

A rapid and efficient autoclave pre-treatment to extract iodine-129 from urine samples for AMS analysis

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Abstract

A new method was developed to extract ^{129}I from urine samples and then measure it by AMS. The samples were pre-treated in an autoclave with hydrogen peroxide to remove unwanted compounds from the urine samples and were acidified with nitric acid, followed by precipitation of iodine as silver iodide (AgI) for measurement by AMS. This new procedure is substantially faster than previous methods for the extraction of iodine from urine and results in less chemical waste. The efficiency and reproducibility of this method were evaluated by using ^{125}I as a yield tracer, eventually giving a recovery above 99%. To achieve this, several iterations of the method were required. The method was then successfully applied to measure $^{129}\text{I}/^{127}\text{I}$ isotopic ratios and ^{129}I concentrations in 25 human urine samples. The AMS results for ^{129}I in urine ranged 3.3×10^6 atoms/L to 884×10^6 atoms/L and the isotope ratio ($^{129}\text{I}/^{127}\text{I}$) in human urine ranged from 7.38×10^{-12} to 3.97×10^{-10} with a median of 1.29×10^{-10} . This new method will be useful for investigations into the sources of iodine in the human diet and their relative importance for iodine sufficiency.

1. Introduction:

^{129}I is a long-lived radioisotope with a half-life of 16.14 million years (García-Toraño et al. 2018). It is produced naturally by the nuclear interaction of cosmic rays with xenon in the upper atmosphere and by the spontaneous fission of uranium-238 in minerals (Raisbeck and Yiou 1999). Since 1945, the concentration of ^{129}I in the environment has increased sharply due to anthropogenic nuclear activities such as nuclear weapons testing, nuclear fuel reprocessing plants (NFRP), and a minor contribution from nuclear power plant (NPP) accidents and emissions. A study by Zhang et al. 2013 differentiated $^{129}\text{I}/^{127}\text{I}$ ratios into three categories: pre-nuclear era natural levels with $^{129}\text{I}/^{127}\text{I}$ ratios of 10^{-12} , modern background $^{129}\text{I}/^{127}\text{I}$ ratios ranging from 10^{-11} to 10^{-9} , and contaminated areas that have $^{129}\text{I}/^{127}\text{I}$ ratios from 10^{-8} higher. As a mobile long-lived radioisotope in the hydrosphere and atmosphere, iodine is distributed throughout the environment and can easily enter the food chain through the hydrosphere, atmosphere, and geosphere. (Zhang and Hou 2013; Jabbar, Wallner, and Steier 2013). Since ^{129}I is a fission product, it is a very good indicator of the global dispersion of nuclear emissions, without being itself a dangerous radioisotope.

High ^{129}I concentrations can be measured by activity counting methods such as liquid scintillation counting (LSC), γ -spectrometry, or neutron activation analysis (NAA) as well as mass spectrometry techniques such as Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) and Accelerator Mass Spectrometry (AMS). Of these methods, AMS is the only one that can measure ^{129}I in environmental and biological samples at the low abundances typically found outside of contaminated areas.

Applications of ^{129}I are typically limited to environmental investigations such as its use as a tracer in oceanography, groundwater dating or of nuclear emissions. Due to the sensitivity of AMS, there is an opportunity to expand the use of ^{129}I applications into new fields such as biomedical

applications or nutrition, which have been investigated less frequently. One hurdle to expanding ^{129}I applications is extracting ^{129}I from biological samples for AMS analysis. Due to its low concentration in the environment, ^{129}I analysis requires chemical separation that must consider many factors such as sample type, sample size, and iodine chemical species (Zhao et al. 2009; Zhang and Hou 2013; Jabbar, Wallner, and Steier 2013). ^{129}I pre-treatment procedures such as UV-irradiation, caustic fusion, acid decomposition, oxidant decomposition, alkaline leaching, ashing, combustion, and microwave digestion have all been used with varying degrees of complexity and success, to prepare ^{129}I samples for AMS analysis (Herod et al. 2014; Gómez-Guzmán et al. 2011; Muramatsu et al. 2008; Zhao et al. 2009; Zhang and Hou 2013).

Regarding ^{129}I in urine, there is only one study by (Hou et al. 2003) that measured ^{129}I in three urine samples, using neutron activation analysis (NAA). (Hou et al. 2003) used three to four liters of urine and compared ^{129}I in animal thyroid and human urine. To date, no studies have measured ^{129}I in urine by accelerator mass spectrometry.

Urine is a human waste product that is typically excreted in a range of 600 ml to 2000 ml daily and is a common clinical sample. In general, 95% of urine is water, and 5% is dissolved solutes (Strasinger.2008). Urine contains a high concentration of urea, organic substances such as creatinine, and inorganic elements, which are primarily chloride followed by sodium, potassium, and trace amounts of others (Strasinger.2008).

