

Inductively Coupled Plasma Mass Spectrometry as a Tool for Addressing Elemental Speciation in Legislation

Shona McSheehy, Torsten Lindemann, Meike Hamester

Thermo Fisher Scientific, Bremen, Germany



Overview

Purpose: Elemental species specific legislation leads to the necessary development and introduction of reliable analytical methods for accurate and precise measurement.
Methods: Chromatographic techniques coupled to ICP-MS are employed for species specific detection at ultra-trace concentrations.
Results: Species such as bromate, chromate, methylmercury, tributyl tin and pentabromodiphenyl ether are measured at ppb or sub-ppb levels by HPLC- or GC-ICP-MS.

Introduction

Over the last decade, elemental speciation has become an established field of analysis as the scientific community recognizes that total element concentrations cannot provide the information necessary to draw valid conclusions in a number of scientific domains. The need to define and measure chemical species of an element lies in the fact that physicochemical factors such as toxicity, bioavailability, mobility and reactivity are dependent on the specific form of an element. Initial speciation research, which highlighted the significance of chemical species with respect to toxicity, sparked the introduction of a number of legislative directives and recommendations for the protection of the environment and our population. Conforming to new legislation specific to chemical species now preoccupies a number of industries and the table in Figure 1 summarizes some of the directives that require speciation analysis. Gas chromatography (GC) and high performance liquid chromatography (HPLC) are the techniques of choice in modern speciation analysis due to their high resolution and the ease with which they are coupled to ICP-MS, allowing for on-line separation and detection. ICP-MS instruments with quadrupole analyzers (ICP-Q-MS) are the most popular for speciation analysis due to their robust nature, small footprint, low cost (relative to high resolution instruments) and their adaptability to changing configurations (e. g. converting from total element analysis to a coupled technique). The mass range accessible to quadrupole ICP-MS instrumentation has been growing continually since the advent of collision/reaction cell technology (CRC). Sector field ICP-MS (ICP-SF-MS), also known as high resolution ICP-MS offers a higher sensitivity and specificity than ICP-Q-MS, thus making it the instrument of choice in speciation analyses where limits of detection (LODs) required are part-per-trillion (ppt) or sub-ppt level or where the analyte in question is a challenge for ICP-Q-MS.

Methods

Speciation analysis is performed in three distinct stages: sample preparation, separation of the chemical species and detection. Due to the wide range of sample matrices and chemical species, a number of different methodologies for sample preparation and species separation have been developed. For each of the chosen applications, some background to the application, the sample preparation, instrumental parameters and the analytical methodologies are outlined.

FIGURE 1. Summarized list of legislation specific to elemental species

Element	Matrix	International Agency/Directive	Legislation
Hg	Fish	US EPA Criterion document 823-R-01-001	Criterion limit value of 0.3 mg/kg of MeHg ⁺ (MeHg ⁺ expected to be without appreciable risk to human health)
	Water	EU Water Framework Directive 2000/60/EC	Maximum of 0.05 µg/kg mercury and its compounds in inland waters (priority hazardous substances)
Sn	Water	EU Water Framework Directive 2000/60/EC	Maximum of 0.0002 µg/kg TBT in inland waters (priority hazardous substance)
	Fuels	USEPA CFR 40, Part 80 Regulation of Fuels and Fuel Additives EC directive 2003/17/EC Automotive Fuel Quality	Maximum of 15 mg S/kg for highway diesel engines and 30 mg S/kg for gasoline Maximum of 50 mg S/kg in fuel with phase in of 10 mg S/kg fuel
Cr	Toys	2008/0018 proposed amendment to 88/378/EEC	Migration limits of 0.04 mg/kg hexavalent chromium from hard or brittle toys
	Automobiles	EC directive 2000/53/EC	Maximum of 2 g of Cr(VI) per end-of-life vehicle
	Cement	EC directive 2003/53/EC	Maximum of 2 mg/kg Cr(VI) in cement or cement preparations which will come into contact with skin
Br	Workplace Atmospheres	USEPA and ACGIH	Threshold Limit value of 0.05 mg soluble Cr(VI)/m ³ air and 0.01 mg insoluble Cr(VI)/m ³ air
	Drinking water	EU Directive 98/83/EC, US EPA Disinfectants and Disinfection Byproducts Rule (Stage 2 DBP rule)	Maximum contaminant level (MCL) of 10 µg/L bromate (EPA) Minimum reporting level (MRL) of 1 µg/L bromate
Br	Water	EU Water Framework Directive 2000/60/EC	Maximum of 0.0005 µg/kg Pentabromodiphenyl ether

