APPLICATION NOTE

HPLC-ICP-MS

Author:

Eve M. Kroukamp

PerkinElmer, Inc.
Woodbridge, ON, Canada

Characterization of Arsenic Species in Apple Juice using a NexSAR HPLC-ICP-MS Speciation Analysis Ready Solution

Introduction

Apple juice is a healthy, and often preferred, alternative to artificially flavored and carbonated drinks for many parents to give to children, posing as a valuable source of vitamins, minerals and fiber.[1] However, concern has been raised over the presence of arsenic in apple juices[2] due to high natural abundances and the historical use of arsenic in pesticides, where arsenic from such applications can persist in soil for decades after its use. This is of special concern in exposures involving children, since children elicit a more severe dose-response than adults.[3]

Although the information gained from the total analysis of arsenic can be useful, it may also provide a biased view about toxicity since different chemical forms of arsenic have differing levels of toxicities. Speciation studies, in contrast, are able to provide a wealth of information about toxicity as this relates to chemical species present, and the relative abundance of each species. For arsenic, the inorganic forms (arsenite [As III] and arsenate [As V]) are most toxic, whereas methylated forms of arsenic (dimethylarsinic acid [DMA], monomethylarsonic acid [MMA], etc.) are less toxic. Consequently, the action limit for inorganic As in apple juice set by the FDA is 10 µg/L,[4] where concentrations above this have been found to invoke a toxicological response in children.[1,2,3,5]

PerkinElmer®
For the Better

To separate the different chemical species of As, chromatographic techniques, such as HPLC, are often employed, where ICP-MS is the detector of choice for many analytical laboratories. This is due to its wide linear dynamic range, low detection limits, and ability to resolve complex interferences. For analytes other than As, the additional capability of ICP-MS to resolve different isotopes of elements empowers users to employ isotope dilution methods, which can greatly improve analytical accuracy.

A challenge faced by HPLC-ICP-MS users is that the majority of applications utilize salt buffers in strongly acidic [6,7] or strongly basic [9] mobile phases so as to achieve baseline resolution of the different chemical species of the element of interest. In steel-based systems, such extreme conditions may cause corrosion of pump and autosampler hardware over long-term use, where this effect will be more pronounced in components which make contact with the mobile phase. Although these systems can be somewhat passivated by flushing with 20% HNO_3, corrosion is still possible.[10] Any rust formed may become dislodged, blocking and sometimes irreversibly damaging expensive columns. This can be effectively prevented when using an HPLC system which has an inert, metal-free fluid path which would eliminate the issue of rust formation. Moreover, the use of this design would ensure that backgrounds are low for other analytes which are typically of analytical interest in trace speciation applications, such as Cr and Fe.

An additional challenge for users is that despite good pump practice involving flushing the pump with a weak organic solution at the end of each day, salt crystals may still form behind the pump seals. This can cause damage to the seal, affecting analytical results, and increasing maintenance costs and instrument downtime. This issue can be effectively mitigated by using an HPLC pump which has post-seal wash capabilities, thereby actively washing behind the pump seal, and reducing seal wear and tear.

In this study, four arsenic species in commercial apple juices were characterized using an HPLC method which was developed to achieve the fast and reproducible separation and analysis of low concentrations of As species. The reputable and verified method developed by Ernstberger *et al.*[7,8] was used to quantify the various arsenic species commonly found in apple juice (As III, As V, DMA, MMA) and thereby evaluate their potential toxicity. This analysis was performed using a PerkinElmer NexSAR™ HPLC-ICP-MS Solution, which comprises an inert NexSAR Speciation Analysis Ready HPLC system coupled to a NexION® ICP-MS, where the elution was thermostatically controlled using a NexSAR Column Oven.

Experimental

Sample Preparation

Calibration standards with concentrations of 0.1, 0.5, 1, 5, 10, and 20 µg/L were prepared in the mobile phase from the following reagents: As (III) from 999 ± 5 mg/L arsenite (Inorganic Ventures Ltd., Christiansburg, Virginia, USA), As (V) from 1003 ± 6 mg/L arsenate (Inorganic Ventures), DMA from cacodylic acid (≥ 99.0%, Sigma Aldrich, St. Louis, Missouri, USA), and MMA from 1000 mg/L MMA (Chemservice, West Chester, Pennsylvania, USA). These species were selected for evaluation due to their presence in past studies involving the analysis of apple juices.[6,7] Concentrations were chosen to reflect the concentration range around the current action level for inorganic arsenic as set out by the FDA.[4] The analysis took place using an acidic mobile phase (pH 4.0) which has been previously proven to be the most suitable method which accurately reflects the pH of apple juices, while also ensuring the integrity of the various arsenic species.[7]