This paper aims to demonstrate a reliable and reproducible method for the measurement of ^{129}I in human urine, by using thermal-pressure digestion for AMS and by relating the result to iodine sources in the diet in a preliminary sense. The initial results from the method are presented and compared to those of (Hou et al. 2003). The efficiency and reproducibility of this method were evaluated using ^{125}I as a tracer of recovery, and a discussion on the factors that affect the

extraction efficiency and AMS measurements is presented. This study is a first step towards understanding the sources of ^{127}I and ^{129}I in the human diet using ^{129}I as a nutritional tracer. This procedure is envisaged to potentially expand the applications of ^{129}I into non-environmental fields.

2. Methods:

2.1 Chemicals and Instruments

Urine samples were digested in 60 ml borosilicate glass tubes in an autoclave (Tuttnauer Brinkmann). ^{125}I tracer solution (37 MBq) was ordered from PerkinElmer and diluted in 14.9 M NH_4OH to give an activity of 20,000 Bq/ml for use as a recovery tracer. All solutions were prepared using 18 M Ω de-ionized water. The parameters used for autoclave digestion are given in Table 1.

A PerkinElmer Wizard 2 Automatic Gamma Counter (2470-0020) with two well type NaI detectors was used to measure ^{125}I to quantify the extraction efficiency. A 3MV HVE tandem AMS system was used to measure the concentrations of ^{129}I in the AgI extracted from the urine at the Andre E. Lalonde Accelerator Mass Spectrometry Laboratory. The technical details of the ^{129}I measurement by AMS are described in (Francisco et al. 2020) and ion source parameters are given in Table 2. An Agilent 8800 ICP-MS triple quadrupole was used to measure the concentrations of ^{127}I .

Table 1: Autoclave parameters

Parameter	Value
<i>Program</i>	Wrapped Instruments and Porous Loads
<i>Temperature</i>	250°F (121°C)
<i>Digestion Time</i>	20 min
<i>Dry time</i>	60 min

Table 2: AMS ion source parameters (adapted from Francisco et al. 2020)

Parameter	Value
<i>Cesium reservoir temperature</i>	80 °C
<i>Target Voltage</i>	7.0 kV
<i>Target current</i>	0.5–1.5 mA
<i>Extraction Voltage</i>	28.0 kV
<i>Extraction current</i>	0.2 mA
<i>Ionizer current</i>	18 A
<i>Vacuum (mbar)</i>	0.5–1.0 mBar

2.2 ^{127}I analysis by inductively coupled plasma mass spectrometry.

The most commonly used method to measure iodine in urine is direct dilution and ICP-MS analysis. In this procedure, we considered FDA guidelines for method validation (FDA 2018), and we applied a modified procedure of (Allen et al.1990) as follows: 1 g of urine sample was mixed with 9 grams of 1% 0.292 M ammonium hydroxide (NH_4OH) to obtain a 1/10 dilution. To avoid any sample memory during the analysis, the samples were diluted and additional 50 times. Indium-115 and tellurium-205 were used as internal standards to obtain optimal precision and to determine the accuracy of the ICP-MS instrument. Seven standards (0ppb), (1.56ppb), (3.125ppb), (6.25ppb), (12.5ppb), (25ppb), and (50ppb) were used to develop a calibration curve to determine ^{127}I in urine. The limit of detection was 0.0445 ppb, and the limit of quantification was 0.148 ppb.

2.3 Sample collection and preparation

Human urine samples were collected in sterile, dry, 200 ml polypropylene, disposable containers from 27 individuals living in Ottawa, Ontario, Canada. Their ages ranged from 3 years old to more than 66 years old (Table 3). Ethics approval was obtained from the University of Ottawa, and participants were provided with consent forms and surveys about their eating

habits (Figure 1). The participants were advised to fill the container in the early morning as morning urine typically has a higher specific gravity, i.e., less water and more solute than urine from other times of day (Strasinger 2008). The minimum urine volume required to perform the experiments was 100 ml.

Sample Type: <input type="checkbox"/> Urine				
SECTION 1: Donor Information				
Sample's Lab code: U2017-09-			UOH number:	
Age	3-18	19-40	41-65	>66
	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
SECTION 2: Donor Diet				
	Daily (more than once a week)	Weekly (once a week)	Monthly (once a month)	None
Using Salt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Using Dairy products (Milk, Yogurt, Cheese,etc)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Eating Sea Food (Fish/Shrimp/Calamari,etc)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Eating Bread	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Eating Vegetables	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Eating Processed meat (Bacon/Sausage/Burger,etc)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Eating Seaweeds	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
For more datils, please read the Consent form.				

