



Natural uranium isotopes determination in 24h-urine samples from exposed workers to enriched uranium

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ABSTRACT

Individual monitoring of occupationally exposed workers to enriched uranium is mandatory, due to its radiotoxicity. The aim of this work is to determine if the use of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and an estimation method for ^{234}U activity, could supply the information obtained by Alpha Spectrometry (AS), which is the analytical technique commonly employed in this scenario. A total of 105 samples of 24 h-urine from exposed workers were analyzed by both techniques and the obtained results were compared. ^{235}U activity results were only quantifiable in 7 samples by AS (0.07–0.30 mBq/d) while ICP-MS quantified it in most of the cases. ^{238}U activity was measurable in 88 samples by AS and ICP-MS with wide range of results (0.11–2.4 mBq/d). For these isotopes no significant dosimetry differences were found between both techniques. However, ^{234}U activities results obtained by AS ranged from 0.15 to 7.6 mBq/d and the isotopic ratio of $^{234}\text{U}/^{238}\text{U}$ were highly variable. ^{234}U estimation was done with the average of these isotopic ratios and ^{235}U and ^{238}U ICP-MS results. Significant differences between estimated results and the ones obtained by AS were observed. Therefore, ICP-MS cannot supply the information given by AS, although can complement it. New studies must be started in order to obtain better and faster results applying radiochemical separation and ICP-MS measurement and studying the real contribution of diet in final uranium dose assessment.

1. Introduction

Uranium has three different natural isotopes (^{238}U , ^{235}U , ^{234}U), which have the same chemical behavior but differ in their radioactivity properties. The specific activity of ^{234}U is higher than the other radionuclides (^{234}U : 230.4 mBq/ng; ^{235}U : 0.0798 mBq/ng; ^{238}U : 0.0124 mBq/ng) (Craft et al., 2004; Wolfram Alpha.). It implies that ^{234}U , the minor isotope in weight, is the most unstable and the most radiotoxic.

Uranium is classified as radiotoxic, as alpha particles emitted during uranium disintegrations can induce harmful effects (Thorne and Wilson, 2015). The toxicity of this metal depends on a great number of variables like the species presents in environment, the incorporation route to the organism, the particle size, the proportion of the different natural isotopes or the exposition type (chronical or acute) (Agency for Toxic Substances and Disease Registry (ATSDR).; Thorne and Wilson, 2015). For this reason, to perform a correct dosimetry assessment, it is mandatory to carry out an adequate characterization of the scenario of interest. Also, the occupational surveillance of workers exposed to ionizing radiation is essential for radiological protection assurance and

compliance with current regulations (Sierra et al., 2007).

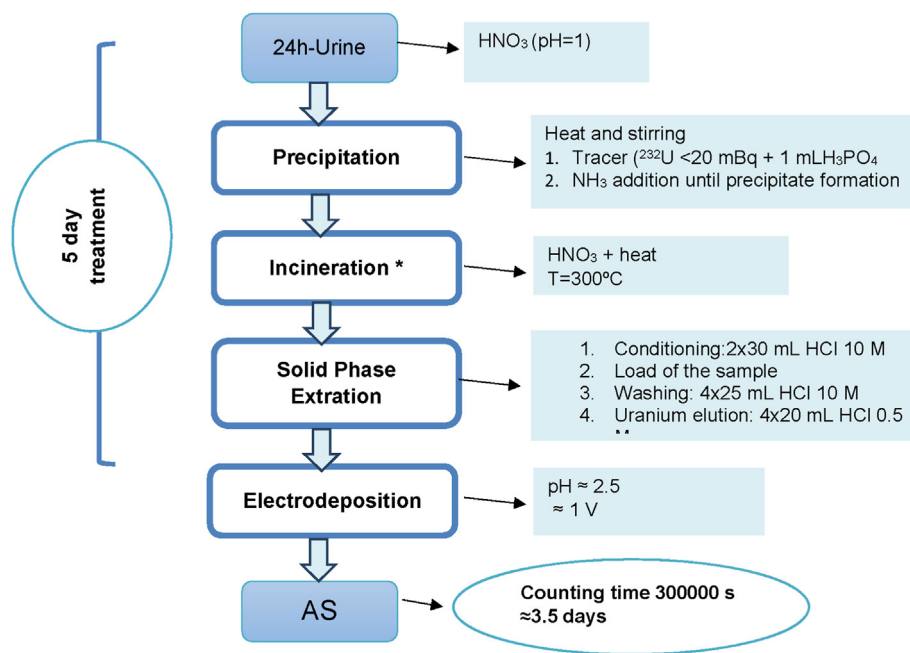
The present study is focused on Spanish Juzbado facility of ENUSA Industrias Avanzadas S.A., involved in the fabrication of nuclear fuel elements for Spanish Nuclear Power Plants with a 5% maximum enrichment in ^{235}U . Its workers, exposed to enriched uranium, are susceptible to internal contamination. Specifically, uranium is enriched in ^{235}U between 3% and 5%, which implies isotopic ratios between ^{234}U and ^{238}U from 3 to 5. The established individual monitoring program is performed with the results of the three natural uranium radioisotope activities determined by AS in 24 h-urine samples and is carried out in Bioelimination Laboratory of CIEMAT (Madrid, Spain). Bioelimination Laboratory is authorized to perform in-vitro bioassays with KPA, AS and other analytical techniques by the Spanish regulatory body (CSN, Nuclear Safety Council). Moreover, this laboratory is accredited according to ISO/IEC 17025 (Lopez et al., 2016).

