation of actinides from one another. Traditional actinide chemical separations used for alpha spectrometry and ICP-MS techniques involved time-consuming, multi-stage, labor-intensive procedures.[2–5, 17–19] Extraction Chromatography (EC), pioneered by Horwitz et al.[20, 21] is now the dominant actinide separation technique, as opposed to older co-precipitation, solvent extraction, and ion exchange methods. Multiple column EC for sequential separation of actinides is the most commonly applied solution, but single column separation offers more rapid separation and simplified procedures. An in-line EC chemical separation hyphenated with ICP-MS (EC-ICP-MS) is another approach to substantially reducing sample analysis time while providing the advantages of a self-contained, automated analysis system that generates a minimal amount of waste. Mahan et al.[15] reported an in-line actinides separation method that uses a single column for the first time, but they were unable to completely separate individual actinides to remove potential interferences and had a relatively high limit of detection (LOD) considering their large urine analysis volumes.

The selection of methods and techniques for actinides separation and detection is, to some extent driven by the analytical cost per sample. Most of the existing methods use large amounts of relatively expensive columns for the separations, because these columns are normally for one-time per sample use only. An Eichrom resin column that can be reused multiple times will not only decrease the analytical cost per sample, but also improve the analysis speed. There are two types of ICP-MS instruments used for actinides analysis. One is the more common quadrupole ICP-MS (ICP-MS), and the other is the more expensive, complex sector field (SF) high resolution SF-ICP-MS. Quadrupole ICP-MS analysis requires less rigorous sample preparation since it can tolerate a higher percentage of total dissolved solids in solution, but it has lower sensitivity compared to SF-ICP-MS. In contrast, an SF-ICP-MS needs more rigorous sample preparation but has greater sensitivity. Because of lower cost and greater availability, performing actinides analyses using quadrupole ICP-MS is desirable, but measures are needed to obtain sensitivity and LODs comparable to those of SF-ICP-MS. Lowering LODs is very important because most actinides have extremely low Clinical Decision Guide (CDG) urine concentrations (protective action levels for radioisotopes in urine, derived from National Council on Radiation Protection and Measurements [NCRP] Report #161[22, 23]). Previous methods have reported higher LODs (nanogram levels) that do not meet our CDG requirements for actinides in urine.[17, 18] Accurately determining the actinides at these ultra-trace levels in urine using the traditional techniques with small “spot” urine volumes (e.g., ≤20 mL) is challenging. For a selected technique, the LOD can be improved by increasing the volume of sample used. For example, Hang et al.[14] manually injected a 25 mL urine sample, and Shi et al.[12] used up to about 1,400 mL urine, which represents a daily excretion. Unfortunately, large volumes of urine are not expected to be available in emergency response sample collections, and the urine that

is available may have to be shared for analysis by other analytical methods for other analytes during the initial phase of the response.

The aim of this study was to develop a rapid, reliable method that identifies and quantifies actinides in urine samples and optimizes the interactive factors of throughput, specificity, precision, LOD, and accuracy. The amount of urine sample used in the method will directly affect the throughput, LOD, and accuracy. The larger the volume of urine, the longer sample loading, processing, and pre-concentration times. This will result in lower throughput but better LODs and higher accuracy.

Another goal was to develop a method that could be used simply and effectively in an emergency response. This new method requires a minimal urine sample volume of 0.5 mL with quick and simple sample pretreatment protocols. Actinide separation is conducted in-line through a single reusable column (more than 200 times), and the detection is performed using an ICP-MS. Finally, the study aimed to create a method that could be adapted or modified to analyze many other isotopes of the five actinides americium (Am), plutonium (Pu), neptunium (Np), thorium (Th) and uranium (U), since there is no addition of tracer/spike actinides to the samples themselves.

EXPERIMENTAL SECTION

Reagents, Standards and Materials.

