# Total Arsenic and Arsenic Speciation Analysis in Rice

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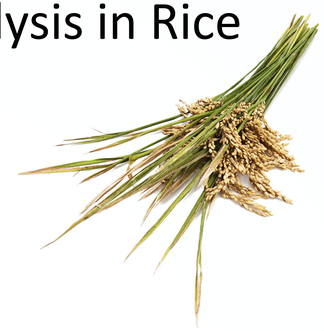

## Introduction & Background

Arsenic (As) is a naturally-occurring environmental contaminant and known carcinogen. The U.S. Environmental Protection Agency's (EPA) and the World Health Organization's (WHO) limit for arsenic concentrations in drinking water is 10 parts per billion (ppb)[1-2].

While the effects of exposure to arsenic from drinking water are well-known, the effects of exposure to arsenic-contaminated food are less clear.

The U.S. EPA Reference Dose (RfD) for oral exposure to "inorganic" arsenic is 300 ng As/kg bodyweight/day[3].

Many recent studies have shown that rice can contain relatively high levels of arsenic, possibly putting rice-consuming populations at risk[4-6].

In response to the growing concern about arsenic species levels in rice, Brooks Rand Labs developed an analytical method to measure arsenic in rice. Because only the inorganic species of arsenic, and not the organic species, are considered highly toxic, speciation analyses are important for determining the threat to human health from arsenic in rice.

In this work, we compare measurements produced by Hydride Generation Atomic Absorption (HGAA) spectroscopy to measurements produced by High Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS) for measuring arsenic species in rice.

We measured the concentrations of the two inorganic arsenic species, trivalent arsenic (As(III)) and pentavalent arsenic (As(V)), and two organic arsenic species: monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA).

The arsenic concentrations found in rice in this study were not sufficiently elevated to threaten the health of the average U.S. citizen based on their average daily consumption (see Table 1). However, in populations that consume more significant amounts of rice, rice consumption can contribute to well over half of a person's daily dietary exposure to arsenic, and possibly exceed safe levels[7].

It is also important to note that arsenic concentrations found in rice do not account for all arsenic exposure, because people are exposed to arsenic from more than one source, such as rice and drinking water.

| Average Bodyweight | Average Daily Rice Consumption | Rice As Concentrations Required to Exceed EPA RfD |
|---|---|---|
| Female - 75.4 kg | 33.7 g | 671.5 ppb |
| Male - 88.7 kg | | 789.9 ppb |

Table 1: The concentrations of inorganic arsenic in rice that would be required to exceed the EPA RfD for the average U.S. citizen.

## References

1. U.S. Environmental Protection Agency (http://water.epa.gov/lawsregs/rulesregs/sdwa/arsenic/regulations.cfm).
2. World Health Organization5 (http://www.who.int/water_sanitation_health/dwq/arsenicun5.pdf).
3. U.S. Environmental Protection Agency (http://www.epa.gov/ttnatw01/hlthef/arsenic.html).
4. Jackson, B.; Taylor, V.; Karagas, M.; Punshon, T.; Cottingham, K. Arsenic, Organic Foods, and Brown Rice Syrup. *Environmental Health Perspectives* **2012**, *120*, 623–626.
5. Stone, R. Arsenic and Paddy Rice: A Neglected Cancer Risk? *Science* **2008**, *321*, 184–185.
6. Meharg, A. A.; Williams, P. N.; Adomako, E.; Lawgali, Y. Y.; Deacon, C.; Villada, A.; Cambell, R. C. J.; Sun, G.; Zhu, Y.-G.; Feldmann, J.; Raab, A.; Zhao, F.-J.; Islam, R.; Hossain, S.; Yanai, J. Geographical Variation in Total and Inorganic Arsenic Content of Polished (White) Rice. *Environ. Sci. Technol.* **2009**, *43*, 1612–1617.
7. Meharg, A. A. Arsenic in rice – understanding a new disaster for South-East Asia. *Trends in Plant Science* **2004**, *9*, 415–417.
8. Huang, J.-H.; Fecher, P.; Ilgen, G.; Hu, K.-N.; Yang, J. Speciation of arsenite and arsenate in rice grain – Verification of nitric acid based extraction method and mass sample survey. *Food Chemistry* **2012**, *130*, 453–459.

## Methods

**HGAA (EPA Method 1632)**

Extract 0.5 g sample in 2M HCl overnight at 80 °C.

Collect separate aliquots for As(III) and As(V) determination.

**As(III)** Adjust pH to 1.5.

**As(V)** Adjust pH to 6.

Derivatize with $NaBH_4$.

Purge with He onto cryotrap.

Thermally desorb into $H_2$/air flame quartz furnace AA spectrometer.

**HPLC-ICP-MS**

Extract 0.25 g sample in 50% (v/v) $CH_3OH$ for 90 min at 95 °C.

Centrifuge to consolidate solids.

Add fresh 50% (v/v) $CH_3OH$ to plug, mix, repeat extraction step.

Add supernatant from first extraction back into mixture, dilute to final volume with 50% (v/v) $CH_3OH$.

Syringe filter at 0.45 µm (PES membrane).