Four popular, commercially produced apple juices were purchased at a local grocery store. No consideration was made for the length of time these juices were on the shelf. This is because the various chemical species of arsenic are known to be in equilibrium, and stable in commercial-grade apple juices over extensive periods of storage time.[6,7] Juices were well-shaken prior to sampling to ensure homogeneity, and 50 mL of each apple juice was filtered through 0.45 µm PTFE filters (hydrophilic, Millex, Sigma Aldrich) to remove unwanted particulate matter. Analyses were performed on undiluted samples.

During this work, the calibration standards were run, and the four apple juice samples, which had been decanted into a number of different plastic HPLC vials, were repeatedly analyzed. A blank sample was analyzed after every apple juice sample to check for carryover between samples. Due to the absence of a certified reference material, accuracy was ensured through spiking of the samples with 2 µg/L and 10 µg/L of each species (As III, As V, DMA, MMA).

Instrumentation

All analyses were performed using a NexSAR Speciation Analysis Ready HPLC system (PerkinElmer Inc., Shelton, Connecticut, USA) comprised of the NexSAR 200 Inert HPLC Pump, NexSAR Cooled Inert Autosampler, NexSAR Solvent Tray with Degasser, and NexSAR Column Oven (PerkinElmer Inc.). The system was coupled to a NexION ICP-MS (PerkinElmer Inc.). Details pertaining to the HPLC and ICP-MS conditions are shown in Tables 1 and 2, respectively, and were based on previous work.[7] During method development, m/z 75 and 77 were monitored to check for the presence of $^{75}ArCl^+$ interference on $^{75}As^+$. Since no $ArCl^+$ could be detected, the samples were analyzed in Standard mode for the rest of the study. All analyses and the collection of data were performed using Clarity™ software version 8.1.

Table 1. NexSAR Inert HPLC System Conditions.

Parameter	Value
Chromatography	Reversed-phase Ion-pairing Chromatography
Mobile Phase	Ion-pairing Reagent
pH	4.0
Separation Scheme	Isocratic
Injection Volume	20 µL
LC Vials	HPLC Tested PP Vials, 1.5 mL

Table 2. NexION ICP-MS Instrument Conditions.

Parameter	Value
Nebulizer	MEINHARD® plus Glass Type C
Spray Chamber	Glass Cyclonic
RF Power	1600 W
Injector	2.0 mm ID Quartz
Nebulizer Flow	Optimized for <2% oxides
Mode	Standard
Dwell Time	100 ms
Sampling Rate	10 points/sec

Results and Discussion

The correlation coefficients for the standards (0.1 – 20 µg/L) of As V, MMA, As III, and DMA were 0.99990, 0.99999, 0.99999, and 0.99998 respectively (Figure 1), where Figure 2 shows the overlap of the calibration standards. The latter (Figure 2) demonstrates the reliable and consistent flow rate delivered by the pump and shows that there is excellent reproducibility of the retention times, regardless of the concentration.

Owing to the inert fluid path of the NexSAR HPLC system, the chromatogram baseline is negligible for As, where the signal-to-noise ratio (S/N) for the 0.1 ppb standard ranged between 12 and 7 for the various chemical species of As. This allows for the detection of As at levels far below the FDA action limits and also ensures that chemical species, which were present in low abundances – such as MMA – can be easily quantified. This is important since the detection of chemical species, which are present in low concentrations, provides a more holistic view of toxicity. Moreover, in studies where mass-balance equations are used, being able to quantify trace-levels of analytes would greatly improve analyte recoveries, thereby improving the overall quality of such results.

