Handbook of Hyphenated ICP-MS Applications

First Edition
August 2007

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Introduction

Hyphenated techniques involving ICP-MS are among the fastest growing research and application areas in atomic spectroscopy. This is because, by itself, ICP-MS does not give information on the chemical or structural form of the analytes present (since all forms of the analytes are converted to positively charged atomic ions in the plasma). However, as an excellent elemental analyzer, it also performs as a superb detector for chromatography. Hyphenated ICP-MS is achieved through the coupling of the ICP-MS to a separation technique – normally a chromatographic separation. In this way, target analytes are separated into their constituent chemical forms or oxidation states before elemental analysis (Figure 1). The most common separation techniques are gas chromatography (GC) and high-performance liquid chromatography (HPLC), which includes ion chromatography (IC), but, other separation techniques, such as capillary electrophoresis (CE) and field flow fractionation (FFF), are also used.

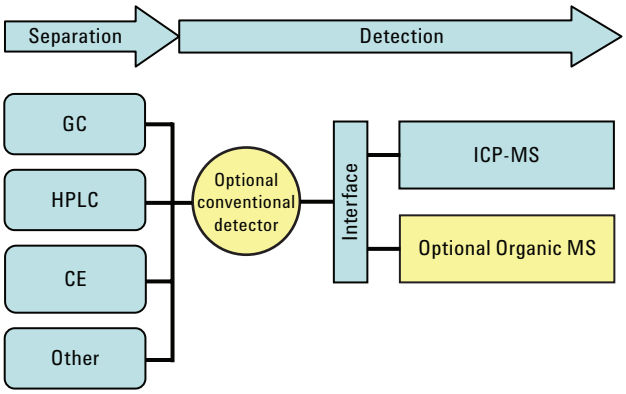


Figure 1. *Schematic of generic hyphenated system.*

This handbook specifically addresses the use of ICP-MS as an elemental detector for GC, LC, IC, and CE, though the same principles would apply to other similar techniques. Because of its ability to accurately distinguish isotopes of the same element, particularly now that collision/reaction cell (CRC) technology has all but eliminated interferences, ICP-MS is also capable of isotope dilution (ID) quantification.

Applications of hyphenated ICP-MS fall into the general category termed speciation analysis. In all cases, the fractionation device (chromatograph or other) is used to separate the species from each other and the matrix, and the ICP-MS is used to detect the species of interest. The analyte species may be as simple as elemental ions of various oxidation states in solution, or as complex as mixtures of pesticides or biomolecules. In all cases though, the ICP-MS is simply acting as an elemental detector. The fractionation device serves to separate the various components in the sample before detection as well as providing additional information in the form of retention time. Often this combination is sufficient to identify and quantify the target analytes. However where accurate retention time data is not available, analysis of standards or the use of additional mass spectrometric techniques can provide further confirmation of identification.

Elemental speciation is important in many application areas and is becoming particularly important in the environmental, food, and clinical industries. This is because, for many elements, properties such as those listed below depend on the species or chemical form of the element present in the sample.

- Toxicity or nutritional value
- Environmental mobility and persistence
- Bioavailability
- Volatility
- Chemical reactivity

A common example would be the measurement of Cr(VI) (toxic) and Cr(III) (essential nutrient) as opposed to total Cr in environmental samples. Similar examples of elemental speciation include As(III)/As(V), Se(IV)/ Se(VI), and other elements that can exist at different stable oxidation states. Furthermore, arsenic and selenium in particular also commonly exist in various organic forms which can significantly affect the traits listed above.

In the case of more complex molecules such as pesticides or biomolecules, the ICP-MS is able to identify and quantify the presence of a particular element or elements in molecular chromatographic peaks. When used in conjunction with organic MS techniques, this technique can permit quick screening for molecules (peaks) containing specific elements in a complex mixture, prior to analysis by organic MS. With modern, integrated systems and software, simultaneous analysis by ICP-MS and organic (for example, electrospray ionization [ESI]) MS is also possible, using a split flow from a single chromatographic device.

In addition to the more conventional liquid phase separations (HPLC and IC, for example), ICP-MS is also an excellent detector for separations carried out by GC. While other element-specific detectors exist for GC, none posses the elemental coverage, sensitivity, or specificity of ICP-MS. Examples of ICP-MS in molecular speciation are many and cover a broad variety of applications:

- Total sulfur and sulfur species in hydrocarbon fuels
- Organotin species in marine sediments and biota, consumer goods, and drinking water
- Mercury species in fish, industrial discharges, and petroleum processing
- Arsenic species in marine algae, food products, and drinking water
- Brominated and phosphorus-based flame retardants in consumer goods
- Phosphorus and sulfur in biological samples
- Protein- and peptide-bound metals

- Pesticides and herbicides
- Chemical warfare agents
- Volatile organohalides in air samples

In some cases, it is the presence of the target element that is important, (for example Cr(III) or Cr(VI)). In other cases, the element or elements are a simple way to identify and quantify a molecule present in a complex mixture (for example using P as a means of quantifying organophosphorus compounds).

This handbook is divided into sections based on the chromatographic component of the hyphenated ICP-MS system. Each section is composed of "application briefs" which outline typical or interesting applications for that technique. The application briefs are deliberately short, showing only general conditions and outlining results. Specific details for each application can be found in referenced publications in each section.

General Requirements

All hyphenated ICP-MS systems require that a few simple conditions are met.

- The connecting interface (transfer line) must transmit the fractionated sample quantitatively from the separation system (called a chromatograph from this point forward) to the plasma of the ICP-MS in a form that the plasma can tolerate.
- The temporal resolution of the sample components must not be unacceptably degraded.
- The chromatograph should communicate with the ICP-MS to allow synchronous separation and detection.
- The ICP-MS must be capable of transient signal acquisition at sufficient sampling frequency and over sufficient dynamic range to accommodate the resolution of the chromatograph and the required number of elements or isotopes per peak over their ranges of concentrations.

A good rule of thumb for chromatographic detectors applies here. In order to achieve accurate and precise peak integration, approximately 10 samples (scans) must be acquired for a typical Gaussian peak. Very narrow peaks will require a higher sampling frequency than wider peaks. As a quadrupole mass spectrometer, the ICP-MS sampling frequency is dependent on the scan speed of the quadrupole, the number of masses scanned, and the dwell time for each mass. Typically, since the number of elements or isotopes in hyphenated work is small, sufficient scan speed is not a problem. It must be possible to tune the ICP-MS under plasma conditions similar to those encountered during the chromatographic run. Generally, this entails introducing the tuning element(s) via the chromatographic interface. In general, using an ICP-MS as a detector for chromatography is a simple matter of connecting the outlet of the column to the sample introduction system of the ICP-MS. If the sample is gaseous, as in GC, the transfer line should be passivated and heated to eliminate sample degradation and condensation and will terminate directly into the ICP torch. If the sample is a liquid, the transfer line will likely terminate in a nebulizer in order to generate an aerosol compatible with the plasma. This may require either a split flow or makeup flow in order to match the chromatographic flow with the nebulizer and plasma requirements. Depending on the total sample flow and choice of nebulizers, the use of a spray chamber may or may not be necessary.

HPLC-ICP-MS

HPLC-ICP-MS Introduction

High-performance liquid chromatography (HPLC) is used to describe any chromatographic technique where analytes dissolved in a liquid mobile phase are separated based on their interactions with the mobile phase and a stationary phase contained in a column. This would include both reverse- and normal-phase HPLC, size exclusion chromatography (SEC) and ion exchange chromatography. HPLC (or IC)-ICP-MS is used for the analysis of nonvolatile compounds or ions in solution. The solution can be aqueous, organic, or a mixture of both. It is this flexibility in the choice of both stationary and mobile phases, including gradient techniques where the mobile phase changes composition during the chromatographic run, which makes HPLC such a powerful separation technique for many applications. As an HPLC detector ICP-MS is the only universal, element-specific detector available for liquid chromatography and, as such, has many applications.

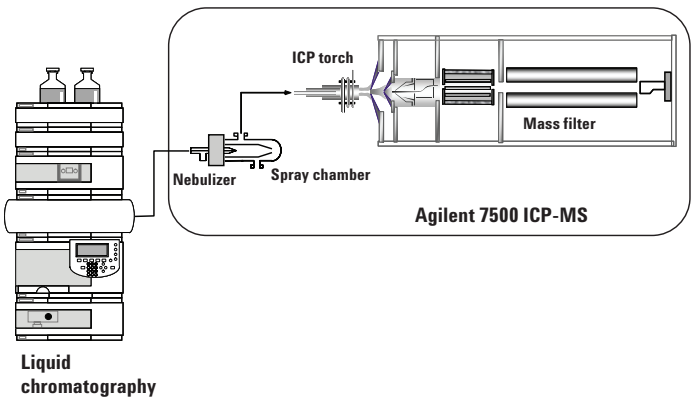
Combined with molecular mass spectrometry, ICP-MS can provide a powerful screening tool for metallic markers in biological compounds. It is also a powerful detector for specific nonmetals including sulfur and phosphorus.

Ion chromatography is a specialized form of HPLC designed to separate ionic species. It is typically used in the separation of cations (most metal ions in solution), though some metals exist as stable anions (usually oxyanions) in solution as well. The hardware is fundamentally similar to HPLC, though allowances are made for acidic or basic aqueous mobile phases, which could damage metal components in the HPLC. High background from dissolution of metal components can also be a problem. As a result, ion chromatographs rely on the use of polymeric or passivated components that are in contact with the mobile phase. Interfacing the IC to the ICP-MS is quite simple, since typical flows for IC are compatible with normal ICP-MS nebulizers. In addition, the sample handling components of the ICP-MS are already designed for acidic or caustic sample types. Since the ICP-MS is not a conductivity detector, special techniques to suppress the conductivity of the mobile phase that are required for normal ion chromatography are not necessary with IC-ICP-MS.

When used with ion chromatography, ICP-MS can provide positive elemental confirmation in addition to retention time. The Agilent LC connection kit supplies all the components and documentation necessary to interface an Agilent or other HPLC or IC to the Agilent 7500 Series ICP-MS.

Matching the Column Flow to the Nebulizer/ Spray Chamber

Matching the optimum column flow with the optimum nebulizer flow is critical to achieve both efficient separation and sample nebulization. Since the ICP-MS can tolerate nebulizer flow rates from near zero to in excess of 1 mL/minute, the nebulizer is generally selected to match the column flow. Any nebulizer has a range of flows over which it produces the highest proportion of fine droplets in the aerosol. This is critical since fine droplets are more efficiently transported through the spray chamber and atomized and ionized in the plasma. Therefore, a nebulizer that has an optimum flow rate at or near the optimum column flow should be selected. For typical HPLC flows of 100 µL/min to 1 mL/min, conventional concentric nebulizers, either in glass, quartz, or fluoropolymer work very well. At significantly higher flows, some of the sample will need to be split off prior to the nebulizer. This can be accomplished through the use of a low dead volume "Tee" near the nebulizer. In this case, a self-aspirating nebulizer must be used to avoid the need for a peristaltic pump, which would introduce unacceptable dead volume. As long as the column flow is larger than the nebulizer self-aspiration rate, there will be positive flow at the split outlet to drain. At the other extreme, if the column flow is extremely low as in the case of nano-LC, then a makeup flow may need to be added to the column flow in order to meet the flow requirements of the chosen nebulizer. While it may seem that this will result in a loss of sensitivity through dilution, in fact this is not necessarily the case. Since the ICP-MS is measuring analyte mass, not concentration, the presence of the makeup flow may not affect the result. There may be some loss, however, depending on the transport efficiency of the nebulizer/spray chamber selected. This configuration has the additional benefit of being able to add a post-column internal standard to the flow, which can be used to correct for instrument drift or matrix effects due to gradient elutions or for isotope dilution calculations. The makeup flow, with or without internal standard, can be supplied by a peristaltic pump, or, if higher precision is desired, by a piston-type LC pump or syringe pump.



HPLC-ICP-MS for Analysis of Chemical Warfare Agent Degradation Products

Douglas D. Richardson, Baki B.M. Sadi, and Joseph A. Caruso, Department of Chemistry,
University of Cincinnati, Cincinnati, OH, USA

Introduction

Phosphorus-containing nerve agents and their degradation products present difficulties for ultra-trace analysis due to their high polarity, low volatility, and lack of a good chromophore. Direct analysis of chemical warfare agent (CWA) degradation products can provide an indirect technique for CWA detection. Previous studies have successfully utilized methods such as GC-MS, ion mobility/mass spectrometry (IMMS), and LC-MS for the analysis of organophosphorus-containing degradation products with detection limits in the ng/mL range. However, considering the lethal doses, lower detection limits in the pg/mL range are desirable. When coupled to HPLC, ICP-MS can provide the necessary selectivity and sensitivity to meet the desired limits.

Table 1. *HPLC and ICP-MS operating parameters*

HPLC	
HPLC	Agilent 1100 with binary pump, vacuum degasser, diode array detector
LC column	Alltima C8, 100 Å, 3.2 mm x 150 mm, 5 µm
Guard column	Alltima C8, 7.5 mm x 3.0 mm, 5 µm
Buffer	50 mM ammonium acetate; 2% methanol; 5 mM myristyltrimethylammonium bromide, pH 4.85
Flow rate	0.5 mL/min
Injection volume	100 µL

ICP-MS	
ICP-MS	Agilent 7500ce
Forward power	1500 W
Nebulizer	Glass micro-concentric
Carrier gas flow	1.2 L/min
Aux gas flow	1.0 L/min
Spray chamber	2 °C
Sampling depth	6 mm
Dwell time	0.1 s
Isotopes	^{31}P and $^{47}PO^{+}$
ORS mode	He collision with KED

Hardware Setup

Reversed phase, ion-pairing HPLC coupled to collision/reaction cell ICP-MS was used. Conditions are listed in Table 1.

Standards and Reagents

The three chemical warfare degradation products (ethyl methylphosphonic acid [EMPA], isopropyl methylphosphonic acid [IMPA], and methylphosphonic acid [MPA]) used were obtained from Cerilliant (Austin, TX) as 1 mg/mL certified reference materials (CRMs). The remaining reagents were of analytical grade, prepared fresh daily through dilution of stock standards with DDI (18 MΩ) water.

Results

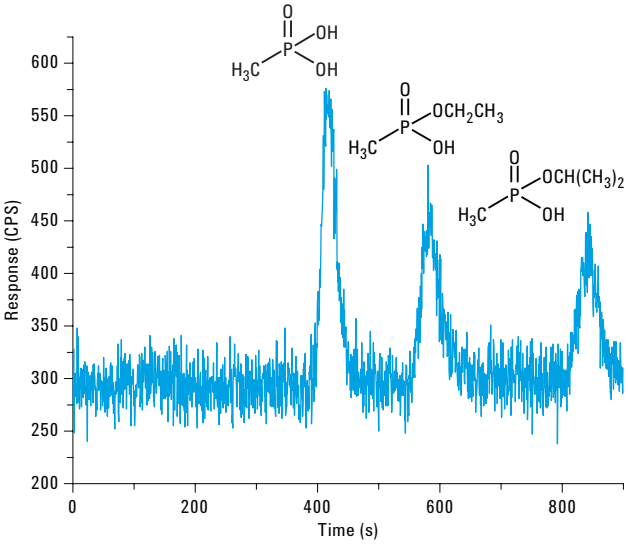
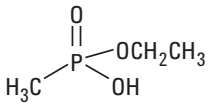
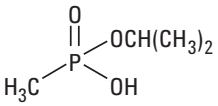
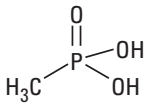


Figure 1. *Separation of MPA, EMPA, and IMPA in a standard mixture.*

Table 2. *Chemical warfare degradation product detection*

Chemical warfare degradation products	Analytical method	Detection limits ng/mL
 **EMPA**	Ion Mobility Mass Spectrometry[a]	560–1700[5]
	LC-ESI-TOF	80–1000[3]
 **IMPA**	Electrophoresis Microchip with Contactless Conductivity Detector	48–86
 **MPA**	RP-IP-HPLC-ICPMS	0.139–0.263

Conclusions

RP-IP-HPLC-ICPMS can provide rapid, sensitive detection of CWA degradation products at analytically useful levels superior to other techniques.

For Additional Information

Agilent Application Note: "Ultra-Trace Analysis of Organophosphorus Chemical Warfare Agent Degradation Products by HPLC-ICP-MS," 5989-5346EN.

First published in: *Journal of Analytical Atomic Spectrometry,* vol. 21, 396-403 (2006).

Analysis of Glyphosate, Gluphosinate, and AMPA by Ion-Pairing LC-ICP-MS

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Introduction

Glyphosate (Roundup®) and the related compound gluphosinate are among the most widely used of nonselective herbicides. They act by inhibiting the synthesis of specific amino acids. AMPA (aminomethylphosphonic acid) is the major metabolite. While LC separation of these compounds is fairly straightforward, specific, sensitive detection has been problematic due to poor ionization characteristics in LC/MS. Detection of phosphorus using collision cell ICP-MS to eliminate the common interferences from NO^+ and NOH^+ when coupled to HPLC can provide a simple, highly sensitive method of analysis for these compounds. See Figure 1.

Hardware Setup

HPLC Conditions: Agilent 1100 liquid chromatograph equipped with a binary HPLC pump, autosampler, vacuum degasser, thermostatted column compartment and diode array detector. The HPLC system was connected to the ICP-MS using the Agilent LC connection kit (G1833-65200). A C8 column (ZORBAX SB-C8, 4.6 x 150 mm, 5 µm, Agilent Technologies) was used for separation. The column temperature was maintained at 30 °C for all experiments.

ICP-MS: Agilent 7500c ICP-MS was used for detection. Instrument operating conditions are shown under Methods.

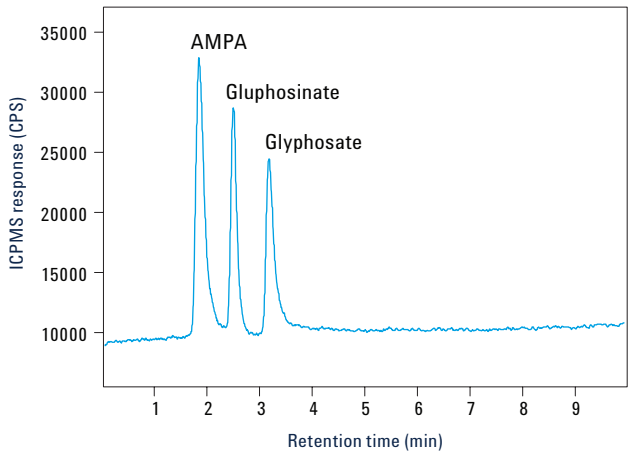

Figure 1. *Chromatogram of the herbicides gluphosinate, glyphosate and metabolite AMPA, (concentration x, y, z).*

Standards and Reagents

Deionized water (18 MΩ cm), NanoPure treatment system (Barnstead, Boston, MA, USA) was used in all standards and in buffer preparation. Commercial chemicals were of analytical reagent grade and were used without further purification. Aminomethyl-phosphonic acid (AMPA), N- (phosphonomethyl) glycine (glyphosate), gluphosinate, ammonium acetate, and tetrabutyl-ammonium hydroxide were purchased from Sigma.

Methods

Table 1. *HPLC and ICP-MS operating parameters.*

ICP-MS Parameters	
Forward power	1500 W
Plasma gas flow rate	15.0 L/min
Carrier gas flow rate	1.11 L/min
Sampling depth	6 mm
Sampling and skimmer cones	Nickel
Dwell time	0.1 s per isotope
Isotopes monitored	^{31}P
Nebulizer	Glass concentric
Spray chamber	Scott double-pass
Cell gas	He
Flow rate of cell gas	1.5 mL/min

HPLC Parameters	
Column	ZORBAX SB-C8, 4.6 x 150 mm, 5 µm
Mobile phase	50 mM ammonium acetate/acetic acid buffer 5 mM tetra-butylammonium as ion pairing reagent 1% methanol pH = 4.7
Flow rate	1.0 mL/min
Temperature	30 °C
Injection volume	100 µL

Results

Table 2. *Limits of detection for phosphorus in AMPA, gluphosinate, and glyphosate.*

	AMPA	Gluphosinate	Glyphosate
Regression coefficient	0.999	0.998	0.999
LOD (conc)	25 ppt	27 ppt	32 ppt
LOD (amount)	2.5 pg	2.7 pg	3.2 pg
RSD, retention time, n = 8	1.1 %	0.8 %	1.2 %

Conclusions

When coupled with ion-pairing HPLC, the Agilent 7500c ICP-MS, using ORS technology to remove interferences on phosphorus, can provide a superior detection system for the phosphorus-containing herbicides and their metabolites.

For Additional Information

Baki B.M. Sadi, Anne P. Vonderheide, and Joseph A. Caruso. Analysis of phosphorus herbicides by ion-pairing reversed-phase liquid chromatography coupled to inductively coupled plasma mass spectrometry with octopole reaction cell, *Journal of Chromatography A,* Volume 1050, Issue 1, 24 September 2004, Pages 95-101.

Analysis of Methyl Mercury in Water and Soil by HPLC-ICP-MS

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Introduction

Mercury can exist either in the elemental or alkylated form. Biological activity will typically methylate mercury to either methyl mercury (MeHg) or, less commonly, di-methyl mercury. The different chemical forms of mercury have different toxicities, with MeHg species being 10 to 100 times more toxic than inorganic mercury compounds. As a result, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently recommended that the Provisional Tolerable Weekly Intakes (PTWI) of MeHg be reduced to 1.6 µg per kg body weight per week, down from 3.3 µg per kg body weight per week.

The simultaneous determination of inorganic and organic mercury is difficult because the typical concentration of MeHg is much lower than that of inorganic mercury. The most common methods for mercury speciation are gas chromatography (GC) or high-performance liquid chromatography (HPLC) coupled with a mercury-specific detector (fluorescence, photometry, or other elemental detector). The low concentration of mercury in natural waters leads to the need for very large sample volumes to be processed. A preconcentration step is usually necessary because the reporting limit required is often below the sensitivity of the detector used.

Instrumentation

The aim of this work was to evaluate HPLC-ICP-MS in terms of its sensitivity and specificity for the determination of MeHg. An Agilent 1100 LC was coupled to an Agilent 7500a ICP-MS using the LC-ICP-MS Connection Kit (G1833-65200).

HPLC Column

For best results, the HPLC column (ZORBAX Eclipse XDB-C18, 2.1 mm x 50 mm, 5 µm) should be preconditioned by pumping HPLC-grade methanol at 0.4 mL/min for at least 2 hours, and then conditioned with eluent at the same flow rate for at least half an hour.

Results and Discussion

A series of calibration standards was prepared from 10 ng/L to 100 µg/L by diluting a mixed Hg species stock solution (1.0 µg/mL Hg for Hg^{2+}, MeHg, and ethyl-Hg, in pure water). A 20-µL injection loop was used throughout except for the 10-ng/L data, which was obtained using a 100-µL loop. The peak areas were integrated for different concentration levels of three mixed Hg species. The linear range of the calibration curves (Figure 1) for Hg speciation by the HPLC-ICP-MS method was at least four orders. This range covers expected real sample levels, and so the method is appropriate for direct determination of water samples without the application of complicated preconcentration procedures.

Chromatographic Separation of Hg Species in 3% NaCl

In order to prove the applicability of the method to high-matrix sample analysis, the stock Hg species solution was also diluted into 3% NaCl (w/v in water) to obtain 100 ng/L MeHg, ethyl-Hg, and Hg^{2+}. The solution was filtered through a 0.45-µm membrane before analysis. A 20-µL injection loop was used for the measurement. The chromatogram was overlaid with the chromatogram of the pure water diluted solution at the same concentration, as shown in Figure 2. The peak areas of the Hg species in 3% NaCl were also integrated, and the recoveries were between 90% and 110% relative to standards in pure water. This demonstrates that the method is suitable for even high-matrix samples, such as seawater.

Application to Soil Samples

When the HPLC-ICP-MS method is applied to solid samples, such as tissues, soils, or sediments, sample preparation is necessary. The extraction of Hg species from the solid samples is a crucial step due to the presence of mercury in environmental samples at low levels, and the Hg species, especially MeHg is easy to lose or transform to other species. A simple extraction method based on dilute hydrochloric acid was used. The spike recoveries of the soil samples were between 80% and 120%. Further testing of the method and the MeHg-containing reference soil sample are planned for future work.