2. Materials and methods

The analytical techniques employed in this study are based in two

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* Organic matter destruction is accomplished after performing several times the combination of two steps: addition of nitric acid to dissolve the residue and its evaporation until complete dryness following by the calcination of the dried residue at 300°C. Note that incineration implies more than one day.

Fig. 1. Sample treatment carried out in AS procedure. Adapted from (Sierra et al., 2017).

different principles. AS detects alpha particles emitted during radioactive decays, being able to distinguish them according to the particle energy. ICP-MS atomizes the sample, generates ions, separates them by mass/charge ratio and determines their intensity. Thus, AS determination is based on the radioactivity properties of the analytes and the intensity depends on the isotope activity, while ICP-MS results depend mainly on the mass of the isotopes of interests. In both cases it is possible to perform isotopic analysis, but the analytical characteristics, sample treatment and the limitations are different.

2.1. Reagents and materials

All reagents used in AS determinations were of analytical grade. Anion exchange resin AG1-X8 (100-200 dry mesh, chloride form) was purchased from Bio-Rad Laboratories (California, USA). ^{232}U reference solution used as tracer was certified by Ionizing Radiation Metrology Laboratory of CIEMAT. All the solutions needed were prepared with distilled water.

ICP-MS calibration and verification standards were prepared by dilution from a natural uranium reference standard certified by IRMM (Institute for Reference Materials and Measurements). A 2 ng/L indium solution, coming from a 1000 µg/mL, commercial standard certified by VWR International LTD (Leicestershire, UK), was used as internal standard. All the solutions and the standards were prepared with ultrapure distilled water (18 MΩ, < 12ppb TOC) and subboiling distilled nitric acid was employed.

2.2. Instrumentation

AS measurements were done with Canberra Alpha Analyst integrated instrument Model 7200 (Meriden, CT, USA), equipped with Canberra, Model A-450-18-AM, passivated implanted planar silicon detectors of 450 mm² active area and alpha resolution (FWHM) of 18 KeV at 5.48 MeV. The software which was employed was Genie 2000.

In ICP-MS technique the determinations were carried out with a Thermo Element XR double focusing magnetic sector field ICP mass spectrometer (Thermo Fischer Scientific, Bremen, Germany) equipped with a glass chamber and a pneumatic concentric nebulizer (Micromist model of 0.2 mL/min) coupled with an ASX-520 autosampler (TELEDYNE CETAC Technologies, Omaha, EEUU). The software which was employed was ELEMENT.

Creatinine determination in the samples was done using a Zuzi 4211/20 UV spectrophotometer (AUXILAB, S.L., Navarra, Spain).

2.3. Sample conservation and quality verification

When 24-h urine samples were received in Bioelimination Laboratory, sample volume was recorded, and two aliquots of 5 mL were taken from each sample. One of them was acidified with 0.5 mL of nitric acid for ICP-MS determination and the other one was used to verify sample quality with creatinine determination. The rest of the sample was acidified with 10% nitric acid and stored refrigerated until AS analysis was performed.

