

**Final report for Arizona Water Institute Grant AWI-07:  
Collaborative Approach to Analyzing Emerging Contaminants  
in Water**

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**Synopsis:** Researchers at the University of Arizona (UA), Arizona State University (ASU), Northern Arizona University (NAU), and the Arizona Department of Health Services (ADHS) were funded in 2006 by AWI to establish a collaboration dedicated to water chemical analysis. This work was intended to provide a keystone for current and future programmatic needs in water quality. The principal focus of this collaboration was on separation, detection and quantification of organic and inorganic contaminants, particularly trace chemicals of emerging concern. One goal of the work was to develop our capabilities to measure the composition of aqueous samples in detail, including the form (speciation) and concentration of dissolved and colloidal constituents, even those occurring at very low concentrations. The need for such understanding is evident in a growing, western state where complex physical, chemical and social issues all surround water use and re-use. Partnering of the universities with ADHS in this effort should ideally continue and have lasting statewide impacts. The collaboration to date has resulted in the results described herein.

The AWI funds in this project were divided evenly between UA and ASU to provide partial support for two analytical chemistry staff positions (Marisa Masles and Mary Kay Amistadi). These individuals bridged the gap between university personnel and ADHS staff, and developed organic and inorganic methods, respectively. Analyst Masles of ASU worked closely with ADHS scientist Matt Rosenow to develop the described methods on analysis of trace organic contaminants (endocrine disruptors, pharmaceuticals and personal care products – EDC/PPCPs) using liquid chromatography tandem mass spectrometry (LCMS/MS). Analyst Mary Kay Amistadi of UA focused on the development of metal(loid) speciation analyses using high performance liquid chromatography (HPLC) coupled to inductively coupled plasma mass spectrometry (ICP-MS). Importantly, this 2006 AWI grant seeded new funding from the National Science Foundation's Major Research Instrumentation (NSF-MRI) program. The 2007 NSF funding to UA has funded the purchase of LC-MS/MS, GC-MS/MS and HPLC-ICP-MS capabilities that will significantly improve universities-wide analytical capabilities, and this will foster a meaningful continuation of collaboration with ADHS on technical approaches.

This report provides details on the development of the analytical methods for (i) organic and (ii) inorganic contaminants in water. Hence, the principal objectives of the proposed work have been met. Specifically:

- Analytical methods for a wide range of endocrine disrupting compounds, pharmaceuticals and personal care products have been tested so that these compounds can be quantified at environmentally relevant concentrations;
- The trained technician remains at ASU and is available to conduct these studies, and the staff at ADHS trust the technician to work independently at their facility. They continue to collaborate to analyze samples and expand the types of chemicals that can be detected.
- ASU purchased an automated solid-phase extraction system that allows concentration of samples at the university, followed by analysis at ADHS.

- A strong collaborative interaction between ADHS and state universities has been established, and ADHS has been part of subsequent proposals applying these analytical methods.

The AWI funding supported Marisa Masles (ASU) and Mary Kay Amistadi (UA) in their focus on methods development for EDC/PPCP compounds and metal(loid) contaminants, respectively. The results of this work is described below.

## **1. Detection and Measurement of EDC/PPCP Target Compounds**

The universities worked with a small working group of faculty, ADHS staff and state representatives to develop a target list of compounds for analysis. Standards were obtained for these compounds and LC/MS/MS methods were developed. Table 1 summarizes the organic compounds, their classification or use in society and related LC/MS/MS conditions. Several internal standards from NIST were also used to confirm the results and provide data on recovery and accuracy that is needed for future proposals.

The LC/MS/MS instrument is operated in three modes (APCI Positive, ESI Negative, and ESI Positive). These were selected to maximize separation and detection of the EDC/PPCP compounds. Specific operating conditions are described in Table 2. The instrument used comprised an Agilent 1100 series HPLC with a G1379A degasser, a G1376A capillary pump, and a G1367A WPALS auto sampler. The column used was a Phenomenex Synergi Max-RP C12, 250mm X 4.6mm, 4 micron. The mass spectrometer was an Applied Biosystems API 4000 Triple Quadrupole. The specific liquid chromatography parameters and method is shown in Figures 1 and 2.

A standard operating protocol was prepared for use of the automated extraction system that ASU purchased with internal funds, separate from AWI funds (Appendix A).

### **Analysis**

Calibration curves are developed at part-per-billion levels (ppb, ug/L). “Real” samples are concentrated 1000x by solid phase extraction into methanol. Thus the reporting range by the developed methods are in the 2 to 10 parts-per-trillion-levels (ppt, ng/L) in “real” water samples. Representative calibration curves for several hormones are presented in Figure 3. The regression coefficients are generally >0.99 and indicate excellent analytical capabilities. The average accuracy was 99.97% with a standard deviation of 9.9% for 42 hormone samples. Calibration curves for other compounds were equal to the quality of the hormones.

Table 1 – List of EDC/PPCPs and LC/MS/MS operating conditions

Ionization Source	Compound	Class/Use	Quantifier Ion	Qualifier Ion(s)
			Q1/Q3	Q1/Q3
<b>APCI Positive</b>	Estradiol	Steroid/Estrogen	255.3/159.2	255.30/133.1
	Ethinyl Estradiol	Steroid/Synthetic Estrogen	279.2/133.0	279.2/159.1
	Progesterone	Steroid/Estrogen	315.3/97.3	315.3/109.0, 315.3/109.2
	Testosterone	Steroid/Androgen	289.3/97.3	289.3/109.2, 289.3/123.3
<b>ESI Negative</b>	Cotinine	Personal Care Product/Nicotine metabolite	177.2/80.2	177.2/98.3
	Diclofenac	Pharmaceutical/Anti-arthritis	294.3/250.0	294.3/214.0
	Dilantin (Phenytoin sodium)	Pharmaceutical/Anti-convulsant	251.4/102.0	251.4/180.0
	Ibuprofen	Pharmaceutical/Analgesic	205.1/159.0	205.1/161.0
	Naproxen	Pharmaceutical/Analgesic	229.0/169.0	229.0/185.1, 229.0/140.9
	Sucralose	Personal Care Product/Sweetener	395.3/359.0	397.2/361.1
	Tetrabromobisphenol A	Personal Care Product/Flame retardant	442.9/239.0	442.9/102.9
	Triclosan (Ingasan)	Personal Care Product/Antibiotic	287.2/34.9	287.2/241.1
	Warfarin	Pharmaceutical/Anti-coagulant	307.3/161.0	307.3/250.0, 307.3/117.0
	<b>ESI Positive</b>	Acetaminophen	Pharmaceutical/Analgesic	152.1/110.2
Atrazine		Pesticide	216.1/174.2	216.1/104.2
Caffeine		Personal Care Product/Stimulant	195.2/138.3	195.2/110.0
Carbamazepine		Pharmaceutical/Anti-seizure	237.3/194.0	237.3/179.3
DEET		Personal Care Product/Insect Repellent	192.1/119.3	192.1/91.3
Diazepam		Pharmaceutical/Muscle relaxant	285.2/193.3	285.2/154.0, 285.2/222.0
Diuron		Pesticide	233.3/72.3	233.3/159.9
Erythromycin-H2O		Pharmaceutical/Antibiotic	716.5/158.3	716.5/558.6
Fluoxetine		Pharmaceutical/Anti-depressant	310.3/44.2	310.3/148.3
Hydrocodone		Pharmaceutical/Analgesic	300.3/199.2	300.3/171.3, 300.3/128.3
Imazamox		Pesticide	306.4/261.2	306.4/245.2
Imazthapyr		Pesticide	290.3/245.2	290.3/177.2
Meprobamate		Pharmaceutical/Anti-anxiety	219.2/158.3	219.2/97.3
Oxybenzone		Personal Care Product/Sunscreen	229.3/151.2	229.3/105.1
Pentoxifylline		Pharmaceutical/Blood thinner	279.4/138.2	279.4/99.2
Primidone		Pharmaceutical/Anti-convulsant	219.2/162.1	219.2/91.3
Prometryne		Pesticide	242.2/157.9	242.2/200.3
Sulfamethoxazole		Pharmaceutical/Antibiotic	254.3/156.2	254.3/108.1
Trimethoprim		Pharmaceutical/Antibiotic	291.3/123.3	291.3/230.4, 291.3/261.2
<b>INST/Surrogate</b>		Acetaminophen-D4		156.2/114.1
	Cotinine-D3		180.3/80.10	180.3/101.2
	Diazepam-D5		290.3/198.4	290.3/154.0, 290.3/227.4
	Estradiol-D5		260.3/161.10	260.3/135.10
	Fluoxetine-D6		316.2/44.2	316.2/154.2
	Hydrocodone-D6		306.3/202.3	306.3/174.3, 306.3/128.3

