

# High Sensitivity Arsenic Speciation: HPLC Sector Field ICP-MS

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## Key Words

- ELEMENT 2
- Arsenic
- Chromatography
- High Resolution ICP-MS
- HPLC
- Speciation

## Introduction

The toxicological and biological properties of elements, as well as their mobility and bioavailability, can depend on the species in which the elements occur. In the case of arsenic for example, arsenite is more toxic than arsenate, monomethylarsonic acid and dimethylarsinic acid while arsenobetaine is non-toxic. It is more important therefore, to determine the concentrations of the different species rather than just the total elemental concentration. The coupling of High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) and Capillary Electrophoresis (CE) with ICP-MS are accepted hyphenated techniques for such speciation analysis. There are many examples of Sector Field ICP-MS coupled with HPLC<sup>[1,2,3]</sup>, GC<sup>[4,5]</sup> or CE<sup>[1,6,7]</sup> for speciation analysis.

One of the main characteristics of Sector Field ICP-MS, in addition to the high mass resolution, is the outstanding sensitivity and signal-to-noise ratio. By coupling the Thermo Scientific ELEMENT 2 Sector Field ICP-MS with chromatography, these advantages result in extremely low detection limits, leading to a new dimension in speciation analysis.

This application report describes the simple on-line coupling of HPLC with the ELEMENT 2 Sector Field ICP-MS for the analysis of the five arsenic species shown in Table 1. All species are separated in an isocratic HPLC mode within twelve minutes. Detection limits are between 0.3 to 1.6 pg g<sup>-1</sup> and are significantly lower than those for quadrupole based ICP-MS<sup>[8]</sup>. Aspects of the flexibility of sector field ICP-MS from the use of different resolution modes are also described.

As(III)	Arsenite	AsO <sub>2</sub> <sup>-</sup>
As(V)	Arsenate	H <sub>2</sub> AsO <sub>4</sub> <sup>-</sup>
MMAA	Monomethylarsonic acid	CH <sub>3</sub> AsO(OH) <sub>2</sub>
DMAA	Dimethylarsinic acid	(CH <sub>3</sub> ) <sub>2</sub> AsOOH
AsB	Arsenobetaine	(CH <sub>3</sub> ) <sub>3</sub> As <sup>+</sup> CH <sub>2</sub> -COO <sup>-</sup>

Table 1: Investigated arsenic species.

## Experimental

The Thermo Scientific ELEMENT 2 Sector Field ICP-MS was coupled to an HPLC system with a short piece of PEEK™ capillary and standard LC fittings. Figure 1 shows the experimental setup. The HPLC parameters used in this study are shown in Table 2. The instrument settings of the ELEMENT 2 are listed in Table 3.

Some features of the ELEMENT 2 particular for speciation analysis are:

- The plasma interface is maintained at ground potential. This enables easy coupling of HPLC<sup>[1,2,3]</sup>, GC<sup>[4,5]</sup>, CE<sup>[1,6,7]</sup> or laser<sup>[9,10]</sup> sample introduction systems.
- Due to the high accelerating voltage of 8000 V, excellent focusing properties and high ion transmission, the ELEMENT 2 possesses an outstanding sensitivity of > 10<sup>6</sup> cps/ng g<sup>-1</sup> <sup>115</sup>In. In combination with the low dark noise of < 0.2 cps, this leads to extremely low limits of detection, which are needed for determining the low concentrations of elemental species in biological and environmental samples.
- The new magnet technology of the ELEMENT 2 incorporates a high power Field Regulator that enables full mass range analyses with a duty cycle of > 90%<sup>[9,10]</sup>. When the elements of interest are within a relative mass range of 30% (e.g., <sup>75</sup>As and <sup>77</sup>Se), duty cycles of > 99.9% are achievable.
- Fast peak jumping (e.g. across 10% of peak width) results in high precisions, important e.g. for on-line isotope dilution.
- A simultaneous dual-mode detector with a linear dynamic range of > 10<sup>9</sup>, enables the accurate detection of low and high abundant elemental species.
- A robust plasma with a high tolerance to organic solvents allows the use of organic solvents in the mobile phase<sup>[3,11]</sup>.
- The ELEMENT 2 sample introduction system is compatible with flow rates from standard-bore, narrow-bore and capillary Liquid Chromatography: uptake rates from a few μL/min to 1 mL min<sup>-1</sup> are possible.
- High mass resolution is the only method to definitively find and identify interferences and to therefore avoid possible misidentification of species not containing the elements of interest.

