Application Note
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Automated Sequencing of Elemental Speciation Methods Using HPLC-ICP-MS with a Quick Change Valve Head

Multi-element speciation analysis with unattended switching of columns and mobile phases

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Introduction

ICP-MS is the technique of choice for trace multi-element analysis in many industries and sample types. But the mobility, bio-availability, and toxicity of several elements, including As, Hg, Cr, Se, Br, I, Sn, Pb, and Sb, depends on the element's chemical form or species (*1–6*). Because of these differences, speciation analysis—where the different chemical forms are separated chromatographically before measurement by ICP-MS—may be required to give a complete assessment of risk. Speciation analysis is especially important for samples where safety is of concern, such as foods, environmental samples, and consumer goods.

To ensure the safety of consumers and the environment, many industries and products are subject to regulations or guidance relating to the speciation of elements. For example, in the case of arsenic, the World Health Organization (WHO) recommends a maximum content of inorganic arsenic (iAs) of 10 µg/kg (ppb) in food and drinking water (*2*). The European Union (EU) and United States Food

and Drug Administration (US FDA) have each published the maximum levels of iAs in fruit juice and rice products. In August 2020, the FDA finalized their guidance for industry on iAs in rice cereals for infants, setting the "action level" limit at 100 µg/kg (ppb) iAs (*3*). Mercury species, particularly methylmercury, are subject to legislation in many countries, including China, Japan, and Philippines. The WHO specifies a maximum guideline concentration of 10 µg/L for bromate in drinking water (*2*). There are also regulations in place to control the level of hexavalent chromium (Cr(VI)) in products such as drinking water, cement, leather, and children toys. Cr(VI) is hazardous, while Cr(III) is an essential nutrient, so speciation analysis is necessary to determine the concentrations of the different forms to ensure compliance with regulations.

To meet current and potential future regulatory requirements, laboratories need fast, automated, reliable, and flexible methods for the determination of various species of multiple elements. High performance liquid chromatography (HPLC) connected to ICP-MS is the most widely used technique for elemental speciation analysis due to its selectivity and sensitivity (*4–10*). Agilent ICP-MS and ICP-QQQ instruments easily link with Agilent HPLC systems through optimized interfaces and integrated software control (*7*). The coupled systems are controlled from the Agilent ICP-MS MassHunter software, simplifying the workflow from method development, data acquisition, and data reporting.

HPLC-ICP-MS methods are typically used to detect species that can be separated on a single column, which often means they are limited to measuring the different forms of a single element. However, labs often need to perform speciation analysis of several elements in each sample, such as monitoring As, Sn, and Hg species in seafoods. Some different element species can be measured successfully using the same column and mobile phase, but this approach may lead to compromised conditions and degraded performance. Labs may also need to run multiple speciation methods on different sample types or assess multiple columns and mobile phases during method development. In these cases, automating a sequence to switch between different speciation methods using an HPLC-ICP-MS system fitted with a Quick Change valve can increase productivity and add flexibility, while also allowing unattended overnight operation. Agilent offers a range of column selector valve heads that can switch between 2, 4, 6, and 8 column positions. A solvent selection valve is also available, to accommodate switching between up to 12 different mobile phases.

Benefits of integrated, HPLC-ICP-MS with a Quick Change valve head include:

- Automated sequencing of multiple speciation methods using different columns and mobile phases. This approach saves time, reagents, and cost, as well as reducing errors by minimizing sample handling.
- Independent control of up to eight speciation methods with customized queue order provides flexibility in operation. All methods can be run in one unattended sequence utilizing automated switching of columns and solvents.
- Support for method development, especially to identify the best column or mobile phase for the separation of target species. A routine analysis can be run in sequence with methods that are under development.

In this study, tests were run to assess the flexibility, performance, and stability of the HPLC-ICP-MS system with a Quick Change valve head for the automatic sequencing of three separate speciation methods. The HPLC-ICP-MS system was used for the measurement of inorganic arsenic, methylmercury, and bromine and iodine species using methods developed in previous studies (*8–10*).

