Fast Analysis of Arsenic Species in Wines using LC-ICP-QQQ

Application Note

Food and Beverages

Authors

Courtney Tanabe[1-2], Susan E. Ebeler[1-2], Jenny Nelson[1-3]

[1]Food Safety and Measurement Facility, University of California, Davis, USA

[2]Department of Viticulture and Enology, University of California, Davis, USA

[3]Agilent Technologies, Inc., Santa Clara, USA

Introduction

Arsenic (As) occurs naturally in the environment but human activity has contributed to the levels found in some locations. Man-made sources of As include industrial processes such as mining, smelting and power generation, as well as agricultural pesticides and timber preservatives [1]. Once contamination has occurred, As persists in the environment for decades. For example, the widespread use of As-containing agrochemicals ceased in the 1970s, but lead and calcium arsenate levels remain high in some soils. As can be absorbed from soil and water into crops. In the case of wine, the As content can also be affected by the wine making processes.

Arsenic exists in multiple forms in foods and beverages and not all forms have the same toxicity. The inorganic forms of As (iAs), comprising As(III) (arsenite) and As(V) (arsenate), are the most toxic, and are categorized as class 1 carcinogens. In contrast, arsenobetaine (AB), the most abundant form of As in fresh seafood, is essentially non-toxic to humans. Due to the high variability in the toxicity of the

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different species of As, and the potential health threat of iAs, it is important to determine the levels of the individual species in foodstuffs –and not just the total As concentration. The US Food and Drug Administration (FDA) has established an action limit for iAs in apple juice of 10 µg/kg (ppb) [2] but there are currently no regulations in the US controlling the As content of wine. Canada (Vintners Quality Alliance VQA, Ontario) and Europe (International Organisation of Vine and Wine, OIV) have set maximum acceptable limits for total As in wine of 100 and 200 µg/L (ppb), respectively [3, 4].

Arsenic contamination of food is of great public interest. There is a clear demand for rapid and reliable screening methods to accurately determine the levels of iAs in food and drink to support existing and future regulations. One of the most useful and reliable approaches uses high performance liquid chromatography (HPLC) to separate the species, which are then quantified by inductively coupled plasma mass spectrometry (ICP-MS) [5].

The methodology described here is based on a previous As speciation method developed by Jackson, who coupled HPLC to a triple quadrupole ICP-MS (ICP-QQQ) [6]. HPLC-ICP-QQQ was also used in this study. However, instead of analyzing the iAs species separately, As(III) was intentionally oxidized to As(V) with hydrogen peroxide before analysis [7, 8]. By converting As(III) and analyzing all inorganic species as As(V), this method was able to separate monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) from iAs (as As(V)) in less than 2 minutes. The analysis time is 10 times faster than the current FDA methods used for the speciation of As [9].

In this work, oxygen reaction gas was used in the collision/reaction cell (CRC) of the ICP-QQQ to resolve the spectral interferences on ^{75}As, while maintaining excellent sensitivity. Results are presented that demonstrate the accuracy and reproducibility of the new method. The method was further validated using a wine matrix that was analyzed by two participating laboratories.

Experimental

Standards

The As(III) and As(V) standards were purchased from Spex Certiprep (Christiansburg, VA; Metuchen, NJ, USA). The MMA and DMA standards were purchased from Chem Service (West Chester, PA, USA). An AB standard was also purchased from Chem Service to be used as a flow injection marker (internal standard) for post-column injection. Calibration standards were prepared at 0.1, 0.5, 1.0, 5.0, 10 and 20 µg/L (ppb) for each of DMA, MMA, and total iAs (sum of As(III) and As(V)).

Samples

Five different California wines were used for the validation (V) study. Each wine represented one of the five main styles of wine: red, white, rosé, sparkling, dessert. Five additional California wines were analyzed for a commercial market basket (MB) study. Details of the wine style, cultivar, growing region, vintage, and alcohol content for all samples are given in Table 1.

Table 1. Wine style, cultivar, regional origin, vintage, and alcohol content of the wine samples for the validation and commercial market basket studies.

