Arsenic speciation analysis in apple juice using HPLC-ICP-MS with the Agilent 8800 ICP-QQQ

Application note

Food and beverages

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Introduction

The presence of potentially toxic elements and compounds in foodstuffs is of intense public interest, and so food producers as well as regulators require rapid, reliable screening methods to accurately determine the levels of such contaminants in food and drink. In the case of arsenic (As), concentration levels in foods may be increased through the historical use of As-containing agrochemicals such as lead hydrogen arsenate (lead arsenate) or calcium arsenate. These compounds were used for much of the 20th century as a pre-harvest pesticide to control pests of fruit crops (primarily apples), such as coddling moth, apple maggots, and fruit fly. While widespread use of these pesticides ceased in 1970, lead and calcium arsenate are stable and persist in soil, so may still affect crops grown in contaminated soil long after application has ceased.

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As speciation is important in food safety because the toxicity of the element is strongly dependent on the chemical form or species in which it occurs. Inorganic arsenic species, arsenite (As(III)) and arsenate (As(V)) are known to be highly toxic and carcinogenic, whereas the organic As species monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are less toxic, and arsenobetaine (AB) is considered to be non-toxic. Levels of inorganic As are therefore routinely monitored in many sample types including drinking water, food and beverages, pharmaceuticals, and petrochemicals.

Separation of the various As-containing species by HPLC followed by detection using ICP-MS is well established as the analytical method of choice for many sample types, including drinking water and urine [1].

In this study, a novel sample preparation method involving simple filtration and dilution in deionized water was developed for fast, routine measurement of low levels of As species in apple juice. The established and proven method from Reference 1 was used to separate and quantify the five As species discussed above in six commercial apple juices, to determine whether this commonly consumed fruit juice contains arsenic species at potentially harmful levels.

The maximum contaminant level (MCL) for total As in drinking water has been established at 10 µg/L by the US Environmental Protection Agency (USEPA). But As in food must be considered as part of the total dietary intake, which is recommended by the USEPA and European Food Safety Authority to be between 0.8 and 8 ug/kg body weight per day. Other dietary sources of arsenic (in a normal diet) include seafood, rice and other sources.

Experimental

Sample preparation

Six different apple juice samples (Apple Juice #1 to #6) were purchased in a Japanese supermarket. The apple juice samples were filtered using two disposable filters (Millex-LH, Millipore, USA, followed by TOYOPAK ODS M, TOSHO, Japan). The Millex-LH filter was used to remove the solid materials contained in the apple juice, and the TOYOPAK ODS M filter was then used to remove non-polar compounds, to avoid overloading the HPLC column. Both filters were washed and activated according to the manufacturers' instructions. After filtration, the apple juice samples were diluted by a factor of two with ultrapure water. Aggressive sample digestion and large dilution factors were avoided in order to minimize species inter-conversion and ensure the lowest possible detection limits in the original samples.

In order to assess the method capability for accurate low-level measurement of As species, the potential for As species contamination during sample preparation was evaluated. Preparation blank samples were prepared as follows to identify any possible contamination arising from the different sample preparation steps:

1. Deionized water blank

2. Millex membrane filter blank

3. TOYOPAK ODS filter blank

4. Method blank (de-ionized water filtered twice as for samples).

All four preparation blank samples were measured using the established chromatography column, mobile phase and HPLC method from Reference 1, and the chromatograms obtained are shown in Figure 1. The chromatograms for the four preparation blanks are shown overlaid with a mixed As species standard (50 ng/L (ppt) each species), confirming that no detectable levels of As were present in the preparation blanks.

It should be noted that the first peak that elutes is arsenobetaine (AB), which is not retained on the column and so elutes in the void volume, where it might also co-elute with other neutral or cationic species that are not retained on the column. While AB can be measured using the LC-ICP-MS method described, the results may be biased if other co-eluting species are present in the sample; but this limitation is not a problem for food safety applications, since AB is considered to be non-toxic even at very high concentrations.