Experimental

Chemicals and reagents

All chemicals and reagents used in this study were bought from Sigma-Aldrich. A 1000 ppm stock solution of As(III) and As(V) was prepared by dissolving the appropriate amount of sodium arsenite and sodium arsenate (ACS grade, purity >99%) into 1% HNO_3. Methylmercury(II) (chloride) (ACS grade, purity >99.5%) was used to prepare a 100 ppm stock solution. Separate solutions of bromide, bromate, iodide, and iodate were prepared from sodium bromide, sodium bromate, potassium iodide, and potassium iodate, respectively (ACS grade, purity >99%). The appropriate amount of each halide salt was dissolved in Milli-Q de-ionized water (DIW, 18.2 MΩ•cm), to give 1000 ppm stock solutions for each target species. Details of the mobile phases used for the three analytical methods and a column flush method are given in Table 2.

Standard preparation

Three calibration levels were prepared for each set of elemental species at the concentrations given in Table 1, by diluting the respective stock solutions with DIW.

Table 1. Calibration standard levels prepared for As(III), As(V), MeHg(II), Br^-, BrO_3^-, I^-, and IO_3^-.

Calibration Level	Mixture of As(III)/As(V) (ppb)	MeHg(II) (ppb)	Mixture of $Br^-/BrO_3^-/I^-/IO_3^-$ (ppb)
1	0.5/0.5	0.25	0.5/0.5/0.25/0.125
2	1.0/1.0	0.5	1.0/1.0/0.5/0.25
3	2.0/2.0	1.0	2.0/2.0/1.0/0.5

Instrumentation

For this work, an Agilent 1260 Infinity II LC system with a quaternary pump and InfinityLab Quick Change 4-column-selector valve head (p/n G4237A) was used. The HPLC was coupled to an Agilent 7900 ICP-MS using the Agilent LC connection kit (p/n G1833-65200). A schematic of the instrumentation is shown in Figure 1. The appropriate HPLC column and mobile phase for each speciation method were selected and changed automatically during the sequence via the Agilent ICP-MS MassHunter software.

The quaternary pump can handle up to four mobile phases, which was sufficient for the seven species (four elements) measured in this study. For analysis requiring more than four mobile phases, a 12 position/13 port solvent selection valve (p/n G4235A) could be added. Similarly, when labs need to run methods using up to eight different columns, an optional Quick Change 8-column-selector valve head (p/n G4239C) can be used in place of the 4-column-selector valve.

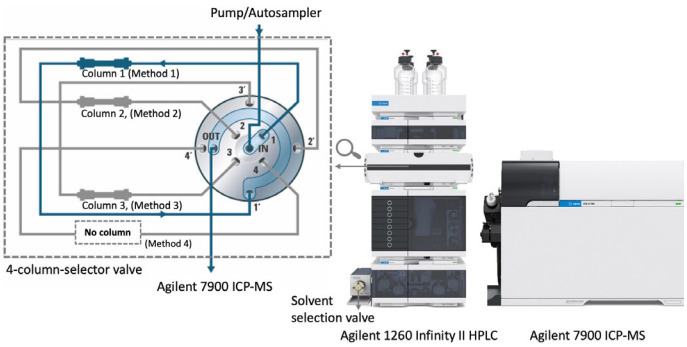

Figure 1. Schematic of the HPLC-ICP-MS system with Quick Change valve head used in this study. The 4-column-selector valve head and quaternary pump enabled automated selection of the different HPLC columns and solvents.

Multi-sequence acquisition method

Three analytical methods separated by wash steps were included in this multimethod speciation sequence, as shown in Table 2. Valve ports 1 to 3 were connected to the respective speciation method columns, while valve port 4 was used to flush out the system using DIW. HPLC and ICP-MS operating conditions and parameters for each of the three speciation methods are given elsewhere (*8–10*). As well as the different elements' acquisition masses, appropriate ICP-MS operating conditions were applied for each HPLC method.