2.4. Analytical procedures

Firstly, it is necessary to ensure that samples are representative to 24-h urine performing creatinine analysis. Creatinine is a natural by-product of the breakdown of muscle tissue that is excreted at a fairly constant rate, normally filtered by the kidneys and excreted in the urine. Creatinine content in urine samples is determined by UV-spectrophotometry after performing Jaffe colorimetric reaction (Folin, 1914). Only samples with creatinine concentration higher than 70% of the reference values are susceptible of analysis. The reference values are established in ICRP 89 (1700 mg/day for men and 1000 mg/day for women) (International Commission on Radiological Protection (ICRP), 2002). Sample treatment realized in AS technique has four steps: precipitation, incineration, solid phase extraction and electrodeposition (Fig. 1).

Table 1
Instrumental parameters employed in ICP-MS analysis.

Plasma		Lenses Power (V)					
Torch (mm)	Ar Flow (L/min)	Normal Resolution		High Resolution			
X axis	5.7	Cooling	15.7	Extraction	−2000	1^{er} Quadrangle	4.19
Y axis	3.6	Auxiliary	0.9	Focus	−866.9	2^o Quadrangle	−6.2
Z axis	−0.6	Sample	1.125	X Deflection	−1.3	Targeting 1^{er} Quadrangle	−9.55
Power applied in plasma: 1278 W				Y Deflection	−1.9	Filter Lenses	−2.5
Power applied in the detector: 2670 V				Shape	125		

Table 2
RSD values for repeatability and reproducibility of ^{238}U and ^{235}U determinations with ICP-MS.

Natural Uranium (mg/L)	^{238}U		^{235}U	
	Repeatability ^a RSD (%)	Reproducibility ^b RSD (%)	Repeatability ^a RSD (%)	Reproducibility ^b RSD (%)
0.005	1–5	8	3–7	7
0.010	3–5	7	2–6	6
0.025	3–6	6	4–8	7

^a **Repeatability** involves the variation of standard measurements during each analysis.

^b **Reproducibility** considers the variation of the standard measurements among all the analyzes performed, including the environmental conditions. The concentration of each standard was obtained using the daily calibration curve.

In order to determine ^{235}U and ^{238}U by ICP-MS technique, a sample aliquot of 5 mL was acidified with 0.5 mL of nitric acid and homogenized. Four replicates of 10 mL were prepared by diluting 500 μL of this acidified aliquot with a 4% (v:v) ultrapure nitric acid solution. Before starting the determinations, instrumental parameters should be optimized to get an adequate signal-noise ratio. Measurement conditions employed in ICP-MS are presented in Table 1. A calibration curve with 9 different concentrations in the range of 0.25 ng/L to 100 ng/L of natural uranium was obtained before each analysis. The acceptance criterion of the calibration curve is defined by the correlation coefficient $r^2 > 0.9990$. Although all the standards were prepared in 4% of nitric acid solution, the instrument was conditioned before each analysis with blanks of urine. After the calibration curve, a verification standard of intermediate concentration was introduced to check the goodness of the calibration curve according to the accuracy and precision criteria (relative bias from -0.15 to $+0.25$ and the standard deviation < 0.2). Different verification standards were also introduced along the sequence of samples to ensure the reliability of the results.

3. Results and discussion

3.1. Study of the analytical methods

The goodness of the two analytical techniques is proved regularly with the participation in international intercomparison exercises, where the results are evaluated taking into account the relative bias (-0.25 , $+0.50$), z-score (≤ 2) and E_n number value ($|E_n| < 1$). Moreover, to verify the quality of the results some parameters were also analyzed during each analysis.

The chemical recovery of AS was obtained in each analysis, ranged from 80 to 100% in nearly 80% of the samples. The minimum detectable activity (MDA) for each isotope was calculated in each measurement, obtaining values between 0.01 mBq/L to 0.2 mBq/L for ^{238}U and from 0.02 mBq/L to 0.2 mBq/L for the other two radionuclides. This variability is caused by the diversity of chemical recoveries and of detector background.

In ICP-MS, ^{238}U and ^{235}U detection limits were calculated for each sequence based on ISO 28218 criteria (International Organization for Standardization (ISO), 2010). The concentration results obtained in ng/L were converted to mBq/L using specific activity values mentioned in

the introduction. Thus, detection limits found were from 0.004 mBq/L to 0.007 mBq/L for ^{238}U and between 0.002 mBq/L to 0.005 mBq/L for ^{235}U . These results showed a higher sensitivity in ICP-MS than in AS for ^{238}U and ^{235}U determinations. Although the detection of ^{234}U with ICP-MS was not possible, detection limit for this radioisotope was calculated employing experimental results obtained in 2014 in the Mass Spectrometry Laboratory of CIEMAT Dosimetry Service. A detection limit value of 3 Bq/L was found for this radionuclide, which confirmed the impossibility of a direct determination of ^{234}U with this method.

