TECHNICAL NOTE 44461

Comparing IC and HPLC systems for speciation analysis – a case study

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Keywords: ion chromatography, high performance liquid chromatography, chromium (III) and (VI), IC-ICP-MS

Goal

This technical note compares the performance of ion chromatography (IC) and high performance liquid chromatography (HPLC) methods for speciation analysis of chromium, including method related benefits and drawbacks.

Introduction

Speciation analysis is a widely accepted tool for the quantitative determination of different chemical forms, or species, of an element. Two of the primary examples of the need to perform speciation analysis are chromium[1] and arsenic[2,3], as both elements may appear as different species (e.g., Cr (III) and Cr (VI) or inorganic and organic arsenic), each with different toxicity and thus, potential related hazards. Another element that requires speciation is bromine, as ozonation of drinking water may lead to the formation of carcinogenic bromate (BrO_3^-) as a byproduct[4].

The different species of arsenic are typically charged depending on the pH, therefore ion exchange chromatography is the preferred technique for separation. In most cases, anion exchange chromatography using an ammonium carbonate-based eluent allows complete separation with narrow peak shapes and excellent signal-to-noise ratios. For chromium, the two relevant species are charged differently, so that, ideally, a mixed-mode resin is used containing separation capacity for both cationic and anionic species. The separation of bromide and bromate (among other brominated organic molecules) can be accomplished using anion exchange chromatography, using, for example, KOH as an eluent (combined with post-column eluent suppression).

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IC systems are designed to separate charged molecules, and are, therefore, the separation instruments of choice for all of the elements mentioned above. As an additional benefit for ICP-MS detection, background contamination is minimized as IC systems have a metal-free flow path.

For Cr speciation, however, other methods, such as ion pairing chromatography, can be applied. As this chromatographic method depends on the use of complexing agents, such as EDTA and organic modifiers, significant spectral interferences will be created affecting the main isotope of chromium, ^{52}Cr. Typical elution conditions for IC and HPLC-based methods for separation of both chromium species of interest are summarized in Table 1. The table also contains information about potential polyatomic interferences of the commonly used isotopes of chromium, which lead to an increased background signal in ICP-MS. Moreover, not all compounds required to prepare the eluent may be available in sufficient purity.

Table 1. Eluent composition, elution conditions, and resulting interferences for IC- and HPLC-based methods for speciation analysis of chromium

Method	Eluent	Elution conditions	Potential interferences
IC (Ion exchange)	2% NHO$_3$	Isocratic	None
HPLC (Ion pairing chromatography)	EDTA in combination with different buffers, such as TBAP in methanol/water	Isocratic or gradient	^{40}Ar^{12}C$^+$ for ^{52}Cr$^+$ ^{40}Ar^{13}C$^+$ for ^{53}Cr$^+$

The choice of the right separation method for a given analytical problem often determines the most appropriate hardware, such as type of chromatography system, column dimensions, stationary and mobile phase. Whereas most applications are based on liquid chromatography (Gas chromatography (GC)-ICP-MS is an alternative for volatile species or species that have volatile derivatives[5]), there is a significant difference between IC and HPLC methods, even though the required hardware can seem almost identical.

Ion chromatography
- Hardware does not use parts made of metal-containing components (e.g., stainless steel or titanium alloys) that come into contact with the eluent. This results in significantly reduced backgrounds due to the absence of metal leaching over time, and assures compatibility with strong eluents such as diluted acids or alkaline eluents, such as hydroxides or carbonates.

- To assure best compatibility with the ICP-MS sample inlet, eluents such as KOH can be neutralized to water using a suppressor before introduction to the plasma source.

- Modern IC systems can deliver elevated pressures (up to 6000 psi) to permit use of higher resolution columns that yield excellent peak separation and short elution time.

High performance liquid chromatography
- Hardware often uses tubing or connectors made of stainless steel or pump heads made of titanium. Bio-inert systems contain significantly fewer parts made of metal to reduce unwanted effects, such as unfolding or degradation of proteins, but do not achieve the low backgrounds observed with IC.