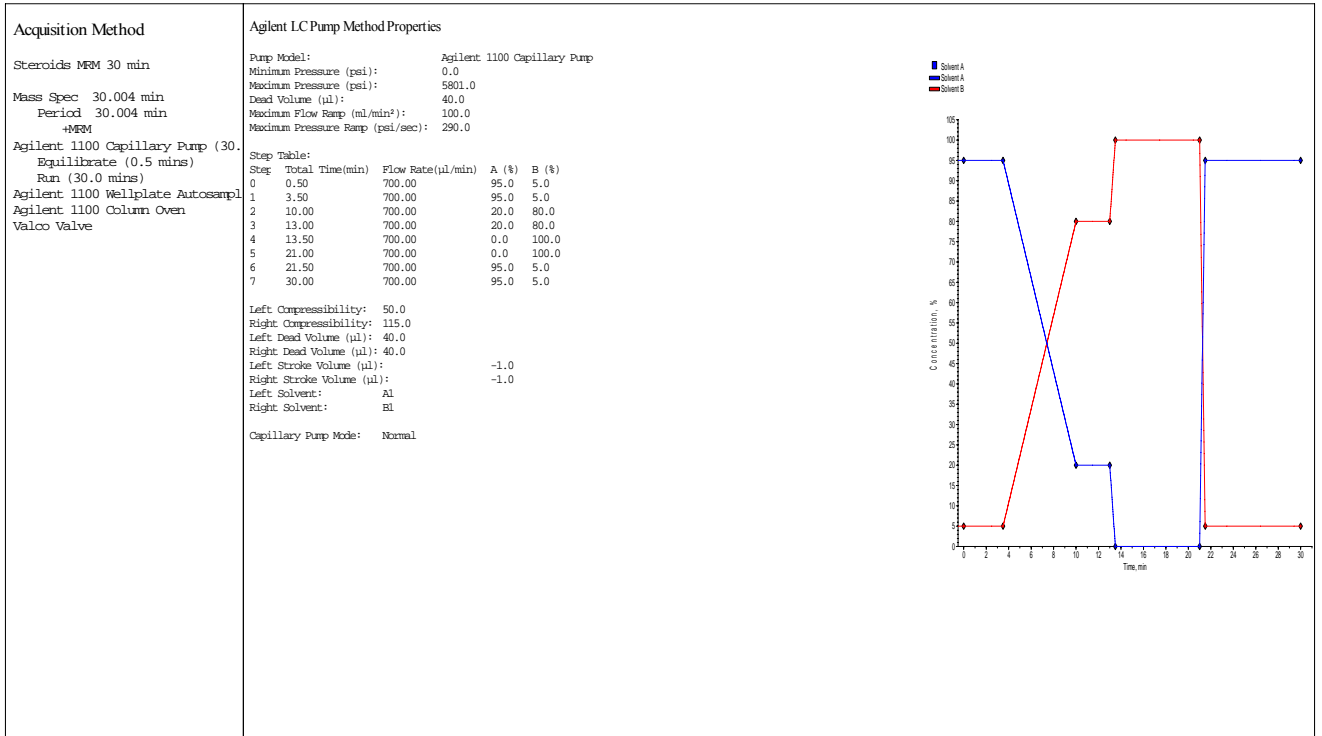
Table 2 – LC/MS/MS operating conditions

<b>APCI Source Parameter Table</b>	
Collision Gas	6
Curtain Gas	14
Ion Source Gas	55
Nebulizer Current	3
Interface Heater	On
Temperature	550

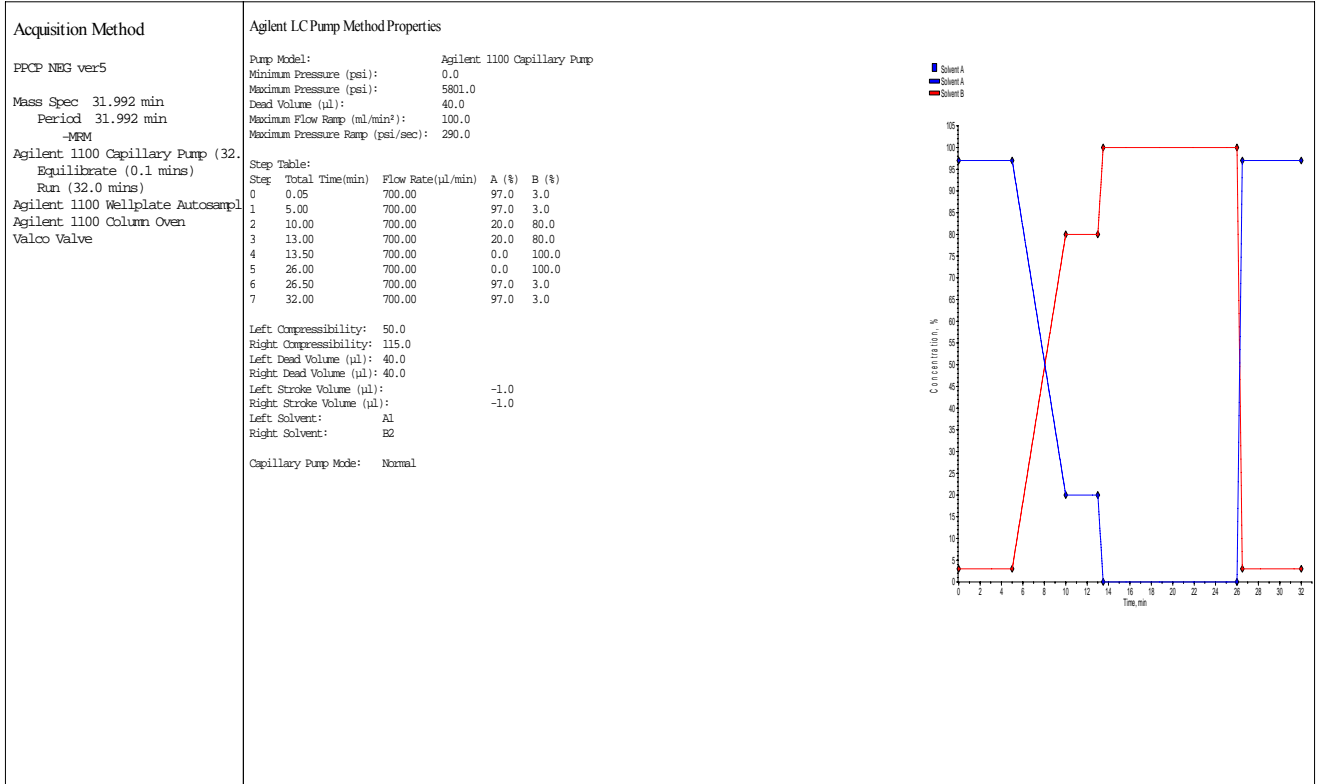
<b>ESI Negative Source Parameter Table</b>	
Collision Gas	6
Curtain Gas	15
Ion Source Gas 1	55
Ion Source Gas 2	60
Ion Spray Voltage	-1500
Interface Heater	On
Temperature	450