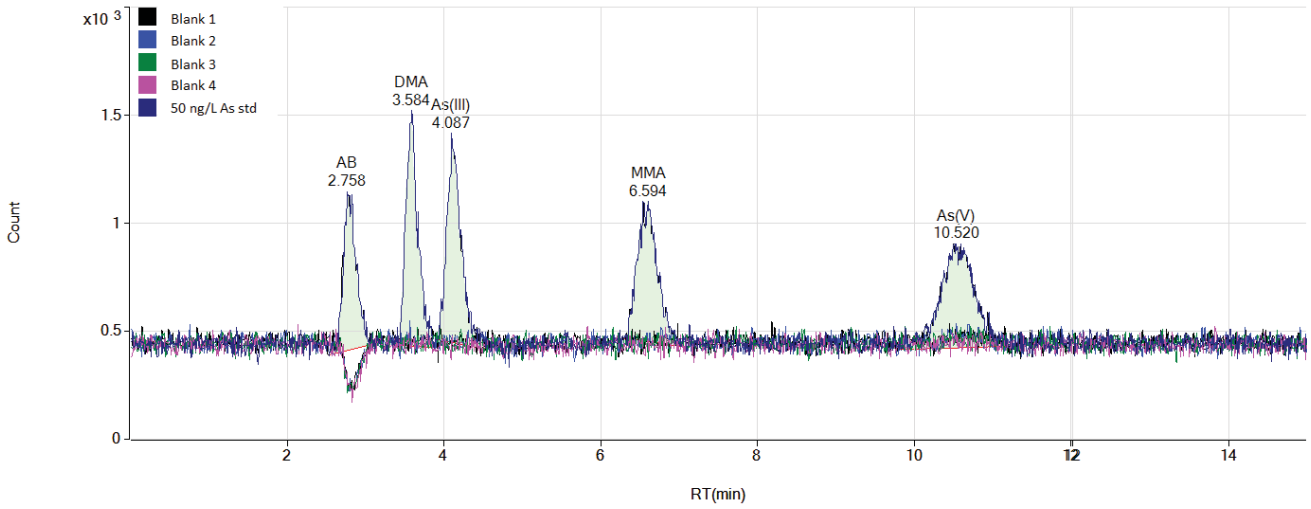

Figure 1. Evaluation of preparation blanks, confirming undetectable levels of As species contamination from the reagents and sample preparation filters. All four blanks are shown, overlaid with 50 ng/L (ppt) mixed As species standard.

For the purposes of food safety, the critical species that must be separated and quantified accurately and at low level are As(III) and As(V), the sum of which can be reported as 'total inorganic arsenic'.

Instrumentation

An Agilent 1290 Infinity LC system comprising a binary pump, autosampler and vacuum degasser was coupled to an Agilent 8800 Triple Quadrupole ICP-MS (ICP-QQQ). An anion exchange guard column (Agilent part number G3154-65002, 4.6 mm id x 10 mm polymethacrylate) followed by As speciation column (Agilent part number G3288-80000, 4.6 mm id x 250 mm polymethacrylate) were used for separation. The columns were maintained at ambient temperature for all experiments. HPLC and ICP-MS parameters are shown in Table 1.

The routine separation and analysis of As species does not require the use of ICP-QQQ to resolve interferences, as potential polyatomic overlaps on As (*m/z* 75) are resolved by the chromatography. As previously reported in Reference 1, the inorganic chloride elutes between the As(III) and MMA peaks and so the $ArCl^+$ polyatomic ion formed from the chloride does not affect any of the As compounds of interest.