Deionized (DI) water of ≥18 MΩ•cm resistivity (from an Aqua Solutions Ultrapure Water System, Aqua Solutions, Inc., Jasper, GA) was used to dilute reagents, standards, and samples throughout the method development. The 3 mol/L HNO_3 and 2 mol/L HCl solutions used were diluted from double-distilled grade concentrated nitric acid ($HNO_{3)}$ and hydrochloric acid (HCl), respectively, (GFC Chemicals Inc., Columbus, OH). The 80 mmol/L oxalic acid used in the elution process was a dilution of 0.8 mol/L Dionex Met Pac oxalic acid eluent (Thermo Fisher™ Scientific, Sunnyvale, CA). 99.999% pure iron (II) sulfate hydrate (Sigma-Aldrich, St. Louis, MO) was used to prepare a final concentration of approximately 0.16 mol/L in a 3% v/v HCl solution, which was used as a redox agent to adjust Np and Pu oxidation states. ^{241}Am, ^{239}Pu, and ^{237}Np were purchased from Eckert & Ziegler Analytics, Atlanta, GA, while ^{232}Th and ^{238}U were purchased from High Purity Standards, Charleston, SC, in natural equilibrium (National Institute of Standards and Technology [NIST] traceable). All five actinides were diluted with 2% v/v HNO_3 to proper concentrations (100 ng/L for ^{241}Am, ^{239}Pu, ^{237}Np; 1,000 ng/L for ^{232}Th and ^{238}U) for use as ICP-MS post-column in-line internal standards. “Base urine” was anonymously collected in Atlanta, GA, during 2010–2012 from a diverse group of male and female adult volunteers, the urine was pooled and stored in refrigerator (< 6 °C). CDC’s Human Subjects Institutional Review Board reviewed and approved the study protocol 3994.

Both the calibrators (1, 3, 8, 20, and 50 ng/L for ^{241}Am, ^{239}Pu, and ^{237}Np and 15, 30, 60, 125, and 300 ng/L for ^{232}Th and ^{238}U) and two daily bench quality control (QC) pools were prepared by spiking base urine with dilution of the five purchased actinides. One reference material of ^{241}Am and ^{239}Pu in base urine was prepared at CDC by using a NIST traceable ^{241}Am Certified Reference Material (CRM) and ^{239}Pu Standard Reference

Material (SRM) from NIST, Gaithersburg, MD. A mix of ^{237}Np, ^{232}Th, ^{238}U made by Oak Ridge National Laboratory (ORNL), Oak Ridge, TN (traceable to NIST) was used for precision and accuracy tests. A base urine and four low concentrations of ^{241}Am, ^{239}Pu, ^{237}Np, ^{232}Th and ^{238}U urine samples were prepared and used for determining the method's LOD. The EC separation column, with an inner diameter of 4.6 mm and length of 50 mm, was packed by Orochem Technology Inc., Napervile, IL with TRU resin (an extraction chromatographic material containing octylphenyl-N, N-di-isobutyl carbamoylphosphine oxide (CMPO) dissolved in tri-n-butyl phosphate (TBP)) (20–50 μm particle size, Eichrom Technology LLC., Lisle, IL).

Sample Preparation.

Sample preparation was developed in CDC, which consists of the following steps. Samples (prepared and saved in refrigerator) were allowed to reach room temperature, then mixed well by simple inversions for 5 seconds before pipetting. Urine sample (0.5 mL) followed by 180 μL concentrated HNO_3 was transferred to a 2 mL screw-capped micro-centrifuge tube which was placed in a sand bath at 85ºC for one hour. The micro-centrifuge tube was removed from the sand bath and cooled at room temperature for at least 30 minutes. 1,070 μL of DI water was added to the tube, and the tube was vortexed with a vortex mixer for 1–2 minutes and centrifuged at 15,000 *g* (RCF) for 5 minutes. 1,350 μL of supernatant was transferred from the tube to a 1.5 mL plastic ion chromatography (IC) vial. 50 μL of 0.16 mole/L iron (II) sulfate was transferred to the IC vial and mixed well by simple inversion approximately 3 times. The processed urine sample then was ready for column separation and ICP-MS detection.

Instrumentation.

