

Rapid determination of five arsenic species in polished rice using HPLC-ICP-MS

Application note

Food safety

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Introduction

Arsenic (As) is a well-known toxic element that can be present in the environment and foodstuffs. Consequently it is a highly regulated element that is widely monitored. However, As speciation analysis rather than total As analysis is important because its biological toxicity is highly dependent on its chemical form. For example, if a seaweed sample is known to contain a high level of As, but mainly in the form of AsB, then there is no potential risk from its consumption as AsB is non-toxic. Among the five major species of arsenic, As(V), MMA, As(III) DMA and AsB, only the two inorganic forms (As(III) and As(V)) are toxic. They are also carcinogenic to humans, so need to be measured, especially in foodstuffs. Arsenic in rice is of special concern in countries where it is consumed daily. Rice plants absorb As from soil and water. Since rice requires a lot of water to grow compared to other plants, the potential for arsenic accumulation is increased.

In this study, we present a rapid method for the determination of the toxic As species in rice using an Agilent 1260 HPLC system equipped with an Agilent ZORBAX SB-Aq column coupled to an Agilent 7900 ICP-MS.



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Experimental

Instrumentation

An Agilent 1260 HPLC system comprising a quaternary pump, autosampler and vacuum degasser was coupled to an Agilent 7900 CP-MS. The column exit was simply connected to the nebulizer of the ICP-MS using PFA tubing. The Agilent ZORBAX SB-Aq (part number 880975-914, 4.6 mm id x 250 mm, 5 μ m) reversed phase column was maintained at room temperature throughout the analysis. The mobile phase was 20 mM citric acid and 5 mM sodium hexane sulfonate, adjusted to pH = 4.3 with sodium hydroxide. Two injection volumes, 5 μ L or 100 μ L, were evaluated. HPLC and ICP-MS parameters are given in Table 1.

The Agilent 7900 ICP-MS with the Octopole Reaction System (ORS⁴) collision/reaction cell (CRC) provides sensitive and specific analysis of As in the presence of multiple interferences. In some regulatory guidance, food samples must be extracted using HCl to simulate gastric conditions. In this case, the formation of polyatomic ions based on ArCl and CaCl may cause spectral interferences on the sole isotope of As at m/z 75. However, operating the 7900 ICP-MS ORS⁴ in helium mode effectively removes these matrix-based polyatomic interferences on As.

Table 1. Operating conditions of the Agilent 1260 HPLC and 7900 ICP-MS

| ICP-MS parameters | |
|--------------------|--|
| RF power | 1550 W |
| Sampling depth | 8 mm |
| Carrier gas | 0.6 L/min |
| Makeup gas | 0.5 L/min |
| Cell gas | He at 4 mL/min |
| KED | 3 V |
| Isotopes monitored | ⁷⁵ As, ³⁶ Cl |
| Dwell time | 1.0 s/ ⁷⁵ As; 0.1 s/ ³⁶ Cl |
| HPLC parameters | |
| Mobile phase | Isocratic, 20 mM C ₆ H ₈ O ₇ and 5 mM C ₆ H ₁₃ NaO ₃ S, adjusted to pH 4.3 with NaOH |
| Column | Agilent ZORBAX SB-Aq |
| Flow rate | 1.2 mL/min |
| Temperature | Ambient |
| Injection volume | 5 μ L or 100 μ L |
| Run time | 4 min |

Chemicals and reagents

Stock standards of As(V), MMA, As(III), DMA and AsB were purchased from NIMC (National Institute of Metrology China, Beijing, China). High purity citric acid ($\geq 99.5\%$), sodium hexane-sulfonate ($\geq 98\%$) and sodium hydroxide ($\geq 99.99\%$) were purchased from Sigma Aldrich Chemicals (UK). Ultra-pure grade HNO₃ (Sigma Aldrich, UK) was used for the sample preparation.

Sample preparation

The polished rice samples were obtained from different locations in the following provinces of China: Jiangsu, Fujian, Liaoning, Chongqing and Jiangxi.

