

Ultra-trace quantification and isotope ratio measurement of U in urine using the X Series ICP-MS

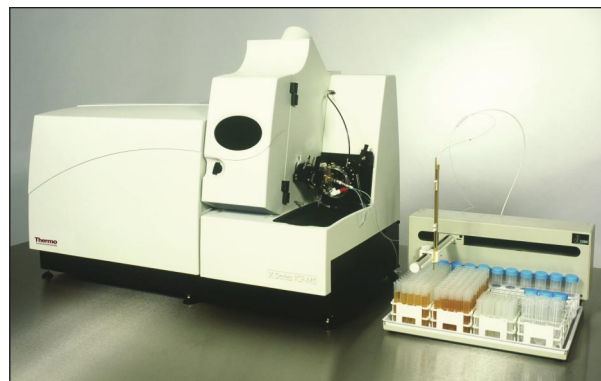
Key Words

- Uranium
- Urine
- Isotope ratio
- Ultra-trace quantitation

Introduction

Uranium is, perhaps somewhat surprisingly, a relatively common element. It is more abundant than silver, mercury and tungsten, and together with thorium, lies at the heart of the nuclear reactions that are believed to generate the Earth's internal heat. It was discovered in 1789 in pitchblende ore by the German chemist, Martin Klaproth, who named it uranium after the planet Uranus, discovered 8 years earlier. Uranium's natural radioactive properties were identified by Henri Becquerel in 1896, the discovery of which jointly earned him the 1903 Nobel Prize for Physics together with Pierre and Marie Curie. Pitchblende is the most common source of uranium, containing 50 to 80% uranium primarily in the form of UO_2 and UO_3 . The largest known concentration of this ore is in the Leopoldville mines, Congo, Central Africa, although smaller deposits exist in the Great Lakes area of Canada, the Czech Republic, Australia and South Africa.

In 1938, the German physicists Otto Hahn and Fritz Strassmann discovered that uranium atoms could be split by bombardment with neutrons to yield energy. This discovery subsequently led to uranium being used first for producing nuclear weapons, then later, as a fuel for nuclear reactors for power generation. The fission process begins with neutron capture by a uranium nucleus, followed rapidly by fission of the nucleus with a corresponding release of a large quantity of energy. Uranium nuclei of odd mass e.g. ^{233}U and ^{235}U can undergo fission by capturing 'slow' neutrons (i.e. those in thermal equilibrium with the surroundings), but to induce the fission process in even-numbered uranium masses (e.g. ^{238}U), 'fast' neutrons with energies in excess of 1 MeV are required. The fission cross section of slow neutrons is larger than that of fast neutrons (so fission is more efficient in the former case), and it is practically simpler to generate slow neutrons. It is for these reasons that nuclear power stations use reactors based on slow neutron fission of odd-numbered uranium masses. Natural uranium contains 99.27% ^{238}U , 0.72% ^{235}U and 0.006% ^{234}U , so is evidently low in abundance of odd-numbered uranium isotopes. Consequently, to optimise the fission process, uranium enriched in ^{235}U is required. This material is generally produced using gas centrifugation of UF_6 . In this process, ^{238}U migrates to the outer edge of the centrifuge (yielding uranium depleted in ^{235}U) and ^{235}U migrates less (yielding uranium enriched in ^{235}U towards the centre of



the centrifuge). The depleted uranium (DU) so formed (a dense and hard material) has been used as a counterweight material (particularly in aircraft, although more modern aircraft now use tungsten based materials) and, more significantly, as the base material (together with titanium) in the penetrator part of armour piercing shells. Natural uranium is not highly radioactive, and depleted uranium even less so (the half-lives for the decay processes $^{238}\text{U} \rightarrow ^{234}\text{Th} + \text{alpha particle}$ and $^{235}\text{U} \rightarrow ^{231}\text{Th} + \text{alpha particle}$ are 4.5×10^9 and 7.0×10^8 years respectively). In addition, the dominant radiation emission from uranium is in the form of alpha particles. These are stopped by clothing or skin and are only of significant concern if high concentrations of intense alpha-emitting materials are ingested or inhaled (in which case DNA damage and fragmentation could occur if the alpha particles reach cell nuclei, possibly resulting in cancer development). So, for the general public, health effects from uranium radiation exposure are in most cases minimal. However, like other heavy metals, such as cadmium, uranium is known to be toxic to the kidneys, where it is transported to before excretion in the urine (which is why urine, rather than blood, is analysed to determine uranium exposure). Consequently, mainly as a result of the use of DU in armour piercing shells in the Gulf War and Balkan conflicts, interest in (and concern about) the possible environmental and health consequences of this material has been growing in recent years. The major health risk associated with the use of DU in the penetrator part of these shells is exposure to DU dust, generated when the shells strike their targets. Inhalation of the dust leads to acute exposure of the lungs and other organs. It has been found that crews of military vehicles hit by DU