Figure 1 shows the flow schematic of actinide analysis by EC-ICP-MS. Table S1 shows instrumental conditions for actinide analysis by EC-ICP-MS. The Ion Chromatography (IC) system used for actinides separation was a Thermo Fisher™ Dionex™ ICS-5000 (Thermo Fisher™ Co., Sunnyvale, CA) which is composed of a thermal compartment (TC), single gradient pump (SP), and model AS-AP autosampler. The Dionex™ ICS-5000 has a poly ether ether ketone (PEEK) flow path capable of operating continuously with highly caustic solutions (see Figure 1). The in-line hyphenated ICP-MS detection system is a PerkinElmer NexION® 300D (PerkinElmer, Waltham, MA) used as the detector for actinide determination. Its unique triple cone interface and Universal Cell™ ion-filtering design is suited for the analysis of difficult matrices such as urine. The "90º" ion path helps keep the instrument clean, minimizing drift and greatly reducing maintenance requirements. The Apex Q, located between the ICS-5000 and the ICP-MS, is a sensitivity enhancing, low-memory desolvating inlet system made by Elemental Scientific Inc. (ESI) and used for sample introduction to the ICP-MS. Two switching valves, EV fluid processors (EV750-100-S2, Valves I & II in Figure 1) made by Rheodyne® are applied to control the flow paths and timing of samples and internal standards. In addition, a Gilson peristaltic pump is used for post-column internal standard injection (see Figure 1).

Instrument parameters for actinides analysis were optimized (Table S1) for concurrent use of the Thermo Scientific™ Dionex™ ICS-5000 and the PerkinElmer (PE) NexION® 300D

ICP-MS. Thermo Scientific™ Dionex™ ICS-5000 parameters are controlled through the Dionex™ Chromeleon™ software and are set up as an AS-AP autosampler sequence method. Basic parameters for NexION® ICP-MS optimization were established and controlled by the ICP-MS software. During data acquisition, the ICP-MS is controlled by PE's Chromera™ method sequence, with Chromeleon™ and Chromera™ synchronized.

Sample Elution Procedure.

Table 1 shows the IC elution program for sequential actinides separation. It consisted of four steps. First, the column was conditioned with 3 mol/L HNO_3 to enhance the maximum retention of actinides on the column after loading the urine sample (time: −1.0 to 2.7 min.). The urine matrix passed through the column and was switched to waste by valve I. Second, Am, Pu, and Np were sequentially eluted from the column using the Dionex™ system's gradient eluent run which changes the eluent composition during the run. The eluent's 2 mol/L HCl content was decreased from 50% to 1%, and its DI water content was increased from 30% to 79%. Third, Th and U were eluted from the column using an isocratic step with the eluent's DI water and 80 mmol/L oxalic acid contents at 80% and 20%, respectively. Fourth, the column was post/preconditioned with DI water to ensure it had the same reproducible performance for the next sample separation. For maximum removal of U from the column, eluent flow rates of 1.0 mL/min and 1.5 mL/min were maintained in the third and fourth steps, respectively, compared with 0.5 mL/min in the first and second steps.

RESULTS AND DISCUSSION

Actinides Chromatographic Sequential Separation.

^{241}Am, ^{239}Pu, ^{237}Np, ^{232}Th, and ^{238}U were sequentially separated, and an example chromatogram is shown in Figure 2. All analyte peaks were adequately resolved, smooth, and symmetric with no significant tailing effects. Tailing factors and resolutions were calculated based on standard HPLC method conventions. Resulting tailing factors were between 1.00 and 1.36 (Table S2), which was within the normally acceptable range of 0.5 to 2.0 for precise, accurate, quantitative measurements. Parameters for resolution of ^{238}U from ^{241}Am, ^{239}Pu, ^{237}Np, and ^{232}Th, as well as for resolution of ^{241}Am from ^{239}Pu, ^{237}Np, ^{232}Th, and ^{238}U were in the range of 2.6–8.6, as shown in Table S3. These values were significantly greater than 1.5, a value which indicates that two peaks were completely resolved at the baseline.[24] As mentioned previously, the purpose of the chromatographic separation was to eliminate potential mass spectral interference from isobaric and/or polyatomic species. However, requiring that all the analytes be completely chromatographically separated from each other was not necessary. Table 2 shows a list of potential mass spectral interferences. It is apparent that most of these interferences were eliminated as long as U is separated from Pu, Np, Th, and Am, since relatively large natural or enhanced amounts of U can result in tailing interference from the ^{238}U mass peak into other mass regions, especially those nearby. Potential organic compounds in a urine matrix that could produce signals that interfere with those from five actinides were broken down by acid digestion during sample preparation. In addition, potential isobaric or polyatomic interferences from impurities, such as Pb, TI, Hg, Bi, Pt, and Au in urine that