The polished rice samples were crushed and sieved through a 30 mesh strainer to make rice flour. 1.00 g of the rice flour sample was weighed into a polypropylene centrifuge tube (50 mL). 15 mL of 0.15 M HNO₃ was added and the sample was vortexed for 30 seconds. The tightly capped tube was left to stand overnight at room temperature; it was then placed in an oven at 90 °C for 2.5 hours where it was agitated for 30 seconds, every 30 minutes. After the extraction had finished, the sample was cooled in a refrigerator at 4 °C. The cooled rice flour suspension was centrifuged at 8000 rpm for 15 minutes and then the supernatant was passed through a 0.22 μ m polyether sulphide (PES) filter. The filtrate was stored at 4 °C and analyzed within 24 hours to minimize any species inter-conversion.

Results and discussion

Separation and quantification

Calibration standard solutions containing the five As species at different concentrations (1 ppb, 5 ppb, 10 ppb, 50 ppb, 100 ppb and 200 ppb) were prepared and analyzed. The chromatogram for the 10 ppb calibration standard (5 μ L injection) shows the five As species are well separated in 4 minutes (Figure 1). The results were used to create calibration curves for the As species, as shown in Figure 2.

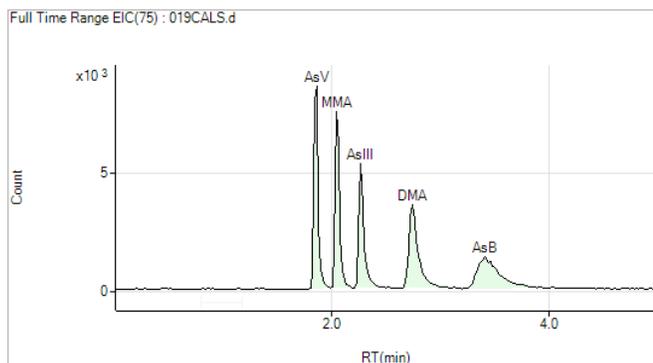


Figure 1. Chromatogram of a 10 ppb As (conc. as As element) species mixture standard (5 µL injection)

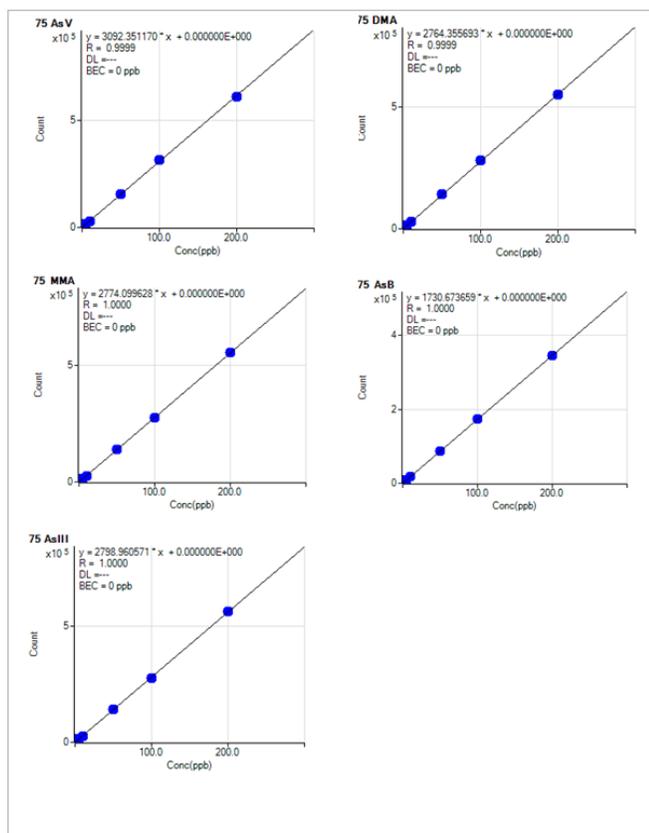


Figure 2. Calibration graphs for As(V), MMA, As(III),DMA and AsB showing excellent linearity.

Detection limits

Detection limits (DLs) for each arsenic species were calculated as the concentration equivalent to three times the peak-to-peak baseline noise (S/N) of the standard chromatogram. The results are summarized in Table 2. As shown, the DL of the five As species were between 0.106 ppb and 0.667 ppb with a 5 µL injection and 0.010 ppb and 0.042 ppb with a 100 µL injection.

