FEASIBILITY STUDY OF A SCREENING METHOD FOR PLUTONIUM IN URINE BY Q–ICP–MS

Petrus Philippus Pieterse

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Masters of Science.

Johannesburg, 2017

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Masters of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other degree or examination at any other University.

Petrus Philippus Pieterse

Signed on the 3rd day of June, 2017 in Johannesburg.

ABSTRACT

The monitoring of personnel for exposure to radioactive elements is a regulatory requirement for any nuclear installation and due to the nature of the research, the need for a non-invasive procedure for the monitoring for exposure to plutonium has been identified at the South African Nuclear Energy Corporation (NECSA). Historically, analyses of short-lived radioactive elements were performed by α-spectrometry. But due to the well documented drawbacks of the older generation of α-spectrometer analysis, Quadrupole Inductively Coupled Mass Spectrometry (Q-ICP-MS) was suggested as an alternative technique.

A feasibility study was undertaken to determine whether sub-trace concentrations of plutonium in urine can be determined using a modern, standard Q-ICP-MS. A simplified One Variable at a Time (OVAT) approach was used to establish optimal analytical conditions starting from instrument setup and ending in sample preparation.

The optimum sample preparation determined required 50 mL urine which underwent co-precipitation, wet-ashing and solid phase extraction using Tetra Valent Actinide (TEVA)-resin. Calibration standards ranged from 5 to 100 pg L^{-1} and the optimised method produced LOD's of 0.2 pg L^{-1} and LOQ's of 0.5 pg L^{-1}. Sample intra-assay precision at 0.5- and 15 pg L^{-1} were 11.3% and 4.43% respectively, in the urine matrix. The bias was 8% and 0% at the same concentrations. The method was evaluated using samples from an international proficiency study and two out of the three tests that could be quantified passed specification. According to ISO 13528 a method is acceptable if two out of three tests pass.

The method has been verified and a modern, standard Q-ICP-MS has been proven to be a suitable alternative to α-spectrometry with respect to sub-trace plutonium analysis.

ACKNOWLEDGEMENTS

First off I would like to thank my wife for being very patient. All the weekends being a single parent and waking up alone gave me the time I needed to complete this project. And for that I will always be grateful.

Next I would like to thank my supervisor Prof. Hlanganani Tutu for guiding me through the project. Under his guidance I learned about-, and worked a lot on my own shortcomings.

Also thanks to the Trace Lab section-head Mrs Magda Gerber because even under a difficult procurement environment managed to buy consumables required for the project. Without you the project would have taken a lot longer.

I would also like to thank my senior colleagues Mr Mike Britton and Mrs Belinda Leigh-Hickman who used their personal time to improve the quality of the dissertation through comments and suggestions.

A special thanks to my colleague Mr Josias Mokgawa. Working on weekends or after hours was a lot easier when you joined me.

Thanks to all my friends and family who showed interest in the project.

A final thanks to NECSA for giving me the opportunity to further my studies through the Study Assistance Scheme (SAS)

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LIST OF ABBREVIATIONS

AMS:	Accelerator Mass Spectrometry
CPS:	Counts per second
DF-SF-ICP-MS:	Double focusing sector-field ICP-MS
DoE:	Design of experiment
EIE:	Easy ionised elements
GDMS:	Glow Discharge Mass Spectrometry
ICP-OES:	Inductively Coupled Plasma-Optical Emission Spectrometry
ICP-MS:	Inductively Coupled Plasma-Mass Spectrometry
IP:	Ionisation potential
IS:	Internal standard
LOD:	Limit of detection
LOQ:	Limit of quantification
MC-ICP-MS:	Multi-collector ICP-MS
NECSA:	Nuclear Energy Corporation of South Africa (Pty) Ltd
OVAT:	One variable at a time
ORS:	Octapole reaction system
PAL:	Pelindaba Analytical Labs
Q-ICP-MS:	Quadrupole Inductively Coupled Plasma-Mass Spectrometry
RIMS:	Resonance Ionisation Mass Spectrometry
SF-ICP-MS:	Sector-field ICP-MS
SHEQ:	Safety, Health and Environment Quality
SIMS:	Secondary Ionisation Mass Spectrometry
TDS:	Total dissolved solids
TEVA:	Tetra Valent Actinides
TIMS:	Thermal Ionisation Mass Spectrometry
TRU:	Trans Uranium elements
UTEVA	Uranium and Tetra Valent Actinides

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CHAPTER ONE– Introduction

1.1 Background

When uranium is placed in a nuclear reactor, a fraction of the ^{238}U absorbs a neutron and is converted to ^{239}U. ^{239}U undergoes β-decay to form ^{239}Np which also undergoes β-decay to form ^{239}Pu (http://nuclearweaponarchive.org/Library/Plutonium/ Accessed: 05/01/2016). The reaction chain can be summarised as follows:

$$^{238}_{92}U + {}^{1}_{0}n = {}^{239}_{92}U \xrightarrow{\beta-decay} {}^{239}_{93}Np \xrightarrow{\beta-decay} {}^{239}_{94}Pu$$

Scientists at the Nuclear Energy Corporation of South Africa (NECSA) are developing a process to recover uranium from ^{99}Mo process waste residue (Stassen, 2015) which contains trace amounts of plutonium. Personnel on this project have to be monitored for exposure to plutonium. It is important for accurate bioassay of plutonium because it is a radioactive element that undergoes spontaneous fission and is a strong alpha emitter (Voelz, 2000). Because of this, external exposure to radiation is not a big concern as even a sheet of paper (or 3-5 cm of normal atmosphere) is enough to absorb the emitted He-particle.

Plutonium analyses are normally performed by α-spectrometry, which is still the preferred method in use at NECSA. Two major drawbacks of this method are:

- the cost of the analysis due to tedious sample preparation
- the time it takes to produce a reliable result due to longer counting times for low-activity radionuclide's (Ting et al.,2003; Bouvier-Capley et al.,2004).

Counting times can be anything from 1 day to a week per sample (Bouvier-Capley et al., 2004). Having similar sensitivity and higher sample throughput, techniques such as Thermal Ionisation Mass spectrometry (TIMS), Resonance Ionisation Mass Spectrometry (RIMS) and Accelerator Mass Spectrometry (AMS) are reliable as alternatives to α-spectrometry (Pappas et al., 2004) but these instruments are not readily available. During the last few decades, ICP-MS has started to dominate the

market for the analysis of long-life radio-nuclides (Hernandez-Mendoza et al., 2011). Limits of detection for different analytical techniques are shown in Figure 1.1.

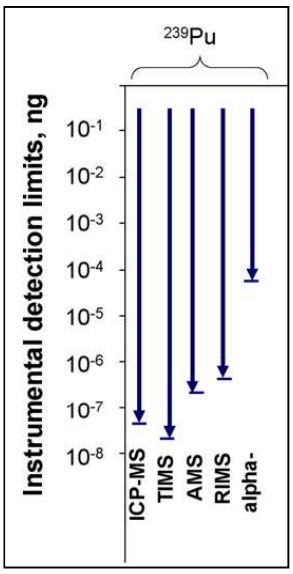

Figure 1.1 : LOD's for different analytical techniques (Boulyga, 2011)

ICP-MS has the advantage of lower LOD's, and decreased analysis times (Ting et al., 2003; Bouvier-Capley et al., 2004). The main concerns for ICP-MS are the spectral- and non-spectral interferences from the heavy urine matrix and the presence of uranium which could interfere with the analysis of plutonium by the following means:

- peak tailing from the $^{238}U^{+}$ isotope if its relative concentration is significantly higher than that of plutonium
- the polyatomic interference from uranium hydride, $^{238}UH^{+}$ (Pappas et al., 2004).

In most references, one or more sample preparation steps are used to reduce or eliminate possible interferences. Sample preparation of urine requires separation from the matrix by alkaline co-precipitation of the actinides using $Ca_3(PO_4)_2$ (EICHROM Technologies, 2000; Wyse et al., 1994; Kuwabara et al., 2002) and/or separation of Pu by using TEVA columns (EICHROM Technologies, 2000; Pappas et al., 2004),

TRU-resin (Wyse et al., 1994; Kuwabara et al., 2002; Epov et al., 2005), Dowex 1X2 and 1X8 Anion Exchange resin (Hernandez-Mendoza et al., 2011) or Chelex-resin.

In this study, a Q-ICP-MS was systematically optimised starting from sensitivity to increase the CPS/ppb, and ending with precision to reduce the method imprecision. Once the instrument was considered optimal, the focus moved to sample preparation. Systematic evaluation and optimisation for sample preparation started with different sample preparation procedures, and ended with additional tests to evaluate the robustness of the process. During sample preparation evaluation, it was established that solid-phase extraction is unavoidable when the lowest possible Limit of Detection (LOD) and Limit of Quantification (LOQ) is required. This required additional evaluation to ensure that during the solid-phase extraction, as much as possible sample matrix (U included) is removed prior to recovering the adsorbed Pu.

1.2 Research Question

The research was based on addressing one main question. That is:

Can a standard, modern Q-ICP-MS analyse Pu at concentrations as low as 0.2 pg L^{-1} as required by the NECSA SHEQ department and similarly to the current α-spectrometric technique?

1.3 Research Hypothesis

The research was based on the hypothesis that:

Ho: A standard, modern Q-ICP-MS cannot analyse Pu concentrations equal to or lower than 0.2 pg L^{-1}.

Ha: A standard, modern Q-ICP-MS can analyse Pu concentrations equal to or lower than 0.2 pg L^{-1}.

1.4 Research Aim & Objectives

i) The aim of the study is to determine whether a standard, unmodified Q-ICP-MS can analyse Pu concentrations similar to or lower than that of α-spectrometry.

The Aim was achieved by addressing the following objectives:

- To determine optimal settings for highest instrument sensitivity.
- To determine optimal sample preparation by balancing the method concentration factor and analysis practicality.
- To establish accuracy and precision over the analytical working range to determine method LOD/LOQ.
- To verify the method using samples from an international proficiency scheme spiked with Pu.

1.5 Significance of Research

There are two aspects to the significance of the research:

1) To determine whether a standard Q-ICP-MS using modern technology can achieve similar LOD's compared to that of modified systems or specialized instruments.

2) To determine whether a drastic reduction in the typical sample volume to be processed will still allow quantification of Pu at the same LOD and other figures of merit as α-spectrometry. This will result in quicker turnaround time, less sample load on the laboratory and more convenient handling of samples.

1.6 Structure of Dissertation

In chapter 1 the general background was given with respect to how plutonium is formed inside a nuclear reactor, the health risk associated with possible exposure, the drawbacks with the current analytical capability of NECSA, a quick glance at historical developments and how the feasibility study was conducted. The Literature

Review is presented in chapter 2 where the references and the stated analytical performances were scrutinized and compared to each other as to give an indication of what can be expected from this study. The Research Methodology is presented in chapter 3 and covers the theoretical framework, design of experiment, materials and reagents. Data gathered as well as deductions made from them will be presented in Chapter 4. The conclusion and final recommendations will be presented in the final chapter, Chapter 5.

CHAPTER TWO –Literature Review

2.1 Radiological hazard of plutonium

It is when plutonium is either inhaled, ingested or absorbed through a wound where it enters the bloodstream and up to 80% of the retained plutonium is deposited in the lungs, liver or bone (Voelz, 2000). That is why lung, liver and bone cancers are the main concern from plutonium exposure (Burns, 2002). It is important to emphasis the *retained* plutonium because the radiological hazard depends on how the plutonium enters the body as well as the particle size. Ingested plutonium passes through the gastrointestinal tract with as little as 0.05% and 0.001% retained for soluble- and insoluble compounds respectively (Burns, 2002). Only 2% of the retained plutonium leaves the body through the urinary tract. The rest is excreted via faeces.

Inhalation of plutonium particles holds the largest risk. The particle size dictates how easily it is absorbed unto the lungs. Particles that are larger than 10 μm pose minimal risk as they are swallowed and pass through the gastrointestinal tract. Between 1- and 10 μm of the particles are retained a little longer, but are also removed through the throat via cilia(hair like structures) (Burns, 2002). Particles smaller than 1 μm are transported by even the smallest of pathways, where they make their way to the lungs and deposit on the alveoli. These particles have the longest retention coefficients (Voelz, 2000).

Every time a plutonium molecule decays, an α-particle is emitted. And for every α-particle, a path of ion pairs is left in its wake as illustrated by Figure 2.1. If enough cells are damaged, tissues may be affected and organs may be impaired.

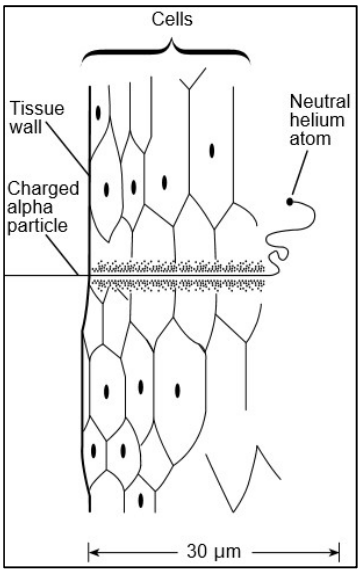

Figure 2.1: The movement of an ejected α-particle through cells (Voelz, 2000)

2.2 Instrumentation

2.2.1 Introduction

Q-ICP-MS is a hyphenated technique which uses a high temperature Ar-plasma for an ion source (which was already in use with ICP-OES), and a quadrupole (used in GC-MS) as a mass filter (Skoog et al, 2007). Since its introduction in the early 1980's, ICP-MS has rapidly developed and started to dominate the analytical market as it can deliver minimal detection limits, has isotopic capability and has the highest throughput (Thomas, 2004).

2.2.2 Principles of analysis

The sample (typically as a liquid) is transported into the nebulizer where the liquid is converted into a fine mist. The mist is carried by Ar-gas into the Ar-plasma where

elements undergo several processes until they are ionised. The cations (as part of the ion beam) are bent and focused by the lens before entering the quadrupole. In the quadrupole the ions are separated based on their *m/z* ratio where *m* is the atomic mass and *z* is the charge (usually +1) (Agilent,2005). The detector measures the signal strength of each ion and generates a mass spectrum. The whole process from sample uptake to signal generation is summarised by Figure 2.2.

