Feasibility Study for the Determination of Arsenic Species in Nutritional Products and Raw Ingredients by HPLC-ICP-MS

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Introduction

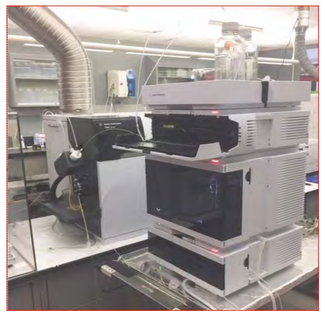
Overview and Importance. Hyphenated techniques utilizing high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) for elemental speciation is one of the fastest growing areas of research in atomic spectroscopy. Elemental speciation plays an important role in food safety. Arsenic is found in the natural environment (soil, water and air) and is a well known human carcinogen. The determination of the total form of arsenic only tells part of the story. For example, the toxicity of arsenic is strongly affected by its chemical form. The inorganic forms of As (arsenite (As (+3)) and arsenate (As(+5)) are generally more toxic than the organic forms of arsenic (dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA)). Other organic forms of arsenic such as arsenocholine (AsC) and arsenobetaine (AsB) are relatively non-toxic in humans. Therefore, their concentrations were not determined in this study.

Objective. To demonstrate the applicability of Agilent 7900 ICP-MS combined to the Agilent 1260 HPLC for the determination of arsenic species. In this study, we report the results obtained from Abbott Nutrition (AN) and UC-Davis laboratories for the determination of four arsenic species (arsenite (As+3), arsenate (As+5), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA)) in raw ingredients and milk based products by using HPLC-ICP-MS

Experimental

Instrumentation and chromatographic conditions

Figure 1. HPLC-ICP-MS: Agilent 7900 ICP-MS and 1260 HPLC at AN



- **At UC-Davis an Agilent Triple Quadrupole ICP-MS (run in single quad mode) was employed**

Table 1. Agilent 7900 ICP-MS operating parameters

Plasma mode	Kinetic Energy Discrimination
Radio Frequency Power	1550 W
Radio Frequency Matching	1.8 V
Sample Depth	10.0 mm
Nebulizer Gas	1.08 L/min
Nebulizer Pump	0.1 rps
Auxiliary Gas	0.90 L/min
Cooled Spray Chamber	2 °C
Collision Reaction Cell Gas	He (≥ 99.9999 %)
He Flow	4.3 mL/min
Plasma Gas	Argon

Experimental

Table 2. Agilent 1260 HPLC operating parameters

Analytical Column	PRP-X 100 10µm 250x4.6 mm
Column Temperature	20 °C
Injection Volume	100 µL
Total Acquisition time	20 min
Draw Speed	100 µL/min
Eject Speed	400 µL/min
Flow rate	0.5 mL/min

Table 3. Gradient settings

Time (min)	Mobile Phase (%) 0.5 mM ammonium citrate dibasic (pH=4.5)	15 mM ammonium citrate dibasic (pH=8)	Flow Rate (mL/min)	Max. Pressure Limit (bar)
0	100	0	1.0	400
2	100	0	0.50	400
2.01	100	0	0.50	400
3	0	100	0.50	400
3.01	0	100	0.50	400
10	0	100	0.50	400
10.01	100	0	0.5	400
12	100	0	0.50	400

Table 4. Microwave extraction operating parameters

Parameters	Value
100 % power, W	1600
Ramp to temperature, min	10
Hold time, min	60
Temperature, ºC	90
Cool down, min	20

Calibration Solutions

- Working standards (UC Davis) - 0 µg/kg, 0.4 µg/kg, 0.5 µg/kg, 1 µg/kg, 5 µg/kg, 10 µg/kg, 20 µg/kg; prepared from intermediate solutions
- Working standards (AN) - 0 µg/kg, 0.2 µg/kg, 1 µg/kg, 5 µg/kg,10 µg/kg, 25 µg/kg; prepared from intermediate solutions

Sample Material Used

1. NIST SRM 1568b – Rice Flour
2. NIST SRM 1549 – Non-Fat Milk Pwd
3. NIST SRM 1849a – Infant formula Pwd
4. NIST SRM 1643e – Trace Element in Water
5. Whey Protein Concentrate
6. EV4H2R – Infant Formula RTF
7. Acid Casein
8. Maltodextrin
9. Milk Protein
10. Rice Syrup
11. Cocoa Pwd

Results

Calculation of LOD and LOQ

- The instrument LOD ((3 x SD) + mean of the baseline noise) was obtained for each arsenic specie. The SD and baseline for the instrument LOD were obtained from 10 microwave extracted blanks.
- The LOQ was obtained by multiplying the instrument LOD by 3.3 or from the blank mean + 10 sigma.
- The LOQ numbers were then multiplied by the dilution factor for liquid (~25) and powder (~50) samples.
- LOQ values of approximately 2.5 ng/g and 5 ng/g (As(+3), DMA, MMA, and As(+5)), were obtained for liquid and powder samples, respectively.