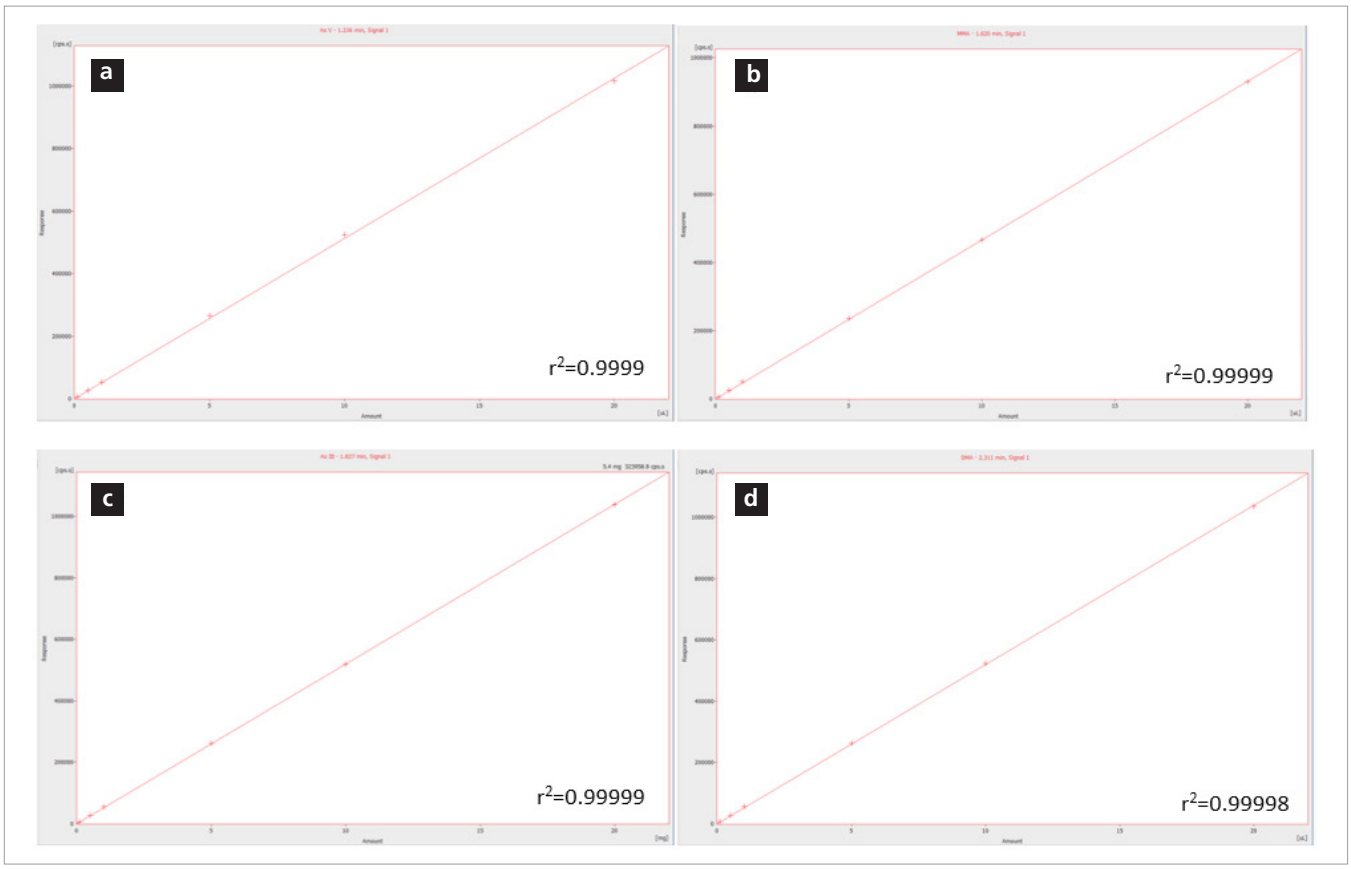

Figure 1. Linear regression of calibration standards ranging in concentration from 0.1-20 µg/L for (a) As V, (b) MMA, (c) As III, and (d) DMA in the mobile phase (pH 4.0) and the respective correlation coefficients.