However, the interest in As speciation in food and beverages extends to the measurement of the toxic inorganic compounds at extremely low concentrations (low ng/L or ppt levels) and the enhanced sensitivity and very low background of the Agilent 8800 ICP-QQQ compared to conventional quadrupole ICP-MS may therefore be beneficial. If slightly higher detection limits are acceptable within the method requirements, the sample preparation and HPLC method described here

Table 1. Operating conditions and parameters of the Agilent 1290 LC and 8800 ICP Triple Quad

1290 Infinity LC	
Condition	**Value**
Column	G3154-65002 (guard column), G3288-80000, 4.6 x 250 mm (analytical column)
Mobile phase	2.0 mM PBS / 0.2 mM EDTA/10 mM CH_3COONa / 3.0 mM $NaNO_3$ / 2% EtOH pH 11.00 adjusted with NaOH
Flow rate	1.0 mL/min
Temperature	Ambient
Injection volume	100 µL
8800 ICP Triple Quad	
Parameter	**Value**
RF power	1550 W
Carrier gas flow rate	1.05 L/min
Spray chamber temperature	2 °C
Sampling depth	10 mm
Exact 1 lens	0 V
Quadrupole mode	Single quad mode
Cell gas mode	No gas

is completely transferable without any modification to the Agilent 7700 Series ICP-MS, which offers around 2x lower sensitivity, but detection limits still in the low tens ng/L (ppt) range.

Results and discussion

Detection limits for each arsenic species were calculated as three times the chromatographic peak-to-peak signal-to-noise (S/N) ratio, alternatively sometimes expressed as the analyte concentration that would give a S/N of 3. The detection limits of all five As species were between 10 ng/L and 22 ng/L as summarized in Table 2. Figure 2 shows the 500 ng/L As species standard used for the S/N and LOD calculation, illustrating the good sensitivity and peak separation of the five As species studied.

The calibration concentration range was from 10 ng/L to 500 ng/L, showing linear response for each As species, as illustrated in Figure 3.

Results of apple juice analysis

The As concentrations determined in the six different apple juices are summarized in Table 3 (the 2x dilution factor has been applied to the results), and the chromatograms are displayed in Figure 4. From the concentration results it is clear that, while the majority of the As was in the toxic inorganic forms, all of the apple juice samples contained much lower levels of total As than the USEPA drinking water limit of 10 µg/L. The chromatograms in Figure 4 are all shown on the same intensity scale, to highlight the different relative concentrations of each species.

Table 2. 3x S/N detection limits for arsenobetaine (AB), dimethylarsinic acid (DMA), As(III) (arsenite), monomethylarsonic acid (MMA), and As(V) (arsenate).
* Arsenobetaine (AB) elutes in the void volume and cannot be reliably quantified in the presence of some other co-eluting species.

Compound	RT (min)	Height	Area	Noise	S/N	LOD (ng/L)	Noise type
AB*	2.823	19584	249584	153	127.99	11.72	Peak-to-peak
DMA	3.602	22117	277103	153	144.54	10.38	Peak-to-peak
As(III)	4.128	18022	265346	153	117.78	12.74	Peak-to-peak
MMA	6.566	14421	299863	153	94.24	15.92	Peak-to-peak
As(V)	10.431	10265	329325	153	67.08	22.36	Peak-to-peak

