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Determination of Arsenic Speciation in Apple Juice by HPLC/ ICP-MS Using the NexION 300/350

Introduction

Recent media coverage has brought the issue of arsenic (As) in apple juice into public awareness. Because arsenic can exist in several forms (some toxic, some non-toxic), it is important to identify the individual forms present in the juice. This is most easily accomplished through the use of liquid chromatography (HPLC), to separate the species, and inductively coupled plasma mass spectrometry (ICP-MS), to detect them. A challenge with juice analysis is the high level of sugars which can affect both the chromatography and the ICP-MS.

This work demonstrates an HPLC/ICP-MS method for the separation and determination of arsenic species in a variety of apple juices.

Experimental**Samples and Sample Preparation**

The apple juice samples were purchased from local grocery stores. Sample preparation involved a 2-fold dilution in the aqueous component of the mobile phase (i.e. without the methanol) with pH adjusted to 7.0 prior to analysis. All quantitative measurements were made against external calibration curves, with calibration standards prepared the same way as the samples.

Instrumental Parameters

Table 1 shows the ICP-MS conditions used for this analysis; the HPLC conditions were derived from previous work.¹ Because no arsenic interferences were found in the apple juice, Standard mode (i.e. no gas was used in the Universal Cell) was used for all analyses.

Table 1. ICP-MS Conditions.

Instrument	NexION® 300D ICP-MS
Nebulizer	Glass Concentric
Spray Chamber	Glass Cyclonic
RF Power	1600 W
Universal Cell Mode	Standard
Isotope	As75
Dwell Time	500 ms

Results and Discussion

From the analysis of several apple juice samples, the following arsenic species were found: trivalent arsenic (As₃), pentavalent arsenic (As₅), monomethyl arsenic (MMA), and dimethyl arsenic (DMA). These species were the focus of further investigation.

Figure 1 shows the chromatogram of a 0.5 µg/L mixed As standard, which demonstrates that the species of interest can be separated in under 4 minutes.

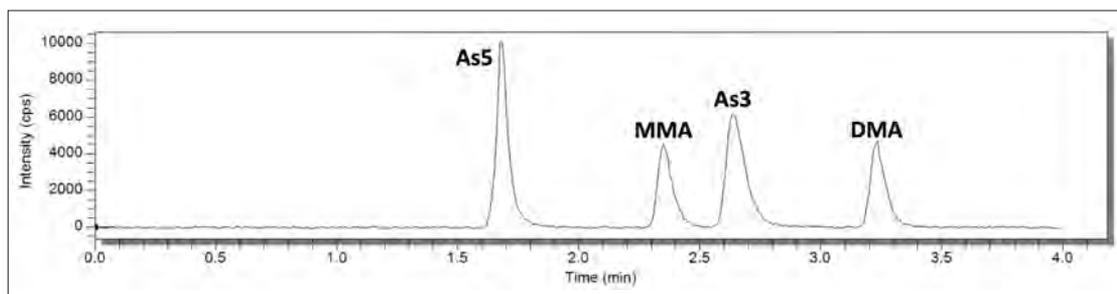


Figure 1. Separation of an arsenic standard containing 0.5 µg/L of each of As₅, As₃, MMA, and DMA.

To explore the effects of the apple juice matrix on the separation, a chromatogram of an arsenic standard was compared to the chromatogram of an apple juice sample. Figure 2 shows the chromatograms of a 0.5 µg/L mixed As standard and an apple juice sample. The juice matrix has a slight affect on the retention times of As₃ and DMA, but does not affect the peak shapes. These retention time shifts can be easily accounted for with appropriate retention time search windows.

