Application Note

Food Testing and Agriculture

# Speciated Arsenic Analysis in Wine Using HPLC-ICP-QQQ

## Validation of an extended FDA Elemental Analysis Manual method


**Authors**

C. K. Tanabe,[1] H. Hopfer,[1,2,3] S. E. Ebeler[1,2,] J. Nelson[1,2,4]

1. Dept. Viticulture & Enology, University of California (Davis), USA

2. Food Safety & Measurement Facility, University of California, (Davis), USA

3. Depart. of Food Science, The Pennsylvania State University, Pennsylvania, USA

4. Agilent Technologies, USA



In 2013, the US Food and Drug Administration (FDA) released Elemental Analysis Manual (EAM) Method §4.10. The method describes the Determination of Four Arsenic Species in Fruit Juice using High-Performance Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry [1]. To extend the method to include wine, a multi-laboratory validation (MLV) of the method was carried out with three US-based laboratories sharing their data [2]. The data shown in this application note is supplementary to the published data. In addition to the paper, this note includes long term stability of the method, and extended quantitative analysis of five commercially available wines. The method required separation and analysis of all target species. This approach differs from another Agilent application note, which focused on the development of a fast method for inorganic arsenic (iAs) [3].

The US Environmental Protection Agency (EPA) set a maximum threshold of total As in drinking water of 10 µg/kg [4]. There is no equivalent US regulation for As in wine. Studies have shown that As in wine can be the result of an accumulation of As in the grapes from the environment [5] or introduced during the wine making process [6].

Regulations in Canada (Vintners Quality Alliance VQA, Ontario) and Europe (International Organisation of Vine and Wine, OIV) specify limits for total As of 100 µg/L and 200 µg/L, respectively [7, 8]. However, the toxicity of As is determined by its chemical form. Because the inorganic forms of As (iAs) are the most carcinogenic, the FDA has established an action limit for iAs in apple juice of 10 µg/kg in 2013 [9]. FDA EAM Method §4.10 details a relatively simple and robust method for the determination of As species in fruit juice using HPLC-ICP-MS [1]. The method describes a procedure to determine iAs (the sum of arsenite, As(III), and arsenate, As(V)); dimethylarsinic acid (DMA); and monomethylarsonic acid (MMA). The method also states that a solution containing arsenobetaine (AB) and As(III) is analyzed to demonstrate adequate separation between unretained arsenic-containing species and As(III).

Due to recent media attention on As levels in wine, and the lack of published research on As speciation in wine, extension of EAM §4.10 to include wine is a logical next step.

In this study, EAM §4.10 was modified for the determination of the main organic arsenic species (DMA and MMA) and the more toxic inorganic forms (As(V) and As(III)) in wine using HPLC coupled to a triple quadrupole ICP-MS (ICP-QQQ). The ICP-QQQ was utilized to provide the highest possible sensitivity of all the instruments available in the lab at UC Davis. ICP-QQQ also provides superior resolution of potential spectral interferences, but the potential Cl-based interferences on $^{75}As$ are resolved chromatographically, so QQQ with MS/MS is not essential. This application could also be done on a single quadrupole ICP-MS such as the Agilent 7800 or 7900.

# Experimental

## Reagents

Arsenite (As(III)) and arsenate (As(V)) were bought as 1000 mg/L standard solutions from Spex Certiprep (Metuchen, NJ, USA). Monomethylarsonic acid (MMA, 98.5% purity) and dimethylarsinic acid (DMA, 98.9% purity) were bought from Chem Service (West Chester, PA, USA). Arsenobetaine (AB, purum p.a., ≥95.0%) was bought from Fluka Analytical (Morris Plains, NJ, USA).

## Samples and sample preparation

Five commercially available wine samples were bought from a local store in Davis, California. The wines were selected to represent the main types (and styles) of wine: red (Cabernet Sauvignon), white (Sauvignon blanc), rosé (Zinfandel), sparkling (sparkling white) and fortified (Port-style). To investigate the range of ethanol content that could be analyzed using the method, the alcohol concentrations of the wines selected ranged from 9.5—20% (v/v).

The sample preparation and analysis details were carried out according to the EAM §4.10 method. Each wine sample was diluted five times with de-ionized water and then filtered separately using syringe-filtration (0.45 µm PVDF membrane).

