

ABSTRACT

Background:

Elemental analysis plays a key role in monitoring the health of individuals and populations around the world. The sensitivity and specificity of inductively coupled plasma mass spectrometry (ICP-MS) has allowed precise quantification of a broad range of elements using various sample types. The collection, storage, and transport of samples without preservatives at room temperature while maintaining accuracy would be advantageous for health surveys, specifically in remote areas without refrigeration. We aimed to validate an assay for, and show the stability of, essential and toxic elements in urine and whole blood collected on filter paper.

Materials and Methods:

Our laboratory developed dried urine and blood spot elemental assays using Whatman 903 filter paper for sample collection and a Perkin Elmer NexION 300D ICP-MS with Dynamic Reaction Cell Technology for analysis. We focused on elements that have shown clinical utility in population health surveys and wellness assessments. Analytes selected were iodine, bromine, selenium, arsenic, cadmium, and mercury in dried urine, and zinc, copper, magnesium, selenium, cadmium, lead, and mercury in blood spot.

Dried urine and blood spot samples were extracted in 96-well fritted filter blocks using dilute ammonium hydroxide, L-cysteine, ethylenediaminetetraacetic acid (EDTA), triton-X, and internal standards. Six 6-mm punches and 1.2 mL of extraction solution of were used for dried urine analysis while two 6-mm punches and 0.55 mL of extraction solution were used for blood spot analysis. Dried urine and blood spot assays were run separately on the ICP-MS in kinetic gas mode using helium, and employing a micro-flow pump and nebulizer to make effective use of the small sample volume.

Results:

Accuracy of the method was assessed by spotting onto filter paper available proficiency samples from the Centers for Disease Control and Prevention (CDC) and College of American Pathologists (CAP), which demonstrated excellent agreement with expected concentrations ($R^2 > 0.96$). Elements for which CDC or CAP samples were not available were validated against Seronorm and ClinChek Trace Elements Urine and Whole Blood controls. Recovery was demonstrated by spiking blood and urine samples with a known concentration of analyte; acceptable recoveries of 80-120% were obtained. Linearity was assessed by diluting samples and comparing results to expected concentrations, and found to be acceptable. Limits of quantification were based on analysis of blank and low level samples, and were found to be acceptable. Intra-assay precision was based on 20 sample replicates, and the coefficient of variation was $< 8.3\%$ for all analytes. Inter-assay precision was tested during 12 sample runs over 1 month keeping samples at room temperature to replicate conditions of collection and transport in areas without refrigeration. The coefficient of variation for inter-assay precision was $< 15.1\%$ for all analytes.

Conclusions:

Dried urine and blood spot element analysis using ICP-MS was successfully validated. Demonstrated stability of elements in samples dried on filter paper allows accurate elemental analysis in population studies in remote areas without access to refrigeration, as samples can easily be collected, transported, and stored without the use of preservatives for at least a month.

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Introduction

Elemental analysis is an important tool for tracking occupational, environmental, and dietary exposure of toxic and essential elements in individuals and populations around the world. We developed two methods using inductively coupled dynamic reaction cell plasma mass spectrometry (ICP-DRC-MS) to analyze iodine, bromine, selenium, arsenic, cadmium and mercury using urine dried on filter paper, and zinc, copper, magnesium, selenium, cadmium, lead, and mercury using dried blood spots. A separate method for dried urine creatinine was used to correct for hydration status. Both urine and whole blood are ideal sample types for identifying both recent and past exposure to elements. Benefits include:

- Long term sample stability without preservatives
- Ease and convenience of collection
- Rapid processing and potential for automation
- Small storage footprint
- Reduced shipping costs.

Figure 1. Dried Urine Samples



Figure 2. Dried Blood Spot Samples



Methods/Procedures/Results

Dried Urine and Blood Spot Collection

Human urine and whole blood were collected on Whatman 903 filter paper by dipping the filter paper in a clean urine cup and by lancet finger prick, respectively (Figures 1 and 2). Urine and whole blood external calibrators from Sero, RECIPE, and Bio-Rad, with established values for all elements tested, and multi-element standards obtained from Inorganic Ventures were dried in the same manner. The samples were left to dry for 4 h and stored at room temperature.

Extraction and Element Analysis

Dried urine (6 punches) and dried blood spots (2 punches) were punched using a 6-mm hole punch into a fritted filter block which was placed on a deep 96-well block. For dried urine samples 1.2 mL of extraction solution (dilute ammonium hydroxide, L-cysteine, EDTA, triton x-100) and for dried blood spots 0.55 mL of extraction solution were added plus internal standards (germanium, indium, and iridium), then the block was shaken and centrifuged to produce the extract to be analyzed. A NexION 300D (figure 3) operating under the conditions seen in table 1 was used to analyze the samples. Element masses were selected based on abundance and potential interferences. Run time for each sample was around 3 min. Dried urine and dried blood spots were run separately. Creatinine analysis used a modified version of Jaffe's reaction in a 96-well microtiter plate using the same extract from the element analysis.¹

Assay Validation and Sample Stability

Inter-assay and intra-assay precision, linearity, spike recovery, limit of quantification, and accuracy were tested, with an emphasis on the stability of room temperature inter-assay samples.

