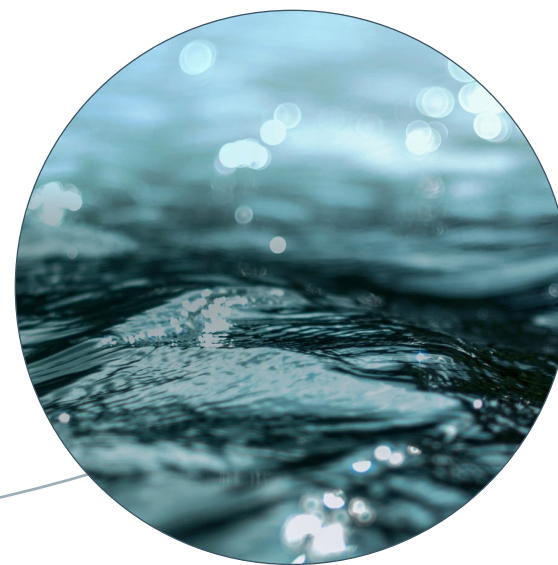


Guide to Speciation

- What is speciation, and the importance of chemical species
- Who should be concerned with speciation testing
- Obstacles to adapting speciation testing



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Guide to Speciation

What is Speciation?

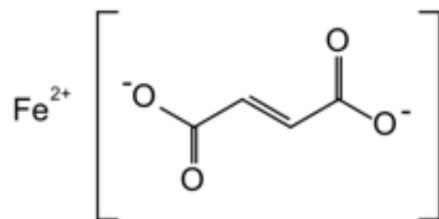
The International Union of Pure and Applied Chemistry (IUPAC) defines speciation as 'the distribution of an element amongst defined chemical species in a system'. Chemical species include isotopic composition, electronic or oxidation states and molecular structures. Speciation analysis is the analytical process of identifying and/or measuring the amounts of one or more individual chemical species in a sample.

Elemental Oxidation States

Z	Element	Negative Oxidation States					Positive Oxidation States					
24	Cr	-4		-2	-1	0	1	2	3	4	5	6
33	As		-3	-2	-1	0	1	2	3	4	5	
34	Se			-2	-1	0	1	2	3	4	5	6
48	Cd			-2		0	1	2				
80	Hg			-2		0	1	2		4		
82	Pb	-4		-2	-1	0	1	2	3	4		

What is the Importance of Chemical Species?

Chemical and elemental species are important in many fields of science and health. Human health and nutrition can be influenced by the presence or absence of certain chemical species such as iron, selenium, arsenic, and mercury. In human nutrition, the different forms of iron can have various amounts of biological activity. Many iron supplements contain these biologically available forms of iron such as ferrous fumarate instead of more biologically inert forms such as ferrous oxide. Selenium species have been associated with the treatment of disease. Studies have shown the value of organoselenium compounds in treatment of tumor growth and the scavenging of free radicals.



Ferrous Fumarate

Guide to Speciation

Toxicity plays a role in the importance of speciation and the need to quantify elemental species. Some species of heavy metals can be highly toxic to humans and animals. For example, organic mercury compounds are more toxic than inorganic mercury compounds. The reverse condition is true for the heavy metal, arsenic, whose organic forms (MMA, DMA, arsenocholine and arsenobetaine) are much less toxic than the inorganic forms (arsine, arsenate and arsenite). A median lethal dose (LD₅₀) of arsenobetaine is over 10000 mg/kg whereas the LD₅₀ of arsine is just mg/kg ppm.

Arsenic Species Toxicity

Arsenic Species		Ld ₅₀ (mg/kg)
Arsenite	As (III)	14
Arsenate	As (V)	20
Arsine	AsH ₃	3
Monomethylarsonic Acid	MMA	700-1800
Dimethylarsinic Acid	DMA	700-2600
Arsenocholine	AsC	> 10000
Arsenobetaine	AsB	> 10000

Chromium can play both a negative and positive role in human health. Traditionally, chromium in the form of trivalent chromium, or Cr (III), has been considered to be a dietary supplement. However, recent studies now contend that it may not play as important a role in human health to warrant supplementation. Another form of chromium, however, has been found to have a definite negative impact on human health. Hexavalent chromium or Cr (VI) is potentially carcinogenic and has an oral toxicity between 50 and 150 µg/kg.

