Dried Urine Analysis of Gadolinium, Thallium, and Uranium by ICP-MS with an Emphasis on Inter-Assay Stability of Samples Kept at Room Temperature

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ABSTRACT

Background:
Gadolinium, thallium, and uranium are non-essential heavy metals that are normally found at part-per-trillion levels in urine. Exposure can come from natural and anthropogenic sources, including industrial pollution, well water, and soil. Gadolinium based contrast agents (GBCAs) used during MRIs are the primary source of gadolinium exposure. Rare and toxic elemental analysis plays an important role in monitoring the heath of individuals and populations around the world. Inductively coupled plasma mass spectrometry (ICP-MS) allows for the precise quantification of multiple elements simultaneously while maintaining sensitivity and specificity. Dried urine on filter paper is simple to collect, can be shipped without preservatives at room temperature, uses minimal storage space, is adaptable for automated assays, and is therefore ideal for at-home and remote collections and large-scale health surveys. We aimed to validate an assay for, and show the stability of, gadolinium, thallium and uranium from urine collected on filter paper.

Materials and Methods:
The dried urine gadolinium, thallium and uranium assay was developed using Whatman 903 filter paper for sample collection and a Perkin Elmer NexION 300D ICP-MS for analysis. Four 6-mm punches of dried urine were extracted in 96-well fritted filter blocks using a 650 µL mixture of dilute nitric acid, triton-X, and indium as an internal standard. Our prior work on essential and toxic elements in dried urine and blood spot made use of the dynamic reaction cell in the NexION 300D, but the sensitivity required for the dried urine gadolinium, thallium, and uranium assay requires analysis in standard mode for low part-per-trillion analysis. Micro-flow pumps along with a low volume nebulizer helped make effective use of the small sample volume.

Results:
Accuracy of the method was assessed by testing trace element urine controls from BioRad, ClinChek, and Seronorm for thallium once dried on filter paper. Since external urine controls for gadolinium and uranium are not available, a spiked liquid urine sample was sent to a clinical reference laboratory and simultaneously run as a dried urine sample for comparison. Recovery was demonstrated using urine samples with a known concentration of analyte; acceptable recoveries of 91-117% (mean 105%) were obtained. Linearity was assessed by diluting samples and comparing results to expected concentrations and was excellent across the assay range. Limits of quantification were based on analysis of blank and low-level samples and were in the low to mid part-per-trillion range. Intra-assay precision was based on 20 sample replicates, and the coeffient of variation was <2.2% for all analytes (mean 1.1%). Inter-assay precision was tested during 12 sample runs over 40 days keeping samples at room temperature to replicate conditions of collection and transport in areas without refrigeration. The coeffient of variation for inter-assay precision was <14.6% for all analytes (mean 7.8%).

Conclusions:
Dried urine analysis of gadolinium, thallium and uranium using ICP-MS was successfully validated. Demonstrated stability of elements in samples dried on filter paper allows for at-home and remote collections without access to refrigeration, as samples can easily be collected, transported, stored, and analyzed without the use of preservatives for over a month.

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Introduction

Gadolinium, thallium, and uranium are heavy metals with no essential role in the human body. Exposure to these elements can be from environmental, occupational or medical sources. Gadolinium is a rare earth element that is primarily used in gadolinium-based contrast agents (GBCAs) for MRIs. Thallium is a common byproduct of coal and cement operations but is also used as a poison due to its lack of color, taste and smell. Uranium is a weakly radioactive element used in nuclear weapons and reactors. Although rare, toxicity caused by these elements comes from their chemical similarities to essential elements like calcium, potassium, and sodium.

We developed a method using inductively coupled plasma mass spectrometry (ICP-MS) to analyze gadolinium, thallium, and uranium using urine dried on filter paper. Creatinine stability information was included, as it is eventually used to correct for hydration status. Dried urine is an ideal sample type for identifying both recent and past exposure. Benefits include:

- **Long term sample stability without preservatives**
- **Collection ease and convenience**
- **Rapid processing and potential for automation**
- **Small storage footprint**
- **Reduced shipping costs.**

Figure 1. Dried Urine Samples

Figure 2. NexION 300D ICP-MS

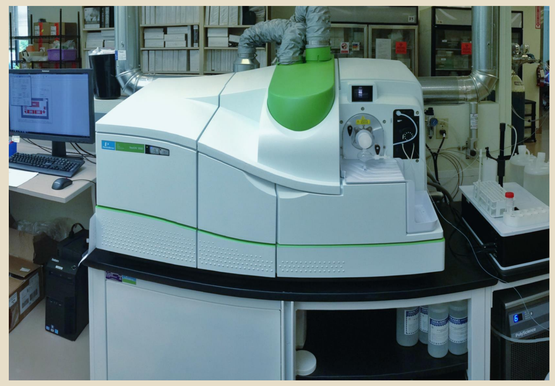


Table 1. ICP-MS Operating Conditions

Component/ Parameter	Type/Value/Mode
Nebulizer	Meinhard Type C
Spray Chamber	Glass Cyclonic PC3
Peristaltic Pump	ESI MP-2
Cones	Nickel
Nebulizer Gas Flow	1.04 L/min
RF Power	1600 W
Cell Mode	Standard
Sample Flow Rate	37.6 µL/min
Rep per sample	5
Sample Flush	60 sec
Wash	60 sec

Methods/Procedures/Results

Dried Urine Collection

Urine was collected from volunteers on Whatman 903 filter paper by dipping the filter paper in a clean urine collection cup or urinating directly on the filter paper (Figure 1). It takes around 2 mL of urine to saturate a filter paper strip. Purchased element reference standards were used to prepare urine calibrators and standards for the assay. Samples, calibrators and standards were left to dry for 4 h and stored at room temperature until analysis.