Table 1. *Working parameters of HPLC.*

HPLC parameters	
Column	ZORBAX Eclipse XDB-C18, 2.1 mm id x 50 mm, 5 µm
Mobile phase	0.06-mol/L ammonium acetate, 5% v/v methanol, 0.1% 2-mercaptoethanol, pH = 6.8
Flow rate	0.4 mL/min
Injection volume	100 µL

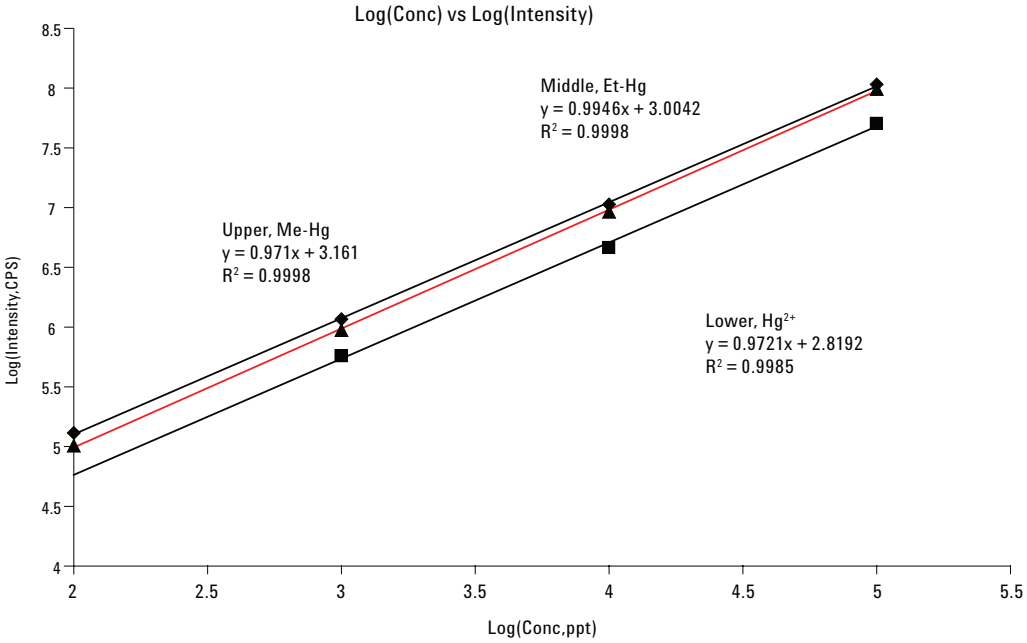

Figure 1. *Calibration curves for MeHg, Hg^{2+} and EtHg.*

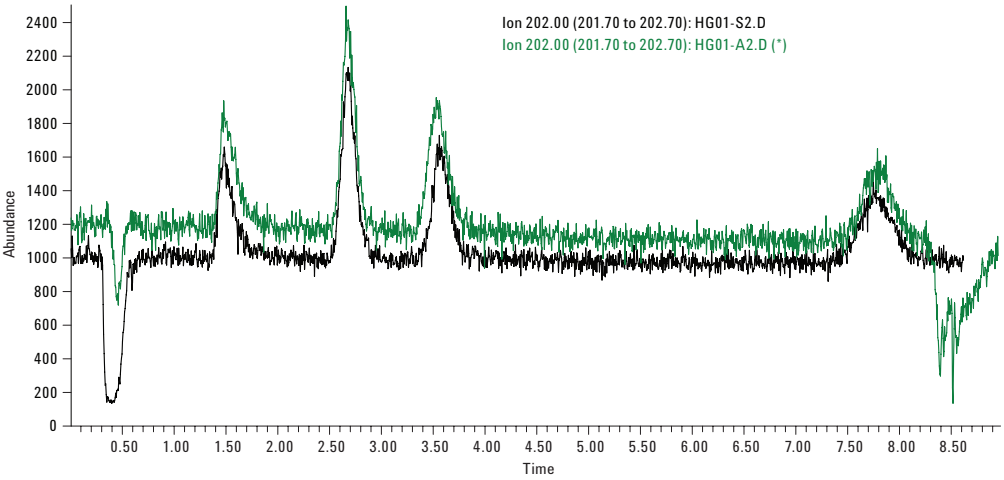

Figure 2. *Overlaid HPLC-ICP-MS ion chromatograms of 100 ng/L Hg species standards in pure water (upper) and in 3% NaCl (w/v, lower) (20 µL loop).*

Conclusions

HPLC-ICP-MS is appropriate for water samples analysis, even when the matrix in the water sample is high. The method detection limits for MeHg, ethyl-Hg, and Hg^{2+} are better than 10 ng/L and meet current regulatory requirements. When the method is applied to soil samples, Hg species extraction by 7.6% HCl is appropriate, with recoveries between 80% and 120%.

For Additional Information

Dengyun Chen, Miao Jing and Xiaoru Wang, "Determination of Methyl Mercury in Water and Soil by HPLC-ICP-MS," Agilent Technologies publication 5989-3572EN.

Agilent ICP-MS Journal May 2005, Issue 23, 5989-2950EN.

Determination of Ceruloplasmin in Human Serum by Immunoaffinity Chromatography and SEC-ICP-MS

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Orr Sharpe and William H. Robinson, Stanford University, Stanford, CA, USA

Introduction

Ceruloplasmin (Cp) is a blue alpha-2 glycoprotein with a molecular weight of 132 kilodaltons (kDa) that binds 90 to 95% of blood plasma copper (Cu) and has six to seven Cu atoms per molecule. The various functions of this protein include ferroxidase activity, amine oxidase activity, superoxidase activity, and involvement in Cu transport and homeostasis. At present there is no standardized reference method for Cp, and the immunologic methods cross-react with apoceruloplasmin (apoCp), which can bias data and deliver higher than expected concentrations for the target protein.

A method for the determination of Cp in human serum at biologically relevant concentrations > 0.01 mg/mL has been developed. Size-exclusion chromatography (SEC) is used to separate Cp from other proteins and from inorganic ions and ICP-MS, to detect Cu isotopes (m/z = 63, 65), and to confirm the identity of Cp using the $^{63}Cu/^{65}Cu$ ratio.

Experimental

Materials

Reconstituted, lyophilized Cp standards purified from human plasma were used in the study. (EMD Biosciences/Calbiochem, La Jolla, CA, USA, and Sigma, Saint Louis, MO, USA). Serum samples from patients with one of four different diseases, including myocardial infarction (MI), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and pulmonary embolism (PE), and normal controls (NC) were obtained from Stanford University (Stanford, CA). All samples were kept frozen at -20 °C until analysis. ERM DA470 is a human serum certified for 15 proteins, including Cp, and was purchased from RTC (Laramie, WY, USA).

Instrumentation

To eliminate possible interference from highly abundant proteins, some of which may bind Cu to form protein-Cu complexes, the serum sample is depleted of albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin by immunoaffinity chromatography using the Agilent 4.6 mm x 100 mm immunoaffinity column prior to SEC. An Agilent 1100 binary liquid chromatography (LC) system was used for the immunoaffinity work. Protein separation was achieved on a silica TSKGel column SW3000 from Tosoh Bioscience (Montgomeryville, PA, USA). All SEC analyses were performed on another Agilent 1100 Series binary HPLC system with diode array detector at 0.3 mL/min flow (0.1 M *tris* -pH 7). The exit from the diode array detector was connected directly to the Agilent 7500ce ICP-MS (MicroMist nebulizer) using polyetheretherketone (PEEK) tubing (60 cm length). The 7500ce was operated in helium collision mode using kinetic energy discrimination (KED) to remove the Na-, Mg- and P-based polyatomic interferences on ^{63}Cu and ^{65}Cu.

Determination of Cp by SEC-ICP-MS

SEC retention times were calibrated using a mixture of standard proteins. Cp eluted at 8.4 minutes, between albumin and IgG. However, its detection in real samples by UV is difficult due to overlap by other serum proteins. Using SEC-ICP-MS, the Cu containing Cp is easily identified. Cp-bound copper is easily distinguished from free Cu by retention time.

Method Performance

The performance of this assay was established with the reference human serum ERM DA470, which is certified for Cp at 0.205 mg/mL. The results, summarized in Table 1, illustrate excellent agreement with the certified values. Method performance data are included in Table 2. Total analysis time is approximately 95 min/sample from start to finish (15 min dilution and filtration, 30 min immunoaffinity chromatography, 20 to 30 min concentration, and 20 min SEC-ICP-MS analysis).

Table 1. *Concentration of Cp in the ERM DA470 reference serum.*

	Certified value (mg/mL)	Measured concentration (mg/mL)	$^{63}Cu/^{65}Cu$
ERM DA470 reference serum (freshly reconstituted)	0.205 (0.011)*	0.208 (5.4%)**	2.1 (3.6%)**

*Uncertainty (mg/mL)

**Average of three determinations; value given in parentheses is the percent coefficient of variation (CV%).

Table 2. *Determination of Cp by SEC-ICP-MS - method performance.*

Method indicator	Value
Detection limit (5-µL injection)	0.01 mg/mL
Dynamic range	0.01 to 5.0 mg/mL (tested only to 5 mg/mL)
Reproducibility	Overall CV: <10%
Accuracy	101% (ERM DA 470)
Cp identification	Retention time plus Cu 63/65 isotope ratio = 2.2 ± 0.1

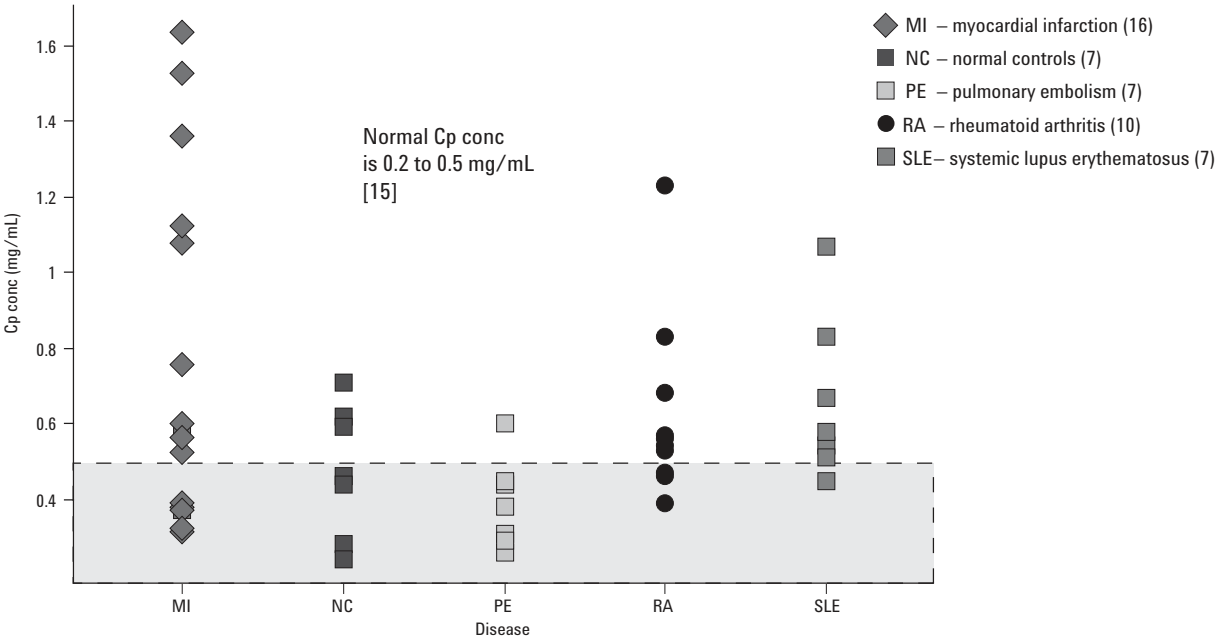

Figure 1. *Cp concentration in human sera from patients with four diseases and from normal controls; numbers in parentheses indicate the number of sera analyzed for Cp. Gray area shows Cp range reported for normal subjects (0.2 to 0.5 mg/mL).*

Results

Forty-seven human sera from patients with one of four different diseases and a set of normal controls were analyzed for Cp by the SEC-ICP-MS method (Figure 1).

Conclusions

Ceruloplasmin in human serum can be accurately determined at physiologically relevant levels using SEC-ICP-MS after cleanup by immunoaffinity chromatography as demonstrated using ERM DA470 reference serum. Initial application of the technique to sera of diseased patients shows a relationship between some diseases and elevated serum Cp concentrations.

For Additional Information

V. Lopez-Avila, O. Sharpe, and W. Robinson, "Determination of Ceruloplasmin in Human Serum by SEC-ICPMS," *Analytical and Bioanalytical Chemistry*, Volume 386, Number 1, Sept. 2006, pp 180-187.

V. Lopez-Avila, O. Sharpe, and W. Robinson,"Determination of Ceruloplasmin in Human Serum by Immunoaffinity Chromatography and Size-Exclusion Chromatography Coupled to ICP-MS," Agilent Technologies publication 5989-5304EN.

Iodine Speciation of Seaweed Using Different Chromatographic Techniques With ICP-MS Detection

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Introduction

Iodine is an essential micromineral for human nutrition, necessary for proper production of thyroid hormones. The recommended dietary allowance (RDA) of iodine is 150 mg per day (mg/d) in the United States and 150 to 200 mg/d in European and other countries. Iodine deficiency leads to various disorders associated with growth and development including "endemic goitres" and cretinism; while excessive iodine intake may cause hyperthyroidism which can also lead to the formation of a goitre, which in turn can lead to retarded brain development and functional impediment. Another important fact associated with consumption of iodine is that, like other elements, bioavailability and toxicity is species dependent. Inorganic forms of iodine, such as iodide and iodate, are less toxic than molecular iodine and some organically bound iodine. Likewise, the bioavailability of organically bound iodine, such as monoiodotyrosine (MIT) and diodotyrosine (DIT), is also less than that of mineral iodide.

Because supplementation of foodstuff with iodine is commonly practiced, total analysis and characterization of iodine species in food supplements is important. Sources include milk, iodized salt, and marine algae, including commercially available seaweed for example, Hizikia (Hiziki), Undaria (Wakame), Laminaria (Kombu) and Porphyra (Nori). Other marine algae used as a food supplement for iodine include Wakame (*Undaria pinnatifida pinnatifida*) and Kombu (*Laminaria digita japonica*).

The aim of this study is an initial characterization and identification of iodine species in commercially available seaweed samples using multidimensional chromatographic techniques coupled to ICP-MS.

Hardware Setup

Chromatographic separations were performed using an Agilent 1100 liquid chromatographic system equipped with an HPLC binary pump, an autosampler, a vacuum degasser, a thermostatted column compartment, and a diode array detector. Chromatographic conditions are summarized in Table 1.

An Agilent 7500ce ICP-MS equipped with a MicroMist nebulizer was used for iodine-specific detection. In order to connect the HPLC to the ICP-MS, the outlet of the UV detector was connected online to the liquid sample inlet of the ICP-MS nebulizer using 300 mm long by 0.25 mm PEEK tubing. For RP-HPLC, online dilution of the chromatographic eluent containing organic solvent was performed to reduce the organic solvent (methanol) load introduced into the plasma. Instrumental operating conditions are summarized in Table 1.

Table 1. *ICP-MS and chromatographic instrumental parameters.*

ICP-MS parameters	
Forward power	1500 W
Plasma gas flow rate	15.0 L/min
Auxiliary gas flow rate	0.87 L/min
Carrier gas flow rate	1.20 L/min
Dwell time	0.1 s per isotope
Isotopes monitored	^{127}I
SEC parameters	
Column	Superdex 75 HR 10/30
Mobile phase	0.03 mol/L Tris-HCl buffer, pH 8.0
Flow rate	0.6 mL/min
Injection volume	100 uL
Ion chromatography parameters	
Column	Ion Pac AS-11 anion exchange column (250 mm x 2.0 mm id x 13 µm)
Mobile phase	0.005 mol/L sodium hydroxide
Flow rate	0.3 mL/min
Injection volume	20 uL
RP-HPLC parameters	
Column	Alltima C18 (150 mm x 4.6 mm, 5 µm)
Mobile phase	(A) 0.01 mol/L Tris-HCl (pH 7.3) (B) 0.01 mol/L Tris-HCl (pH 7.3) and 50% MeOH
Flow rate	0.5 mL/min
Injection volume	50 uL
Make up solution	2% (v/v) HNO_3; 0.5 mL/min
Gradient	0-5 min-100% A to 45% B; 5-8 min-45% B to 85% B; 8-10 min-85% B to 100% B; and 10-40 min-100% B

Samples

Commercially available dried seaweed samples [marine algae Kombu (Laminaria japonica) and Wakame (Undaria pinnatifida)] were obtained from local Asian stores in the USA for total iodine analysis and speciation studies. The dried algae samples were ground in a household coffee grinder.

Results

- Total iodine concentration in samples and extracts – Both types of seaweeds were analyzed for total iodine content by ICP-MS after complete digestion using an MES 1000 closed vessel microwave digestion system (CEM Corp., Matthews, NC, USA). See Table 2.
- Size exclusion chromatography (SEC)-ICP-MS – SEC coupled to ICP-MS was used to investigate the association of iodine with various molecular weight fractions and to separate inorganic iodine from organically bound iodine.
- IC-ICP-MS for speciation of inorganic iodine – Anion exchange chromatography coupled to ICP-MS was used to separate iodide and iodate. See Table 2.
- RP-HPLC-ICP-MS for studying iodine species – Reversed-phase high-performance liquid chromatography (HPLC) coupled to ICP-MS was used for the separation and identification of low molecular weight iodine species in seaweed samples. Identification of iodine species was performed by matching the peak retention times with those of standards.

Table 2. *Inorganic iodine species present in seaweed samples.*

	Total content µg/g (% RSD)	Iodide µg/g	Iodate µg/g
Kombu	4170 (5.6)	3940	Not detectable
Wakame	226 (4.8)	140	4.16

Conclusions

In this study, the applicability of several chromatographic techniques, including SEC, IC-HPLC, and RP-HPLC, coupled to ICP-MS to iodine speciation in seaweed has been demonstrated. Moreover, the use of hyphenated techniques for iodine speciation in seaweed extracts allowed us to obtain important information on the association of iodine to the various matrix components of seaweed. Whereas iodide is about the most predominant species present in Kombu, a more complicated distribution of iodine is present in Wakame seaweed. This study shows that incorporation of iodine in different seaweeds follows different metabolic pathways, notwithstanding that both of them belong to the same class, *Phaeophyceae*. The presence of iodide was proved in Kombu, while in the case of Wakame, monoiodotyrosine and diiodotyrosine are also present and probably bound to the proteins. Since the bioavailability of iodide is better than any other form of iodine, Kombu seaweed would be preferred as a natural dietary supplement.

For Additional Information

Monika Shah, Rodolfo G. Wuilloud, Sasi S. Kannamkumarath, and Joseph A. Caruso. "Iodine speciation studies in commercially available seaweed by coupling different chromatographic techniques with UV and ICP-MS detection," *J. Anal. At. Spectrom.*, 2005, 20, 176-182.

Determination of Organic and Inorganic Selenium Species Using HPLC-ICP-MS

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Introduction

Selenium is important from an ecotoxicological point of view due to the narrow concentration range between its essential and toxic effects. Selenium compounds are distributed throughout the environment as a result of human activities (industrial and agricultural uses) and natural processes (weathering of minerals, erosion of soils, and volcanic activity). In waters, concentrations can vary from 2 ng/L to 1,900 µg/L depending on the system [1]. The natural cycle of selenium shows its existence in four oxidation states (-II, selenide; 0, elemental selenium; +IV, selenite; +VI, selenate) and in a variety of inorganic and organic compounds. The organically bound Se(-II) compounds include seleno-amino acids and volatile forms (dimethylselenide, dimethyldiselenide), which are less toxic relative to other species resulting from detoxification pathways.

Instrumentation

A standard 7500ce ICP-MS equipped with a concentric nebulizer (Meinhard Associates, California, USA) was used for this study. Chromatographic separation was carried out using the Agilent 1100 Series HPLC pump equipped with a variable volume sample loop. The analytical column was a Hamilton PRPX-100, 10 µm particle size, 25 cm length x 4.1 mm internal diameter (id). The chromatographic separation of selenocystine (SeCyst), selenomethionine (SeMet), selenite (SeIV), and selenate (SeVI) was adapted from [2] and performed using a 5 mmol/L ammonium citrate buffer, pH 5.2.

Injection volume was fixed at 100 µL. Methanol (2% v/v) was added to the mobile phase to improve sensitivity [3]. The mobile phase was delivered at 1 mL/min isocratically. The HPLC-ICP-MS interface consisted simply of polyetheretherketone (PEEK) tubing.

Experimental

Total selenium concentration (measured at ^{78}Se isotope) and selenium species concentrations were determined in different mineral and spring waters (Table 1). Results for certified simulated rain water (TM-Rain 95 from National Water Research Institute) are also given. The method was then applied to the mineral and spring water samples previously analyzed for their total selenium content.

Concentrations found in total and speciation analysis are in complete agreement showing the suitability of the method when applied to natural water samples. Although the bromine hydride interference on *m/z* 80 is present, it is separated chromatographically without overlapping with the selenium species. The chromatogram of water sample "C" (Figure 1) shows bromine elutes after the selenate peak.

Selenate, commonly found in oxygenated waters, was determined in commercial waters A through D. Selenite was identified in TM-Rain 95 water, which is only certified for its total selenium content. Only water E, a non-commercial ground water, contained both inorganic (selenite and selenate) species. See Figure 2.

Table 1. *Selenium concentrations determined in different natural waters (units: ng(Se)/L).*

Natural water sample	Elemental analysis ^{78}Se	HPLC coupling ^{78}Se		^{80}Se	
		SeIV	SeVI	SeIV	SeVI
TM-Rain 95	622 ± 19*	629 ± 7	< DL	615 ± 8	< DL
A	67 ± 1	< DL	69 ± 2	< DL	72 ± 6
B	142 ± 24	< DL	140 ± 9	< DL	143 ± 4
C	240 ± 20	< DL	232 ± 13	< DL	267 ± 13
D	467 ± 17	< DL	475 ± 4	< DL	492 ± 5
E	1890 ± 160	55 ± 2	1840 ± 30	57 ± 6	1920 ± 20

*Certified value 740 ± 290 ng(Se)/L

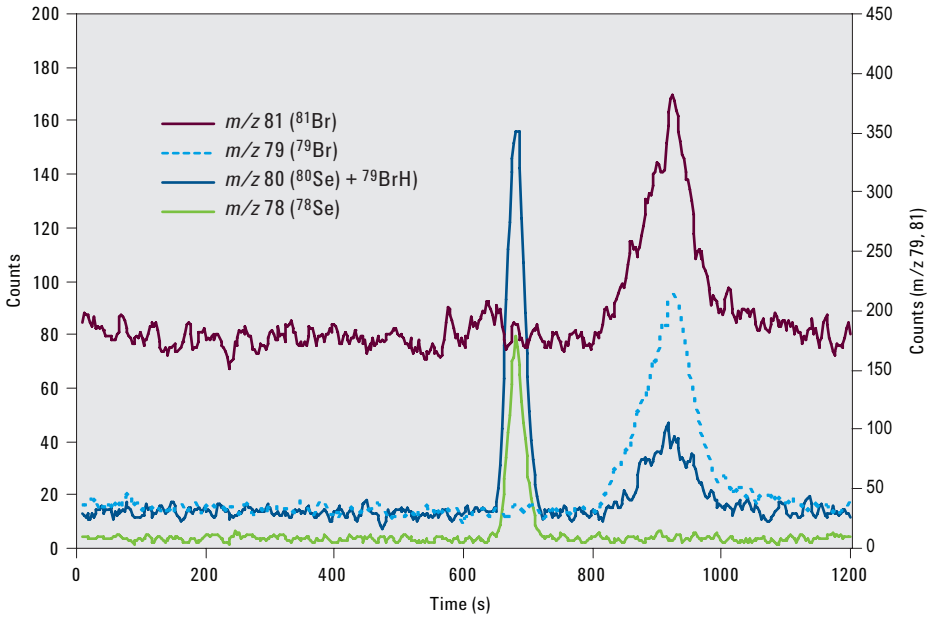

Figure 1. *Chromatogram of natural water "C" showing interference from bromine hydride elutes after the selenate peak.*

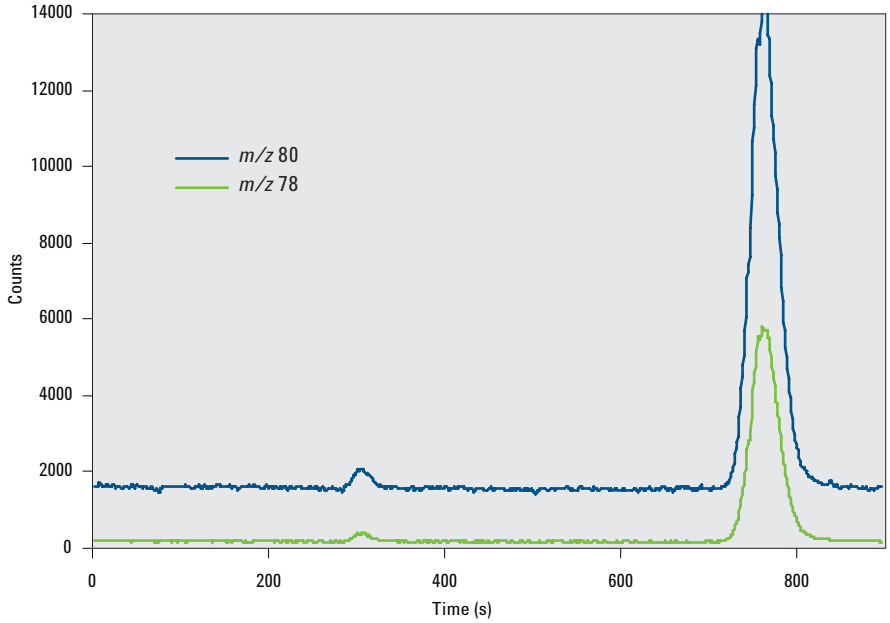

Figure 2. *Chromatogram of Natural Water "E," the only sample to contain both inorganic species. First peak is Se(IV), second peak is Se(VI).*

Conclusions

A hyphenated technique consisting of isocratic HPLC coupled to ICP-MS with optimized collision/reaction cell conditions allows for a quick and precise simultaneous analysis of organic and inorganic selenium species. Moreover as HPLC-ICP-MS coupling is easily automated, it can be considered a robust routine method to monitor selenium species levels in environmental and nutritional samples.

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HPLC-ICP-MS for Preliminary Identification and Determination of Methyl-Selenium Metabolites of Relevance to Health in Pharmaceutical Supplements

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Introduction

Selenium (Se) is an essential trace element with several functions that are relevant to health. While the nutritionally essential functions of Se are understood to be fulfilled by the selenoproteins, dietary Se can be metabolized to low molecular weight species (for example, methyl-Se compounds) that have more recently generated interest because of putative anticancer effects [1]. In contrast to such beneficial effects, at a sufficiently high dose level, Se metabolites can also cause toxicity. Since selenium is declining in the ordinary diet in Europe, efforts have been made to increase Se intake levels, mainly through biofortification of food and the production of pharmaceutical supplements.