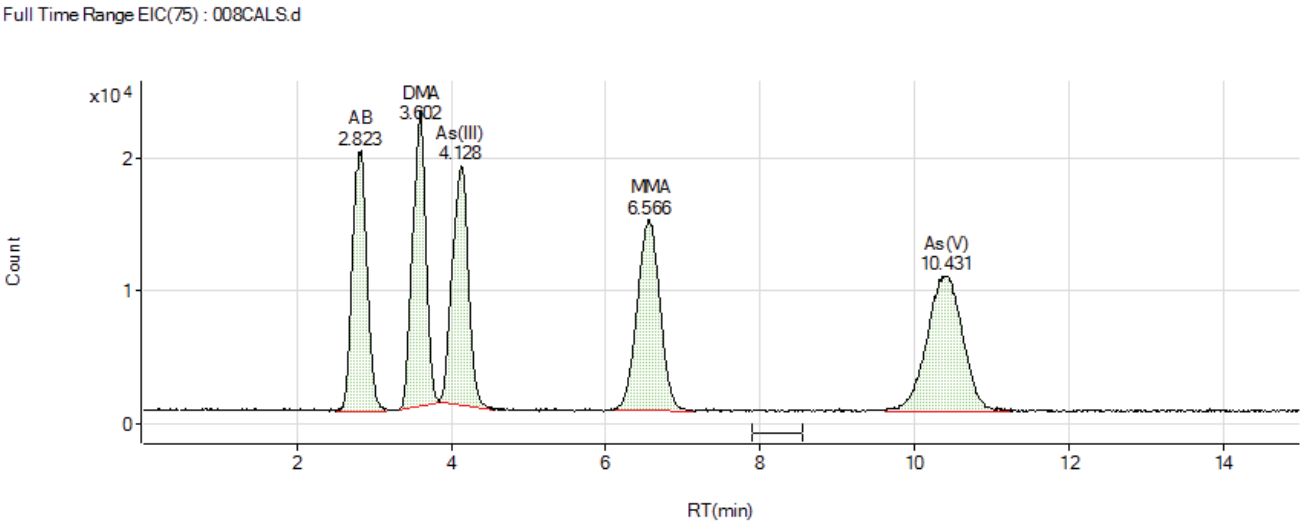

Figure 2. Chromatogram of 500 ng/L (ppt) mixed As species standard showing good sensitivity and peak separation, and noise region used for S/N and LOD calculation

