SmartNotes

Why should I add analytical capabilities to perform speciation in my laboratory?

I have been using ICP-MS a long time for the analysis of trace elements, but I have not employed it for speciation analysis yet. As there is an increased interest in speciation analysis, I would like to know more about the options available.

Why is speciation analysis needed?

If you are familiar with ICP-MS you will know how demanding the analytical needs and expectations for modern ICP-MS laboratories are. The main objective of ICP-MS analyses is to make assessments regarding potentially adverse effects to consumers, product quality, or also process control. However, for some elements an evaluation cannot be made based on the measured concentration of the element alone, since it can be present in different chemical forms, or species, which exhibit different properties with respect to e.g. toxicity or bioavailability.

In these cases, it is necessary "to identify and measure the quantities of one or more individual chemical species in a sample" – in other words, speciation analysis must be performed[1].

One element that is often used as a prime example of the need for speciation analysis is arsenic. Arsenic exhibits differing toxicities between its inorganic and organic forms. Whereas the inorganic forms (As (III) and As (V)) have been labeled as highly toxic, the organic form arsenobetaine has been termed harmless. Another element which requires speciation analysis is chromium. Chromium (III) is an essential element in humans whereas chromium (VI) is a known carcinogen.

This difference in toxicity between the different elemental species is recognized in both environmental and food quality standards globally. Some of the better known regulations demanding maximum allowable concentrations of certain species are:

- Bromate in drinking waters, for example MCL 10 $\mu g \cdot L^{-1}$ in the US, 3 $\mu g \cdot L^{-1}$ in Europe
- Inorganic arsenic in rice, MCL between 0.1 and 0.3 $mg \cdot kg^{-1}$ in the EU depending on product
- Methylmercury in fish, FDA Action level is at 1 $mg \cdot kg^{-1}$
- Organotin in water, allowable average of 0.2 $ng \cdot L^{-1}$ in the EU´s Water Framework Directive

How do I perform speciation analysis?

Speciation is essentially the separation of the individual elemental species, such as separation of chromium (VI) from chromium (III) and their subsequent detection and quantification.

Speciation analysis can therefore be split into two components: separation of individual species from a mixture using a suitable chromatographic technique followed by trace elemental detection and quantification using ICP-MS. Such combined method approaches are also termed "hyphenated techniques", and the exact denomination depends on the chromatographic approach used, e.g. Ion Chromatography – Inductively coupled Plasma Mass Spectrometry (IC-ICP-MS).

Which analytical techniques are available for speciation?

The toolbox for performing speciation analysis contains a variety of different techniques, having their related advantages and drawbacks. The selection of the appropriate technique depends on which elements should be analyzed, and hence the chemical and physical properties of the expected species. A brief selection of the most commonly applied techniques in conjunction with ICP-MS as an element selective detection system are shown in the Table 1.

Table 1. Brief selection of analytical techniques used as hyphenation to ICP-MS.

Analytical Technique	Stationary Phase	Mobile Phase	Elution	Analytes
Ion chromatography (IC)	**Anion exchange:** Cationic groups like $-NR_3^+$	Water based • Inorganic or organic buffers and salts • Diluted Acids and bases	Change of ionic strength and/or pH value of the eluent	Charged and/or polar analytes As (III), As (V) Cr (III) and Cr (VI)
	Cation Exchange: Anionic groups like SO_3H			
High Performance Liquid Chromatography (HPLC)	Non polar: C_8, C_{18} etc.	Water and organic solvents (Methanol, Acetonitrile); Addition of charge pairing agents: Formic Acid (FA), Trifluoroacetic acid (TFA)	**Reversed Phase Chromatography:** Change polarity of mobile phase (e.g. increase percentage of organic modifier)	Polar and non-polar molecules
			Hydrophilic Interaction Chromatography (HILIC): Decrease of polarity of mobile phase (decrease amount of organic modifier)	
		Addition of ion pairing reagents: Tetramethyl ammonium hydroxide (TMAH)	**Ion pairing chromatography:** Decrease amount of ion pairing reagent	Analysis of polar or charged analytes in conjunction with non polar analytes
Gas Chromatography	Vinyl or similar	Carrier gas, He, H_2, N_2	Increase volatility through increase of temperature of the column	Volatile compounds or compounds that have volatile derivatives (e.g. organotin compounds)
Flow-Field Flow Fractionation (AF4)	N/A	Water based, surfactants (e.g. SDS) at low concentrations	Gradual decrease of cross flow as opposing force	Nanoparticles, Polymers, Proteins and Antibodies

What is the difference between using Ion Chromatography (IC) and High Performance Liquid Chromatography (HPLC) for speciation analysis?

The difference between IC and HPLC is basically the mechanism, which is used to separate species. The basic hardware is similar in all cases:

- A pump is needed to provide the constant flow of the mobile phase through the separating column.
- An autosampler draws a given volume of the sample into the injection loop, so that a constant volume of sample is always injected onto the column.
- An injection device, typically a 6 port valve and a sample loop with a well-defined volume.
- A separation device (mostly a column): there is a variety of different chromatographic columns available that offer different separation mechanisms and are thus suited for different types of analyses.