penetrators during the Gulf War subsequently showed DU levels in their physiological fluids above the range of values observed for natural uranium in unexposed individuals. Very high orally administered doses of uranium have been found to cause kidney damage in humans, and other studies have suggested that uranium exposure can also cause liver damage. Uranium accumulates in the renal tubular epithelium which causes cellular necrosis and atrophy in the tubular wall, resulting in decreased re-absorption efficiency in the renal tubule in humans. Uranyl (UO_2^{2+}) ions, like other heavy metal ions, are also known to delay or block cell division, which enhances the effects of cell necrosis. As with many elements, the toxicity of uranium varies according to its chemical form and route of exposure, with more water-soluble hexavalent U compounds, such as uranium hexafluoride, being the most potent renal toxins. In 2000, as part of the National Primary Drinking Water Regulations; Radionuclides, the US EPA set a new Maximum Contaminant Level (MCL) for uranium in drinking water of 30 ng/ml, in response to concern over its potential toxic effects.

Insoluble tetravalent compounds such as uranium tetrafluoride and uranium dioxide are not significant renal toxins, but can cause pulmonary toxicity if inhaled. Ingested uranium is less toxic than inhaled uranium, partly because of the low gastrointestinal absorption of uranium compounds (only < 0.1 – 6% of even the most soluble uranium compounds is absorbed). Highly soluble forms of uranium leave the lungs rapidly and are consequently less likely to affect the lung tissue. However, chronic inhalation exposure to levels of uranium hexafluoride accepted as tolerable, has been shown to cause kidney damage. Since natural uranium has low radioactivity, its renal and respiratory effects are usually attributed to its chemical properties. However, exposure to more radioactive uranium isotopes (e.g. ^{232}U and ^{233}U , and naturally occurring ^{234}U and ^{235}U) has been proposed to result in additive effects of the chemical and radiological toxicity, in some cases. Persistent exposure of uranium miners to the mine atmosphere has been linked to (sometimes fatal) respiratory diseases, although these diseases have been mainly attributed to exposure to other agents such as silica dust, oxide dusts, diesel fumes, radon and its progeny, and cigarette smoking. In fact, although uranium mining definitely elevates the risk of respiratory disease, it has been concluded that uranium itself makes only a minimal contribution to this risk, so kidney damage remains the most significant concern.

This application note describes the development of a method for ultra-trace quantitation and isotope ratio measurement of uranium in urine, which satisfies the challenging analytical requirements for this demanding and important analysis.

Sample and calibration solution preparation

A set of 30 urine samples were provided for analysis using the X Series ICP-MS from Thermo Electron Corporation. The samples were sub-divided into three groups. The first group comprised 10 blank urine solutions, the second comprised 10 samples of the same urine spiked with 9.5 ppt of U and the final group comprised 10 samples of the blank urine spiked with 95 ppt of U. The samples were diluted 1:10 with 1% HNO_3 and spiked with Lu as the internal standard (at 100 ppt). Calibration solutions were prepared in 1% HNO_3 from blank to 23 ppt U and spiked with the Lu internal standard (again, at 100 ppt). Spike recovery tests were also performed on samples A3, A5, B3, B5, C3 and C5. These samples were all diluted 1:10 with 1% HNO_3 , then spiked with U at 10ppt. Lu internal standard was added at 100ppt. Finally as part of the analysis procedure, a drift standard was run multiple times; once in the original calibration block, then after running sample set A, then after set B, again after set C and once more in a re-run of the calibration block at the end of the analysis (measured as unknown samples to test the calibration recovery after running the samples). The 14 ppt U calibration solution was selected to function as the drift standard.

