

# Use of Dual-Selectivity IC-ESI-MS for the Separation and Detection of Anionic and Cationic Arsenic Species

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There are times when an analytical challenge demands a solution that reaches beyond standard methodologies. The separation and detection of the most common, yet chemically diverse, arsenic species is one such challenge. In the present work, separation chemistry, system, and method features are discussed that allow the chromatographic retention of both anionic and cationic arsenic species using mobile phases that are compatible with electrospray ionization-mass spectrometry (ESI-MS) detection. The requirement is that all of the species be well retained in the separation in order to minimize void volume interferences and be detected with the structural information, as provided by ESI-MS.

Arsenic is ubiquitous in the environment, in toxic and nontoxic forms. It occurs in inorganic and organic compounds; in trivalent and pentavalent states; and as anions, cations, zwitterions, and neutral species. In general, methylated and other organoarsenicals are less toxic than inorganic arsenic, and pentavalent arsenic is considerably less toxic than the trivalent state. The speciation of arsenic is important to assess the risk to human health, and since some forms are not considered toxic, the suitability of

certain As-containing foods for human consumption. The inorganic forms of arsenic—arsenite and arsenate—are the usual forms found in drinking water, and the U.S. EPA has set a maximum contamination level (MCL) for total arsenic in drinking water, which took effect in January 2006, at 10  $\mu\text{g/L}$ .<sup>1</sup> Some foods, such as fish and seaweed, can contain organic forms of arsenic resulting from contamination and biological processes. One important form, arsenobetaine, is considered stable, metabolically inert, and relatively nontoxic, but is a common form of arsenic in some foods such as seafood.<sup>2,3</sup> Currently, there are no regulations on the individual arsenic species, only on the total arsenic found.

The five most common arsenic species include arsenite ( $\text{As}^{\text{III}}$ ), arsenate ( $\text{As}^{\text{V}}$ ), monomethylarsonic acid ( $\text{MMA}^{\text{V}}$ ), dimethylarsinic acid ( $\text{DMA}^{\text{V}}$ ), and arsenobetaine ( $\text{AsB}$ ) (see *Table 1*). ICP-MS is the most popular detection scheme for arsenic species, but it detects all species as

As, at  $m/z$  75. Speciation is commonly provided by chromatographic separation using multiple separation columns in order to achieve retention of all five species. The first four species listed above are anions, and the arsenobetaine can be retained as a cation at low pH. Alternately, anion exchange separations are shown in

**Table 2** Separation and detection parameters used in this method for five common arsenic species, sodium, and chloride

Analyte	Separation	ESI-MS Detection	Species Detected
Arsenite	Anion Exchange (with Suppressor)	SIM 107, Neg	$\text{AsO}_2^-$
Arsenate	Anion Exchange (with Suppressor)	SIM 141, Neg	$\text{H}_2\text{AsO}_4^-$
Arsenobetaine	Cation Exchange (No Suppressor)	SIM 179, Pos	$(\text{CH}_3)_3\text{As}^+\text{CH}_2\text{COOH}$
Monomethylarsonic Acid ( $\text{MMA}^{\text{V}}$ )	Anion Exchange (with Suppressor)	SIM 141, Pos	$\text{CH}_3\text{AsO}(\text{OH})_2\text{H}^+$
Dimethylarsinic Acid ( $\text{DMA}^{\text{V}}$ )	Anion Exchange (No Suppressor)	SIM 139, Pos	$(\text{CH}_3)_2\text{AsO}(\text{OH})\text{H}^+$
Sodium	Cation Exchange	SIM 23, Pos	$\text{Na}^+$
Chloride	Anion Exchange	SIM 35, Neg	$\text{Cl}^-$

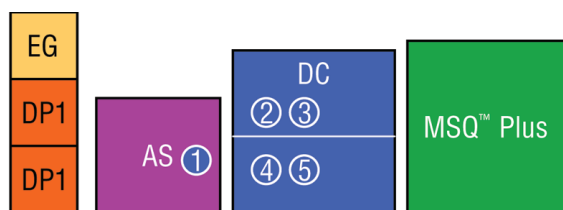
which the arsenobetaine is very close to the column void volume, making it susceptible to matrix interference.<sup>4,5</sup> To date, there is no single chromatographic separation that provides sufficient retention and separation of both anionic and cationic arsenic species using ESI-MS-compatible mobile phases.