could combine with argon and/or hydrogen, were tested by spiking them into base urine. Pb, Tl, Hg, and Pt were spiked at concentrations of 5 μg/L, 0.5 μg/L, 5 μg/L, and 0.05 μg/L, respectively. These spiked impurity concentrations in urine were above the National Health and Nutrition Examination Survey (NHANES) 95th percentiles for urine Pb, Tl, Hg, and Pt concentrations.[25] Although NHANES survey data is not available for Bi and Au, analysis of what was otherwise determined[26, 27] to be high urine concentrations of Bi (5 μg/L) and Au (0.05 μg/L), together with Pb, Tl, Hg, and Pt, produced no apparent interferences in this experiment. Furthermore, this impurity test also demonstrated that no observed organic compounds in the urine matrix could interfere with five actinides. The breakdown of organic materials in the urine matrix by acid digestion thus extended the life span of separation columns, which was further verified in a recent study by Xiao and Jones.[28]

The successful separation of ultra-trace actinides using extraction chromatography is related to column efficiency which is reported as the number (N) of theoretical plates. The greater the number of theoretical plates, the better the potential resolving power of the column. The number of theoretical plates for the actinide isotopes in this study ranges from 4,610 to 18,800 (see Table S4). These numbers are determined by the particle size of the packing materials, the length of the column, and the flow rate of the mobile phase. Peterson et al. have thoroughly examined and discussed these parameters for the TRU column as they affect actinide separation.[29] For this study, the TRU resin with the smallest particle size (20–50 μm) available from Eichrom was selected for column packing. In general, smaller, more densely packed resin improves separation performance in liquid chromatography.

Although a longer column increases the number of theoretical plates and leads to a better separation, sample transport through a longer column takes more time resulting in a reduced sample throughput. We selected a 50 mm column which proved to be effective for separation of the target analyte and still allowed for sufficient sample throughput. Flow rates of both the sample and later the eluents passing through the column are also important parameters for optimization. High flow rates will decrease analysis time but will also decrease the efficiency of sample loading onto the column and reduce peak resolution during elution. Additionally, the flow rate of the eluent from the column must be synchronized with the sample uptake rate of the desolvator/ICP-MS nebulizer; otherwise, the sample may overload the desolvator and/or ICP-MS spray chamber, resulting in distorted or overlapping peaks. Finally, the custom packed TRU resin columns applied in this method were reusable. A typical column maintains the required performance parameters during as many as 200 cycles, including loading and sequential separation of calibrators, QCs, blanks, and samples.

Oxidation State Adjustment.

Actinides retained on the TRU resin column can be present in trivalent (III), tetravalent (IV), pentavalent (V), or hexavalent (VI) oxidation states. They will be eluted in a defined sequence according to their oxidation state with V first, III next, IV next, and VI last, as the concentration of HCl decreases in the gradient elution program.[30] To better remove and simplify potential isobaric and polyatomic interferences, a single oxidation state for each actinide is necessary. Under sample pretreatment conditions used in this method, Am, Th, and U each had only one oxidation state: Am (III), Th (IV), and U (VI). However, Pu exists

as Pu (III), Pu (IV), and Pu (VI), and Np exists as Np (V), Np (IV), and Np (VI). In this method Pu and Np were both required to have tetravalent states, and Pu (III) and Pu (VI) were converted to Pu (IV), while Np (V) and Np (VI) were converted to Np (IV). Iron (II) sulfate and HNO_3 redox agents were selected to adjust the oxidation state of Pu and Np. Resulting chromatograms show that both Pu and Np have unique, tetravalent ion peaks as long as the analysis is completed within ten hours of adding iron (II) sulfate. We observed that an excess of iron (II) sulfate in the sample solution will suppress the ion beam intensity of the ICP-MS, especially for a long sample run (e.g., a batch of over 35 patient samples). Therefore, concentration of iron (II) sulfate in the urine samples, including calibrators and QCs, was kept at less than 0.025 mol/L.