Instrument configuration

The instrument was configured with a Peltier cooled, quartz impact bead spray chamber, Burgener Ari Mist low flow parallel path nebuliser (Figure 1) and standard single piece, 1.5 mm i.d. injector torch. The PlasmaScreen *Plus* and high performance interface (HPI) sensitivity enhancement options were used, to optimise the instrument performance. The instrument was operated in standard mode (i.e. without the use of the collision cell option).

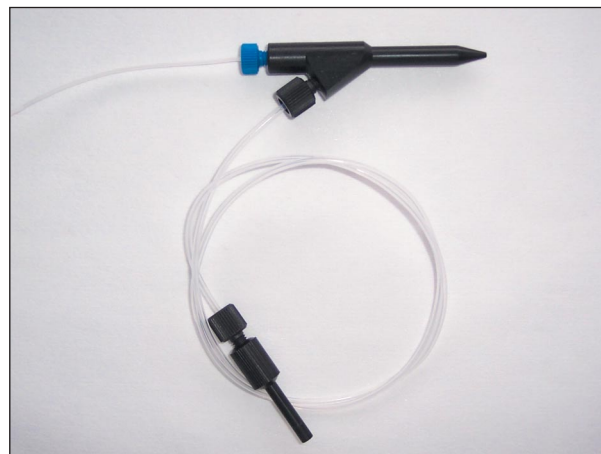


Figure 1: The Burgener Ari Mist parallel path low flow nebuliser.

General analytical conditions

The instrument was operated using the following parameters:

Parameter	Value
Forward power (W)	1400
Nebuliser gas (L/min)	0.90
Auxiliary gas (L/min)	0.60
Cool gas flow (L/min)	13.0

The instrument lens voltages were optimised for maximum sensitivity at uranium. The following acquisition parameters were used:

Sample uptake rate:	0.5 ml/min
Sample uptake/wash time:	60 s for both
Dwell times:	$^{235}\text{U} = 200 \text{ ms}$, $^{238}\text{U} = 2 \text{ ms}$ ^{175}Lu (internal standard) = 3 ms
Quadrupole settle time:	2 ms
Number of sweeps:	400
Number of points per peak:	1
Number of repeats per sample:	5
Total main peak jump run acquisition time:	88 s

Total acquisition time per sample = 10 min

Results and Discussion

The calibrations obtained for the two uranium isotopes are shown in Figures 2(a) and (b), below.

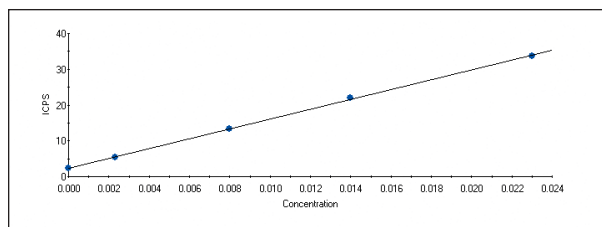


Figure 2(a): ^{235}U calibration, blank to 23 ppt.

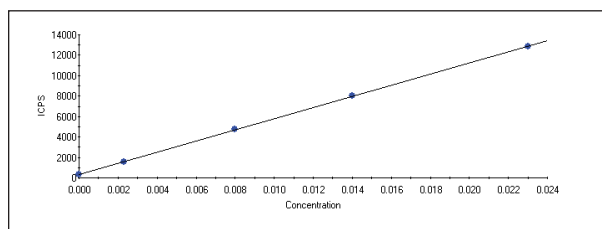


Figure 2(b). ^{238}U calibration, blank to 23 ppt.

The following figures of merit were derived from these calibrations.

PARAMETER	VALUE	
	^{235}U	^{238}U
Sensitivity (cps/ppt)	1.38	548
Blank equivalent concentration (ppt)	1.7	0.6
Detection limit (3 std. dev. of the blank) (ppt)	0.7	0.1

With the 10-fold sample dilution taken into account, the detection limit of U in the neat urine is equivalent to 7ppt at ^{235}U and 1ppt at ^{238}U . The concentration results obtained for each isotope in each sample measured are given in Table 1.