<b>ESI Positive Source Parameter Table</b>	
Collision Gas	6
Curtain Gas	15
Ion Source Gas 1	60
Ion Source Gas 2	55
Ion Spray Voltage	1500
Interface Heater	On
Temperature	450

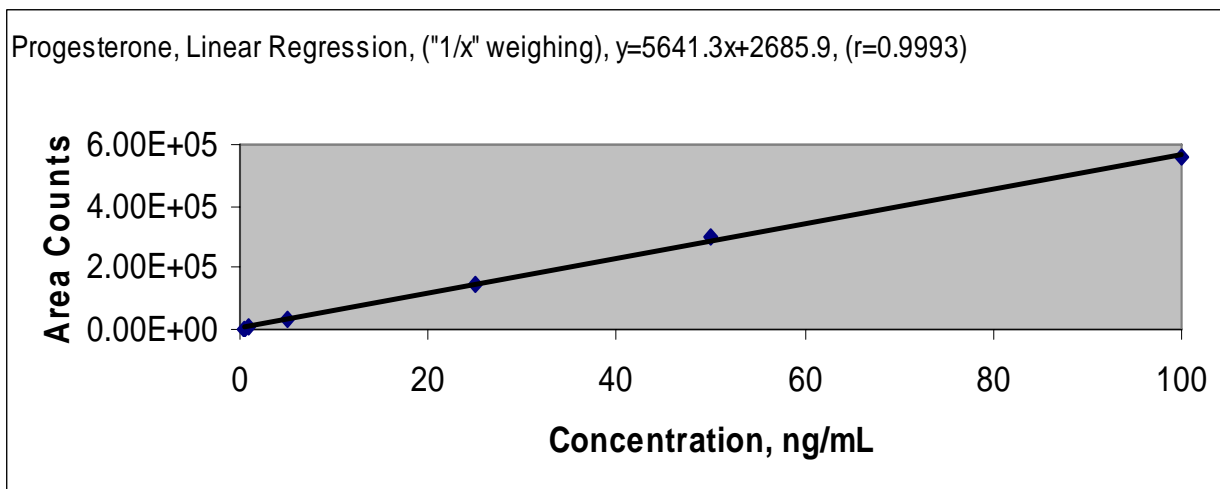
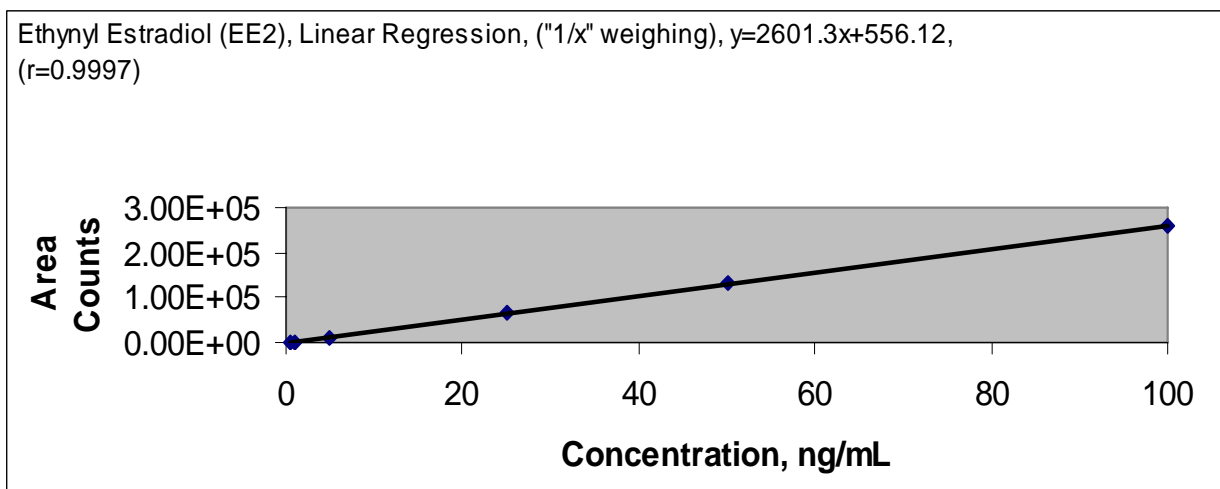
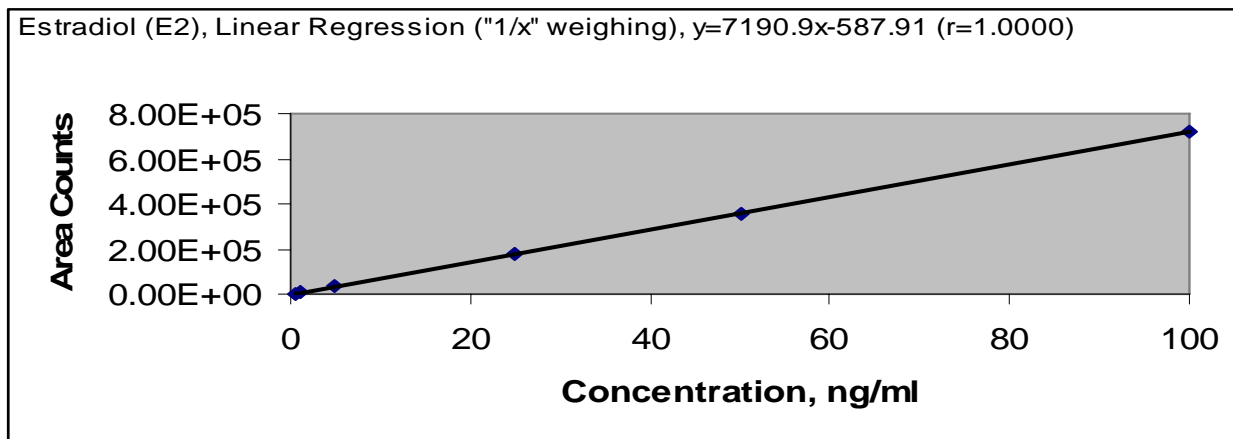
**Figure 1** Liquid chromatography operating conditions