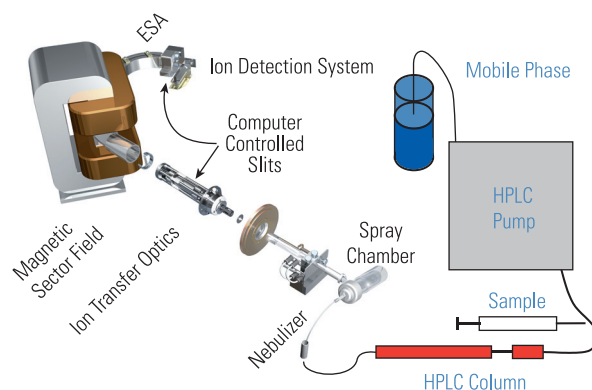


Figure 1: Schematics of HPLC/Sector Field ICP-MS.

Stationary phase:	Hamilton PRP-X 100, 250 x 4.6 mm, 4 $\mu\text{m}$ , with guard column 8 x 3 mm
Mobile phase:	2.6 mM $(\text{NH}_4)_2\text{HPO}_4$ , 1mM EDTA, 1% ethanol, pH 8.2 with $\text{NH}_3$
Sample loop:	200 $\mu\text{L}$
Flow rate:	1 mL $\text{min}^{-1}$

Table 2: HPLC parameters.

Nebulizer:	Quartz concentric
Spray chamber:	Quartz double pass, cooled (2 $^{\circ}\text{C}$ )
Cones:	Nickel
RF power:	1300 W
Isotope monitored:	$^{75}\text{As}$
Resolutions:	low (300) or high (10000)
Mass window:	10% in low, 120% in high resolution
Integration window:	10% in low, 40% in high resolution

Table 3: Instrument settings of the Thermo Scientific ELEMENT 2 as detector.

In order to obtain the highest sensitivity, precision and the best detection limits the following points were considered for this analysis:

- The mass window size was optimized to give best sensitivity and precision.
- The magnet was set to a fixed mass and the As peak was scanned electrically, leading to a duty cycle of > 99.9%.
- 1% ethanol was added to the mobile phase in order to increase the As signal.
- EDTA was added to the mobile phase in order to shorten elution times and to obtain sharper chromatographic peaks with higher intensities.
- The HPLC flow rate used (1 mL  $\text{min}^{-1}$ ) was similar to the normal uptake rate of the quartz concentric nebulizer used.
- The best baseline stability and lowest As background in the HPLC effluent was achieved after repeated flushing of the HPLC column with diluted nitric acid, water and diluted ammonia.
- The mobile phase composition was optimized in high resolution mode by investigating and minimizing background signals and interferences.

### Software Features for Transient Signals

The Thermo Scientific ELEMENT 2 software contains several features for convenient acquisition, display and evaluation of transient signals:

- External devices, e.g. HPLC, GC, CE or laser, can trigger data acquisition of the ELEMENT 2 using contact closure or with a 5V TTL signal.
- All measured transient signals can be monitored online. Figure 2 shows the online chromatogram display after injection of 100  $\text{pg g}^{-1}$  of five arsenic species.
- Additionally, time resolved mathematical calculations of different traces can be displayed, e.g. for on-line isotope dilution.

- Chromatograms can be exported automatically in various user defined file formats:

- ANDI (AIA netCDF)
- ASCII
- GRAMS
- Spectacle
- Xcalibur<sup>®</sup>

- Basic evaluation of transient signals is possible in the ELEMENT software but for more advanced data processing a specialized chromatographic data suite is recommended, e.g. GRAMS/AI from Thermo Fisher Scientific. This software package is a powerful software tool for analyzing and processing chromatographic and spectral data. An example of the data evaluation possible in GRAMS/AI is shown in Figure 3.