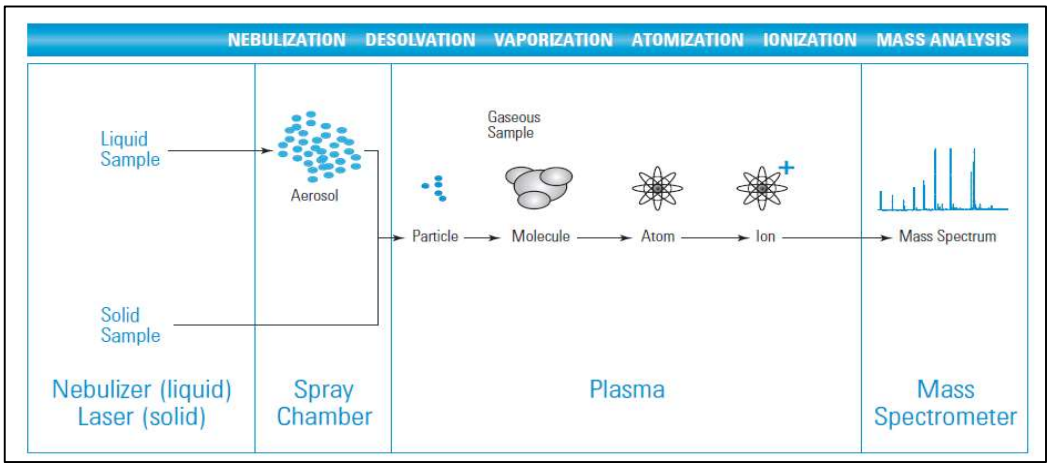

Figure 2.2: Schematic of the processes that lead to signal generation in ICP-MS (Agilent,2005)

2.2.3 Q-ICP-MS components

The instrument used for this study is the Agilent 7700X Q-ICP-MS. Most of the components can be seen in Figure 2.3.

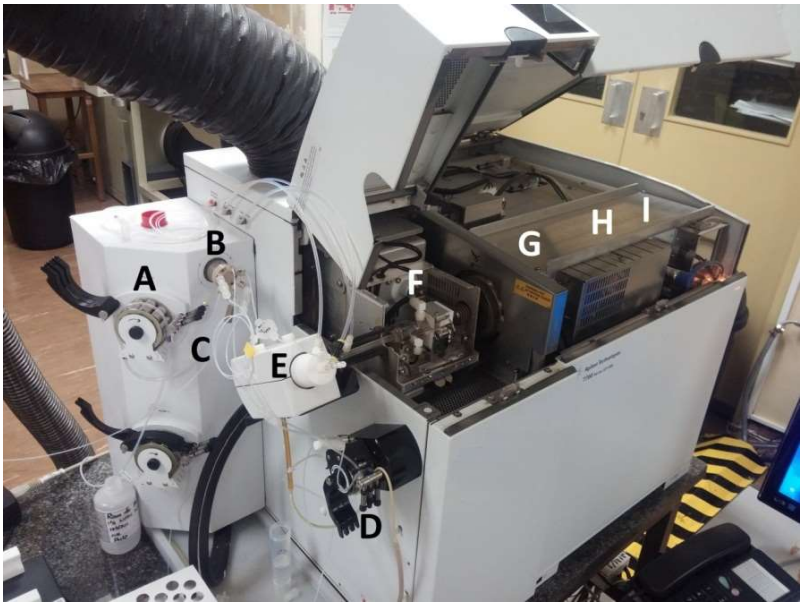

Figure 2.3: Agilent 7700X

2.2.3.1 Peristaltic pumps

The peristaltic pumps (A) consist of 10 rollers which place pressure on flexible tubing with the help of a spring-loaded cam. This allows continuous and pulse-free flow of sample solution (Figure 2.4) to be carried from the sample container to the *Sample Loop*(C) by the peristaltic pump, and from the *Sample Loop* to the nebulizer by the Nebulizer pump (Skoog et al, 2007).

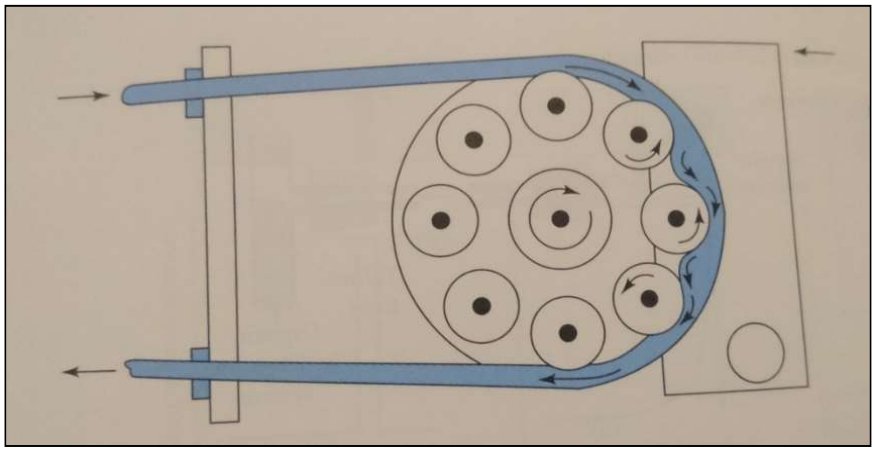

Figure 2.4: The mechanism of a peristaltic pump (Skoog et al, 2007)

2.2.3.2 Nebulizer

Inside the concentric quartz nebulizer (E) (Refer to Figure 2.7), the liquid sample is carried through the inner quartz tube while argon flows at approx. 1 L/min through the outer quartz tube. As the sample flows out of the tip, the high velocity Ar gas converts (nebulizes) the liquid into a fine mist. This mist is then carried into the spray chamber (Agilent Technologies Inc., 2009).

2.2.3.3 Spray chamber

The sample mist droplet sizes vary when the mist is formed in the nebulizer. The spray chamber (E) acts primarily as a droplet size filter where small droplets are transported by the carrier gas into the plasma and large droplets collide with the chamber wall (Figure 2.5) and are drained by one of the 3 channels of the peristaltic pump (Agilent Technologies Inc.,2009). The secondary function of the spray chamber is to reduce any pulses that could have been created by the peristaltic pumps (Thomas, 2004)

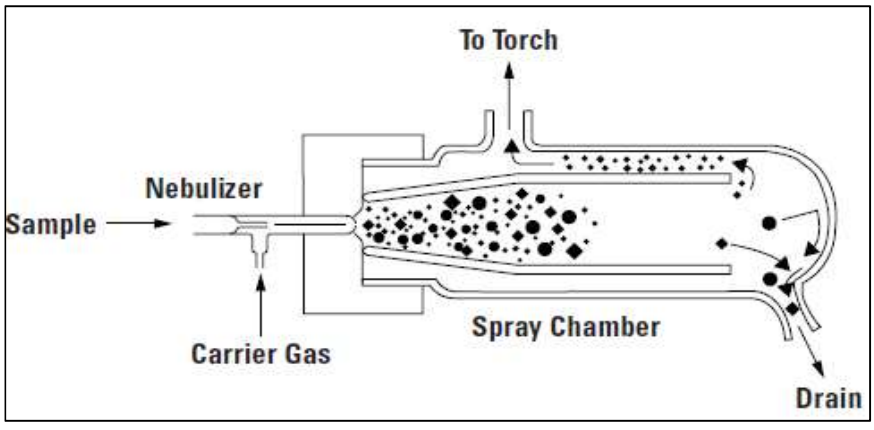

Figure 2.5: Nebulizer and Spray Chamber (Agilent Technologies Inc., 2009)

2.2.3.4 Torch

The sample mist is transported from the spray-chamber to the torch (F) which contains three concentric quartz tubes (Figure 2.6). In the inner tube, the sample aerosol is carried into the plasma with argon carrier gas (approx. 1 L min^{-1}). In the middle tube, argon auxiliary gas flows. The outer tube is where argon coolant gas flows, also called the Plasma gas (at ca 14 L min^{-1}). This serves to stabilize and thermally isolate the plasma (Thomas, 2004).

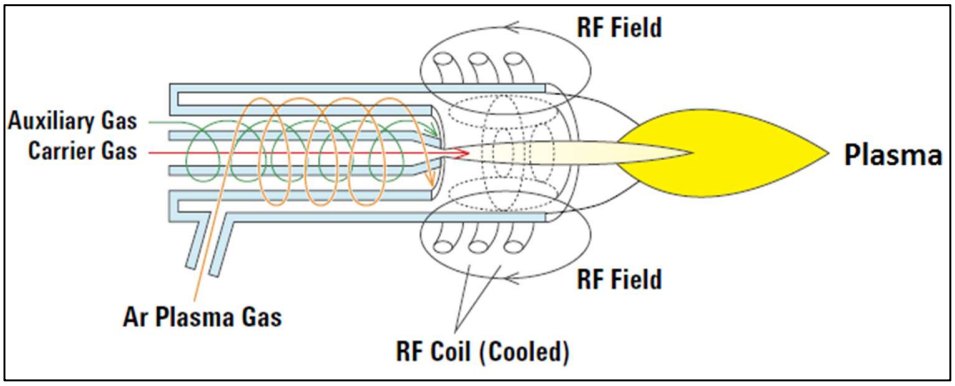

Figure 2.6: ICP Torch and Plasma (Agilent Technologies Inc,2009)

2.2.3.5 Plasma

The plasma normally has an intense white core and a flame like tail, and consists mainly of argon ions and electrons in equilibrium with each other (Skoog et al, 2007). The Ar-plasma allows most elements of interest to be ionized with efficiencies larger than 90% (Agilent Technologies Inc., 2009). The temperature ranges of a plasma are illustrated in figure 2.7.

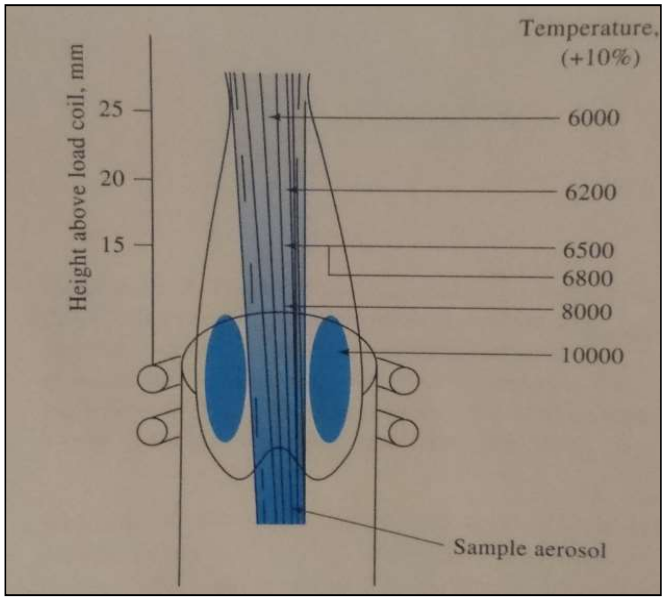

Figure 2.7: The plasma and typical temperature ranges (Skoog et al, 2007)

2.2.3.6 Interface

After the ions have formed in the plasma, they travel a short distance to the interface (G) which is kept under vacuum to ensure the integrity of the ion beam. The ion beam first passes through the sampling cone (orifice approx. 1.0 mm) where it expands. A fraction of the expanded beam is sampled by the skimmer cone (orifice approx. 0.4mm). The skimmer acts as a differential aperture between the interface and intermediate vacuum stages of the instrument. The interface vacuum is sustained by a rotary valve vacuum pump, while the intermediate vacuum is sustained by a turbo molecular pump (Agilent Technologies Inc.,2009).

2.2.3.7 Ion Lenses

The ion beam contains not only analyte ions but also neutral species and photons (Thomas, 2004). The aforementioned two have to be removed in order to get better sensitivity and lower background for more reliable results. For improved beam

bending and focusing, the Agilent 7700x has a 5-piece ion-lens separated by ceramic spacers because each has a different voltage applied to it. The Omega Bias-x lens serves to bend the beam of ions and thus eliminate all the neutral particles (photons are removed at the detector) (Agilent Technologies Inc., 2009). Figure 2.8 shows the different components of the Agilent 7700 lens.

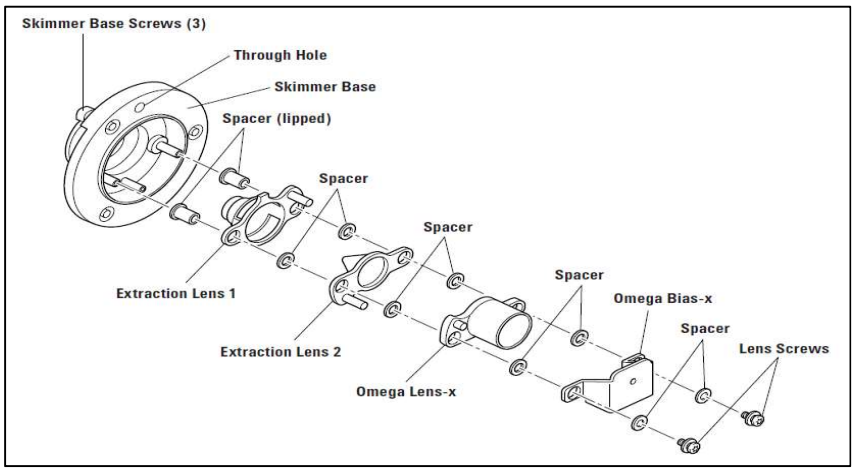

Figure 2.8: Exploded view of the 7700 ion-lens optics (Agilent Technologies Inc., 2009)

2.2.3.8 Collision/reaction cell (ORS)

After the ion beam exits the optic lenses, it enters the collision/reaction cell (ORS). When activated in analysis mode, the cell has a constant stream of gas flowing through it. Helium or hydrogen is commonly used although other gases such as ammonia, oxygen and methane are also used in certain cases. The cell can be used in one of two ways. a) where interfering polyatomic species can be reduced by collision with the gas and b) where analyte/interfering isobaric-, polyatomic species are converted to non-interfering species by reaction with the gas (Thomas, 2004). The collision/reaction cell slightly reduces sensitivity and was not activated for this study.

2.2.3.9 Quadrupole

After the ions exit the ORS, they enter the quadrupole mass filter(H) which consists of four cylindrical rods placed parallel to each other. Opposite pairs are connected electronically to a DC current with polarity alternating at radio frequency(RF) between one set(positive) and the other(negative). As Figure 2.9 illustrates, the rods act as a mass filter when the DC and RF current are varied, allowing only a specific m/z ratio to have a stable trajectory through the rods (Agilent Technologies Inc., 2009).