This article discusses the use of dual-selectivity ion chromatography coupled with electrospray ionization mass spectrometry (IC-MS or IC-MS-MS) to provide retention of all five species and structural information using ESI-MS detection. The use of MS-MS detection is needed for complex matrices, especially in the determination of arsenite. The ICS-3000 ion chromatograph<sup>6</sup> (Dionex Corp., Sunnyvale, CA) was used with carefully selected ion chromatography methodologies to solve a tricky combination of separation and detection requirements posed by this analyte mixture.

An anion exchange separation with electrolytic suppression and a mixed-mode

**Table 1** Chemical structures of five common arsenic species

Arsenite ( $\text{As}^{\text{III}}$ )	Arsenate ( $\text{As}^{\text{V}}$ )	Monomethylarsonic Acid ( $\text{MMA}^{\text{V}}$ )	Dimethylarsinic Acid ( $\text{DMA}^{\text{V}}$ )	Arsenobetaine ( $\text{AsB}$ )
$\begin{array}{c} \text{O} \\    \\ \text{HO}-\text{As}-\text{OH} \\   \\ \text{OH} \end{array}$	$\begin{array}{c} \text{O} \\    \\ \text{HO}-\text{As}-\text{OH} \\   \\ \text{OH} \end{array}$	$\begin{array}{c} \text{O} \\    \\ \text{H}_3\text{C}-\text{As}-\text{OH} \\   \\ \text{OH} \end{array}$	$\begin{array}{c} \text{O} \\    \\ \text{H}_3\text{C}-\text{As}-\text{CH}_3 \\   \\ \text{OH} \end{array}$	$\begin{array}{c} \text{CH}_3 \\   \\ \text{H}_3\text{C}-\text{As}^+-\text{CH}_2\text{CO}_2\text{H} \\   \\ \text{CH}_3 \end{array}$
pKa = 9.29	pKa <sub>1</sub> = 2.26 pKa <sub>2</sub> = 6.76	pKa <sub>1</sub> = 3.6–4.1	pKa = 6.2	pKa = 2.2



Valves: 1, Diverter; 2 and 3 as below; 4 and 5 Injection Valves

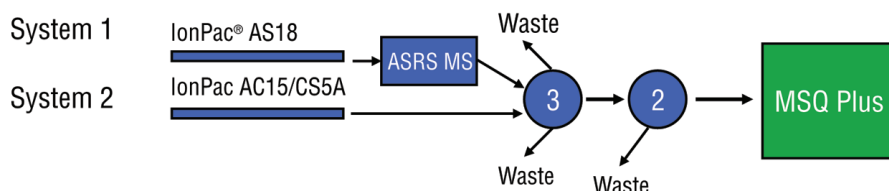


Figure 1 Schematic of the dual-selectivity IC-MS hardware configuration.

anion/cation exchange separation without suppression were combined in one analytical method to provide retention of a model group of five arsenic species that includes anions and a betaine. Within the analyte set, the MMA, DMA, and AsB are best detected by positive-polarity ESI-MS, and the arsenite and arsenate are best detected using negative-polarity ESI-MS. In addition, because the DMA crosses the membrane in the suppressor, it needs to be separated using anion exchange without suppression. Table 2 summarizes the requirements of this analyte set. The overall method illustrates the ability to separate and detect a suite of analytes with diverse acid/base properties from important matrix ions.

## Experimental

### Instrumentation

Figure 1 shows the flow diagram of the dual DS-IC-MS or DS-IC-MS-MS system with shared autosampler, chromatography module, and mass spectrometer. The ion chromatograph used in this work was the ICS-3000, which included a dual pump module with two analytical pumps (DP1 and DP2); an eluent generator (EG); conductivity detector (CD); autosampler (AS), including diverter valve; and a chromatography compartment (DC), including two high-pressure valves and two injection valves. The single-quadrupole mass spectrometer was the MSQ™ Plus (Dionex and Thermo Electron, Santa Clara, CA), and the triple-quadrupole

mass spectrometer was the API 2000 (Applied Biosystems/MDS SCIEX, Foster City, CA). Chromeleon® 6.8 software (Dionex) was used for all instrument control, data collection, and data reduction with the ICS-3000/MSQ Plus system. DCMS Link software version 1.1 (Dionex) was used for instrument control, data collection, and data reduction with the ICS-3000/API 2000 system.

