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Determination of $^{236}U/^{238}U$ and $^{235}U/^{238}U$ isotope ratios in human urine by inductively coupled plasma mass spectrometry[☆]

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ABSTRACT

Isotopic ratios of urinary uranium (U) provide a decisive tool in diagnosing a patient's potential exposure to depleted uranium (DU). This study investigated the measurement of urinary $^{236}U/^{238}U$ and $^{235}U/^{238}U$ ratios of ultra trace concentrations using a sector field inductively coupled plasma mass spectrometer (ICP-MS). Uranium was separated from the urinary matrix and pre-concentrated by co-precipitation with calcium and magnesium after addition of ammonium hydroxide. The precipitates were collected, dissolved and analyzed by ICP-MS. $^{235}U/^{238}U$ and $^{236}U/^{238}U$ ratios were measured using a desolvating sample introduction system and a sector field ICP-MS. These ratios were accurately measured in samples containing as low as 5 ng L^{-1} U. ^{236}U measurement was hindered by abundance sensitivity limitations and uranium hydride (^{235}UH) formation. Measured $^{235}U/^{238}U$ and $^{236}U/^{238}U$ ratios were about 7×10^{-3} and $<5\times10^{-6}$, respectively, in urine samples from patients not exposed to DU. The $^{235}U/^{238}U$ ratio was consistently about 2×10^{-3} in 12 urine samples from patients with embedded fragments of DU, while the corresponding ratio of $^{236}U/^{238}U$ ranged from 9×10^{-6} to 33×10^{-6}. This wide range in $^{236}U/^{238}U$ ratios might suggest different sources of depleted uranium in those DU-urine samples. It is proposed that $^{235}U/^{238}U$ and $^{236}U/^{238}U$ ratios are 7×10^{-3} and $<5\times10^{-6}$, respectively, in urine samples from individuals not exposed to DU, and 2×10^{-3} and $>7\times10^{-6}$, respectively, in urine samples from DU-exposed individuals.

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1. Introduction

Uranium (U) occurs naturally and contains 0.0055% of ^{234}U, 0.72% of ^{235}U, less than 10^{-7}% of ^{236}U, and 99.274% of ^{238}U [1–3]. Depleted uranium (DU) is a byproduct of the ^{235}U enrichment process [4] and is used in military and commercial applications. DU contains approximately 0.0006 to 0.0007% of ^{234}U, 0.2 to 0.4% of ^{235}U, various small amounts of ^{236}U, and 99.6% or more of ^{238}U [3,4]. Depleted uranium contains less ^{235}U than natural uranium and is approximately 40% less radioactive than natural uranium. Because of its high density, availability, and low relative cost, it has been incorporated into munitions as high energy kinetic penetrators and into armor plates for military vehicles. Soldiers in battle are therefore at risk of inhaling DU aerosols, ingesting DU particles, and/or experiencing wound contamination by DU particles and/or embedded fragments. Its use in both military and industry is of interest as a human health concern primarily in terms of its chemical toxicity with less likelihood for effects from radiation [5–8].

Analysis of urinary uranium has been commonly used as a non-invasive test [4,9,10] to monitor occupational and/or environmental exposure to depleted uranium. The determination is done via a two-step process [11] (Fig. 1): 1) analysis of total uranium concentration in urine; 2) if the urinary uranium is above a pre-set level, measurement of uranium isotope ratios is conducted [8,9], with a $^{235}U/^{238}U\approx0.002$ indicative of exposure to DU.

Exposure to depleted uranium might be confirmed if the measured $^{235}U/^{238}U$ ratio is unambiguously different from its natural ratio. However, in some cases the $^{235}U/^{238}U$ isotope ratio cannot conclusively prove DU exposure because the enrichment of ^{235}U in reactor uranium can differ. As an alternative, ^{236}U has been suggested as a second signature of irradiated uranium in ammunition and contaminations [3]. The $^{236}U/^{238}U$ ratio is very low in natural samples but can be

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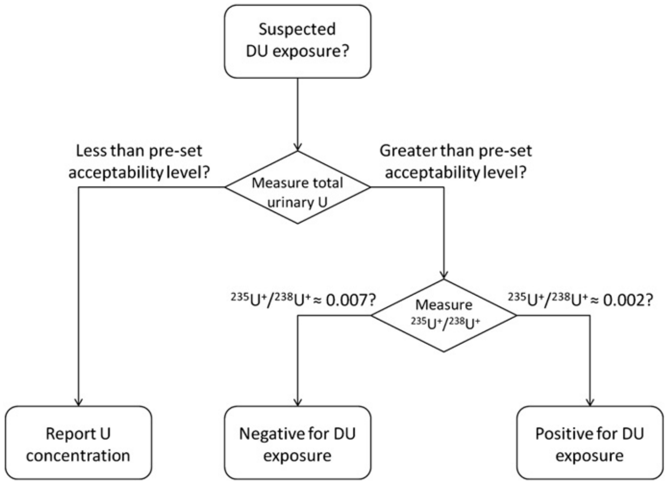

Fig. 1. Schematic process to identify exposure to depleted uranium (DU) based on analysis of urine uranium content includes: 1) measurement of total urinary U, 2) measurement of chosen U isotope ratios, and 3) exposure determination from isotope ratio results.

relatively high in specifically processed nuclear materials [3,12]. Therefore, a $^{236}U/^{238}U$ ratio greater than background level also can suggest DU exposure, since natural uranium contains negligible amounts of ^{236}U [3,12]. It has also been suggested that the $^{236}U/^{238}U$ ratio is a better index than $^{235}U/^{238}U$ in indicating anthropogenic sources of uranium [3].