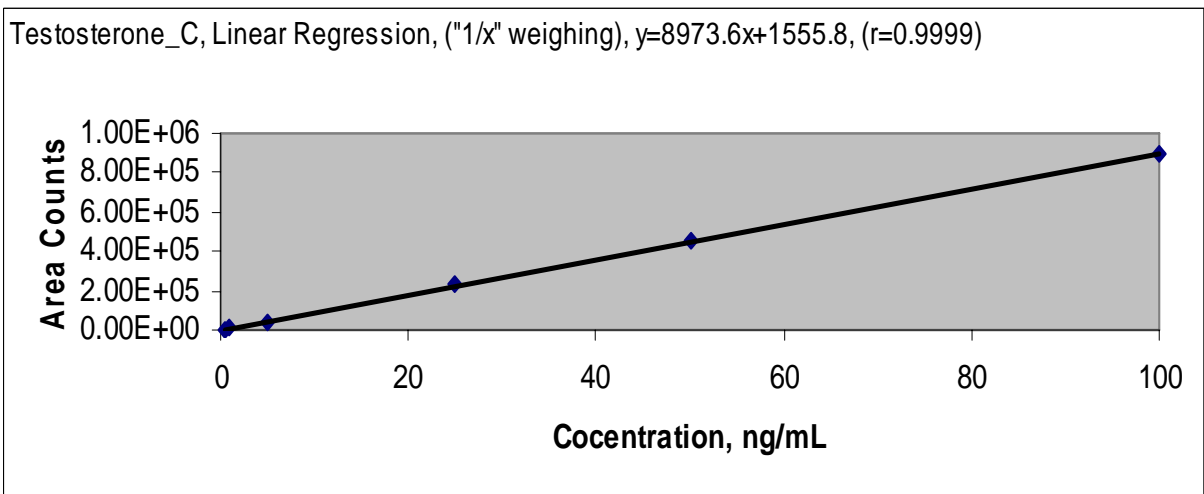
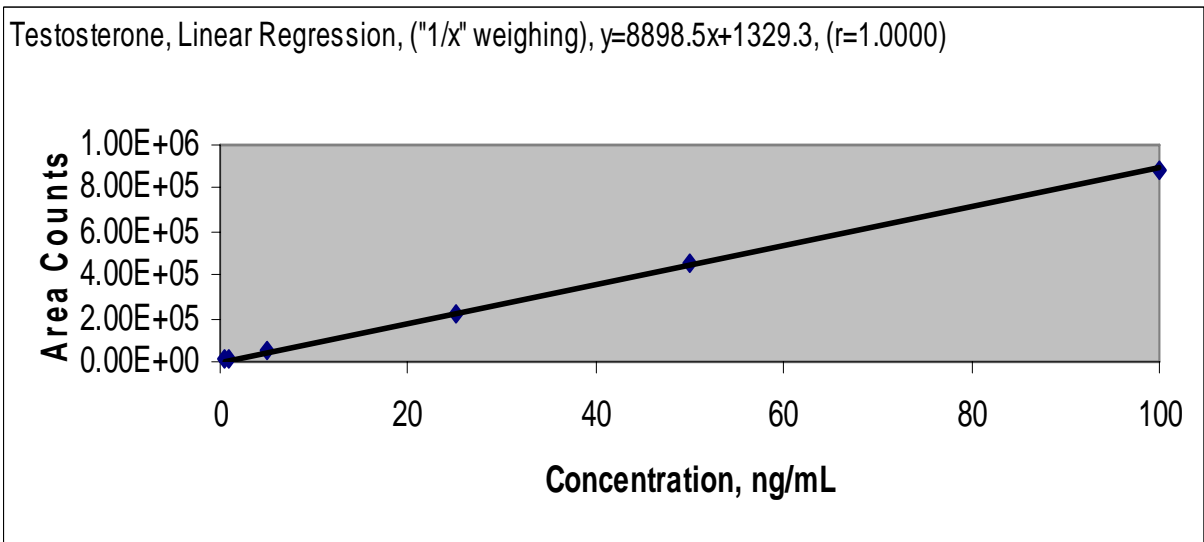
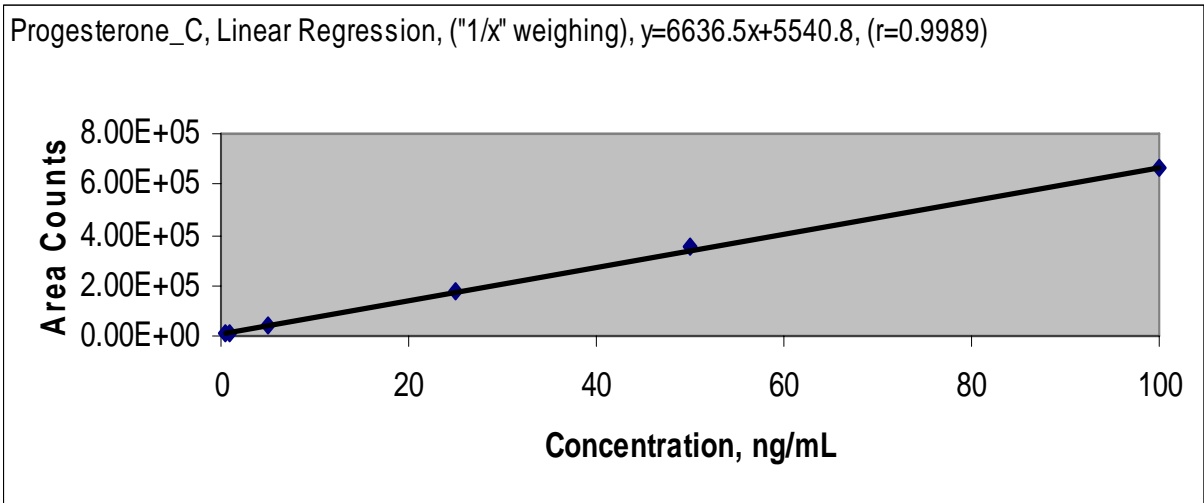


**Figure 2** Liquid chromatography operating conditions

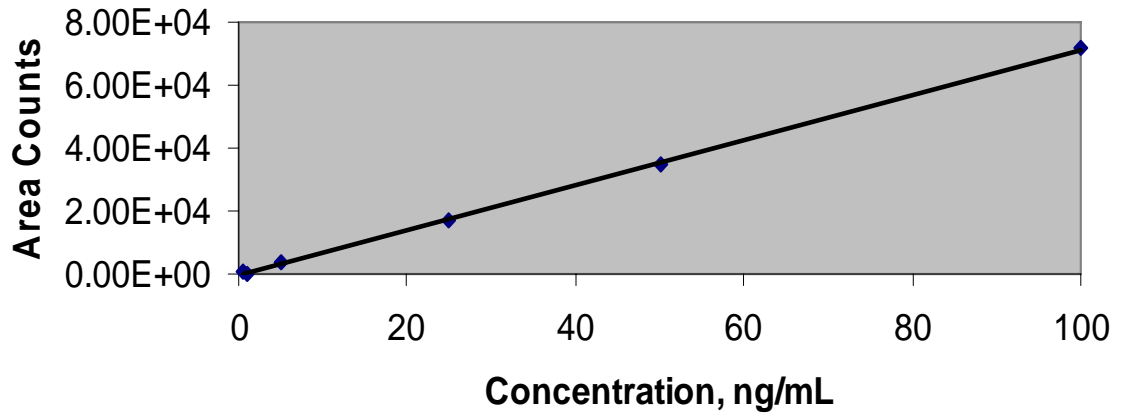


**Figure 3** Calibration curves for hormones





Testosterone\_C2, Linear Regression, ("1/x" weighing),  $y=716.94x-252.12$ , ( $r=0.9997$ )



## **Aqueous Speciation of Trace Elements with HPLC- and HPSEC-ICP-MS:**

Trace elements in water, soil and sediment occur across a range of species that differ in their bioavailability and toxicity. Whereas traditional methods for measuring metal(loid)s in environmental samples generally yield total concentrations, the assessment of element distribution across different species requires analyte pre-separation prior to detection. Coupling detection to on-line pre-separation represents an efficient approach to element speciation of aqueous samples. Methods for doing this are currently under development in the field of environmental analytical chemistry. Development and testing of these analyses for application to a wide range of trace contaminants in water is therefore an important activity within the broader science of water sustainability.

The principal focus of the University of Arizona portion of this AWI grant involved the development of methods for coupling on-line high performance liquid chromatography with inductively-coupled plasma mass spectrometry (HPLC-ICP-MS) for quantitative speciation analysis of inorganic micro-pollutants (principally metals and metalloids). Funding from the TRIF Venture Capital Fund allowed us to acquire a Perkin Elmer Series 200 HPLC to upgrade an existing Perkin Elmer ELAN DRC-II ICP-MS for use in the project. Hardware installation occurred in late August 2007. Preliminary training (ca. 4 hours) was provided at that time, followed by a 3-day training workshop held at the Perkin Elmer Support Center in San Jose, California in mid-October 2007.

Prior to acquisition and installation of the hardware, a review of the literature for existing methods was compiled, and it is attached as an appendix to this report. The review provided an essential starting point for methods development trials. Species of environmental interest that have been effectively separated prior to ICP-MS detection include the redox pairs Cr(III)/(VI), Se(IV)/(VI), Sb(III)/(V), Br(I-)/ Br(V) and As species (III), (V), monomethyl arsenate (MMA), dimethyl arsenate (DMA), and arsenobetaine (AsB). These separations were conducted using column affinity methods. High performance size exclusion chromatography (HPSEC) has also been coupled with ICP-MS to investigate the association of metals with dissolved organic matter (DOM) and with protein fractions. This latter area of research is particularly important to ongoing efforts at UA to focus on the influence of wastewater-derived organic matter effects on metal speciation.