Table 2. Details of the separate methods used in the multimethod acquisition sequence.

Method Description	Detail	Valve Position	Mobile Phase	Column
Speciation 1	Speciation of inorganic As (As(III) and As(V))	1	2.0 mM PBS/0.2 mM EDTA/10 mM CH_3COONa/3.0 mM $NaNO_3$/2% ethanol, pH 11.0 adjusted with NaOH Run time: 10 mins	Anion exchange column, Agilent p/n G3288-80000
Speciation 2	Speciation of MeHg(II) (CH_3Hg)	2	2% methanol /0.5g/L L-Cysteine, pH 2.3 adjusted with HCl Run time: 4 mins	ZORBAX RRHT Eclipse Plus C18, Agilent p/n 959941-902
Speciation 3	Speciation of bromide, bromate, iodide, and iodate	3	5.0 mM NaH_2PO_4/ 15.0 mM Na_2SO_4 / 5.0 mM EDTA Run time: 8 mins	Anion exchange column, Agilent p/n G3268-80001
Wash 4	Flushing of HPLC system	4	DIW Run time: 10 mins	No column required

To demonstrate the flexibility of automated column switching for routine unattended analysis, the three speciation methods were run with a different sequence order over three days, as shown in Figure 2. At the beginning of each day's sequence and after each speciation method, the wash method flushed the HPLC-ICP-MS for 10 minutes using a continuous flow of DIW. For compatible columns and mobile phases, the separate wash method could be eliminated, with blank injections at the start of each batch ensuring stabilization under the new method conditions. Method performance criteria such as linearity, recovery, and precision for the speciation analysis of arsenic, mercury, bromine, and iodine were evaluated over three days.

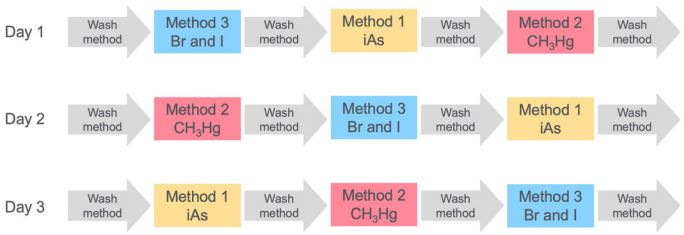

Figure 2. Experimental flowchart for the automated multi-method HPLC-ICP-MS sequences run over three days.

Results and discussion

The performance of the HPLC-ICP-MS system with Quick Change valve head was assessed based on the workflow shown in Figure 2. On each of the three days, the three analytical method batches were run in a different order, interspersed with the wash method. Each method batch consisted of three injections of each of the three calibration levels, plus four injections of the blank, giving 39 injections for the three batches. The addition of the four interspersed wash injections indicated in Figure 2 gave 43 injections in total for each day's sequence. Each sequence was completed within 5.5 hours, regardless of the different method run order.

Using the sequence on day 2 as an example, Figure 3 outlines the automated procedure and queue order that was controlled by the ICP-MS MassHunter software. The illustration in Figure 3 shows how the Quick Change valve-based HPLC-ICP-MS system can be set up for routine, unattended analysis of multiple batches in a user-defined order. Running the different methods sequentially on one HPLC system significantly improves the productivity and turnaround times of speciation analysis, compared to running the methods individually with manual column changeover. The flexibility of the valve-based HPLC-ICP-MS system means that up to eight speciation methods can be run sequentially on one instrument, according to the requirements of the laboratory.

Linearity of calibration

Calibration was carried out by measuring standard solutions of As(III) and As(V), CH_3Hg, and Br^-, BrO_3^-, I^-, IO_3^- in three different batches over three days. Triplicate injections were measured for each calibration level on each day. Excellent linearity was observed for all analytes over the three days. The representative calibration curves for all species obtained on day 1 show excellent linearity, with R greater than 0.9995 (Figure 4).