Analyze by HPLC-ICP-MS.

| | |
|---|---|
| ICP-MS Instrument | PerkinElmer DRC-II |
| RF Power | 1500 W |
| Plasma Gas Flow Rate | 17 L/min |
| Auxiliary Gas Flow Rate | 1.2 L/min |
| Nebulizer Gas Flow Rate | 1.1 L/min |
| Dwell Time | 200 ms |
| Scan Mode | Peak Hopping |
| Sweeps/Reading | 5 |
| Readings/Replicate | 970 |
| Replicates | 1 |
| Autolens | On |
| HPLC | Dionex ICS-5000 |
| Column | Hamilton PRP-X100, 10 µm particle size<br>250 mm x 4.6 mm I.D. |
| Mobile Phase: | A: DI Water<br>B: 80% MeOH<br>C: 20 mM $NaH_2PO_4$ |
| Gradient Program | 0-1.5 min: 0.5 mL/min, 20% A, 75% B, 5% C<br>1.5-4 min: 0.5 mL/min, 95% A, 0% B, 5% C<br>4-6 min: 0.5-1.5 mL/min, 95-0% A, 0%B, 5-100% C<br>6-16.5 min: 1.5 mL/min, 0% A, 0% B, 100% C |
| Mobile Phase Flow Rate | 0.5-1.5 mL/min |
| Injection Loop Size | 1 mL |

Table 2: HPLC and ICP-MS Operating Parameters

## Method Performance

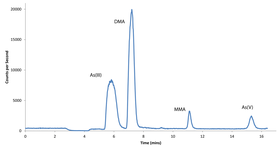


Figure 1: HPLC-ICP-MS Chromatogram

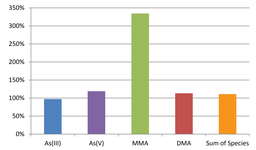


Figure 2: CRM Recoveries

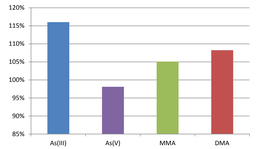


Figure 3: Matrix Spike Recoveries

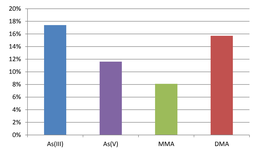


Figure 4: Duplicate Precision

Certified reference material (CRM) NIST 1568A – Rice Flour is only certified for total arsenic, not for individual arsenic species, so the "Sum of Species" value is compared to the certified value for total arsenic. Individual species concentrations are the averages of those given by Huang et al. for arsenic species in NIST 1568A [8].

Our certified reference material recoveries (Figure 2), relative to literature values were within acceptable limits, with the exception of MMA. However, there is a wide range of MMA values reported in the literature for certified reference material NIST 1568A. We calculated our recoveries relative to an average of the values given in Huang et al., which range from 2-15.7 ppb with no clear consensus value.

As a result, the average value we used to calculate our recovery may not be close to the true value. Our results for MMA in this material were highly precise (1.71% relative percent difference), and our sum of species is equal to 110.8% of the certified total arsenic value for this reference material, indicating that our values may be close to the true value.

Matrix spikes were introduced as an aqueous mixed standard to each extraction vial after the addition to 50% CH3OH extractant but prior to heated extraction. The final concentration of the spike in the diluted extractant matrix was nominally 1 ppb. We achieved acceptable matrix spike recoveries for each of the arsenic species (Figure 3).

We also achieved acceptable duplicate precision for each of the arsenic species (Figure 4). However, the lowest duplicate precision measured was in brown and black rice, where duplicate relative percent difference for As(III) and DMA was above 40%. This finding indicates that this method performs less well in samples with high fiber content.

We estimated the detection limits of this method using 1/3 of the peak area of the lowest calibration standard (Table 3).

| Species | Detection Limit (ppb) |
|---|---|
| As(III) | 0.036 |
| As(V) | 0.034 |
| MMA | 0.037 |
| DMA | 0.029 |

Table 3: Detection Limits

## Results & Discussion

Our HPLC-ICP-MS method performed well and was fairly robust across a range of different rice samples.

The HPLC-ICP-MS method generally measured higher concentrations of As(III) (Figure 5) and lower concentrations of As(V) (Figure 6) than the HGAA method. Because the HPLC-ICP-MS method does not require pH manipulation, which can cause species conversion, we believe that it produces more accurate results than the HGAA method.

The HPLC-ICP-MS method achieved complete recovery (107%) of total As from the rice samples (Figure 7).

The wide range of concentrations of individual species in addition to the range of total arsenic concentrations (Figure 8) indicates that speciated analysis of arsenic in rice is critical to protecting human health.

It is interesting to note that while the inorganic arsenic concentrations measured in these rice samples do not threaten to bring most consumers close to their reference dose, they are all above the U.S. EPA drinking water limit of 10 ppb.

More research is needed to determine whether arsenic exposure from rice at this level poses a human health threat.

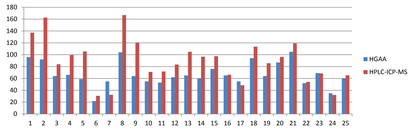


Figure 5: Comparison of As(III) concentrations measured by HGAA and HPLC-ICP-MS

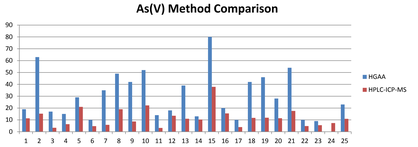


Figure 6: Comparison of As(V) concentrations measured by HGAA and HPLC-ICP-MS

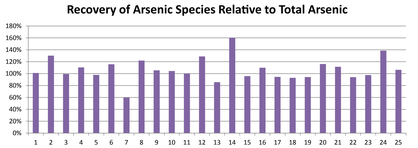


Figure 7: Digestion recovery completeness: comparing the sum of species measured by HPLC-ICP-MS to total As values measured by ICP-DRC-MS after a nitric acid digestion.

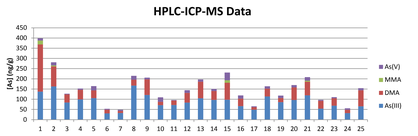


Figure 8: Arsenic speciation results for the U.S. market rice samples (1-24) and Chinese market rice sample (25) analyzed by HPLC-ICP-MS.