Per EAM §4.10, calibration curves were prepared at nominal concentrations of 0.4, 0.5, 1, 5, 10, 20, 40 µg/kg for the four arsenic species: As(III), DMA, MMA, and As(V). However, for this method, a fifth, low-level calibration point was also prepared at 0.1 µg/kg. NIST 1643e Trace Elements in Water standard reference material (SRM), used to assess recovery and stability, was prepared using a 15-fold dilution. All calibration standards and the SRM were prepared in a 3% ethanol solution to approximately match the level of alcohol (carbon matrix) in the diluted wine samples. In addition to the effect that a change in sample viscosity has on sample transport and nebulization, the level of carbon also affects (increases) the degree of ionization of some elements in the ICP, including arsenic. Therefore, sample preparation for carbon-containing matrices should ensure a reasonably consistent level of carbon across all samples and standards, to avoid errors due to variable carbon enhancement in different sample solutions.

## Instrumentation

An Agilent 1260 Infinity LC comprising a binary pump, autosampler, and vacuum degasser was coupled to an Agilent 8800 Triple Quadrupole ICP-MS (ICP-QQQ). HPLC and ICP-QQQ parameters are shown in Table 1.

**Table 1.** HPLC-ICP-QQQ hardware system and operating conditions.

| LC conditions | Value |
|---|---|
| Column | Hamilton PRP-X100 anion exchange (4.1 x 250 mm) column with a matching Hamilton PRP-X100 guard column |
| Mobile phase | Mobile phase, aqueous 10 mM ammonium phosphate dibasic, 1% ethanol, pH 8.25 (±0.05) |
| Flow rate (mL/min) | 1.0 |
| Temperature | Ambient |
| Injection volume (µL) | 100 |
| Column compartment time table for introduction of ISTD | 0.1 min, column position 1, 1.0 min; switch to column position 2, 2.0 min; switch back to column position 1 |
| **ICP-QQQ parameters** | **Value** |
| RF power (W) | 1550 |
| Carrier gas flow (L/min) | 1.0 |
| Spray chamber temperature (°C) | 2 |
| Sample depth (mm) | 8.5 |
| Peristaltic pump speed (rps) | 0.3 (~1.2 mL/min) |
| Scan mode | MS/MS |
| Helium cell gas flow (mL/min) | ~2.0 |

## Results and Discussion

Method blanks (3% ethanol) spiked with low levels of As(III), DMA, MMA, and As(V) were prepared and analyzed for the determination of the detection limits.

Figure 1 shows overlaid chromatograms obtained for the mixed As species standards, demonstrating excellent peak separation of the As species of interest. The calibration curves in Figure 2 show a linear response for each As species across the concentration range from 0.1 to 40 µg/kg.

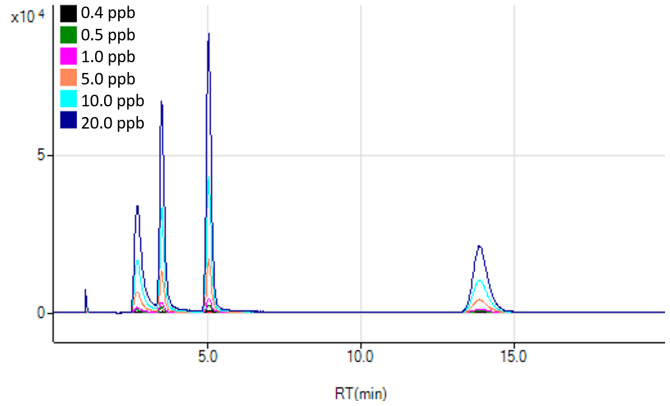



**Figure 1.** Overlaid chromatograms of As species standards at nominal concentrations of 0.4, 0.5, 1, 5, 10, 20 µg/kg showing good peak separation. The 40 µg/kg standard is not shown, to allow the lower concentration levels to be seen.