Sample	Style	Cultivar	Region	Vintage	Alcohol (%v/v)
V-1	Rosé	Zinfandel	Napa and Lodi	NA	9.5
V-2	White	Sauvignon blanc	Oakville/Napa County	2013	13.0
V-3	Sparkling	Sparkling white blend	Sonoma County	NA	12.0
V-4	Dessert	Petite Sirah Port-style	Clarksburg/Yolo County	2012	20.0
V-5	Red	Cabernet Sauvignon	Monterey County	2013	14.5
MB-1	Red	Cabernet Sauvignon	North Coast	2009	13.5
MB-2	Red	Pinot noir	Appellation Central Coast	2004	13.8
MB-3	White	Chardonnay	Santa Barbara County	2013	13.5
MB-4	Rosé	Zinfandel	Napa and Sonoma	2013	10.5
MB-5	White	Chardonnay	Central Coast	2013	13.5

Sample preparation

H_2O_2 was added to all samples at a 1:1 ratio to oxidize As(III) to As(V). Each sample was further diluted with de-ionized water to give a total dilution factor of 5 or 6 (there were no differences in results between the two dilution factors). Each sample was then passed through a 0.45 µm syringe filter to remove any particulates. Samples V-1, V-4, V-5 were spiked in duplicate with all As species at three concentration levels: 5, 10, and 30 µg/kg.

Instrumentation

An Agilent 1260 HPLC fitted with a Hamilton PRP-X100 5 µm 50 x 2.1 mm column was coupled to an Agilent 8800 Triple Quadrupole ICP-MS (ICP-QQQ). The mobile phase was 40 mM ammonium carbonate ($(NH_4)_2CO_3$, trace metal grade 99.999% from Sigma Aldrich) with 3% v/v methanol (Optima LC/MS grade, Fisher Chemical) adjusted to a pH of 9.0 with ammonium hydroxide (Optima Grade, Fisher Scientific). The ICP-QQQ was equipped with a standard sample introduction system comprising a quartz torch with 2.5 mm i.d. injector, a quartz spray chamber, glass concentric nebulizer, and

nickel-tipped interface cones. Peak integration was carried out according to FDA EAM §4.10 and 4.11.15 [9]. The instrument operating conditions are summarized in Table 2.

Table 2. HPLC-ICP-QQQ operating conditions.

ICP-QQQ	
Forward power	1550 W
Sampling depth	8.0 mm
Spray chamber temp.	2 °C
Carrier gas	0.95 L/min
Makeup gas	0.20 L/min
Extract 1	0 V
Octopole bias	–5.0 V
Energy discrimination	–7 V
Cell gas (O_2) flow rate	0.31 mL/min
Scan mode	MS/MS
Q1/Q2 mass	75/91 u
HPLC	
Mobile phase flow rate	0.5 mL/min
Injection volume	5 µL
Sample temperature	4 °C
ISTD injection volume	5 µL

Results and Discussion

Development of a fast method

For this study, the focus of the method development was to reduce the analysis time per sample. In the development of this method, we followed Jackson's use of a small injection volume, short ion-exchange column, oxygen cell gas, and a high mobile phase linear velocity [6].

Figure 1 shows overlaid chromatograms for a representative calibration set of 0.5, 1.0, 5.0, and 20 µg/kg standards. All As species are clearly separated in less than two minutes. Simply by oxidizing As(III) to As(V) and analyzing all iAs in the form of As(V), the analysis time was reduced significantly compared to the current FDA regulatory method [9].

Linear calibrations

The calibration curves for DMA, MMA, and iAs show good linearity (Figure 2). All As concentrations in the wine samples were within the linear range except iAs, which was measured at a maximum concentration of 150% of the highest calibration standard.