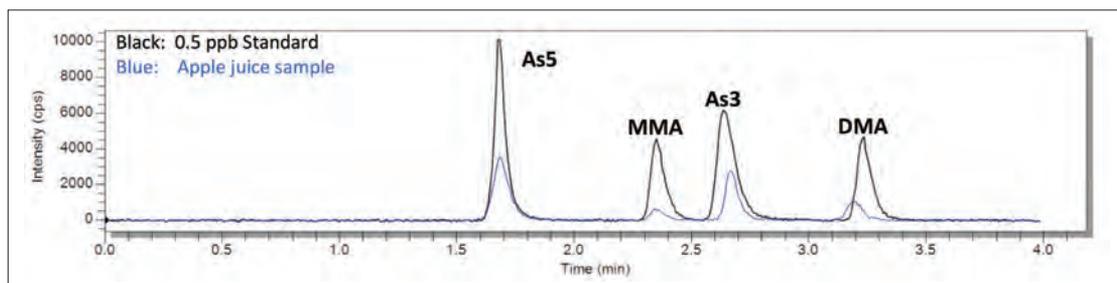


Figure 2. Chromatograms of a 0.5 µg/L mixed As standard (black) and an apple juice sample (blue).

The stability of the separation is demonstrated in Figure 3, which shows the chromatograms of 30 injections of the same apple juice sample over 2.25 hours, along with the concentration of each species.

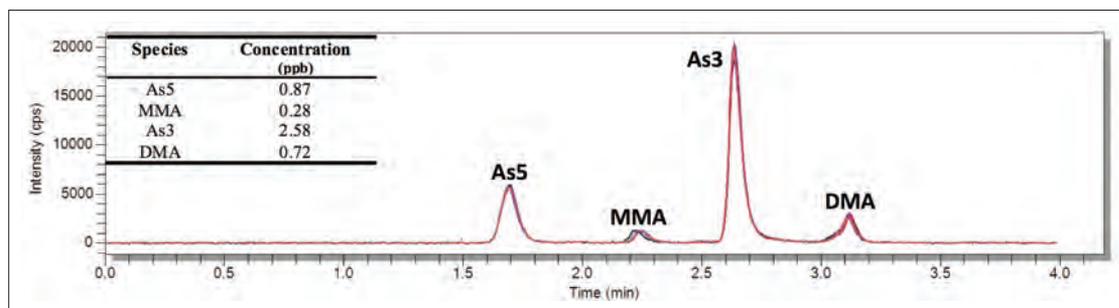


Figure 3. Chromatograms of 30 consecutive injections of the same apple juice sample, along with the concentration of each species.

Figure 4 shows the chromatograms for the calibration standards used in this work. Since the juice samples contain low levels of As, the calibration standards ranged from 0.1-1 µg/L and gave regressions greater than 0.999.

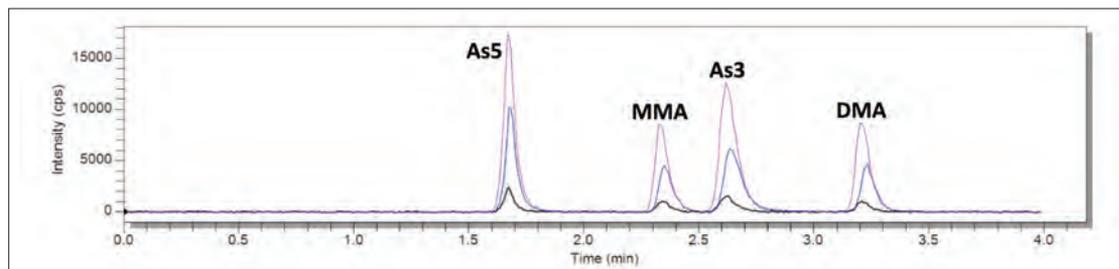


Figure 4. Chromatograms of As calibration standards between 0.1 and 1.0 µg/L.

To test the stability of the method, several samples were measured multiple times. Figure 5 shows the chromatograms and quantitative results from a typical sample over five consecutive injections. The consistency of the results demonstrates the short-term robustness of the method. Long-term robustness is demonstrated in Figure 6, which shows a stability plot over 8 hours, with all concentrations normalized to the first sample. For this test, a number of juice samples were analyzed, with a 0.5 µg/L check standard being analyzed every 6 injections. All results are within 10% of the initial measurement, indicating both the reproducibility of the chromatography and robustness of the NexION ICP-MS.