Knowledge of speciation of selenium in food and food supplements will have implications with respect to the determination of Se requirements and to the investigation of relationships between Se status and health and disease. It will help in the development of safe and effective products and with future regulation of their production and use. Characterization of food and dietary supplements for Se speciation is challenging and demands the development of analytical techniques, such as hyphenated mass spectrometry methods, that allow the measurement and identification of Se chemical forms (species) in a complex sample matrix [2]. The combined application of element-specific MS (ICP-MS) and molecule-specific MS (ESI- or MALDI-MS) with HPLC has become an irreplaceable tool in this field.

In terms of quantifying Se compounds, the attractive features of ICP-MS, such as isotope specificity, versatility, high sensitivity, large dynamic range, and the virtual independence of the signal intensity of the structure of the biomolecule, makes this detector, in combination with a selective chromatographic separation, a potential and unique tool for quantitative Se speciation. ICP-MS, when used in combination with complementary HPLC separation methods, may allow preliminary identification of Se compounds, for which standards are available. Moreover, knowledge of the HPLC-ICP-MS retention times of minor Se-containing compounds in complex matrix samples (for example, food supplements) has been found to be essential to the identification of the Se isotope patterns in the total ion chromatogram (TIC) obtained by HPLC-ESI-MS [3].

In this paper, the potential of the coupling HPLC-ICP-MS for Se speciation analysis in complex samples (for example, dietary supplements) will be illustrated through its application to the measurement and preliminary identification of minor Se metabolites for example, γ-glutamyl-Se-methylselenocysteine (γ-glutamyl-SeMC) in selenized yeast used as the intervention agent in human cancer prevention trials [1].

Experimental

Instrumentation

Extraction of the water-soluble seleno-compounds from yeast was carried out by accelerated solvent extraction (ASE) using a Dionex ASE 200 system (Sunnyvale, CA, USA).

HPLC-ICP-MS measurements were performed using an Agilent Technologies 1100 Series HPLC system for chromatographic separations and an Agilent 7500i ICP-MS for element-specific detection. Reversed-phase HPLC was performed on an Agilent ZORBAX Rx-C8 column (250 mm x 4.6 mm ID, with a particle size of 5 µm). The HPLC column was directly connected to the 100 µL/min PFA microflow concentric nebulizer of the ICP-MS via PEEK tubing (30 cm x 0.1 mm ID). The Agilent Technologies ICP-MS chromatographic software (G1824C Version C.01.00) was used for integration of the chromatographic signal.

Reagents and Samples

Selenium standards (Figure 1) and other chemical substances were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise. L-γ-glutamyl-Se-methylseleno-L-cysteine was purchased from PharmaSe (Lubbock, TX, USA). Single-standard stock solutions (1 mg/g) were prepared and stored as detailed elsewhere [4]. A standard solution of 10 µg/kg of Se in the corresponding mobile phase was prepared from a 1,000 mg/kg Se reference solution (Romil) and used for the daily optimization of the ICP-MS parameters (optimal settings: RF power: 1,300 W; make-up Ar flow rate: 0.31 L/min; nebulizer Ar flow rate: 0.85 L/min; isotopes: ^{77}Se, ^{82}Se, and ^{103}Rh; integration time per mass: 100 ms).

Samples from one batch of SelenoPrecise®, Lalmin™ Se2000 and SelenoExcell™ selenized yeast were supplied by Pharma Nord (Vejle, Denmark), Lallemand Inc. (Montréal, Canada) and Cypress Systems, Inc. (Fresno, CA, USA), respectively. These samples were stored at 4 °C in the dark under dry conditions and thoroughly mixed before sample treatment. The moisture content of these samples was determined using a procedure reported elsewhere [4].

Procedures

Extraction of Se species in water: 0.3 g of Se-yeast was extracted with degassed water using accelerated solvent extraction using the conditions described in a previous work [4].

Se speciation by RP HPLC-ICP-MS: A 50-µL portion of the 1:5 diluted extract was analyzed by ion pairing reversed phase HPLC-ICP-MS at the flow rate of 0.5 mL/min using a water-methanol (98 + 2, v/v) mixture containing 0.1% (v/v) formic acid as the mobile phase. For quantification, calibration was carried out by the standard addition technique at three concentration levels, using peak area measurements of the chromatographic signals by monitoring the ^{82}Se signal. The Se concentration of γ-glutamyl-SeMC in the water-soluble extracts is expressed as average ± SD (n = 3) and referred to as dry sample weight.

Total Se determination of the yeast samples was performed by ICP-MS after microwave acid digestion [4].

Results and Discussion

Two ion-pairing reversed phase HPLC methods (with trifluoroacetic acid [TFA] and formic acid as ion pairing reagents) coupled with ICP-MS were compared for preliminary identification of γ-glutamyl-SeMC in Se-yeast aqueous extracts. The method using formic acid with on-line ICP-MS detection (see conditions above) was preferred for further experiments due to its capability to provide enough retention of target Se species while offering good chromatographic/detection selectivity in the chromatographic region under investigation. Moreover, in comparison with TFA, the use of formic acid is preferable because of its higher compatibility with electrospray ionization (ESI). For chromatographic identification, retention time matching with an authentic standard was used. An alternative, standardless approach based on retention time matching with an aqueous extract from garlic, in which the major species of Se is known to be the γ-glutamyl-SeMC species, was also investigated [4].

The chromatograms of a standard mixture and of 1:5 diluted aqueous extracts from the Se-yeast samples are shown in Figure 1. Assignments based on retention times suggest that the samples seem to contain Se species such as SeMC, selenomethionine (SeMet), and γ-glutamyl-SeMC. For quantification of γ-glutamyl-SeMC, the 1:5 diluted extracts were spiked with γ-glutamyl-SeMC standard of a known Se concentration (see procedures) at three concentration levels. The recovery of added γ-glutamyl-SeMC was 98.9 ± 2.1%. Based on three times the standard deviation for 11 replicate determinations of the reagent blank, the detection limit for γ-glutamyl-SeMC was found to be 8.1 ng/kg.

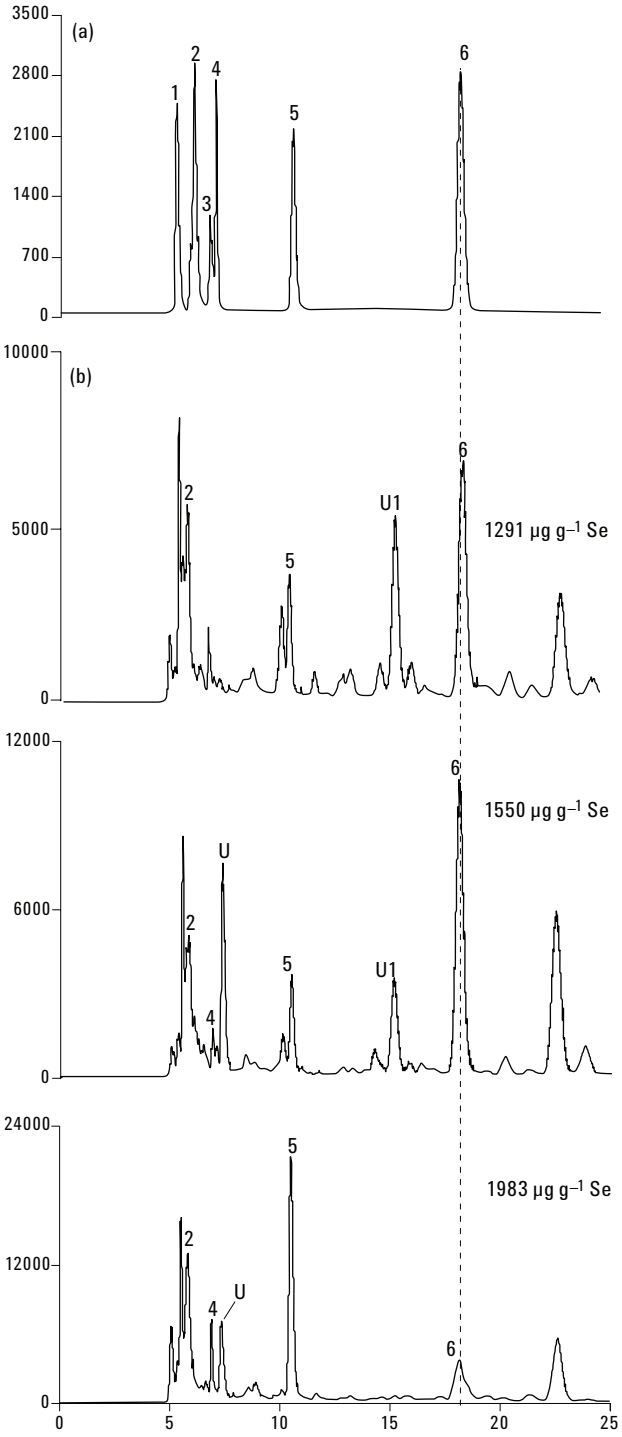

Figure 1. *RP HPLC-ICP-MS chromatograms of (a) a Se standard mixture containing 3 µg/L Se as Se(Cys)2 (peak 1), 2.5 µg/L Se as selenite (peak 2), 5 µg/L Se as SeMC (peaks 3 and 4) and SeMet (peak 5), and 25 µg/L Se as γ-glutamyl-SeMC (peak 6) and (b) the 1:5 diluted Se-yeast water extracts.*

Table 1 summarizes the percentage of the total Se in the water extract, which is associated with γ-glutamyl-SeMC as well as the concentration of Se found as γ-glutamyl-SeMC in the yeast samples analyzed. The results shown in Table 1 and Figure 1 suggest that there is a significant variation of the Se speciation in the water extracts with the variation of the total Se. The numbers in Table 1 also show that the concentration of γ-glutamyl-SeMC decreased not only relatively, but also in absolute terms between concentrations of 1,550 and 1,983 µ/g Se. Since Se-yeast samples with a wider range of total Se concentrations were not available, the results below, while intriguing, should be interpreted with caution regarding the change in selenium distribution upon increase in total Se content. Moreover, further studies should be pursued to elucidate whether or not the differences observed for the speciation of selenium may also be a result of the slightly different methods of yeast enrichment with selenium used by he different manufacturers.

Conclusions

Confirmation of the presence of γ-glutamyl-SeMC (a dipeptide of SeMC and glutamic acid) in the Se-yeast samples analyzed by HPLC-ICP-MS was achieved, for the first time, using the on-line coupling of the chromatographic method developed with ESI MS/MS in selected reaction monitoring mode without the need for extract pretreatment [4]. The presence of γ-glutamyl-SeMC might be relevant to the anticarcinogenic potential of selenized yeast since this species is believed to serve primarily as a carrier of SeMC, which appears to be easily converted in animals and possibly humans to methylselenol. This Se metabolite is thought to be an effective anticarcinogen.

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Table 1. *Percentage of the total Se in the yeast water extract and in the whole yeast sample associated with γ-glutamyl-SeMC and concentration of Se (µg/g) incorporated into γ-glutamyl-SeMC in the yeast sample, as found by RP HPLC-ICP-MS. Precisions are calculated for three independent chromatograms.*

Yeast source	Se concentration of γ-glutamyl-SeMC		
	Water extract (%)[a]	Yeast sample (%)[b]	µg/g
SelenoExcell™ (1291 µg/g Se)	4.4 ± 0.1	0.91 ± 0.04	11.2 ± 0.6
SelenoPrecise (1550 µg/g Se)	7.3 ± 0.2	1.00 ± 0.06	15.7 ± 0.9
Lalmin™ Se2000 (1983 µg/g Se)	2.4 ± 0.1	0.4 ± 0.02	7.2 ± 0.4

[a]Fraction of the total Se in the water extract

[b]Fraction of the total Se in yeast

Determination of Arsenic Species in Marine Samples Using Cation-Exchange HPLC-ICP-MS

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Introduction

A method for the determination of arsenic species in marine samples by cation-exchange HPLC ICP-MS was investigated. A three-step gradient elution of the arsenic species led to the detection of up to 23 different arsenic species in a single analytical run.

Sample Preparation

Freeze-dried samples of marine origin (0.25 g) were extracted three times by mechanical agitation with methanol/water (1 + 1). The three supernatants were combined, evaporated to dryness, and redissolved in 5 mL water prior to analysis.

Instrumentation

An Agilent 7500c ICP-MS was used as an element-specific detector connected to an Agilent 1100 Series HPLC system (degasser, autosampler, and quaternary pump).

A Chrompack Ionospher C column (100 x 3 mm id) was used as the stationary phase and a pyridine solution in 3% MeOH adjusted to pH = 2.7 with formic acid as the mobile phase.

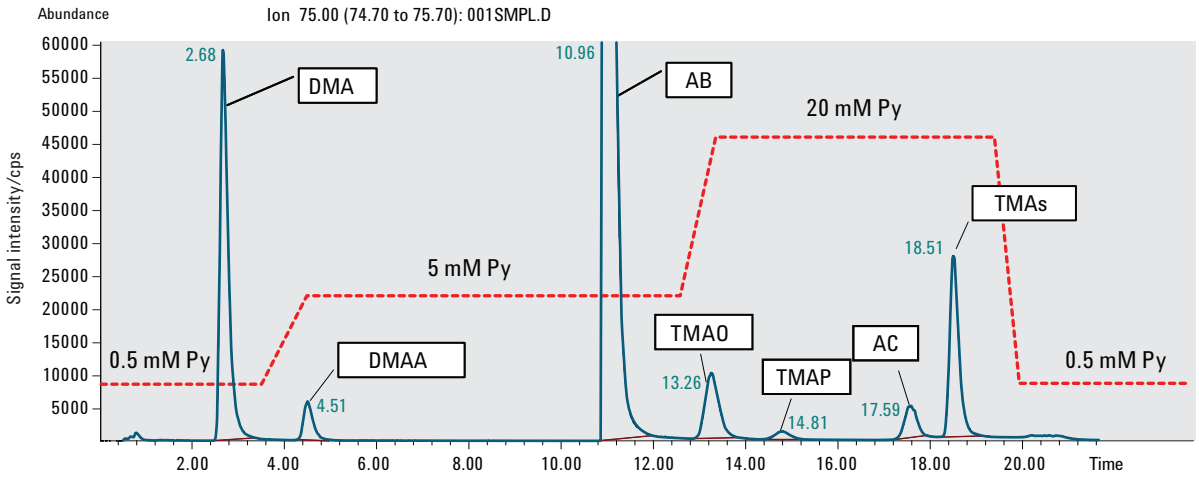
A three-step gradient elution was employed in order to achieve the best possible separation of the arsenic species. Figure 1 shows a chromatogram of a standard solution of the available arsenic species. The three-step gradient elution procedure is illustrated by the red dotted line.

Results and Discussion

The separation/detection capability of the methodology is illustrated in Figure 2, where 23 different arsenic species are detected in one analytical run (25 min) in a scallop kidney. Seven arsenic species have been identified by retention time matching with available standards. As can be seen from the chromatogram, unknown peaks remain. The majority of these peaks are probably arseno-riboside compounds (arsenosugars).

Analysis of Certified Reference Materials

To date, only two reference materials of marine origin have been certified for the content of arsenic species: NRCC DORM-2 Dogfish muscle and BCR627 Tuna. Table 1 shows the results from this work and the certified values. In all cases, good agreement between measured and target values was obtained.



Key:

DMA	Dimethylarsinic acid	TETRA	Tetramethylarsonium ion
AB	Arsenobetaine	DMAA	Dimethylarsinoylacetic acid
TMAO	Trimethylarsine oxide	TMAP	Trimethylarsoniopropionate
AC	Arsenocholine ion		

Figure 1. *Chromatogram of a standard solution. Dotted line illustrates the gradient elution of the arsenic species with the pyridinium mobile phase.*

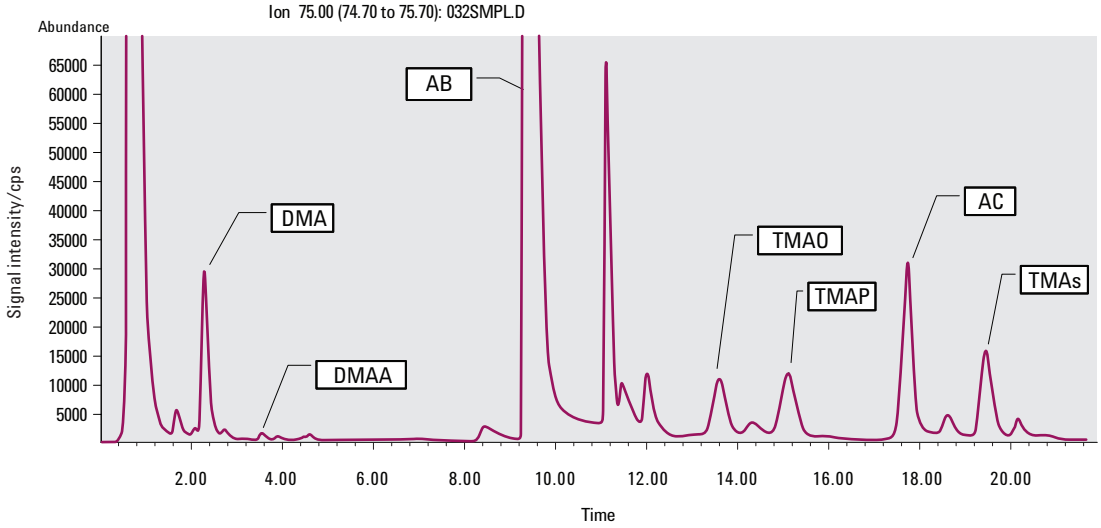


Figure 2. *Chromatogram of a kidney from a scallop.*

Conclusions

An HPLC-ICP-MS method capable of separating 23 arsenic species in one analytical run has been developed. The separation, which was based on cation exchange HPLC, employed a three-step gradient elution and resulted in excellent selectivity. The analysis of the CRMs DORM-2 and BCR627 Tuna fish tissue showed good agreement with certified values and provided a set of values for noncertified arsenic species. The method is useful for future studies of arsenic metabolism in biological samples of marine origin. Several naturally occurring arsenic species were detected but could not be identified in this study due to the lack of available standard substances. In order to characterize these unknowns, further investigation by, for example, ESI-MS/MS will be necessary. Identification of the unknown arsenic compounds will improve our understanding of arsenic-containing natural products and possibly help to elucidate the pathways of transformation of arsenic compounds in the environment.

Table 1. *Results from the analysis of the certified reference materials NRCC DORM-2 (Dogfish Muscle) and BCR627 Tuna. All results in mg (As)/kg ± 95% confidence interval.*

	DORM-2		BCR627 Tuna	
	Cert	Found	Cert	Found
AB	16.4 + 1.1	16.9 + 0.8	3.9 + 0.2	3.7 + 0.2
DMA	–	–	0.15 + 0.01	0.14 + 0.01
TETRA	0.248 + 0.054	0.26 + 0.01	–	–

For Additional Information

Jens J. Sloth, Erik H. Larsen, and Kåre Julshamn, "Determination of organoarsenic species in marine samples using gradient elution cation exchange HPLC-ICP-MS," *J. Anal. At. Spectrom.*, 2003, 18, 452-459.

Routine Determination of Toxic Arsenic Species in Urine Using HPLC-ICP-MS

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Introduction

Arsenic exposure may lead to cancer or other adverse effects, but the toxicity is strongly dependent on the species. Of the five As species most commonly found in human urine, the order of toxicity is: As(III) (arsenite) > As(V) (arsenate) > DMA (dimethylarsinic acid) = MMA (monomethyl arsonic acid) > AB (arsenobetaine).

While HPLC-ICP-MS is well accepted as the analytical technique of choice for As speciation in urine, some remaining difficulties have prevented the technique from becoming routine. These are:

- Finding chromatographic conditions that will separate the five most important species as well as inorganic chloride in a reasonable time, with good retention time reproducibility, dynamic range, and sensitivity.
- Resolving or eliminating the ArCl interference on As that is derived from the high NaCl concentration in urine samples.
- Avoiding clogging of the ICP-MS interface from total dissolved solids (TDS) contained in the urine and HPLC buffers.

Experimental

An Agilent 1100 Series HPLC isocratic pump with autosampler, thermostatted column compartment, and vacuum degasser was coupled to an Agilent 7500ce ICP-MS system fitted with an Agilent MicroMist glass concentric nebulizer. Typical ICP-MS conditions were used for As analysis, including forward power: 1,550 W; sample flow rate: 1 mL/min; and total carrier gas flow: 1.12 L/min. As was monitored at its elemental mass: m/z = 75.

Column Selection

A new anion exchange column was developed and manufactured by Agilent.

Column	G3288-80000 (4.6 mm x 250 mm)
Guard column	G3154-65002

The new Agilent column provides the advantages of excellent resolution of As(III) from both AB and DMA and good separation of MMA from Cl^- under isocratic conditions.

Mobile Phase

The basic mobile phase consisted of:

- 2 mM phosphate buffer solution (PBS), pH 11.0 adjusted with NaOH
- 10 mM, CH_3COONa
- 3.0 mM, $NaNO_3$
- 1% ethanol

Purging the mobile phase with argon during analysis minimized the effects of pH changes due to absorption of atmospheric carbon dioxide.

Interference Removal

The new Agilent G3288-80000 column provides the necessary chromatographic resolution to completely separate inorganic chloride from the arsenic species under isocratic conditions, thereby eliminating the ArCl interference on As. As a result, this method is also suitable for use with non-ORS 7500 ICP-MS systems.

Calculation of Detection Limits

Detection limits for each arsenic species were calculated as three times the chromatographic peak-to-peak signal-to-noise ratio. All species met the goal of < 0.1 µg/L (Table 1).

Table 1. *Calculation of detection limits.*

Species	Height counts	DL (S/N x 3) µg/L
Noise x 3 (average)	117.5	
AB*	2865	0.041
DMAA	3328	0.035
As(III)	2255	0.052
MMAA	1574	0.075
As(V)	1172	0.100

*Arsenobetaine, while well-separated from the four anionic species, elutes with the void volume and may coelute with other neutral or cationic species if present.

Results

The new methodology was applied to the analysis of NIES CRM No.18 urine, using a 5-µL injection of the undiluted sample (Figure 1A). The results agree well with the certified values (AB 66.0 µg/L, DMA 31.0 µg/L). Repeated injections (n = 15) of a 1/10 diluted human urine sample spiked at 5 µg/L demonstrates good long-term stability and the robustness of the method (Figure 1B).

Conclusions

A new HPLC-ICP-MS method capable of separating all five important arsenic compounds in human urine within 12 minutes has been developed through careful, systematic optimization of all parameters, including the development and manufacture of a new column. The method is robust enough for the analysis of undiluted urine with limits of detection of 0.1 µg/L or less for the individual As species.

For Additional Information

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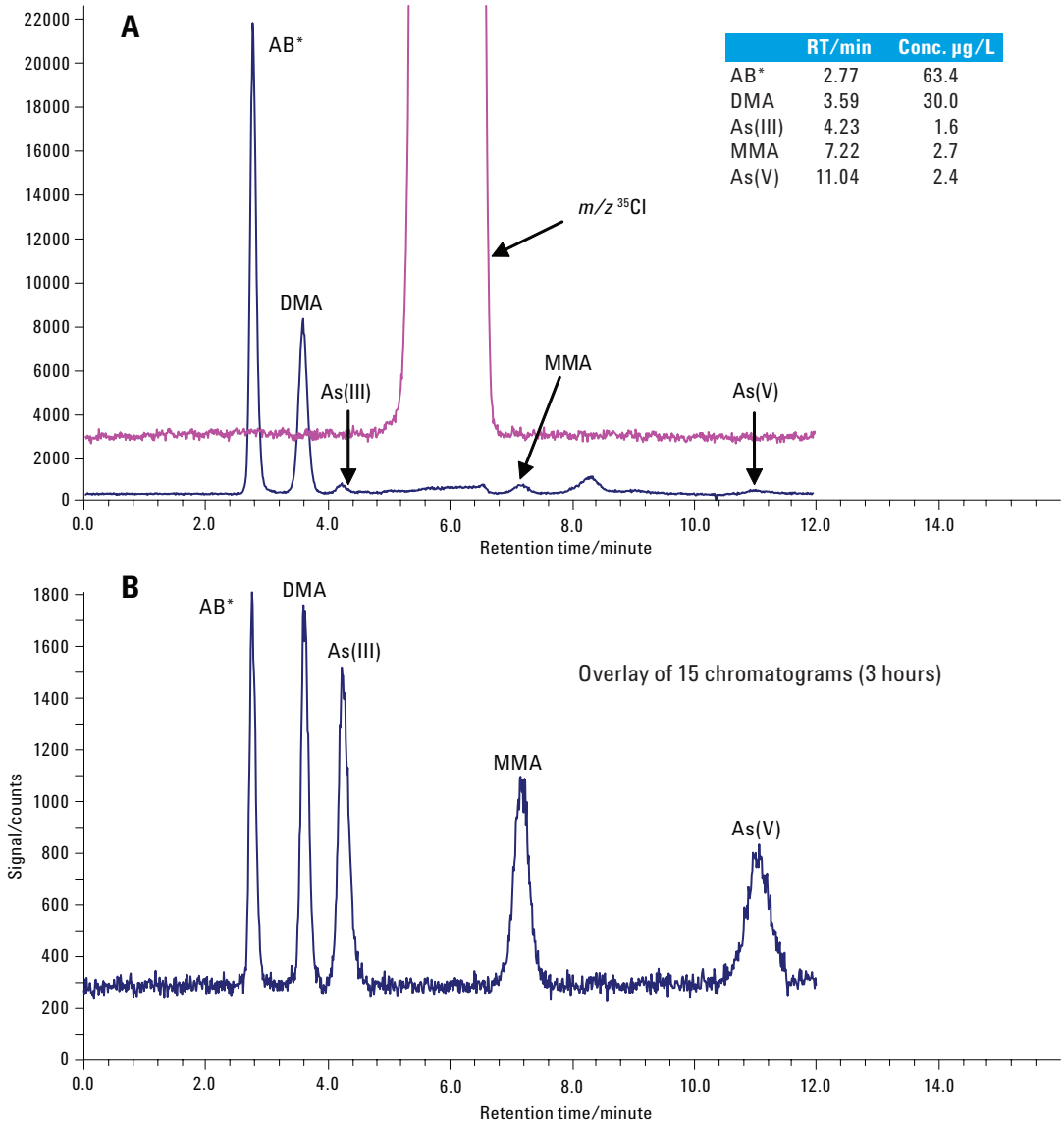

*Arsenobetaine, while well-separated from the four anionic species, elutes with the void volume and may coelute with other neutral or cationic species if present.