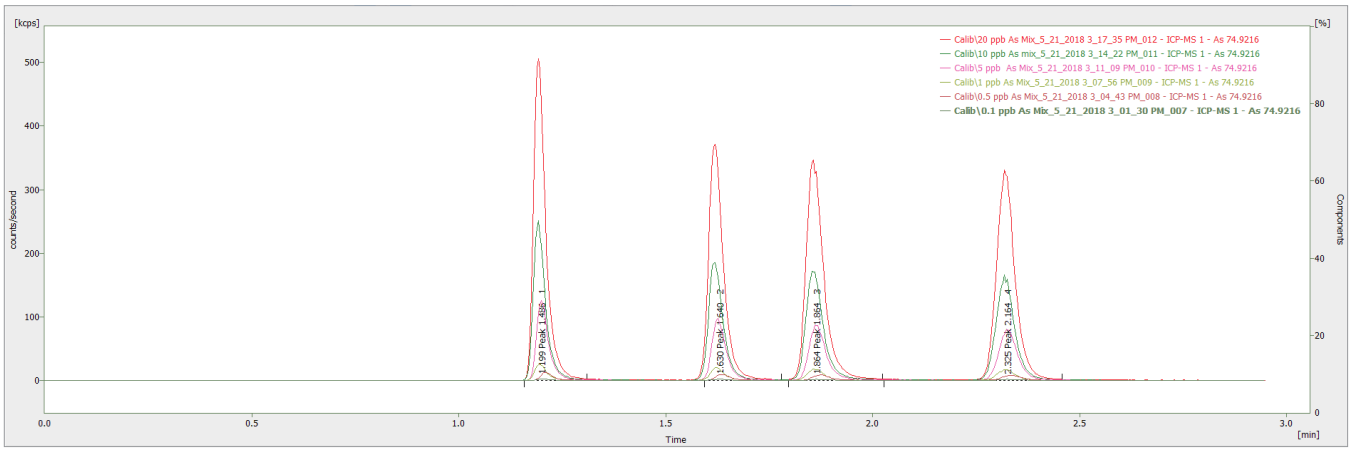

Figure 2. Chromatogram showing overlay of calibration standards (0.1 – 20 µg/L) in the mobile phase at (pH 4.0).

Apple juice is a complex matrix, so achieving reproducible results in undiluted samples can sometimes be challenging. In this study, injections of the same sample from different sample vials were found to exhibit excellent reproducibility (Figure 3) with a percentage relative standard deviation (% RSD) below 2% for all analytes. Moreover, low chromatographic baselines with negligible pulsation for both the samples and calibration standards demonstrates the robustness of the technique in different matrices, and the appropriateness of the HPLC-tested plastic vials for this application. The overlay also shows that injection volumes were highly repeatable, having a near-exact response at each injection. It should be noted that higher quality solvents and chemicals could lower the chromatographic baseline (S/N). Nevertheless, Figure 3 demonstrates that low-ppt analytes can be accurately quantified where the concentrations of As V, MMA, As III, and DMA are 0.94, 0.09, 0.77, and 0.20 µg/L respectively in this particular sample, and the S/N for the lowest concentration analyte (MMA) was 6.

Figure 3 also shows that a complete separation and accurate quantification of the four most common and relevant arsenic chemical species found in apple juices can take place in under three minutes per sample. This elution time is five times faster than traditional techniques, such as anion exchange chromatography, used in arsenic speciation studies. This work was performed without an internal standard and the reproducibility shown demonstrates that there is no need for one; however, if an internal standard is required, previous work has shown that arsenobetaine (AsB) can be used as a suitable internal standard eluting just after three minutes.[7] The analysis of a blank after each apple juice sample indicated that there was no carryover between injections. The absence of carryover is extremely useful, especially in commercial laboratories, where reduced rinsing between samples means less solvent use, lower overheads, and higher productivity.

In order to assess the impact of the apple juice matrix upon analytical accuracy, a low-end (2 µg/L) and high-end (10 µg/L) spike of each arsenic species was added to an undiluted sample. Figure 4 shows the chromatogram for the 2 µg/L and 10 µg/L spikes in comparison to the sample. Spiked samples were found to have similar retention times to the pure sample and exhibited good spike recoveries, ranging between 99-111% for the different arsenic species (2 µg/L spike: 103, 111, 100, 99% recovery and 10 µg/L spike: 106, 103, 111 and 101% recovery for As V, MMA, As III, and DMA respectively). This proves the accuracy of the method across a larger linear dynamic range and shows that samples can be prepared in a relatively simple manner.