EC - European Commission, US EPA - United States Environmental Protection Agency, ACGIH - American Conference of Governmental Industrial Hygienists, TBT - tributyl tin

FIGURE 2. Thermo Scientific Accela U-HPLC coupled to the Thermo Scientific XSERIES 2 ICP-Q-MS



FIGURE 3. Thermo Scientific Trace GC Ultra coupled to the XSERIES 2 ICP-Q-MS



For HPLC-ICP-MS (Figure 2), compatible HPLC mobile phase flow rate allows for a simple coupling connection from the outlet from the HPLC column directly to the ICP-MS nebulizer and chemical species are separated in accordance with their affinity to a mobile and stationary phase component. For GC-ICP-MS (Figure 3), the coupling is achieved by connecting the outlet of the GC column to the ICP-MS torch using a temperature controlled transfer line. Chemical species are then separated according to their boiling point characteristics and their affinity for the stationary phase component.

Results

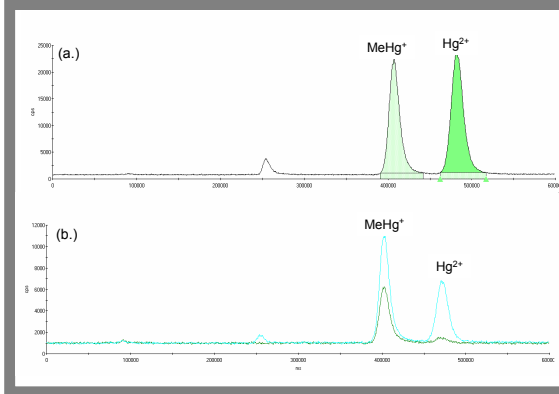
Determination of Methylmercury in Fish Tissues by HPLC-ICP-Q-MS

The alkylmetal, methylmercury (MeHg⁺), generated from biomethylation of inorganic mercury (Hg²⁺) is currently one of the most worrying pollutants in the environment due to its extremely high toxicity, its resistance to degradation and its ability to bioaccumulate in carnivorous fish. MeHg⁺ is presently recognized as a potent neurotoxin, capable of crossing blood-brain and blood-placental barriers, thus, raises issues about the potential danger to the developing nervous system of babies and young children. As fish consumption is the major source of MeHg⁺ for the general population, several national food safety agencies have issued recommendations, particularly aimed at women of child bearing age to limit the consumption of carnivorous fish. Additionally, the US EPA have established a quantitative health risk, a reference dose of 0.1 µg/kg/day. Speciation of mercury in fish starts with extraction of the sample in basic media (TMAH), a process hastened by use of a low power microwave. The extract should be diluted 10-fold prior to injection as high TMAH concentration will disrupt the chromatography. The reversed phase chromatography employed uses an isocratic elution with separation of Hg²⁺ and MeHg⁺ in a 10 minute run (Figure 5a). The ICP-Q-MS parameters and sample introduction are standard, although a high sensitivity interface can be employed to improve LODs. A calibration of the mercury species standards (not shown) was performed from 0.1 to 5 µg/kg. A higher concentration calibration was not employed as mercury is notorious in ICP-MS as an element with a high memory. Fish extracts were diluted appropriately to fall within the calibration. The extract of DORM-2 certified reference material (CRM) (Figure 5b) was diluted 50-fold. The chromatography confirms essentially what we already know, that fish samples tend to contain mercury exclusively

FIGURE 4. Analytical parameters for speciation of mercury by HPLC-ICP-Q-MS

Sample Preparation	0.25 g sample + 5 mL 25 % TMAH; Microwave (CEM Discovery), 2 min, 40 W
HPLC	
Column	Thermo Scientific Hypersil GOLD™, 150 x 4.6 mm, 5 µm
Elution program	Isocratic: 60 mM ammonium acetate, 5% methanol, 0.01% 2-mercaptoethanol
Flow rate	1.5 mL/min
Injection volume	100 µL
ICP-Q-MS	
Operation mode	Standard (no CRC employed)
Sample Introduction	Standard concentric nebulizer with Pellier cooled impact bead spray chamber
Interface	High sensitivity option with negative ion extraction potential (Xs-)