### **Speciation Results:**

We began the speciation work for this project with methods development for As, Se, Cr and Br, and followed that with tests pertaining to speciation by HPSEC-ICP-MS. The resulting methods are discussed individually below:

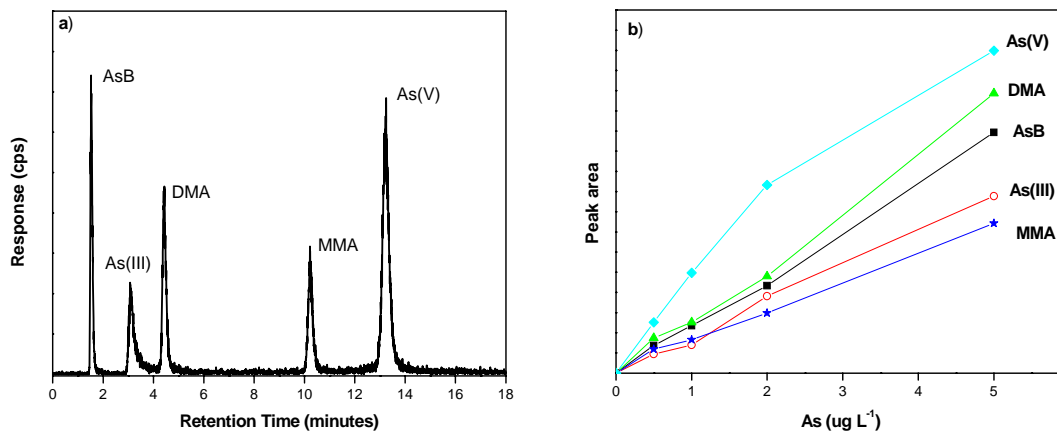
*Arsenic speciation:* The first protocol developed was the gradient separation of the five As species listed above. In the initial trials, several of the elution gradients from the literature were unsuccessful. We suspect the noise to be due to contamination in one or more of the reagents used to prepare the eluents. Since only one isotope of As exists, the potential for interference due to the presence of chloride is significant. The purity

certificate provided had not included a specific analysis for chloride, listing the Cl- concentration only as “less than 1%”.

This concern was addressed in two ways:

- 1) The Elan DRC II is configured with a “dynamic reaction cell” which can be purged with an alternate reaction gas (molecular oxygen in this case) to reduce the interferences caused by the presence argon chlorides. The reaction with O<sub>2</sub> results in the formation of As oxides that can then be detected at m/z 91 (as <sup>75</sup>As<sup>16</sup>O<sup>+</sup>). The flow rate for the cell gas was 0.61mL/min.
- 2) The mobile phase was changed to a gradient using ammonium carbonate from 10 to 50mM at pH 9

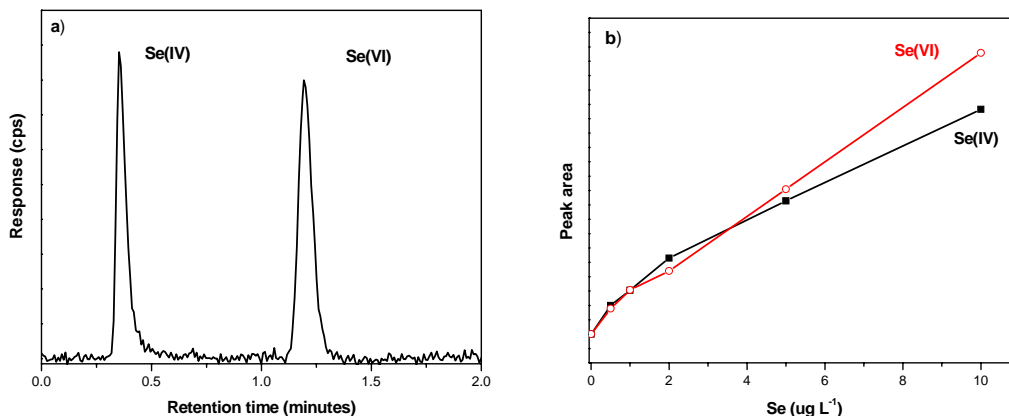
The resulting baseline was much improved, ranging from 0 to 30 cps, with good separation of the 5 species in less than 16 minutes (Figure 4a). The detection limits achieved ranged from 0.89 µg/L for DMA to 1.45 µg/L for As<sup>3+</sup> using a 50 µL injection. A larger injection volume may improve sensitivity. A typical calibration curve for these five species is shown in Figure 4b.



**Figure 4.** Arsenic speciation method results. a) Separation of five arsenic species at 2 ppb. Response measured as AsO at m/z 91 in DRC mode using oxygen as the reaction cell gas. b) Calibration curve from 0.5 to 5 ppb.

Column	Hamilton PRP-X100 column (5 micron; 150mm x 4.6mm)
Mobile phase	Ammonium carbonate at pH 9.0; 10mM to 50mM over 18 minutes
Flow rate	1 mL/min
Column oven temp	35°C
Injection volume	50µL

**Selenium speciation:** The isocratic separation of Se(IV) and Se(VI) was achieved with a C8 column in less than two minutes (Figure 5a). Selenium was monitored at  $^{78}\text{Se}$  using  $\text{NH}_3$  as the reaction gas at a flow rate of 0.25 mL/min in the DRC cell. The detection limits achieved were 1.15  $\mu\text{g/L}$  for  $\text{Se}^{4+}$  and 0.74  $\mu\text{g/L}$  for  $\text{Se}^{6+}$



**Figure 5.** Selenium speciation method results: a) Separation of two selenium species at 2 ppb concentration. Response measured as  $m/z$  78 in DRC mode using  $\text{NH}_3$  as the reaction cell gas at 0.25mL/min. b) Calibration curve from 0.5 to 10 ppb.

Column	Brownlee PECO C8 (3 $\mu\text{m}$ ; 33mm x 4.6mm)
Mobile phase	0.1mM tetrabutylammonium hydroxide (TBAOH), 0.15mM ammonium acetate and 0.15mM EDTA at pH 7.
Flow rate	1.5 mL/min
Column oven temp	35°C
Injection volume	50 $\mu\text{L}$

**Bromine speciation:** It has been found that the use of ozone in water treatment promotes the conversion of naturally occurring bromide ( $\text{Br}^-$ ) into bromate ( $\text{BrO}_3^-$ ), a carcinogen. An isocratic method for the separation of bromate/ bromide species was developed using the anion exchange column Hamilton PRP-X100. This method was found to separate the two species in less than 8 minutes (Figure 6a).

Standards were initially prepared in the mobile phase to range in concentration from 5 to 100  $\mu\text{g L}^{-1}$ . Prior to the speciation run, the mobile phase was analyzed in standard mode at mass 79 for comparison to a blank solution of 1% nitric acid. The resulting signal did not show a significant elevation of background compared to the nitric acid blank (304 vs 300 counts per second). However, during the speciation analysis, the signal-to-noise level was such that the response at 5  $\mu\text{g L}^{-1}$  was not reproducible. Therefore, the calibration curves shown for the bromate and bromide species (Figure 6b) range from 10 to 100  $\mu\text{g L}^{-1}$ . The detection limits for this method were 7.19 and 13.43 $\mu\text{g L}^{-1}$  for bromate and bromide respectively.