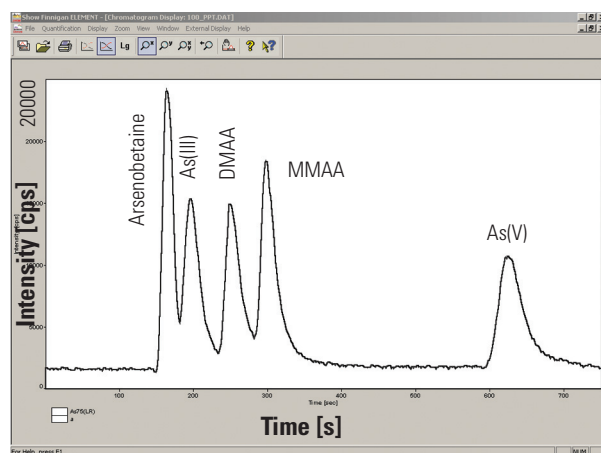


Figure 2: Online chromatogram of a solution containing 100  $\text{pg g}^{-1}$  of five arsenic species.

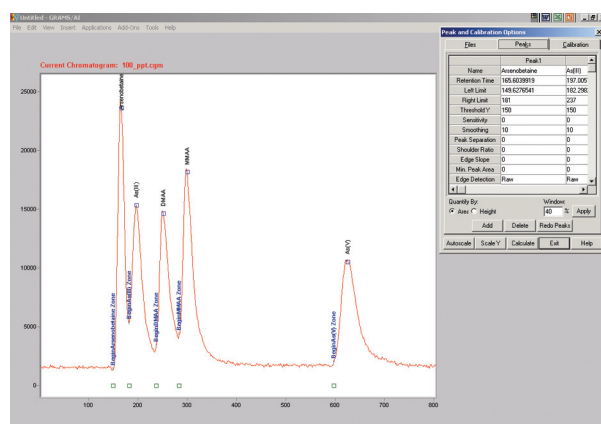


Figure 3: Data evaluation in GRAMS/AI.

### Results and Discussion

The coupling of HPLC with the Thermo Scientific ELEMENT 2 for speciation analysis results in outstanding sensitivity, extremely low detection limits, a wide linear range, good precision and good linearity of calibration curves. The figures of merit for measurements in low mass resolution mode are shown in Table 4. Depending on the arsenic species, a sensitivity of 90 to 240 cps/ $\text{pg g}^{-1}$  As is obtained.

This sensitivity leads to extremely low detection limits, ranging from 0.3 to 1.6 pg g<sup>-1</sup> As. These detection limits are only limited by the chromatographic system (contamination of the mobile phase, washout effects after injection of high concentration samples etc).

Calibration curve parameters are listed in Table 5. The linear dynamic range is between 10 pg g<sup>-1</sup> and 10 ng g<sup>-1</sup> with correlation coefficients of 0.9997 or higher. Signal precisions are better than 5% while reproducibility of retention times is better than 3%. As an example, Figure 4 shows the linearity of the calibration curve for MMAA. The good correlation and precision is due to the good stability of the ELEMENT 2 and to the extremely high sensitivity, which gives good counting statistics even at low concentrations.

	RETENTION TIME [min]	LOD [pg g <sup>-1</sup> As]	SENSITIVITY [cps/pg g <sup>-1</sup> As]
AsB	2.7	0.3	241
As(III)	3.2	1.0	165
DMAA	4.2	1.1	132
MMAA	4.9	0.9	157
As(V)	10.5	1.6	94

Table 4: Figures of merit in low resolution.

	CONCENTRATION [ng g <sup>-1</sup> ]	CORRELATION COEFFICIENT r	RSD [%] HEIGHT*	TIME **
AsB	0.01 -10	0.9999	2.1	1.7
As(III)	0.01 -10	0.9999	4.5	2.1
DMAA	0.01 -10	0.9997	1.9	1.2
MMAA	0.01 -10	0.9999	4.3	1.8
As(V)	0.01 -10	0.9997	4.3	2.5

\* n = 4, 500 pg g<sup>-1</sup> As, \*\* n = 21

Table 5: Calibration parameters.

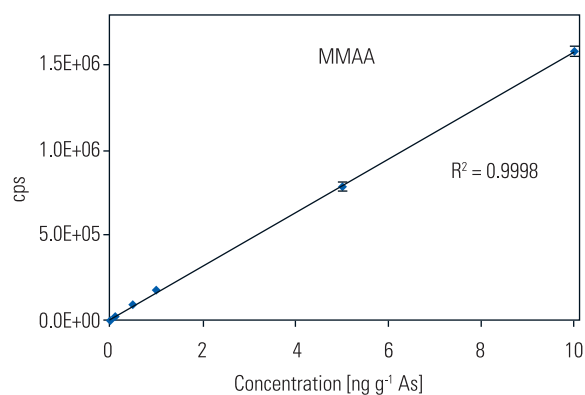


Figure 4: Calibration curve for MMAA.