Chromium Oxidation States

Arsenic Species	
-2	Na ₂ [Cr(CO) ₅]
-1	Na ₂ [Cr ₂ (CO) ₁₀]
0	Cr ₂ (C ₆ H ₆) ₂
1	K ₃ [Cr(CN) ₅ NO]
2	CrCl ₂
3	CrCl ₃
4	K ₂ CrF ₆
5	K ₃ CrO ₈
6	K ₂ CrO ₄

Guide to Speciation

Species toxicity is influenced by three factors: absorption, distribution and biotransformation. If a chemical or elemental species is easily absorbed by an organism, that species becomes more biologically available and therefore potentially toxic. The ability of a chemical species to cross biological barriers (i.e. blood/brain, intestines, and cell membranes) increases toxicity. Finally, if a chemical species can undergo modification, accumulation or excretion from biological systems, toxicity is affected.

Who Should be Concerned with Speciation Testing?

As more data is collected regarding the toxicity of chemical species, it becomes increasingly important to target the forms particular chemicals and elements with the highest potential toxicity. Many fields of science and chemistry are now becoming involved in determining the need for speciation in their testing methods. Environmental laboratories are moving away from testing total chromium and total mercury levels in favor of testing for the more dangerous Cr (VI) and organomercury species. Pharmaceutical and nutraceutical industries are establishing guidelines for the limitation of inorganic arsenic and mercury species under the new USP <232>, <2232> and ICH guidelines.

What are the Obstacles to Adapting Speciation Testing?

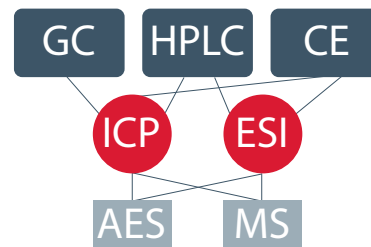
The ability to detect individual chemical and elemental species started almost forty years ago. Despite the value of determining individual chemical species, there are many obstacles to be resolved before the methods for speciation are widely implemented.

One of the first obstacles is sample collection and preservation. The chemical species must be able to be preserved or testing must occur in-situ for accurate results. Chemical species can change states with oxidation or reduction reactions in response environmental factors, microbial activity, preservatives, container interactions, or time. Common sample preparation techniques include the addition of chemical compounds which can cause reactions and change the pH of the samples, which then facilitates the change in state of target elements. Natural processes such as microbial activity, temperature changes, chemical volatility and time, can cause chemical species to change states. Finally, the actual collection vessel can contribute to contamination or facilitate absorption of chemicals into or from the materials of the container and thereby change the distribution of chemical species in the sample.

After a sample is collected, a process of sample preparation or treatment begins to prepare the sample for analytical testing. The sample preparation methods can also be responsible for changes in the distribution of the chemical species. Extraction processes can introduce heat and oxidizers to the sample. This effect can be seen when there is methylation of mercury from sediments during an extraction process. Some chemical compounds need to be derivatized prior to analysis but these derivatization steps can change the chemical state of the sample. One example is the formation of methyl- and ethylmercury in a reaction between inorganic mercury and the sodium tetra-n-propyl borate derivatization agent.