The anion exchange separation used an IonPac AS18 analytical column (250 × 2 mm i.d., Dionex) with AG18 guard column (50 × 2 mm i.d., Dionex). The

mixed-mode separation was accomplished using an IonPac AC15 (50 × 2 mm i.d.) with an IonPac CS5A (250 × 2 mm i.d.) column. The suppressor was the ASRS® MS (Dionex) with external water at 50 mA current. The eluent for the AS18 anion exchange column was a KOH gradient, 6–52 mM in 15 min, 0.3 mL/min. The eluent for the mixed-mode separation was 80 mM formic acid, 0.37 mL/min.

Detection conditions for each analyte were optimized to provide the lowest detection limits within the capability of the single-quadrupole mass spectrometer. These were arsenite, SIM 107, –ESI, 50 V; arsenate, SIM 141, –ESI, 30 V; MMA, SIM 141, +ESI, 60 V; DMA, SIM 139, +ESI, 80 V; and arsenobetaine, SIM 179, +ESI, 70 V. ESI needle voltage was 3 kV. Two injections were made sequentially into two column sets, 13 min apart, and the detection data from the conductivity and ESI-MS detectors were collected in one data file.

### Standards

Arsenic (III) and arsenic (V) stock solutions were obtained from SPEX CertiPrep (Metuchen, NJ). Disodium methylarsenate (MMA) was obtained from Chem Services (West Chester, PA), and cacodylic acid (DMA) was

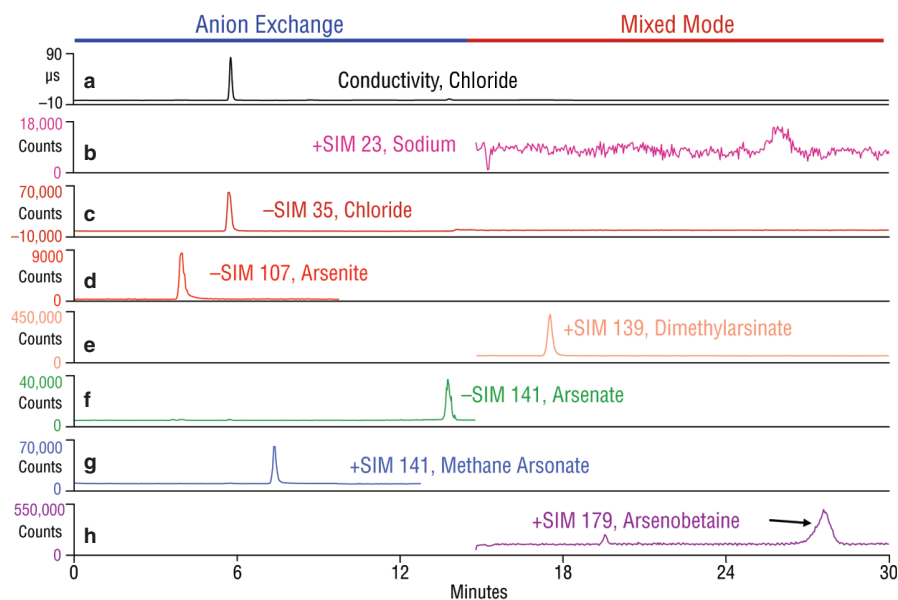


Figure 2 Stacked overlay of detection channels. a) System 1, conductivity, chloride; b) System 2, +SIM 23, sodium; c) System 1, –SIM 35, chloride; d) System 1, –SIM 107, arsenite; e) System 2, +SIM 139, DMA; f) System 1, –SIM 141, arsenate; g) System 1, +SIM 141, MMA; h) System 2, +SIM 179, arsenobetaine (minor peak arsenate); conditions in text.