Table 2. Detection limits (ppb) for As(V), MMA, As(III), DMA and AsB with 5µL and 100 µL injections

| Injection volume (µL) | As(V) | MMA | As(III) | DMA | AsB |
|-----------------------|-------|-------|---------|-------|-------|
| 5 | 0.106 | 0.121 | 0.187 | 0.267 | 0.667 |
| 100 | 0.010 | 0.012 | 0.025 | 0.028 | 0.042 |

Figure 3 shows the chromatogram of a 50 ppt As species standard mixture (100 µL injection) demonstrating the ultra-trace analysis capability of the method.

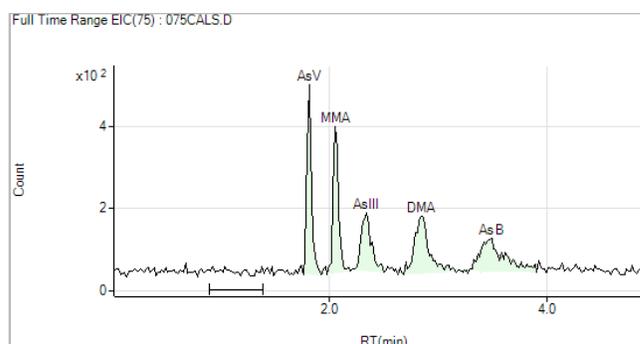


Figure 3. Chromatogram of 50 ppt As species mixture (100 µL injection)

Certified Reference Material (CRM) analysis

The method was applied to the analysis of two rice flour CRMs; NIST 1568a and NIST 1568b. Though only total As content is certified for NIST 1568a, (290 ± 30 ppb), the CRM has been well-characterized in the literature. The two CRMs were extracted following the aforementioned sample preparation method and analyzed for the five As species.

Tables 3 and 4 summarize the quantified results of the inorganic As species, MMA, DMA and the total As content in the two CRMs, the certified values or reported values in the literature. For this study, total As was calculated from the sum of inorganic As, MMA, DMA and AsB. As shown, the quantified inorganic As (iAs) in NIST 1568b and total As in both CRMs are in good agreement with the certified values and within the standard deviation. The quantified results for each species are also in good agreement with reported values in the literature [1-4].

Table 3. Quantification of As species in NIST 1568b Rice Flour^a

| | As(V) ($\mu\text{g}/\text{kg}$) | MMA ($\mu\text{g}/\text{kg}$) | As(III) ($\mu\text{g}/\text{kg}$) | DMA ($\mu\text{g}/\text{kg}$) | iAs ^b ($\mu\text{g}/\text{kg}$) | Total As ($\mu\text{g}/\text{kg}$) |
|-----------------|--------------------------------------|------------------------------------|--|------------------------------------|---|---|
| This study | 62.0 \pm 5.1 | 10.6 \pm 1.3 | 36.8 \pm 5.8 | 179 \pm 7 | 98.8 \pm 3.6 | 288.4 |
| Certified Value | | 11.6 \pm 3.5 | | 180 \pm 12 | 92 \pm 10 | 285 \pm 14 |

^a value is mean \pm SD of ten injections, ^binorganic arsenic: sum of As(III) and As(V)

Table 4. Comparison of quantified As species in NIST 1568a Rice Flour determined in this study compared to values from the literature^a. The certified total As content of NIST 1568a is 290 \pm 30 ppb.

| | As(V) ($\mu\text{g}/\text{kg}$) | MMA ($\mu\text{g}/\text{kg}$) | As(III) ($\mu\text{g}/\text{kg}$) | DMA ($\mu\text{g}/\text{kg}$) | iAs ^b ($\mu\text{g}/\text{kg}$) | Total As ($\mu\text{g}/\text{kg}$) |
|------------|--------------------------------------|------------------------------------|--|------------------------------------|---|---|
| Ref. [1] | 44 \pm 2 | 12 \pm 0.8 | 52 \pm 1 | 173 \pm 2 | 100 | 281 \pm 2 |
| Ref. [2] | 50.3 \pm 2.9 | 14.9 \pm 3.9 | 63.4 \pm 3.5 | 144 \pm 4.5 | 113.7 | 272.8 \pm 9.9 |
| Ref. [3] | 53.7 \pm 3.3 | 14.8 \pm 1.8 | 54.7 \pm 1.4 | 165 \pm 8 | 108.4 | 288.2 |
| Ref. [4] | | 13.6 \pm 2.5 | | 168.0 \pm 2.2 | 104.3 \pm 4.4 | 286.1 \pm 3.8 |
| This study | 64 \pm 5 | 14 \pm 2 | 57 \pm 4 | 140 \pm 4 | 121 | 275 \pm 4 |

^a value is mean \pm SD of ten injections, ^binorganic arsenic: sum of As(III) and As(V)

Spiked sample recovery

Spiked sample recovery was checked for the five As species. A mixture of As(V), MMA, As(III), DMA and AsB was spiked into three rice sample extracts at 1 ppb, 5 ppb and 10 ppb. Three replicates were prepared (spike 1, 2 and 3). As shown in Table 5, good recoveries ranging from 85% to 110% were achieved for all species, at all concentrations, in each of the spiked solutions.