SAMPLE ID	^{235}U		^{238}U	
	CONC. (PPT)	+/- 1SD	CONC. (PPT)	+/- 1SD
A0	56.03	38.34	24.58	0.61
A1	22.08	14.61	7.33	0.36
A2	21.61	1.32	10.11	0.86
A3	20.04	0.91	8.27	0.62
A4	16.89	1.91	7.09	0.96
A5	13.07	2.32	7.07	0.28
A6	12.03	1.91	6.05	0.62
A7	13.85	1.84	7.05	0.25
A8	9.8	1.04	6.17	0.76
A9	15.52	8.75	6.17	0.29
B0	24.14	1.93	17.72	0.71
B1	22.62	1.1	16.47	0.76
B2	21.51	3.16	16.62	1.12
B3	22.59	0.94	17.2	0.95
B4	18.54	2.3	14.8	0.67
B5	31.29	1.14	24.02	0.46
B6	21.77	2.38	15.75	0.98
B7	22.48	8.41	14.67	0.61
B8	20.38	3.56	15.27	0.48
B9	22.91	9.46	14.72	0.49
C0	101.9	3.17	102.3	1.75
C1	103.5	7.52	98.3	1.45
C2	98.51	3.98	98.08	1.94
C3	103.3	6.18	100.2	1.47
C4	101.5	9.78	96.49	1.21
C5	100.2	0.98	98.04	2.4
C6	97.13	1.86	97.98	2.07
C7	95.78	5.68	98.03	2.93
C8	109.5	18.06	96.76	1.95
C9	96.94	2.06	98.79	2.69

Table 1: Uranium concentration (in ppt) calculated for ^{235}U and ^{238}U in each sample (dilution corrected).

Table 1 shows that the U concentration reported using the ^{235}U isotope is greater than that for ^{238}U for the A and B sample sets in particular. The reason for this is that in these samples, most of the U present is natural U, which has a higher level of ^{235}U than the depleted U used to prepare the spiked samples and calibration solutions (see later in this note). Samples containing natural U therefore show a positive bias on their measured U concentration if ^{235}U data alone are used. Since the ^{238}U abundance in both the depleted U used in this work and natural U is very similar, this bias is not apparent in the ^{238}U data. In addition, ^{238}U is far more abundant (and therefore sensitive) than ^{235}U , so higher accuracy is achieved with this isotope. For these reasons, total U concentration results based on ^{238}U are more reliable and should be used for quantitative measurement of U.

The data in Table 1 show that sample A0 was an outlier for the blank urine samples and sample B5 was an outlier for the 10ppt U in urine samples. With these data excluded, the average values for the A, B and C samples, based on the ^{238}U isotope measurement were as shown in Table 2 below.

SAMPLE I.D.	AVERAGE CONCENTRATION (PPT)	STD. DEV.	% RSD
A	7.3	1.3	17.63
B	15.9	1.1	7.16
C	98.5	1.7	1.71

Table 2. Average concentrations measured for samples A, B and C (dilution corrected), based on ^{238}U . Data for outliers A0 and B5 excluded.

Taking the average value of sample A as being representative of the blank urine U concentration, Table 3 below shows the blank corrected results and recovery relative to the expected concentrations of 9.5 ppt (B) and 95 ppt (C) for the average B and C sample set results.

SAMPLE I.D.	AVERAGE CONCENTRATION (PPT)	EXPECTED CONCENTRATION (PPT)	% RELATIVE ACCURACY
B	8.7	9.5	91
C	91.2	95	96

Table 3. Blank corrected (based on sample A) results for samples B and C (^{238}U data).

The data presented in Table 3 demonstrate that results consistent with the spiked U quantity were achieved in both the B and C sample sets, at measured U concentrations (after the 1:10 dilution) of only 0.95 and 9.5 ppt respectively. The results of the spike recovery tests are summarised below (Table 4).