Figure 1. *(a) Undiluted 5-µL injection of NIES CRM No.18 urine standard. (b) Reproducibility of 15 x 1/10 human urine samples (spiked 5 µg/L).*

Application of ICP-MS to the Analysis of Phospholipids

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Introduction

Phospholipids are the main constituents of membranes in all types of prokaryotic and eukariotic cells. Due to their complexity and heterogeneity in biological samples, qualitative and quantitative analyses of membrane phospholipids in cellular extracts represent major analytical challenges, mainly due to the requirement for suitable and sensitive detection methods. ICP-MS is a suitable detector for selective determination of phospholipids, which all contain phosphorus. However, the determination of phosphorus and its compounds by an ICP-MS is not an easy task because phosphorus has a high ionization potential and, consequently, is poorly ionized in the plasma. Additionally, it suffers from polyatomic interferences at *m/z* ratio 31 from $^{12}C^{1}H^{316}O+$, $^{15}N^{16}OH$, $^{15}N^{16}O$, and $^{14}N^{17}O$. Phospholipids are extractable with organic solvents; therefore, liquid chromatography with an organic mobile phase was used for separation of different lipid species.

Experimental

Reagents and Sample Preparation

A standard mixture of six phospholipids was prepared by diluting each standard in a chloroform/methanol mixture (2/1, v/v).

1,2-dioleoyl-phosphatidic acid monosodium salt ($C_{39}H_{72}O_8PNa$, DOPA)

1,2-dioleoyl-phosphatidylcholine ($C_{44}H_{84}NO_8P$, DOPC)

1,2-dioleoyl-phosphatidylethanolamine ($C_{41}H_{78}NO_8P$, DOPE)

1,2-dioleoyl-phosphatidylglycerol sodium salt ($C_{42}H_{78}O_{10}PNa$, DOPG)

1,2-dioleoyl-phosphatidylserine sodium salt ($C_{42}H_{77}NO_{10}PNa$, DOPS)

Phosphatidylinositol sodium salt isolated from bovine liver ($C_{47}H_{82}O_{13}PNa$, PI)

The concentrations of each expressed as phosphorus were as follows: 3.3 mg/L DOPA, 2.9 mg/L DOPG, 2.7 mg/L PI, 3.1 mg/L DOPE, 3.0 mg/L DOPS, and 2.9 mg/L DOPC.

Chromatographic System

HPLC separations were carried out using an Agilent 1100 chromatographic system equipped with a thermostatted autosampler (variable injection loop 0 to 100 µL), YMC Pack Diol-120 column (250 x 4.6 mm, 5 µm) (Kyoto, Japan) maintained at 50 °C and a flow rate of 0.6 mL/min. The composition of mobile phase A was acetone/hexane/acetic acid/triethlyamine (900/70/14/2 [v/v]) and the composition of mobile phase B was methanol/hexane/ acetic acid/triethlyamine (900/70/14/2 [v/v]). The following gradient elution program was used: 95% of A at 0 min, 82% of A at 40 min, 55% of A at 42 min, 40% of A at 44 min, 40% of A at 49 min, 95% of A at 49.5 min, and 95% of A at 58 min.

Detection System

The 0.6 mL/min flow from the HPLC column was split to approximately 130 µL/min before reaching the 7500c ICP-MS via self-aspiration using a PFA 100 nebulizer. To prevent deposition of carbon on the interface cones, an optional gas (20% oxygen in argon) was introduced. Since added oxygen promotes corrosion of interface cones, a platinum sampler cone was used. The detection was carried out by recording *m/z* ratio 31 at scan rate of 0.3 s per point.

The system was optimized by pumping mobile phase A containing 2 mg/L of phosphorus as a DOPE. The following optimized conditions were used for the detection of the phospholipids:

Plasma gas	15 L/min
Auxiliary gas	1.0 L/min
Carrier gas	0.50 L/min
Optional gas flow rate	24% (of carrier gas flow rate)
RF power	1600 W
ORS gas (helium)	4.0 mL/min
Spray chamber temperature	25 °C; and sample depth (torch-interface distance) 10 mm

All chromatograms were smoothed before integration.

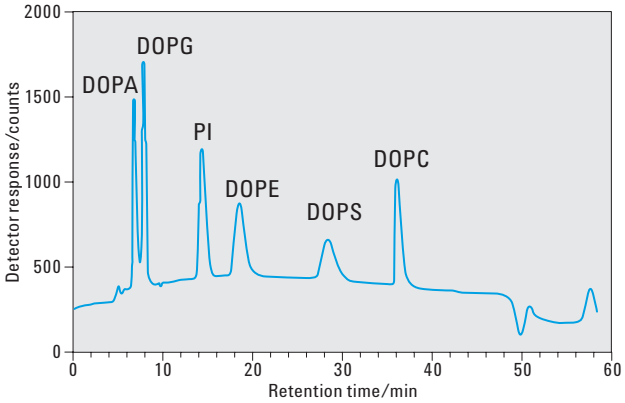

Figure 1. *Separation of six chemically defined phospholipids in standard mixture on YMC Pack Diol-120 column (250 x 4.6 mm, 5 µm) with ICP-MS detection of phosphorus at m/z ratio 31 (5 µL injected, 0.6 mL/min, each peak corresponds to ~15 ng of phosphorus).*

Results and Discussion

Chromatographic Separation

Successful chromatographic separation of six standard phospholipids was achieved by utilization of modified conditions described by Sas et al. [1]. A typical chromatogram is presented in Figure 1. Almost all six phospholipid standards were baseline separated.

The usefulness of the developed method for analysis of phospholipids was demonstrated on a complex lipid extract from yeast. Each identified compound was quantified by using the calibration curves given in Table 1. The results are presented in Table 2 as peak areas and as calculated masses and concentrations of each identified compound in the sample extract. It should be noted that all masses and concentrations are expressed as phosphorus and that peak coeluting with chemical class of phosphatitylcholine (PC) was integrated and considered as belonging to PC class.

Table 1. *Calibration parameters (expressed as mass of phosphorus).*

Compound	Retention time/min	Calibration curve	R	Linear range/ng	LOD/ng	Reproducibility* (%)
DOPA	6.7	A = 18000 x m – 830	0.9998	1.6-16	0.36	±6
DOPG	7.8	A = 23400 x m – 5650	0.9997	1.4-14	0.21	±5
PI	14.2	A = 25000 x m – 850	0.9999	1.4-55	0.54	±7
DOPE	18.3	A = 21000 x m – 10400	0.9999	3.0-61	1.2	±7
DOPS	28.1	A = 16500 x m – 18600	0.9998	3.0-59	1.2	±16
DOPC	35.9	A = 19500 x m – 230	0.9999	1.5-59	0.50	±14

* At lowest point of calibration curve.

Table 2. *Peak areas, calculated masses and concentrations, relative amounts of identified compounds and semi-quantitatively determined relative amounts of all phospholipids in yeast lipid extract (all values are expected as phosphorus).*

Class	Peak area/ 103 units	Mass/ng	Concentration/ mg 1^{-1}	Relative amounts* (%)	Semi-quantiative relative amounts** (%)
PA	65.5	3.7	0.74	1.6	1.2
PI	783	31	6.3	13	15
PE	1440	69	14	29	27
PS	150	10	2.0	4.3	2.8
PC	2380	120	24	51	44
X_1	400	–	–	–	7.6
X_2	45.6	–	–	–	0.9

* Relative amounts of identified compounds.

** Relative amounts determined bt semi-quantitative procedure.

Theoretically, the response of an ICP-MS is element dependant, meaning it is the same for all compounds regardless to their structure. The determined response factors, which are part of calibration curves in Table 1, have values from 16,500 to 25,000 peak area units per ng of phosphorus. Deviations from theory are expected, since we used a gradient elution program. This gives a different matrix composition for each compound, resulting in different nebulization and ionization efficiencies. Therefore, simplification of the quantification procedure by using a calibration curve based only on one compound should be used with caution and considered in the field of semi-quantitative analysis.

In cases when we are interested only in obtaining approximate ratios between classes of phospholipids in the sample, only peak areas without any calibration can be used. To show the usefulness of such a quick semiquantitative analysis, a yeast lipid extract was treated in that way. Peak areas of all peaks found in the chromatogram were summed; their relative amounts were calculated and are presented in Table 2. Compared to literature data [2], this semiquantitative approach gives good agreement.

Conclusions

Collision/reaction cell-ICP-MS has been shown to be a suitable detector for selective determination of phospholipids following separation of different lipids by LC. To reduce polyatomic interferences at *m/z* ratio 31 (for example, CH_3O^+) and to improve detection limits, helium was used as a collision gas within the ORS cell. The achieved absolute detection limits were between 0.21 and 1.2 ng of phosphorus and were superior to those obtained by an evaporative light-scattering detector, which provides an alternative detection system for lipid analysis.

The usefulness of the developed method was demonstrated by analysis of lipid extracts from the yeast Saccharomyces cerevisiae.

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Chromium Speciation in Natural Waters by IC-ICP-MS

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Ed McCurdy, Agilent Technologies, Stockport, United Kingdom

Introduction

Measurement of total chromium doesn't always tell the whole story. The anionic, hexavalent form of the element is toxic, while in its cationic trivalent oxidation state, chromium is an essential element for human nutrition. Methods to establish the potential toxicity of Cr must therefore determine the concentration of Cr(VI), rather than simply total Cr.

Separating and detecting Cr is challenging because the common forms of Cr in natural samples such as water are chromate (CrO_4^{2-}) for Cr(VI) and chromic ion (Cr^{3+}) for Cr(III). Chromate is an anion and the chromic ion is cationic, so a single ion exchange method will not work for both forms under the same conditions. A further problem is that Cr(III) is the most stable oxidation state in samples such as water, whereas Cr(VI) ions are strong oxidizing agents and are readily reduced to Cr(III) in the presence of acid or organic matter. Consequently, great care must be taken during sample collection, storage, and preparation, to ensure that the Cr species distribution present in the original sample is maintained up to the point of analysis.

This novel method, developed at Agilent, uses an optimized sample stabilization method, in which the samples were incubated at 40 °C with EDTA, which forms an anionic complex with the Cr(III), allowing a single chromatographic method to be used to separate the Cr(III)EDTA complex and the Cr(VI).

The Agilent 7500ce Octopole Reaction System (ORS) ICP-MS allows Cr to be measured with high accuracy and good sensitivity, using the main isotope at mass 52, by removing interferences from ArC and ClOH. The sample preparation method, column type, and chromatographic conditions used for Cr speciation are shown in Table 1.

The non-metal ion chromatography (IC) pump (Metrohm 818 IC Pump) was used to deliver the mobile phase, but the sample loop was filled and switched using the optional Integrated Sample Introduction System (ISIS) of the Agilent 7500ce ICP-MS. While this configuration maintains the high precision and relatively high pressure of the IC pump, it also provides a much simpler and lower cost alternative to a complete IC or HPLC system, since only the IC pump module is required in addition to the ICP-MS system.

Table 1. *Chromatographic conditions for Cr speciation.*

Sample Preparation	
Reaction temp	40 °C
Incubation time	3 h
EDTA concentration	5 ~ 15 mM pH 7 adjust by NaOH
Chromatographic conditions	
Cr column	Agilent p/n G3268A, 30 mm x 4.6 mm id
Mobile phase	5 mM EDTA (2Na), pH 7, adjust by NaOH
Flow rate	1.2 mL/min
Column temperature	Ambient
Injection volume	50 ~ 500 µL

Results and Discussion

Under the conditions described in Table 1, with ICP-MS detection using the Agilent 7500ce in H_2 cell gas mode to remove the ArC and ClOH interferences on Cr at mass 52, detection limits (DLs) of < 20 ng/L were obtained for the individual Cr species, as shown in Table 2.

Many international regulations for hexavalent Cr specify a maximum allowable concentration of 1 µg/L, with a required DL of one-tenth of this level (100 ng/L), and even the small sample volume injection of 100 µL easily meets these requirements. However, increasing the injection volume to 500 µL allowed the DLs to be reduced to 13.2 ng/L for Cr(III) and 15.8 ng/L for Cr(VI).

In order to test the suitability of the method for real-world sample types, the method was applied to the determination of both Cr species in spiked and unspiked mineral water samples.

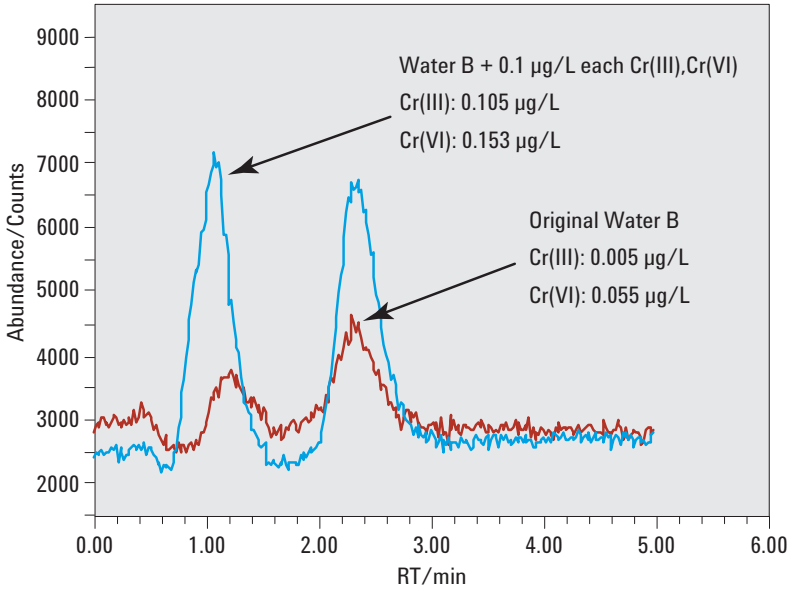
One mineral water sample analyzed was a French mineral water, referred to as mineral water B, which has among the highest levels of calcium and sulfates of any commonly available mineral water (over 450 mg/L Ca and more than 1,000 mg/L sulfates). Mineral water B was analyzed with and without a spike of the two Cr species and the spike recovery was assessed. The results

for the measured samples are shown in Figure 1. The major element composition of the mineral water is shown below the chromatogram, illustrating the very high mineral levels. Despite these high major element levels, the optimized sample prep and chromatographic method gave good chromatographic separation and identification for both Cr species.

The ability to recover low concentration spikes for both Cr species in such a high matrix sample indicates the effectiveness of the optimized method for sample stabilization, which ensures that a high enough concentration of EDTA is available for complete complexation of the Cr(III) species, even in the presence of a high level of competing ions. Furthermore, the accurate recovery of low concentration spikes of both species indicates that potential problems of species interconversion (reduction of Cr[VI] to Cr[III]) were avoided through the selection of an appropriate pH for the samples and the mobile phase, together with the use of EDTA in the mobile phase as well as for sample stabilization. See Table 2.

For Additional Information

For a full account of this application see: "Ion Chromatography (IC) ICP-MS for Chromium Speciation in Natural Samples," Agilent Technologies publication 5989-2481EN.



Na	7.3 mg/L
Ca	91.0 mg/L
Mg	19.9 mg/L
K	4.9 mg/L

Figure 1. *Major element composition (mg/L) and chromatogram for spiked mineral water B.*

Table 2. *Detection limits for Cr species by IC-ICP-MS.*

	Retention time/min		Peak area/counts		DL (S/N = 3) ng/L	
Inject/µL	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)	Cr(III)	Cr(VI)
50	0.79	2.09	1082295	914804	69.5	139.4
100	0.79	2.09	1704312	1525147	43.4	82.8
250	0.85	2.21	4939876	4546219	17.5	28.5
500	0.97	2.39	10268086	9398651	13.2	15.8

Multi-Element Speciation Using Ion Chromatography Coupled to ICP-MS

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Introduction

ICP-MS has been shown to be a powerful tool for the measurement of ionic species of single elements separated by ion chromatography (IC). However, simultaneous speciation of multiple elements has been challenging for a number of reasons. Chromatographic elution and separation of elements with widely varying ionic properties can be difficult, such as when some of the species exist as cations while others are anionic. Furthermore, detection limits can be compromised when polyatomic interferences overlap one or more of the analytes of interest. By combining simple anion chromatography with chelation using EDTA, species preservation and efficient separation of multiple species of 13 elements was achieved simultaneously under a single set of conditions. If necessary, polyatomic interferences that would normally interfere with the measurement of several elements, including Cr, As, and Se, can be eliminated using the Octopole Reaction System (ORS) of the Agilent 7500ce ICP-MS.

Hardware Setup

The IC-ICP-MS system (Figure 1) consists of a nonmetal IC pump (Metrohm), the Agilent Integrated Sample Introduction System (ISIS), and an Agilent 7500ce ICP-MS. The IC pump was used to deliver high-pressure mobile phase to the anion exchange column via the ISIS high-pressure 6-port valve and sample loop.

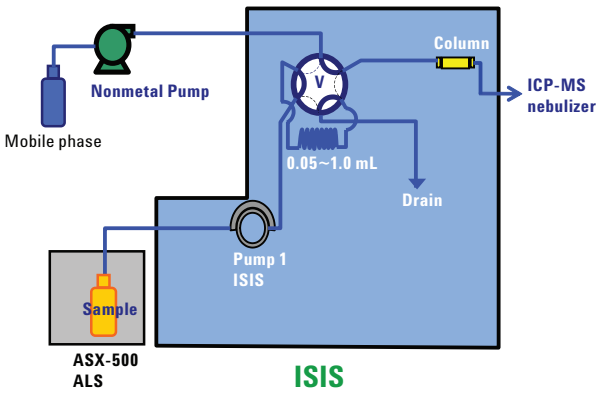

Figure 1. *Schematic of the IC-ICP-MS arrangement.*

Methods

Standards and samples were prepared by chelation with EDTA [1, 2, 3]. Chelation with EDTA serves two purposes. First, it converts all ionic species to anions, allowing separation by simple anion-exchange. Second, it helps to maintain the original species composition. This is particularly important for elements like chromium, which can exist as stable anions but are quite labile cations in nature. The rate of formation of stable Cr(III)-EDTA complex was found to be highly temperature dependent (Figure 2), and was determined to be 50 °C.

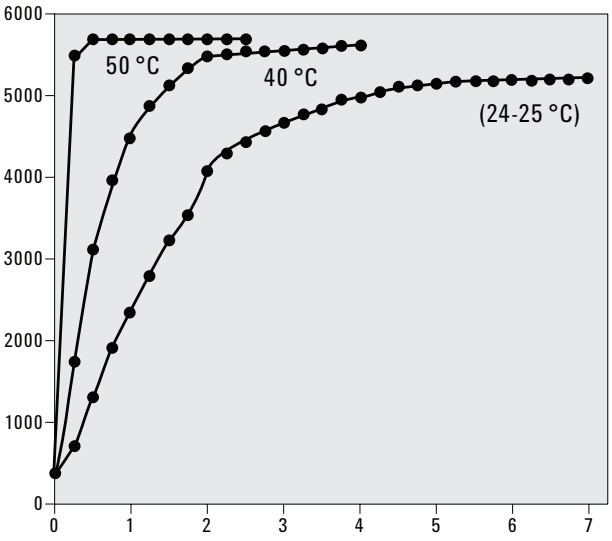

Figure 2. *Rate of formation of stable Cr(III)-EDTA complex as a function of time and temperature.*

Table 1. *Ion chromatography and ICP-MS conditions.*

Ion Chromatography	
IC column	Excelpack ICS-A23
Mobile phase	3.0 mM Na_2CO_3
Eluent flow	1 mL/min
Injection volume	50 µL
Run time	20 min
ICP-MS	
Isotopes acquired	^{31}P, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{66}Zn, ^{75}As, ^{78}Se, ^{79}Br, ^{127}I, ^{182}W, ^{208}Pb
Acq mode	Time resolved
Dwell time	0.1 s/point
RF power	1450 watts
Sample depth	8.5 mm
Carrier gas flow	1.1 L/min
Nebulizer	MicroMist
ORS mode	No gas mode

Results

Separation and detection of 20 species from 13 different elements was obtained in less than 20 minutes (Figure 3). Detection limits are approximately 0.5 µg/L for the most of elements.

Conclusions

Since ICP-MS is able to differentiate ionic species by their elemental mass spectra in addition to their retention time, it is not necessary to chromatographically resolve all species from each other as it would be with conductivity detection. This permits simultaneous analysis of multiple species under rapid, simple conditions that may not separate all species in time.

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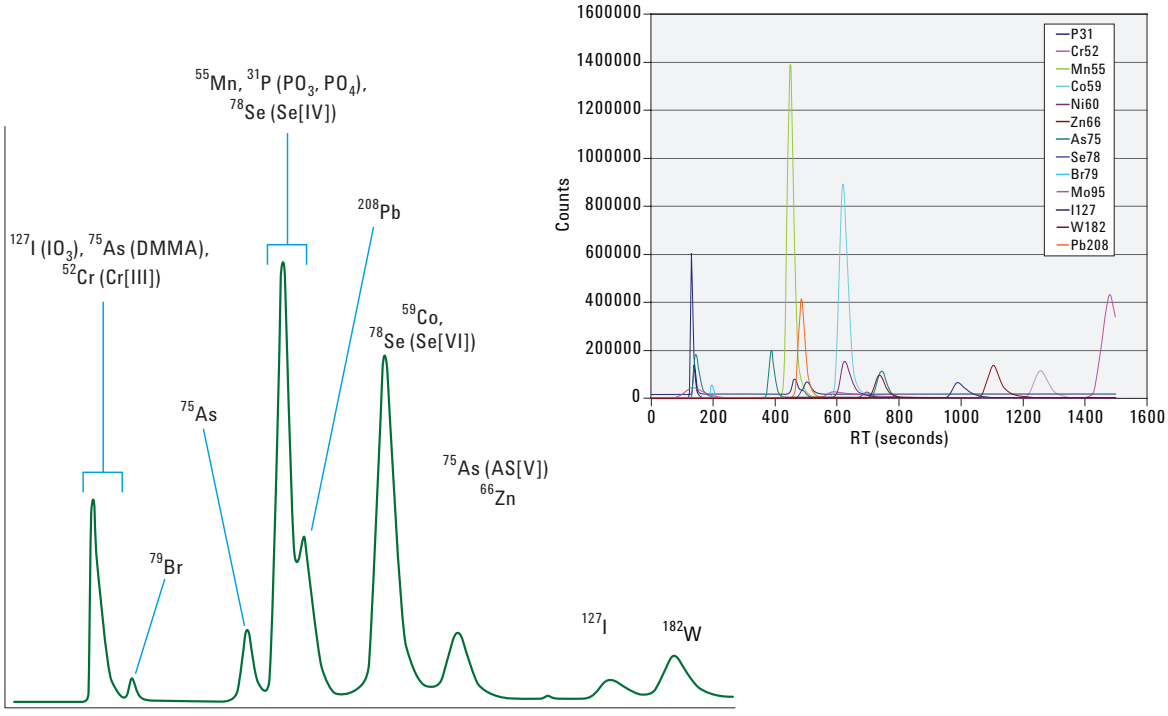

Figure 3. *Total ion chromatogram depicting simultaneous speciation of multiple elements. Inset shows extracted ion chromatograms of the 13 elements measured. Because of the elemental specificity of ICP-MS, it is not always necessary for each species to be chromatographically resolved.*

Determination of Trivalent and Hexavalent Chromium in Pharmaceutical, Nutraceutical, and Biological Matrices Using IC-ICP-MS

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Introduction

Chromium is a transition metal that can form complexes in four different valence states (CrIII, CrIV, CrV, and CrVI). However, the two most stable oxidation states, and therefore the most commonly detected, are complexes of trivalent and hexavalent chromium. Trivalent chromium (CrIII) has been characterized as an essential nutrient involved in carbohydrate and lipid metabolism, while hexavalent chromium (CrVI) is considered highly toxic.

Cr(VI) complexes, such as chromate $(CrO_4)^{2-}$, at physiological pH easily penetrate cell membranes through the sulfate and phosphate anion channels. It is accepted that for Cr(VI) to exert its cytotoxic and carcinogenic effects, it has to be reduced inside the cell. In contrast, cellular membranes are normally impermeable to Cr(III) cations, which can penetrate the cell membrane only under specific conditions.

After recent studies suggesting ingestion of Cr(VI) may cause chromate-induced cancers, there has been a renewed interest in the ability to separate, identify, and quantify trivalent and hexavalent chromium in a broad range of sample matrices. Additionally, the use of biological agents, such as bacteria or plants for bioremediation of chromium-polluted soils and water, is an active area of research. In the environment, Cr(VI) salts do not bind to constituents in the soil or readily precipitate from water. This creates the need to find naturally occurring organisms to reduce the Cr(VI) species to Cr(III) in the aqueous environment. Filamentous fungi have been shown to cause the complete reduction of Cr(VI) to Cr(III), without accumulating chromium in the biomass.