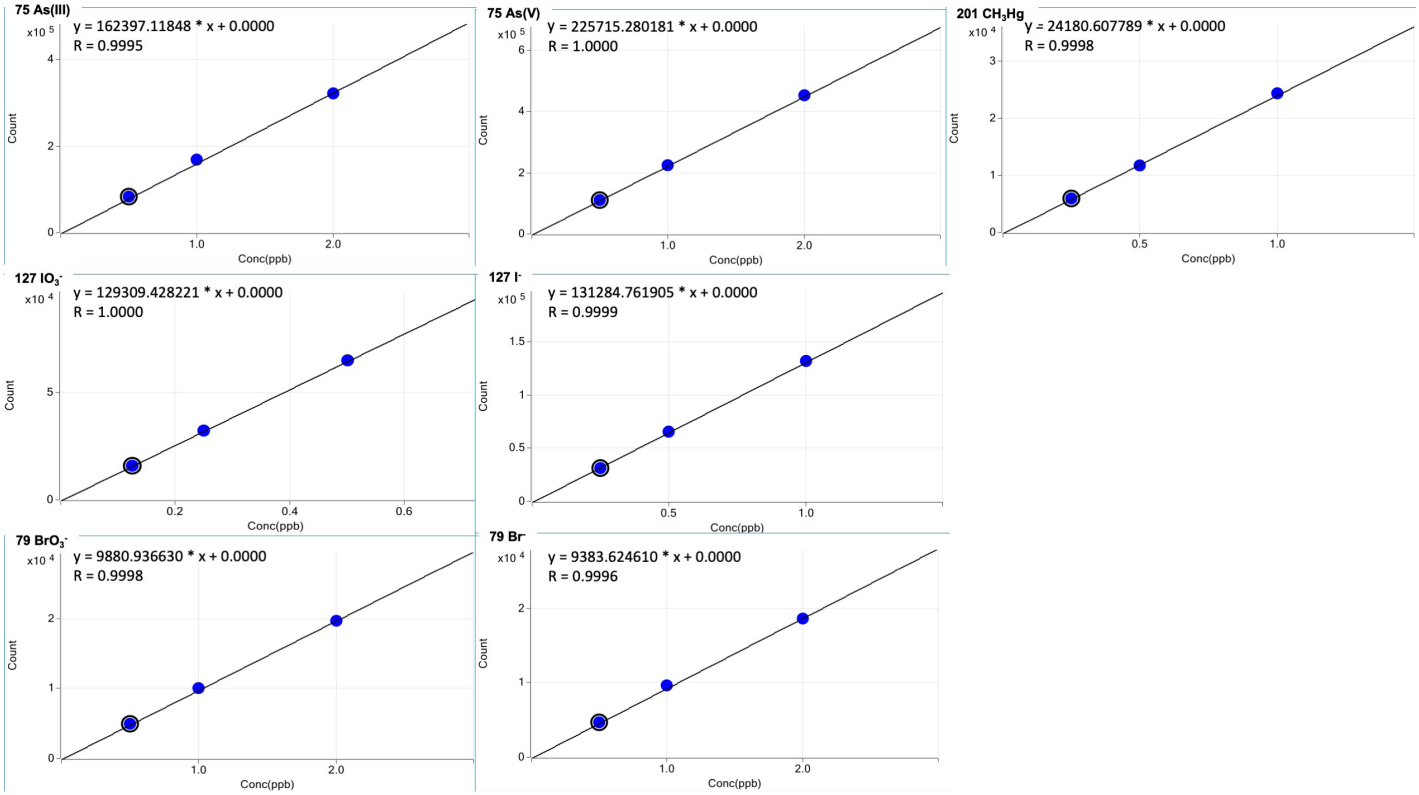

Figure 4. Calibration curves showing excellent linearity. Top: As (III), As(V), and CH_3Hg; Middle: IO_3^- and I^-; Bottom: BrO_3^- and Br^-.