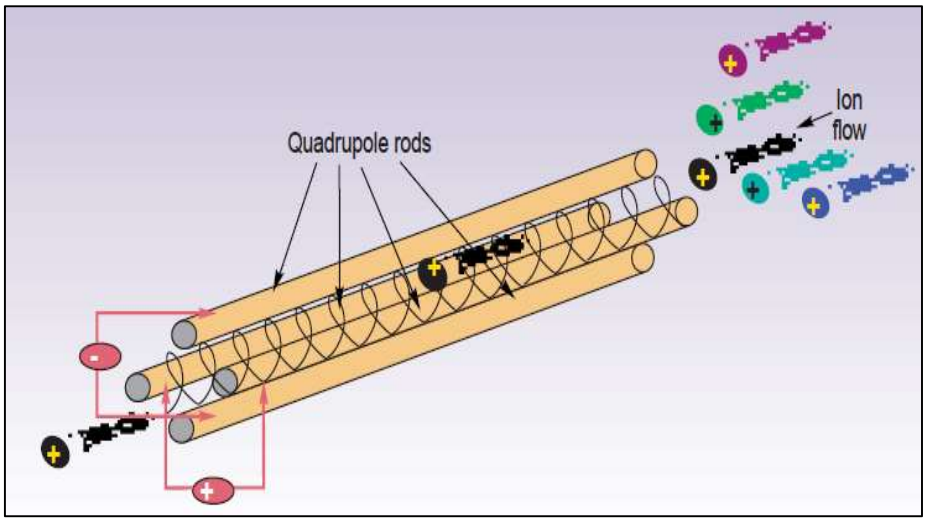

Figure 2.9: The quadrupole filtering a single *m/z* ratio (Thomas ,2004)

Lower DC and RF voltages are selected for lighter *m/z* ratios and higher voltages for larger *m/z* ratios. The resolution of a quadrupole is expressed as:

$$R = \frac{f^2 L^2}{V} \qquad (2.1)$$

f is the frequency, L is the quadrupole length and V is the velocity of the ion (Agilent Technologies Inc., 2009). The disadvantages are that the quadrupole cannot

distinguish isobaric - and polyatomic interferences from ions of the analyte with the same *m/z* ratio.

2.2.3.10 Electron Multiplier (Detector)

The electron multiplier (I) consists of multiple dynodes. The ion hits and ejects electrons from the initial dynode, which are attracted to the next dynode where more electrons are ejected. At the end, a large amount of electrons is generated for each ion that hits the initial dynode (Agilent Technologies Inc., 2009). The detector is usually placed off axis to the quadrupole so as to reduce the background noise arising from photons and other stray radiation (Thomas, 2004). The 7700 detector consists of two modes. *Pulse* mode for low concentrations which uses all the dynodes for electron multiplication, and *analog* mode for high concentrations where approx. half the dynodes are used for multiplication. The two modes are not linear and a P/A-factor has to be measured in order to establish linearity over 10 orders of magnitude (Agilent Technologies Inc., 2009).

2.3 Limit of detection

The LOD of an analytical method is important because it gives some information about parameters such as accuracy, precision and sensitivity. These parameters are determined by both instrumental technique and sample preparation. It is therefore important to investigate what LOD's had been previously reported in the literature and how the LOD's were improved over time. From the literature there are four main factors that affect the LOD of an analytical method namely: the type of instrument used, modifications to the instrument (to further boost sensitivity), sample volume treated and sample purification.

2.3.1 Type of instrumentation

Work performed by Epov et al. (2005) shows the difference in the capabilities of a Q-ICP-MS and a sector field inductively coupled plasma mass spectroscopy (SF-ICP-MS). LOD's were determined by each of these instruments using the conventional pneumatic nebulizer. The Q-ICP-MS's LOD was determined to be 230 pg L^{-1} while the Double Focusing SF-ICP-MS (DF-SF-ICP-MS) reached a LOD of 5 pg L^{-1}. The DF-SF-ICP-MS is superior because it has higher sensitivity ($1x10^6$ CPS/ppb) and lower background with the help of ion-transfer optics, a magnetic field mass filter and electrostatic analyser. Hernandez-Mendoza et al., (2011) also compared LOD's from three different techniques. The α-spectrometry method produced an LOD of 0.05 pg L^{-1}, SF-ICP-MS was 0.01 pg L^{-1} and AMS was 0.005 pg L^{-1}. This partly contradicts the publication by Boulyga (2011) which indicates that AMS cannot report LOD's as low as those for ICP-MS. One of the early publications on the analysis of plutonium in urine by Wyse and Fischer (1994) employed a matrix purification step using TRU-resin and reported a LOD of 100 pg L^{-1}. For comparison more recent LOD's have been established as 0.19pg L^{-1} (Epov et al, 2005). A similar volume of sample was processed, but slightly different sample preparation was employed. Samples were microwave digested before matrix purification by TRU-resin. The main factor determining improvement in the LOQ was the technology. In 10 years the LOD has been reduced from 100 pg L^{-1} to 0.19 ng L^{-1}, approx. 500 times lower. The instrumentation used by Epov et al. (2005) was a SF-ICP-MS coupled with a desolvating sample introduction system. The Pelindaba Analytical Laboratories (PAL) currently only have a standard Q-ICP-MS, and it would be beneficial to determine how a modern Q-ICP-MS method with no modifications compares to these published methods.

2.3.2 Instrument modification

With the exception of Hang et al. (2004), all of the references relating to plutonium analysis in urine used modified systems to increase sensitivity and by extension, lower the LOD's. These include systems with an extra primary pump to increase vacuum (Baglan et al., 2000), replacing the pneumatic nebulizer with an ultra-sonic nebulizer (Wyse and Fisher, 1994; Kuwabara et al., 2001), or replacing the standard sample introduction with a desolvating introduction system (Ting et al., 2003; Zoriy et al., 2005; Pappas et al., 2004). Epov et al. (2005) also illustrated the effect of adding a desolvating introduction system to both Q-ICP-MS (Figure 2.10) and DF-SF-ICP-MS (Figure 2.11) compared to standard systems. This results in an average LOD reduction of approximately 5x which agrees with comments by Wyse and Fisher, (1994).

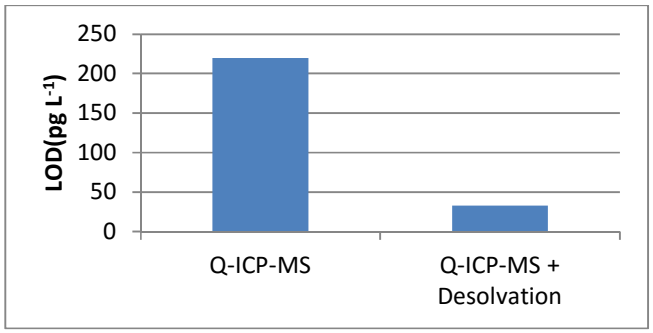

Figure 2.10: Comparison of LOD by addition of desolvating introduction system for Q-ICP-MS

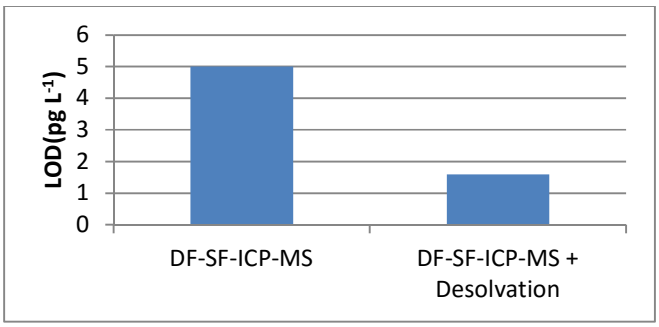

Figure 2.11: Comparison of LOD by addition of desolvating introduction system for DF-SF-ICP-MS

2.3.3 Sample volume

There is a correlation between the volume of sample processed and the LOD. Only a few publications described sample treatment on sample volumes less than 50 mL with higher LOD's than those obtained with larger volumes. For example, Pappas et al. (2004) reported a LOD of 0.19 pg L^{-1} by treating 1 mL of sample compared to 0.01 pg L^{-1} from Hernandez-Mendoza et al. (2011) who treated 370 mL for the same instrument. Ting, et al. (2003) reported a LOD of 4.7 pg L^{-1} by processing a sample of 10 g (≈10 mL assuming density = 1 g mL^{-1}) compared to Kuwabara et al., (2001) who reported 0.13 pg L^{-1} by treating 1000 mL urine. The higher the sample volume prepared, the lower the concentration of plutonium that can be detected. For this reason, most publications use sample volumes greater than 500 mL (Kuwabara et al., 2001; Baglan et al., 2000; Zoriy et al., 2005; Sherrod et al., 2008). For the scope of this study, 50 mL was selected as the sample volume as it is sufficient volume for a 10x concentration factor, yet sufficient for a spot-sample. PAL does not have the capacity or equipment to process 1 L samples.

2.3.4 Sample purification

ICP-MS analysis suffers both spectroscopic and non-spectroscopic interferences (Hernandez-Mendoza et al., 2011), especially in a heavy urine matrix. Spectroscopic interferences occur where atomic-or molecular ions formed in the plasma, have the same atomic mass (or more specifically *m/z* ratio) as the element of interest. This will interfere with the analysis by causing an increase on the measured m/z ratio which will result in false concentration determinations (Evans and Guglio, 1993). For actinides in urine, the spectroscopic interferences resulting from uranium have been thoroughly investigated and if plutonium is expected in the urine, the uranium will also be present in greater concentrations. Uranium form the $^{238}UH^{+}$ ion which is isobaric to ^{239}Pu (Wyse et al., 1994; Baglan et al., 2000; Ting et al., 2003; Zoriy et al., 2005; Epov et al., 2005; Maxwell et al., 2011). Pappas et al. (2004) also reported that

samples containing organic matter also causes a polyatomic interference on the m/z=239 signal. Non-spectroscopic interferences can either enhance or reduce the analyte signal by affecting properties such as sample transport, ionisation, or ion throughput (Evans and Guglio, 1993). An example of non-spectroscopic interference is the suppression of signal when analysing undiluted urine or with a small dilution factor (Wyse et al., 1994; Hang et al., 2004; Pappas et al., 2004; Zoriy et al., 2005). Because of a combination of very low concentrations of plutonium and the interferences from the urine matrix, emphasis has to be placed on sample preparation as this is what determines whether the method will be successful or not. Ting et al. (2003) reported, as illustrated by Figure 2.12, there is a trade-off between the time spent on removing the matrix, and the LOD that can be achieved.

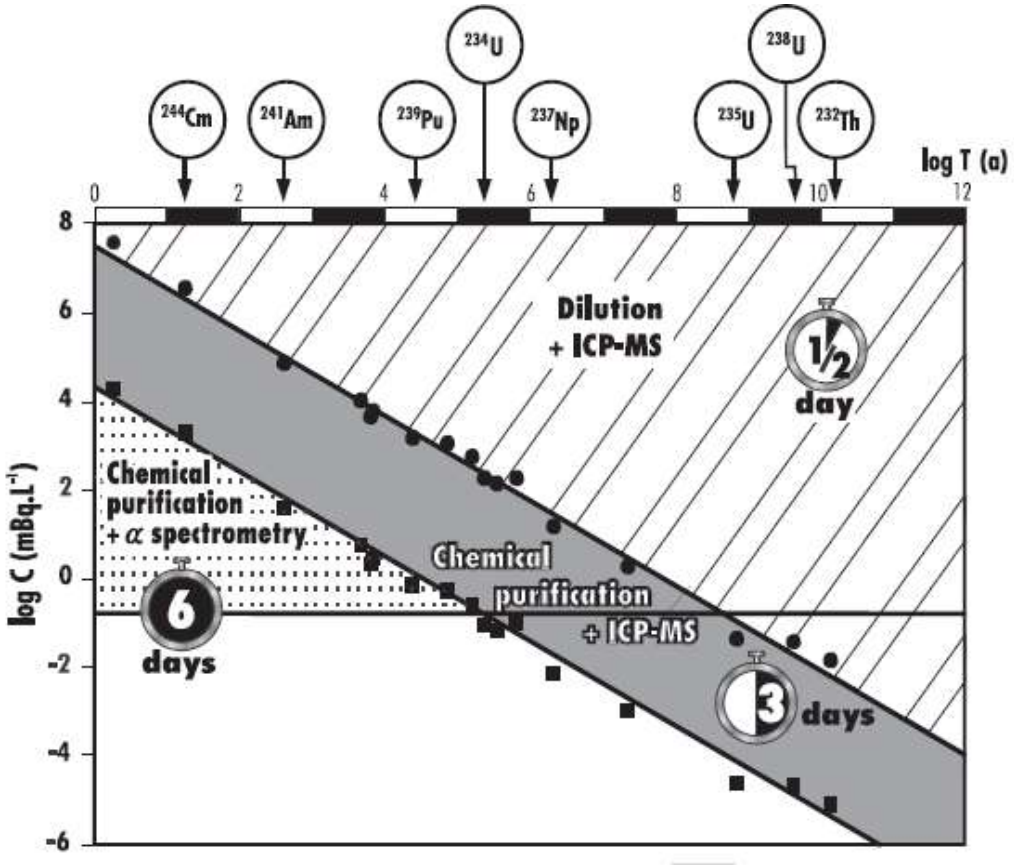

Figure 2.12: LOD dependence on sample preparation for different radionuclide's. (Bouvier-Capley et al., 2004)

They reported an LOD of 230 pg L^{-1} for a dilution as compared to 4.7 pg L^{-1} for a microwave acid digested sample when using a SF-ICP-MS. Kuwabara et al., (2001) reported an LOD of 0.13 pg L^{-1} using a less sensitive Q-ICP-MS, but spent considerably more time on sample preparation. Samples were co-precipitated, microwave oven digested, solid-phase separated and microwave acid digested again prior to analysis.

2.4 TEVA-resin for actinide analysis

For this application where the plutonium is the only analyte of interest, TEVA is the more suited resin as opposed to Uranium and Tetra Valent Actinides (UTEVA)-resin or Trans Uranium elements (TRU)-resin (as per email correspondence with Steffen Happle: Eichrom method specialist). The **Te**tra-**V**alent **A**ctinides selective resin is the quaternary ammonium salt (Aliquat 336) with the structure: $(C_8H_{17}/\ C_{10}H_{21})_3\text{-}N^+\text{-}CH_3$.