Experimental

This method exploits the ability of EDTA to form metal complexes. At pH 7, Cr(III) exists as a hydrated chromic cation (Cr^{3+}), while Cr(VI) exists as the chromate anion $(CrO_4)^{2-}$. Under these conditions a Cr(III)-EDTA complex is weakly retained on an anion exchange column amidst a strongly retained chromate anion, resulting in a resolution number greater than 1.5. Incubation times and temperatures were optimized for the greatest Cr(III) response, in conjunction with the concentration of the EDTA complexing solution, in an attempt to minimize sample preparation.

A powdered pharmaceutical sample was submitted to our laboratory for total chromium and chromium speciation analysis. The company wanted to determine which and how much of each chromium species, Cr(III) and Cr(IV), was present in its finished product. A similar determination was performed on over-the-counter nutraceutical samples from GNC (General Nutrition Center) consisting of powdered tablets containing 200 µg of chromium picolinate and over-the-counter gel caps (oil-based), also containing 200 µg of chromium picolinate. The tablets were manufactured in 1999, while the gel caps were manufactured in 2006. Standards and samples were prepared under the same conditions (see Table 1).

The pharmaceutical and nutraceutical samples were first dissolved in 200 mL of the 15 mM EDTA solution and allowed to incubate for 15 minutes in a 60 °C circulating water bath. Standards were prepared in double-distilled deionized water and diluted in a manner to maintain and minimize a change in the 15 mM EDTA concentration and incubated for 15 minutes in the 60 °C circulating water bath.

Fungal growth media were analyzed to illustrate the efficiency and reductive capability of the fungi to bioremediate 50 ppm of spiked Cr(VI) (ongoing research). Multiple samples of 500 mL aliquots of fungal growth medium solution were first frozen on dry ice and then lyophilized. After lyophilization, 500 µL of 15 mM EDTA solution was added to each sample and allowed to incubate for 30 minutes in a 60 °C circulating water bath for maximum Cr(III) response.

Monitoring chromium isotopes ^{50}Cr, ^{52}Cr, and ^{53}Cr, allowed for unambiguous chromium identification. ^{54}Cr was also monitored; however, it is a minor isotope (2.3%) and suffers from an isobaric interference from iron. Quantifications were attained by extracting the ^{52}Cr isotope trace and integrating peak areas with the chromatographic data analysis software (Figure 1). The Metrohm 818C isocratic pump and six-port valve were controlled by the Integrated Sample Introduction System (ISIS) software. Both the ISIS and chromatographic software options are integrated into the ICP-MS ChemStation software.

Table 1. *Chromatographic and ICP-MS conditions for Cr(III) and Cr(VI) speciation.*

IC Conditions	
LC pump	Metrohm 818C isocratic pump (PEEK)
Column	Agilent Technologies G3268A, 30 mm x 4.6 mm id
Mobile phase	5 mM (disodium) EDTA at pH 7 adjusted with NaOH
Flow rate	1.2 mL/min
Injection volume	100 µL PEEK sample loop
ICP-MS Conditions	
	MicroMist nebulizer (Glass Expansion) with Scott spray chamber 7500ce (Agilent Technologies) 3.5 mL/min H_2 collision gas Masses monitored (*m/z*): 50, 52, 53, 54
Sample Incubation	
Reaction temperature	60 °C (water bath or microwave)
Incubation time	15 min
EDTA concentration	15 mM at pH 7 adjusted with NaOH
Sample matrix	Pharmaceutical, nutraceutical, fungi (growth media)

Results and Discussion

Under the conditions previously stated, the LOD ($\sigma = 3$) for Cr(III) and Cr(VI) were 0.10 µg/L and 0.15 µg/L, respectively. The LODs are based upon a 100-µL PEEK sample loop and 15- and 30-minute incubation times (for drug samples and fungal samples, respectively). The LODs could easily be improved by using a larger sample loop. Table 2 illustrates the method's reproducibility for both response and retention time.

Table 2. *Cr(III) and Cr(VI) 50 ppb standard (n = 7).*

	Cr(III) Area	Cr(VI) Area	Cr(III) RT	Cr(VI) RT
Average	1452262	1965195	0.932 min	4.420 min
Std Dev	21065	42945	0.003 min	0.021 min
% RSD	1.5	2.2	0.3	0.5

Conclusions

By optimizing the incubation times and temperatures, along with the EDTA concentration, a sample preparation time of only 15 minutes for the drug samples and 30 minutes for the fungal samples was achieved. Stable and reproducible separation, identification, and quantification of Cr(III) and Cr(VI) were accomplished in under 7 minutes using this method. This brings the total time per sample to less than 25 minutes for the drug samples and less than 40 minutes for the fungal samples, while attaining low, reproducible LOD for each species.

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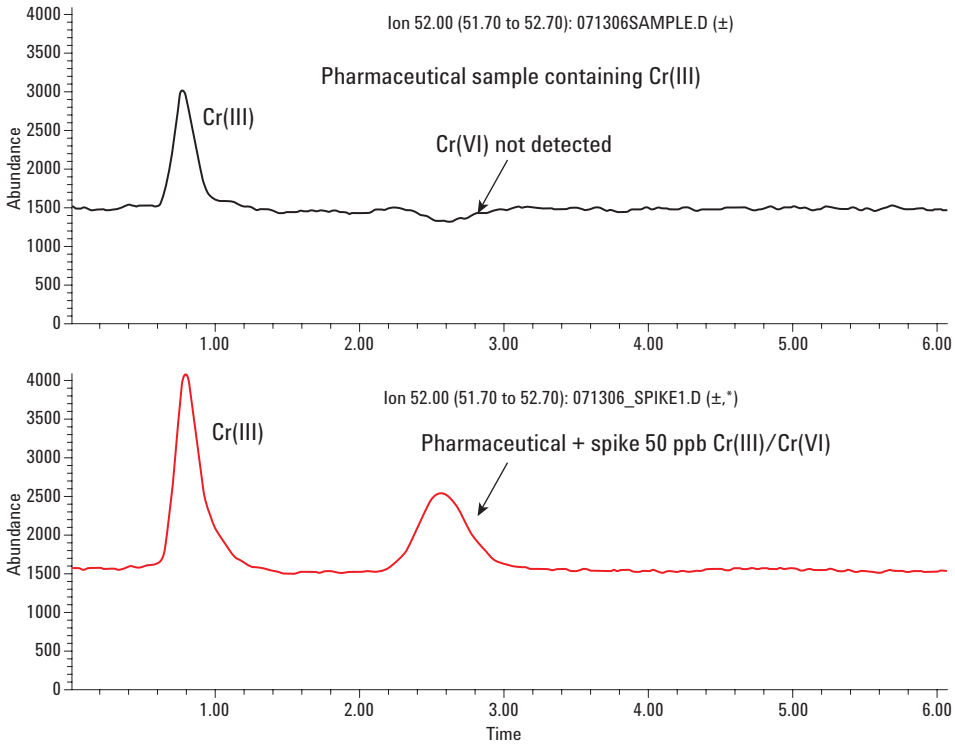

Figure 1. *Chromatograms showing separation and identification of Cr(III) and Cr(VI).*

Determination of Iodine Species Using IC-ICP-MS

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Introduction

Iodine is an essential micronutrient in mammals, necessary for proper production of thyroid hormones. A deficiency leads to various disorders associated with growth and development. Iodized salt is a common source of iodine, but excessive intake can lead to the development of high-iodine goiters, which in turn can lead to retarded brain development and functional impediment. The bioavailability and toxicity of iodine, like other essential elements, is species dependent. Inorganic iodide and iodate are less toxic than molecular iodine and some organically bound forms of the element. Likewise, the bioavailability of organically bound iodine is also lower than that of mineral iodine [1,2].

Instrumentation

An Agilent 7500a ICP-MS was coupled to an Agilent 1100 HPLC with ICS-A23 ion chromatography column (available only in Japan; Cr speciation column, G3268-80001, may work with some modified conditions) and ICS-A2G guard column. The mobile phase was 0.03 mol/L ammonium carbonate solution (pH 9.4).

Species Stability of Iodide and Iodate

In order to choose a suitable storage medium, pure water, the mobile phase, 0.01% KOH, and 0.1% KOH were tested. Interestingly, iodide was unstable and lost in pure water and the mobile phase medium when the stock solution was diluted or kept for a long time. Dilution factors from 10 to 10,000 and storage from one to five days were tested. However, iodide was found to be stable in 0.01% KOH and 0.1% KOH. No stability problems were observed with iodate in different media. In order to minimize any potential high-matrix effects, 0.01% KOH was selected as the storage medium for field sampling.

Linear Range and Detection Limits

The linear range of iodate was more than four orders of magnitude from 5 nmol/L to 50 µmol/L. Linear regression was investigated for all species and r^2 was found to be 0.9999. The repeatability (n = 7) for 50-nmol/L injections of iodate and iodide was 2.1% and 3.3%, respectively (Figure 1).

Application of Method

The method described is being used in conjunction with total iodine measurements by ICP-MS in a survey of various groundwater samples. Preliminary findings have identified that some samples contain iodine as iodide and iodate in which the sum of the two inorganic iodine species does not differ significantly from the total iodine concentration. In some cases, the sum of the two inorganic iodine species differs significantly from the total iodine concentration directly determined by ICP-MS. It is believed that this difference is due to the presence of organoiodine compounds. The theory was tested using SEC-ICP-MS.

A representative chromatogram is shown in Figure 2. The results suggest that organoiodine and iodide were the main species in these unusual groundwater samples.

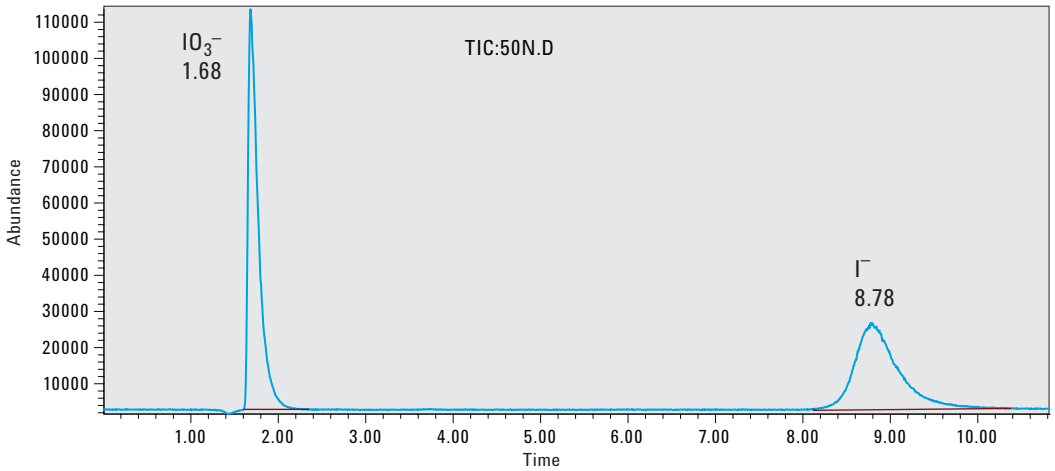

Figure 1. *IC-ICP-MS chromatogram of 50 nmol/L iodate and iodide standard solution (0.01% KOH medium, mobile: 0.03 mol/L ammonium carbonate).*

Conclusions

We have successfully developed a method to separate inorganic iodine anions using HPLC with detection using ICP-MS. The use of 0.01% KOH stabilizes the solutions and provides for species independence in terms of calibration. The method is being applied to a survey of ground-water samples, and for the majority of samples it is very successful. Some samples behave differently and there is evidence (using SEC-ICP-MS) that in these cases iodine is present in the form of organoiodine compounds.

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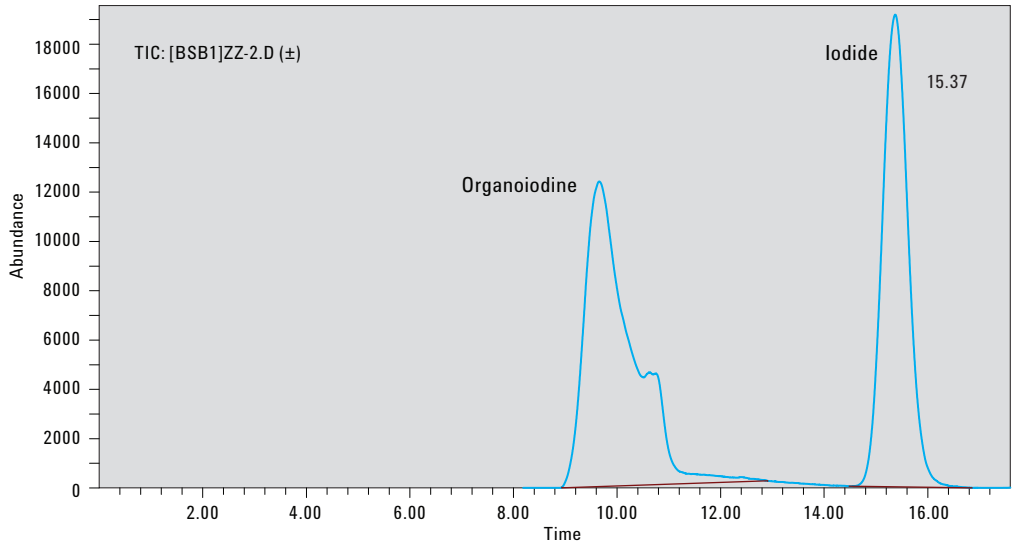

Figure 2. *SEC-ICP-MS chromatogram of organoiodine and iodide from a groundwater sample. Mobile phase: 0.03 mol/L ammonium carbonate, pH 9.4.*

GC-ICP-MS

GC-ICP-MS Introduction

GC-ICP-MS is used for the analysis of volatile organic or organometallic compounds when no other GC detector can provide the required elemental or isotopic specificity or sensitivity. Furthermore, because of the generally higher resolution of GC compared with LC, it is sometimes advantageous to create volatile derivatives of otherwise non-volatile compounds for analysis by GC. When used as a detector for GC, ICP-MS provides several other advantages over alternative elemental detectors.

- ICP-MS is almost universal (only hydrogen, helium, argon, fluorine, and neon cannot be directly measured).
- ICP-MS can tolerate a wide range of GC carrier gases and flows.
- ICP-MS permits the use of compound independent calibration, which is useful for screening or when standards are expensive or unavailable.
- ICP-MS does not typically suffer from suppression of analyte response due to coeluting compounds.
- ICP-MS is capable of isotope dilution quantification.

The Agilent GC-ICP-MS interface consists of a heated, passivated transfer line and a special torch with a heated injector tube. In this way, the sample is maintained at constant high temperature from the end of the chromatographic column in the GC oven to the tip of the ICP injector (Figure 1).

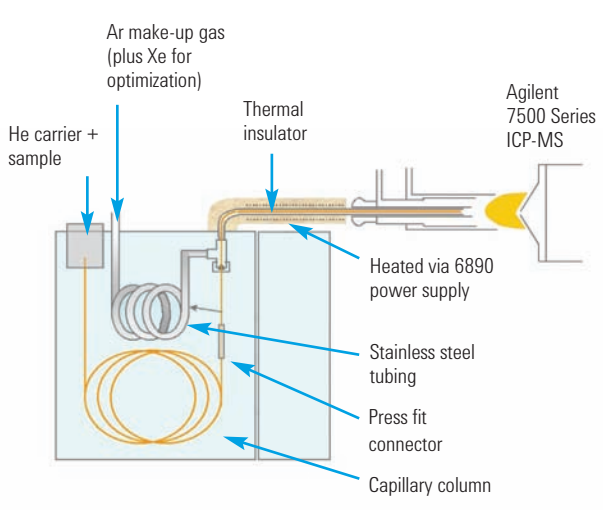

Figure 1. *Schematic diagram of Agilent GC-ICP-MS system.*

Tuning and Optimization of ICP-MS for GC Applications

Tuning the ICP-MS requires optimization of the plasma for efficient ion production, ion optics, and torch position for best sensitivity, octopole reaction cell (if equipped) for optimum interference removal, quadrupole for mass resolution and mass calibration, and detector for sensitivity and linear dynamic range. In conventional ICP-MS, these conditions are met by aspirating an aqueous tune solution containing several elements upon which the system is optimized. However in the case of GC-ICP-MS, the liquid sample introduction system is not fitted and the plasma conditions are sufficiently different that a solution-based tune would not be appropriate. In this case, tuning and optimization must be carried out using a gaseous tune sample. Normally, this is accomplished through the addition of 0.05% – 0.1% xenon in helium or argon, either in the GC carrier gas or in the argon makeup gas. Since Xe is composed of nine isotopes between masses 124 and 136 and ranging in abundance from ~0.1 to 26% relative abundance, it provides numerous good tuning points. Since it is introduced with the GC carrier gas, it is especially useful in optimizing the horizontal and vertical torch positions, which are critical to optimum sensitivity due to the narrow injector diameter of the GC torch. If a wider range of masses is needed, other tuning gases can be used, or, alternatively, plasma background masses such as 38 and 78 can be used.

Since the Agilent GC-ICP-MS interface does not rely on introduction of a wet aerosol for tuning or operation, optimization is somewhat different from typical wet plasma conditions. First, because no water or acids are being continuously introduced, polyatomic interferences, particularly oxides, are mostly eliminated. Second, without the introduction of cooling water, much lower plasma power is required for complete ionization, even of high IP elements. Typically, optimum performance is achieved with plasma power set between 600 and 700 watts.

Use of Optional Gases

In addition to Xe for tuning, the addition of other gases to the sample flow can have benefits. Adding a small amount of oxygen can be used to prevent carbon deposits on the interface components (primarily the cones) by oxidizing elemental carbon to carbon dioxide. Optional gases, including oxygen and nitrogen, have also been shown to enhance the sensitivity for several common analyte elements, including Sn, As, Se, and others. These gases are typically added via a Tee into the argon makeup gas line. Their flow can be controlled by either the auxiliary mass flow controller on the ICP-MS or by an optional mass flow control channel on the GC.

Analysis of Polybrominated Diphenyl Ether (PBDE) Flame Retardants by GC-ICP-MS

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Introduction

PBDEs are widely used flame retardants added to many common household products, including textiles, mattresses and furniture, and electronic devices. Their similarity in structure to PCBs and dioxins has raised concerns about health risks associated with their use. Recently some classes of these compounds have been banned in Europe in response to Restriction of Hazardous Substances (RoHS) regulations and and voluntarily removed from production in the US. However, some classes are still in use, and the compounds are widely distributed within the environment. Gas chromatography is typically used in the separation of these compounds, since the large number of possible congeners (209) makes LC separation impractical. However, the low volatility, high molecular weight, and fragile nature of some congeners make GC analysis difficult. In particular, identifying trace levels of PBDEs in the presence of other halogenated compounds is difficult with conventional GC detectors.

Hardware Setup

The analytical system consisted of an Agilent 6890N GC interfaced to an Agilent 7500a ICP-MS using the Agilent GC-ICP-MS interface. GC and ICP-MS conditions are summarized in Table 1. A short 5 M x 0.25 mm x 0.25 µm Agilent DB-5MS column was used. The GC was equipped with the optional three-channel auxiliary EPC module to control the addition of optional gases, including oxygen and helium. Oxygen is added to the plasma gas to burn off carbon deposits on the sample and skimmer cone. Helium is added to the carrier gas to enhance the sensitivity for bromine. 100 ppm Xe in He was used as an alternate GC carrier gas supplied to the GC via a manual switching valve to allow either pure He or Xe in He to be used. Xe is used to tune the ICP-MS for maximum sensitivity and can also be used as an online internal standard.

Standards and Reagents

PBDE standards were purchased from AccuStandard Inc. (New Haven, CT, USA) and diluted into either semiconductor-grade xylene or pesticide-grade isooctane. No certified standard reference materials are currently available for PBDE compounds in real matrices.

Method

Table 1. *Method parameters for the separation of PBDEs using the GC with ICP-MS detection.*

GC	
Injection	Split/splitless - 1µL
Oven program	80 °C (1 min), 20°/min –> 320 °C (5 min)
Carrier gas	He at 7 mL/min
Transfer line temp	250 °C
ICP injector temp	280 °C
ICP-MS	
Isotopes acquired	79, 81
Acq mode	Time resolved
Dwell time	0.1 s/point
RF power	650 watts
Sample depth	7 mm
Carrier gas	1.05 L/min
Extract 1	–180 V

Results

GC-ICP-MS as described is capable of rapid, sensitive detection of PBDEs, including the difficult-to-analyze deca-bromo congener (BDE-209), see Figure 1 and Table 2. Analysis times of less than 12 minutes with detection limits of ~150 fg on column (0.15 ppb) can be achieved.

Conclusions

ICP-MS is the ideal GC detector for PBDEs and other bromine-containing volatile organics. It is sensitive, selective, and can tolerate a wide range of GC carrier gases and flows. Very high GC flows allow rapid elution of deca-BDE, which improves recovery.

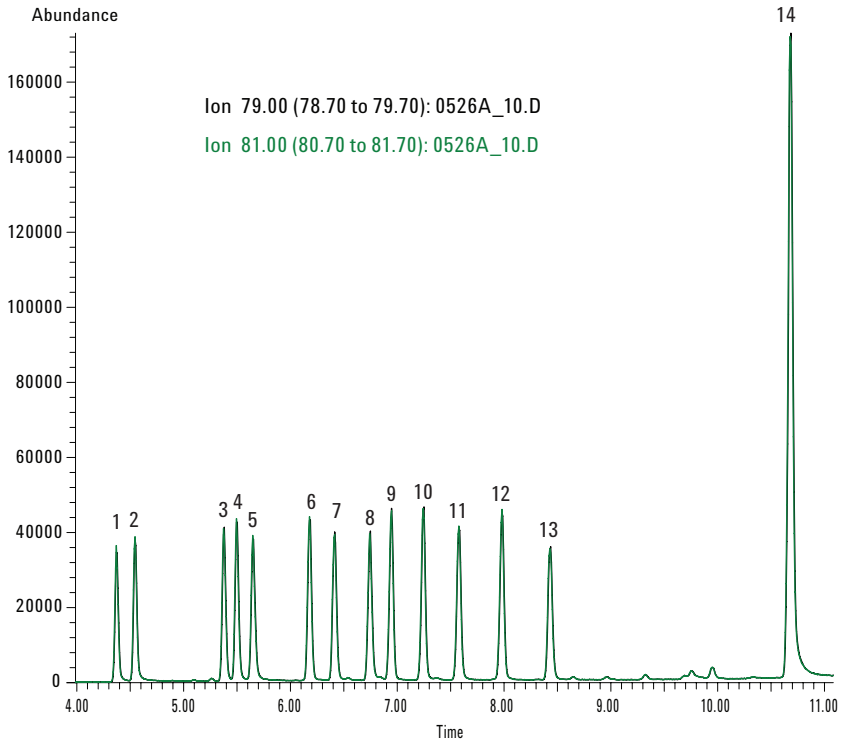

Figure 1. *GC-ICP-MS chromatogram of 10 ppb PBDE standard mix.*

Table 2. *PBDE peak identification.*

Peak	Congener (2.5 mg/mL)
1	2,2′,4-TriBDE (BDE-17)
2	2,4′,4-TriBDE (BDE-28)
3	2,3′,4′,6-TetraBDE (BDE-71)
4	2,2′,4,4′-TetraBDE (BDE-47)
5	2,3′,4,4′-TetraBDE (BDE-66)
6	2,2′,4,4′6-PentaBDE (BDE-100)
7	2,2′,4,4′5-PentaBDE (BDE-99)
8	2,2′,3,4,4′-PentaBDE (BDE-85)
9	2,2′,4,4′,5,6′-HexaBDE (BDE-154)
10	2,2′,4,4′,5,5′-HexaBDE (BDE-153)
11	2,2′,3,4,4′,5′-HexaBDE (BDE-138)
12	2,2′,3,4,4′,5′,6-HeptaBDE (BDE-183)
13	2,3,3′,4,4′,5,6-HeptaBDE (BDE-190)
14	DecaBDE (BDE-209) (12.5 mg/mL)

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Analysis of Sulfur in Low-Sulfur Gasoline by GC-ICP-MS

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Introduction

Sulfur in motor fuels has been implicated in global warming and acid rain. It is also a catalyst poison for automobile catalytic converters and refinery catalytic crackers. Reducing total sulfur in motor fuels has become a critical air pollution control goal worldwide. The USEPA tier-2 guidelines beginning in 2004 mandate an average sulfur standard of 30 ppm and a cap of 80 ppm total sulfur by 2007. The European Union announced in December of 2002 that new regulations would require full market availability of sulfur-free fuels, defined as containing less than 10 parts per million (ppm) sulfur content, by January 1, 2005. GC-ICP-MS has the capability to meet current and projected detection limits for both total sulfur in reformulated gasolines and other motor fuels as well as individual sulfur species. Additionally, GC-ICP-MS can identify and quantify other volatile organometallic species in fuels.

Hardware Setup

An Agilent 6890 gas chromatograph with split/splitless injector was coupled to an Agilent 7500a ICP-MS using the Agilent GC-ICP-MS interface. GC and ICP-MS conditions are summarized in Table 1.

Standards and Reagents

Calibration was based on a multi-level analysis of thiophene and 2-methyl thiophene spiked into 3:1 isooctane/toluene obtained from Ultra Scientific. Calibration levels ranged from 2.5 ppm per compound to 500 ppm per compound (Figure 1). Because GC-ICP-MS is capable of compound-independent calibration, it was not necessary to calibrate every possible sulfur compound separately. The sulfur response factor for any compound(s) can be determined from a single compound. In this case, the response factors from thiophene were used and confirmed by those from 2-methylthiophene.