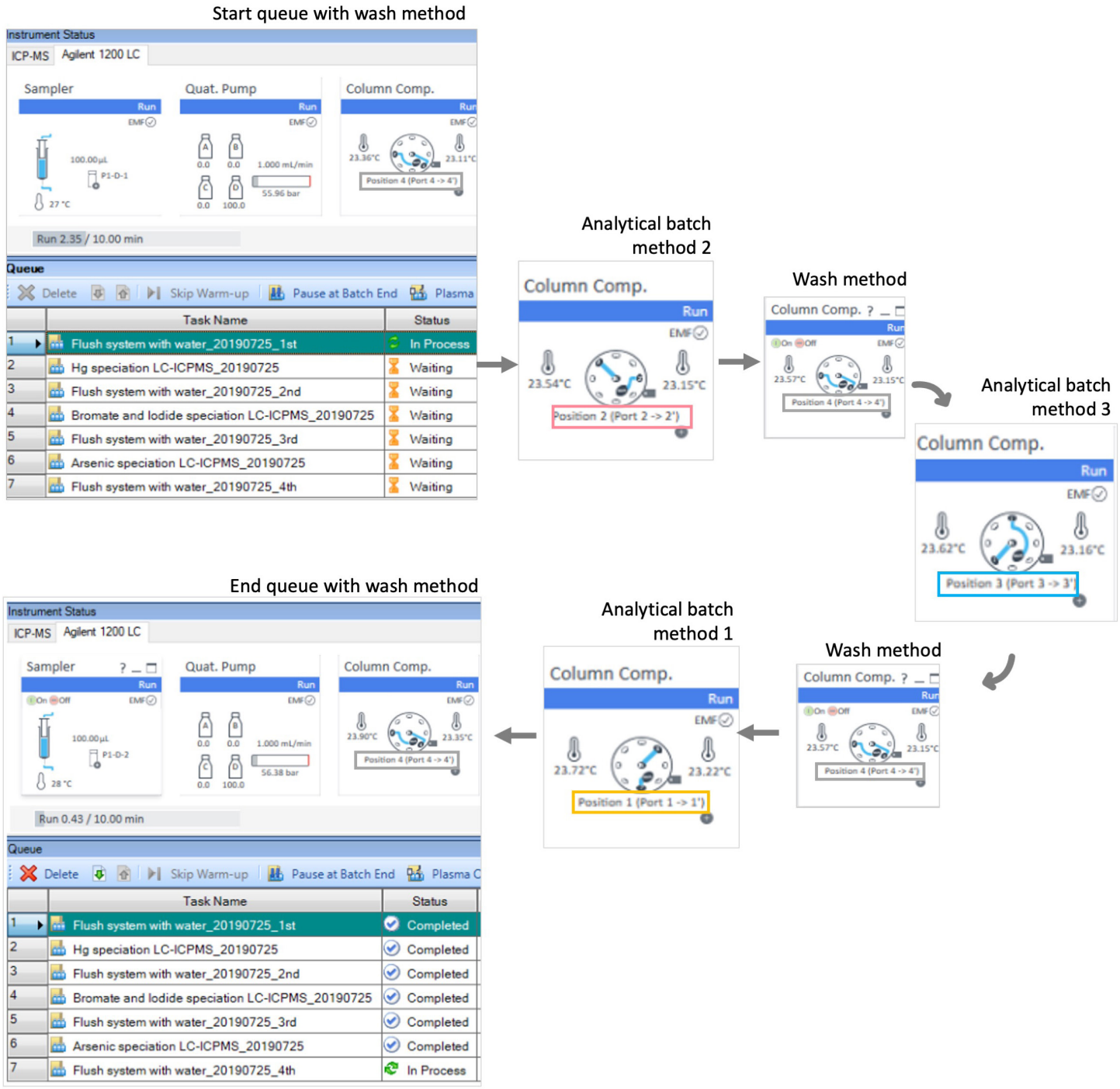

Figure 3. Schematic showing the automated HPLC-ICP-MS procedure controlled by ICP-MS MassHunter software.