Internal Standard and Chemical Yield.

To quantitatively determine ultra-trace levels of actinides in urine samples, tracer spikes are typically required to calculate the chemical yields of analytes from the separation process. Isotopes of each of the analytes (that are not expected to be in the samples) are normally selected to be tracer spikes. However, in this study, no tracers were added to the samples. In some cases, no suitable isotope is available for use as a tracer; for instance, there is no long-lived neptunium isotope other than ^{237}Np. As previously mentioned, this method may have universal application for analysis of most isotopes of the five actinides. Using isotopes of these actinides as tracers, such as using ^{243}Am as tracer for ^{241}Am, will limit the capability of determining tracer isotopes (e.g., ^{243}Am) that could appear in the sample as target analytes.

Chemical yields for column separation of the five actinides were optimized during method validation and verified during ruggedness testing. All calibrators and samples in any given run were analyzed using the same column. The column performance was uniform and reproducible throughout method validation runs. The five analytes themselves (^{241}Am, ^{239}Pu, ^{237}Np, ^{232}Th, and ^{238}U) were used as internal standards by post-column injection into the ICP-MS to compensate for instrument drift. Chemical yields were calculated based on the ratios of sample peak area to the post-column internal standard peak area. The resulting average yields over five different concentration levels were in a range of 84.6% to 92.7%, as shown in Table S5. Note that since the matrix matched calibration standards and QC materials (as opposed to the post column internal standards, which are only intended to correct for beam current variation) pass through the TRU column, they do not correct for column recovery.

Sample Carryover and Linearity.

The TRU resin column is reusable in this method, and single columns are used throughout whole analytical batches which may consist of 200 samples, including calibrators, QCs, and blanks. Therefore, it is important that no carryover occurs from one sample to another throughout the full range of sample and calibrator analyte concentrations. An experiment was conducted to monitor the change in concentration detected for blanks, low, and high calibrators for forty consecutive measurements. The sample sequence included 10 cyclic measurements of a blank, a low calibrator, a blank, and a high calibrator. The concentrations of the low and high calibration standards were 1 ng/L and 50 ng/L, respectively, for ^{241}Am,

^{239}Pu, and ^{237}Np, while the concentrations of the low and high calibration standards were 15 ng/L and 300 ng/L, respectively, for ^{232}Th and ^{238}U. As shown in Figures S1, S2, S3, S4 and S5 for ^{241}Am, ^{239}Pu, ^{237}Np, ^{232}Th, and ^{238}U, respectively, the experimental results demonstrate that there is no tendency of concentration increase in the blanks for any of the five actinides. This indicates no significant carryover between samples over the calibration range and intended measurement range during consecutive sample measurements.

Linearity was assessed as an indicator of the method's ability to obtain results that are proportional to known analyte concentrations. Five calibrators with concentrations of 1, 3, 8, 20, and 50 ng/L for ^{241}Am, ^{239}Pu, ^{237}Np, and 15, 30, 60, 125, and 300 ng/L for ^{232}Th and ^{238}U were used. The calibration model was simple linear regression with correlation coefficients of greater than 0.999 for this method.

Precision and Accuracy.

The precision of this method was evaluated based on short- and long-term measurements of low (Level I) and high (Level II) concentrations of actinides. For the short-term measurements, the low and high calibrators were used for the evaluation, which included ten replicates each of low and high calibrators within a typical 12 hour run. In contrast, the low and high QC were measured for long term precision evaluation, which consisted of more than 40 analytical runs within a six-month period. Two concentration levels of QC samples were measured twice in each analytical run: once at the beginning and once at the end. The results for both short- and long-term measurements are shown in Table 3. Th and U had larger Relative Standard Deviations (RSDs), as compared with Am, Pu, and Np for Concentration Level I solutions in both short- and long-term measurements. This is due to the natural existence of Th and U which are detectable in most U.S. population urine samples. As expected, short term RSDs were larger for Concentration Level I than for Concentration Level II solutions. Short term precision is normally better than long term precision, and as shown in Table 3, this proved true for this method, except in Concentration Level I for uranium. This is probably because of natural U abundance and its dominant impact on the statistics. Short term precision (in terms of RSD) was in a range of 1.8% to 6.5% for Am, Pu, Np, and Th, whereas U RSD was 12.4%. Long term precision (over a six-month period) remained below 11% RSD for all actinides.