Table 1. *GC and ICP-MS operating parameters.*

Instrumentation	
GC	Agilent 6890 GC
Inlet	Split/splitless
Detector	Agilent 7500a ICP-MS
Column	30 M x 0.25 mm id x 0.25 µm HP-5
GC Conditions	
Inlet temperature	250 °C
Injection volume	1 µL
Injection mode	Split 1:50
Carrier gas	Helium
Carrier gas flow	2.5 mL/min (constant flow mode)
Transfer line temperature	250 °C
Oven temperature	40 °C /4 minutes, 20 °C/min to 250 °C, program hold for 1 min
ICP-MS Conditions	
Forward power	700 watts
Sample depth	13 mm
Carrier gas flow	1.1 L/min
Extract 1	–150 V
Extract 2	–75 V
Aux gas	He, 10 mL/min added to Ar carrier
Injector temperature	260 °C

Results

Chromatograms of three standard reference gasolines and a standard reference diesel are shown in Figure 2. Comparison with quantitative results for total sulfur compared favorably with those obtained by x-ray fluorescence [1]. Single compound detection limits for thiophene and 2-methyl thiophene are less than 5 ppb. When translated to total sulfur in gasoline, the detection limit is approximately 0.1 to 0.5 ppm.

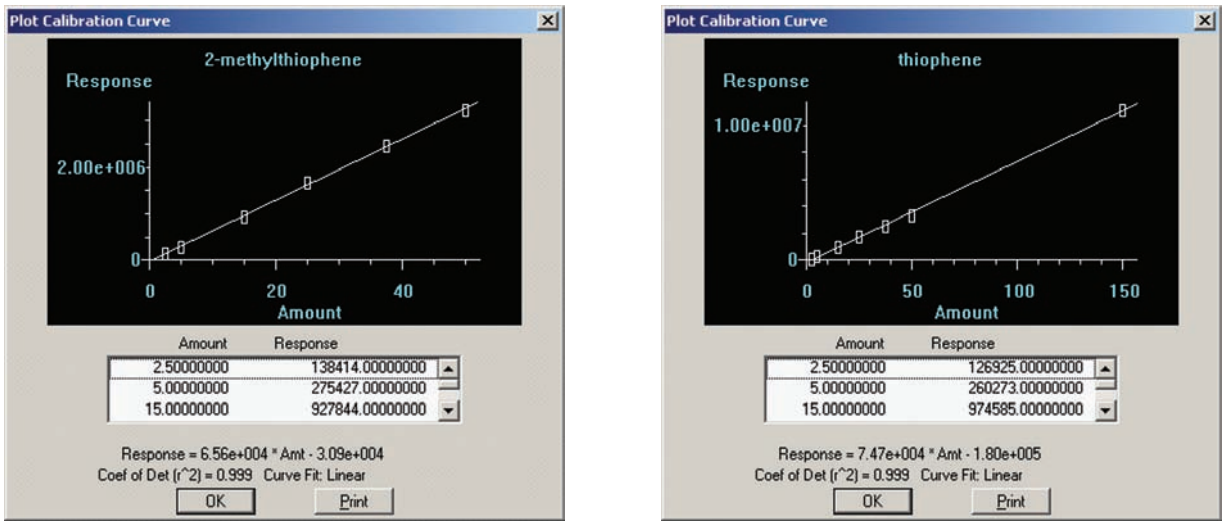

Figure 1. *Calibration curves, thiophene and 2-methylthiophene in 3:1 isooctane:toluene.*

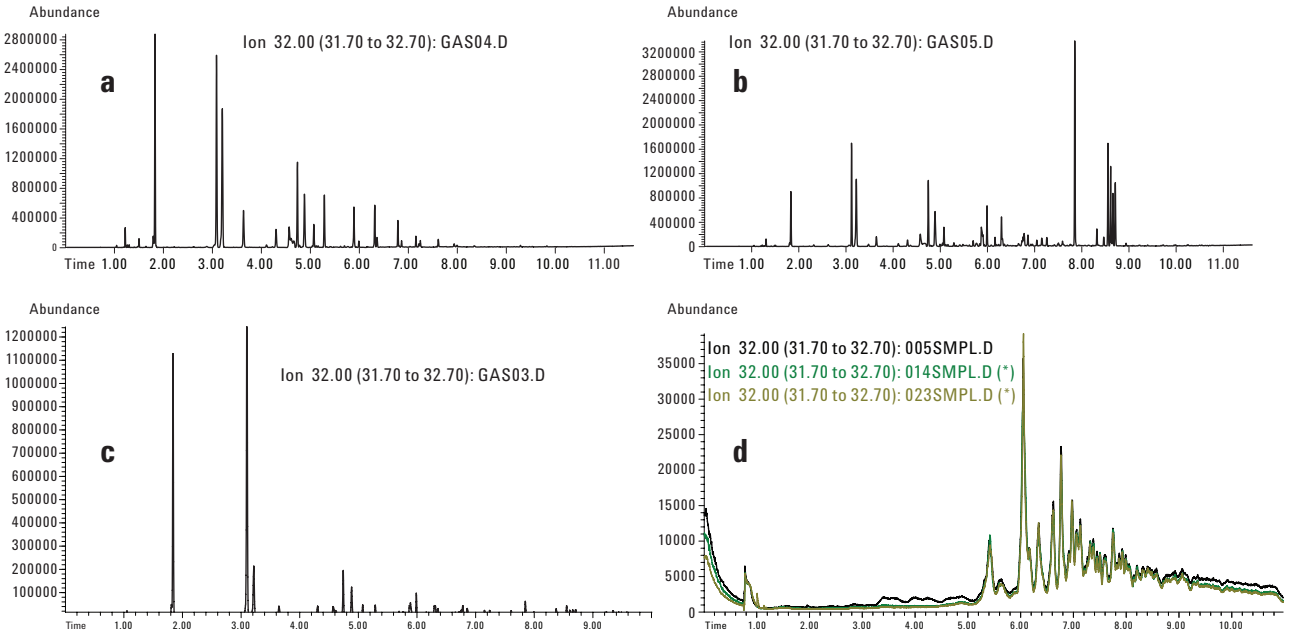

Figure 2. *a) ASTM-Fuel-QCS02, conventional gasoline QC sample, ~330 ppm total sulfur. b) ASTM Round Robin Gasoline Standard #2, not certified for sulfur. c) CARB low-sulfur reformulated gasoline, ~55 ppm total sulfur. d) Low-sulfur diesel diluted to ~5.6 ppm total sulfur, analyzed in triplicate.*

Conclusions

GC-ICP-MS offers significant advantages over other techniques for the analysis of total sulfur and sulfur species in motor fuels. These include high sensitivity, wide dynamic range, freedom from interferences and suppression, ability to use compound-independent calibration as well as the ability to simultaneously monitor other elements.

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For Additional Information

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Combining GC-ICP-MS and Species-Specific Isotope Dilution Mass-Spectrometry (SS-IDMS)

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Introduction

The toxic effects of organotin compounds in the environment have been well documented [1] and have led to extensive research into analytical methodologies for their determination in a variety of matrices. The widespread use of organotin compounds in pesticides and antifouling paints and as heat and light stabilizers in PVC products has resulted in their detection in most marine and freshwater sediments and in open-ocean waters [2]. In recent years, the focus of research in organotin analysis has begun to include matrices with human health implications, such as seafood [3], artificial matrices such as PVC pipes used for drinking water distribution [4], and human blood [5] and liver samples [6]. Currently, a wide range of methods is being used for the extraction, separation, and detection of organotin compounds, and significant variation in the results can be obtained by different methodologies [7, 8].

Generally, the separation method of choice has been gas chromatography (GC), which allows for the analysis of many different groups of organotin compounds (for example, butyl-, phenyl-, octyl-, and propyl) in a single analysis after derivatisation [9]. GC separation has been successfully coupled to a variety of detectors, such as atomic absorption spectroscopy (AAS) [10, 11], atomic emission detection (AED) [12], microwave-induced plasma atomic emission detection (MIP-AED) [13, 14], and, more recently, inductively coupled plasma mass-spectrometry (ICP-MS) [15, 16]. All of these detectors can offer sufficient detection limits for organotin analysis.

However, the derivatization required for GC analysis can result in variation in yields between species and in terms of efficiency depending on matrix components. The use of SS-IDMS can effectively eliminate the bias that can be introduced by the derivatization step, and a number of high-throughput laboratories are now beginning to use the technique for this purpose. GC-ICP-MS has the unique potential to facilitate simultaneous multi-elemental speciation analysis, because species of several elements, such as Se [17], Pb [18], Hg [19], and Sn have volatile forms and could be analysed in a single analysis.

Species-specific isotope dilution mass-spectrometry (SS-IDMS) for organometallic speciation studies has been made possible by the synthesis of organometallic molecules containing an isotopically enriched hetero-atom [20]. Because IDMS has the capability to overcome shortfalls of analytical methods, such as analyte losses, analyte breakdown, or incomplete recovery (provided that complete equilibration between the spike and the inherent analyte has been achieved), the application to speciation methodologies can help reduce the uncertainties associated with such methods. SS-IDMS and GC-ICP-MS have previously been applied to the speciation of methylmercury [19] as well as organotin compounds [16].

The same approach for isotope dilution analysis was used for TBT and DBT in sediment as that described for nonspeciation IDMS measurements described by Catterick et al. [21]. This IDMS methodology relies on the approximate matching of the isotope ratios in both the sample (R'_B) and the calibration solution (R'_{Bc}). As described in reference 21 , this matching approach negates errors associated with mass-bias effects, detector dead-time, and the characterization of the spike. Because the concentration of the spike is eliminated from the calculation of the mass fraction in the sample (Equation 1), the time-consuming reverse isotope dilution mass spectrometry (RIDMS) used to characterize this value is not required.

Experimental

To carry out the IDMS analysis, alternating injections of the sample (SB) and calibration solutions (MB) were made. In order to obtain sufficient data for the calculation of meaningful uncertainty budgets, each sample extract was injected four times and bracketed by a total of five injections of the calibration blend. For each injection of the calibration blend, the measured isotope amount ratio (R'_{Bc}) was calculated from the ratio of the peak areas of ^{120}TBT and ^{117}TBT. The isotope amount ratio (R'_B) was also calculated in the same way for each sample injection. For the measured isotope amount ratio of the calibration blend (R'_{Bc}), the average of the two ratios measured before and after each sample blend isotope amount ratio (R'_B) were taken. The average of the four mass fractions was then reported as the mass fraction obtained for the blend analyzed. The final mass fraction was recalculated back to the original sample and corrected for moisture content.

The chromatographic peaks were integrated manually using the Agilent ICP-MS chromatographic software. The mass fraction obtained from the measurement of each sample blend injection was then calculated according to:

$$w'_X = w_Z \cdot \frac{m_Y}{m_X} \cdot \frac{m_{Zc}}{m_{Yc}} \cdot \frac{R_Y - R'_B \cdot \frac{R_{Bc}}{R'_{Bc}}}{R'_B \cdot \frac{R_{Bc}}{R'_{Bc}} - R_Z} \cdot \frac{R_{Bc} - R_Z}{R_Y - R_{Bc}}$$

R'_B	Measured isotope amount ratio of sample blend
R'_{Bc}	Measured isotope amount ratio of calibration blend
R_{Bc}	Gravimetric value of the isotope amount ratio of calibration blend
R_Z	Isotope amount ratio of primary standard Z (IUPAC value)
R_Y	Isotope amount ratio of spike Y (value from certificate)
w'_X	Mass fraction of Sn in sample X obtained from the measurement of one aliquot
w_Z	Mass fraction of Sn in primary standard Z
m_Y	Mass of spike Y added to the sample X to prepare the blend B
m_X	Mass of sample X added to the spike Y to prepare the blend B
m_{Zc}	Mass of primary standard solution Z added to the spike Y to make calibration blend Bc
m_{Yc}	Mass of spike Y added to the primary standard solution Z to make calibration blend Bc

The isotope amount ratios of the primary or natural TBTCl standard was calculated on the basis of the representative isotopic composition of Sn according to IUPAC. For the spike TBTCl, the isotopic composition was obtained from the certificate supplied with the ^{117}Sn enriched material from AEA Technology plc (UK).

Results

Table 1 shows the data obtained for the analysis of DBT and TBT in the CRM PACS-2 and Figure 1 shows a chromatogram obtained for this CRM. The comparison between the measured values and the certified concentrations for this reference material are in good agreement. A second evaluation of the GC-ICP-IDMS methodology was performed by comparing the data for the same samples when measured by LC-ICP-IDMS.

Table 1. *TBT and DBT determined in PACS-2 (fresh bottle) by ASE and GC-ICP-IDMS analysis.*

Analysis	DBT (ng/g Sn)	TBT (ng/g Sn)
1	1084	885
2	1081	879
3	1086	872
4	1097	869
Mean	1087	876
Expanded uncertainty (k = 2)	77	51
Certified concentration	1090	980
95% confidence interval	150	130

The comparative data provided for analysis of the same sediment extracts by HPLC-ICP-IDMS and GC-ICP-IDMS shows that the type of chromatographic separation used has no significant influence on the mass fractions determined. Therefore, the GC and HPLC methods can be used as an independent check on one another.

Table 2. *Comparative TBT data for the analysis of two different sediments (PACS-2 [old bottle] and P-18/HIPA-1) by both methods.*

All as ng/g Sn*	PACS-2 (old)	P-18/HIPA-1
LC-ICP-IDMS	828 ± 87	78.0 ± 9.7
GC-ICP-IDMS	848 ± 39	79.2 ± 3.8

*The values represent the mean result and their associated expanded uncertainty with a coverage factor of k = 2.

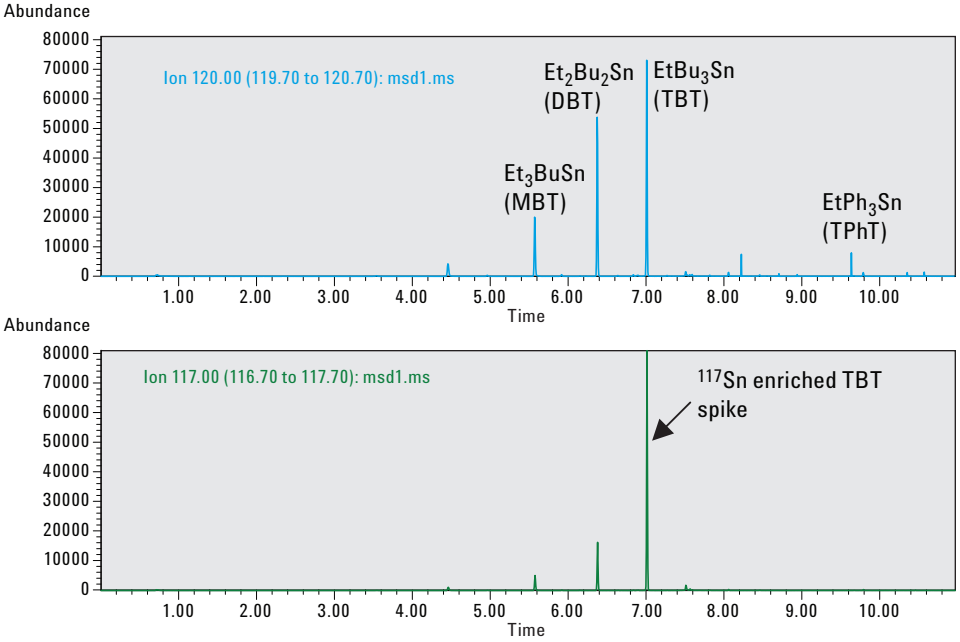

Figure 1. *GC-ICP-MS chromatogram of a sediment extract after ethylation.*

However, GC-ICP-MS analysis yields greater sensitivity with method detection limits based on sediment analysis (0.03 pg TBT [as Sn]) being two orders of magnitude better than achieved by HPLC-ICP-MS. Typical isotope amount ratio precisions achieved by GC-ICP-IDMS were superior by 1.5- to 2-fold compared to HPLC-ICP-IDMS. This difference is reflected in the expanded uncertainty associated with the final results, which are greater by HPLC-ICP-MS (10%) compared to GC-ICP-IDMS (5%) for analysis of the same extracts. It has been shown that the use of additional gases with GC-ICP-MS analysis can enhance the detection limits for a range of organotin compounds, and the addition of 0.1 L min^{-1} N_2 showed increases in peak areas of up to 150-fold compared to no gas addition.

The precision of the isotope amount ratio measurements for both methods can be compared for repeat injections of the mass-bias calibration blend solutions and the sample blends. Typical percent relative standard deviations achieved by HPLC-ICP-IDMS range from 0.3% to 1.4% for R'_B and from 0.7% to 1.3% for R'_{Bc}. For GC-ICP-IDMS the respective precisions achieved range from 0.5% to 1.0% for R'_B and 0.4% to 0.7% for R'_{Bc}. This data suggests that GC-ICP-MS can provide isotope amount ratios, which are 1.5- to 2-fold superior to HPLC-ICP-MS. This is partly explained by differences in the chromatographic separations and different plasma conditions used. The peak area integration by GC-ICP-MS is more reproducible than for HPLC-ICP-MS because the peaks are narrower, there is no significant background noise for the isotopes monitored, and the peaks do not suffer from tailing as much as the HPLC peaks. These differences in peak area integration are then reflected by the respective isotope amount ratio precision data for R'_B and R'_{Bc}, which shows GC-ICP-MS to have lower maximum RSD values (1.0% and 0.7%, respectively) compared to HPLC-ICP-MS (1.4% and 1.3%, respectively).

Conclusions

The combination of GC-ICP-MS and SS-IDMS can provide a number of advantages for organotin speciation analysis. On the one hand the GC-ICP-MS approach provides excellent sensitivity and chromatographic resolution for a wide range of organotin species whilst the SS-IDMS approach can either be used for high-accuracy high-precision analysis as shown above, or it can simply be used to eliminate the potential for bias due to differences in derivatization efficiency for different species or in different sample matrices that may otherwise lead to a significant bias in the result.

Acknowledgement

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Determination of Phosphoric Acid Triesters in Human Plasma Using Solid-Phase Microextraction and GC-ICP-MS

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Baiba Cabovska, Laboratory of Applied Pharmacokinetics and Therapeutic Drug Monitoring, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Introduction

Although phosphoric acid triesters are used as flame retardants and plasticizers in a variety of products, some of the alkyl phosphates like tris(2-chlororethyl) phosphate show neurotoxic and carcinogenic properties. Similarly, aryl phosphates such as triphenyl phosphate and 2-ethyl-hexyl diphenyl phosphate show allergenic and hemolytic effects. Analysis of these species in human blood plasma is gaining increasing attention due to their possible leaching from the plastic plasma collection bags.

Sample Preparation

Solid-phase microextraction (SPME) was utilized as a sample preparation step for extraction and preconcentration of phosphate esters from the human plasma samples stored in conventional polyvinylchloride plasma bags.

Instrumentation

An Agilent 6890 Series GC system for the separation of the species was connected via a heated GC-ICP-MS interface to an Agilent 7500cs ICP-MS with Octopole Reaction System collision/reaction cell. Separation of phosphoric acid esters was performed on a 30 M x 0.320 mm id x 0.25 µm DB-5 capillary column (Agilent Technologies, Folsom, CA, USA). The presence of phosphoric acid triesters in human plasma was further validated by SPME GC time-of-flight high-resolution mass spectrometry (GC-TOF-MS) using a Micromass GCT orthogonal time-of-flight mass spectrometer coupled to the Agilent 6890 GC.

Results and Discussion

To check the performance of the method, SPME analysis of spiked plasma samples containing known amounts of phosphoric acid ester standards was performed. The assay was linear ($r^2 > 0.993$) over a concentration range of 0.1 to 50 ng P/mL for each phosphoric ester studied (see Table 1). The detection limits were 50 ng/L for tripropyl phosphate, 17 ng/L for tributyl phosphate, 240 ng/L for *tris*(2-chloroethyl) phosphate, and 24 ng/L for triphenyl phosphate. Recovery of triphenyl phosphate increased from 5 to 66% after deproteinization of plasma samples while that for tripropyl, tributyl, and *tris*(2-chloroethyl) phosphates was in the range of 35%, 43%, and 49%, respectively, after sample deproteinization at 10 ng/mL of spiked concentration. Note that such a low analyte recovery is commonly encountered in drug determination from plasma due to considerable binding with the plasma proteins. The precision of the method was obtained by consecutive analysis of 10 replicate spiked plasma samples at 1 ng P/mL. The relative repeatability was below 15% for all the analytes. Validation of the method could not be performed due to lack of commercially available certified reference material for determination of analytes in the plasma matrix.

Application to Human Plasma Samples

Human plasma collected from a plasma bag was analyzed for the organophosphate esters. Presence of tributyl phosphate and tri-phenyl phosphate was detected in the plasma that was exposed to the polyvinyl chloride plasma collection bag for a two-week period (Figure 1b), while these compounds were absent in

Table 1. *Analytical performance characteristics of the phosphoric acid triester detection in human plasma.*

Phosphoric acid triesters	Limit of detection (ng P/L)	Retention time (min)	r^2	Method precision (%)	Recovery (%)
Tripropyl phosphate	50	3.36 ± 0.03	0.998	8	35
Tributyl phosphate	17	4.48 ± 0.01	0.999	11	43
Tris(2-chloro ethyl) phosphate	240	4.98 ± 0.01	0.993	7	49
Triphenyl phosphate	24	7.21 ± 0.04	0.995	14	66

the same plasma that had not been stored in the conventional plastic storage bags (Figure 1a). Levels of triphenyl phosphate were in the range of 0.2 ng P/mL, while that of tributyl phosphate was close to the detection limit of the method (0.02 ng P/mL). Both ^{31}P GC-ICP-MS and GC-TOF-MS chromatograms for the nonspiked human plasma are presented in Figure 1. Presence of triphenyl phosphate in natural plasma stored in polyvinylchloride bags was confirmed with high-resolution TOF-MS measurements. The identity of the triphenyl phosphate was verified by retention time matching, correct isotope pattern (M^{o+} and $[M-H]^+$) and accurate mass measurements (within 1 mDa accuracy) as seen in Figure 1. However, presence of tributyl phosphate could not be confirmed through GC-TOF-MS due to its very trace levels in the plasma samples.

Conclusions

Application of SPME in conjunction with GC-ICP-MS proved to be a very promising analytical method for determination of trace amounts of phosphoric acid esters in complex biological samples such as human plasma. The developed method is relatively simple, sensitive, reasonably fast, and solvent free.

Low detection limits obtained in the parts-per-trillion range also assisted in determination of triphenyl phosphate in human plasma previously stored in conventional plasma storage bags. Levels of this were found to be three orders of magnitude lower than its haemolytic EC20 value. Combination of GC-ICP-MS with GC-TOF-MS helped to confirm the presence of this species in plasma collected from the bag. The presence of this is attributed to the fact that triphenyl phosphate is applied as nonflammable plasticizer in polyvinyl chloride bags. Despite previous reports of large levels of ethylhexyl diphenyl phosphate in plasma stored in polyvinyl chloride bags, no evidence of this compound was found in our study.

For Additional Information

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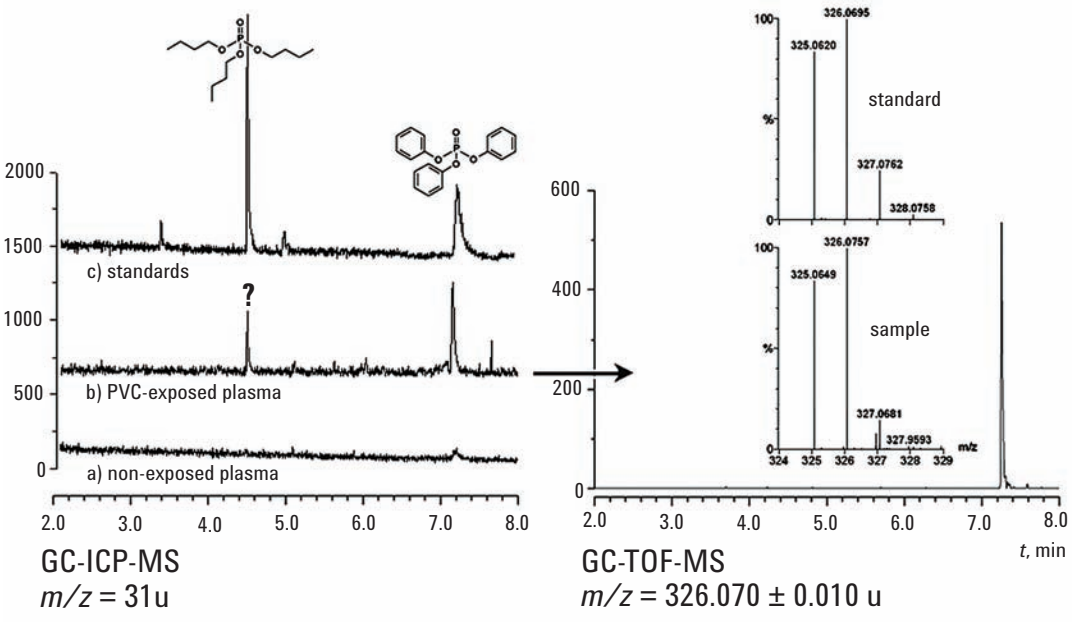

Figure 1. *Analysis of phosphoric acid triesters in human plasma with GC-ICP-MS (left) and GC-TOF-MS showing extracted ion chromatogram for triphenyl phosphate and Mo^+ and $[M-H]^+$ ions for sample and standard (right). (a) Native human plasma, (b) human plasma that has been stored in a polyvinyl chloride bag, and (c) human plasma spiked with 1 ng(P)/mL (1 ppb) of phosphoric acid triesters. SPME extraction was performed after sample deproteinization, and addition of 0.70 g NaCl at pH 7.0. Extraction was carried out with a 65-µm PDMS-DVB fiber for 30 min at 40 °C.*

Analysis of Methylmercury and Inorganic Mercury (Hg^{2+}) in Biological Tissue by Isotopic Dilution GC-ICP-MS

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Introduction

The predominant natural source of environmental mercury is from volcanic activity. However, human exposure to heavy metals, including mercury, has risen dramatically in the last 50 years as a result of an exponential increase in the use of heavy metals in industrial processes and products. From a human toxicology perspective, methylmercury ($MeHg^+$) is the chemical species of greatest interest. The major form of Hg is elemental, which is oxidized to inorganic mercury, then methylated, and finally incorporated as $MeHg^+$ by fish. The principal sources of exposure to mercury in everyday life are from fish consumption, dental amalgams, skin-lightening cosmetic creams, and occupational environments. Once elemental Hg has been absorbed by the body, it is oxidized to inorganic divalent Hg.