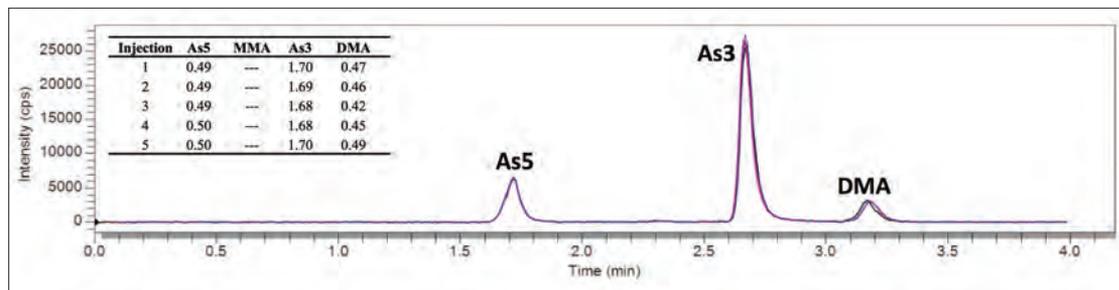


Figure 5. Chromatograms and concentration results from five consecutive injections of the same apple juice sample (units are µg/L).

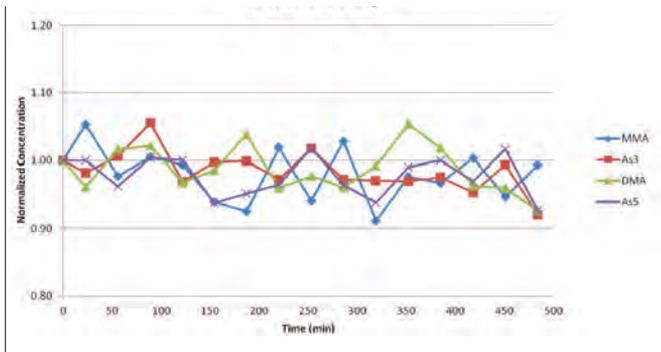


Figure 6. Eight-hour stability plot of a 0.5 µg/L check standard analyzed after every 6 injections of apple juice samples – all concentrations are normalized to the initial result.

Table 2 shows the results obtained from running a variety of different apple juice samples. Table 3 demonstrates the accuracy of the method by comparing the total arsenic values for eight samples obtained both by summing the speciation results (accounting for difference in concentrations between the arsenic species and arsenic as a component of the species) and ICP-MS measurement of total arsenic (i.e. ICP-MS analysis without the HPLC).

Table 2. Results from Various Apple Juice Samples (all values in µg/L).

Sample	As5	MMA	As3	DMA
1	1.69	–	2.07	0.85
2	0.95	–	0.21	0.62
3	2.17	1.21	1.17	0.50
4	2.02	–	1.88	0.69
5	0.79	0.36	2.37	0.67
6	0.56	0.19	0.40	0.31
7	0.47	0.44	0.82	0.45
8	0.79	–	3.23	0.92

(all values in µg/L).

Sample	Sum of Species	Total
1	4.24	4.22
2	1.55	1.49
3	4.14	4.26
4	4.18	4.27
5	3.95	3.67
6	1.46	1.23
7	1.88	1.77
8	4.52	4.73

Conclusions

This work has demonstrated a simple, rugged, fast method for arsenic (As) speciation in apple juice samples. To further validate the method, the analyses were repeated over multiple days on different instruments with columns from different lots. The similar results (not presented) verify the robustness of the method.

References

S. Miyashita, M. Shimoya, Y. Kamidate, *et. al.* **Chemosphere** 75 (2009), 1065-1073.