### Use of High Resolution for Arsenic Speciation

With the Thermo Scientific ELEMENT 2, high mass resolution is used to separate interferences from target isotopes by their small difference in mass. This is of particular use in chromatographic analyses for investigating the background signal and identifying interferences from the mobile phase, which would otherwise unnecessarily increase detection limits. For example, Figure 5 shows a high mass resolution spectrum of the column effluent at m/z 75.

This spectrum shows the common ArCl polyatomic interference as well as a second, more intense interference: both interferences are resolved from the As peak by the use of high mass resolution. Such analyses will indicate whether As contamination, or an interference – e.g. caused by components in the mobile phase or sample – is the source of the ‘background’. This information would be helpful in order to reduce background during method development.

Due to the very good mass stability<sup>[12]</sup> and high sensitivity of the ELEMENT 2, it is possible to routinely perform arsenic speciation analyses in high mass resolution mode. This is advantageous over measurements in low resolution when either the mobile phase or sample generates polyatomic interferences. Through the use of high mass resolution, misidentification of non-arsenic containing species is avoided and background from interferences is eliminated. Figure 6 shows an online chromatogram of 1 ng g<sup>-1</sup> As of each species acquired in high resolution and demonstrates the large reduction of background.

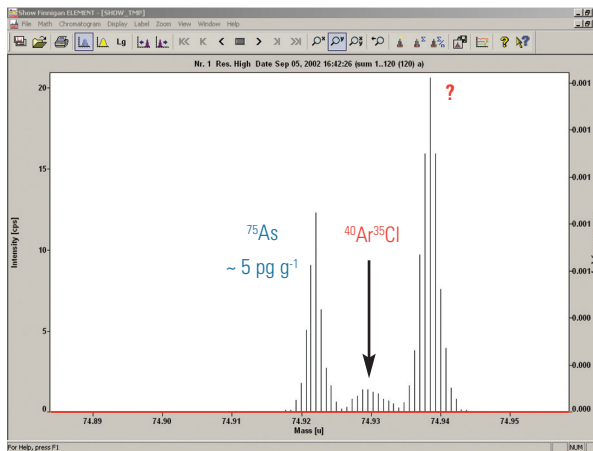


Figure 5: Interferences in the mobile phase become apparent in high resolution.

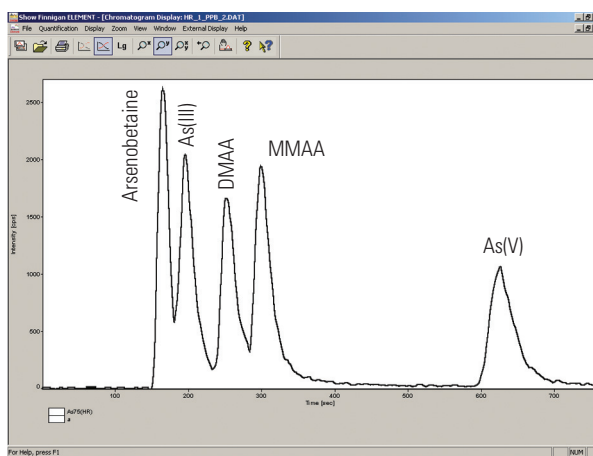


Figure 6: Online chromatogram of 1 ng g<sup>-1</sup> As in high resolution.

## Conclusions

- HPLC coupled to Sector Field ICP-MS is a powerful tool for speciation analysis.
- Outstanding sensitivity of 90 to 240 cps per pg g<sup>-1</sup> As is obtained, resulting in high precision even for low abundant species.
- Due to the high sensitivity and low background noise, extremely low detection limits of between 0.3 to 1.6 pg g<sup>-1</sup> As are achievable.
- High mass resolution is the only method to definitively find and identify interferences and to therefore avoid possible misidentification of non-arsenic containing species.

The methods mentioned in this report can be downloaded from the Customer Information Service section CIS at [www.thermo.com](http://www.thermo.com)

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