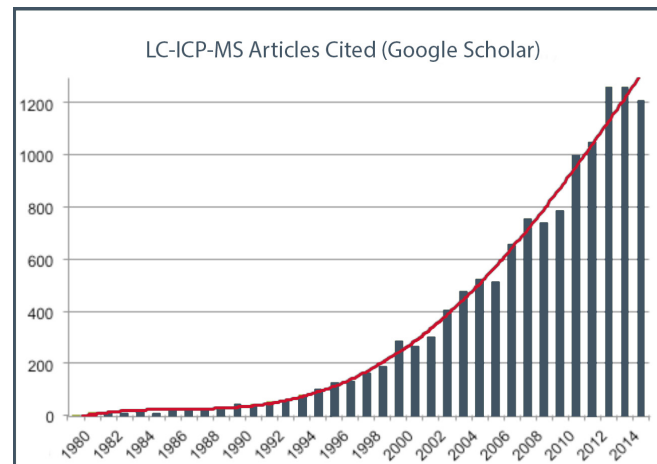
Guide to Speciation

The next step of an analysis procedure is then some type of instrumental or chemical analysis to determine identification and quantitation of target chemicals. There are a number of various methods for the separation of samples into their component states or species. These methods often couple a chromatographic or separation process to an ionization or electromagnetic process then to a spectrometry process. The most common separation processes are gas chromatography (GC), liquid chromatography (LC) and capillary electrophoresis (CE). These separation techniques allow each state or species to be measured, as a single peak or unit in time. The samples are then ionized or subjected to a magnetic field or charge by an inductively coupled plasma (ICP) or electrospray ionization process (ESI) before detection using a measurement and detection system such as mass spectrometry (MS) or optical or atomic emission spectroscopy (OES or AES).



Separation Process

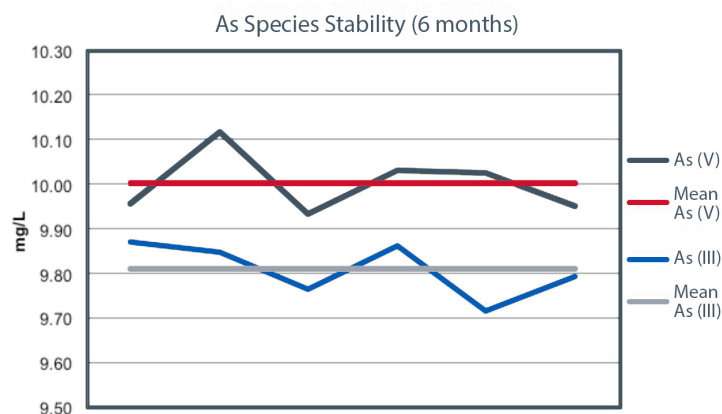
The fastest growing technology for speciation analysis is LC-ICP-MS which uses a liquid chromatography system coupled to an ICP-MS. This technology, which started in the 1980's, has seen slow growth over the past few decades only to explode into the analytical world within the last fifteen years. In 2000, there were just under 300 academic articles citing the LC-ICP-MS technique. By the end of 2014, there were over 1200 articles citing the use of LC-ICP-MS.



Guide to Speciation

Despite the advances in speciation analysis, the techniques themselves still pose some challenges to integrating speciation analysis into routine laboratory testing. The first hurdle is often cost. The costs associated with a hyphenated system combine all the costs of each individual system and then increase the costs of training and operation. There are many technical challenges for each type of system which can increase the difficulty of obtaining accurate speciation results. For example, chromatographic and separation techniques are dependent upon the resolution and separation of all the components. These techniques can be challenged by co-eluting compounds and loss of species or compounds to unseparated areas of the analysis. Many times the column yield of these techniques needs to be checked by mass balance of the total elemental concentration. These chromatography techniques can also cause distribution changes. In HPLC, some species of antimony and arsenic can transform via the oxygen present in mobile phases to other states. In GC, phenyl mercury salts can change to diphenyl mercury.

Two of the final obstacles are in regard to regulations and reference materials. It has only been within the last few years that the techniques of speciation analysis have started to appear in regulations and methodologies. Currently, most of the regulations are only concerned with Cr (VI) and organotin compounds. Newer regulations, such as the USP <232>, USP <2232> and ICH are incorporating analyses for arsenic and mercury species. These regulations are in a state of development and flux and it will be some time before the final methods are implemented. Once those regulations and methods are finalized it will then be a challenge to find standards and reference materials for all of the various chemical and elemental species covered under the directives. The true value of a standard or reference material will be the stability, homogeneity and accuracy of its stated chemical species distribution.



Arsenic Species Stability

Arsenic Species	Mean	StD	% RSD
As (III)	9.809	0.062	0.631
As (V)	10.002	0.069	0.692

Guide to Speciation

Spex CertiPrep, the leader in the field of inorganic standards for over sixty years, has taken the lead in now developing high quality speciation standards for this new and growing analytical field. Our speciation standards are tested using our own LC-ICP-MS to ensure the high quality, accuracy and stability found in all Spex CertiPrep standard products.

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