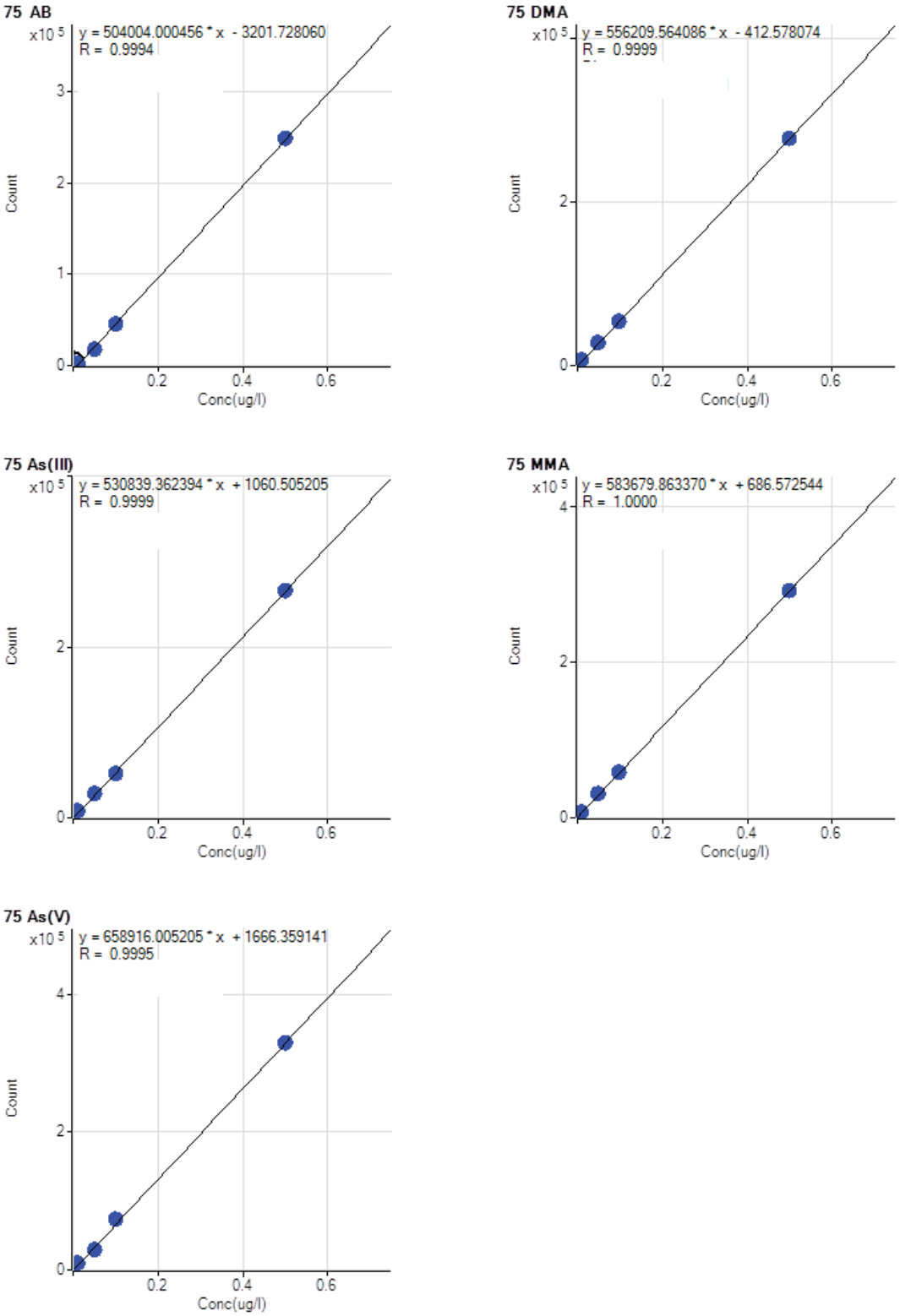

Figure 3. Calibration graphs for AB, DMA, As(III), MMA, and As(V)

Table 3. Quantitative results (µg/L) for all five As species in six commercial brands of apple juice measured by LC-ICP-QQQ

		Concentration µg/L						
Sample name	**Dilution**	**AB**	**DMA**	**As(III)**	**MMA**	**As(V)**	**Inorganic As**	**Total As**
Apple Juice 1	2	0.069	0.196	0.704	0.033	0.631	1.335	1.600
Apple Juice 2	2	0.066	0.037	0.062	0.006	0.008	0.070	0.173
Apple Juice 3	2	0.063	0.292	0.847	1.633	0.827	1.674	3.662
Apple Juice 4	2	0.052	0.276	1.014	1.475	1.977	2.991	4.794
Apple Juice 5	2	0.067	0.225	1.196	0.795	0.724	1.920	3.007
Apple Juice 6	2	0.043	0.254	1.218	0.005	0.095	1.313	1.610