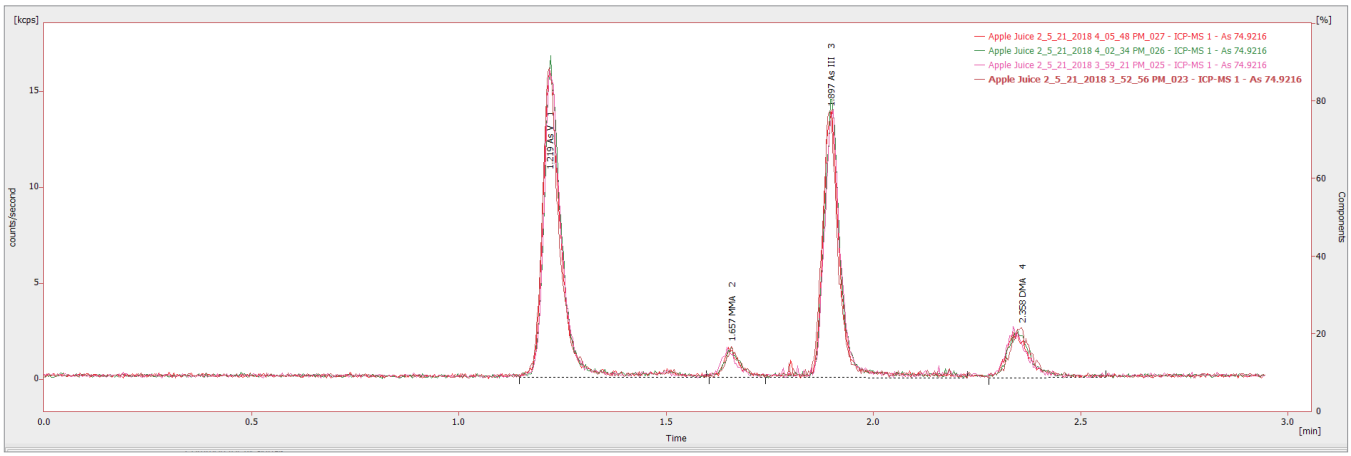

Figure 3. Chromatogram showing four injections (20 µL) of an undiluted apple juice sample from different sample vials.

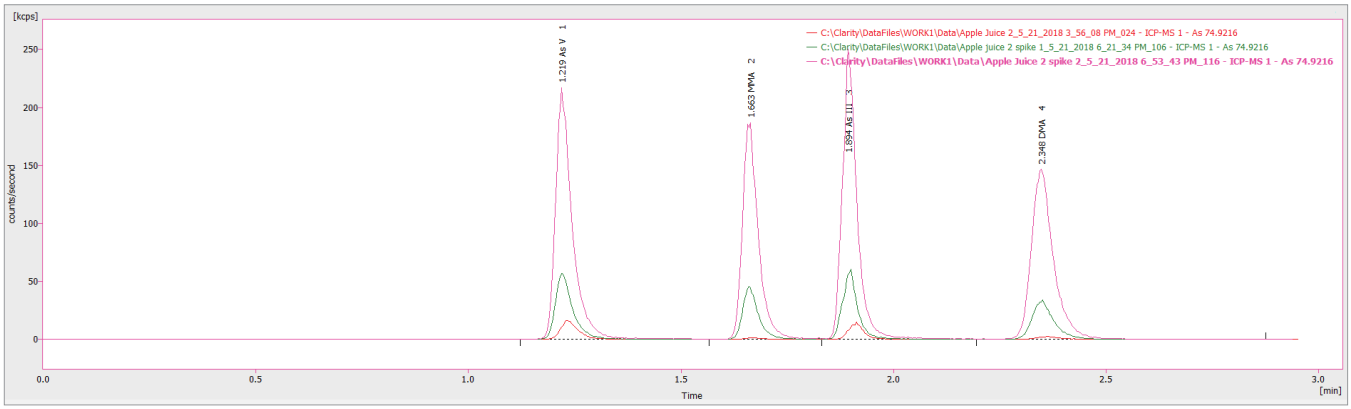

Figure 4. Chromatogram showing an undiluted apple juice sample as well as low-end (2 µg/L) and high-end (10 µg/L) spikes of this sample.

Figure 5 shows the results from the samples collected. As found in previous studies, the dominant forms of arsenic are the inorganic forms As III and As V, where DMA was the third most abundant and MMA was only present in a few samples.[6,7] It is clear that each of the apple juices have different concentrations of the various chemical species of arsenic, however all samples ranged from 16-34% of the action level of inorganic arsenic[5] and therefore is below the toxicological concern threshold as set by the FDA.[4]

Conclusion

This study has shown that the use of the reversed-phase ion-pairing method allows for the complete separation and accurate quantification of the main arsenic species in commercially available apple juices, including the primary toxic forms (As III and As V), in under three minutes. This work was performed using a NexSAR HPLC-ICP-MS Speciation Analysis Ready Solution, where concentrations were in the µg/L and ng/L range. The concentrations of inorganic As in all the apple juice samples analyzed in this study were under the FDA recommended action limit of 10 µg/L.[4]

The strength of the proposed methodology and reliability and robustness of the hardware were demonstrated through repeatability, carryover, and spike recovery studies. Moreover, the inert fluid path of the NexSAR Pump and Autosampler, and the post-seal wash of the pump, ensure peace of mind that samples can be run routinely at a low pH (pH 4.0 in this study), and with salted buffers, without being at risk for corrosion and significant seal damage.