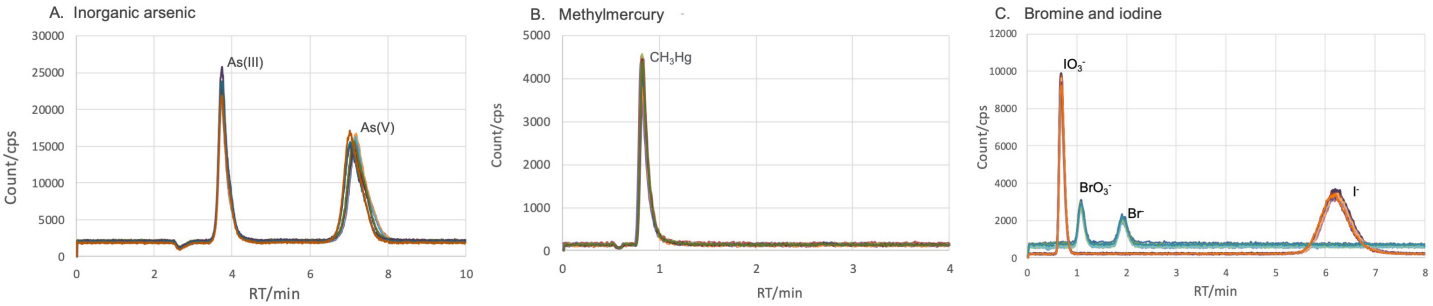

Figure 5. Overlaid chromatograms of A: inorganic arsenic, B: methylmercury, and C: bromine and iodine species obtained from nine injections of level-3 calibration standards. Shows excellent stability of retention times and peak areas over three days. n=9 for each chromatogram.

Day to day stability of measured elemental species

Figure 5 shows overlaid chromatograms of the nine injections of the level-3 calibration standard for iAs, CH_3Hg, IO_3^-, I^-, BrO_3^-, and Br^- measured over three days. Within each day's sequence, all species were separated and measured using the optimum column and mobile phase conditions, switched automatically during the run. The excellent reproducibility over three days demonstrates the stability and applicability of the multibatch HPLC-ICP-MS method with Quick Change valve.

Recovery of standard solutions

The recoveries of all elemental species were based on the ratio of the calculated concentration from the calibration curves relative to the expected concentration at the three concentration levels. The recoveries for all species measured over three days were within 90–110%, as shown in Figure 6. The results confirm the stability and accuracy of the data produced by the Quick Change valve-based HPLC-ICP-MS system.

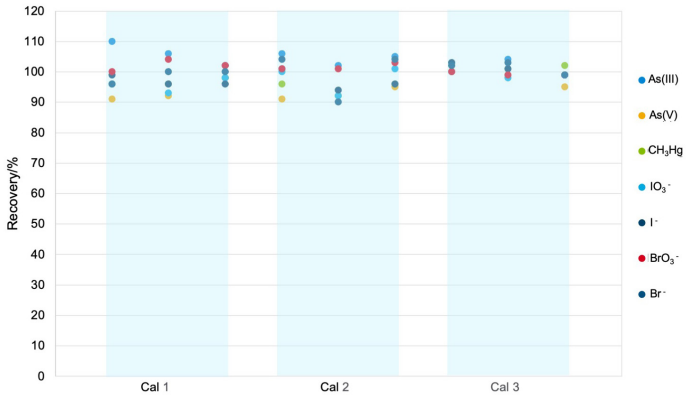

Figure 6. Recovery values for all elemental species measured at three concentrations over three days.