FIGURE 5. Chromatography measured at ²⁰²Hg of (a.) standard mercury species at 5 µg/kg (with software applied peak integration) and (b.) dogfish extract (DORM-2 CRM) – green trace and the same extract with a standard mercury species spike at 1 µg/kg – turquoise trace



in the MeHg⁺ form due to bioaccumulation. The DORM-2 CRM serves to validate the method, and the measured MeHg⁺ concentration of 4.27 µg MeHg⁺/g is in good agreement with the certified value of 4.47 µg MeHg⁺/g. An instrumental LOD of 0.05 µg MeHg⁺/kg gives a method LOD of 10 µg MeHg⁺/kg of fish, 30-fold lower than the criterion limit set by the US EPA.

Determination of Bromate in Drinking Waters by HPLC-ICP-Q-MS

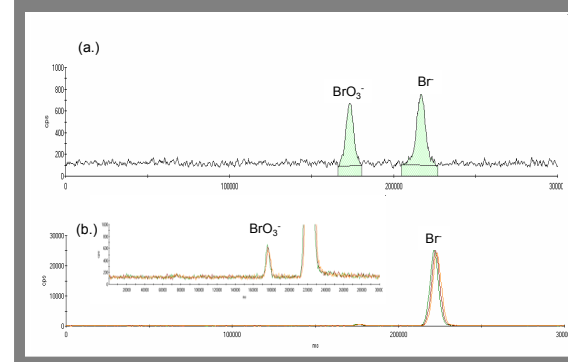
Bromine is naturally present in the earth's crust and seawater in a range of chemical forms. In natural waters (including drinking water) it is present as the bromide ion (Br⁻) that is essentially non-toxic. During the ozonation process (the most commonly used method for the purification of drinking water in the US) the bromide may be converted into bromate (BrO₃⁻) that is recognized as a highly carcinogenic compound. Both the European Drinking Water Directive (98/83/EC) and the United States EPA Disinfectants and Disinfection Byproducts Rule (Stage 2 DBP rule) stipulate a maximum contaminant level (MCL) of 10 µg/bromate L in drinking waters. The EPA also requires a minimum reporting level (MRL) of 1 µg bromate/L.

No sample preparation is required before the HPLC-ICP-MS analysis. An isocratic elution separates Br⁻ and BrO₃⁻ in under 5 minutes (Figure 7a) and 1 µg/L of both bromine species can be easily seen above the baseline, clearly allowing the detection and quantification of bromine species at sub-ppb levels. The ICP-Q-MS can be operated in standard or CRC mode, the advantage of CRC being simultaneous monitoring of both Br isotopes (⁷⁹Br and ⁸¹Br), the ⁸¹Br isotope otherwise being inaccessible in standard operating conditions due to the ArArH⁺ interference. The overlay of chromatograms from triplicate injections of tap water spiked with 1 µg bromate/L are shown in Figure 7b. This confirmed the absence of

FIGURE 6. Analytical parameters for speciation of bromine by HPLC-ICP-Q-MS

Sample Preparation	-
HPLC	
Column	Thermo Scientific BioBasic AX (100 x 4.6 mm, 5 µm) and guard column (10 x 4.6 mm, 5 µm)
Elution program	Isocratic: 10 mM ammonium carbonate (pH 8)
Flow rate	1 mL/min
Injection volume	100 µL
ICP-Q-MS	
Operation mode	CRC (CRC employed with 7mL/min 7% H ₂ in He, no energy discrimination)
Sample Introduction	Standard concentric nebulizer with Pellier cooled impact bead spray chamber
Interface	Matrix tolerant interface (Xi)

FIGURE 7. Chromatography measured at ⁷⁹Br of (a.) standard bromine species at 1 µg/L (with software applied peak integration) and (b.) overlapped triplicate injections of tap water spiked with 1 µg bromate/L



BrO₃⁻ in the tap water (where matrix effects could theoretically cause co-elution) and ensure the ability of the developed chromatographic method for determination and quantification of Br species in the sub-ppb range. LODs of less than 5 ng/L of both bromide and bromate were possible with the ICP-Q-MS employed in CRC mode.