Figure 1: An example of the questionnaire given to all participants

Age (years)	Number
3 to 18	4
19 to 40	14
41 to 65	8
> 66	1
Total number	27

Table 3 Participants demographic information

2.3 Extraction of ^{129}I from urine for AMS analysis

Four progressive iterations were required to develop the optimal procedure to extract ^{129}I from the urine samples. In order to improve the yield of iodine and to minimize the chemical waste, the fourth iteration that uses less HNO_3 and less NH_4OH was applied to all samples for the extraction to analyse the samples by AMS.

In the fourth, and final, extraction procedure, 25g of urine plus 5 g of hydrogen peroxide were treated in the autoclave. After digestion in the autoclave, twenty milligrams of sodium carbonate Na_2CO_3 was added and heated under hot plate for 10 min to remove any residual hydrogen peroxide. The samples were then acidified using 0.2ml of 15.66M HNO_3 to lower the pH to around one, and two milligrams of iodine as NaI carrier with a $^{129}\text{I}/^{127}\text{I}$ ratio of 1.4×10^{-14} was added. To precipitate a sample of AgI for AMS, 200 μl of 0.1M AgNO_3 was added. The samples were left for 12 hours in darkness to allow the precipitate to settle. The precipitate was collected and washed in de-ionized water and 1 ml of 14.99 M ammonium hydroxide NH_4OH was added to dissolve any co-precipitating AgCl . The samples were centrifuged and the supernatant removed. The precipitate was washed in de-ionized water and centrifuged. The samples were dried in the oven at 60°C for overnight. The AgI precipitate was then prepared for AMS analysis by mixing it with pure niobium powder in a ratio of 1:1 or 1:2 by volume and pressing the mixture into a copper cathode to make the AMS target. A flow chart of the method is shown in Fig 2.

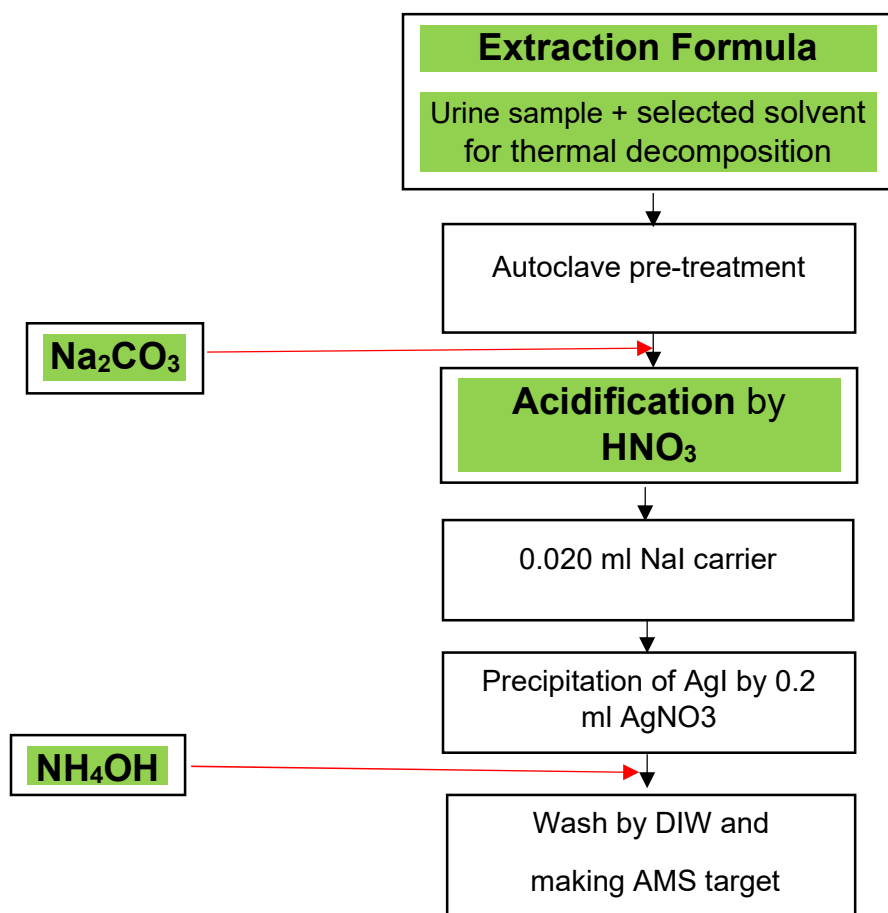


Figure2. Flow chart showing the chemical extraction of ^{129}I in the urine. Steps in the bold were varied among the iterations.