Figure 2.13 shows how the TEVA columns can aid the separation of uranium and plutonium with a suitable rinse solution.

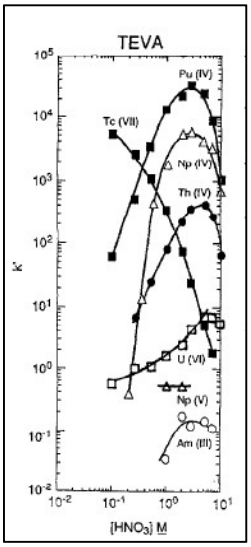

Figure 2.13: Dependence of retention factor (k') for different acid concentrations

The most basic steps required to perform actinide separation on a TEVA column were attained from EICHROM Technologies (2001), and EICHROM analytical procedure ACU02. A co-precipitation step is used to remove the plutonium from the liquid phase. The heavy urine matrix can then be discarded as the supernatant. The samples are then placed on a hotplate with HNO_3 and H_2O_2 to remove any remaining organic matter. A valence adjustment step is performed to convert all the plutonium to the tetravalent species, which has the highest retention factor on TEAV-resin, before being passed through the TEVA-packed column. The loading solution should be more acidic (>1M HNO_3) to retain the plutonium. A suitable solution is passed through the column to strip the plutonium from the resin and then analysed via Q-ICP-MS.

2.5 Current actinide analysis trends

Every year, NECSA participates in an international actinide proficiency scheme in which labs are requested to submit information about the method used. The following data (figure 2.14 – figure 2.17) was obtained from the 2016 Procorad results where at least 65 laboratories participated to show current practices and trends. Figure 2.14 shows that wet assay is still the predominant sample treatment, used by 70% of the participating laboratories.

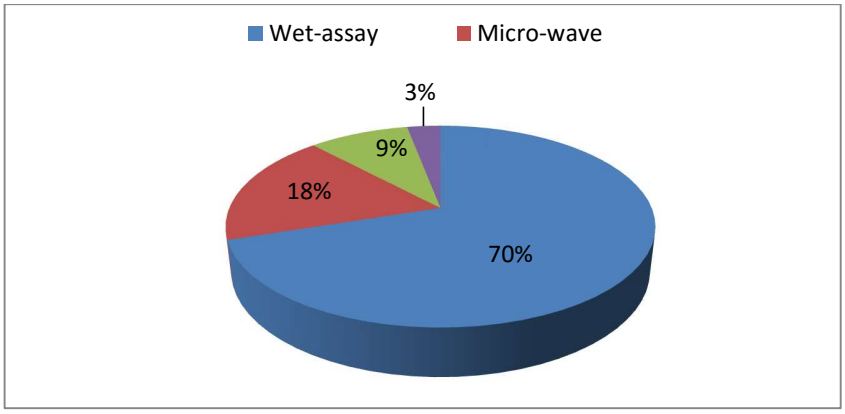

Figure 2.14: The preferred sample mineralization for actinide analysis in 2016

Figure 2.15 shows the preferred co-precipitation step. The $(Ca)_3(PO_4)_2$ should have been included in the total for Alkaline earth precipitation. This study will utilize the predominant co-precipitation step, used by ≈ 64% of the laboratories.

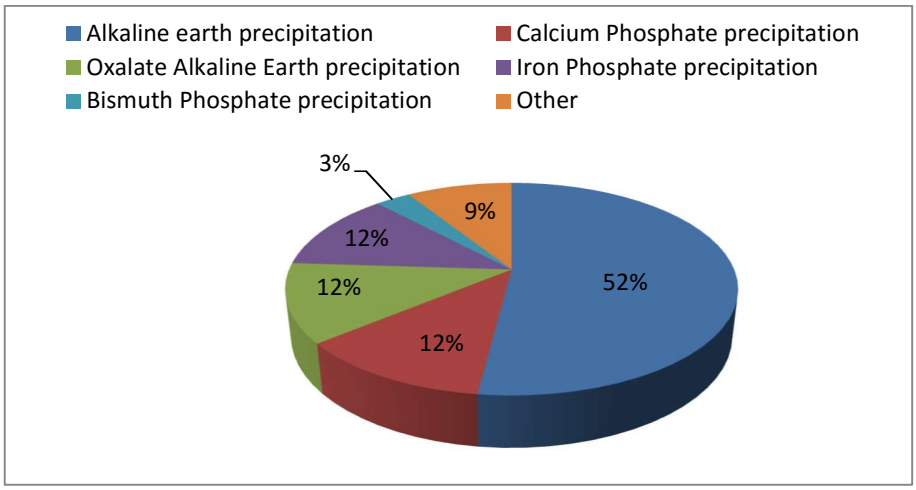

Figure 2.15: The preferred sample co-precipitation for actinide analysis in 2016

Figure 2.16 shows the predominant actinide separation step. This study will use the predominant actinide separation step used by 40% of the participating laboratories.

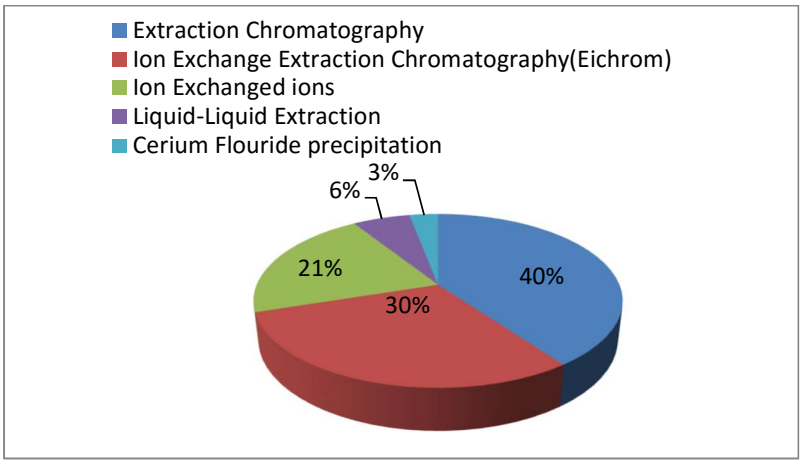

Figure 2.16: The preferred method for chemical separation for actinide analysis in 2016

Even though the Procorad indicated that ICP-MS is a viable method to analyse low levels of plutonium, the market is still dominated by radio-analytical methods as illustrated by figure 2.17.

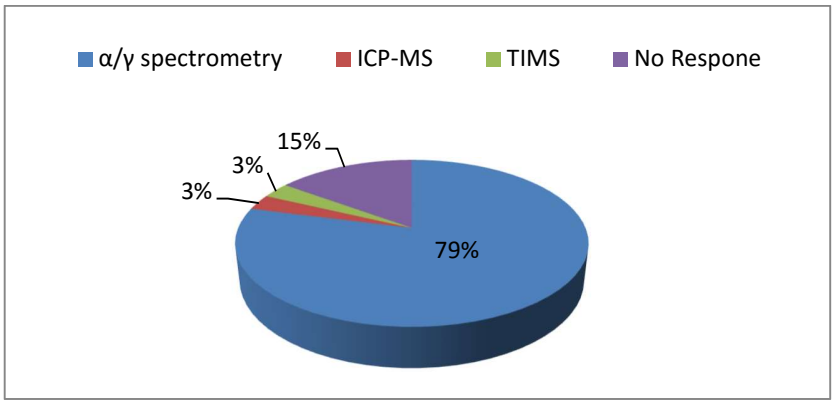

Figure 2.17: The preferred technique for actinide analysis in 2016

It is not clear which type of ICP-MS was employed, but it is expected that very few, if any, were Q-ICP-MS.

2.6 Brief Chapter review

The radiological hazards of plutonium, principles of analysis and the components of the analytical instrument (Agilent 7700) were discussed in detail. The main factors that affect the LOD have been investigated, and the analysis of sub-trace plutonium in urine using a standard Q-ICP-MS is possible when treating a large volume of urine. However, instrument modification or specialized instrumentation is still preferred over standard ICP-MS instruments and this is supported by the 2016 Procorad report where only 3% of at least 65 laboratories are using ICP-MS internationally. This study aims to determine whether a standard Q-ICP-MS can measure on par with α-spectrometry, and in the process to fill the knowledge gap where no studies have been performed on a standard Q-ICP-MS using low volume sample in order to assess the potential of modern technology.

CHAPTER THREE–Research Methodology

3.1 Theoretical Framework

The research was conducted from a technological point of view. It was based on the premise that due to technological improvements, it is possible for a standard, modern Q-ICP-MS to report LOD's/LOQ's similar to or better than those achieved by previous publications on Q-ICP-MS when modified or specialist equipment was required to match α-spectrometry.

3.2 Research Methodology

Quantitative research was employed with an experimental design approach which is explained in section 3.3. This was the most suitable method because the study took place in a laboratory environment and quantitative data was generated.

3.3 Design of experiment (DoE)

Because this is a feasibility study, a simplified approach to the classical OVAT (one variable at a time) DoE will be used. The OVAT is where one factor is studied to determine optimal parameters, whilst other factors are kept constant (Antony, 2003)

The experimental design approach involved the deliberate change of certain variables while keeping other variables constant and then assessing the outcome to determine the significance of each variable with respect to the outcome (Wrexhem, 2012). The same reference states that if a laboratory exercises strict control, then the experiment has objectivity and increased repeatability. It is also easy for another laboratory to repeat the experiment and verify the findings.

The disadvantages are that not all factors can be controlled or accounted for (Wrexhem, 2012). An example for this experiment would be that not every person has the same concentration of Ca^{2+} or PO_4^{3-} in their urine. Thus precipitation kinetics will differ from person to person. Another disadvantage from the same reference is that some of the experiments might require an artificial situation which deviates from reality. This could lead to analyte behaving differently than it would under normal circumstances. An example of this would be where a blank urine sample is spiked with Pu, or more precisely inorganic $PuNO_3$. The difference is that when Pu is passed through the body naturally, the largest fraction of urinary Pu is found in the citrate form (Popplewell et al., 1975). Organic plutonium citrate is a larger molecule than inorganic plutonium nitrate which could also affect sample stability and/or co-precipitation.

The DoE can be subdivided into the following experiments, in which a variable is evaluated that will determine the success or failure of the feasibility study. In chronological order:

3.3.1 Instrument optimisation

- Overall instrument sensitivity: The instrument was tuned in two ways. First a *General Tune* to establish a general sensitivity. Then again in High Mass Tune to give preference to heavier atomic masses. The higher the sensitivity, the lower the concentration that can be detected.
- Integration times: Different integration times was assessed to determine how the precision increases over the working range. Higher precision produces lower LOD's/LOQ's.

3.3.2 Sample preparation optimisation

- Sample pre-analysis treatment: A blank urine sample underwent various pre-analysis treatments ranging from direct analysis to co-precipitation, wet-ashing and column separation. This determined to what extent the urine

matrix interferes with plutonium measurement and what the minimum sample preparation required was.

- Elution optimisation: Using references as a guide, column separation was investigated followed by elution studies. This section was split into two parts namely uranium elution (washing) and plutonium elution (stripping) from a TEVA column.

3.3.3 Evaluation of the established method

- The LOD and LOQ that established the lowest concentrations that can be reported with acceptable accuracy and precision
- Accuracy: Replicate analyses which determined intra-assay accuracy across the working range
- Precision: Replicate analyses which determined intra-assay precision across the working range
- Method verification: Analysis of international proficiency scheme samples that contain ^{239}Pu. The result from the Q-ICP-MS was statistically compared to the consensus concentration from all the participating laboratories internationally.

Should the method prove to be feasible, then a two factorial or even full factorial design as described by Ebrahimi-Najafabadi et al. (2014) and Anthony (2003) would be recommended for future studies.

3.4 Materials and Reagents

EMSURE® nitric acid (65%w/w), EMSURE® hydrofluoric acid (40%w/w), EMSURE® hydrogen peroxide (30% w/w) were purchased from Merck, Germany.

18.2 MΩ cm^{-1} ultra-pure deionised water was obtained from a Millipore Direct-Q (Merck, Germany) water purification system. 15 and 50 mL polypropylene tubes were obtained from LASEC (Greiner Bio-One, Germany). High purity Nalgene bottles were purchased from AEC Amersham, SA. An aqueous stock solution of $^{239}PuNO_3$ (7.14 GBq g^{-1}) for calibration and $^{242}PuNO_3$ (26.8 GBq g^{-1}) for internal standard, were obtained from AEC Amersham, Johannesburg. Calcium nitrate (98%), potassium nitrite (95%) and ammonia (30% w/w) were purchased from Merck, Midrand. Ferrous sulphate heptahydrate (99.5%), di-hydrogen ammonium phosphate (99.0%) and aluminium nitrate nonahydrate were purchased from Merck, Midrand. Columns with 2 mL TEVA (100-150 µm particle size) were obtained from Eichrom Technologies, USA.

3.4.1 Preparation of co-precipitation reagents

Solutions of $Ca(NO_3)_2$ and $(NH_4)H_2PO_4$ were prepared by dissolving 0.5 g of each salt separately in 5 mL ultra-pure water. Fresh solutions were prepared for every experiment.

3.4.2 Preparation of valence-adjustment reagents

The $Fe(II)SO_4$ reducing solution was prepared by weighing 0.22 g of $Fe(II)SO_4.7H_2O(s)$ and dissolving it in 1 mL ultrapure water. The KNO_2 oxidizing solution was prepared by weighing 0.27 g $KNO_2(s)$ and dissolving it in 1 mL ultrapure water. Fresh solutions were prepared for every experiment.

3.4.3 Preparation of solutions for column separation.

The column load solution was prepared by dissolving 93.75g of $Al(NO_3)_3.9H_2O(s)$ in 125 mL water, adding 34.7 mL of HNO_3 and diluting to 250 mL with ultra-pure

water. The column rinse solution was prepared by pipetting 3.5 mL of HNO_3 into 100 mL of water and then diluting it to 250 mL with ultra-pure water. The column strip solution was prepared by pipetting 1.8 mL of HNO_3 and 2.2 mL HF to 100 mL of water followed by dilution to 250 mL with ultra-pure water. The column storage solution was prepared by pipetting 1.8 mL of HNO_3 into 100 mL of water and then diluting it to 250 mL with ultra-pure water.