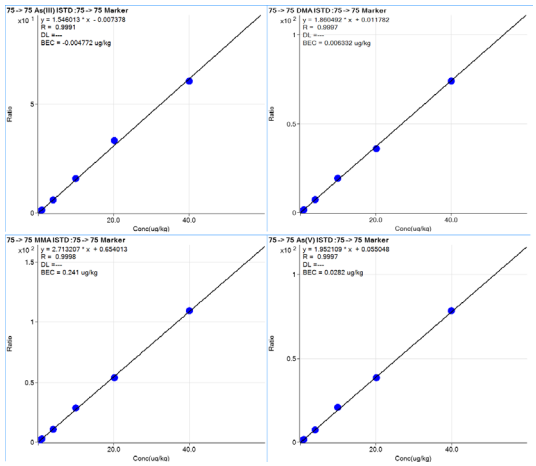


**Figure 2.** Calibration graphs for As(III), DMA, MMA, and As(V).

The limits of detection (LOD) for the As species in wine were calculated as described in the FDA's Elemental Analysis Manual Section 3.2 [1]. The limits of quantification (LOQ) for each species were calculated as LOQ = Dilution Factor (DF) x 30 x σ. The LOQs for As(III) and As(V) were 1.18 and 1.35 µg/kg, respectively. The LOQ for total inorganic arsenic (calculated from the SD of the sum of the integrated peak areas for As(III) and As(V) in each repeat of the low standard) was 2.53 µg/kg. The LODs and LOQs determined for the species DMA, MMA, and total iAs (sum of As(III) and As(V)) using the optimized method are given in Table 2. Results are reported for iAs since the current regulations only specify iAs, and not the individual species As(III) and As(V).

**Table 2.** LODs and LOQs for DMA, MMA, and iAs.

| | LOD, µg/kg | LOQ, µg/kg |
|---|---|---|
| DMA | 0.17 | 1.3 |
| MMA | 0.15 | 1.2 |
| iAs | 0.17 | 1.4 |

The iAs LOQ is well within the FDA's 10 µg/L level of concern for iAs in juice samples. The sensitivity of the method is therefore sufficient to determine iAs in solution following a five-fold dilution of the samples.

### Quantitative results

The five wines included in the MLV were analyzed in the lab at UC Davis using LC-ICP-QQQ and the results are shown in Table 3. The average percent recovery of the sum of the species compared to the total As present in the samples (determined using direct analysis without HPLC separation) was calculated using the mass balance approach. The percent recovery for all samples was between 91–107%. The results were found to be in good agreement with the results obtained from the other laboratories taking part in the MLV study [2].

**Table 3.** Quantitative results for the five wines analyzed at UC Davis as part of the MLV study. Average ± 1σ, n=3 for the individual species.

| Wine sample | % Ethanol (v/v) | DMA µg/kg | MMA µg/kg | iAs µg/kg | Sum of species µg/kg | Total As µg/kg | Mass balance % |
|---|---|---|---|---|---|---|---|
| Red (Cabernet) | 9.5 | 0.81 ± 0.1* | <LOD | 14.4 ± 1.0 | 15.2 ± 1.1 | 15.3 ± 1.2 | 99 |
| White (Chardonnay) | 13 | 0.74 ± 0.04* | <LOD | 10.7 ± 0.2 | 11.4 ± 0.2 | 11.1 ± 0.8 | 103 |
| Rosé (Zinfandel) | 12 | 0.75 ± 0.1* | <LOD | 9.2 ± 0.4 | 9.9 ± 0.4 | 9.3 ± 1.1 | 107 |
| Sparkling wine | 20 | 1.7 ± 0.1 | <LOD | 2.1 ± 0.3 | 3.8 ± 0.3 | 3.6 ± 0.3 | 105 |
| Port-style wine | 14.5 | 0.45 ± 0.01* | <LOD | 1.5 ± 0.3 | 2.0 ± 0.3 | 2.2 ± 0.1 | 91 |