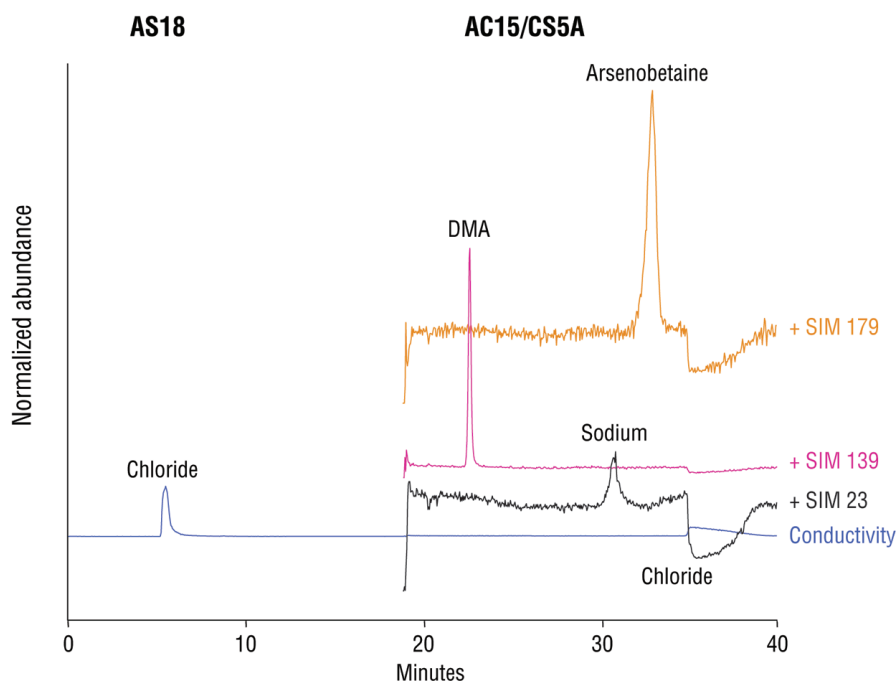


Figure 3 Overlay of detection channels for chloride, arsenobetaine, DMA, and sodium. Conditions same as Figure 2.

obtained from **Sigma-Aldrich** (Milwaukee, WI). The arsenobetaine was purchased from the European Commission (Reference Material no. 626). Deionized water (18 megohm-cm, Milli-Q, **Millipore**, Bedford, MA) was supplied to the eluent generator, CR-ATC device (**Dionex**), and suppressor, and was used to prepare all standards and samples.

## Results and discussion

### Separations

1. *Anion exchange with suppression.* The IonPac AS18 column is a latex-based, hydroxide-selective anion exchange column with 75  $\mu\text{Eq}/\text{column}$  in the 250  $\times$  2 mm format. The anion exchange phase is selective for hydroxide because the quaternary ammonium anion exchange sites bear an alkanol group that increases the hydration of the phase. Hydroxide selectivity is important since it allows use of a hydroxide-eluting ion that can be neutralized by a cation exchange suppressor (to water) so that the mobile phase entering the ESI-MS contains only water rather than nonvolatile hydroxide. The selectivity of the AS18 also permits elution of the trivalent arsenate ion while still retaining arsenite. The monomethylarsenate has intermediate selectivity on this column but is well separated from common sample matrix anions including chloride and sulfate. Chloride is also well separated from arsenite, which is important because chloride can cause significant signal suppression of coeluting analytes. The potassium hydroxide eluent produced by the eluent generator for this separation is suppressed to water using the electrolytic membrane suppressor, ASRS MS. The suppressor allows the use of a nonvolatile eluent, KOH, for the separation, by replacing the  $\text{K}^+$  with  $\text{H}^+$ . The resulting neutralization reaction presents water to the ESI interface, thus avoiding clogging from salt buildup.
2. *Mixed anion/cation exchange without suppression.* Arsenobetaine is a zwitterion; thus the best opportunity for retention is as a cation at acidic pH. Dimethylarsinic acid can cross the membrane of the suppressor during the neutralization process and cannot be determined using the suppressed AS18 system, even though separation is by anion exchange. AsB and DMA separately require cation exchange and anion exchange with no suppression for analysis. The IonPac CS5A