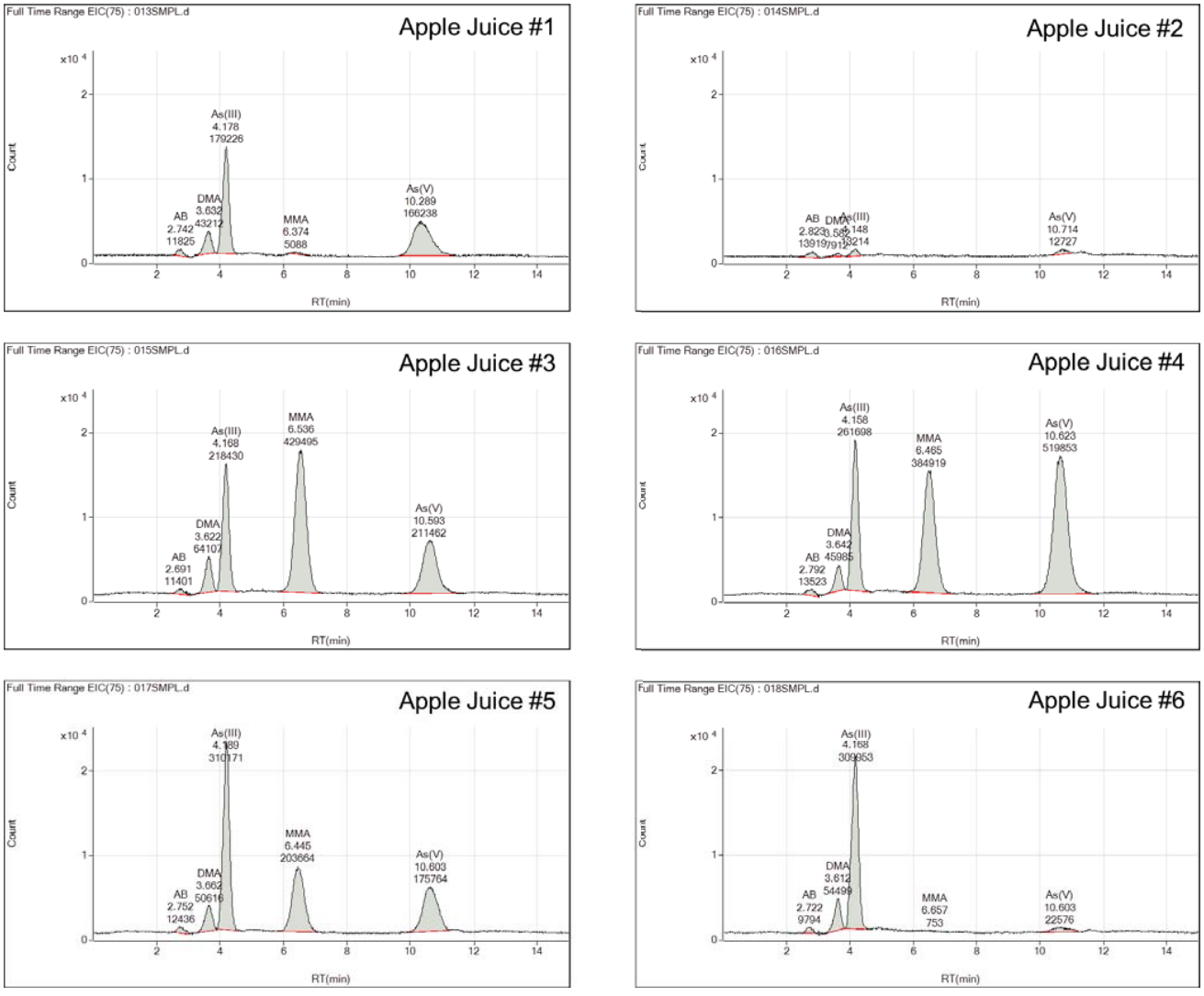

Figure 4. Chromatograms of As species in six apple juice samples

Although As species were found in all six apple juices, both the total As concentration and the relative concentrations of the different species varied among them. However the total concentration of arsenic (sum of all species) was below 5 µg/L in all tested juice samples, a level less than half the USEPA maximum contaminant level for drinking water (10 µg/L). The total inorganic As (sum of As(III) and As(V)) was less than 3 µg/L in all samples, and below 2 µg/L in five of the six apple juice samples measured.

Spike recovery test and reproducibility

In order to validate the method performance in real samples, a spike recovery test was performed using the mixed As species standard solution. Apple juice sample #1 was spiked with the As species standard at a level of 500 ng/L in the 2x diluted sample. The spiked sample was analyzed repeatedly as an unknown, with seven separate injections being measured in total. Table 4 shows the retention time (RT) and concentration results for all the As species, indicating the excellent reproducibility of both the RT and concentration for the seven separate analyses. The %RSDs for all As species were less than 0.5% for retention time and less than 1.6% for concentration. The overlaid chromatograms of all seven injections are shown in Figure 5.

Table 4. Results of spike recovery (n = 7) of 1 µg/L standard added to the Apple Juice #1

Sample name	AB		DMA		As(III)		MMA		As(V)	
	RT (min)	Conc. (µg/L)	RT (min)	Conc. (µg/L)	RT (min)	Conc. (µg/L)	RT (min)	Conc. (µg/L)	RT (min)	Conc. (µg/L)
Apple Juice 1 Spike 1	2.77	0.848	3.62	1.112	4.19	1.606	6.43	0.980	10.62	1.524
Apple Juice 1 Spike 2	2.76	0.862	3.61	1.116	4.19	1.632	6.43	0.996	10.63	1.560
Apple Juice 1 Spike 3	2.77	0.872	3.61	1.125	4.19	1.621	6.41	1.007	10.62	1.551
Apple Juice 1 Spike 4	2.77	0.886	3.61	1.122	4.18	1.632	6.41	1.003	10.63	1.554
Apple Juice 1 Spike 5	2.78	0.882	3.61	1.134	4.19	1.643	6.41	1.008	10.65	1.561
Apple Juice 1 Spike 6	2.78	0.873	3.61	1.146	4.17	1.637	6.37	1.016	10.68	1.597
Apple Juice 1 Spike 7	2.78	0.881	3.60	1.145	4.17	1.651	6.35	1.018	10.69	1.588
Average	2.78	0.872	3.61	1.128	4.18	1.632	6.41	1.004	10.65	1.562
Standard deviation	0.0076	0.0133	0.0058	0.0135	0.0096	0.0148	0.0306	0.0130	0.0291	0.0242
%RSD	0.28	1.53	0.16	1.20	0.23	0.91	0.48	1.29	0.27	1.55