Table 5. Results of spiked sample recovery with 5 μL injection

| Sample name | Spike Conc. | As(V) | | MMA | | As(III) | | DMA | | AsB | |
|-----------------|-------------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|
| | | Conc.(ppb) | Rec.(%) |
| Rice # 1 | | 0.48 | – | N.D. | – | 4.60 | – | 0.48 | – | N.D. | – |
| Spike 1 | 1 ppb | 1.38 | 89.2% | 1.02 | 101.5% | 5.65 | 105.6% | 1.54 | 106.1% | 1.03 | 103.2% |
| Spike 2 | | 1.43 | 94.5% | 0.96 | 96.2% | 5.51 | 90.8% | 1.42 | 93.9% | 1.07 | 106.5% |
| Spike 3 | | 1.47 | 98.1% | 0.95 | 94.3% | 5.52 | 92.2% | 1.45 | 97.4% | 0.98 | 98.4% |
| Rice # 2 | | 1.14 | – | N.D. | – | 5.82 | – | 1.32 | – | N.D. | – |
| Spike 1 | 5 ppb | 5.52 | 87.6% | 4.96 | 99.2% | 10.98 | 103.2% | 6.23 | 98.3% | 4.82 | 96.3% |
| Spike 2 | | 5.65 | 90.1% | 4.77 | 95.3% | 10.88 | 101.3% | 6.45 | 102.6% | 4.97 | 99.4% |
| Spike 3 | | 5.77 | 92.5% | 4.34 | 86.7% | 11.35 | 110.6% | 6.62 | 106.1% | 5.13 | 102. % |
| Rice # 3 | | 0.28 | – | N.D. | – | 3.44 | – | 0.73 | – | N.D. | – |
| Spike 1 | 10 ppb | 9.65 | 93.7% | 9.68 | 96.8% | 13.35 | 99.1% | 9.72 | 89.9% | 8.91 | 89.1% |
| Spike 2 | | 9.89 | 96.1% | 10.08 | 100.8% | 12.97 | 95.3% | 10.29 | 95.6% | 9.54 | 95.4% |
| Spike 3 | | 9.53 | 92.5% | 10.62 | 106.2% | 13.10 | 96.6% | 10.65 | 99.2% | 10.48 | 104.8% |

Precision

Precision of the method was checked by repeatedly analyzing a rice sample, with ten separate injections. Table 6 shows the concentration results for all the As species, indicating the excellent reproducibility for the ten separate analyses. The %RSDs for all As species were less than 4%, and less than 2% for inorganic As.

Table 6. Results of reproducibility of rice # 4 (n = 10) with 5 μL injection

| Sample | As(V) ($\mu\text{g}/\text{kg}$) | MMA ($\mu\text{g}/\text{kg}$) | As(III) ($\mu\text{g}/\text{kg}$) | DMA ($\mu\text{g}/\text{kg}$) | AsB ($\mu\text{g}/\text{kg}$) | iAs ($\mu\text{g}/\text{kg}$) |
|-------------|--------------------------------------|------------------------------------|--|------------------------------------|------------------------------------|------------------------------------|
| Rice # 4-1 | 56.10 | N.D. | 107.32 | 22.29 | N.D. | 163.42 |
| Rice # 4-2 | 55.23 | N.D. | 110.39 | 24.74 | N.D. | 165.62 |
| Rice # 4-3 | 56.97 | N.D. | 107.30 | 23.10 | N.D. | 164.27 |
| Rice # 4-4 | 57.68 | N.D. | 108.51 | 24.19 | N.D. | 166.18 |
| Rice # 4-5 | 57.16 | N.D. | 108.46 | 23.64 | N.D. | 165.62 |
| Rice # 4-6 | 56.10 | N.D. | 108.52 | 24.82 | N.D. | 164.62 |
| Rice # 4-7 | 56.43 | N.D. | 108.89 | 23.96 | N.D. | 165.32 |
| Rice # 4-8 | 57.27 | N.D. | 107.90 | 22.37 | N.D. | 165.17 |
| Rice # 4-9 | 56.18 | N.D. | 109.56 | 22.70 | N.D. | 165.73 |
| Rice # 4-10 | 56.71 | N.D. | 108.76 | 23.35 | N.D. | 165.47 |
| Average | 56.58 | – | 108.56 | 23.52 | – | 165.14 |
| % RSD | 1.3% | – | 0.9% | 3.9% | – | 0.5% |