2.4 Blank

Several blanks were used during this analysis. Deionized water was used as a processed blank during the digestion and extraction chemistry procedure, giving isotopic ratio. In AMS run, sodium iodide (NaI) blank carrier, and niobium powder blank were used and their total counts and ratio were constant every ten samples. In ICP-MS run, 1% NH_4OH was used as blank and its concentration was constant every ten samples too. This indicates that no sample cross contamination occurs during the run in both instruments.

Results and Discussion

3.1 Experimental optimization

3.1.1 Optimization of iodine extraction from urine

Biological samples, such as urine, contain variously abundant components and elements, generally termed matrix, which often affect extraction recovery and reproducibility for rare isotopes. In order to measure ^{129}I in urine samples by AMS, the sample preparation should recover sufficient iodine through reduction of these matrix effects. In the case of iodine in urine, sample matrix is usually eliminated either by acid digestion or alkaline ashing (May et al. 1997). In this method, autoclave pre-treatment is used as an alternative to the conventional extraction techniques for ^{129}I , which typically use strong acids or bases to digest the sample (May et al. 1997; Zhao et al. 2009). For the digestion, autoclave and microwave provide the same outcome of thermal breakdown (Taylor et al. 1996). However, the autoclave is generally more affordable than lab microwaves. The ability of the autoclave to break down organic matter is well known and it can be used to digest organic matter and compounds such as urine, if it is used with a proper digestion solvent (Jeffries, Dieken, and Jones 1979; Crowther 1978; Navarrete-López et al. 2012). Using an autoclave to extract iodine from samples is uncommon in the literature (Haldimann, Eastgate, and Zimmerli 2000); therefore, the evaluation of this technique with respect to iodine recovery was necessary to ensure all iodine remained in the sample. The recovery of iodine during autoclave pre-treatment was tested using the addition of a known amount of ^{125}I to determine the efficiency of the procedure.

3.1.2 Chloride interference

In the first and second iterations, potassium persulfate was the digestion solvent used. For the first iteration, 0.4 g of urine was mixed with 1 g of potassium persulfate then dissolved in 20 g of de-ionized water and digested in the autoclave. The preliminary AMS results showed that ^{129}I concentration was low and too close to the AMS background to obtain reliable data. Therefore, a second iteration with increased sample volume was attempted to increase the AMS signal. A 4 g urine sample was mixed with 1 g potassium persulfate then dissolved in 200 g of de-ionized water and digested. The sample was precipitated using the method described above; however, the resulting precipitate was initially white and became gray over time. A portion of this gray precipitate was collected and analyzed using a scanning electron microscope which showed a significant amount of chlorine relative iodine suggesting that AgCl was coprecipitating with AgI (Table 4). This resulted in poor currents in the AMS ion source (Brunzel 2013).

<i>Sample 1</i>	Al	Cl	Ag	I	O	K	S	Total %
Spectrum 1	0.48	22.71	66.01	5.48	5.32			100
Spectrum 2	0.00	22.45	67.20	5.37	4.98			100
Spectrum 3	0.00	16.30	72.06	6.29	5.34			100
Spectrum 4	19.27	17.62	42.79	0.00	20.31			100
<i>Sample 2</i>								
Spectrum 1		18.74	59.78	3.22	10.53	4.23	3.49	100
Spectrum 2		18.74	63.80	3.23	9.17	2.43	2.63	100

Table 4: Scanning Electron Microscope results show chloride interferences for two samples from iteration two.

Units in %

The results of these initial experiments raise a number of questions regarding the efficiency of digestion solvent, the required sample volume and how to eliminate interference from coprecipitation of AgCl.

3.1.3 Iodine loss due to volatilization

To resolve the problems identified in the first two iterations, potassium persulfate was replaced with hydrogen peroxide as the solvent, and the ratio of sample volume to solvent was changed to 5 ml urine + 0.5 ml H₂O₂ (10:1) in order to reduce dilution. 20 mg sodium carbonate, Na₂CO₃, was added after the autoclave digestion to remove residual hydrogen peroxide (Wu and Englehardt 2012). 3 mL of ammonium hydroxide was added at intervals before drying to eliminate chloride. A portion of the precipitate was collected and analyzed using a scanning electron microscope which showed a large reduction in the chloride interference (Table 5). However, both gamma spectrometry of the ¹²⁵I tracer and AMS of ¹²⁹I results showed that there was poor recovery despite these changes. We hypothesize that the loss of iodine from samples was related to the addition of nitric acid after the autoclave pre-treatment, which oxidized the iodine into the volatile I₂ species. Therefore, the acid volume was decreased in the fourth iteration from 1 milliliter to 0.2 millilitres to reduce iodine volatilization.