Table 5. Retention times and recovery for four arsenic species

Specie	RT (min)	Theoretical Conc. (ng/g)	Measured Conc. (ng/g)	Recovery (%)
As+3	5	20.1	20.2	100.5
DMA	8.1	20	18.9	94.5
MMA	9.5	19.9	19.6	98.5
As+5	11.7	20.1	21.1	105.0

Figure 2. Separation of the four arsenic species in working standards

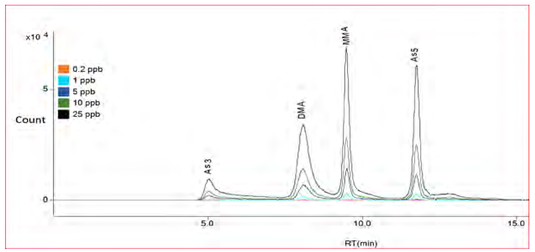


Figure 3. Calibration curves for As(+3), DMA, MMA, and As(+5)

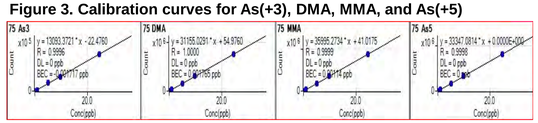


Table 6. Average spike recovery of 5 µg/kg As(+3), DMA, MMA, and As(+5) in NIST SRM 1849a

	As+3	DMA	MMA	As+5
Average (n=3)	100 %	104 %	105%	101 %
Range	97-102 %	103-105 %	104 -105 %	93-109 %

Table 7. Results from analysis of NIST SRMs for As species obtained at AN and UC Davis laboratories

		DMA			MMA			iAs			Speciation Total			Total As (Microwave Digestion)		
NIST SRM	Lab	Certified (ng/g)	Exp. (ng/g)	Rec. (%)	Certified (ng/g)	Exp. (ng/g)	Rec. (%)	Certified (ng/g)	Exp. (ng/g)	Rec. (%)	Certified (ng/g)	Exp. (ng/g)	Rec. (%)	Certified (ng/g)	Exp. (ng/g)	Rec. (%)
1568b	UC Davis	180 ± 12	173.0 ± 1.3	96	11.6 ± 3.5	14.9 ± 0.03	129	92 ± 10	98.1 ± 1	107	285 ± 14	286.1 ± 0.7	100	285 ± 14	278.9 ± 5.9	98
	AN		178.1 ±4.9	99	11.6 ± 3.5	10.1 ± 0.3	87	92 ± 10	92.3 ± 2.5	100	285 ± 14	280 ± 1	98	285 ± 14	277.9 ± 4.1	98
1549	UC Davis	NR	NR		NR	NR		NR	NR		1.9*	NR		NR	Diluted too high (~400x)	
	AN	NR	NR		NR	NR		1.9*	2.04 ± 0.05		1.9*	2.04 ± 0.05		1.9*	2.04 ± 0.05	107
1849a	UC Davis	NR	NR		NR	NR		NR	25.0 ± 0.4		NR	25.0 ± 0.4		NR	Diluted too high (~400x)	
	AN	NR	NR		NR	NR		NR	26.7 ± 1.0		NR	26.7 ± 1.0		NR	24.8 ± 2.1	92.9
1643e	UC Davis	NR	NR		NR	NR		58.98 ± 0.70	57.7 ± 1.0	98	58.98 ± 0.70	57.7 ± 1.0	98	58.98 ± 0.70	NR	NR
NR- Not reported					SRM 1549 - NonFat milk Powder			*Information value only- not certified by NIST								
SRM 1849a - Infant Formula Powder					SRM 1643e -Trace Element in Water			iAs = As(+3) + As(+5)								

Table 8. Quantitative results for the seven samples analyzed at UC Davis and AN. Average ± 1σ, n=2 for the individual species

Laboratory	Sample	iAs	DMA	MMA	Sum of Species
UC Davis	Whey Protein Conc.	26.57 ± 0.61	<LOD	<LOD	26.57 ± 0.61
AN		25.93 ± 0.49	<LOD	<LOD	25.93 ± 0.49
UC Davis	Cocoa Powder	31.88 ± 4.25	<LOD	<LOD	34.06 ± 4.23
AN		32.92 ± 1.28	LOD	<LOD	32.92 ± 1.28
UC Davis	Acid Casein	20.29 ± 1.09	<LOD	<LOD	20.29 ± 1.09
AN		22.35 ± 1.17	<LOD	<LOD	22.35 ± 1.17
UC Davis	Maltodextrin	27.87 ± 5.77	<LOD	<LOD	28.00 ± 5.75
AN		28.56 ± 0.76	<LOD	<LOD	28.56 ± 0.76
UC Davis	Milk Protein	18.02 ± 1.25	<LOD	<LOD	18.02 ± 1.25
AN		62.26 ± 5.20	<LOD	<LOD	62.26 ± 5.20
UC Davis	Rice Syrup	28.94 ± 0.73	13.88 ± 0.99	<LOD	42.83 ± 1.23
AN		27.76 ± 1.50	14.53 ± 1.13	<LOD	42.29 ±1.88
UC Davis	EV4H2R	15.62 ± 0.32	<LOD	<LOD	15.62 ± 0.32
AN		15.79 ± 0.41	<LOD	<LOD	15.79 ± 0.41

Conclusions

- Based on these results the Agilent 1260 HPLC combined with an Agilent 7900 ICP-MS is well suited for arsenic speciation in raw ingredients and nutritional products.
- Adequate sensitivity was demonstrated to meet regulatory requirements.
- Good accuracy, precision, and comparable results were obtained within and between AN and UC Davis laboratories.
- Inorganic arsenic (As(+3) and As(+5) species were the predominant species found in the raw ingredients and milk based samples
- The next step will be to develop a validated method for all four Arsenic species.

Acknowledgements

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