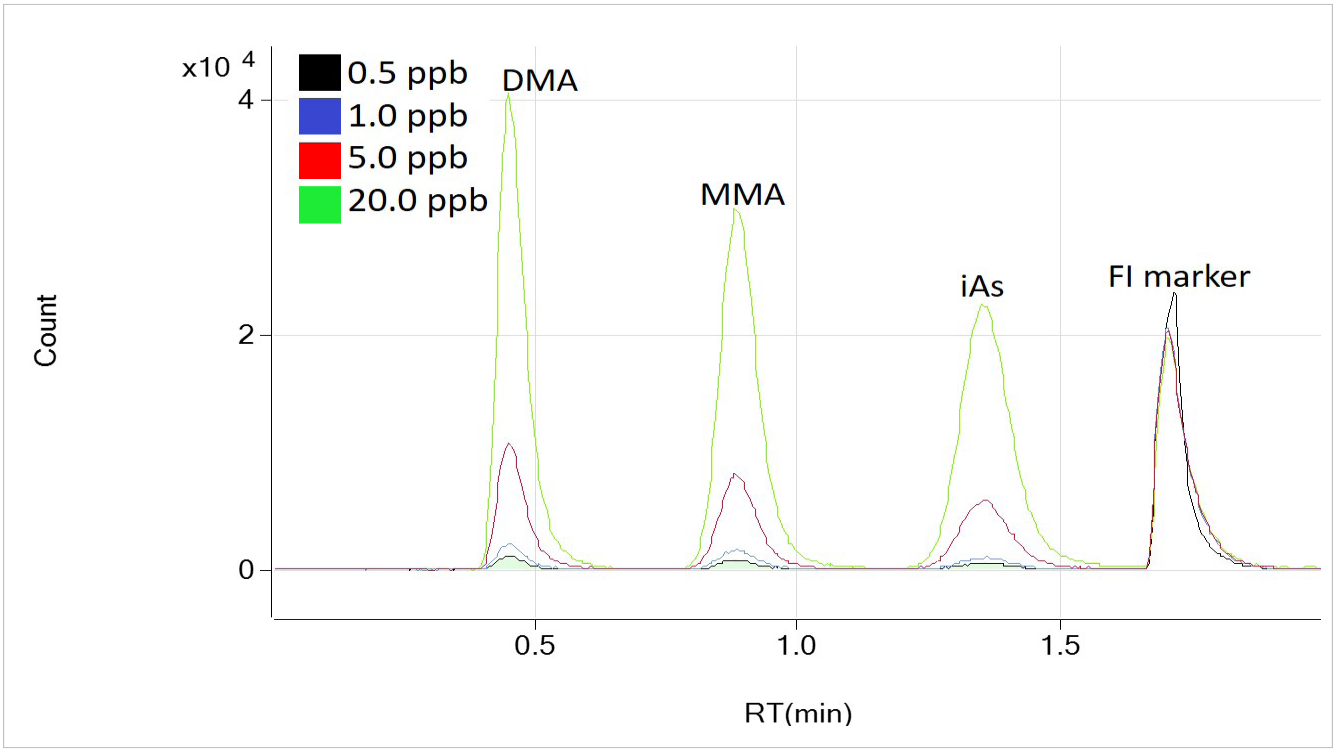

Figure 1. Overlay of the 0.5, 1.0, 5.0, and 20.0 µg/kg calibration standards. An AB internal standard (flow injection marker; fourth peak) was added post column via an external switching valve.