- Some separations will require the addition of ion pairing agents in order to achieve charge separation or neutralization (example: Cr speciation). This will lead to increased backgrounds through interferences.

Experimental
Materials and methods
A Thermo Scientific™ iCAP™ RQ ICP-MS system was used for determination of background levels in different eluents after they had passed through either an IC or an HPLC system. The instrument was tuned on each measurement day to achieve the best sensitivity across the mass range and effective suppression of occurring spectral interferences. For IC, a Thermo Scientific™ Dionex™ Integrion™ HPIC™ system was used. The IC system was compared against a standard HPLC system. Please note that the use of this eluent may be outside of working specifications of typical HPLC systems (typically between pH 1-13). The time-dependent evaluation of eluent contamination was accomplished either online or offline (fraction collection).

The Dionex Integrion IC system was connected to a Thermo Scientific™ Dionex™ AS-AP Autosampler that was configured for fraction collection into 10 mL Dionex AS-AP Autosampler vials to collect a fraction of the eluent every 15 min for quantitative evaluation using ICP-MS. The HPLC system was coupled online to the ICP-MS, and data acquired over a period of 15 min were averaged for comparison. Following system start-up, each chromatography system was run for ~10 min prior to the start of sample collection/analysis. To ensure that liberated metals were not trapped (and thereby not detected) prior to reaching the ICP-MS systems, no column was connected during the experiment. Instead, a pressure coil (made of inert polyetheretherketone (PEEK)) was placed in the flow path to assure real-life system working conditions (i.e., back pressure was ≥2000 psi (138 bar)). Additional detection systems in the flow path for both systems (e.g., a conductivity detector for IC and a DAD detector for HPLC) were bypassed. The experiments are summarized in Table 2.

Results and discussion

To compare IC and HPLC systems for their suitability for speciation analysis, a direct comparison for the likelihood and extent of contamination was performed for an IC system and an HPLC system. The analyte selected was chromium, where the two main species (Cr (III) and Cr (VI)) can effectively be separated using an isocratic elution with dilute nitric acid using a column with exchange capacity for both cationic as well as anionic species[6]. This method achieves consistent separation of the two species in less than three minutes and detection limits in the low $ng \cdot L^{-1}$ range. The resulting levels of different trace elements were monitored in a time-dependent manner and the results for key elements are shown in Table 3.

As the data show, the observed levels of contamination are typically lower for most elements with the IC system. For the HPLC setup, higher levels of eluent contamination are observed on the timescale of this experiment. Especially for metals such as Ti, Cr, V, and Mo, commonly used as additives to stainless steel. The significant difference in the results for chromium suggests that this application is ideally executed on a dedicated IC system. In some cases, e.g., for Co and Ni, comparable levels are observed for both systems. It has to be noted that the IC system also showed sporadic eluent contamination, for example, ^{57}Fe was detected.

Table 2. Experiments performed to assess IC vs. HPLC systems for their suitability for chromium speciation

Instrument	Flow rate / Eluent	Sampling
Dionex Integrion IC	0.3 mL·min⁻¹; 0.4 N HNO₃	Fraction collection (every 15 min)
HPLC System		Online (data averaged over 15 min)

Table 3. Time-dependent concentrations of different trace elements in the eluent collected from both IC and HPLC hardware