Inductively coupled plasma mass spectrometry (ICP-MS) is widely used to determine the concentration of urinary uranium because it displays low detection limits, simple spectra, requires minimum sample preparation (dilution is usually sufficient for urinary analysis), nine orders of magnitude dynamic range, fast analysis time, isotope specific detection, and relatively low cost [10,13–15]. Thermal ionization mass spectrometry (TIMS) [16] also provides accurate and precise measurements, but is not practical for large number of patient screenings since extensive sample preparations are required [2,16–18]. Alpha and gamma particle spectrometries have also been used [19–22] but require long counting times, removal of uranium from the matrix and display detection limits 2–3 orders of magnitude worse than ICP-MS.

Isotope ratio analysis of urinary uranium is challenging due to ultra-trace uranium concentrations (median urinary uranium measured in our lab is 5 ng L^{-1} ($N=3364$)) [9,23–25] and urine's complex matrix (Na, Mg, K, Ca, P, Cl, S, and dissolved organic materials). Other successful methods have been primarily developed for samples with elevated and/or spiked uranium concentrations [23,26] and have focused on the $^{235}U/^{238}U$ ratio. Reproducible measurements of $^{236}U/^{238}U$ have been reported in spiked urine with multicollector ICP-MS [10] but not of unspiked patient samples with a sector-field ICP-MS and sequential single channel detection.

Current methods adopt different sample preparation schemes to separate and pre-concentrate uranium from urine [10,11,23,26,27]. These include dilution, microwave digestion, ion-exchange chromatography, and uranium precipitation. Co-precipitation can simultaneously pre-concentrate and separate uranium from the urinary matrix. Uranium may be co-precipitated with calcium and magnesium when the pH is elevated above 9 by adding ammonium hydroxide (NH_4OH). Use of NH_4OH as opposed to other precipitation agents such as calcium phosphate ($Ca_3(PO_4)_2$ [10] has several advantages: 1) high purity ammonium hydroxide is readily available, 2) urine contains sufficient amounts of Ca (40–200 mg L^{-1}) and Mg (20–100 mg L^{-1}) for co-precipitation when the pH of urine is carefully adjusted to above pH 9, and 3) use of NH_4OH to adjust the pH is also applicable to previously acidified samples.

In this study, $^{235}U/^{238}U$ and $^{236}U/^{238}U$ ratios in urine were measured by co-precipitating uranium, matrix removal by decanting, re-dissolving with dilute acid, and measuring by sector field ICP-MS. To our knowledge, this is the first study describing the analysis of total urinary uranium and uranium isotopic ratio from unspiked patient urine samples using ICP-sector field-MS and co-precipitation procedures.

2. Materials and methods

2.1. Reagents

Deionized water (>18.2 MΩ cm^{-1}) was made by using a Milli-Q Academic system (Millipore Corp., Molsheim, France). Dilute nitric acid was made from "Optima" grade nitric acid (Fisher Scientific, PA 67–70%) and stored in 2 L FEP bottles. Ammonium hydroxide was "Optima" grade (Fisher Scientific). The uranium contamination in concentrated NH_4OH was determined by neutralizing 1 mL of concentrated NH_4OH with 1 mL of concentrated HNO_3, followed by 1:10 dilution in water. The uranium concentration in NH_4OH was determined by ICP-MS to be <0.01 ng L^{-1} and was in agreement with the certificate of analysis (<0.1 ng L^{-1}). Therefore, the uranium contribution from 1 mL of ammonium hydroxide was considered to be negligible.

2.2. Equipment

Polypropylene centrifuge tubes (Becton Dickinson, Franklin Lakes, NJ), were filled with 20% (v/v) nitric acid, soaked for 72 h, triple rinsed with de-ionized water and dried overnight. A Finnigan Element 2 ICP-SF-MS (Thermo Electron, Bremen, Germany) was used in "low" resolution ($M/\Delta M=300$, 10% valley definition) mode. The sample introduction system included an SC-E2 sample changer and either a quartz concentric nebulizer and cyclonic spray chamber or a desolvating APEX Q sample introduction system (Elemental Scientific, Omaha, NE). The APEX Q system consisted of a heated cyclonic spray chamber and a Peltier-cooled multipass condenser. Typical conditions, methods, and tune parameters used to optimize the ICP-MS instrument are listed in Table 1.

2.3. Preparation of uranium isotope ratio working standard

Primary isotope standards were obtained from the Institute for Reference Materials and Measurements (IRMM) inter-laboratory

Table 1
Typical parameters for measurement total uranium and uranium isotopic ratios using Thermo Finnigan Element2 ICP-SF-MS.

Instrument parameters			
Plasma power	1250 W		
Cool gas	16.5 L min^{-1}	Aux gas	0.9 L min^{-1}
Torch	Standard	Cones	Nickel
Sample tubing	ID 0.38 mm	Pump rotation	20 rpm
Sample flow rate	0.26 mL min^{-1}	Volume/sample	2.0 mL
Uptake time	1′30″	Measure time	5′13″
Wash time	2′	Total time/sample	9′15″
ESI APEX Q sample introduction			
Nebulizer	100 mL micromist	Spray chamber	Heated cyclonic
Sample gas	0.77 L min^{-1}	Gas 2	0.30 L min^{-1}
Direct nebulization			
Nebulizer	GE AR30-1-FM04E	Spray chamber	Tracey cyclonic
Sample gas	1.00 L min^{-1}		
Method parameters			
Runs × passes	7 × 200	Setting time (ms)	1
Isotope	^{235}U	^{236}U	^{238}U
Mass window	10	10	10
Sample time (ms)	20	20	4
Sample per peak	50	50	50
Detection mode	counting	counting	counting