column is a mixed-mode anion/cation exchange column with 10  $\mu\text{Eq}/\text{column}$  of anion exchange capacity and 5  $\mu\text{Eq}/\text{column}$  of cation exchange capacity. This capacity enables both anions and cations to be retained on the CS5A phase. The formic acid eluent supplies formate as the anionic eluting ion and hydronium ion as the cationic eluting ion. Chloride elutes very close to arsenobetaine using a CS5A column and is well known to cause ESI signal suppression of coeluting anions. The separation of chloride from arsenobetaine is not adequate using CS5A alone, and signal suppression is observed on the SIM +179 channel during the elution of chloride. The additional anion exchange capacity in the form of an AC15 column added enough retention of the chloride to allow adequate separation of chloride and arsenobetaine.

### Detection

Using ESI-MS detection, the arsenicals can be detected as  $\text{M}^-$ ,  $(\text{M}+\text{H})^+$ ,  $(\text{M}+\text{H}\cdot\text{xH}_2\text{O})^+$ , etc., or as fragments.<sup>7</sup> Figure 2 shows the overlay of the separation and detection of the arsenic standards using these conditions. Arsenite is retained by anion exchange on the AS18 column (in the void volume of the AC15/CS5A system) and can be detected as the  $\text{H}_2\text{AsO}_3^-$  ion or as the  $\text{AsO}_2^-$  ion with better sensitivity. Arsenate is retained by anion exchange on both ion exchange systems and is detected with the best sensitivity as the  $\text{H}_2\text{AsO}_4^-$  ion. It can also be detected as an acid-water adduct in positive mode at  $m/z$  179. The monomethylarsenate is retained as an anion on the AS18 column but is detected with good sensitivity as the protonated species using +ESI. Arsenobetaine is protonated in 80 mM formic acid eluent, and is therefore retained by cation exchange on the AC15/CS5A column set. It is detected as a cation since the As bears a permanent positive charge. Figure 3 shows the separation of arsenobetaine from sodium and chloride using this column set. The DMA is retained as an anion and detected as a cation, but since it can cross the membrane of a suppressor, it is determined using the nonsuppressed AC15/CS5A system. The dips in the signals on all channels (signal suppression) are caused by the elution of chloride.

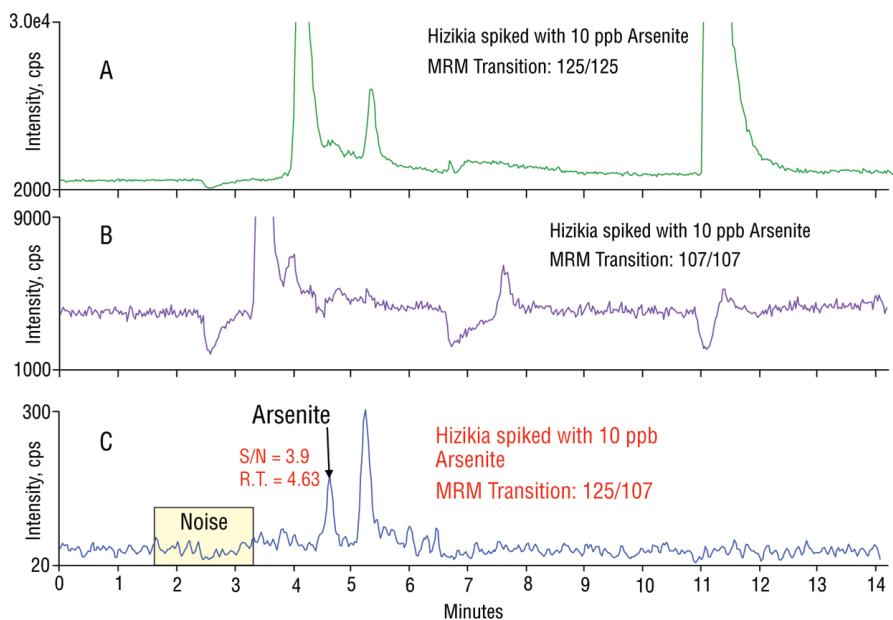


Figure 4 Stacked overlay of MRM channels for IC-MS-MS showing 10- $\mu\text{g/L}$  spike of arsenite into methanol/water extract of *Hizikia fusiforme*. a) MRM 125/125, b) MRM 107/107, c) MRM 125/107 showing detection of 10  $\mu\text{g/L}$  arsenite with S/N of 3.9. Separation conditions the same as Figure 2, except acetonitrile was added postcolumn at a flow rate of 0.2 mL/min.