- Intra-assay precision was based on 20 sample replicates, and the CV was $< 8.3\%$ for all analytes.
- Inter-assay precision for both low and high samples for each analyte was tested during 12 sample runs over 1 month, keeping samples at room temperature to replicate conditions of collection and transport in areas without refrigeration. The CV for inter-assay precision was $< 15.1\%$ for all analytes and can be seen in table 2.
- Results for a single dried urine and dried blood spot sample kept at room temperature and analyzed for 1 month can be seen in figures 4 and 5.
- Recovery was demonstrated by spiking blood and urine samples with a known concentration of analyte; acceptable recoveries of 80-120% 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 human analysis as seen in table 2.
- Proficiency samples from the Centers for Disease Control and Prevention (CDC) and College of American Pathologists (CAP) were spotted on to filter paper and analyzed to confirm accuracy, which demonstrated excellent agreement with expected concentrations ($R^2 > 0.96$). Elements for which CDC or CAP samples were not available were validated against Seronorm and ClinChek Trace Elements Urine and Whole Blood controls.

Table 2. Inter-Assay Precision and Limit of Quantification

		Inter-Assay (One Month) n=12		LOQ Conc.
		CV [Avg. Low Conc.]	CV [Avg. High Conc.]	
Dried Urine	Iodine ($\mu\text{g/L}$)	8.4% [41.0]	5.1% [514.6]	1.6
	Bromine ($\mu\text{g/L}$)	5.2% [1468]	5.9% [14772]	31.3
	Selenium ($\mu\text{g/L}$)	5.2% [51.1]	3.7% [667.4]	2.3
	Arsenic ($\mu\text{g/L}$)	7.4% [5.2]	3.3% [567.4]	0.3
	Cadmium ($\mu\text{g/L}$)	9.6% [0.32]	4.7% [9.94]	0.08
	Mercury ($\mu\text{g/L}$)	8.7% [0.60]	6.0% [11.09]	0.05
	*Creatinine (mg/mL)	4.9% [0.38]	5.9% [1.8]	0.01
Blood Spot	Zinc (mg/L)	6.5% [3.21]	4.7% [8.17]	0.21
	Copper (mg/L)	6.9% [0.42]	4.2% [1.75]	0.03
	Magnesium (mg/L)	12.4% [21.7]	6.6% [66.3]	3.3
	Selenium ($\mu\text{g/L}$)	8.9% [38.2]	8.7% [437.0]	9.4
	Cadmium ($\mu\text{g/L}$)	9.7% [2.37]	8.8% [8.36]	0.22
	Lead ($\mu\text{g/dL}$)	5.1% [1.87]	5.7% [40.10]	0.23
	Mercury ($\mu\text{g/L}$)	11.6% [0.64]	15.1% [13.1]	0.11

Figure 3. NexION 300D ICP-DRC-MS



Table 1. ICP-DRC-MS Operating Conditions

Component/Parameter	Type/Value/Mode
Nebulizer	ESI PolyPro ST
Spray Chamber	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	He (3.5 mL/min)
Sample Flow Rate	37.6 $\mu\text{L}/\text{min}$
Rep per sample	5
Sample Flush	60 sec
Read Delay	15 sec
Wash	60 sec

Figure 4. Dried Urine Inter-Assay Stability

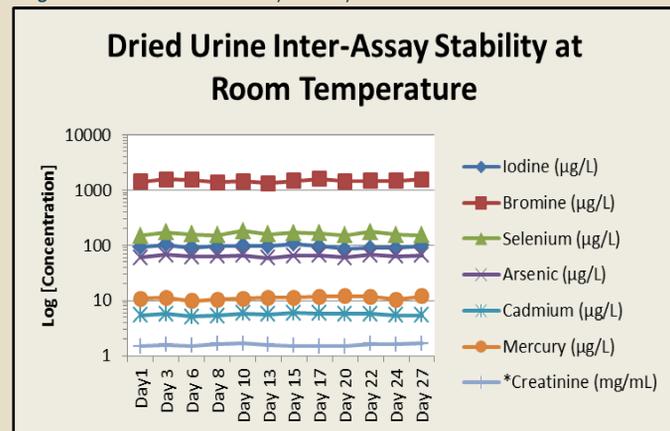
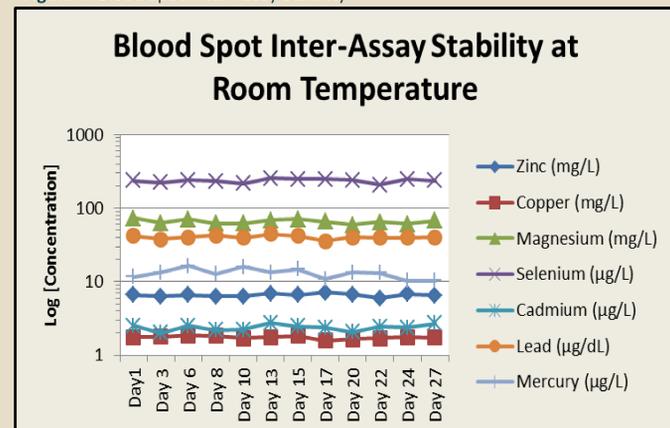


Figure 5. Blood Spot Inter-Assay Stability



Discussion/Conclusion

- Validation of the dried urine and blood spot essential and toxic element methods was successful.
- Dried samples are stable for at least 1 month at room temperature.
- Dried samples are ideal for both individual analysis and large population studies in both urban and remote areas due to simple collection that is stable during shipping without the need for preservatives or refrigeration and reduced storage space.
- 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.
- Proper collection of dried blood spots is essential, as double spotting or small blood drops can affect results.
- Dried sample analysis has changed the way samples can be collected, transported, processed, and stored, paving the way for a new era in laboratory analysis.

Reference

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