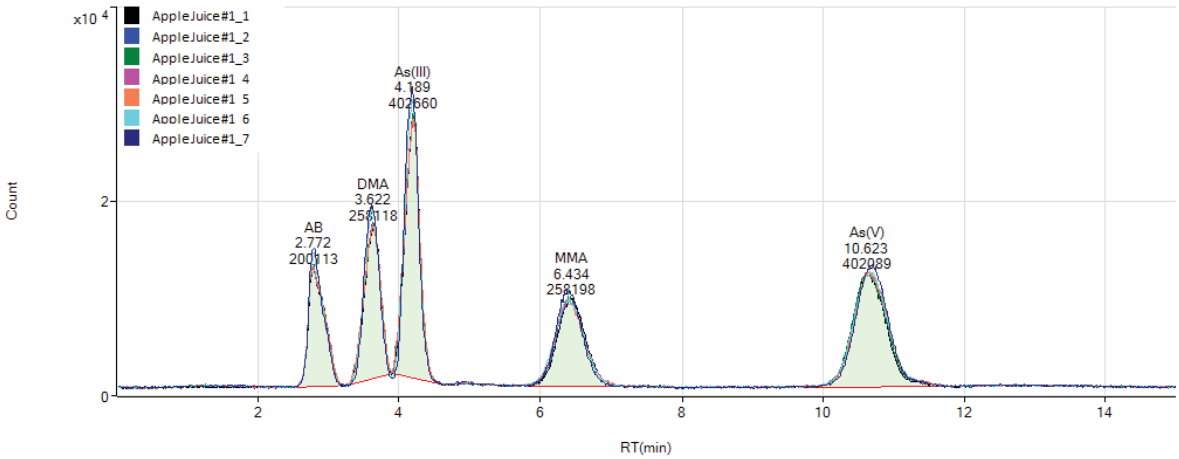

Figure 5. Seven overlaid chromatograms of Apple Juice #1, spiked with 500 ng/L As standard

Conclusions

Five arsenic species including the toxicologically relevant inorganic forms As(III) and As(V) were determined at low- and sub-µg/L concentrations in commercially available apple juice, using an Agilent 1290 Infinity LC coupled to an Agilent 8800 ICP-QQQ. Detection limits of between 10 and 20 ng/L (ppt) were obtained for all As species following a simple filtration and 2x dilution of the apple juice samples. Arsenic (As) species were detected in all of the apple juice samples measured, although the concentrations of the different species varied among the brands. None of the samples analyzed in this study contained more than 5 µg/L total As, and the level of inorganic As (sum of the As(III) and As(V) species) was below 3 µg/L in all samples and below 2 µg/L in five of the six brands analyzed.

On the basis of this small sample size of apple juice brands available in Japan, our data supports the current US Food and Drug Administration (FDA) advice that commercial apple juice does not pose a significant risk to health from raised levels of inorganic As. Our results for apple juice available in Japan confirm the findings of the FDA's monitoring program in the US, that the level of inorganic As is below the 10 µg/L drinking water limit in all apple juice samples tested.

Reference

1. Tetsushi Sakai and Steve Wilbur, Routine Analysis of Toxic Arsenic Species in Urine Using HPLC with ICP-MS, Agilent publication 5989-5505EN.

www.agilent.com/chem

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