Gas chromatography is typically used in the separation of Hg compounds in biological samples, since the high volatility and high fat matrix make analysis by LC difficult. Moreover, GC-ICP-MS, specifically when using a dry plasma (that is, no water vapor is introduced to the ICP), is a highly specific, high-sensitivity technique with negligible matrix effects.

Instrumentation

The analytical system consisted of an Agilent 6890N GC interfaced to an Agilent 7500ce ICP-MS using the Agilent GC-ICP-MS interface and high-sensitivity (cs) lenses. A 30 M x 0.25 mm x 0.25 µm Agilent HP-5 GC column was used. The GC was equipped with the optional three-channel auxiliary EPC module to control the addition of the optional gases. Oxygen was added to the plasma gas to burn off carbon deposits on the sample and skimmer cones (Pt). 50 ppm Xe in Ar was used as an additional GC carrier gas. It was supplied to the GC from a Tee connector positioned between the end of the capillary column and the start of the transfer line to increase the carrier gas velocity and minimize residence time in the transfer line. Xe was used to tune the ICP-MS for maximum sensitivity. It can also be used as an online internal standard.

Standards and Reagents

$MeHg^+$ and inorganic mercury (Hg^{2+}) standards (IRMM, Belgium, and Eurisotope, Paris), were diluted into Milli-Q water (18.2 MΩ), spiked with $^{202}MeHg^+$ and $^{199}Hg^{2+}$, derivatized with $NaBPr_4$ (Sigma-Aldrich, France) and then sonic-extracted into pesticide-grade hexane. Because there are no available certified standard reference materials (CRMs) for mercury speciation compounds in blood samples, DORM-2 fish (IRMM) was used as a CRM. Figure 1 shows chromatograms obtained following the analysis of DORM-2 using the operating parameters given in Table 1.

Method

Table 1. *GC and ICP-MS operating parameters.*

GC	
Injection	Split/splitless - 2 µL
Oven program	50 °C (1 min), 25°/min ' 220 °C (4 min)
Carrier gas	He at 2 mL/min
Transfer line temp	250 °C
GC injector temp	220 °C
ICP-MS	
Isotopes acquired	124, 198, 199, 200, 202
Acqired mode	Time resolved
Dwell time	0.03 sec/point
RF power	1050 watts
Sample depth	8.5 mm
Carrier gas	0.45 L/min
Extract 1	4 V

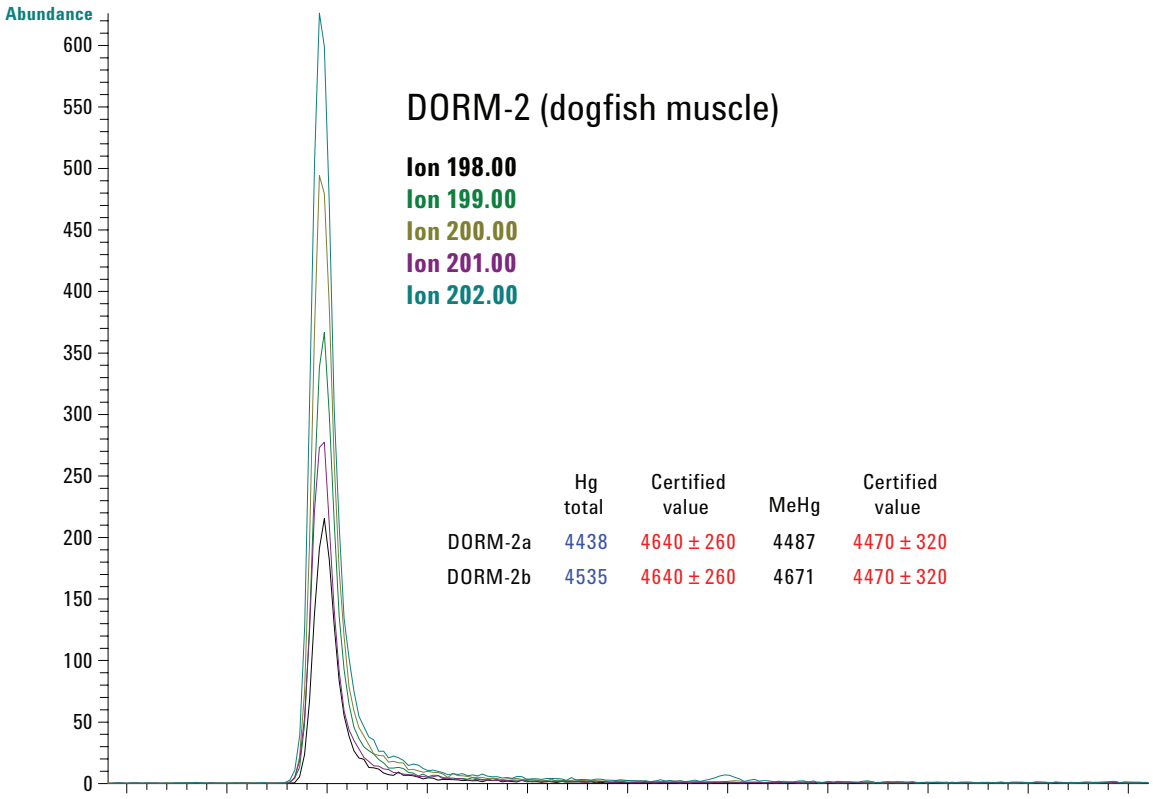

Figure 1. *Extracted ion chromatograms (overlaid) for mercury in DORM-2 dogfish muscle.*

Results

GC-ICP-MS is capable of rapid, sensitive detection of $MeHg^{+}$ and Hg^{2+}. Analysis times of less than 12 minutes with detection limits of approximately 3 fg on column for aqueous samples and 50 fg (0.05 ppb) for blood with a sample aliquot of 0.25 mL can be achieved.

Conclusions

ICP-MS is the ideal GC detector for elemental speciation of volatile and semivolatile organometallic compounds. It is sensitive, selective, and can tolerate a wide range of GC carrier gases and flows. Isotope dilution (ID) allows quality control and improves recovery. Limits of detection for GC-ID-ICP-MS are about 20 times better than HPLC and 100 times better than previous-generation GC-ICP-MS.

Reference

"Methylmercury ($MeHg^{+}$) and Inorganic Mercury (Hg^{2+}) Determination in Blood using GC-ICP-MS," Winter Plasma Conference, January 2005

CE-ICP-MS Introduction

While capillary electrophoresis (CE) (also known as Capillary Zone Elecrophoresis or CZE) is not strictly a chromatographic technique, it functions in much the same manner for the separation of mixtures into individual components (see Figure 1). CE differs from chromatography in how this is achieved. Chromatographic systems rely on partitioning of analytes between a stationary phase (usually a packed or coated column) and a flowing mobile phase (gas or liquid). CE uses a high-voltage electric potential applied across a narrow, fluid-filled capillary to induce the migration of charged species in the capillary according to their net charge, size etc.

CE is applicable to a wide range of analytes from small cations to large biomolecules. It can be coupled to ICP-MS in much the same way as nanoflow HPLC. The primary differences are the high voltages across the capillary, which must be taken into consideration, as well as the very low flow rate (nL/min) and lack of a high-pressure pump. Because of these, CE always requires the addition of a pumped makeup flow in order to achieve efficient nebulization. In order to minimize sample dilution and loss of analyte in a drained spray chamber, CE is most effectively coupled with a microflow nebulizer and direct coupling to the torch through an undrained spray chamber. Depending on the configuration, CE has the ability to achieve high-resolution separations based on charge, molecular weight, hydrophobicity, or other properties. In addition to potentially very high resolution, CE has the advantage over HPLC in being relatively simple and inexpensive. Disadvantages include very low capacity (nL sample size) and associated dynamic range limitations.

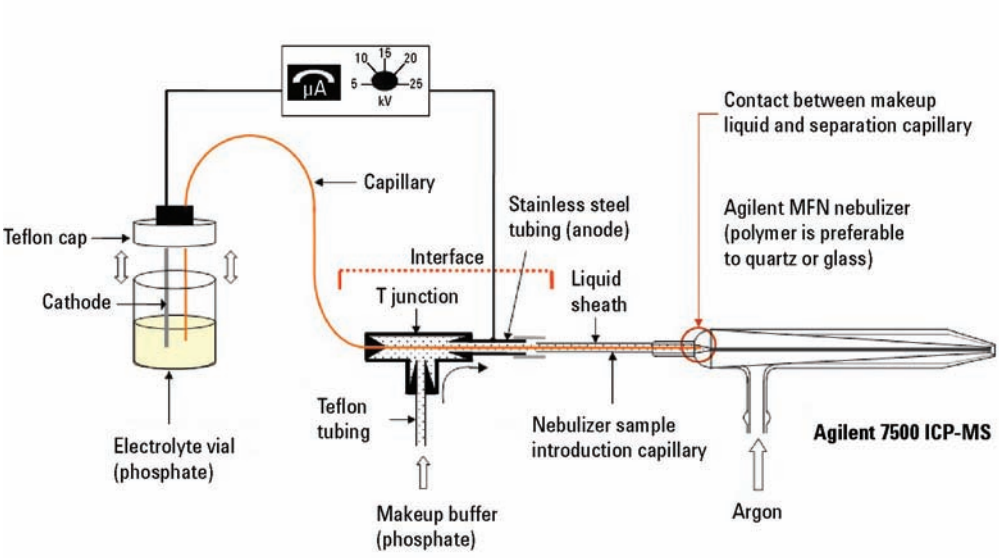

Figure 1. *Schematic of CE-ICP-MS.*

Determination of Roxarsone and Its Transformation Products Using Capillary Electrophoresis Coupled to ICP-MS

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Introduction

Roxarsone (3-nitro-4-hydroxyphenyl-arsonic acid) is one of the most widely used growth-promoting and disease-controlling feed additives in the United States. Many broiler chickens are fed roxarsone to promote weight gain and control parasites. Most of the roxarsone is believed to be excreted unchanged, and the resulting arsenic-containing waste is commonly recycled as fertilizer. Once in the environment, roxarsone can easily degrade into much more mobile and toxic arsenic (As) species. While HPLC coupled to ICP-MS has been used for the determination of As species including roxarsone degradation products, it is limited in its resolution. Capillary electrophoresis (CE) has the advantages of simple hardware and high efficiency. When coupled with ICP-MS for detection, CE-ICP-MS can provide a sensitive, highly selective method for the determination of roxarsone and its transformation products.

Instrumentation

A Beckman P/ACE 5500 Capillary Electrophoresis unit was coupled to an Agilent 7500c ICP-MS using the Burgener MiraMist CE nebulizer (Figure 1). 30 kV was applied to achieve electrophoretic separation through a 75-µm x 93-cm uncoated, fused silica capillary. Capillary temperature was set at 22 °C. Because of the very low CE flow (ca 181 nL/min), makeup flow is required to achieve efficient nebulization while providing closure of CE electrical circuit during separation. Makeup flow was set to 20 µL/min and consisted of 1% nitric acid with 3% methanol containing germanium as the internal standard at 100 ng/mL. CE and ICP-MS conditions are shown in Table 1.

Table 1. *CE-ICP-MS conditions.*

Capillary Electrophoresis	
Voltage	30kV
Capillary	75-µm id x 93-cm fused silica
Running buffer	20 mM sodium phosphate, pH 5.7
Pre-analysis rinse	0.1 M sodium hydroxide (3 min), running buffer (3 min)
Post-analysis rinse	0.1 M sodium hydroxide (3 min), DI water (3 min)
ICP-MS	
Forward power	1350 W
Nebulizer	Burgener MiraMist CE
Plasma gas flow	14.8 L/min
Auxiliary gas flow	0.92 L/min
Carrier gas flow	1 L/min
Make-up gas flow	0.25 L/min
Sample depth	6.3 mm
Make-up flow	20 µL/min
Masses monitored	72, 75
Integration time	0.70 sec per point

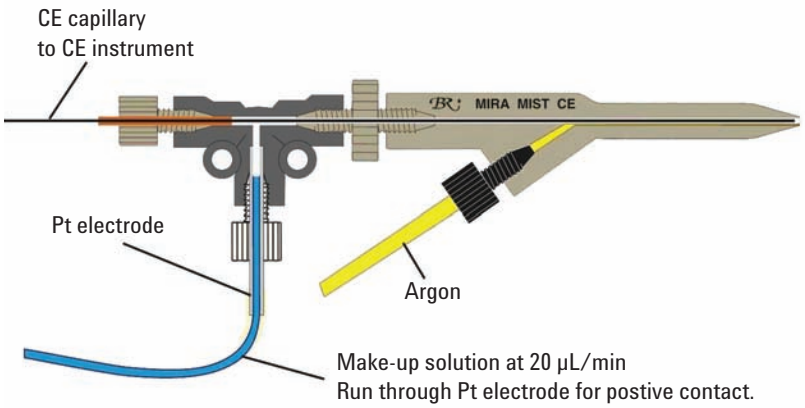

Figure 1. *Cutaway view of Burgener MiraMist CE interface.*

Standards and Reagents

Stock solutions (100 mg/L As) of arsenite (As(III)), arsenate (As(V)), dimethylarsinate (DMA), monomethylarsonate (MMA), 3-amino-4-hydroxyphenylarsonic acid (3-AHPAA), 4-hydroxy-phenylarsonic acid (4-HPAA), ortho-arsanilic acid (o-ASA), and roxarsone were prepared in deionized water (18 MΩ resistivity) and diluted into working standards in 10% running buffer in DI water.

CE-ICP-MS Operation

Initially, the ICP-MS was tuned and optimized for response at *m/z* 75 with the CE disconnected by introducing a 10-µg/L solution of arsenate via a syringe pump. The CE capillary was preconditioned with 0.1 M NaOH followed by a rinse with DI water. After connecting the CE to the ICP-MS via the MiraMist CE nebulizer, the analytical run consisted of the following steps.

- Column is prerinsed for 3 minutes each with 0.1 M NaOH and 20 mM phosphate buffer
- 10-second pressure sample injection
- 30-minute separation at 30kV
- Nebulization and detection by ICP-MS

Results

Up to eight arsenic species in standards were separated by CE-ICP-MS in about 25 minutes with sensitivity superior to CE with UV detection (Figure 2). Absolute limits of detection (3 σ) based on a 30-nL injection volume were calculated as approximately 55 to 130 fg as arsenic.

Conclusions

Taking into account the small injection volume (low nL) when compared to other techniques such as HPLC (µL), the sensitivity using CE-ICP-MS is extremely high. However, migration time reproducibility, especially when running extracts of manure samples, was poorer than hoped. One cause is due to incomplete ionization of the silica capillary walls at the buffer pH of 5.7, which can affect the electro-osmotic flow. A second cause is matrix effects from high concentrations of dissolved solids in the samples. Additional sample cleanup, including protein precipitation, significantly improved the migration time reproducibility. Internal standard correction was also shown to improve retention time reliability.

For Additional Information

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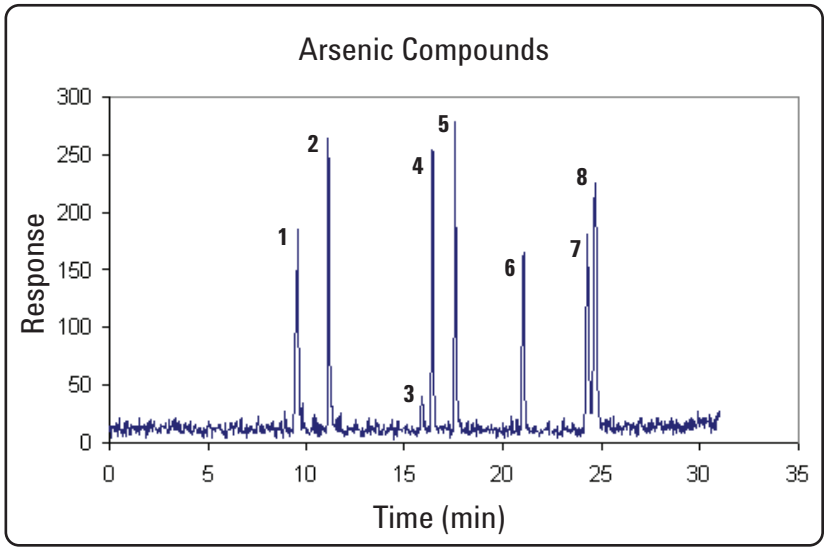
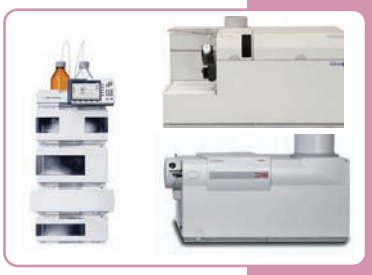
Notice: Although this work was reviewed by USEPA and approved for publication, it may not necessarily reflect official Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.



Arsenic Species
(in order of elution)

1. As(III)
2. DMA
3. 3-AHPAA
4. 4-HPAA
5. o-ASA
6. MMA
7. 3-NHPAA
8. As(V)

Figure 2. *Electropherogram of standard containing eight As species (20 ng/mL).*



Multi-MS

Multi-MS Introduction

As a chromatographic detector, ICP-MS's strengths are also its weaknesses. As an elemental mass spectrometer, it possesses excellent sensitivity and specificity for individual isotopes. For the same reasons, by itself it is incapable of providing molecular or structural information. While the addition of retention or migration time data supplied by a coupled fractionation device can provide additional information, positive identification generally requires the analysis of standards, which may not always be available. Identification of unknown molecular species is the strength of molecular mass spectrometry including GC/MS and the various forms of LC/MS. These molecular mass spectrometers can provide information on structure via fragmentation information as well as molecular weight. However, due to the complexity of molecular mass spectra, particularly in the case of complex and incompletely resolved sample mixtures, it is often difficult to locate the compound(s) of interest in the resulting data. In this case, ICP-MS, with its ability to see the elemental needle in the molecular haystack, is a useful complement.

By coupling both ICP-MS and for example, Electrospray ionization (ESI)-MS to an LC, either in parallel (Figure 1) or as separate experiments, the ICP-MS can be used to locate target compounds based on unusual or unique elemental components. For example, ICP-MS can easily locate all the selenium-containing peaks from a complex mixture of peptides. Once the desired peaks have been located, the task of characterizing them becomes much simpler.

For these reasons, the simultaneous hyphenation of HPLC to both ICP-MS and ESI/MS has become a powerful tool in the identification and characterization of biomolecules.

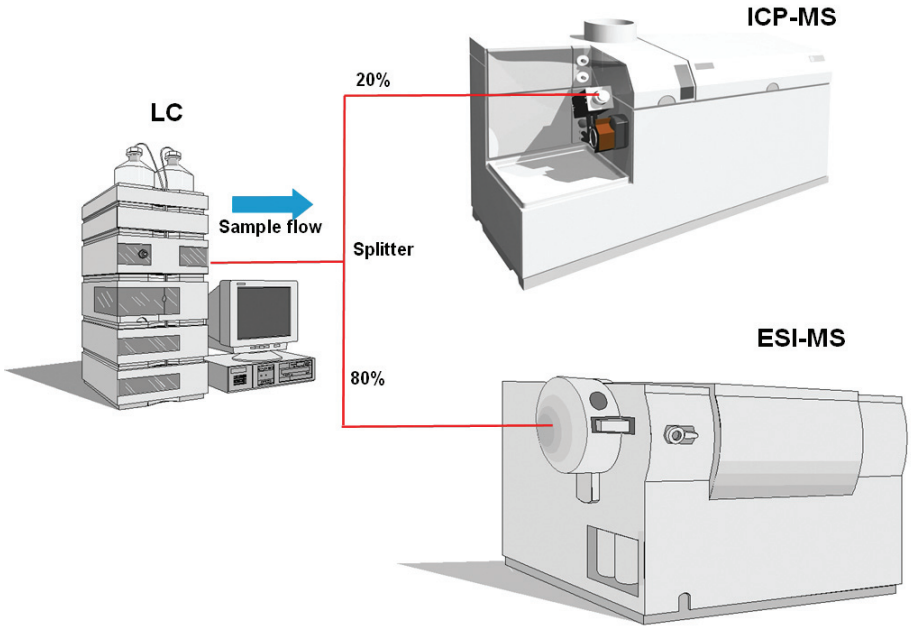


Figure 1. *Schematic showing a typical LC-multi-MS arrangement.*

Arsenic Metabolites in the Urine of Seaweed-Eating Sheep Using Simultaneous LC-ICP-MS/ES-MS

Jörg Feldmann, Helle R. Hansen, University of Aberdeen, College of Physical Sciences, Aberdeen, Scotland, UK

Introduction

Sheep on a small island north of the Scottish mainland live almost entirely on seaweed, which contains enormous amounts of arsenic in the form of arsenosugars. They eat about 35 mg arsenic daily. Since the toxicity of arsenic in foodstuff depends on its molecular form or species, it is necessary to study the metabolism of arsenic in its many forms. Initial studies [1] showed that most of the arsenosugars are bioavailable and are metabolized to many different arsenic-containing species in urine. These studies were done by conventional speciation methods using HPLC-ICP-MS. However, only the main metabolite, dimethylarsinic acid, could be identified by retention time comparison with a standard. Although the Trace Element Speciation Laboratories Aberdeen (TESLA) group was in possession of more than 15 different arsenic standards, none of them gave exact retention time matches with the seven to eight unknown major metabolites. Fraction collection after anion exchange chromatography and the use of electrospray mass spectrometry (ES-MS) did not result in any successful identification.

When ICP-MS (Agilent 7500c) and ES-MS (Agilent 1100 Series) experiments were performed using identical chromatography, we were able to overlay the arsenic peaks from the ICP-MS (*m/z* 75) with that of certain *m/z* channels of the ES-MS; coeluting peaks could be identified easily. The ICP-MS signal gave the window in which an arsenic-containing compound must elute, and the ES-MS signal gave the possible molecular mass and fragmentation information.

Breakthrough

Real advances were made when the HPLC (Agilent 1100 Series) was simultaneously coupled online to the ES-qMS (Agilent 1100 Series) and the Agilent 7500c ICP-MS: HPLC-ICP-MS/ES-MS. The HPLC is connected to a microsplitter, which splits the flow into 75% ES-MS and 25% ICP-MS. The asymmetric split compensates for the differences in the sensitivity of the two detectors. The peaks and the exact time of the ICP-MS signal (*m/z* = 75) define the envelope in which molecular fragments from the arsenic metabolites are produced. This reduces the screening to less than 1/50 of the total chromatogram and makes it possible to identify arsenic-containing masses in the ESI spectrum. Otherwise (since arsenic is monoisotopic), no identifiable elemental isotope pattern can be recognized among the thousands of masses generated by the ES-MS. Using this technique, it was not long before most of the arsenic metabolites were identified and quantified [2,3]. Among the newly identified metabolites was the first arsenothiol compound found in a biological sample (dimethylarsenothioyl acetic acid), and also the new compound dimethylarsinoyl acetate (see Figure 1) [4,5,6,7]. Today the ES-qMS has been replaced by an Agilent ion trap-ES-MS, which has been used to identify larger molecules containing arsenic, such as arseno phytochelatin-3 or arsenic triglutathione in plants and for the identification of mercury and organomercury biomolecular species.

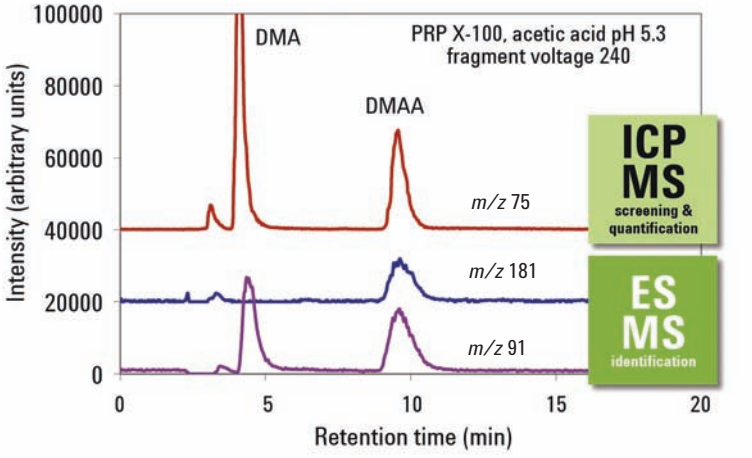


Figure 1. *First identification of dimethylarsinoyl acetate (DMAA) using HPLC-ICP-MS/ES-MS. The main metabolite dimethylarsinic acid (DMA) is also shown.*

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Determination of Unstable Arsenic Peptides in Plants Using Simultaneous Online Coupling of ES-MS and ICP-MS to HPLC

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Introduction

The determination of arsenic species in plants is necessary for various reasons. On the one hand, one would like to know the toxicity of arsenic when the plants are used as food or feed [1]. The Chinese government has released import guidelines for grains such as rice in which only inorganic arsenic is considered, which makes speciation analysis obligatory. On the other hand, the determination of arsenic species is key to understanding the uptake of arsenic from soil into the roots as well as the translocation of arsenic from the roots into the grains. In the past, only the traditional separation after a water-based extraction gave a hint that methylated arsenic species are only minor constituents while inorganic arsenic species (arsenite and arsenate) are paramount. These extractions are, however, only quantitative when rather strong extraction media are used, such as 1 *M* trifluoroacetic acid (TFA). The separation was classically done on a strong anion exchange column (PRP-X100). Although the uptake of those compounds from soil is dependent on chemical species, it cannot, however explain the variation of translocation rates in the different plants. Since plant physiologists proposed that weak, low-molecular-weight complexes with arsenic are transported into the vacuoles, it is important to detect such compounds in the plants. When this happens, the arsenic is no longer in a form that can be transported in the sap to the grain. In order to understand this process, the weak arsenic complexes need to be identified and quantified.

