FAST ARSENIC SPECIATION ANALYSIS OF WINES AND RICE WITH LC-ICP-QQQ

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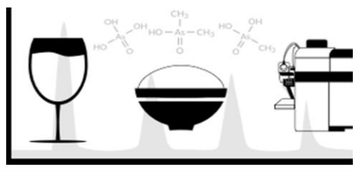
Introduction

This fit-for-purpose method was designed in response to recent and proposed food standards, both international and national, that limit inorganic arsenic rather than total, organic, or individual arsenic species such as arsenite (As^{III}) and arsenate (As^{V}). In this method, As^{III} is intentionally oxidized to As^{V} with H_2O_2 during sample preparation, converting all inorganic arsenic (the sum of As^{III} and As^{V}) to the As^{V} form. Arsenic species were separated in less than 2 minutes using a short, narrow bore, 5 μm chromatography column. This analysis time is 10x faster than the current FDA regulatory method. The use of O_2 reaction gas with inductively coupled plasma triple quadrupole mass spectrometry (ICP-QQQ with MS/MS capability) avoided spectral interferences and dramatically increased sensitivity, allowing for low volume injections. The small injection volume and modified mobile phase composition mitigate non-spectral interferences such as carbon enhanced ionization. Furthermore, the shortened analysis time significantly increases sample throughput. Validation data from two laboratories demonstrate the method's accuracy and reproducibility of both wine and rice matrices in a single analytical batch.
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

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.

Sample	Style	Cultivar	Region	Vintage	Alcohol (%v/v)
V-1	Rose	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	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	Rose	Zinfandel	Napa and Sonoma	2013	10.5
MB-5	White	Chardonnay	Central Coast	2013	13.5

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

Sample Preparation

- 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)).
- 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 wine sample was then passed through a 0.45 μm syringe filter to remove any particulates. Samples V-1, V-4, V-5 were spiked with all As species in duplicate at three concentration levels: 5, 10, and 30 μg/kg.

Data Collection

8800/8900 ICP-MS/MS (Agilent)

- Concentric micromist, double-glass spray chamber at 2ºC
- 1550 W RF power, 1.8 V RF matching voltage, 8 mm sampling depth, 0.95 mL/min Ar carrier gas, 0.20 mL/min Ar makeup gas, O_2 flow (0.31 mL/min)

Results and Discussion

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. Figure 1 shows a representative calibration set of overlaid chromatograms for the 0.5, 1.0, and 5.0 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.

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

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.

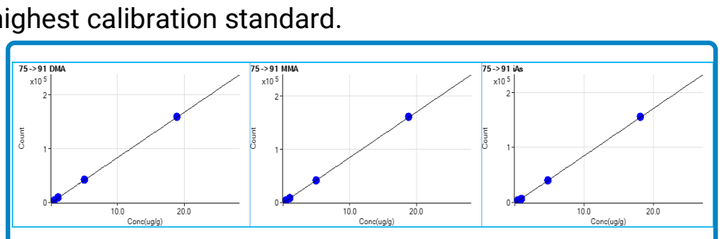


Figure 2. Calibration curves for DMA, MMA, and iAs.

The limits of detection (LOD) and limits of quantitation (LOQ) given in Table 2 are based on repeated measurements of the 0.05 μg/kg (ppb) mixed standard, n=15.

	LOD, μg/kg	LOQ, μg/kg	Estimated wine LOQ, μg/kg (6 x dilution)	Estimated rice LOQ, μg/kg (50 x dilution)
DMA	0.018	0.175	1.1	8.8
MMA	0.026	0.258	1.5	12.9
iAs	0.022	0.221	1.3	11.0

Table 2. Limits of detection and quantification estimated from repeated measurements of a 0.05 ppb mixed standard (N=15)

Results and Discussion

Table 3: Comparison of data measured at 2 labs using the fast LC method to reference FDA method 4.10 for 5 wine samples.