Precision

Method precision was calculated using within-day (intraday) repeatability (RSD_r) and between-day (interday) reproducibility (RSD_{iR}) based on the variation of peak area measurements of the three repeat injections of each calibration level. Intraday repeatability, denoted RSD_r was determined by calculating the percent relative standard deviation (%RSD) of peak area using three injections of each calibration standard performed on the same day. The RSD_r values for all species were ≤ 8%.

Interday reproducibility, denoted RSD_{iR}, was measured as %RSD of peak area from a total of nine injections per concentration level measured over three days. RSD_{iR} values for all elemental species were ≤10%, as shown in Table 3. The between-day reproducibility was not affected by the queue order of the speciation methods, which demonstrates the robustness of the automatic valve-based HPLC-ICP-MS method.

Table 3. Between-day reproducibility of peak area for all elemental species measured at three concentration levels.

Calibration Level	RSD_{iR} of Peak Area (%)						
	As(III)	As(V)	CH_3Hg	IO_3^-	BrO_3^-	Br^-	I^-
Cal 1	2	6	7	5	4	3	5
Cal 2	4	6	9	8	6	10	8
Cal 3	3	4	7	5	5	5	4

Carryover assessment

The effectiveness of DIW to remove any residue or contaminants from the LC flow path after each speciation method was evaluated. The chromatograms in Figure 7, which were obtained during three separate wash-method runs on day 2, show that carry over between the different speciation methods was reduced to acceptable levels of <2% in each run.

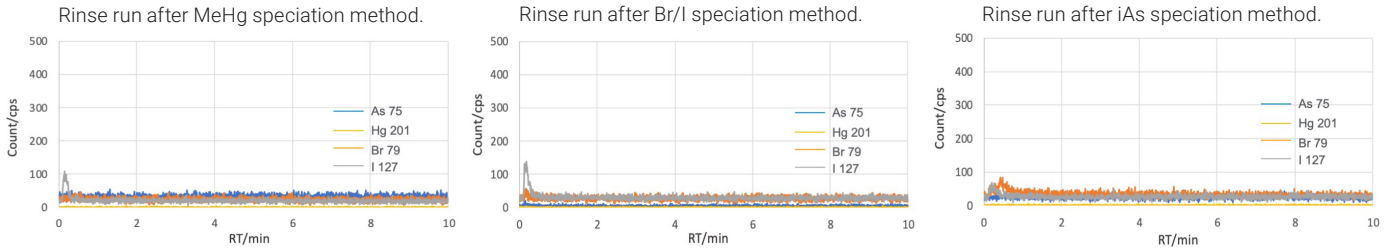

Figure 7. Background signals in wash methods confirm minimal carryover between analytical method runs on day 2.

Conclusion

Multiple speciation analysis methods requiring different columns and mobile phases were run automatically using an Agilent 1260 Infinity II LC coupled to an Agilent 7900 ICP-MS. The HPLC was fitted with an Agilent Quick Change column selector valve head, which eliminated the need to manually disconnect and reconnect different columns.

Three speciation methods were run enabling the measurement of As(III) and As(V); CH_3Hg; and Br^-, BrO_3^-, I^-, IO_3^-. ICP-MS MassHunter was used to control the column selector valve, selecting the queue order of the three speciation methods and the wash cycle through the automatic positioning of the appropriate column. Good levels of precision, recovery, repeatability, and reproducibility were achieved for the multiple speciation methods run each day, and using different queue orders of the methods over three days.

Once an analyst has set up the columns, prepared the mobile phases, and loaded the samples into the autosampler, the HPLC-ICP-MS with Quick Change valve head can run up to eight speciation methods. Multiple speciation methods can be applied sequentially to the same samples, or different sample batches can be analyzed sequentially using different methods. For applications where multi-element speciation is of interest, automating switching between different methods improves productivity, shortens turnaround time, and reduces running costs—important considerations for many laboratories.

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DE44180.0443634259

Printed in the USA, January 22, 2021
5994-2943EN

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