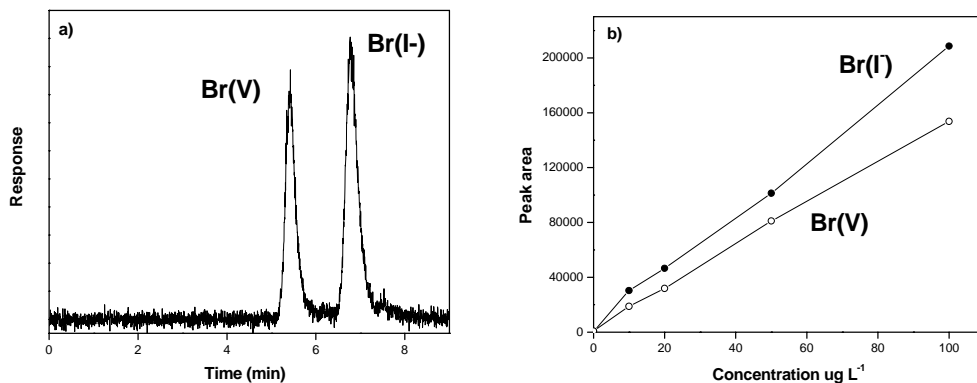


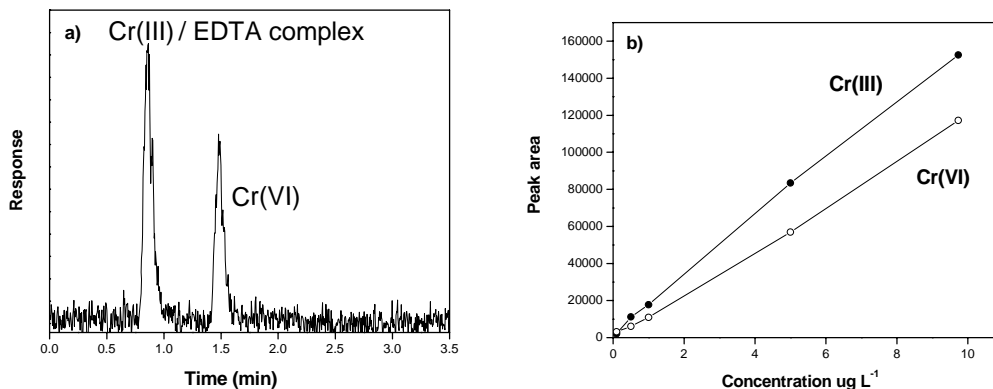
Figure 6. Bromide speciation on Hamilton PRP-X100 column. Response measured at  $m/z$  79 in standard mode. a) Separation of bromate and bromide species in water matrix at  $20\mu\text{g L}^{-1}$ ; b) Calibration curve from 10 to 100 ppb.

Column	Hamilton PRP-X100 column (5 micron; 150mm x 4.6mm)
Mobile phase	18mM nitric acid with 34 mM ammonium hydroxide at pH 4
Flow rate	1.5mL/min
Column oven temp	35°C
Injection volume	100 $\mu\text{L}$

Though the linear response appears satisfactory, it will be necessary to further investigate the cause on the background noise to achieve an improved signal-to-noise response in order to increase the sensitivity of this method.

Chromium speciation: The isocratic separation of chromium species Cr(III) and Cr(VI) was performed on a C8 cartridge column using an ion-pairing technique. It was found that the pH of the eluent solution is critical to the success of the separation. This protocol allowed for separation of the two species in less than 3 minutes (Figure 7a).

The mass-to-charge signal response was measured at mass 52, using DRC mode of the instrument. The use of the alternate gas ( $\text{NH}_3$ ) reduces interferences such as those posed by argon-carbon ( $40+12$ ) at this  $m/z$ , allowing the user to collect data at an isotope of higher abundance. A calibration curve ranging from  $0.1$  to  $10\mu\text{g L}^{-1}$  is shown in Figure 4b. A test solution containing both species at  $1\mu\text{g L}^{-1}$  in a water matrix was analyzed, and the detection limits calculated from 10 replicated injections were  $0.35\mu\text{g L}^{-1}$  for Cr(III) and  $0.36\mu\text{g L}^{-1}$  for Cr(VI).



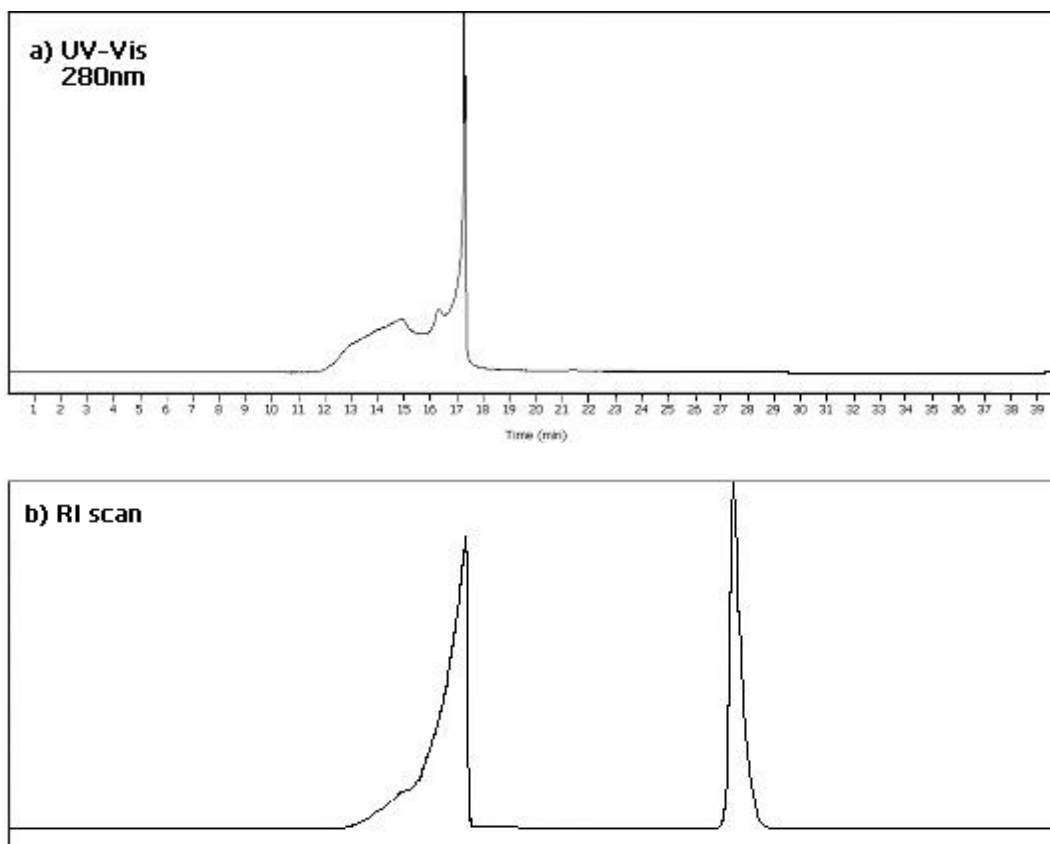
**Figure 7.** Chromium speciation method results: Response was measured as m/z 52 in DRC mode, using ammonia as the alternate gas at 1.5mL/min. a) Separation of trivalent and hexavalent species in water matrix at  $1\text{ug L}^{-1}$ ; b) Calibration curve from 0.1 to  $10\text{ug L}^{-1}$ .

Column	Brownlee PECO C8 (3 $\mu\text{m}$ ; 33mm x 4.6mm)
Mobile phase	1mM tetrabutylammonium hydroxide (TBAOH) and 0.6mM EDTA plus 2% methanol at pH 6.9
Flow rate	1.5 mL/min
Column oven temp	35°C
Injection volume	100 $\mu\text{L}$

**HPSEC-ICP-MS:** The method currently under development uses size exclusion chromatography (SEC) coupled with ICP-MS to investigate the association of various metals with dissolved organic matter (DOM) and with protein or other biomolecular fractions. This latter area of research is particularly important to ongoing efforts at UA to focus on the influence of Central Arizona Project (CAP) and wastewater-derived organic matter effects on metal complexation. Both CAP water and treated wastewater effluents contain DOM at concentrations elevated above native groundwater in the Tucson basin. The goal is to allow us to quantify aqueous phase metals into various DOM-bound and free pools.