Sample	Al	Cl	Ag	I	O	C	Total %
Spectrum 1	0.00	0.00	39.29	45.03	2.62	13.06	100
Spectrum 2	0.00	0.00	38.85	44.35	3.11	13.69	100

Table 5: SEM results show the absence of chloride interferences in the precipitate. Units are %..

3.1.4 Effect of autoclave digestion and solvent on ¹²⁵I recovery

In order to determine if there was any loss of iodine in the autoclave, ¹²⁵I was added as a yield tracer. Using ¹²⁵I as a quantitative recovery tracer for ¹²⁷I and ¹²⁹I has been reported in detail elsewhere (Herod et al. 2014). Briefly, the method uses the coincidence sum gamma counting method to determine absolute sample activity (Horrocks and Klein 1974). The use of ¹²⁵I as a quantitative tracer of iodine recovery enabled us to determine if digesting the samples in

the autoclave led to a loss of iodine. The data in Table 6 confirm that heat and pressure in autoclave digestion has no effect on ^{125}I recovery and, therefore, is not expected to cause a loss of ^{129}I or ^{127}I from the samples. In the first iteration, potassium persulfate was used as a digestion solvent because the persulfate digestion is known as a safe oxidizing agent used in iodine analysis (Pino, Fang, and Braverman 1996) but it requires more water and produces substantial chemical waste. Thus, another safe oxidizing agent such as hydrogen peroxide was used as a digestive solvent along with an increase in the amount of urine sample. The results of the ^{125}I yield tracer for each solvent after digestion in the autoclave and show that the recovery obtained from H_2O_2 was superior to that of $\text{K}_2\text{S}_2\text{O}_8$. As a result, H_2O_2 was chosen as the solvent for the optimized procedure as it is both more efficient and produced less waste.

	Total Activity (Bq)	Recovery % to Pure ^{125}I
Pure ^{125}I	2372.9	100
Blank(air)	0	0
Mean value of ^{125}I standards (n=4)	2381	100.3
Mean value of ^{125}I in urine with $\text{K}_2\text{S}_2\text{O}_8$ (n=3)	2157.4	90
Mean value of ^{125}I in urine with H_2O_2 (n=3)	2509.9	105
		Recovery % to standard
Mean value of ^{125}I in urine with $\text{K}_2\text{S}_2\text{O}_8$ (n=3)	2157.4	90.6
Mean value of ^{125}I in urine with H_2O_2 (n=3)	2509.9	105

Table.6: Analytical results of ^{125}I yield for the two-solvents (n=6) used in the extractions after autoclave digestion compared to mean values of ^{125}I in water (n=4) without autoclave digestion.

3.1.5 Comparison of ^{125}I recovery for each iteration

The ^{125}I recovery for each iteration were compared and the results are summarized in Table 7. The second iteration was not tested as it was identical to the first except for an increased volume of the sample and volume of solvent.

Iteration	Total activity (Bq)	Recovery (%)	% Recovery Error
First			
	8765.6	42.4	0.00020
	8206.8	39.7	0.00021
	8351.5	40.4	0.00021
Third			
	584.6	2.8	0.00069
	536.8	2.6	0.00073
	555.2	2.7	0.00071
Fourth			
	20739.9	100.4	0.00013
	20718.1	100.3	0.00013
	20667.2	100	0.00014

Table.7: A comparison table of the iterations in terms of ^{125}I recovery shows that the fourth extraction procedure has more sensitivity and reproducibility (n=9).

The first iteration had a poor recovery of ^{125}I of less than 50% due to the low sample volume and interference from chloride in the samples, while the third experiment had very poor recovery of ~3% due to volatilization of iodine from the sample during acidification. The loss of iodine from samples in the first and third iteration is not related to autoclave heat and pressure as the results in Table 6 showed no loss of ^{125}I following the autoclave digestion step. The fourth iteration decreased the amount of acid used and as a result an excellent recovery of ^{125}I , exceeding 99%, was obtained.

3.2 ^{129}I and ^{127}I concentrations in urine

The fourth iteration of the method was successfully applied to measure ^{129}I in urine samples from 25 people. The AMS results for ^{129}I in urine ranged from 3.3×10^6 atoms/L to 884×10^6 atoms/L with a median of 108.7×10^6 atoms/L. The results of ^{127}I in the urine sample ranged from 24.1 $\mu\text{g/L}$ to 3544 $\mu\text{g/L}$ with a median of 160.4 $\mu\text{g/L}$. The $^{129}\text{I}/^{127}\text{I}$ ratio ranged from 7.38 x

10^{-12} to 3.97×10^{-10} with a mean of 1.36×10^{-10} and a median of 1.29×10^{-10} . All results are summarized in Table 8.