* Value between LOD and LOQ

## Long-term stability

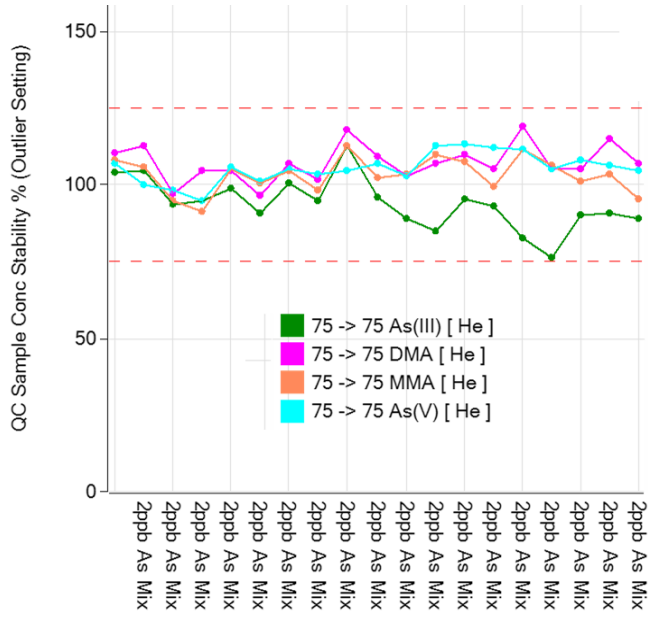



**Figure 3.** Stability plot of the 2-ppb mixed As species standard solution, analyzed over 96 hours (four days).

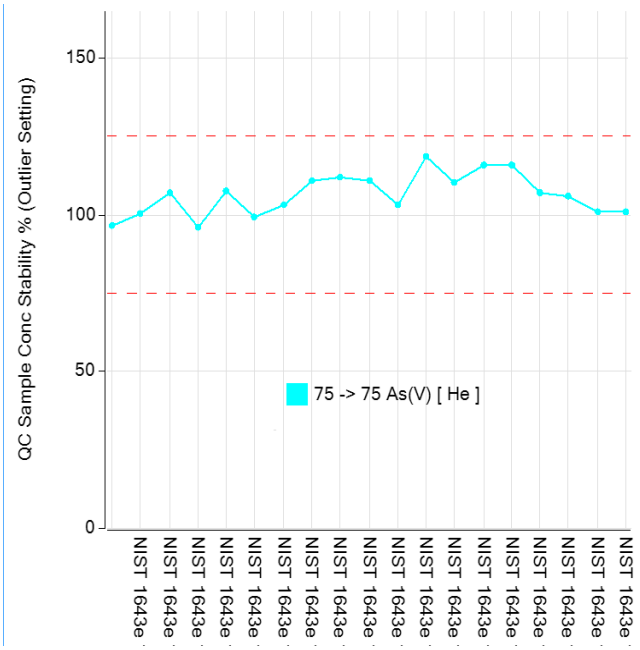



**Figure 4.** Stability plot of As in NIST 1643e spiked with ethanol and analyzed over four days.

To test the stability of the ICP-QQQ over an extended sampling period of 96 hours (four days), the wine samples were measured repeatedly in a continuous sequence. Two quality control (QC) samples—a 2-ppb mixed As species standard solution and NIST 1643e spiked with 3% ethanol—were analyzed after every 10 wine samples. The instrument was not recalibrated during the continuous analytical run. The plots shown in Figures 3 and 4 show exceptional stability was achieved over the course of the validation stability test.

## Results of additional market basket wine analysis

In addition to the five wines used in the MLV study, an extra 60 wines were analyzed as part of the method validation [2]. In this study, a selection of previously untested wines (S1 to S5) were analyzed. The results shown in Table 4 are consistent with the published data from the reference paper [2]. Most of the As was in the more toxic, inorganic forms. While four of the five wine samples contained levels of total As higher than the EPA drinking water limit of 10 µg/L, the levels in all five wines were below the 100 and 200 ug/kg limits for total As in wine set in Canada and Europe, respectively. However, the measured concentrations for iAs in four out of five of the wines exceeded the FDA's action limit of 10 µg/kg for iAs in apple juice.

**Table 4.** Quantitative results (µg/kg) for As species in five commercially available wines measured by LC-ICP-QQQ.

| Wine Sample | iAs | DMA | MMA | Sum of Species |
|---|---|---|---|---|
| S1 | 17.13 ± 0.22 | 0.83 ± 0.03 | <LOD | 17.96 ± 0.13 |
| S2 | 7.49 ± 0.15 | 0.30 ± 0.06 | 0.77 ± 0.32 | 8.56 ± 0.17 |
| S3 | 14.63 ± 0.40 | 0.80 ± 0.08 | <LOD | 15.43 ± 0.24 |
| S4 | 25.03 ± 0.89 | 0.69 ± 0.26 | 0.47 ± 0.12 | 26.19 ± 0.42 |
| S5 | 23.45 ± 1.12 | 0.32 ± 0.05 | <LOD | 23.77 ± 0.59 |

## Spike recovery test

Table 5 shows the spike recoveries for the MLV samples fortified at levels of approximately 5, 10, and 30 µg/kg for DMA, MMA, and iAs (the iAs spike concentration was the sum of As(III) and As(V) each spiked at 50% of the levels shown). The average recoveries of DMA, MMA, and iAs measured using LC-ICP-QQQ were 99, 92, and 104%, respectively. All the recoveries are within the FDA's EAM acceptability criteria of 100 ± 20% for iAs, DMA, and MMA [1].