Extraction and Element Analysis

Care must be taken to prevent contamination of the filter card and supplies/equipment used for sample processing must be free of all analytes of interest. Dried urine strips were punched using a 6-mm hole punch (4 punches) into a fritted filter block which was placed on top of a deep 96-well block. For dried urine samples, 0.65 mL of extraction solution (dilute nitric acid, triton x-100, indium internal standard) was added, then the block was shaken and centrifuged to produce the extract to be analyzed. A NexION 300D ICP-MS (Figure 2) operating under the conditions seen in Table 1 was used to analyze the samples. Element masses of Gd155, Gd157, Tl205, U238, In115 were selected for analysis based on abundance and potential interferences. Run time for each sample was around 3 min.

Creatinine Assay

Creatinine analysis used a modified version of Jaffe's reaction[1] in a 96-well microtiter plate utilizing the same extract from the element analysis.

Assay Validation and Sample Stability

• Intra-assay precision was based on 20 sample replicates, and the CV was <2.1% for all analytes.

• Inter-assay precision for both low and high samples for each analyte was tested during 12 sample runs over 40 days, keeping samples at room temperature to replicate conditions of collection and transport in areas without refrigeration. The CV for inter-assay precision was <14.6% for all analytes and can be seen in Table 2. Figure 3 shows the minimal variation in analyte concentrations over 40 days at room temperature.

• Recovery was demonstrated by spiking urine samples with a known concentration of analyte; acceptable recoveries of 91-117% (mean 105%) were obtained.

• Linearity was assessed by diluting samples and comparing results to expected concentrations and found to be acceptable. Limits of quantification (LOQ) were based on analysis of blank and low-level samples and were found to be sufficient for analysis (Table 2).

• Accuracy for thallium was completed using trace element controls from BioRad, ClinChek, and Seronorm that were dried on filter paper. Since external urine controls for gadolinium and uranium are not available, spiked liquid urine samples were sent to a clinical reference laboratory and simultaneously run as dried urine samples for comparison. All results were acceptable.

Table 2. Intra-Assay and Inter-Assay Precision and Limit of Quantification

	Intra-Assay n=20		*Inter-Assay (40 Days) n=12*		
	CV [Avg. Low Conc.]	**CV** [Avg. High Conc.]	**CV** [Avg. Low Conc.]	**CV** [Avg. High Conc.]	***LOQ***
Gadolinium (µg/L)	**2.1%** [0.372]	**0.9%** [25.269]	**5.4%** [0.334]	**7.9%** [23.853]	**0.032**
Thallium (µg/L)	**1.3%** [0.174]	**0.6%** [8.992]	**3.1%** [0.176]	**5.9%** [8.350]	**0.006**
Uranium (µg/L)	**1.8%** [0.162]	**0.9%** [2.268]	**14.6%** [0.147]	**11.3%** [1.963]	**0.019**
Creatinine* (mg/mL)	**2.3%** [0.393]	**1.7%** [2.927]	**7.2%** [0.309]	**6.9%** [2.650]	**0.050**

Discussion/Conclusion

• Validation of the dried urine gadolinium, thallium, and uranium method was successful, and samples were found to be stable for at least 40 days at room temperature.

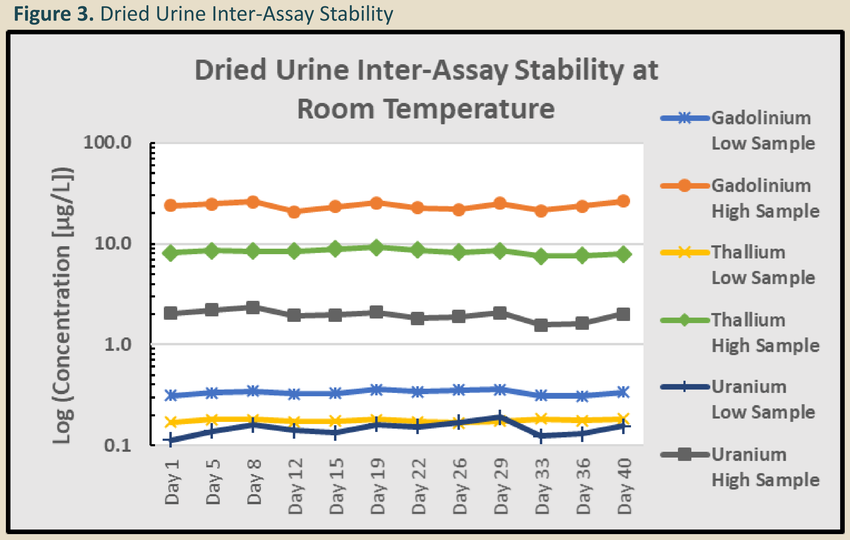
• ICP-MS allows for analysis of elements from urine dried on filter paper into the low parts-per-trillion.

• Dried urine is ideal for individual patient analysis and large population studies in both urban and remote areas due to simple collection and sample stability during shipping without the need for preservatives or refrigeration.

• Low extraction volume produces minimal waste.

• Dried samples (urine, blood, serum, sweat, etc.) are changing the way samples are collected, transported, processed, and stored, paving the way for a new era in laboratory analysis.

Figure 3. Dried Urine Inter-Assay Stability



References: 1) Jaffé M. Ueber den Niederschlag welchen Pikrinsaure in normalen Harn erzeugt und uber eine neue Reaction des Kreatinins. Z Physiol Chem 1886;10:391-400.