Moreover, in ICP-MS a study of the repeatability and reproducibility was carried out. The parameter employed in this study was the Relative Standard Deviation (RSD) and was determined for the three different concentrations of verification standard used (0.005 mg/L; 0.010 mg/L and 0.025 mg/L of natural uranium). In this work, considering the concentration of the standards used, RSD values equal or less than 5% were considered as excellent and the limit of the acceptance was established in 10%. The range of RSD values found for both repeatability and reproducibility were collected in Table 2.

ICP-MS is an attractive alternative technique to AS because it has lower detection limits for ^{235}U and ^{238}U isotopes, only an aliquot of sample volume is required and, therefore, offers the possibility of repeating the measurement. Other advantages of ICP-MS over AS are the lack of sample treatment and the analysis speed. However, ICP-MS could only supply AS information if two circumstances would happen: ^{238}U and ^{235}U results obtained with both techniques are comparable, and ^{234}U is estimated adequately with the information acquired by ICP-MS.

3.2. Activity results

Most of the samples (80) measured by AS presented activities of ^{235}U below the MDA and only one sample corresponded with a positive control, requiring dosimetry evaluation. The range of activities found for this isotope with AS in the seven quantifiable samples was 0.07–0.3 mBq/L. On the other hand, ICP-MS could detect and quantify ^{235}U in most of the cases, presenting results close to quantification limit and agreeing with AS results. Moreover, a comparison study considering the seven quantifiable samples by the two methods was done with Student t-test, finding no significant statistical differences.

In relation to ^{238}U activity results, this isotope could not be detected

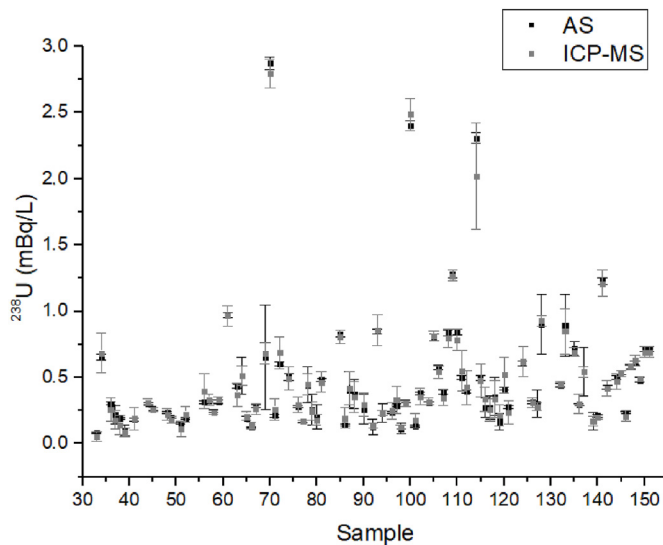


Fig. 2. ^{238}U activities and the correspondent expanded uncertainty ($k = 2$) (International Organization for Standardization (ISO) and International Electrotechnical Commission (IEC), 2008), obtained by ICP-MS and AS.

by AS in three samples and by ICP-MS in only one case, due to the lower detection limit. The results obtained in 88 samples with quantifiable amounts of this isotope by both techniques (Fig. 2) show a great variability of activities between samples, being 40 positive controls. Another relevant fact is that the amount of activity found for ^{238}U is higher than the one obtained for ^{235}U . As shown in Fig. 2, there are not significant differences between the activities found by both techniques, something which is also corroborated by Student *t*-test.

The same variability can be found in ^{234}U activity results by AS in the range of 0.15–7.6 mBq/L. It was not quantifiable only in four samples. Moreover, only eight cases did not require a dosimetric evaluation for this isotope. These results indicated that in general, the isotope present in highest activity was ^{234}U following by ^{238}U and the inferior was ^{235}U . This fact agrees with the composition of natural uranium: around 48.2% of ^{238}U ; 2.2% of ^{235}U and 49.6% of ^{234}U (Craft et al., 2004; Wolfram Alpha.) and the last two isotopes are higher in enriched uranium.

3.3. Calculated $^{234}\text{U}/^{238}\text{U}$ ratio with Alpha Spectrometry measurements

In order to propose an experimental method to estimate ^{234}U values from ^{238}U obtained in ICP-MS measurements, it is necessary to study the ratio between the activities of ^{234}U and ^{238}U obtained by AS. In this study, only the samples with quantifiable activity amounts for both isotopes could be considered. $^{234}\text{U}/^{238}\text{U}$ ratio was calculated in each sample with AS activity results and the uncertainty was obtained as the combination of the two isotopes uncertainties (Fig. 3).