**Table 5.** Average spike recovery results for duplicate analyses of five samples spiked at 5, 10, and 30 µg/kg with DMA, MMA, and iAs. n=30.

| | DMA | MMA | iAs |
|---|---|---|---|
| Average spike recovery, % | 99 | 92 | 104 |
| Recovery range | 93 – 107 | 72 – 119 | 97 – 114 |

## Conclusions

The As speciation results obtained using an Agilent 1260 Infinity LC coupled to an Agilent 8800 ICP-QQQ were used as part of an MLV to validate the extension of Elemental Analysis Manual Method §4.10 to include wine. The method was optimized for the analysis of four arsenic species including the toxicologically relevant inorganic forms, As(III) and As(V).

In addition to the data published as part of the MLV, five more wines were analyzed. The total As levels of the five wines were between 8.56 and 26.19 µg/L. These levels are below the Canadian and European regulatory limits for total As in wine of 100 and 200 µg/kg, respectively. The average percentage of As found in the form of iAs in the five wine samples was 95%.

## References


1. S. D. Conklin, K. Kubachka, N. Shockey, Elemental Analysis Manual for Food and Related Products, 4.10 HPLC-ICP-MS As Species in Fruit Juice (Ver. 1; 2013), http://www.fda.gov/EAM (accessed September 2017)

2. C. K. Tanabe, H. Hopfer, S. E. Ebeler, J. Nelson, S. D. Conklin, K. M. Kubachka, and R. A. Wilson, ***J. Agric. Food Chem***., **2017**, 65 (20), pp 4193–4199

3. C. K. Tanabe, S. E. Ebeler, J. Nelson, Fast Analysis of Arsenic Species in Wines using LC-ICP-QQQ, Agilent publication, 2017, 5991-8454EN

4. ***National Primary Drinking Water Regulations***. US E.P.A, In Title 40, EPA, Ed. 2014; Vol. AE 2. 106/3:40/

5. D. Bertoldi, R. Larcher, M. Bertamini, S. Otto, G. Concheri, G. Nicolini, ***J. of Agri and Food Chem.,*** **2011**, 59, 7224–7236.

6. M. V. Aguilar, M.C. Martinez, T.A. Masoud, Z. ***Lebensm-Unters-Forsch.,*** **1987**, 185, 185–187.

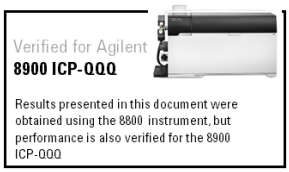
7. Canada Ontario, V. Q. A., Wine Standards. 1999 http://www.vqaontario.ca/Regulations/Standards (accessed August 2017)

8. OIV–Compendium of International Methods of Analysis. Maximum acceptable limits of various substances contained in wine, 2011 issue. Europe

9. US Department of Health and Human Services Food and Drug Administration Center for Food Safety and Applied Nutrition, Guidance for Industry Arsenic in Apple Juice: Action Level (draft), 2013. https://www.fda.gov/RegulatoryInformation/Guidances/ucm360020.htm (accessed September 2017)


## More Information

For a full account of this study, see Courtney K. Tanabe, Helene Hopfer, Susan E. Ebeler, Jenny Nelson, Sean D. Conklin, Kevin M. Kubachka, and Robert A. Wilson, Matrix Extension and Multilaboratory Validation of Arsenic Speciation Method EAM §4.10 to Include Wine, ***J. Agric. Food Chem***., **2017**, 65 (20), pp 4193–4199, DOI: 10.1021/acs.jafc.7b00855




**www.agilent.com/chem**



Printed in the USA, February 26, 2018
5991-8833EN


Agilent
Trusted Answers