Although no SRM with required, certified concentrations of actinides in a urine matrix were available, two reference materials with known values from different sources were used for comparison. CDC prepared one reference material in urine containing a NIST ^{241}Am CRM and NIST ^{239}Pu SRM 4330B. ORNL provided another reference material in urine which contained ^{237}Np, ^{232}Th, and ^{238}U traceable to NIST. Five replicate analyses of each reference material were performed, and the results, including the calculated bias and the recoveries compared with the known standard target value, are listed in Table 4. These results demonstrate that the accuracy of this method is within requirements established by the Division of Laboratory Sciences, with all measurement bias values within a range from −2.4 to 5.3%.

Limit of Detection (LOD) and Sensitivity.

Method LODs were determined by using an approach of considering both Types I and II error recommended by Clinical Laboratory Standards Institute,[31] which is required by our Division of Laboratory Sciences at CDC. The blank of base urine and four different low concentration urine pools close to the LOD were measured in 20 runs within a three-month period for the LOD determination. The LODs were calculated according to the following formula: $Conc_{LOD}$ = [mean b + 1.645(Sb + int)]/[1–1.645(slope)], which is the level where the standard curve meets the upper 95% CI of the blank signal. Where mean b = blank average, Sb = standard deviation of blank average, int = intercept of the equation, Slope = slope of the equation. The resulting LODs are in Table 5. The high LODs for ^{232}Th and ^{238}U (4.5 ng/L and 2.4 ng/L) as compared with those for ^{241}Am, ^{239}Pu, and ^{237}Np (0.015, 0.022 and 0.039 ng/L) were due to natural U and Th intake daily from drinking water and food. The National Health and Nutrition Examination Survey (NHANES) 95th percentile for U in urine of the total U.S. population for year 2015–2016 is 31 ng/L.[25] Th had a 95th percentile concentration of 3.09 ng/L.[32]

Method sensitivity was determined by the sample preparation “efficiency” and performance of the EC-ICP-MS system. Use of the Apex Q high stability, low memory desolvating inlet system, as well as efficient actinide separation and matrix removal in this study dramatically enhanced actinide sensitivities. This method’s actinide LODs were lower than other quadrupole ICP-MS methods [13, 17] that report LODs of a few ng/L. This method’s LODs are at least ten times lower than those obtained using a Sector Field (SF) ICP-MS,[18] after converting LOD unit for the analyzed different sample matrix. The LODs for ^{239}Pu and ^{241}Am obtained in this study are approximately 7–10 times lower than the LODs using a quadrupole ICP-MS, reported by Hang et al.[14], while the LODs for ^{237}Np, ^{232}Th, and ^{238}U are comparable. To further compare actinide sensitivities with previous methods reported in the literature, LODs were converted to absolute amounts of the analytes in pico-grams and listed in Table 5. For this method, equivalent absolute LODs were calculated based on the 0.5 mL urine used for sample preparation, the dilution factor, and the volume of processed sample injection.

It is worth mentioning that the method LODs would be significantly improved by increasing the volume of urine sample used. Without changing the size of digestion vessel, it would be flexible to increase the urine sample to up to 1 mL, which would require a proportional increase in concentrated nitric acid in the process of acid digestion. However, the increase in volume of urine sample would result in a longer time for sample digestion, sample loading, and elution; thus, it extends the overall time of per sample analysis as well as reduces the life span of EC separation column from degradation. Considering the priority of a rapid and high throughput to meet CDC’s mission in public health, 0.5 mL of urine sample is an appropriate and large enough volume for this method.