The nature of the analyte determines the applicable separation mechanism, and hence influences the column selection as well as the applicable mobile phase. Field Flow Fractionation and all derived techniques can be considered as an exception, as the sample is separated without interaction to a stationary phase, but only based on diffusion in a narrow channel. In addition, techniques based on electrophoresis (capillary electrophoresis, gel electrophoresis) are also used for speciation analysis, but represent only niche applications.

What is the advantage of IC over HPLC for speciation analysis using ICP-MS?

Due to the ionic nature of the most commonly investigated species, ion chromatography hyphenated to inductively coupled plasma mass spectrometry (IC-ICP-MS) is usually the method of choice. The striking advantage of this combination is that modern IC systems are completely metal free, eliminating any background contamination that might be introduced via the chromatographic system. Additionally, IC has the advantage that also the commonly applied mobile phases (eluents) are water based and are thus easily compatible with the inductive coupled plasma ion source. In contrast, HPLC is using mobile phases using organic modifiers such as methanol or acetonitrile, so that the use of oxygen addition is mandatory.

Can Gas Chromatography be used for speciation analysis?

The hyphenation of gas chromatography to ICP-MS is a very powerful tool for the speciation analysis of volatile compounds, such as organotin compounds or mercury and its alkylated derivatives. Due to the usually very short peak width in GC (2-3 seconds), very good signal to noise ratios can be achieved. However, sample preparation tends to be a bit more laborious, and as mentioned the use of GC is limited to the analysis of compounds which are volatile or can be converted into such compounds.

Can I control my IC, HPLC or GC using the Thermo Scientific Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software?

The Qtegra ISDS Software can be ordered with the optional ChromControl Plug-in that allows you to fully control chromatography systems inside the ICP-MS operating software. Thanks to the modular structure of the Qtegra ISDS Software, switching from one application to another, e.g. from total element quantification using a conventional autosampler to speciation analysis using ion chromatography, is possible just in a few clicks. The use of different evaluation methods within the Qtegra ISDS Software LabBooks gives you the same “look and feel” for data acquisition and data evaluation, so that the amount of training is minimal when switching to speciation analysis.

The ChromControl Plug-in allows to manually control the chromatographic system in the Dashboard View of Qtegra ISDS Software, e.g. for column conditioning or cleaning. For method simplest method creation, a wizard is available that guides through all relevant steps. For data acquisition, the Qtegra ISDS Software LabBook is all that is needed, since all method details and the sample list are stored inside, so that there is no need for a second PC or a trigger cable. A dedicated shutdown feature turns off all analytical systems in the case of an unexpected shutdown, so that speciation analysis can as well be run unattended in the night.

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All products from the Thermo Scientific™ Chromatography product portfolio (IC, HPLC, GC) can be controlled using the ChromControl Plug-in for Qtegra ISDS Software. Up to five independent instruments can be controlled, if various techniques are used in a laboratory (e.g. one IC System and a GC etc.).

Additionally, some systems which have been discontinued may still be supported through the ChromControl Plug-in (e.g. the Thermo Scientific™ ICS-3000 Series Ion Chromatography System).

How are chromatographic devices connected with the ICP-MS?

For liquid chromatography (IC and HPLC), connection is straightforward, as the column outlet is directly connected to the nebulizer of the ICP-MS instrument. This can be done without any special precautions (such as turning off the nebulizer gas flows) because of the robust free running RF generator of the Thermo Scientific™ iCAP™ Q and Thermo Scientific™ iCAP™ Qnova Series ICP-MS.

Figure 1. The Thermo Scientific iCAP RQ ICP-MS connected to a Thermo Scientific TRACE 1310 GC with the dedicated Thermo Scientific GCI 100 Interface.

For GC, a special interface, the Thermo Scientific™ GCI 100 Interface, is available (Figure 1). The GCI Interface consists of a heated transfer line, allowing to avoid condensation of previously separated compounds through lower temperatures outside of the GC oven, which would lead to poor separation quality. It is easy to handle and can be installed and uninstalled quickly if the GC system is only occasionally used for GC-ICP-MS analysis.

Conclusions

Adding analytical solutions to perform speciation analysis allows you to expand your laboratory capabilities and improve the assessment of levels of toxic elements in samples, which can potentially have adverse effects on consumers and product or process quality.

From a technical standpoint speciation analysis is essentially the separation of the individual elemental species, their subsequent detection and quantification and it can be performed by coupling a suitable chromatographic technique followed by trace elemental detection and quantification using ICP-MS. Depending on the nature of the species under scrutiny, the right separation technique can be selected and easily integrated with existing ICP-MS instrumentation.

Dedicated hardware such as IC or HPLC coupling kits and the Thermo Scientific GCI Series Interface enables seamless integration of the two analytical techniques. Software can also be integrated in the new workflow with the optional Thermo Scientific Qtegra ISDS Software ChromControl Plug-in, allowing intuitive operation and simplified data acquisition and evaluation.

References

1. D. M. Templeton, F. Ariese, R. Cornelis, L.-G. Danielsson, H. Muntau, H. P. van Leeuwen, R. Lobinski, IUPAC Guidelines for Terms Realted to Speciation of Trace Elements, Pure Appl. Chem., 72/8 (2000), 1453-1470

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