3.4.4 Preparation of calibration standards

Intermediate standards were prepared by diluting the ^{239}Pu to a concentration of 10 ng L^{-1}, and the ^{242}Pu to a concentration of 20 ng L^{-1} using 1% HNO_3. The calibration standards were prepared by mass to increase the accuracy, for example an empty tube was tared and the required aliquot of ^{239}Pu pipetted before being weighed. The standards were then diluted with 1% HNO_3 and a fixed volume of ^{242}Pu was added to each tube before being weighed again. The concentration for each calibration standard was calculated by the following equation:

$$Pu_{std} = Pu_{intermediate} * \left(\frac{m_{aliquot}}{m_{total\ vol.}}\right) \quad (3.1)$$

Where $Pu_{intermediate}$ = Concentration of the intermediate standard in pg L^{-1}.

$m_{aliquot}$ = Mass of the aliquot in grams

$m_{Total\ Vol.}$ = Mass of the total volume in grams

An example of the calculation spreadsheet is attached as appendix L.

3.4.5 Sample Preparation (Final Procedure)

3.4.5.1 Co-precipitation

A 50 mL aliquot was measured into a 50 mL Falcon tube. 50 µL of tracer was added to each sample followed by 1 mL of $CaNO_3$ and 1 mL of $(NH_4)_2HPO_4$. The sample was mixed thoroughly and 5 mL of 30% NH_3 added. The samples were left to stand for at least 2 hours before being centrifuged at 5000 rpm for 5 minutes. The supernatant liquid was decanted, 50 mL water added to each tube and shaken to mix thoroughly before being placed back into the centrifuge for 5 minutes. The sample was removed and the supernatant liquid discarded leaving only the precipitate.

3.4.5.2 Wet-ashing

The precipitate was dissolved in 2 mL HNO_3 and transferred to a 50 mL glass beaker. The tube was rinsed with another 2 mL HNO_3 aliquot which was transferred to the corresponding beaker. 1 mL of H_2O_2 was added to each beaker which was then placed on a hotplate. The samples were evaporated to dryness, preventing baking. Volumes of 1 mL of HNO_3 and 1 mL H_2O_2 was added to each beaker and evaporated again. This step was repeated twice for the precipitate to be pure white. Samples were removed from the hotplate and allowed to cool to room temperature.

3.4.5.3 TEVA Column separation

The white precipitate was dissolved in 3 mL of the column load solution. 200 µL of the $Fe(II)SO_4$ reducing solution was added to each sample and left for 15 minutes. 200 µL of the KNO_2 oxidizing solution was added to each sample and left for another 15 minutes. 3 mL of 2 M HNO_3 was passed through the column followed by the sample. The sample beakers were rinsed with 3 mL 2 M HNO_3 and also passed through the column. The columns were then rinsed with 20 mL of 0.2 M HNO_3 and

all the solutions collected up to this point discarded. The plutonium was stripped with 5 mL 0.1 M HNO_3:0.2 M HF solution and the eluate collected for analysis by Q-ICP-MS.

3.5 Brief Chapter review

The quantitative research method with an experimental approach was employed, using the OVAT system to systematically evaluate and optimise the analytical method. The preparation of the reagents and calibration standards was relatively straight forward but because of the very low concentrations of analyte, the calibration standards have to be prepared by mass. The sample preparation required more effort to remove as much as possible urine matrix which affected the plasma conditions and by extend, affected the analysis.

CHAPTER FOUR – Results and Discussion

4.1 Instrument Optimisation

4.1.1 Sensitivity

In order to increase the sensitivity and lower the background signal, a manual tune of instrument parameters had to be performed using a tune solution which contained 1 µg L^{-1} ^{7}Li, ^{89}Y and ^{205}Tl, representing light-, medium- and heavy elements. The instrument was auto-tuned with preference to the heavy masses, monitored by the ^{205}Tlsignal. During auto tuning, the instrument gradually varies parameter after parameter and determines which settings allow for the highest sensitivity for the heavy masses. Auto tune is limited only to the lens- and cell parameters, meaning that the sample depth and nebulizer pump settings have to be changed manually. For comparison, a general tune report was generated (Appendix A). When the ideal settings were established, to ensure that precision and peak definition were not compromised (even if sensitivity increased), a second tune in standard mode was run and is attached (Appendix B). The two modes are compared in table 4.1.

Table 4.1: Difference between the standard-, and high mass tune mode

Parameter	Standard tune	Custom tune
Sample Depth (cm)	8	6
Nebulizer Pump (RPM)	0.1	0.2
Extract 1(V)	0	-4.4
Extract 2(V)	-200	-200
Omega Bias(V)	-80	-65
Cell Entrance(V)	-30	-40
Cell Exit(V)	-30	-40
Deflect(V)	9.4	14
Omega Lens(V)	9	13
Plate Bias(V)	-40	-60
OctP Bias(V)	-8.0	-8.0
OctP RF(V)	180	200
Energy Discrimination(V)	4.5	4.5

The RF Power, Carrier gas flow rate and Spray chamber temperature were kept constant at 1550 W, 1.1 L min^{-1} and 2 °C respectively. Table 4.2 summarises the performance differences between the two methods

Table 4.2: Performance difference between standard and custom tune

Parameter	Standard tune	Custom tune	Improvement factor
Heavy mass Sensitivity (CPS/ppb)	5805	25629	4.41
% RSD	3.13	2.29	1.37
Background (CPS)	46.7	6.00	7.78

The custom tune for heavy masses resulted in a slight increase in precision, but significantly increased the sensitivity and reduced background relative to the standard tune. The LOD will be reduced by approximately the same factor as for the increase in sensitivity.

4.1.2 Precision

Increasing the integration times does not increase the sensitivity but does increase the precision due to counting statistics. Three different integration times, 10, 20 and 30 seconds were used and the calibration statistics obtained indicated the optimal dwell time. Integration times exceeding 30 seconds were not evaluated due to sample volume restrictions. Data is summarised in Tables 4.3 to 4.5.

Table 4.3: Instrument response for calibration standards at 10 seconds integration time

Pu Standards pg L^{-1}	Pu CPS	Pu %RSD	Lu-IS CPS
0	9.79	15.1	279806
1	10.8	12.3	281022
5	10.6	10.7	282175
10	12.9	9.01	282616
50	23.9	6.40	281388
100	41.8	5.83	280964
500	170	1.80	283546

Table 4.4: Instrument response for calibration standards at 20 seconds integration time

Pu Standards pg L^{-1}	Pu CPS	Pu-RSD	Lu-IS CPS
0	9.83	10.1	285896
1	9.19	8.03	286514
5	11.2	8.75	285888
10	12.2	6.19	286788
50	27.1	4.26	287521
100	41.0	3.53	283216
500	174	1.4	286323

Table 4.5: Instrument response for calibration standards at 30 seconds integration time

Pu Standards pg L^{-1}	Pu CPS	Pu-RSD	Lu-IS CPS
0	9.58	5.53	277971
1	9.95	7.55	278365
5	10.7	6.61	276744
10	11.9	4.42	276879
50	25.6	3.14	278181
100	41.2	2.77	270944
500	170	1.02	276643

Calibration curves were constructed by plotting the ratio of the sample CPS and the internal standard against the concentration of plutonium standard. The calibration curves for Table 4.3 to 4.5 produced Figure 4.1to 4.3.

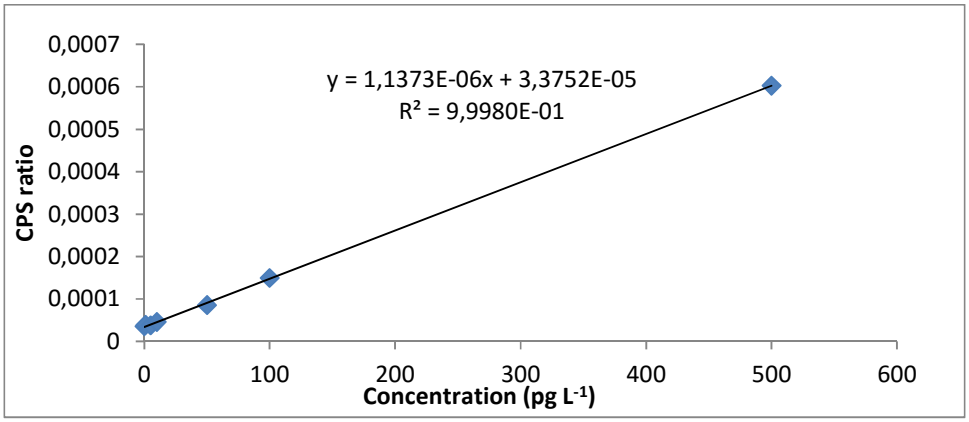

Figure 4.1: Calibration curve for 10s integration time

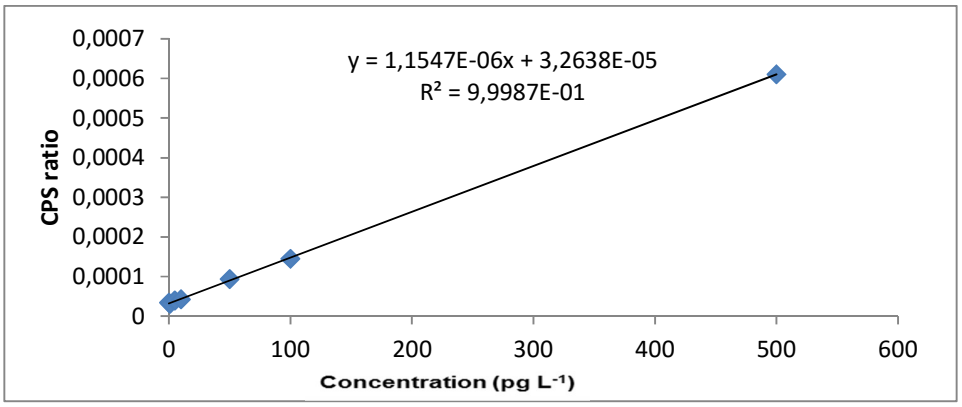

Figure 4.2: Calibration curve for 20s integration time

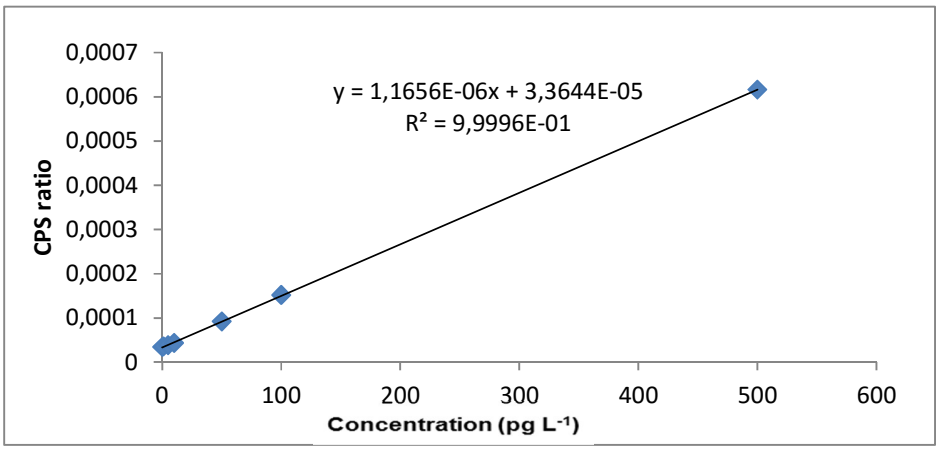

Figure 4.3: Calibration curve for 30s integration time

When visually inspecting the calibration curves, there seemed to be little difference. But increasing integration times improve the counting statistics, and by extension, the precision. When plotting the % RSD as a function of concentration, the following figure was obtained:

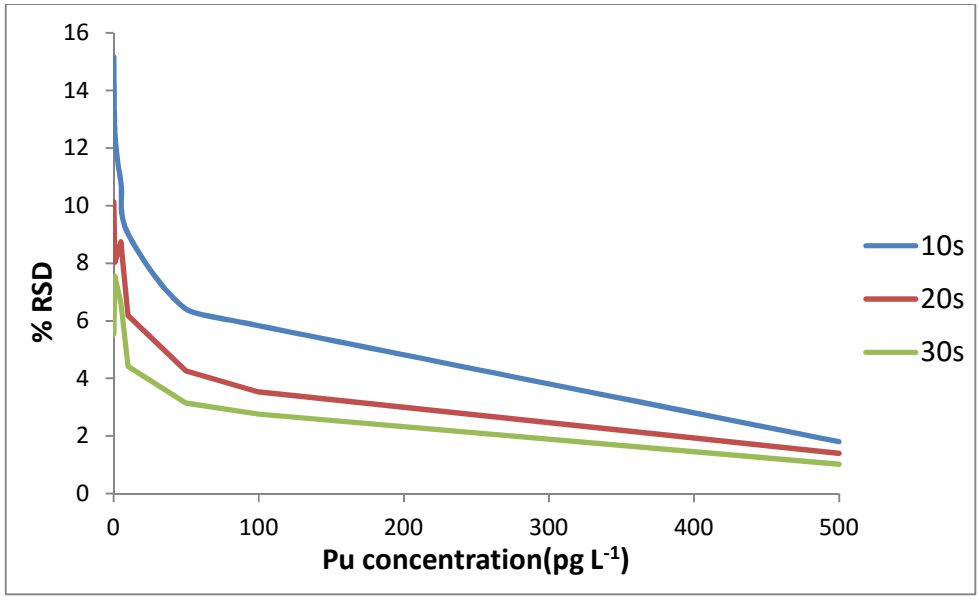

Figure 4.4: %RSD's for calibration standards for different integration times

The increase in integration time from 10s to 30s significantly increased the overall precision especially at the lower concentrations (<100 pg L^{-1}). There was a diminishing increase in precision from 20s to 30s compared to the increase from 10s to 20s. Regression statistics were performed on the 3 calibration sets and attached as appendix D, E and F. From the calibration statistics, the following data was obtained:

Table 4.6: Regression parameters for different integration times

Integration Times	Regression error($S_{y/x}$)	Uncertainty in intercept(S_a)	Slope(b)	Regression LOD*
10	3.25E-06	1.41E-06	1.14E-06	4.08
20	2.67E-06	1.16E-06	1.15E-06	3.33
30	1.46E-06	6.34E-07	1.17E-06	1.79

* Where the regression LOD approximation is defined as LOD = $3.3S_a/b$ according to de Beer, 2011

From Table 4.6 and Figure 4.4, it is evident that higher integration times lead to higher overall precision especially at lower concentrations. Because the precision increases, regression statistics also gradually increase, lowering the LOD approximation from 4.08 pg L^{-1} to 1.79 pg L^{-1}.