CONCLUSIONS

This article presents a method using in-line extraction chromatographic separation, coupled with a more common quadrupole ICP-MS detection system, for automated, high-throughput, simultaneous determination of five actinides (Am, Pu, Np, Th and U). This method is

appropriate for the rapid identification and quantification for selected actinides in urine in case of a radiological incident requiring emergency response. This is also a Clinical Laboratory Improvement Act (CLIA) certified method conducted in the CDC radiation laboratory. Method development and optimization aimed to prioritize and balance three factors: LOD, throughput, and accuracy. This method's major advantages include (1) simplicity and speed of sample pretreatment and single column separation (e.g., total of 23-minute analysis per sample); (2) minimal (0.5 mL) sample volume requirement; (3) a reusable column (up to 200 sample analyses without degradation); (4) relative high sensitivity; (5) good precision and accuracy; and (6) low LODs that achieved our Method Quality Objectives (MQO). Our analytical results demonstrate that this method is useful for rapidly detecting ultra-trace Am, Pu, Np, Th, and U to determine if a single actinide or multiple actinides have been exposed to a population following a radiological or nuclear incident.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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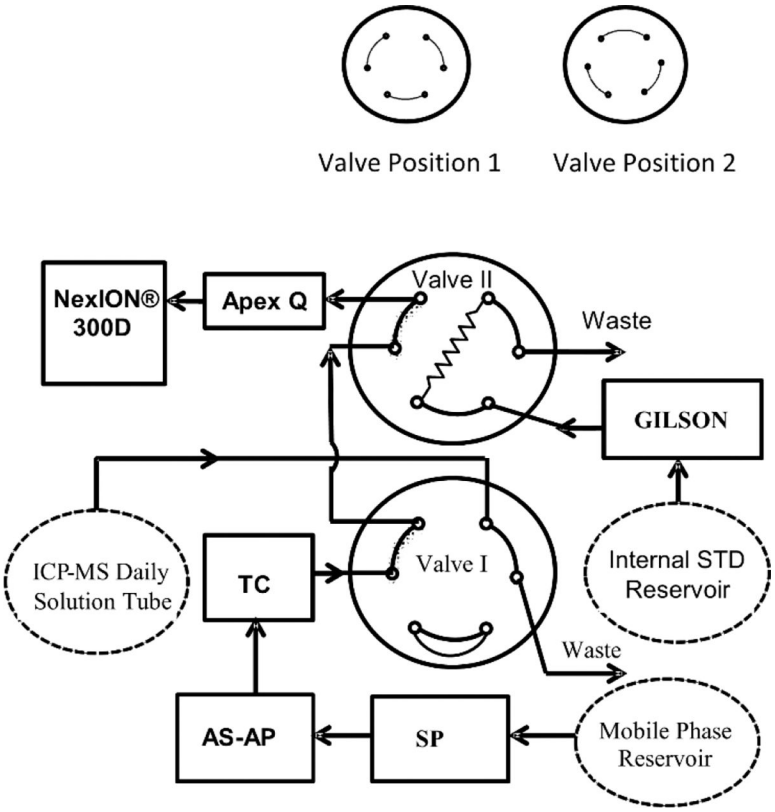

Figure 1.

Flow schematic of actinides analysis by EC-ICP-MS. EC-ICP-MS: Extraction Chromatography Quadrupole Inductively Coupled Plasma Mass Spectrometry; Gilson: Peristaltic pump; TC: Thermal compartment; AS-AP: Autosampler; SP: Single gradient pump.

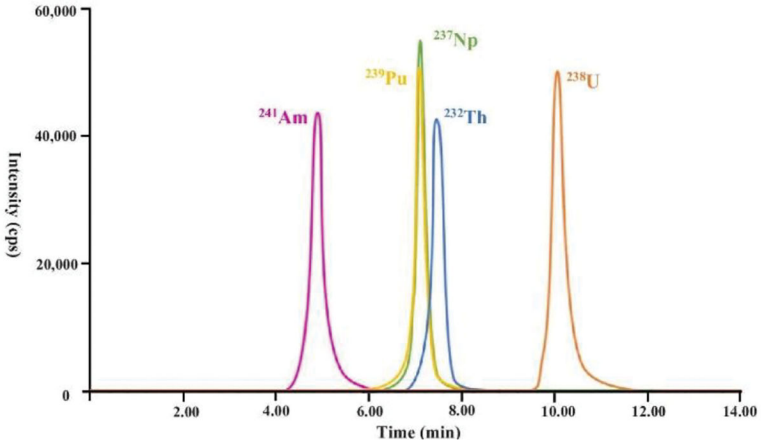

Figure 2.