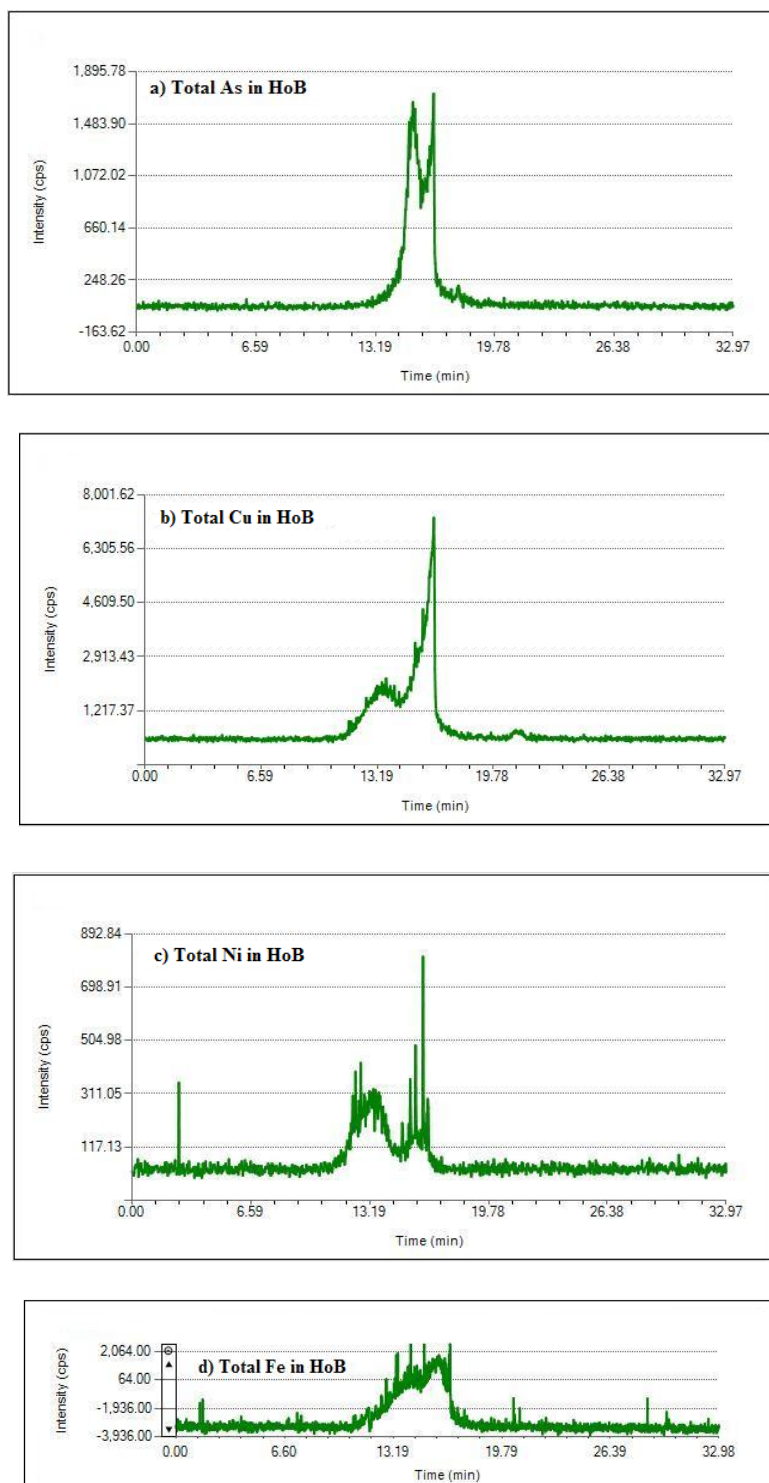
The original SEC method was developed for the Waters HPLC system in our lab, using dissolved organic matter extracted from wastewater solids. The method uses two SEC columns in series: the MCX 100,000 angstrom followed by the 1000 angstrom column (Polymer Standard Service, Warwick, RI). Previous work showed that this configuration could provide a linear range of molecular weights from 208 to 149,000 Daltons using polystyrene sulfonate standards (PSS).

This column set was transferred to the Perkin Elmer HPLC system. Using a mobile phase of nanopure water, the UV-Vis (280nm) and RI detectors show good separation of peaks, tentatively identified as the protein (early peak) and carbohydrate (the later peak) components of a DOM sample (Figure 8). These assignments are based on the UV-absorbing properties of proteins and the non-chromophoric behavior of polysaccharides.



**Figure 8.** HPSEC separation on Perkin Elmer HPLC showing results of sequential scan on two detectors. Sample was HoB (hydrophobic base) fraction from wastewater DOM. a) UV-Vis scan at 280nm; b) RI scan.

The next phase of this work (currently underway) requires synchronizing the LC output with that of the ICP-MS, and performing calibrations and sample analyses for the desired analytes. Preliminary results for several analytes are shown below in Figure 9. Since the data observed with the UV and RI detectors produced an initial peak between 12 and 17 minutes for the UV adsorbing molecules, these results suggest that these metals are more closely affiliated with these molecules (proteins). No peaks were detected in the ICP-MS response in the range of 27 to 29 minutes where the RI had shown evidence of polysaccharide fractions.



**Figure 9.** HPSEC-ICP-MS separation on Perkin Elmer ELAN using HoB (hydrophobic base) fraction from wastewater DOM. a) As measured as AsO at m/z 91 in DRC mode with O<sub>2</sub> as cell gas; b) Cu measured at m/z 63; c) Ni measured at m/z 60; d) Fe measured at m/z 56 in DRC mode (NH<sub>3</sub> as cell gas)

Column	MCX GPC columns 100,000 and 1000angstrom 8x300 in series
Mobile phase	Nanopure water
Flow rate	1.0 mL/min
Column oven temp	25°C
Injection volume	50µL

*Future work:* The same C8 column used above for Cr and Se separations has been used by other researchers for other LC-ICP-MS methods such as the separation of As(III) and As(V). When only these two species are desired, this short column can provide good separation with a mobile phase of 1mM TBAH and 0.5mM EDTA at pH 7. Initial trials on this method showed good separation in less than 2 minutes (data not shown), though detection limits need improvement.

The separation of antimony species Sb(III)/(V) will require the purchase of a different column set (TSKgel IC-AP). This protocol could be developed in the future if there is interest from other users.

## Appendix A

### Protocol for Solid Phase Extraction using Caliper Life Sciences AutoTrace SPE Workstation

1. Turn on AutoTrace Workstation.
2. Turn on nitrogen. Adjust workstation gas pressure, if necessary, to 6-8psi. The gas pressure control knob can be found in the panel on the left side of the instrument.
3. Check solvent reservoirs. Only use HPLC grade solvents. The solvent lines are designated as follows:  
  
Solvent #1 MTBE  
#2 Methanol  
#3 MTBE/Methanol (90/10)%  
#5 Water
4. Check/empty waste containers. One container is designated for aqueous waste, the other for solvents.
5. Load elution rack with empty, clean vials. Fill clean sample containers with 1000mls of water sample. (Samples should be GF/F filtered prior to extraction). Load Waters Oasis HLB SPE cartridges, pressing lever down until you hear a click and a green light comes on. Note: from 1 to 6 samples can be run at a time.
6. Open AutoTrace SPE workstation software by double clicking icon on desktop. Under Login enter "admin" for User ID and "admin" for Password.
7. Under Serial-Op select "Utilities" and "Purge Solvents". Click "Ok" when the "code=8192" box appears. This code indicates that the method has been successfully downloaded to the AutoTrace Workstation. Press continue on the keypad to start the method. (Solvents should be purged at the beginning of the day before the first run or whenever solvents are changed.)
8. Under Methods, load "Clean Sample Path". Double click on method to open it. Click on Edit to enter number of samples and save method. Place sample lines into a beaker of nanopure water. Select the method under Serial-Op. (If the method editor window is open, the current method is downloaded to the AutoTrace.) Click "Ok" when "code=8192" appears and press continue. (Use "Clean Sample Path" between sample batches to prevent contamination.)
9. Under Methods, double click "Steroid/PPCP" to open method. Click on "Edit" to make changes to method. Enter number of samples and check to see that method steps are correct. Under "Parameters" check flow rates. Save method when done.