4.2 Sample Preparation Optimisation

4.2.1 Minimum sample preparation

Urine samples were obtained from individuals whom could not have been exposed to Pu containing matrices. These samples were mixed and served as a sample blank. The blank sample was prepared in four different ways and then analysed (starting with the quickest and easiest and ending with the slowest and more difficult process). The purpose for the experiment was to determine whether modern technology is more robust to changes in the sample matrix, and to determine what the minimum sample preparation is in order to obtain reliable results. Results are presented in Table 4.7:

Table 4.7: Pu concentration and IS CPS for a blank urine sample prepared by different preparations

Sample preparation	Pu(pg L^{-1})	Lu-IS CPS
Undiluted	1038	56826
10X -Diluted	331	162274
Co-precipitate-1(No wet ash, No column separation)	10.1	252849
Co-precipitate-2(Wet ash, Column separation)	< 1.00	261589

As the intensity of sample preparation increased, lower concentrations of Pu could be reported. The undiluted and diluted urine samples had the same interferences, but the diluted samples were a lesser load on the plasma. Signal suppression was observed, which was in agreement with what Hang et al., (2004), Pappas et al., (2004) and Zoriy et al., (2005) reported. Evans and Giglio (1993) described that organic matter can reduce the temperature of the plasma because of the energy required to dissociate molecular species like C_2. Changes in the plasma temperature affect ionisation efficiency. Wyse et al. (1994) reported that with a direct injection nebulizer, urine can be analysed directly. This is however not possible because the interaction between the plasma and the heavy urine matrix cannot be changed. What can be changed is what goes into the plasma, lessening the sample load for a clean mass spectrum and robust plasma. This is supported by results for the co-precipitate 1 and 2. The sample that was not wet-ashed still contained some organic matter compared to the sample that was wet-ashed. Co-precipitate-1 showed slight signal suppression as well as a slight increase in the CPS on the *m/z* =239. The slight signal suppression did not increase the Pu-measurement after correction with internal standard. This indicates that the non-spectroscopic interference should be negligible, and the interference is due to a spectroscopic interference. This agreed with what Pappas et al. (2004) stated. They observed that organic content can cause a low interference at m/z 239. This experiment has confirmed that full sample purification (preparation) has to be performed in order to analyse plutonium accurately in a urine matrix.

4.2.2 Elution optimisation

The ideal column separation would completely remove the uranium (to remove $^{238}UH^{+}$ interference) and then selectively elute the plutonium in the smallest possible volume. A solution spiked with uranium was treated and passed through a column packed with TEVA resin to determine the elution profile. Data is summarised in Table 4.8 and illustrated in Figure 4.5.

Table 4.8: Uranium rinsed from TEVA resin with 2 M HNO_3

Cumulative volume (mL)	^{238}U(CPS)
5	8376
10	8007
15	7768
20	1411
25	1077
30	667
35	475
40	271

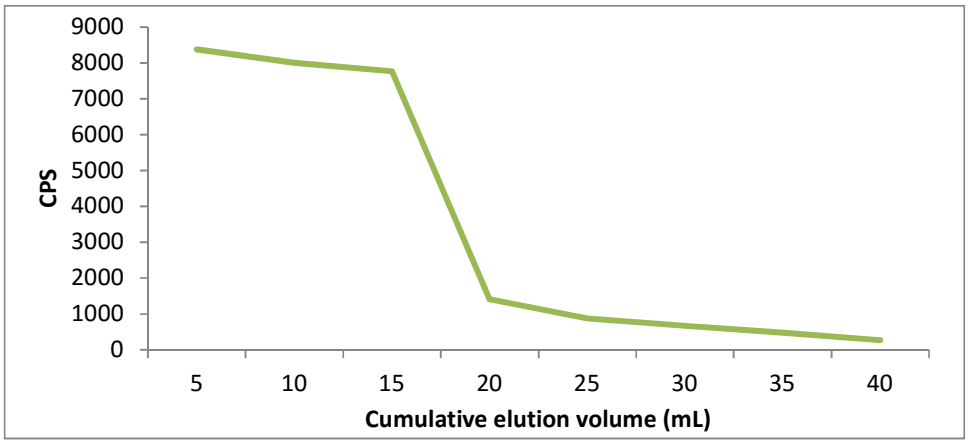

Figure 4.5: Elution of uranium from TEVA-resin

20 mL of 2 M HNO_3 was sufficient to reduce the uranium signal to below 5000 CPS where the $^{238}UH^+$ interference was negligible.

For plutonium elution, 0.5 mL of a 10 ng L^{-1} Pu solution was loaded onto the column and the column washed with 3 mL fractions of 0.1 M HNO_3:0.2 M HF. The fractions were analysed separately and the results presented in table 4.9 and figure 4.6.

Table 4.9: HF assisted removal of plutonium from TEVA-resin

Cumulative volume (mL)	^{239}Pu (CPS)
3	239
6	12.0
9	10.5
12	11.2

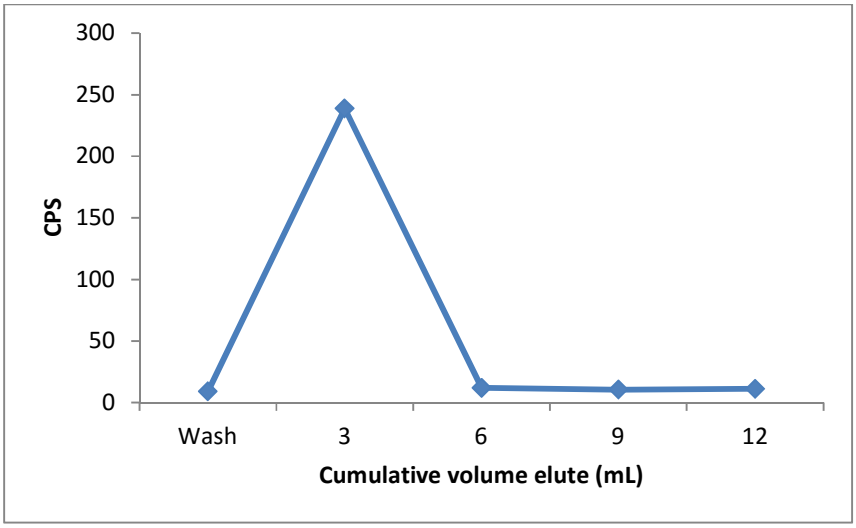

Figure 4.6: HF assisted elution of plutonium from TEVA resin

The rapid elution of plutonium from the column was possible as fluoride (from the HF acid) is efficient in forming stable complexes with the actinides, in this case plutonium. Because the plutonium is in a stable complex, it cannot re-enter the

stationary-phase. An initial sample of 50 mL will be processed and eluted with 5 mL 0.1 M HNO_3:0.2 M HF, yielding a concentration factor of 10. Sample LOD's can then be reported ca 10x lower.

4.2.3 Additional Tests

4.2.3.1 Baking

Wyse and Fisher (1994) mentioned that during sample wet-ashing, sample baking should be avoided as this causes plutonium species to become refractory. They gave no indication of how and to what extent the results are affected. Three urine samples spiked with 10 pg L^{-1} of Pu were taken through the whole process. One sample was removed from the hotplate just before dryness, another slightly baked (slight yellow discoloration of the white precipitate), and another excessively baked (yellow to brown discoloration of white precipitate). Samples were analysed and the % recovery calculated.

Table 4.10: Effect of baking on Pu recovery

Sample #	Description	Measured Pu (pg L^{-1})
1	No baking	9.65
2	Slight baking	7.09
3	Excessive baking	3.34

By plotting the recoveries, Figure 4.7 was obtained:

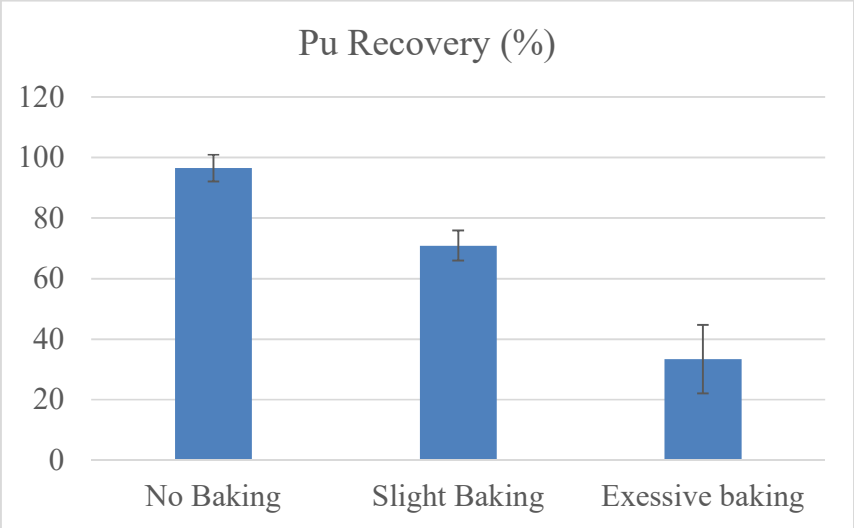

Figure 4.7: Effect of baking on Pu-recovery

It is clear that even slight baking will affect the results significantly. For the highest possible recovery, even slight baking should be avoided.

4.2.3.2 Interferences

The half-lives of ^{239}Np and ^{239}U is 2.36 days and 23.5 minutes respectively. Isobaric interference from these elements were considered negligible because of their extremely short half-lives. It can be assumed that almost all of the ^{239}Np and ^{239}U will decay to ^{239}Pu.

^{238}U presents the biggest problem because if ^{239}Pu is present in the urine, it is highly probable that ^{238}U will also be present at significantly higher concentrations which could cause polyatomic interference. Because uranium has similar chemical and physical properties will also co-precipitate and elute along with the plutonium. Although the method has been shown to effectively reduce the ^{238}U to a concentration whereby interferences are negligibly small, it is still necessary to monitor the uranium signal and determine the extent of the interference to accommodate any unforeseen

circumstances. The combined effect of $^{238}UH^{+}$ formation and UH-tailing was determined by analysing uranium standard solutions and measuring the resultant pseudo plutonium concentration. The results are presented in Table 4.11.

Table 4.11: Extent of uranium induced interferences.

U Spike (ng L^{-1})	Measured Pu (pg L^{-1})
10	0
100	6.12
1000	86.3

Figure 4.8 below show the linear relationship between the Uranium concentration and the measured pseudo plutonium concentration. And for ^{238}U below 10 ng L^{-1}, uranium induced interferences have no observable effect at *m/z* = 239. The 10 ng L^{-1} uranium solution gave an instrument response of 5000 CPS at *m/z* = 239.

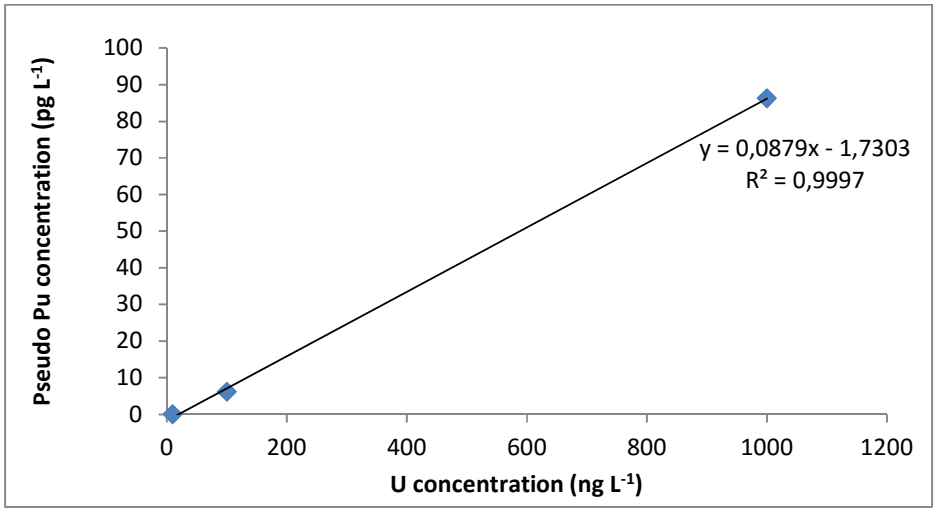

Figure 4.8: Linear relationship between the uranium concentration and the increased plutonium measurement

For uranium concentrations above 10 ng L^{-1}, inter-elemental correction will have to be done. The trend line equation can be used:

$$Pu_{True} = Pu_{Measured} - Pu_{Amplified} \quad (4.1)$$

$$\text{Where: } Pu_{Amplified} = [0.087*U_{Conc}]-1.730 \quad (4.2)$$

4.2.3.3 Sample stability

All analyses performed on fresh urine samples yielded tracer (^{242}Pu) recoveries in the region of 70-80%. Analysis on older acidified urine samples which had a known concentration of ^{239}Pu, repeatedly yielded tracer recoveries of 20-30%. This could possibly be due to the co-precipitation step being affected by the stabilizing agents and pH of the urine samples. Fresh and unpreserved urine produced a less dense, soft solid during co-precipitation, while the older acidified urine samples produced a compact solid. Initial analysis of the older acidified urine samples also gave under-recoveries for ^{239}Pu even when correction by tracer element was done. Pappas et al. (2004) reported that "*analytical results depend on the time of shaking and vigour before removal of an aliquot*" for an older sample. Re-analysis of an aliquot from a vigorously shaken sample produced satisfactory results.

4.3 Final Method Evaluation

4.3.1 LOD

Normally the LOD would be expressed as 3.3*standard deviation of at least 10 blanks but for this study however, the theoretical LOD was calculated from a practically established LOQ. This results in higher confidence when reporting LOD and LOQ results. Theoretically:

$$LOD = 3.3*S_a/b \quad (4.3)$$

$$LOQ = 10*S_a/b. \quad (4.4)$$

Where Sa is the standards uncertainty (or standard error on excel) for the intercept, and b is the slope from regression statistics. By making S_a/b the subject of the formulas and substituting the equations, we get the following:

LOD = (3.3/10) * LOQ

= 0.33*0.5 *Note: refer to 4.3.2 for determination of LOQ*

=0.16 pg L^{-1}

≈ 0.2 pg L^{-1}.