The results showed a significant heterogeneity, therefore the samples can be classified into four different groups:

- **Samples from natural uranium exposure:** 10 samples presented a proportion of these isotopes similar to natural uranium. Besides, in these cases ^{238}U activity is in the range of 0.15–0.84 mBq/L, being inside the range considered as natural background of uranium ((International Organization for Standardization (ISO) and International Electrotechnical Commission (IEC), 2012). Thus, in these samples the possibility of enriched uranium intake is discarded.
- **Samples from natural and enriched uranium exposures:** 30 samples presented a $^{234}\text{U}/^{238}\text{U}$ ratio between natural uranium proportion and the proportion expected in the scenario of interest (3–5%). The values found in this group ranged from 1.9 to 2.9. Exposed workers

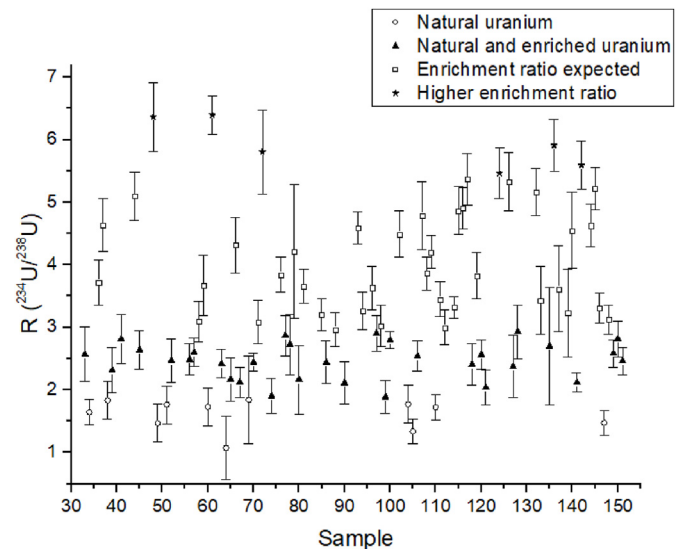


Fig. 3. Ratio of $^{234}\text{U}/^{238}\text{U}$ activities and the corresponding combined uncertainty obtained by AS.

to enriched uranium, as population in general, can incorporate natural uranium from the environment and/or the diet. In this way, the existence of this group of samples is justified by the incorporation of both natural and enriched uranium.

- **Samples from enriched uranium with expected ratio (3–5%):** This is the majority group, composed by 39 samples, corresponding with 56% of the cases with enriched uranium incorporation. This fact shows that it might be possible to perform an adequate ^{234}U estimation with ICP-MS information. However, it could not be ignored that the number of cases with expected ratio is minimum compared with the total of samples considered in this study (105).
- **Samples from enriched uranium with ratios higher than expected:** This group is formed by 6 samples, with ratios between 5.5 and 6.4. In these cases, results of initial controls were analyzed, observing that $^{234}\text{U}/^{238}\text{U}$ ratios were also high. This implies that some uranium intakes had occurred before Bioelimination Laboratory started the occupational surveillance of these workers. Until 2014 annual individual monitoring of ENUSA workers was carried out by in-vivo measurements by Whole Body Counting in CIEMAT and by in-vitro bioassays measurements by KPA in ENUSA facility.

3.4. Estimation of ^{234}U activity from ICP-MS information and comparison with the results obtained by AS

The diversity observed in the isotopic ratio values and the lack of the compliance of the expected range of ratios, make it difficult to estimate the activity of ^{234}U . In order to calculate the ^{234}U activity satisfactorily, independently of the isotope proportion, the estimation was performed with the average isotope ratio of all the results exposed before. The obtained average ratio, 3.3, is inside the expected range (3–5). The estimation of ^{234}U activity was performed in 103 cases; in all the samples with quantifiable amounts of ^{238}U by ICP-MS. ^{234}U estimated activities and AS obtained results are shown in Fig. 4.