Chromatogram of ^{241}Am, ^{239}Pu, ^{237}Np, ^{232}Th, and ^{238}U spiked in base urine separated using a Dionex ICS-5000 with a HPLC column containing TRU resin.

Table 1.

Extraction chromatography elution program.

Time (min)	Flow rate (mL/min)	HNO_3 3 mol/L (%)	HCl 2 mol/L (%)	DI H_2O (%)	Oxalic acid 80 mmol/L (%)	Valve I position	Valve II position	Curve
−1.0	0.5	100				2	1	-
2.7	0.5	100				2	1	5
3.1	0.5		50	30	20	1	1	9
7.0	0.5		1	79	20	1	1	9
15.5	1.0			80	20	1	1	5
17.0	1.0			80	20	1	2	5
17.5	1.5			100		1	2	5
19.6	1.5			100		2	1	5

Table 2.

Actinides potential spectral interferences.

Mass number	Isobars	Polyatomic ions
232	^{232}U, ^{232}Th	
237	^{237}Np	^{236}NpH
238	^{238}U, ^{238}Pu	^{237}NpH
239	^{239}Pu	^{238}UH
241	^{241}Pu, ^{241}Am	^{240}PuH

Table 3.

Repeatability results of actinides measurement for concentration level I and II standards over short- and long-term periods.

		Concentration Level I (ng/L)					Concentration Level II (ng/L)				
		^{241}Am	^{239}Pu	^{237}Np	^{232}Th	^{238}U	^{241}Am	^{239}Pu	^{237}Np	^{232}Th	^{238}U
Short term(n=10)*	*Average*	0.96	0.98	1.09	14.2	15.8	49.0	48.5	50.0	297	301
	SD	0.06	0.04	0.07	0.93	1.96	2.1	0.9	1.3	10.0	12.8
	RSD %	5.8	4.0	6.4	6.5	12.4	4.3	1.8	2.7	3.4	4.2
*Long term**(n=40)*	*Average*	2.03	1.81	1.97	39.8	39.2	30.4	25.6	28.2	198	187
	SD	0.16	0.13	0.15	4.3	4.0	2.5	1.7	1.9	15.5	17.6
	RSD %	7.9	7.2	7.6	10.8	10.2	8.1	6.8	6.6	7.8	9.4

* Measurements were within 5 hours.

** Measurements were within 6 months.

Table 4.

Measurement of two reference materials of ^{241}Am and ^{239}Pu, and ^{237}Np, ^{232}Th, and ^{238}U.

Replicate	^{241}Am* (ng/L)	^{239}Pu* (ng/L)	^{237}Np** (ng/L)	^{232}Th** (ng/L)	^{238}U** (ng/L)
1	25.0	20.6	20.3	123	120
2	24.8	21.2	19.9	128	120
3	25.5	22.0	20.6	135	122
4	26.4	22.0	20.8	139	131
5	26.2	22.3	18.4	129	117
Average	25.6	21.6	20.0	131	122
SD	0.7	0.7	1.0	6.3	5.5
Target value	24.30	22.00	20.35	125	125
Bias	1.3	−0.40	−0.35	5.6	−3.0
Bias (%)	5.3	−1.8	1.8	4.5	−2.4

*Urine reference material made at CDC by spiking NIST ^{241}Am SRM (4322C) and NIST ^{239}Pu SRM (4330B) in base urine.

**Mix of 3 actinides prepared by ORNL from NIST traceable SRM.

Table 5.

Limit Of Detection (LOD) results for spiked ^{241}Am, ^{239}Pu, ^{237}Np, ^{232}Th and ^{238}U in base urine.

	^{241}Am	^{239}Pu	^{237}Np	^{232}Th	^{238}U
LOD (ng/L)	0.015	0.022	0.039	4.5	2.4
Equivalent absolute LOD (pg)	0.004	0.006	0.011	1.2	0.6
C/P CDG[*]	0.733 (pg/L)	13.8 (pg/L)	812 (pg/L)	1.00 (μg/L)	9.09 (μg/L)

[*] Urine output levels expected at 5 days post intake for children or pregnant woman (C/P) at the National Council on Radiation Protection & Measurements (NCRP) Report No. 161 I Clinical Decision Guide (CDG)