The sample LOD of 0.2 pg L^{-1} agrees with the LOD approximation made in Table 4.6 which was from a regression curve. LOD approximation was established as 1.79 pg L^{-1}. Because the sample is concentrated by a factor 10, LOD approximation = 1.79/10 = 0.18≈ 0.2 pg L^{-1}.

4.3.2 LOQ

For every concentration, a 200 mL urine sample was spiked with a calculated concentration of ^{239}Pu and divided into four 50 mL aliquots before being purified and analysed. For the LOQ, the lowest concentration that conforms to analysis specification will be the LOQ which can be reported with acceptable accuracy and precision. The specification was set at 50% bias for accuracy and <30% RSD using requirements from NECSA Safety, Health, Environment and Quality (SHEQ) Department as a guide.

Table 4.12: Accuracy and precision for various spiked concentrations (n=4)

^{239}Pu Spike (pg L^{-1})	^{239}Pu measured (pg L^{-1})	% RSD	% Bias
0.10	0.00	N/A	ND
0.25	0.40	23.7	60
0.50	0.54	11.3	8

From the results in table 4.12, the concentration that meets both the precision and accuracy requirements is 0.5 pg L^{-1}.

4.3.3 Accuracy and Precision

Urine samples collected from healthy individuals that could not have been exposed to Pu-containing matrixes was collected, mixed and split into two 200 mL aliquots. The aliquots were then spiked with 0.5- and 15 pg L^{-1} ^{239}Pu, respectively. Each sample was divided into four 50 mL aliquots, purified and analysed separately so as to produce signals in the low and high region of the working range. Note that the % accuracy is expressed in terms of the recovery of the initial concentration ^{239}Pu added to the samples. This should not be confused with the method efficiency (Refer to appendix G). Data for the spiked samples is summarised in table 4.13.

Table 4.13: Accuracy and precision for Pu in urine on both sides of working range (n=4)

^{239}Pu Spike (pg L^{-1})	^{239}Pu measured (pg L^{-1})	% RSD	% Accuracy
0.5	0.54	11.3	108
15	15	4.43	100

4.3.4 Method verification

Three samples were obtained that formed part of an international Procorad *Proficiency testing* scheme. According to the ISO 13528 standard, an analytical method is acceptable if 2 of the 3 following tests fall within specification. The 3 tests are as calculated as follows:

- Bias : $100 - [\left(\frac{X}{\mu}\right) * 100]$ (4.5)

  Where X = Calculated Pu concentration

  μ = Consensus/True value

- Z-Score : $\left(\frac{|X-\mu|}{sd}\right) * 100$ (4.6)

  Where X = Calculated Pu concentration

  μ = Consensus value

  sd = Standard deviation of the consensus value

- En (Normalized error) : $\left(\frac{|X-\mu|}{\sqrt{U_{Lab}^2+U_{Ref}^2}}\right)$ (4.7)

  Where X = Calculated Pu concentration

  μ = Consensus value

  U_{Lab}^2 = Expanded uncertainty (95%) of the measured value

  U_{Ref}^2 = Expanded uncertainty (95%) of the consensus value

That the method is currently not validated and thus has no expanded uncertainty values thus normalized error can therefore not be calculated. The consensus results are expressed a mBq per sample and this method reports pg L^{-1}. The conversion was calculated as follows:

$$Pu_{(mBq\ per\ sample)} = (Pu_{(ICP)} * Pu_{(Sp\ Act)}) * \frac{Sample\ Vol_{(mL)}}{1000\ mL} \quad (4.8)$$

Where: $Pu_{(ICP)}$ = Measured ^{239}Pu concentration from Q-ICP-MS in pg L^{-1}

$Pu_{(Sp\ Act)}$ = Specific activity for 239Pu = 2.296 mBq pg^{-1}

Sample $Vol_{(mL)}$ = Total volume of original sample.

All procorad samples submitted has a total volume of 500 mL. The results obtained for the analysis of these samples are presented in table 4.14.

Table 4.14: Results for the Procorad proficiency samples

Procorad sample	Measured result (pg L^{-1})	Converted result (mBq per sample)	Consensus result (mBq per sample)
A	2.4	2.8	2.2
B	4.3	4.9	4.4
C	0.2	0.3	0.0

For Consensus results, refer to appendix I-K. Since sample C is a blank sample, it would not be considered when performing the evaluation calculation.

Table 4.15: Summary of the evaluation criteria for the proficiency samples

Tests	Criteria	Sample A	Sample B	Status
Bias (%)	-25 to 50%	16.7	11.4	Pass
Z-score	-3 to 3	2.1	1.5	Pass

The results obtained by the Q-ICP-MS method fell within specification for at least 2 out of the 3 tests and thus the method was regarded as having been verified sufficiently to be acceptable internationally.

4.3.5 Creatinine normalisation

A single 50 mL sample is viewed as a spot sample and the measured Pu concentration does not give sufficient information since it is influenced by the total volume of urine excreted in a 24-hour cycle. By normalising with creatinine, it was not necessary to collect a full 24-hour urine sample. By assuming that the average male (weighing 70 kg) and female (weighing 60 kg) excretes roughly 1575 and 1050 mg creatinine per day, respectively (Horowitz, 2014), and the average volume excreted is 1600 mL and 1200 mL respectively (ICRP, 2003), the samples can be normalised to Pu concentration per mass creatinine. The average man, with an average creatinine excretion through an average urine volume, will excrete 0.049 g creatinine per 50 mL, and a female 0.044 g per 50 mL.

Creatinine normalised values were obtained from the following calculations.
Equation 4.9 shows the conversion of the concentration of Pu in 1 L to concentration per gram of creatinine. Equation 4.10 shows the conversion from concentration per gram of creatinine to radioactive counts (in mBq) per gram of creatinine.

$$Pu_{(pg\ per\ g)} = \frac{Pu_{(ICP)} * \frac{50\ mL}{1000\ mL}}{C} \qquad (4.9)$$

Where: $Pu_{(ICP)}$ = Measured ^{239}Pu concentration from Q-ICP-MS in pg L^{-1}.

C= Grams of creatinine per 50 mL urine (different for males and females).

$$Pu_{(mBq\ per\ g)} = \frac{\left[\left(Pu_{(ICP)} * Pu_{(Sp\ Act)}\right) * \frac{50\ mL}{1000\ mL}\right]}{C} \quad (4.10)$$

Where: $Pu_{(ICP)}$ = Measured ^{239}Pu concentration from Q-ICP-MS in pg L^{-1}

$Pu_{(Sp\ Act)}$ = Specific activity for 239Pu = 2.296 mBq pg^{-1}

C= Grams of creatinine per 50 mL urine (different for males and females).

The creatinine normalised reporting limits and ranges are summarised in Table 4.16 and 4.17 for males and females, respectively.

Table 4.16: Creatinine normalised measurement parameters for males

Parameter	Pu(pg g^{-1})	Pu(mBq g^{-1})
LOD	0.2	0.5
LOQ	0.5	1.2
Working range	0.5-102	1.2-234

Table 4.17: Creatinine normalised measurement parameters for females

Parameter	Pu(pg g^{-1})	Pu(mBq g^{-1})
LOD	0.2	0.5
LOQ	0.6	1.3
Working range	0.6-114	1.3-260

4.4 Brief Chapter Review

The instrumentation was successfully tuned for increased sensitivity and precision at large *m/z* ratios. The minimum sample preparation was a full purification step including co-precipitation, wet-ashing and solid-phase separation leading to a sample

concentration factor of 10. The method LOD and LOQ were established as 0.2 and 0.5 pg L^{-1} respectively. Method accuracy, i.t.o. bias has been established as 8% for 0.5 pg L^{-1} and 0% for 15 pg L^{-1} and method precision was 11.3% and 4.43% RSD for the same concentrations. The method was verified using samples from an international proficiency study.

CHAPTER FIVE–CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study has shown that it is possible to analyse for ^{239}Pu concentrations in urine samples at concentrations as low as 0.2 pg L^{-1} using an unmodified Q-ICP-MS, thus leading to the rejection of the null hypothesis (Ho) that had been proposed. The sensitivity of the instrument was increased by a factor of 4. Sample purification (using the adsorbent column) was shown to be important in reducing interferences and improving the LOD by a factor of 10. It was also possible to reduce uranium to a level where $^{238}UH^{+}$ interferences were considered negligible. Acceptable bias (of 50%) and precision (<30% in terms of % RSD's) were obtained over the analytical working range. Furthermore, the increase in sensitivity due to optimisation made it possible to reduce the sample volume required from > 500 mL to just 50 mL. The method was verified using samples from an international proficiency scheme spiked with Pu and the results were satisfactory. The turnaround time from sample preparation to analysis was reduced significantly compared to α-spectrometry. The method also meets all the requirements of the NECSA SHEQ department. However, the method robustness can be improved as was noted by the loss of analyte when shaking, baking was carried out on the samples. The method also requires fresh solutions to be prepared as Fe (in the reagents) tends to oxidise quickly upon extended exposure to the atmosphere. The presence of ^{239}U and ^{239}Np was not ascertained in the study, but it may be important to determine them in future studies. The creatinine normalisation was also done with an average creatinine per sex. But the values can vary drastically depending on water intake (Horowitz, 2014). For more accurate analysis or future applications, it might be necessary to quantify the creatinine. Some of the recommendations that can be followed to further improve the method and increase its feasibility are mentioned below.

5.2 Recommendations

5.2.1 Reducing the total sample preparation time

Additional tests that were not part of the scope of the feasibility study include reducing the standing time during the co-precipitation step in 15 minute intervals to determine at which point the yield reaches maximum as well as reducing the standing time during the valence adjustment steps in 2 minute intervals to determine optimum yield. These tests can form part of a multi-variate experimental design for the method validation process.

5.2.2 Further reduction of LOD's

In order to report even lower LOD's, method or instrument adjustments can be made to increase either the concentration factor or the abundance sensitivity (CPS/ug L^{-1}). From the least expensive to the most expensive they are:

- Increase the sample volume. The higher the sample volume processed, the higher the concentration factor. By using 50 mL sample, the concentration factor is 10. By using 1000 mL, effectively 20 times lower concentrations can be reported with the same accuracy.

- Install a different sample introduction system. By installing an ultrasonic nebulizer, the transport efficiency is increased to 15% compared to 1-2% for pneumatic nebulizers as reported by Wyse and Fisher (1994) This will allow analysing of concentrations 7 to 15 times lower with the same precision.

5.2.3 Analysis of multiple actinides

To expand this method to include multiple actinides, the elution volumes per actinide should be 5 mL. By implementing a 30s integration time, the Q-ICP-MS uses 4.5 mL per actinide including a tracer (^{242}Pu) and uranium monitoring.

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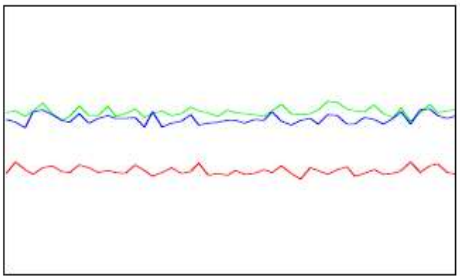
Appendix A-Standard Tune (Quartz introduction system)

Tune Report

Batch Folder D:\Data\2015\AUG\U discrete Sample throughput 06-08-2015 B.b
Acq. Date-Time 8/8/2015 5:41
Report Comment
Instrument Name G3281A JP10290508

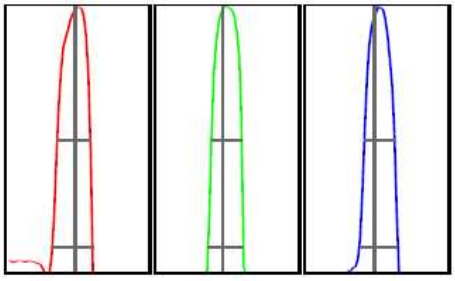
[No Gas]



Mass	Range	Count	RSD%	Background
7	5000	1940	3.860	1.800
89	20000	12151	2.728	15.700
205	10000	5805	3.129	46.700

Ratio 156/140 0.901 % **Ratio** 70/140 1.139 %

Integration Time [sec] 0.1 **Sampling Period [sec]** 0.311



Mass	Peak Height	Axis	W-50%	W-10%
7	1925.87	7.00	0.68	0.830
89	12043.04	88.95	0.61	0.724
205	5896.58	205.00	0.61	0.802

Integration Time [sec] 0.1 **Acquisition Time [sec]** 22.74 **Y Axis** Linear

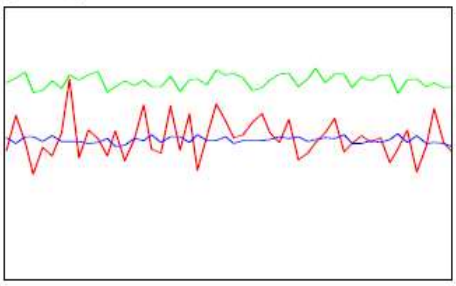
Tune Parameters
Plasma Paramters

Appendix B-Heavy mass specific Tune (Quartz Introduction system)

Tune Report

Batch Folder D:\Data\2015\Development\Plutonium in urine Discrete 8-08-15.b
Acq. Date-Time 8/8/2015 6:04
Report Comment
Instrument Name G3281A JP10290508

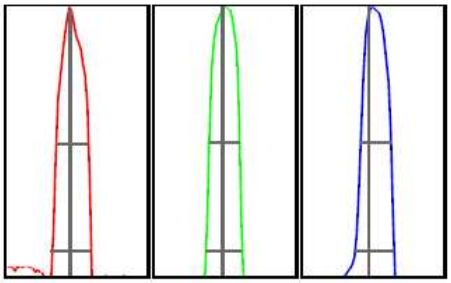
[No Gas]



Mass	Range	Count	RSD%	Background
7	500	261	13.803	0.600
89	20000	14615	3.283	2.700
205	50000	25692	2.285	6.000

Ratio 156/140 1.385 % **Ratio** 70/140 0.383 %

Integration Time [sec] 0.1 **Sampling Period [sec]** 0.311



Mass	Peak Height	Axis	W-50%	W-10%
7	289.54	6.90	0.67	0.811
89	14451.04	89.00	0.62	0.758
205	25564.20	204.95	0.62	0.816