Style	Cultivar	% ethanol	DMA		iAs		Total As	
			Reference	Measured	Reference	Measured	Reference	Sum Species
Rosé	Zinfandel	9.5	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%)
White	Sauvignon blanc	13	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%)
Sparkling	Sparkling white blend	12	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%)
Dessert	Petite Sirah	20	1.7 ± 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%)
Red	Cabernet Sauvignon	14.5	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%)

Table 4: Analyses of rice reference materials. Uncertainty shown as 1 standard deviation (N = 3). % recovery shown in parentheses.

Rice	DMA		MMA		iAs		Sum	
	Reference	Measured	Reference	Measured	Reference	Measured	Reference	Measured
NIST 1568b	180 ± 12	195 ± 4 (109%)	11.6 ± 3.5	14.9 ± 0.9 (128%)	92 ± 10	105 ± 1 (114%)	285 ± 14	315 ± 3 (110%)
NMIJ 7503a	13.3 ± 0.9	15.4 ± 0.1 (116%)	None reported	< LOD	84.1 ± 3*	79 ± 4 (94%)	98 ± 7	94 ± 4 (96%)
NMIJ 7532a	18.6 ± 0.8	18.7 ± 1.3 (101%)	None reported	2.2 ± 1.9	298 ± 8	277 ± 12 (93%)	320 ± 10	297 ± 12 (93%)
ERM BC-211	119 ± 13	146 ± 3 (123%)	None reported	19.9 ± 0.6	124 ± 11	124 ± 2 (100%)	260 ± 13	290 ± 5 (112%)

* NMIJ 7503a iAs uncertainty estimated as the square root of the sum of squares of the AsIII and AsV uncertainties.

Method validation in a wine matrix

Table 3 shows results using the fast LC method compared to the current FDA method 4.10 matrix extension method which includes wine as a validated matrix. DMA and iAs results from the fast speciation method agreed within ± 13% of the reference method results. All MMA results were less than the LOD for both methods and are not included in table 3, but 1 ppb fortified portion recoveries ranged from 90% to 107%. The fortification solution included As^{III}, As^{V}, DMA and MMA. The sum of arsenic species agreed with the total arsenic to within 10%.

Results and Discussion

We injected a 1 ppb standard mix in 30% ethanol and did not observe non-spectral interference, e.g. carbon enhanced ionization, and the baseline was stable. Ethanol likely did not cause matrix effect because the injection volume was small and the mobile phase contains 3% methanol which overwhelms any effect from the ethanol injection.

Method Validation in a rice matrix

Species recoveries ranged from 93% to 123% of their certified values when concentrations were above LOQ. ERM BC211 DMA recovery was biased high in all 3 replicates but the z-score was 2.1. Z-scores are the number of standard deviations from the mean and are more informative than % recovery. Z-scores between -3 and +3 are sufficient for regulatory purposes. Although NIST 1568b MMA recovery was 128%, the measured concentration was < LOQ and the z-score was 0.94. Z-scores for all species and sum of species were less than 2.7 when concentrations were greater than LOQ.

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 above the LOQ of 1.1 μg/kg.

Overall, the concentration of iAs ranged from 1.7 ± 0.3 to 32.9 ± 0.8 μg/kg (which is above the FDA's action limit for iAs in apple juice of 10 μg/kg). The sum of 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.

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 and five market basket 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%)

Conclusions

The method presented here has been shown to work for both wine and rice matrices. Runtime has been decreased to 2 minutes, which is a factor of 10x faster than previous methods. The method also showed improved sensitivity and limits of detection and quantification compared to the current FDA method. Small injection volumes and addition of methanol in the mobile phase increased robustness to non-spectral interferences and makes this method more suitable for wine speciation. If needed, analysis time and resolution could be further improved by decreasing injection volume, increasing mobile phase flow rate and using a small volume, fast-washout spray chamber.

Acknowledgments and References

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