Experimental

Our studies identified that fresh plants can be extracted with 1 *M* formic acid at 4 °C and the analysis has to be performed within a few hours after extraction, then arsenic-polypepetides such as arsenic glutathione and arsenic phytochelatin complexes can be identified. This identification in a crude plant extract is only possible using mild chromatographic conditions such as size-exclusion or reverse-phase chromatography. Since the complexes are still of low molecular mass (< 1.5 kilodalton [kDa]), C_{18} columns using methanol gradients as mobile phase are ideal for the separation of those complexes from the inorganic and organometallic species. The identification can be achieved by coupling both mass spectrometers (ICP-MS and ES-MS) simultaneously online to the HPLC via a flow splitter from Upchurch. Approximately 20% of the flow goes to the ICP-MS (equipped with a microflow nebulizer) and the rest is transported to the ES-ion trap MS. When looking at the *m/z* 75 trace of the ICP-MS, it becomes apparent that the peptide-bound arsenics are well separated from the inorganic and methylated arsenic species, which elute near the void. Considering the slight variability of the arsenic sensitivity with the change of methanol in the eluent, the ICP-MS signal gives a good account of the quantities of the arsenic species in the extract.

Peptide Identification

If we used basic ES-MS with the crude plant extract, the identification of peptides would be a very laborious task. Having the ICP-MS signal available, only the retention times where arsenic signals appear have to be checked for coelution of any molecular mass (molecular fragments or protonated molecular mass in the positive mode). When the ES-MS was set to selected *m/z* of suspected complexes as seen in Figure 1, identification of the complexes was based on molecular mass coeluting with an arsenic signal of the ICP-MS (same retention time and peak shape).

However, unequivocal identification could only be obtained when full mass spectrum and MS/MS experiments were carried out on the $M\text{-}H^+$ signal (Figure 2). If the fragmentation pattern is consistent with that of the expected fragmentation of the peptides, the unstable arsenic peptide complex occurring in the plant extract can be positively assigned, as has been done for ferns, grass [2], and sunflower [3].

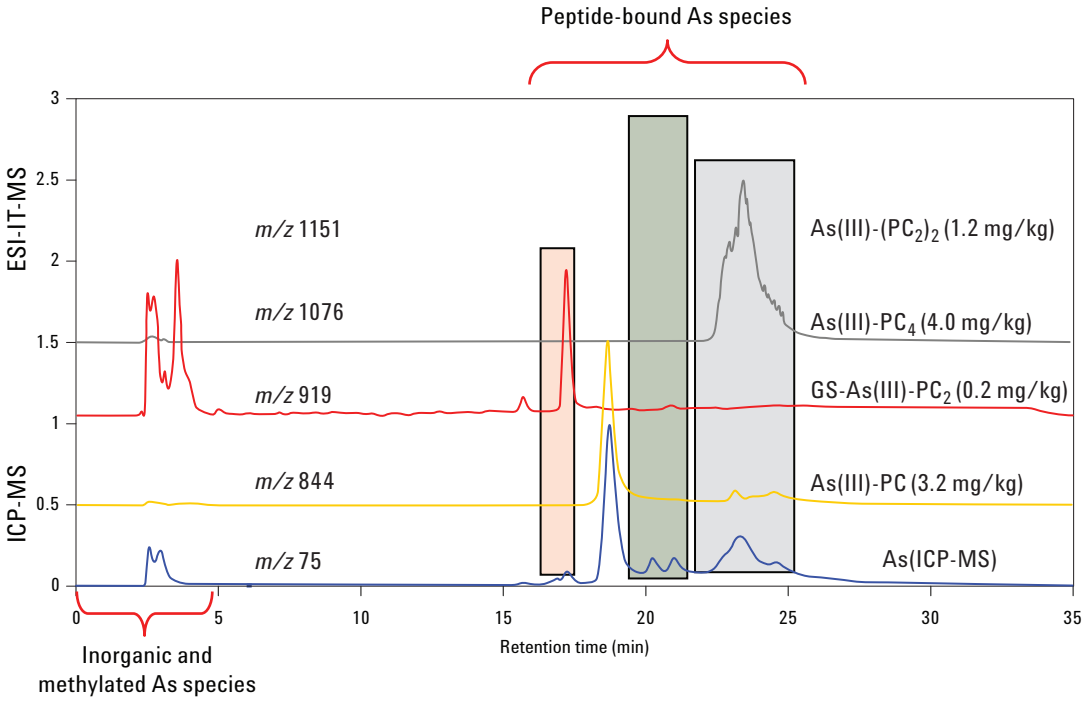

Figure 1. *Overlaid ion chromatograms of from electrospray MS and ICP-MS showing phytochelatin arsenic complexes.*

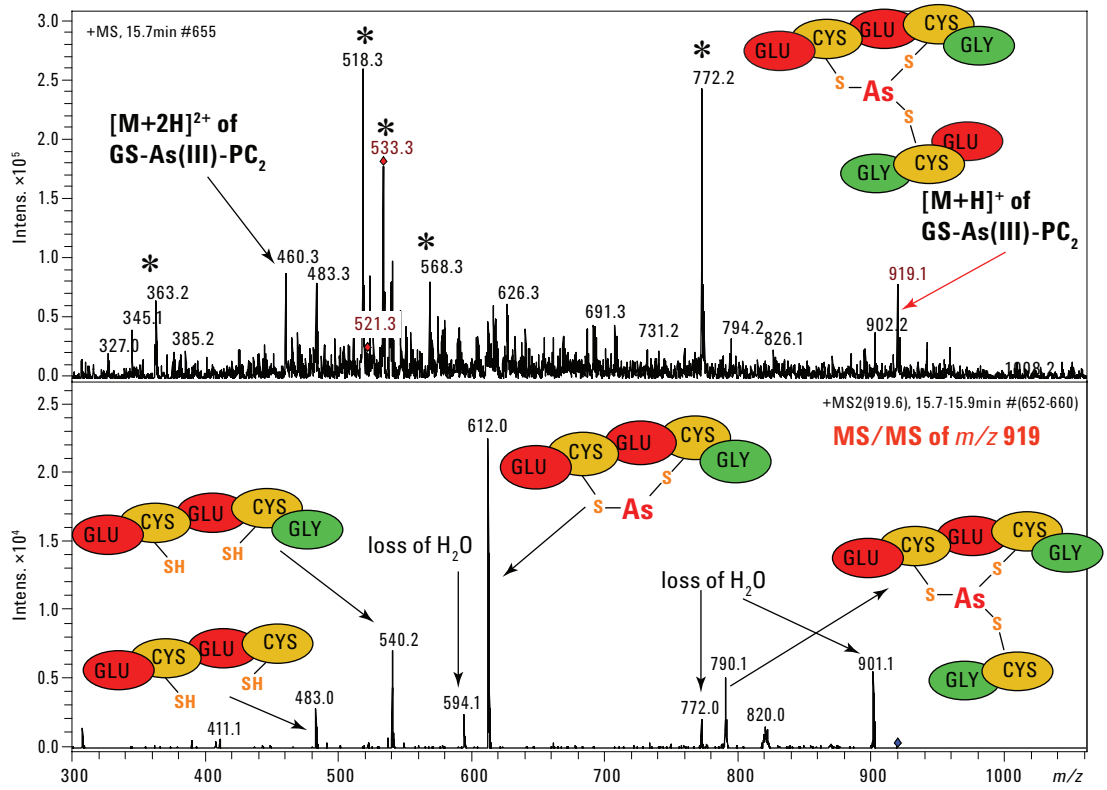

Figure 2. *ESI mass spectrum and MS/MS results from the M–H⁺ signal of GS-As(III)-PC₂.*

Conclusions

It is believed that arsenic peptide complexes trap arsenic in the roots and prevent them from being transported to the grain. The simultaneous online use of molecular and elemental mass spectrometry as detectors for HPLC has one disadvantage: the use of compromised conditions for the mobile phase, which can be used for ICP-MS and ES-MS. Otherwise, the simultaneous use saves analysis time, which is crucial when unstable compounds are investigated, and the mass spectra can be overlaid directly and ambiguities concerning a possible shift in retention time can be cancelled out [4].

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Phosphorylation Profiling of Tryptic Protein Digests Using Capillary LC Coupled to ICP-MS and ESI-MS

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Introduction

The reversible phosphorylation of proteins at the amino acid residues serine, threonine, and tyrosine is an important dynamic process in eukaryotic living systems that affects the structure of proteins, their catalytic activity during physiological processes, protein-protein interactions or the regulation of gene expression, and protein synthesis.

Traditional approaches to identifying protein phosphorylation have included incorporation of radioactive ^{32}P followed by gel electrophoreses or thin-layer chromatography, the use of phosphospecific antibodies, or high-resolution mass spectrometry. More recently, chromatographic or electrophoretic separation techniques such as liquid chromatography (LC) or capillary electrophoresis (CE) combined with element- or molecule-specific detection techniques such as ICP-MS or electrospray ionization (ESI) mass spectrometry have proven to be powerful complementary tools in biochemical analysis.

While ESI gives detailed information about the molecules under investigation, ICP-MS opens up the possibility of obtaining qualitative and quantitative information on the elemental stoichiometry of a molecule. Because of its high selectivity for elements and its nearly compound-independent ionization process, especially when using low-flow or nearly matrix-free separation techniques such as capillary-LC, ICP-MS is useful for rapid prescreening of tryptic digests for phosphorylated peptides before their final characterization by ESI-MS-MS or ESI-TOF-MS.

Handling the low solvent flow rate of capillary LC, which is normally around 4 µL/min, and controlling the background at the mass of phosphorus are the most critical issues in coupling capillary LC to ICP-MS for phosphorylation mapping. This approach offers the possibility of preselecting certain phosphorylated peptides for further analysis and detection of phosphorylated peptides, which have poor ionization properties in ESI.

Instrumentation

An Agilent 1100 Series capillary LC system was used for all experiments. Capillary-LC and nano-LC analysis were performed on an Agilent ZORBAX 300 SB-C18 column combined with a 300 SB-C18 precolumn. All connections were made of PEEK-coated silica tubing and zero dead-volume fittings. All LC modules were arranged to achieve the lowest possible dead volume between the cap-LC pump and the ICP-MS.

A modified capillary electrophoresis interface (CEI) 100 nebulizer (Cetac, Omaha, NB, USA) was used combined with a homemade 4-mL spray chamber with an injector tube extension used for direct nebulization inside the ICP torch (in-torch nebulization [ITN]).

An Agilent 7500cs ICP-MS system operating in helium collision mode was used as an element-specific detector. An Applied Biosystems API 4000 triple-quadrupole mass spectrometer was used as molecule-specific detector during the capillary LC-ESI-MS-MS experiments

Sample Preparation

The protein (beta-casein, 2 mg) was dissolved in 1 mL 50 mmol/L NH_4HCO_3 solution and used as stock solution for all further experiments. Trypsin was weighed and diluted with cold water (4 °C) to a final concentration of 100 µg/mL trypsin and 5 mmol/L $CaCl_2$. Before digestion, the proteins were denatured at 90 °C for 20 min. For digestion, 100 µL denatured protein stock solution and 50 µL trypsin solution were mixed in 1-mL vials. Digestion was carried out at 37 °C overnight. Formic acid (100%, 5 µL) was added to stop the activity of the enzyme. Finally, the digest was centrifuged at 25,000 g at 4 °C for about 5 min. The supernatant was pipetted into sealed 100-µL microvials that fit into the Agilent autosampler.

Results and Discussion

Figure 1 shows the comparison of the ^{31}P ICP-MS trace and the corresponding ESI-TIC of the beta-casein digestate. The ICP-MS peak for ^{31}P at approximately 40 minutes corresponds to the singly phosphorylated peptide FQ-pS-EEQQQTEDELQDK with a theoretical isotopic molecular weight of 2061.829 Da (as $[M+H]^+$), which is known from the literature.

Estimation of the Detection Limits for Phosphorus

To estimate the detection limit for phosphorus under Cap-LC conditions, flow-injection analysis of phosphorus standards of different concentrations was performed under isocratic conditions (90% A [0.05% TFA in water] and 10% B [0.05% TFA, 20% H_2O, 80% methanol]) at a flow rate of 4 µL/min. According to the IUPAC guidelines, a detection limit of 1.95 µg/L phosphorus corresponding to 1.95 pg P absolute was obtained.

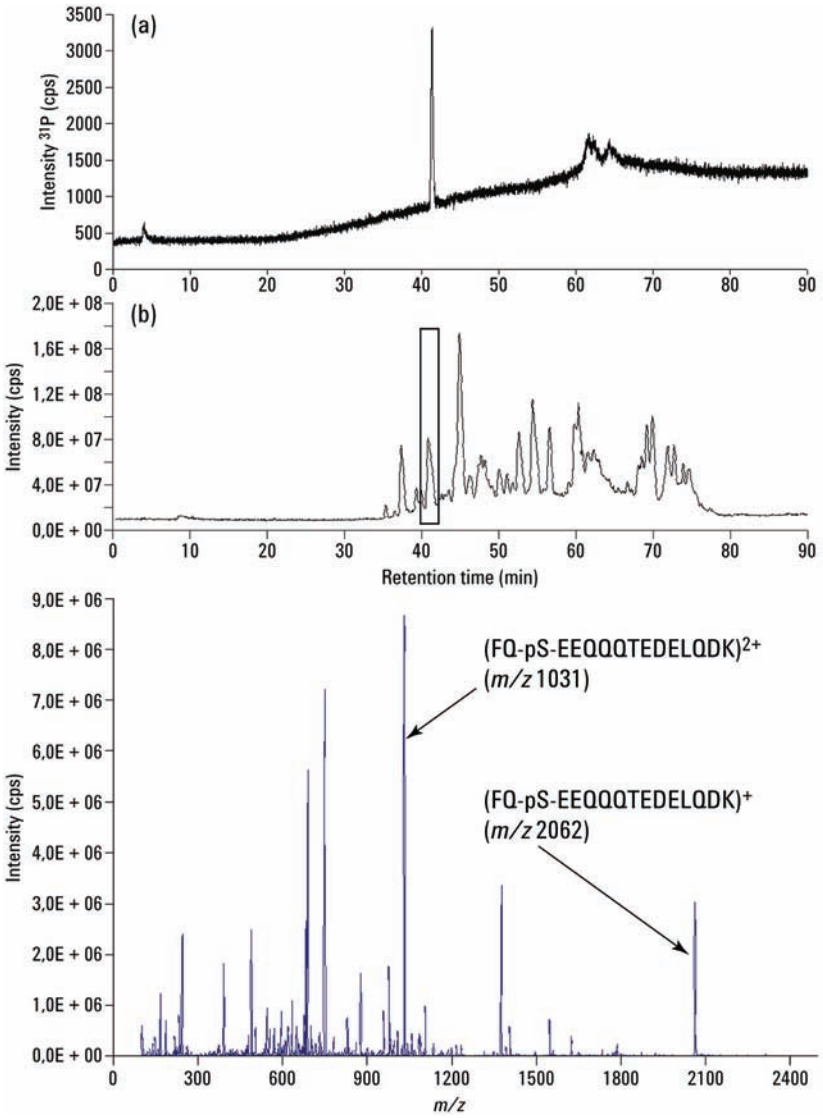

Figure 1. *(a) ^{31}P and ESI-TIC peaks of beta-case in separated by Cap-LC at 4 µL min-1 flow rate. (b) Data deconvolution of the ESI-TIC peak corresponding to the ^{31}P peak in Figure 1a. Reprinted with permission of ABC.*

Conclusions

The successful application of a new, high-efficiency capillary interface for collision cell ICP-MS has provided a sensitive complementary tool for use in connection with existing MS techniques in the field of protein phosphorylation analysis. The setup applied, consisting of a modified capillary electrophoresis nebulizer and a specially designed spray chamber that allows direct nebulization inside the ICP torch, takes into account the requirements when combining a low-flow separation technique with ICP-MS as element-specific detector, namely 100% transport efficiency, good nebulization stability, and minimized dead volumes. Because of the low solvent flow rates used and the high transport efficiency of the nebulizer, CC-ICP-MS has high compatibility with the organic gradient conditions usually used in protein and peptide analysis.

The optimized setup has been successfully applied to the phosphorylation screening of beta-casein, which has been used as a model protein. Additional ESI-MS-MS experiments proved the presence of a phosphorylated peptide under the identified peak.

The micronebulizer-based hyphenation of cap-LC to collision-cell ICP-MS, in general, opens up new application fields that are currently not accessible to element-specific detection techniques because of the use of highly organic mobile phases or the limited sensitivity or chromatographic resolution of conventional "large-bore" techniques. The proposed setup could be a sensitive alternative to already established HPLC techniques, especially for determination of other hetero-element-containing compounds, such as pesticides or pharmaceuticals.

More Information

D. Pröfrock, P. Leonhard, W. Ruck, and A. Prange, "Development and characterization of a new interface for coupling capillary LC with collision-cell ICP-MS and its application for phosphorylation profiling of tryptic protein digests," *Anal Bioanal Chem* (2005) 381:194-204.

Other Speciation Techniques

Other Hyphenated ICP-MS Techniques Used for Speciation

In addition to the more common chromatographic techniques already described, many other possibilities exist and are (or will be) used to separate analyte species prior to elemental analysis by ICP-MS. In fact, any technique that can be used to separate a sample into its constituent components, either in time (as in chromatographic techniques), or in space (as in slab gel electrophoretic techniques), can probably be interfaced to an ICP-MS. Only the simple four requirements listed in the introduction need be met.

When the sample components elute from the fractionation system in a flowing stream of liquid such as in field flow fractionation (FFF), interfacing to the ICP-MS follows the same rules as any chromatographic technique (see General Requirements on page 5). In fact, FFF has shown itself to be a simple, versatile technique for the separation of components ranging from macromolecules to nanoparticles, based on a wide variety of physiochemical properties. Interfacing FFF to ICP-MS is straightforward and is beginning to see increased use, both as the versatility of FFF and the power of ICP-MS as a detector are realized.

Distillation is another category of simple fractionation techniques, commonly used in the chemical and hydrocarbon industries for separating components from a mixture based on differences in boiling point. Various forms include fractional distillation, steam distillation, vacuum distillation, azeotropic distillation, and others. Interfacing a distillation system to an ICP-MS varies with the size and type of system, but typically involves a simple transfer line (with or without flowing liquid makeup) between the outlet of the still and the ICP-MS nebulizer or torch.

Other techniques, where the sample is fractionated in space rather than in time, include solid-phase chromatographic and electrophoretic techniques. Solid-phase chromatographic techniques include paper and thin-layer chromatography, both one- and two-dimensional. Solid-phase electrophoretic techniques include slab gel electrophoresis and are commonly used for the separation of bio macro molecules, including peptides, and proteins, and nucleic acids. In these cases, the separated analytes are immobilized on or in a stationary medium (paper, silica, alumina or polyacrylamide or starch gels) after either one- or two-dimensional separation. Commonly, the "plate" is treated to render the analyte components visible through a staining procedure, allowing the analytes to be manually selected for transport to the ICP-MS. Transfer may involve simply removing the "spot" from the plate, dissolving or desorbing it in the appropriate buffer, and analyzing by conventional ICP-MS. More elegantly, the analyte spot(s) on the plate can be ablated or vaporized using a laser and transported to the ICP-MS as an aerosol in a flowing stream of argon using a technique called laser ablation. Alternatively, the entire plate can be "scanned" by slowly moving the laser across the surface while the ICP-MS measures and records the elemental composition constantly as a function of time. The result is a two-dimensional map of the elemental composition of the plate. In this case, staining is not necessary, since the entire plate is analyzed.

A related technique, while not strictly a separation technique, has been called metal imaging mass spectrometry (MIMS). In this case, the various metal "analytes" are fractionated biologically into various regions of a biological organ, depending on their biological function or state of disease. The organ is frozen and thin sections prepared, which can be analyzed by scanning laser ablation much the same way as a chromatographic or electrophoretic slab. The result is a two-dimensional "picture" of the elemental composition of the organ.

As old separation techniques find new applications and new separation techniques continue to emerge, interfacing them to ICP-MS as the "universal elemental detector" will continue to play an important role in the science of speciation analysis.

Analysis of Copper- and Zinc-Containing Superoxide Dismutase by Isoelectric Focusing Gel Electrophoresis Laser Ablation ICP-MS

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Introduction

Metals ions play an important role in biological systems. They can act as a catalytic center in numerous biochemical reactions and can be involved in enzyme regulation and gene expression. The characterization of metal-protein complexes is a challenging task that requires the development of analytical methodology capable of their separation and detection prior to identification by molecular mass spectrometry. For this purpose, the most powerful separation technique used in proteomics, that is, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) cannot be applied because of the noncovalent binding between metals and proteins. The objective can be achieved by combining a non-denaturing (to avoid metal-protein dissociation and metal loss) gel electrophoresis and a highly sensitive, quantitative and isotope-specific detection technique. ICP-MS using laser ablation micro-local sampling was investigated as a suitable detection technique for mapping trace elements in gel electrophoresis.

Sample

The Cu-Zn superoxide dismutase (SOD) was purchased from Sigma (Sigma-Aldrich, Saint Quentin Fallavier, France) and was dissolved in Milli-Q water. The SOD isoforms were separated using a nondenaturing separation protocol on a BioRad isoelectric focusing system. After separation, the proteins were photochemically stained according to the enzyme activity.

Instrumentation

A nanosecond laser CETAC LSX 100 (CETAC, Omaha, NE) was interfaced to an Agilent 7500cs ICP-MS (operating conditions are given in Table 1). Prior to introduction into the plasma, the aerosol produced via laser ablation was mixed with the nebulizer-induced aerosol (2% HNO_3) in a two-inlet torch. 5% O_2 was added into the spray chamber in order to prevent accumulation of carbon on the interface cones. Signal acquisition was performed in transient signal mode.

Table 1. *Experimental parameters for LA-ICP-MS.*

Laser	**Cetac LSX 100**
Wavelength	266 nm
Laser energy	0.7–0.8 mJ
Frequency	20 Hz
Pulses duration	8 ns
Scan speed	60 µm s^{-1}
Defocus	0
ICP-MS System	**Agilent 7500cs**
Nebulizer	MicroMist
Nebulizer gas flow rate	0.48 mL min^{-1}
Carrier gas flow rate (ablation cell)	0.5 mL min^{-1}
Optional gas	5% O_2 (spray chamber)
Cones	Pt
Torch	1 mm id
Isotoped monitored	^{63}Cu, ^{65}Cu, $^{6}4Cu$, ^{66}Cu
Dwell time	150 ms

Results

Staining the native isoelectric focusing (IEF) gel strip revealed the separation of different isoforms of the enzyme (Figure 1). Scanning the IEF strip using LA-ICP-MS identified Cu and Zn in one of these isoforms.

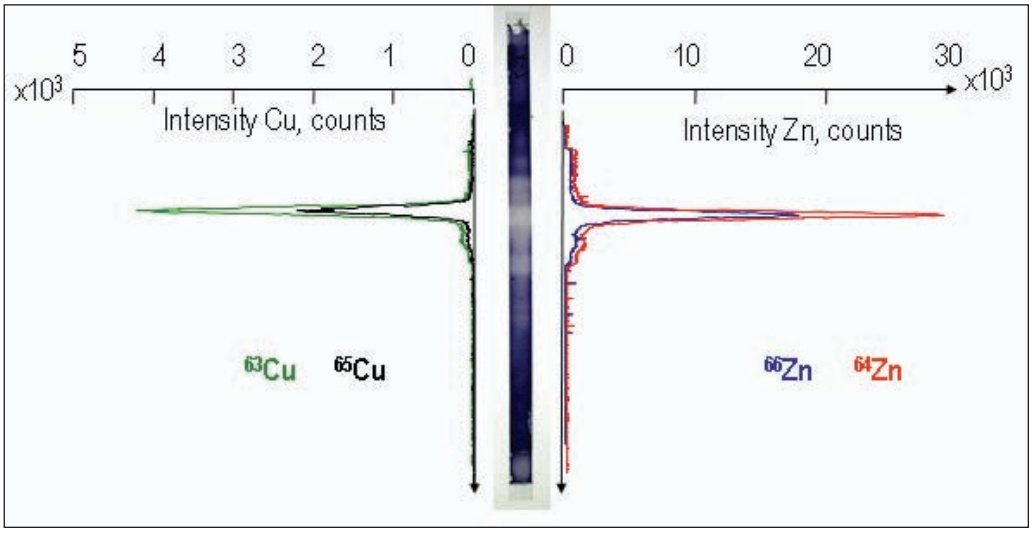

Figure 1. *Imaging of Cu and Zn in SOD separated by IEF and analyzed by LA-ICP-MS.*

Conclusions

Laser ablation ICP-MS is an attractive technique for the detection of metals present in gel electrophoresis owing to its sensitivity, speed, robustness, and multielement detection capability. It allows direct screening of trace elements in polyacrylamide gels without the need for a reaction/derivatization step. The signal obtained is directly proportional to the quantity of the element present in the gel.

Reference

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