Extraction steps for the “Steroid/PPCP” method are as follows:

1. Process “x” samples using the following method steps:
2. Condition column w/5.0 ml Solvent 1 into Solvent waste
3. Condition column w/5.0 ml Solvent 2 into Solvent waste
4. Condition column w/5.0 ml Solvent 5 into Aqueous waste
5. Load 1000ml of sample onto column
6. Rinse column w/5.0 ml of Solvent 5 into Aqueous waste
7. Dry column with gas for 40 min
8. Soak and Collect 2.5 ml fraction using Solvent 3
9. Collect 2.5 ml fraction into sample tube using Solvent 3
10. Soak and Collect 2.5 ml fraction using Solvent 2
11. Collect 2.5 ml fraction into sample tube using Solvent 2
12. End

Flow Rates:

Conditioning Flow:	10ml/min
Load Flow:	10ml/min
Rinse Flow:	10ml/min
Elute Flow:	5ml/min
Conditioning air push:	10ml/min
Rinse air push:	10ml/min
Elute air push:	5ml/min

10. When extraction is complete, release HLB cartridges and dispose. Turn off nitrogen gas.

**Appendix B: Review of recent literature featuring hyphenated techniques of high performance liquid chromatography (HPLC) and size exclusion chromatography (SEC) coupled with ICP-MS for investigation of metal species.**

Technique	Analyte/matrix	Column	Eluent	LC conditions	Isotopes/Species	References
SEC-ICP MS	Metals in blood serum and hemolysate	TSK G3000SW	TRIS buffer 0.1M pH 7.2	0.5mL min <sup>-1</sup> isocratic	<sup>208</sup> Pb, <sup>54</sup> Fe, <sup>63</sup> Cu, <sup>64</sup> Zn, <sup>24</sup> Mg	Geraken and Barnes (1991)
HPLC-ICP MS	As species in urine	Anion exchange: PRP-X-100 Cation exchange: Supelcosil LC-SCX	Phosphate buffer 30 mmole/L pH6.0 Pyridine 20mmole/L pH2.7	1.3ml min <sup>-1</sup> isocratic	As(III), (V), MMA, DMA AsB	Feldman et al (1999)
HPLC-ICP MS	Cd in plant tissue	Supelcosil SAX1	Phosphate buffer 5-26 mmole/L pH6.2	1.0ml min <sup>-1</sup> gradient	<sup>114</sup> Cd Arsenosugars,	Szpunar et al (2000)
SEC-ICP MS	As in marine algae and cell cultures	HR 10/30	1% Acetic acid pH3	0.6ml min <sup>-1</sup> isocratic	As(III), (V), MMA, DMA	
HPLC-ICP MS	Fe in meat	TSK gel 2000SW	TRIS 0.1M pH 7.2	1.0ml min <sup>-1</sup> isocratic	<sup>56</sup> Fe	Harrington et al (2001)
HPLC-ICP MS	As species in urine	Anion exchange: PRP-X-100	Tartaric acid 15mM pH 2.9	1.5ml min <sup>-1</sup> isocratic	As(III), (V), MA(III), MMA(V), DMA(V), AsB	Chen et al (2002)
HPLC-ICP MS	Se species in urine with ion-pairing	C8 Alltima	Hexanesulfonic acid 2mmole/L pH2.5 0.4% acetic acid 0.2% TEA, 5% MeOH	0.9ml min <sup>-1</sup> isocratic	AdoSeMet Me-SeMet TMSe	Wrobel et al (2003b)
SEC-ICP MS	Metals in humic substances from compost extracts	Superdex Peptide HR 10/30	CAPS buffer 10mM pH 10.3	0.7ml min <sup>-1</sup> isocratic	<sup>59</sup> Co, <sup>60</sup> Ni, <sup>63</sup> Cu, <sup>66</sup> Zn, <sup>208</sup> Pb	Wrobel et al (2003a)

HPLC-ICP MS	Iodinated compounds from earthworms	Supelco C18	Formic acid 0.01M/ Methanol (5 to 80%)	0.8ml min <sup>-1</sup> gradient	<sup>127</sup> I	Duckett et al (2003)
SEC-ICP MS	Ni from hyperaccumulating plants	Superdex Peptide HR 10/300	NH <sub>4</sub> acetate 5 mM pH7	0.75ml min <sup>-1</sup> isocratic	<sup>58, 60, 61, 62, 64</sup> Ni	Vacchina et al (2003)
SEC-ICP MS	Metals in DOM fractions from natural waters	YMC-Pack Diol 300	KClO <sub>4</sub> 10 mM pH 7.0	0.5ml min <sup>-1</sup> isocratic	<sup>51</sup> V, <sup>52</sup> Cr, <sup>56</sup> Fe, <sup>59</sup> Co, <sup>60</sup> Ni, <sup>63</sup> Cu, <sup>66</sup> Zn, <sup>98</sup> Mo, <sup>111</sup> Cd, <sup>140</sup> Ce, <sup>208</sup> Pb, <sup>232</sup> Th, <sup>238</sup> U	Wu et al (2004)
SEC-ICP MS	Metals in fulvic acids	Superdex Peptide HR 10/300	TRIS-HCl 50mM pH8.0 CAPS 10mM pH10.0 ACN 10%;0.1% TEA pH6.0	0.6ml min <sup>-1</sup> isocratic	<sup>24</sup> Mg, <sup>44</sup> Ca, <sup>53</sup> Cr, <sup>55</sup> Mn, <sup>56</sup> Fe, <sup>59</sup> Co, <sup>63</sup> Cu, <sup>66</sup> Zn, <sup>75</sup> As, <sup>77,78,82</sup> Se, <sup>95</sup> Mo, <sup>107</sup> Ag, <sup>127</sup> I, <sup>202</sup> Hg, <sup>208</sup> Pb	Grant et al (2004)
SEC-ICP MS and CE-ICP MS	Se associated with proteins from nuts	Superdex 200  Superdex 75  Superdex Peptide HR	NH <sub>4</sub> bicarb 150mM pH 7.8 TRIS/HCl 50mM pH 8.0 TRIS/HCl 50mM pH 8.0	0.5ml min <sup>-1</sup> isocratic 0.7ml min <sup>-1</sup> isocratic 0.6ml min <sup>-1</sup> isocratic	<sup>77,78,82</sup> Se	Kannamkumarath et al (2005)
SEC-ICP MS	DOM extracts from sediment (Ni,U)	Superdex Peptide HR 10/300	NH <sub>4</sub> Cl 10mM pH6.0		<sup>27</sup> Al, <sup>54,57</sup> Fe, <sup>55</sup> Mn, <sup>58,60,62</sup> Ni, <sup>238</sup> U	Jackson et al (2005)
HPLC-ICP MS	Sb and As in urine	TSKgel IC-AP	EDTA 20mM pH4.7	0.25 mL min <sup>-1</sup> isocratic	Sb(III)/Sb(V) As(III)/As(V) Fe(II)/Fe(III) Mn(II)/Mn(IV)	Mitsunobu et al (2006)
SEC-ICP MS	Cr in compost	TSK G3000SW	NH <sub>4</sub> NO <sub>3</sub> 0.1M pH7.4	0.5ml min <sup>-1</sup> isocratic	Total Cr, (VI), (III)	Laborda et al (2007)

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