SAMPLE I.D.	MEASURED VALUE (PPT)	SPIKE AMOUNT (PPT)	UNSPIKED SAMPLE RESULT (PPT)	% RECOVERY
A3 spike	10.86	10	0.83	100
A5 spike	10.64	10	0.71	99
B3 spike	12.18	10	1.72	105
B5 spike	13.71	10	2.40	113
C3 spike	19.91	10	10.02	99
C5 spike	20.58	10	9.80	108

Table 4: Spike recovery test results (^{238}U data).

Quantitative recoveries were achieved for all 6 spiked samples, at the 10 ppt added U level. The results of the drift standard analysis (14ppt) are summarised below (Table 5) and the re-run of the calibration set (measured as unknown samples) are compared with the original calibration data in Table 6.

SAMPLE I.D.	MEASURED RESULT (PPT)	% RELATIVE TO INITIAL CALIBRATION RESULT
14ppt U - initial cal. result	14.13	100
14ppt U - after sample set A	14.02	99
14ppt U - after sample set B	13.93	99
14ppt U - after sample set C	14.04	99
14ppt U - cal check	14.26	101

Table 5: Drift standard analysis results (^{238}U data).

SAMPLE I.D.	INITIAL CALIBRATION RESULT (PPT)	RE-RUN VALUES (PPT)	% RELATIVE TO INITIAL CALIBRATION RESULT
2ppt U	2.34	2.20	94
8ppt U	8.11	7.97	98
14ppt U	14.13	14.26	101
20ppt U	22.88	22.71	99

Table 6: Comparison of the complete calibration set measured as unknown samples at the end of the run versus the original calibration values (^{238}U data).

Tables 5 and 6 illustrate that the instrument performance has remained consistent throughout the entire run (a time period of 9 hours). Finally, the non-blank corrected $^{235}\text{U}/^{238}\text{U}$ ratios for each sample are shown below (Table 7(a) to (c)). Note that the calibration and spike solutions were prepared from a commercially available U standard that is depleted in ^{235}U , leading to a lower $^{235}\text{U}/^{238}\text{U}$ than is observed with natural U. This reinforces the earlier observation that determination of the total U concentration in urine is more reliable using the less variable (in terms of isotopic abundance) ^{238}U data.

SAMPLE I.D.	$^{235}\text{U}/^{238}\text{U}$	STD. DEV.	% RSD
A0	0.00609	0.00332	54.45
A1	0.00753	0.00274	36.34
A2	0.00615	0.00033	5.32
A3	0.00669	0.00042	6.23
A4	0.0067	0.00059	8.82
A5	0.00593	0.00041	6.92
A6	0.00622	0.00021	3.4
A7	0.00609	0.00025	4.06
A8	0.0057	0.00038	6.65
A9	0.0069	0.0019	27.54
Mean	0.0064		
Std dev	0.0005		
RSD %	8.6		

Table 7(a): Isotope ratio results for samples A0 to A9.

SAMPLE I.D.	$^{235}\text{U}/^{238}\text{U}$	STD. DEV.	% RSD
B0	0.00442	0.00027	6.03
B1	0.0045	0.00024	5.4
B2	0.00434	0.00027	6.16
B3	0.00436	0.00021	4.89
B4	0.00437	0.00027	6.08
B5	0.00409	0.00015	3.56
B6	0.00457	0.00047	10.26
B7	0.00488	0.00103	21.06
B8	0.00449	0.00045	10.1
B9	0.00491	0.00111	22.64
Mean	0.004493		
Std dev	0.0002		
RSD %	5.5		

Table 7(b): Isotope ratio results for samples B0 to B9.

SAMPLE I.D.	²³⁵ U/ ²³⁸ U	STD. DEV.	% RSD
C0	0.00277	0.00009	3.08
C1	0.00291	0.00017	5.96
C2	0.0028	0.00014	4.94
C3	0.00286	0.00017	6.09
C4	0.00291	0.00024	8.12
C5	0.00284	0.00006	2.23
C6	0.00277	0.00007	2.36
C7	0.00273	0.00011	3.94
C8	0.0031	0.00044	14.28
C9	0.00274	0.00008	2.74
Mean	0.002843		
Std dev	0.0001		
RSD %	3.9		

Table 7(c): Isotope ratio results for samples C0 to C9.