Sample ID	^{127}I concentration $\mu\text{g/L}$ with standard error ± 0.24	Corrected ^{129}I Concentration 10^6 atm/L	Error of ^{129}I Concentration 10^6 atm/L	$^{129}\text{I}/^{127}\text{I}$ ratio 10^{-10}
1	225.6	238.3	6.65	2.23
2	79.7	61.8	2.74	1.64
3	3543.9	884.0	20.22	0.56
4	85.0	109.5	2.56	2.72
5	94.4	3.3	2.33	0.07
6	1197.0	798.6	18.31	1.41
7	207.2	217.3	4.63	2.21
8	339.7	318.4	7.51	1.98
9	94.8	16.1	1.01	0.36
10	255.4	40.2	1.76	0.33
11	24.1	15.3	1.28	1.33
12	143.1	87.7	2.49	1.29
13	139.6	180.2	3.61	2.72
14	482.5	84.5	2.63	0.37
15	394.8	340.6	17.05	1.82
16	185.9	264.6	5.65	3.00
17	338.8	108.7	2.66	0.68
18	122.6	52.2	2.61	0.90
19	81.0	12.4	3.36	0.32
20	160.4	50.8	2.78	0.67
21	131.0	32.1	2.23	0.52
22	83.3	156.6	3.25	3.97
23	102.3	14.2	0.87	0.29
24	441.8	231.0	8.21	1.10
25	222.7	157.5	4.40	1.49

Table 8: Concentration of ^{129}I and ^{127}I in human urine from ICP-MS and AMS (n=25)

Urinary iodine is highly variable from day to day (Zimmermann 2008). The results in Table 8 show the concentration of ^{127}I on the day of collection. Figure 3 shows the frequency distribution of ^{127}I in the samples. One sample had a very low ^{127}I concentration of $24.1 \mu\text{g/L}$ which could be related to the age of that particular participant, who was 3 years old. Six samples were

within mild deficiency concentration range (50-99 $\mu\text{g/L}$), eleven samples were within the optimal concentration range (100-299 $\mu\text{g/L}$), and five samples were within the risk of hyperthyroidism concentration range (300-499 $\mu\text{g/L}$). There were two samples which significantly exceeded the high concentration range (^{127}I above 500 $\mu\text{g/L}$) and exceeded 1000 $\mu\text{g/L}$. It is likely these extremely high values are due to thyroid diseases or iodine supplements taken by those participants. The variables ^{127}I and ^{129}I were found to be positively correlated when these high samples were included, with a Pearson $r = 0.84$ ($n=25$). If we consider the ^{127}I values above 500 $\mu\text{g/L}$ as outliers and eliminate those samples from the Pearson correlation analysis, the variables ^{127}I and ^{129}I were still found to be significantly correlated with a Pearson $r = 0.56$ ($n=23$). In both scenarios, the ^{129}I concentration had a statistically significant correlation with the ^{127}I concentration (Fig 4). This positive correlation suggests that may be a common source of ^{129}I and ^{127}I in the human body. In environmental samples, including those from other studies analysing ^{129}I in North America, ^{129}I and ^{127}I are rarely well correlated due to their differing sources (Herod et al. 2016; Fehn 1999). This is the first time such a correlation has been measured in humans and suggests that ^{129}I could be a potential tracer of dietary ^{127}I as it appears the integration of these two iodine isotopes is occurring in nature prior to their consumption by humans. Nutritional literature generally presumes that most ^{127}I in the human body is from iodized salt (Pandav et al. 2013; Fields and Borak 2009; Gunnarsdottir and Dahl 2012) and salt iodination programs have been extremely successful in reducing hypothyroidism due to lack of sufficient dietary iodine, particularly in developing nations (Mannar and Dunn 1995; de Benoist et al. 2004). However, these results could indicate that the source of iodine in humans is mixed as opposed to being solely from iodized salt and that whatever the principal sources of iodine to humans is, they include ^{129}I as well. It is clear from this data that additional dietary sources of

iodine may be important contributors of both ^{129}I and ^{127}I and their importance as iodine sources should be the subject of further study.

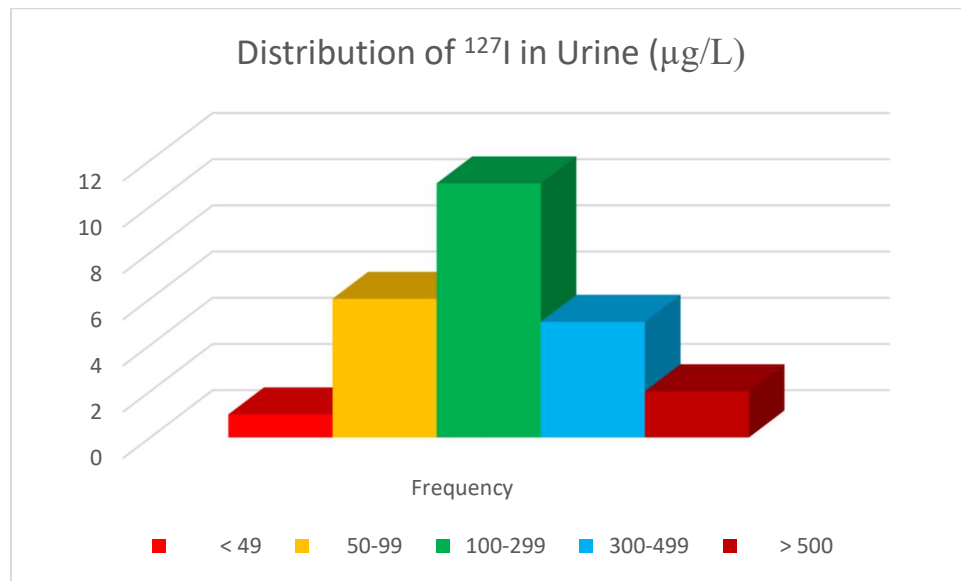


Figure 3: Frequency distribution of ^{127}I concentration data based on 5 categories. Less than 49 is mild deficiency, 50-99 is moderate deficiency, 100-299 is optimal concentration, 300-499 is risk of hyperthyroidism concentration, and > 500 is high concentration.

^{129}I vs ^{127}I in Urine

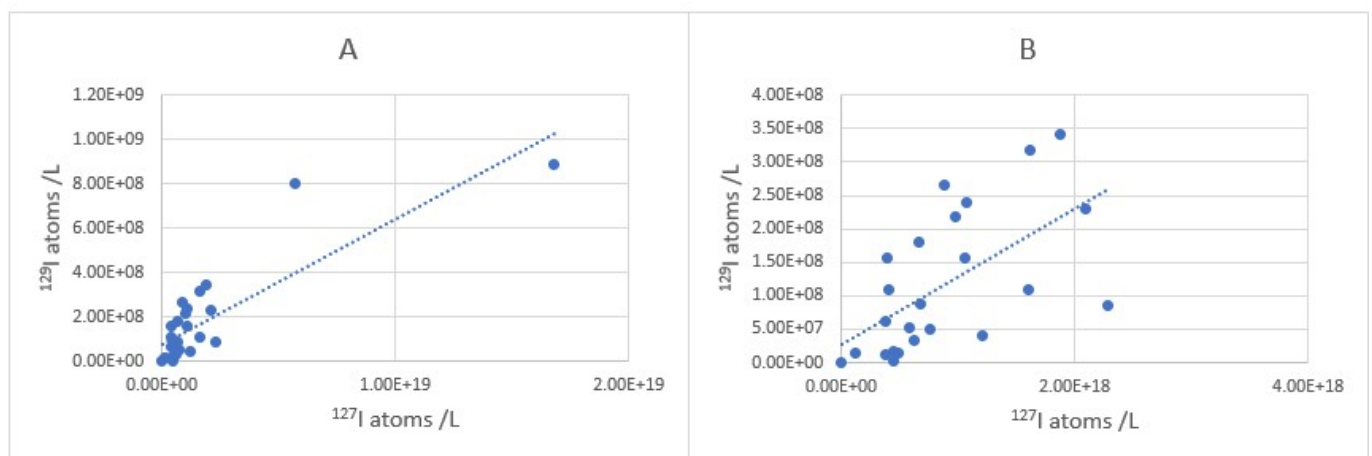


Figure 4: Linear regressions showing correlation of ^{129}I concentration expressed as atoms/L with ^{127}I atoms/L
(A) $R^2 = 0.701$, $p < 0.01$, $n = 25$, (B) $R^2 = 0.32$, $p < 0.01$, $n = 23$.

The main sources of ^{129}I in the environment are from human nuclear activities specifically, nuclear fuel reprocessing plants in Europe and to a lesser degree, Asia. The release of ^{129}I from all sources, dominated by fuel reprocessing, is estimated at 5.21×10^{11} Bq/year, giving an estimated dose equal to 3.36×10^{-6} mSv/year (Sunny et al. 2014). Although there is no radiation risk for human health at this current environmental dose level, the accumulation of ^{129}I in the human body should be investigated as it may be used as a tracer. To do so, it is necessary to establish a biological background level of ^{129}I and the $^{129}\text{I}/^{127}\text{I}$ ratio in humans. The $^{129}\text{I}/^{127}\text{I}$ ratios in our data ranged from 7.38×10^{-12} to 3.97×10^{-10} with median of 1.29×10^{-10} . These values fall within the present background level in the (Zhang and Hou 2013) study. Our ratios are considerably lower than those measured by Hou et al., 2003, where the median ratio in human urine was 1.87×10^{-8} . However, only 3 samples of urine were measured in (Hou et al. 2003) and cannot be used to establish a robust background level. Nevertheless, as the three urine samples were obtained from three males in Zealand, Denmark it is highly probable that the source of these urine samples is responsible for the higher values of $^{129}\text{I}/^{127}\text{I}$. In Denmark, the ^{129}I concentration is heavily influenced by nearby contributors of ^{129}I , such as discharges from the nuclear fuel reprocessing plants at Sellafield and La Hague with a small contribution in the past from Chernobyl. Also, the concentration of ^{129}I in this area is influenced by a secondary emission of ^{129}I from surface seawater in the North Sea (Kadowaki et al. 2018). In comparison, the source of human urine samples in this study was from Ottawa, Canada, where is the emission of ^{129}I is much lower. The primary source of ^{129}I in North America today is wet deposition of ^{129}I from long-range atmospheric transport of ^{129}I from European fuel reprocessing, recycled ^{129}I from

weapons testing and ocean volatilization (Wetherbee et al. 2012). It is well known that the atmospheric deposition of ^{129}I in North America is much lower than western Europe, and the ^{129}I concentration in North American rivers and rain reflects this (Santschi and Schwehr 2004; Sheppard and Herod 2012). ^{129}I has been investigated in several sites in Ontario and around Ottawa. For instance, the ^{129}I concentration was measured in Sturgeon Falls groundwater, snow, soil, and grass. The ^{129}I concentration ranges from 1.5×10^5 atoms/L to 1.9×10^6 atoms/L in groundwater, 8.5×10^7 atoms/L in snow, 4.3×10^8 atoms/g in the soil, and 7.5×10^7 atoms/g in grass (Renaud et al. 2005). These ^{129}I concentrations are very similar to the data presented in this paper. This convergence in concentrations between the Ontario environment and urine of people who live in Ontario supports the earlier assumption that the higher values of $^{129}\text{I}/^{127}\text{I}$ of urine in (Hou et al. 2003) compared to this study is related to the location and suggests that there is a strong influence on place of residence to the concentration of ^{129}I and the $^{129}\text{I}/^{127}\text{I}$ ratio in people.

4. Conclusions

Iodine-129 was successfully extracted from human urine by using hydrogen peroxide as a digestive solvent and treated by autoclave. Autoclave was chosen because it is available in most labs and provides the same outcome of thermal breakdown as microwave (Taylor et al. 1996) with low cost. Inorganic ^{125}I was successfully used as a quantitative recovery tracer. Our extraction technique determined the optimal conditions needed to extract ^{129}I from urine efficiently. It was found that a smaller volume of acid (0.2 ml HCl) after autoclave digestion is the optimal volume for iodine recovery to avoid loss by volatilization. After setting the optimal conditions for extracting ^{129}I from urine, 25 urine samples were analyzed, and the results were compared to three urine samples from Denmark (Hou et al. 2003). It was found that urine from people in the Ottawa area had a mean $^{129}\text{I}/^{127}\text{I}$ ratio of 1.36×10^{-10} whereas the Danish samples

had a mean ratio of 1.87×10^{-8} . This difference is due to the much closer proximity of Denmark to sources of ^{129}I emission, such as nuclear fuel reprocessing facilities and shows the important influence of place of residence on ^{129}I concentrations and the $^{129}\text{I}/^{127}\text{I}$ ratio in people. The concentration of ^{127}I and ^{129}I in Ottawa urine samples were significantly correlated. This is evidence that both iodine isotopes share a common source of intake into the human body. The ability to measure ^{129}I in human urine expands the application of ^{129}I in the biomedical field and its use as a tracer. This new and reliable method will enable the investigation of the sources of iodine in the human diet and their relative importance for iodine sufficiency. Also, this procedure can help to evaluate ^{129}I exposure directly in the people whom live near or downstream from a contaminated area such as nuclear reprocessing plants.

Conflict of Interests

The authors declare that they have no conflict of interest.

Human Rights:

All procedures followed were in accordance with the ethical standards of the Office of Research Ethics and Integrity committee in University of Ottawa, Canada. Informed consent was obtained from all participants for being included in the study.

Animal Rights:

This article does not contain any studies with animal subjects performed by the any of the authors.

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