Commercial rice sample analysis

The As concentration was determined in the 20 different commercial rice samples and the results are summarized in Table 7. As shown, As(V), As(III) and DMA were found in all 20 rice samples. The inorganic As and DMA content varied significantly among the rice samples, likely reflecting the As concentration in the growing environment of the original plant. Although the concentration of toxic inorganic As varied among the samples, all of the rice samples contained lower limit than the upper level of 0.2 mg/kg recommended by Codex Alimentarius Commission.

Table 7. Quantitative results ($\mu\text{g}/\text{kg}$) for all five As species in 20 rice samples

| Sample name | As(V) | MMA | As(III) | DMA | AsB | iAs |
|-------------|-------|------|---------|--------|------|--------|
| Rice # 1 | 7.27 | N.D. | 68.97 | 7.16 | N.D. | 76.24 |
| Rice # 2 | 17.15 | N.D. | 87.23 | 19.77 | N.D. | 104.38 |
| Rice # 3 | 4.26 | N.D. | 51.57 | 10.93 | N.D. | 55.83 |
| Rice # 4 | 56.10 | N.D. | 107.32 | 22.29 | N.D. | 163.42 |
| Rice # 5 | 17.31 | N.D. | 147.2 | 26.38 | N.D. | 164.73 |
| Rice # 6 | 12.52 | N.D. | 75.82 | 8.79 | N.D. | 88.35 |
| Rice # 7 | 7.14 | N.D. | 53.84 | 2.67 | N.D. | 68.94 |
| Rice # 8 | 14.4 | N.D. | 106.14 | 27.27 | N.D. | 120.54 |
| Rice # 9 | 14.49 | N.D. | 72.24 | 29.51 | N.D. | 86.73 |
| Rice # 10 | 13.73 | N.D. | 101.68 | 39.41 | N.D. | 115.41 |
| Rice # 11 | 20.50 | N.D. | 87.04 | 285.21 | N.D. | 107.55 |
| Rice # 12 | 22.78 | N.D. | 70.94 | 160.77 | N.D. | 93.72 |
| Rice # 13 | 23.74 | N.D. | 122.19 | 34.53 | N.D. | 145.94 |
| Rice # 14 | 13.85 | N.D. | 59.79 | 361.82 | N.D. | 73.64 |
| Rice # 15 | 16.38 | N.D. | 91.34 | 83.42 | N.D. | 125.38 |
| Rice # 16 | 18.48 | N.D. | 110.58 | 32.25 | N.D. | 129.06 |
| Rice # 17 | 10.83 | N.D. | 61.56 | 6.69 | N.D. | 72.39 |
| Rice # 18 | 16.96 | N.D. | 105.14 | 209.49 | N.D. | 122.1 |
| Rice # 19 | 36.04 | N.D. | 97.17 | 176.12 | N.D. | 133.22 |
| Rice # 20 | 16.35 | N.D. | 74.55 | 587.49 | N.D. | 90.9 |

Conclusions

The separation of five arsenic species in polished rice was carried out using an Agilent 1260 Infinity LC coupled to an Agilent 7900 ICP-MS. The method is rapid - all five species including the toxicologically relevant inorganic forms As(III) and As(V) were determined in 4 minutes, with excellent sensitivity, accuracy and precision. Any potential interferences on As, such as ArCl^+ , were removed by the ORS⁴ cell operating in helium mode.

Inorganic arsenic and DMA species were detected in all of the twenty rice samples at various concentrations. However, none of the samples contained more than Codex Alimentarius Commission's recommended limit of 0.2 mg/kg.

The validated methodology outlined here is suitable for the rapid determination of As(V), MMA, As(III), DMA and AsB at trace levels in rice and other food samples.

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