Troubleshooting the Transformation of Arsenic Species in Urine by ICP-MS

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Problem

- While preparing a set of four calibrators for six arsenic species in blank human pooled urine, one of the arsenic species (arsenobetaine, AsB) had poor recoveries (0~30%) in calibrators 1-2 along with the presence of an unexpected arsenic peak
- Another arsenic species (arsenocholine, AsC) had lower than expected recoveries in calibrator 1-2 (~72%)
- Only calibrator 4 had acceptable recoveries (within 10% of target) for AsB and AsC
- The remaining arsenic species included dimethylarsonic acid (DMA), monomethylarsonic acid (MMA), AsV and AsIII and had an average recovery of 93 ± 3% to target across the four calibrators

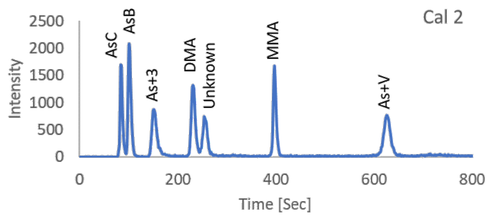


Figure 1. Chromatogram of calibration level 2 containing 15 µg/L each of AsC, As+3, DMA, MMA, and As+V. AsB concentration should be at 50µg/L but shows much lower recovery along with an unidentified peak eluting slightly after DMA.

	Target [µg/L]		%Recovery					
Cal	AsB	All other analytes	AsC	AsB	AsIII	DMA	MMA	AsV
1	5	5	72%	-4%	92%	96%	98%	87%
2	50	15	73%	29%	92%	94%	95%	94%
3	150	50	79%	87%	90%	91%	92%	93%
4	450	150	90%	93%	93%	92%	95%	97%

Table 1. Arsenic species in calibrators prepared with blank pooled human urine.

Method Information

- 50µL urine diluted with 950µL diluent + internal standard (Sb) solution
- Agilent 1260 Infinity II LC
- Agilent 7700 ICP-MS
- PRP-X100 Anion Exchange HPLC column (150x4.6mm), with KrudKatcher Classic HPLC in-Line filter
- MPA – 20mM Ammonium Carbonate + 3% Methanol, pH 8.7
- MPB – 50mM Ammonium Carbonate + 3% Methanol, pH 8.0
- 12min analytical step gradient, Flow 1mL/min
- Column oven 20°C
- 50µL injection volume

Certain **bacteria** found in pooled urine were capable of **degrading and transforming** organic **arsenic species**.

Troubleshooting Steps

- Spike AsB and AsC into 4 additional blank urine pool lots (Lot#4 was the same lot used in the original calibration preparation)
- Two of the four lots showed As degradation after left on benchtop for 12h
- Literature search revealed microorganisms are capable of AsB biotransformation*

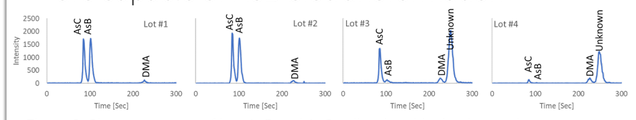
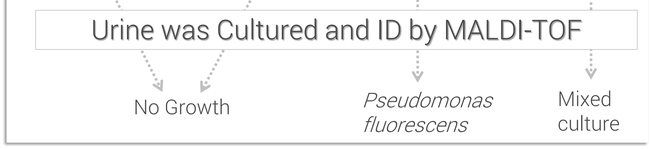


Figure 2. Chromatograms of low AsB and AsC spikes into blank urine pools



- Isolated and inoculated bacteria into sterile urine spiked with AsB and AsC
- Urine samples were left on benchtop and tested by ICP-MS at 12h (and 2weeks for mixed isolates)

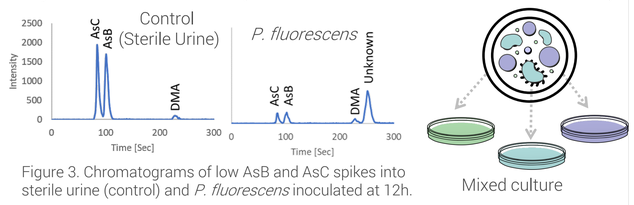


Figure 3. Chromatograms of low AsB and AsC spikes into sterile urine (control) and *P. fluorescens* inoculated at 12h.

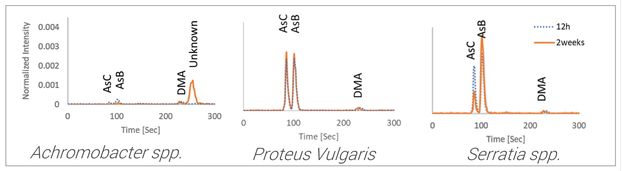


Figure 4. Chromatograms of low AsB and AsC spikes into sterile urine inoculated with the isolates from the mixed culture urine at 12h and 2week time points.

	%Recovery 12h (2weeks)			
	AsC [µg/L]	%Rec	AsB [µg/L]	%Rec
Target	5.0	--	5.0	--
P. fluorescens				
Control	5.3	106%	5.2	104%
P. Fluorescens	0.9	18%	1.0	20%
Mixed Culture Isolates				
Control	4.6 (4.6)	92 (92)%	5.2 (5.7)	104 (114)%
Serratia spp.	4.1 (1.7)	82 (34)%	5.8 (8.2)	116 (164)%
Proteus Vulgaris	4.4 (4.5)	88 (90)%	4.9 (5.5)	98 (110)%
Achromobacter spp.	0.2 (0.0)	4 (0)%	0.6 (0.0)	12 (0)%

Table 2. Arsenic species concentrations in inoculated human urine at various time points.

Outcome

- The calibrators were prepared in freshly prepared synthetic urine instead of human pooled urine to avoid preparing calibrators in urine contaminated with bacteria

* Harrington, C.F., Brima E.I., & Jenkins, R.O. (2008). Biotransformation of arsenobetaine by microorganisms from the human gastrointestinal tract. Chemical Speciation & Bioavailability, 20:3, 173-180.

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