Linearity over the range 5–100  $\mu\text{g/L}$  and reproducibility values in water were obtained using this method with a single-quadrupole mass spectrometer. Minimum detection limits in deionized water were calculated using external standard quantification since internal standards are as yet unavailable. The MDL calculation used seven replicates and the standard student's *t* test calculation. The  $R^2$  values for all five analytes in deionized water were 0.999, and the %RSD ranged from 1.7% for DMA to 7.7% for arsenobetaine. Method detection limits were determined for all species in both deionized water and a drinking water matrix of 100 mg/L each of chloride, carbonate, and sulfate. The MDLs are: arsenite, 10 and 20  $\mu\text{g/L}$  in the two matrices; arsenate, 8 and 5  $\mu\text{g/L}$ ; arsenobetaine, 15 and 20  $\mu\text{g/L}$ ; MMA, 4  $\mu\text{g/L}$  in both matrices; and DMA, 1  $\mu\text{g/L}$  in both matrices.

The authors applied this methodology to a very complex matrix, a methanol/water extract of *Hizikia fusiforme*. In very com-

plex matrices, it is necessary to use ESI-MS-MS detection in order to minimize interferences from multiple species. A significant example of the interferences that are found using selected ion monitoring (SIM)-only detection is shown in Figure 4. The figure shows detection using an MRM 107/107, 125/125, and 125/107 for 10  $\mu\text{g/L}$  arsenite spiked into the seaweed extract. It is evident that the 10  $\mu\text{g/L}$  arsenite is not identifiable in this matrix without use of the 125/107 MRM transition. An extraction procedure and internal standard are under development for this type of sample.

## Summary

The use of both suppressed anion exchange and nonsuppressed mixed anion/cation exchange separations in combination with positive- and negative-polarity electrospray ionization MS allows the determination of a diverse set of arsenicals. The system hardware and software provide the opportunity

to make sequential injections into two separation schemes and to collect data in one analytical method using ESI-MS detection. Judicious combinations of separation chemistries provide for retention of all species and separation from important matrix components including sodium and chloride.

## References

1. U.S. Environmental Protection Agency—Arsenic and Clarifications to Compliance and New Source Monitoring Rule. [www.epa.gov/safewater/arsenic/pdfs/quickguide.pdf](http://www.epa.gov/safewater/arsenic/pdfs/quickguide.pdf).
2. Sakurai, T.; Kojima, C.; Ochiai, M.; Ohta, T.; Fujiwara, K. Evaluation of in vivo acute immunotoxicity of a major organic arsenic compound arsenobetaine in seafood. *Immunopharmacology* **2004**, *4*, 179.
3. Le, X.C.; Lu, X.; Li, X. Arsenic speciation. *Anal. Chem.* **2004**, *76*(1), 26A–33A.
4. Schaeffer, R.; Soeroes, C.; Ipolyi, I.; Fodor, P.; Thomaidis, N.S. Determination of arsenic species in seafood samples from the Aegean Sea by liquid chromatography-(photo-oxidation)-hydride generation-atomic fluorescence spectrometry. *Anal. Chim. Acta* **2005**, *547*, 109–18.
5. IonPac AS7 Product Information Bulletin, Dionex Corp., Sunnyvale, CA, 2003.
6. Jack, R. A full-featured, dual ion chromatography system. *Am. Lab.* **2006**, *36*(3), 24–9.
7. Florencio, M.H.; Duarte, M.F.; de Betencourt, A.M.M.; Gomes, M.L.; Vilas Boas, I.F. Electrospray mass spectra of arsenic compounds. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 469–73.

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