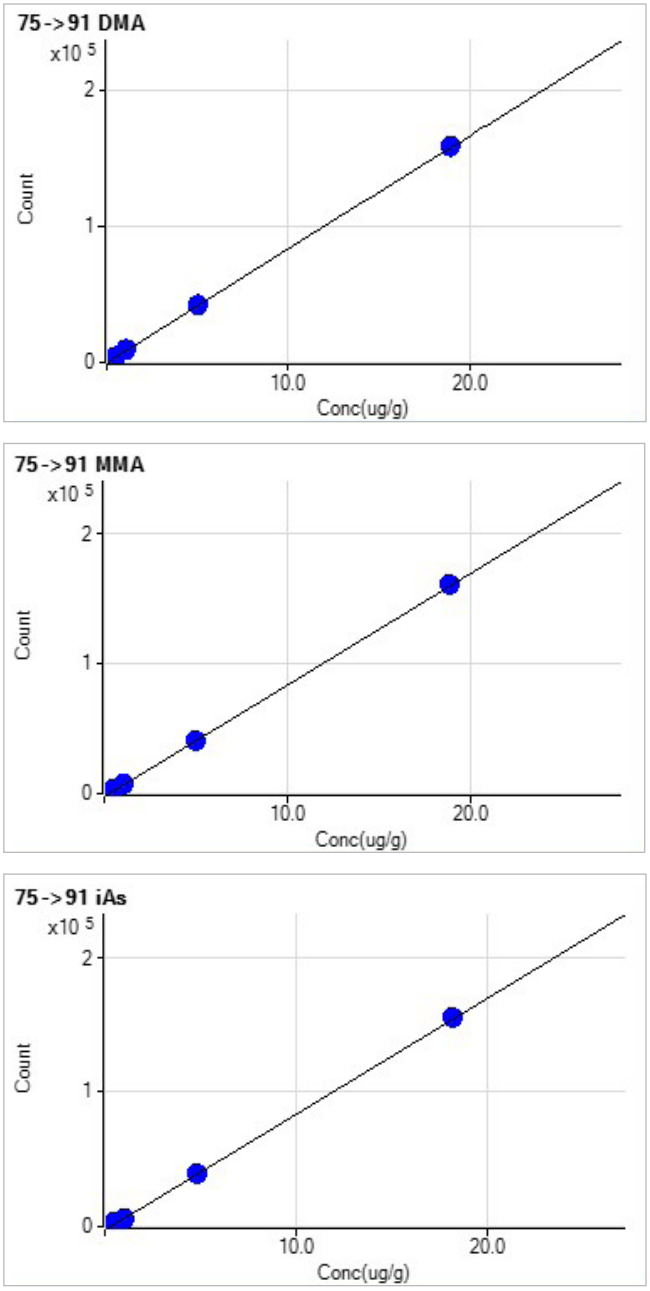

Figure 2. Calibration curves for DMA, MMA, and total iAs (sum of converted As(III) and As(V)).

Detection limits

The limits of detection (LOD) and limits of quantitation (LOQ) given in Table 3 are based on repeated measurements of the 0.05 µg/kg (ppb) mixed standard, n=15.

Table 3. LODs (3 sigma), LOQs (30 sigma), and estimated wine LOQ.

	LOD (µg/kg)	LOQ (µg/kg)	Estimated wine LOQ, (6 x dilution) µg/kg
DMA	0.018	0.175	1.1
MMA	0.026	0.258	1.5
iAs	0.022	0.221	1.3

Spike recoveries

Samples V-1, V-4, V-5 were spiked in duplicate with each species (DMA, MMA, and total iAs as As(V)) at 5, 10, and 30 µg/kg. The averaged recoveries for all As species at the three different fortification levels were 100 ± 3% (Table 4).

Table 4. Percent recovery (mean and range) for three spiking levels of DMA, MMA and iAs in wines V-1, V-4 and V-5.

	DMA	MMA	iAs
Average, %	102	97	99
Range, %	97–107	91–102	95–103

Quantitative results

All 10 wines were analyzed using the new HPLC-ICP-QQQ method. Table 5 lists the measured concentrations for DMA and iAs. All MMA values were below the calculated LOD (0.026 µg/kg) and could not be quantified. The measured concentrations using the new method were compared to the values obtained using the FDA EAM §4.10 extension method [10]. The agreement between the measurements was mostly within ±10%. iAs represented the majority of As in all wines, while only one wine sample (MB-3) contained DMA levels significantly above the LOQ of 1.1 µg/kg. A chromatogram of V-1 is shown in Figure 3.

Overall, the concentration of iAs ranged from 1.7 ± 0.3 to 32.9 ± 0.8 µg/kg (the latter being above the FDA's action limit for iAs in apple juice of 10 µg/kg). The sum of all As species (Table 5) ranged from a low of 2.2 ± 0.3 µg/kg to a high of 32.9 ± 0.8 µg/kg, which is under the Canadian limit of 100 µg/L and OIV limit of 200 µg/L.

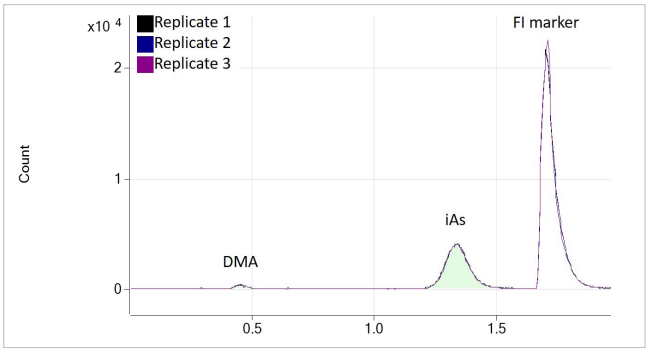

Figure 3. Chromatogram showing the overlay of the three replicates of wine sample V-1.

Table 5. Results from the fast and fit-for-purpose analysis method (measured at two different labs) compared to the FDA EAM §4.10 extension results for the five validation (V) and five market basket (MB) wines. % Recovery (shown in parentheses) calculated as "Measured" divided by "EAM §4.10" and "Sum of Species" divided by "Total".