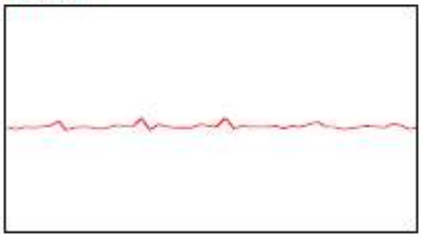
Integration Time [sec] 0.1 **Acquisition Time [sec]** 22.74 **Y Axis** Linear

Appendix C-Heavy mass specific Tune (PFA Introduction)

Tune Report

Batch Folder D:\Data\2016\0-Development\PhilipDissertation\Pu In urine 19-10-16_Final Proffeciency samples!.b
Acq. Date-Time 10/19/2016 13:21
Report Comment
Instrument Name G3281A JP10290508

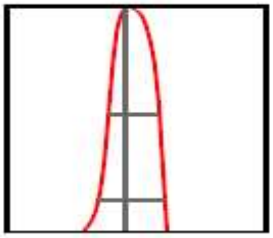
[No Gas]



Mass	Range	Count	RSD%	Background
205	50000	23574	2.282	7.000

Ratio 156/140 10.896 % **Ratio** 70/140 2.316 %

Integration Time [sec] 0.1 **Sampling Period [sec]** 0.1002



Mass	Peak Height	Axis	W-50%	W-10%
205	23986.89	204.90	0.61	0.799

Integration Time [sec] 0.1 **Acquisition Time [sec]** 7.38 **Y Axis** Linear

Appendix D- Regression statistics for 10s integration times

Regression Statistics	
Multiple R	0.9998976
R Square	0.9997953
Adjusted R Square	0.9997543
Standard Error	3.25E-06
Observations	7

ANOVA

	df	SS	MS	F	Significance F
Regression	1	2.58E-07	2.60E-07	24419.3	2.04E-10
Residual	5	5.28E-11	1.10E-11		
Total	6	2.58E-07			

	Coefficients	Standard Error	t Stat	P-value
Intercept	3.38E-05	1.41E-06	23.9417	2.37E-06
X Variable 1	1.14E-06	7.28E-09	156.267	2.04E-10

Appendix E- Regression statistics for 20s integration times

Regression Statistics	
Multiple R	0.9999332
R Square	0.9998663
Adjusted R Square	0.9998396
Standard Error	2.67E-06
Observations	7

ANOVA

	df	SS	MS	F	Significance F
Regression	1	2.66E-07	2.70E-07	37396.8	7.02E-11
Residual	5	3.55E-11	7.10E-12		
Total	6	2.66E-07			

	Coefficients	Standard Error	t Stat	P-value
Intercept	3.26E-05	1.16E-06	28.219	1.05E-06
X Variable 1	1.15E-06	5.97E-09	193.382	7.02E-11

Appendix F- Regression statistics for 30s integration times

Regression Statistics	
Multiple R	0.99998
R Square	0.999961
Adjusted R Square	0.999953
Standard Error	1.46E-06
Observations	7

ANOVA

	df	SS	MS	F	Significance F
Regression	1	2.71E-07	2.70E-07	126629	3.33E-12
Residual	5	1.07E-11	2.10E-12		
Total	6	2.71E-07			

	Coefficients	Standard Error	t Stat	P-value
Intercept	3.36E-05	6.34E-07	53.0283	4.51E-08
X Variable 1	1.17E-06	3.28E-09	355.849	3.33E-12

Appendix G- Erroneous data: Pu determination with Uranium removal, without tracer.

The following results were obtained when spiked urine samples were prepared in replicate and analysed. The procedure was: Co-precipitation, wet-ashing, solid phase extraction. After column load, 20 mL 2 M HNO_3 was passed through the column to strip any matrix elements as well as uranium. Plutonium was then stripped with 5 mL 0.1 M HNO_3+ 0.2 M HF.

Spiked sample replicates read as follows:

Table G1: The measured values from spiked urine samples

	Pu-spike (pg L^{-1})	
	1	**15**
Urine spike	0.77 ± 0.12	10.28 ± 0.7

By modifying the data, the following table was obtained:

Table G2: Analysis parameters for urine matrix across the working range.

	Pu-spike (pg L^{-1})	
Parameter	**1**	**15**
Method efficiency (%)	77.3	68.6
Precision (%)	11.6	4.63

The under-recoveries have led to the transition from Lu as internal standard to Pu^{242} as an internal standard. Because the ^{242}Pu undergoes the same procedure as ^{239}Pu, it corrects more efficiently for ^{239}Pu loss from co-precipitation up to solid-phase extraction

Appendix H- Erroneous data: Pu determination without complete Uranium removal, with tracer.

The following results were obtained when spiked urine samples were prepared in replicate, ^{242}Pu internal standard added and analysed. The procedure was: Co-precipitation, wet-ashing, solid phase extraction. After column load, 10 mL 0.5M HNO_3 was passed through the column to strip any matrix elements. Plutonium was then stripped with 5 mL 0.1 M HNO_3+ 0.2 M HF.

Table H1: The measured values from spiked urine samples

	Pu-spike (pg L^{-1})	
	1	**15**
Urine spike	1.42 ± 0.13	15.3 ± 0.9

By modifying the data, the following table was obtained:

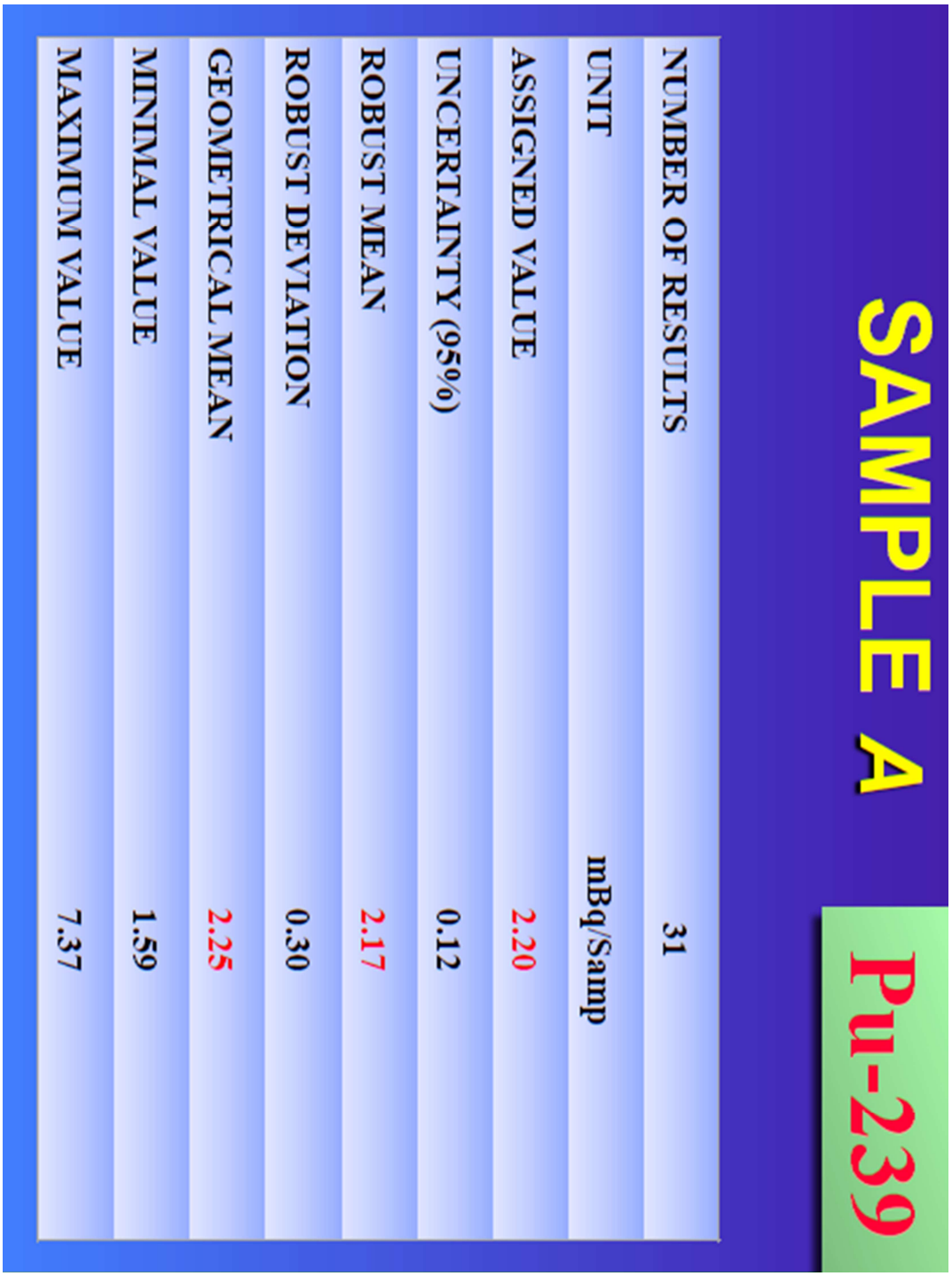
Table H2: Analysis parameters for urine matrix across the working range.

	Pu-spike (pg L^{-1})	
Parameter	**1**	**15**
Bias (%)	42	2
Precision (%)	9.15	6.03

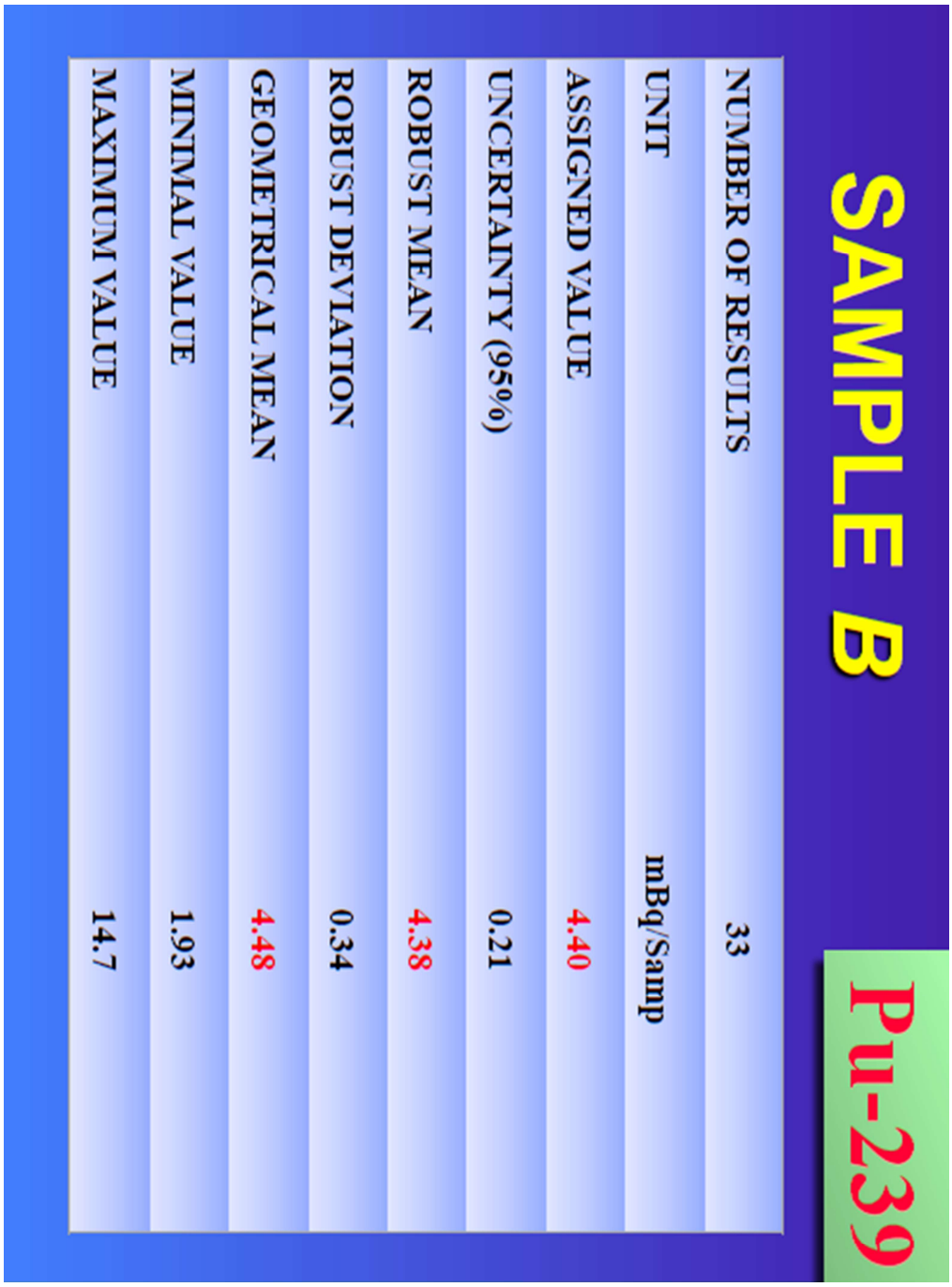
From the data there is a clear spike for the ^{239}Pu. The interference was more pronounced on the low spike. The suspect was determined to be uranium from either contaminated glassware or incomplete column separation. Samples generated ^{238}U CPS >15 000CPS and it has been established that uranium CPS should be kept < 5000CPS. Thus there was clear evidence of uranium induced interferences. As

preventative action, all glassware was left overnight in 10% HNO_3 and rinsed three times with ultra-pure water. Column rinse was additionally changed from 10 mL to 20 mL to ensure that if there was higher than expected uranium, most would be removed.

Appendix I- 2016 Procorad Sample A ^{239}Pu results



Appendix J- 2016 Procorad Sample B ^{239}Pu results



Appendix K -2016 Procorad Sample C ^{239}Pu results

Appendix L –Example of calibration standard calculation form.

Analysis Date : 2016/07/25

Pu Stock Conc : 1 µg/L

	Aliquot(g)	Total mass(g)	Calc Conc	
Pu Intermediate	0,1023	10,2882	9,94	ng/L
5 pg/L Conc	0,0054	9,7486	5,5	pg/L
10 pg/L Conc	0,0102	9,9385	10,2	pg/L
20 pg/L Conc	0,0197	10,2026	19,2	pg/L
50 pg/L Conc	0,0491	9,8727	49,5	pg/L
100 pg/L Conc	0,0998	9,9619	99,6	pg/L