Table 7(a) shows that even at a U concentration of only around 1 to 2 ppt in the diluted urine, reasonable ²³⁵U/²³⁸U ratio precision (<10% RSD, n = 10) was obtained. It should be noted that this precision was achieved with a ²³⁵U signal at this concentration level of only 4 counts per second (a value still at least 40x larger than the instrument background level of < 0.1 cps). Increasing the number of sweeps made per repeat to a value greater than the 400 used may have further improved the precision, but the best precision achievable is ultimately determined by Poisson counting statistics and as such eventually becomes independent of further increases in the data acquisition time per sample. In summary, increasing the number of sweeps extends the total acquisition time with little or no improvement in precision, so an acceptable compromise was selected for this work. Table 7(a) also suggests that the U level detected in the un-spiked urine samples had arisen mainly from natural U as opposed to arising solely from a depleted U source, since the measured ²³⁵U/²³⁸U ratio of 0.0064 ± 0.0005 (not corrected for mass bias) is closer to the natural ²³⁵U/²³⁸U ratio of 0.007253 ± 0.000002 than to the equivalent ratio for depleted U. In samples B and C, that had been spiked with a commercial U standard, the ²³⁵U/²³⁸U ratio changed significantly from the un-spiked samples, indicating that the source of U in these samples was not entirely natural. As the spiked U concentration is increased, the measured ²³⁵U/²³⁸U ratio shifts from the natural value of 0.00725 to a value of around 0.00284, highlighting the (already, in this case, known) fact that the source of the spiked U in the samples was depleted in ²³⁵U. The results in Tables 7(a) to (c) demonstrate that using the X Series ICP-MS, even at the low ppt U levels monitored in this study, sufficiently large changes in the measured ²³⁵U/²³⁸U ratio are observed when depleted U is present to state with certainty that the sample contains U from non-natural sources. It should be noted that to improve the accuracy of these isotope ratio measurements (with respect to the mean measured value and its relation to the true value), correction for mass bias is required. Without mass bias correction, larger uncertainties would need to be applied to the measured isotope ratios to ensure that the

uncertainty range encompassed the true value. This would slightly degrade the level at which confident differentiation between samples can be achieved, but since the difference in ratio between DU and natural U is large, differences would still be visible at the ppt levels of U measured in this work.

Conclusions

The X Series ICP-MS is an excellent tool for trace U determination and isotope ratio measurement in urine and other biological samples. The instrument is also equally capable of quantifying uranium in environmental samples, such as waste waters, soils and drinking water, making it a powerful tool for monitoring and controlling uranium distribution in and discharge to the environment. With its high sensitivity and corresponding industry-leading signal to background specification, this robust, easy to use instrument is capable of routinely measuring U in urine at ppt levels in more than 100 samples per day. Measurement of other trace elements of medical interest in clinical samples can also be readily achieved using the X Series ICP-MS, following a simple sample preparation step (a single dilution step applicable for all liquid clinical samples is all that is required (see Application Note AN_E0601)). Through the combination of the X Series ICP-MS PlasmaScreen *Plus*, collision cell technology and Xi interface options, interference-free, simultaneous determination of all elements of clinical interest in a wide range of clinical samples can now be rapidly and effectively performed.

1. Agency for Toxic Substances and Disease Registry (ATSDR) Public Health Statement for Uranium, (see <http://www.atsdr.cdc.gov/toxprofiles/tp150-c2.pdf>)
2. Maximum Contaminant Level for Radionuclides in Drinking Water, current US EPA regulations (2000) (see <http://www.epa.gov/safewater/standard/pp/radnucpp.html>)
3. IUPAC Isotopic Composition of the Elements, Rosman, K.J.R. and Taylor, P.D.P, 1997 (available from <http://www.iupac.org/reports/1998/7001rosman/iso.pdf>)

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Australia
+61 2 9898 1244

Austria
+43 1 333 50340

Belgium
+32 2 482 30 30

Canada
+1 800 532 4752

China
+86 10 5850 3588

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+33 1 60 92 48 00

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+49 6103 4080

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