Sample	DMA (µg/kg)		iAs (µg/kg)		Total As (µg/kg)	
	EAM §4.10	Measured	EAM §4.10	Measured	Total	Sum of Species
V-1	0.81 ± 0.1*	0.72 ± 0.04 (89%)	14.4 ± 1.0	16.0 ± 0.5 (111%)	16.5 ± 0.02	16.7 ± 0.5 (101%)
V-2	0.74 ± 0.04*	0.72 ± 0.06 (98%)	10.7 ± 0.2	11.4 ± 0.4 (107%)	12.6 ± 0.16	12.1 ± 0.3 (96%)
V-3	0.75 ± 0.1*	0.83 ± 0.04 (111%)	9.2 ± 0.4	9.5 ± 0.6 (103%)	10.4 ± 0.11	10.3 ± 0.5 (99%)
V-4	1.70 ± 0.1	1.86 ± 0.06 (109%)	2.1 ± 0.3	2.3 ± 0.4 (109%)	4.5 ± 0.01	4.1 ± 0.4 (92%)
V-5	0.45 ± 0.01*	0.47 ± 0.04 (105%)	1.5 ± 0.3	1.7 ± 0.3 (113%)	2.4 ± 0.03	2.2 ± 0.3 (90%)
MB-1	<LOD	<LOD	30.2 ± 1.3	32.9 ± 0.8 (109%)	34.4 ± 0.4	32.9 ± 0.8 (96%)
MB-2	0.33 ± 0.04*	<LOD	7.57 ± 0.49	9.1 ± 0.4 (120%)	9.1 ± 0.3	9.1 ± 0.4 (100%)
MB-3	0.71 ± 0.08*	1.1 ± 0.0 (155%)	24.64 ± 0.40	27.6 ± 0.7 (112%)	28.9 ± 0.9	28.6 ± 0.7 (99%)
MB-4	1.16 ± 0.09*	1.0 ± 0.1 (86%)	26.3 ± 0.89	27.5 ± 0.9 (105%)	27.9 ± 0.9	28.5 ± 0.9 (102%)
MB-5	<LOD	<LOD	3.5 ± 0.25	4.5 ± 0.1 (129%)	4.7 ± 0.1	4.5 ± 0.1 (96%)

Average ± 1σ, n=3. *Indicates value between LOD (0.17 µg/kg) and LOQ (1.3 µg/kg) for EAM §4.10 method. Refer to Table 3 for Measured LODs and LOQs.

Conclusions

This note describes a simple, robust, and fast HPLC-ICP-QQQ method to measure the sum of the most toxic inorganic As species (As(III) and As(V)) and two organic As species in under two minutes. By oxidizing As(III) to As(V) with H_2O_2 during sample preparation, total iAs can be determined as As(V), leading to a much faster separation of the species of interest in wine samples. The narrow bore column and 0.5 mL/min flow rate provided excellent sensitivity which allowed low volume injections to be used. Compared to the current FDA method for the determination of As in wines, sample run times were 10x faster with improved limits of detection and quantification.

In this study, total As concentrations ranged from 2.2 to 32.9 µg/kg, which is well below the limit defined in regulations set in Ontario, Canada (100 µg/kg) and the maximum level established by the International Organisation of Vine and Wine in Europe (200 µg/kg). However, iAs was the predominant species present in the wines, and five of the wines tested contained iAs at concentrations that exceeded 10 µg/kg, which is the FDA's action limit for iAs in apple juice.

The results obtained using the new fast and fit-for-purpose method were in good agreement with data obtained using the FDA's EAM §4.10.

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More Information

For a full account of this study, see Patrick J. Gray, Courtney K. Tanabe, Susan E. Ebeler, and Jenny Nelson, A fast and fit-for-purpose arsenic speciation method for wine and rice, *J. Anal. At. Spectrom.*, **2017**, 32, 1031–1034; DOI: 10.1039/C7JA00041C

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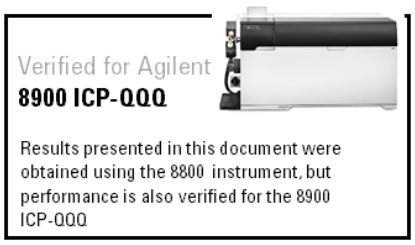

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