References

1. Gerhauser C. 2008. Cancer Chemopreventive Potential of Apples, Apple Juice, and Apple Components, New York: *Planta Medica* DOI: 10.1055/s-0028-1088300.
2. Wilson D, Hooper C, Shi W. 2012. Arsenic and Lead in Apple Juice: Apple, Citrus and Apple-Base, *Journal of Environmental Health,* 75: 14-21.
3. de Burbure C, Buchet JP, Leroyer A, Nisse C, Haguenoer JM, Mutti A, Smerhovsky Z, Cikrt M, Trzcinka-Ochocka M, Razniewska G, Jakubowski M, Bernard A. 2006. Renal and Neurologic Effects of Cadmium, Lead, Mercury, and Arsenic in Children: Evidence of Early Effects and Multiple Interactions at Environmental Exposure Level, *Environmental Health Perspective,* 114: 584-590.

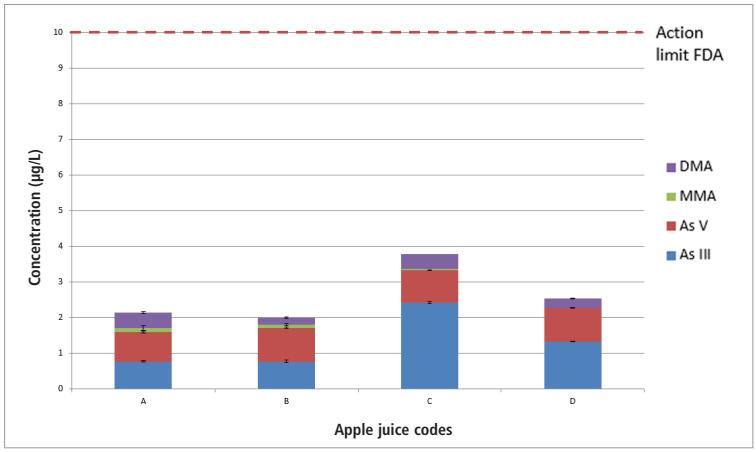

Figure 5. Averaged concentrations of As III, As V, MMA, and DMA in four commercially produced apple juices compared to the action limit of 10 µg/L, where the error bar shows the standard deviation (SD) across replicate analyses.

Consumables Used

Component	Description	Part Number
Reversed Phase Column	4.6 mm I.D x 250 mm, 5 µm	N8145326
HPLC Vials	HPLC Tested Plastic Vials, 1.5 mL PP	N9301736
PEEK Tubing	Yellow, 0.007" ID, 1/16" OD (5 feet)	N9302678
PEEK Fittings	Fingertight for 1/16" OD PEEK Tubing	09920513
Nebulizer Connector	Column-to-Glass Concentric Nebulizer Connector	N8152484

4. FDA. 2013. Supporting Document for Action Level for Arsenic in Apple Juice. FDA-2012-D-0322.
5. Bhattacharya P, Welch AH, Stollenwerk KG, McLaughlin MJ, Bundschuh J. 2007. Arsenic in the Environment: Biology and Chemistry, *Science of the Total Environment,* 379: 109-120.
6. Neubauer K, Perrone P, Reuter W. 2012. Determination of Arsenic Speciation in Apple Juice by HPLC/ICP-MS. *PerkinElmer Application Note.*
7. Ernstberger H, Neubauer K. 2015. Accurate and Rapid Determination of Arsenic Speciation in Apple Juice, *PerkinElmer Application Note.*
8. Ernstberger H, Neubauer K. 2015. HPLC, ICP/MS Sniff Out Arsenic in Apple Juice: The Use of Ion Pairing Chromatography with Cation Pairing Reagent Enables Faster Run-Time and Lower Detection Capability during the Analysis of Arsenic in Apple Juice. *Chromatography Techniques,* p10.
9. Heitland P, Köster HD. 2008. Fast Determination of Arsenic Species and Total Arsenic in Urine by HPLC-ICP-MS: Concentration Ranges for Unexposed German Inhabitants and Clinical Case Studies. *Journal of Analytical Toxicology 32:* 308-314.
10. Liquid Chromatography Problem Solving and Troubleshooting. 1994. *Journal of Chromatographic Science* 32:524 https://doi.org/10.1093/chromsci/32.11.524

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

014359_01 PKI