The existence of differences between estimated activities and the ones measured by AS is easily appreciable in Fig. 4. This variability has been verified with Student *t*-test, obtaining significant statistical differences in 56% of the cases. Additionally, an absolute bias study was performed to evaluate the deviation of the estimated activity respect AS results: 8 samples had values lower than 5%; 8 cases between 5–10%; 25 samples between 10–25%; 34 cases between 25–50% and 27 samples higher than 50%. According to ISO acceptance criteria for radioassay (International Organization for Standardization (ISO), 2010),

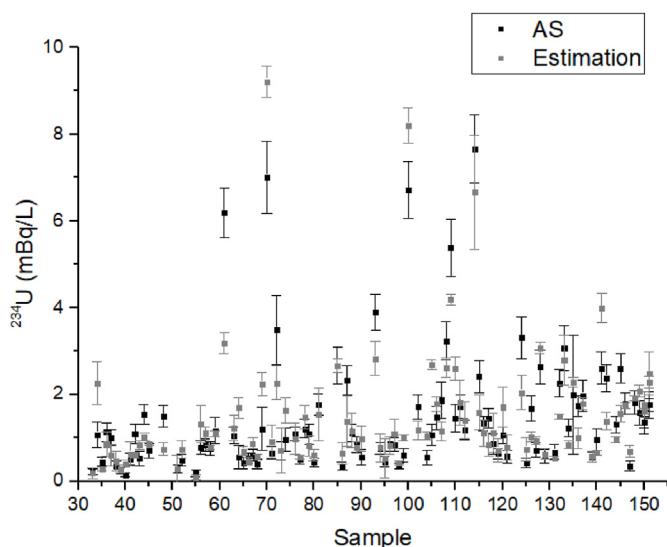


Fig. 4. ^{234}U ICP-MS activities estimated and ^{234}U activity results obtained by AS, with the combined uncertainty in each case.

the estimated activity would be rejected in 44 cases. Therefore, in 55% of the samples the estimation of ^{234}U presents an assumable deviation from the corresponding activity found by AS.

4. Conclusions

An internal dosimetry monitoring program of occupationally exposed workers to enriched uranium must select the most adequate analytical technique to perform the in-vitro measurements. In this work both Alpha Spectrometry and ICP-MS techniques have been studied in more than 100 urine samples.

The mass proportion of ^{238}U allows the quantification of this isotope by ICP-MS and AS, despite its low specific activity (0.0124 mBq/ng). The uranium isotopic composition analysis of the samples shows that ^{238}U activity is highly diverse and no statistical significant differences were found in the results of both techniques.

Due to the low mass proportion of ^{234}U , this radioisotope could not be detected by ICP-MS, while its high specific activity (230.4 mBq/ng) allows its detection by AS. Besides, the results were higher than ^{238}U activities, finding only four cases of 105 with concentrations of ^{234}U below MDA. This fact highlights the importance of ^{234}U quantification in the scenario of study.

^{235}U is one of the minor isotopes of uranium in mass proportion and has a low specific activity (0.0798 mBq/ng). Thus, in the analyzed samples this isotope is the one with less activity; only 7 samples were quantified by AS, whereas ICP-MS allowed the quantification in most of the cases. Both methodologies provided concordant results of this isotope in all cases, revealing the minimal health implication of ^{235}U in this specific enriched uranium scenario.

$^{234}\text{U}/^{238}\text{U}$ isotopic ratio study presented strong variation, finding some samples with an isotopic proportion of natural uranium and others with enriched uranium of higher enrichment than expected. This heterogeneity and the lack of precise information about the enriched uranium existing in the scenario of interest, hinder the ^{234}U estimation performed. This variability could explain the lack of consistency between ^{234}U ICP-MS estimation and ^{234}U AS measurement. Thus, it can be concluded that using the ^{234}U estimation method in this scenario, ICP-MS technique cannot supply the isotopic information given by AS. The importance of ^{234}U radiotoxicity in this case makes AS the reference technique in the studied scenario of chronic intake of enriched uranium.

New studies related to dietary contribution of natural uranium in

the area and initial control measurements of new workers are now in progress in order to describe better this specific exposure and to improve the internal dose assessment of these workers.

Moreover, other studies are focused on improving ICP-MS detection limit for ^{234}U by increasing the volume of sample used in the analysis and applying pre-concentration processes or radiochemical separation prior to the measurement.

CRedit authorship contribution statement

P. Albendea: Conceptualization, Methodology, Validation, Formal analysis, Writing - original draft. **I. Sierra:** Validation, Investigation, Writing - review & editing, Visualization, Supervision, Project administration. **C. Hernández:** Resources, Writing - review & editing, Visualization. **A.I. Barrado:** Resources. **A. Yllera:** Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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