Determination of Tin and Mercury Species in Inland Natural Waters by GC-ICP-SF-MS

The Water Framework Directive (EU 2000/60/EC) (WFD) will soon have limits for mercury and its compounds' and tributyl tin (TBT) in inland waters of 50 ng/L and 0.2 ng/L respectively. The extremely low levels, especially for TBT demand an appropriately sensitive technique for measurement. GC-ICP-Q-MS is a technique which can offer high specificity and sensitivity and is the current method of choice for simultaneous speciation of Hg and Sn in environmental matrices. Further improvement in sensitivity can be offered by ICP-SF-MS (Figure 8). A GC-ICP-SF-MS (Figure 9) was used to quantify tin and mercury species in inland waters. Samples were collected from recreational ponds, a recreational lake and a nature reserve. 250 mL of water was collected from the edge of the water mass in 250 mL PFA bottles. No preservation or stabilization agent was added to the samples. Samples were stored in the fridge at 4 °C until preparation and analysis (parameters shown in Figure 10). Chromatography for tin, from one of the sample sites (Figure 11) show the presence of tin species eluting earlier than the butyl tins; these are possibly ethyl or propyl tins. The samples investigated were all found to contain lower MeHg⁺ levels than the LOD, and MBT was the only tin species significantly different from the LOD. Spike recoveries experiments, performed at 2.5 ng/L showed accurate but imprecise recoveries.

FIGURE 8. Sensitivity comparison of GC with ICP-Q-MS vs ICP-SF-MS

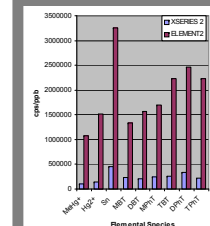


FIGURE 9. Trace GC Ultra coupled to the Thermo Scientific ELEMENT 2 ICP-SF-MS

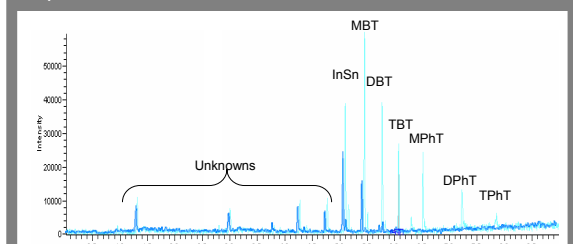


FIGURE 10. Analytical parameters for speciation of tin and mercury by GC-ICP-SF-MS

Sample Preparation	40 mL sample transferred to a clean dry 50 mL headspace vial. Spikes for recovery measurements were made at this point. 5 mL ammonium acetate buffer at pH 4.9, 1 mL hexane and 1 mL 1% NaBr ₂ were added to the vials. The vials were sealed and agitated during 5 minutes. The hexane was transferred to a GC vial. A centrifugation step was sometimes necessary to help in separating the phases
GC	
Column	Thermo Scientific Tr-5, 30 m x 0.25mm ID, 25 µm
GC oven parameters	50°C (1 min), ramp at 30°C/min to 300°C (1min)
Flow rate	3 mL/min He
Injection volume	1 µL (PTV, splitless)
ICP-SF-MS	
Operation mode	Low resolution
Sample Introduction	Dual introduction system: GC and standard concentric nebulizer with cyclonic spray chamber
Sample Gas Flows	Nebulizer 350 mL/min Ar, Additional Gas 600 mL/min Ar
GC transferline	320°C isothermal
Interface	X cones

Sub-ppt (< 1 ng/L) LODs for all species investigated and 0.45 ng/L for TBT indicate that although this analytical method is more than appropriate for the mercury species, the LOD for TBT is difficult to achieve. Initial GC-ICP-SF-MS results are promising for this application, but many aspects of the sample preparation must be improved (cleaner reagents, higher sample volume, larger injection volume, more standards) for a more reproducible methodology.

FIGURE 11. Chromatography measured at ¹¹⁹Sn of inland water – blue trace and the same sample with a standard multi-tin species spike at 2.5 ng/L – turquoise trace



Conclusions

The methods shown illustrate the advantages of ICP-MS for the speciation of trace metals in a number of matrices, with established methods providing accurate and precise results and addressing legislation:

- Commercially available coupling kits for simple connectivity solutions with GC and HPLC
- Many methods already established and validated – possible to address different legislation with just one technique
- Techniques show good specificity and sensitivity that give LODs low enough to conform to legislation
- ICP-SF-MS will be an added advantage for legislation that requires ultra-low LODs

Acknowledgements

We would like to thank Dr. Aleksandra Polatajko, currently affiliated with the University of Köln for the bromate in drinking water determinations

This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries.