Determination of Iodine, Bromine, Selenium and Arsenic by ICP-DRC-MS using Urine Dried on Filter Paper

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Introduction

Iodine and selenium are trace elements essential for thyroid hormone synthesis. Iodine is incorporated into thyroid hormones, while selenium is a key component at the catalytic site of several selenoproteins involved in protecting the thyroid gland from free radicals produced during thyroid hormone synthesis, and in peripheral tissue conversion of thyroxine to triiodothyronine.[1-2] Bromine and arsenic are antagonists of thyroid hormone synthesis: the former competes with iodine in the thyroid, while the latter forms a tight complex with selenium, preventing its incorporation into selenoproteins.[3-6] To better understand the interplay of bromine on iodine and arsenic on selenium, we developed a new method to measure these four elements in urine dried on filter paper. We also used the method to determine whether convenient morning and night urine collections could replace four collections taken throughout the day.

Materials and Methods

Multi-Element and Creatinine Standards
Iodine, bromine, selenium and arsenic liquid standards [Perkin Elmer] were combined and diluted with 0.1% ammonium hydroxide to obtain a liquid, multi-element standard solution containing 10000 µg/L iodine, 100000 µg/L bromine, 1000 µg/L selenium, and 1000 µg/L arsenic. A liquid creatinine standard solution was prepared from anhydrous creatinine [Sigma] diluted with 0.1% ammonium hydroxide to 4 mg/mL.

External Controls
External trace element controls were obtained from NIST, SeroNorm, and ClinChek. Creatinine controls were obtained from BioRad and Audit.

Sample Preparation
Twenty five volunteers collected four spot urine samples on Ahlstrom grade 226 filter paper (morning, lunch, afternoon and night) during a 24-hour period. Liquid multi-element and creatinine standards, external controls and 0.1% ammonium hydroxide blanks were dried on filter paper. Six 6.0 mm diameter disks of dried standards, controls, blanks and patient urine samples were punched (Wallac DBS Puncher; PerkinElmer) into a 96-well filter block (1 mL wells with 20 µm frit; Nunc) and extracted with 1000 µL of 0.1% ammonium hydroxide containing germanium as an internal standard. The fritted block was then centrifuged at 3000 rpm into a deep 96-well plate (2.2 mL wells; VWR) to obtain the extract used for analysis.

Materials and Methods Cont.

Multi-Element Assay
Extracted multi-element standard was serially diluted with extracted 0.1% ammonium hydroxide blank to create the standard curve. Standards, external controls, blanks and patient urine samples were added to the ICP-MS auto-sampler. Multi-element analysis was performed on a Perkin Elmer NexION 300D inductively coupled plasma mass spectrometer (ICP-MS) with Dynamic Reaction Cell (DRC) technology. Instrument conditions are seen in tables 1 and 2.

Creatinine Assay
Creatinine analysis was completed using a modified version of Jaffe's reaction in a 96-well microtiter plate.[7] Extracted creatinine standard was serially diluted with extracted 0.1% ammonium hydroxide blank to create the standard curve. Standards, external controls, blanks and patient urine samples were added to a 96-well microtiter plate. Next, alkaline picrate (2.5 M sodium hydroxide mixed with 1 wt.% solution picric acid [Sodium hydroxide and 1 wt.% solution picric acid; Sigma–Aldrich] and deionized water) was added to all wells. The plate was mixed by gentle shaking at room temperature for 30 min before reading absorbance at 490 nm.

Table 1. Element Mass Examined

Element	Mass Examined (amu)
Iodine	127
Bromine	79
Selenium	78
Arsenic	75
Germanium	72

Table 2. ICP-DRC-MS Operating Conditions

Component/ Parameter	Type/Value/Mode
Nebulizer	ESI MicroFlow PolyPro ST
Spray Chamber	Perkin Elmer Glass Cyclonic
Peristaltic Pump	ESI MP-2
AutoSampler	ESI SC-2
Cones	Nickel
Plasma Gas Flow	18 L/min
Auxiliary Gas Flow	1.2 L/min
Nebulizer Gas Flow	1.04 L/min
RF Power	1600 W
KED Cell Gas	Helium (3.5 mL/min)
Sample Flow Rate	37.6 µL/min
Replicates per sample	5
Sample Flush	60 sec
Read Delay	15 sec
Wash	45 sec
Dwell Time	50 ms

Results

External controls (Table 3), intra- and inter-assay percent variations (Table 4), spike recoveries (Table 5), detection limits and limits of linearity (Table 6) were used to check the validity of the iodine, bromine, selenium and arsenic assay and creatinine assay. Inter-assay variations were tested twelve times over a period of one month at room temperature (21C), refrigerated (6C), and frozen (-20C) to determine if dried urine samples are stable at these conditions for at least a month. Iodine, bromine, selenium and arsenic results in µg/L were divided by their respective creatinine results in mg/mL to correct for patient hydration status. After creatinine correction, we compared median values (Table 7) and the average of all 4 spot urine collections to averaged morning and night spot collections (Fig 1A-D).

Table 3. External Controls

	Iodine (µg/L)			Bromine (µg/L)		
External Control	Experimental	Expected	Ctrl Range	Experimental	Expected	Ctrl Range
NIST 2670a Toxic Elements Level 1	92.6	88.2	87.1-89.3			
SeroNorm Trace Elements Level 1	81.3	84.0	72.0-96.0	2238.3	2200.0	NA
ClinChek Trace Elements Level 1	122.6	115.0	92.0-138.0			
NIST 2670a Toxic Elements Level 2	88.9	88.2	87.1-89.3			
SeroNorm Trace Elements Level 2	271.2	304.0	260.0-348.0	2161.4	2200.0	NA
ClinChek Trace Elements Level 2	508.9	501.0	401.0-601.0			
	Selenium (µg/L)			Arsenic (µg/L)		
External Control	Experimental	Expected	Ctrl Range	Experimental	Expected	Ctrl Range
NIST 2670a Toxic Elements Level 1	8.0	8.0	5.0-11.0	3.0	3.0	NA
SeroNorm Trace Elements Level 1	16.5	13.9	8.3-19.5	87.8	79.0	47.0-111.0
ClinChek Trace Elements Level 1	26.6	29.3	23.4-35.2	40.4	44.6	35.7-53.5
NIST 2670a Toxic Elements Level 2	208.1	229.5	221.2-237.8	219.8	220.0	210.0-230.0
SeroNorm Trace Elements Level 2	74.2	70.1	41.9-98.3	192.3	184.0	110.0-258.0
ClinChek Trace Elements Level 2	75.0	79.0	63.2-94.8	78.4	84.5	67.6-101.0

	Creatinine (mg/mL)		
External Control	Experimental	Expected	Ctrl Range
BioRad 376 Lyphochek Quant Urine Control Level 1	0.772	0.786	0.629-0.943
BioRad 377 Lyphochek Quantitative Urine Control Level 2	2.379	2.300	1.84-2.76
BioRad 397 Liquichek Urine Chemistry Control Level 1	0.613	0.632	0.506-0.758
BioRad 398 Liquichek Urine Chemistry Control Level 2	1.320	1.420	1.13-1.7
Audit Urine Chemistry Control Level 1	0.714	0.678	0.542-0.814
Audit Urine Chemistry Control Level 2	1.692	1.640	1.31-1.97

Table 4. Intra-assay and Inter-assay variations

	No. of Runs	No. of Samples	Iodine Range (%CV)	Bromine Range (%CV)	Selenium Range (%CV)	Arsenic Range (%CV)	Creatinine Range (%CV)
Intra-assay	20	6	1.9-3.4	2.2-3.9	2.9-4.8	2.5-5.3	2.1-4.4
Inter-assay Room Temp	12	6	3.8-10.1	2.7-7.4	3.4-8.3	3.3-14.2	2.7-6.7
Inter-assay Refrigerated	12	6	3.9-11.7	2.7-5.0	3.9-6.3	3.7-16.3	3.1-4.3
Inter-assay Frozen	12	6	4.0-10.3	3.4-5.1	3.5-5.6	3.0-12.4	2.4-4.5

Table 5. Spike Recovery

Recovery	No. of Spiked Samples	Iodine Recovery Range (%)	Bromine Recovery Range (%)	Selenium Recovery Range (%)	Arsenic Recovery Range (%)	Creatinine Recovery Range (%)
Low Spike	6	95.9-131.3	90.7-103.8	80.6-106.0	92.4-112.3	73.2-102.7
Medium Spike	6	96.1-100.9	94.0-112.3	84.7-112.9	91.2-109.8	87.0-101.9
High Spike	6	93.9-98.8	97.9-111.4	82.0-108.9	94.1-108.1	85.0-99.7

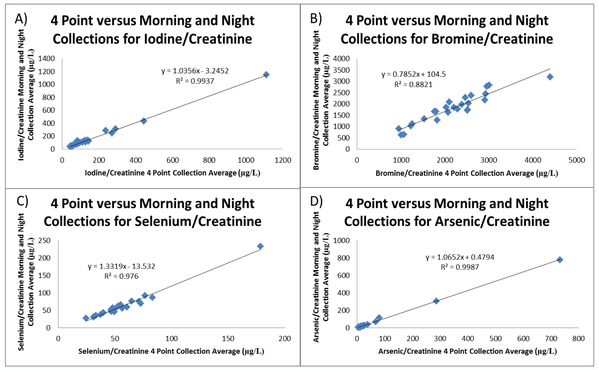
Table 6. Limit of Detection and Linearity

	Iodine (µg/L)	Bromine (µg/L)	Selenium (µg/L)	Arsenic (µg/L)	Creatinine (mg/mL)
Limit of Blank	1.4	10.4	0.9	0.7	0.010
Limit of Detection	2.3	29.1	2.7	1.7	0.016
Limit of Linearity	2.1	21.0	3.0	0.2	0.060

Table 7. Median Values for 4-Point versus Morning and Night Collections

Median (µg/g)	No. of Patients	I/ Creatinine	Br/ Creatinine	Se/Creatinine	As/Creatinine
4 Point Collection	25	110.8	2206.4	49.4	12.6
Morning + Night Collection	25	111.6	1788.7	54.6	13.2

Figure 1A-D. 4 Point versus Morning and Night Collections



Summary of Conclusions

Validation of the iodine, bromine, selenium and arsenic assay by ICP-DRC-MS and a creatinine assay by a modified version of Jaffe's reaction, using urine dried on filter paper, was successful. All analytes are stable on filter paper for at least a month at room temperature, refrigerated and frozen, making urine dried on filter paper ideal for a wide range of shipment and storage conditions. Although the study population was small, it was demonstrated that averaged morning and night collections are relatively equivalent to four averaged collections throughout the day, once creatinine is used to correct for patient hydration status. Further studies will be needed to determine if morning and night collections can replace 24-hour collections, which are often troublesome and inconvenient for the patient. The methods described are ideal for large population studies and clinical testing investigating the role of iodine, selenium and their antagonists, bromine and arsenic, in thyroid hormone synthesis and function.

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