Element	Technique	Concentration in eluent [ng·mL⁻¹]							
Time elapsed [min]		0	15	30	45	60	90	120	150
^{48}Ti	IC	<1	<1	<1	<1	<1	<1	<1	<1
	HPLC	<1	10.8	10.4	10.0	9.7	9.1	8.7	8.8
^{51}V	IC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	HPLC	n.d.	1.21	1.22	1.10	1.04	1.01	0.93	0.95
^{52}Cr	IC	<1	<1	<1	<1	<1	<1	<1	<1
	HPLC	<1	7.1	9.5	8.4	8.2	7.7	7.5	7.0
^{57}Fe	IC	2.80	22.7	5.0	4.4	4.0	4.5	3.8	30.1
	HPLC	<1	44.4	33.1	52.6	39.7	31.2	26.3	23.2
^{59}Co	IC	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	HPLC	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
^{60}Ni	IC	<1	<1	<1	<1	<1	<1	<1	<1
	HPLC	<1	<1	<1	<1	<1	<1	<1	<1
^{75}As	IC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	HPLC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
^{95}Mo	IC	<0.1	<1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	HPLC	<0.1	<1	<1	<1	<1	<1	<1	<1

It is possible that this contamination may have resulted from the process of fraction collection or from the sample containers used in this study. Further investigation will be directed to determine the origin of the observed background. For some elements, a time-dependent behavior of elution was observed for the HPLC system under test in this study, leading to a reduction in the observed levels over time. Generally, metal alloys are known for forming a passive oxide layer on the surface that protects the core of the metal against corrosion when in contact with certain solutions and chemicals. For stainless steel, the passive oxide layer mainly consists of a Cr-oxide, but also Fe and Ni can be present in different ratios. The ratio and the depth of the oxide layer depends on the properties of the solution (e.g., pH, oxidizing/reducing potential, temperature, duration) with which it is in contact. This indicates that the passivating layer has not reached a steady state, or in other words, the HPLC system has not yet been fully equilibrated with the eluent in the 150 min of this study. The dependency of the passivating layer from the composition of the eluent, however, means that not only a leaching of metals is likely to be observed when running an IC method on an HPLC system, but also a potential change of the leaching behavior can be expected when switching to a different eluent/mobile phase for other applications. However, in no case did the observed levels of these elements reach the levels observed with a dedicated IC system. As an example, the time-dependent elution of chromium is shown in Figure 1.
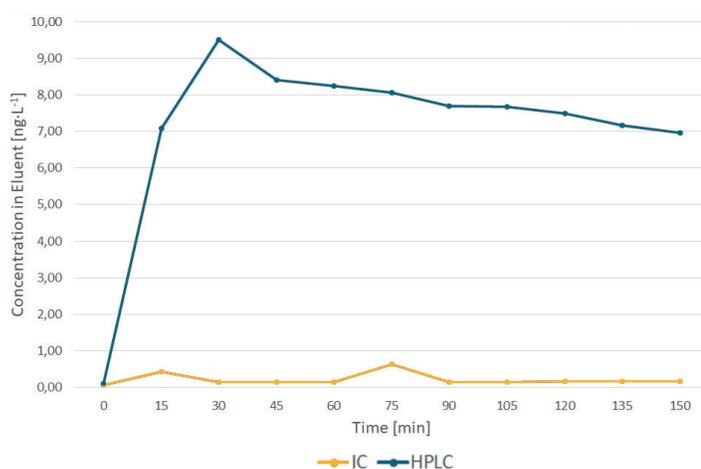
Figure 1. Time-dependent elution of ^{52}Cr as a contaminant in an IC and HPLC system

Conclusion

Eluent contamination with trace elements is significantly increased for a variety of elements on an HPLC system compared to an IC system. This is most likely caused by metal-containing parts in the flow path of the eluent/mobile phase, including the pump heads, connectors, and tubing. For some elements such as chromium or vanadium, a time-dependent wash out was observed in the first two hours of operation; however, equivalent background levels between the IC and HPLC systems were not achieved. Systemic accumulation of metal contamination after long-term usage of an HPLC system with a typical IC eluent was not evaluated here, but, based on the current findings, it would likely be significant, ultimately degrading system performance. To avoid elevated backgrounds caused by leaching of metals from hardware components, chromatographic methods based on ion-exchange chromatography should be run on a dedicated IC system.

References

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6. Specification Sheet Thermo Scientific™ Dionex™ IonPac™ AS7 IC Column; Thermo Fisher Scientific

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