

Expanding Selenium Speciation in Environmental Waters

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- ▶ HPLC method development takes time
- ▶ HPLC method development requires chromatography expertise
- ▶ The HPLC method will drive the speed of analysis and overall success and is therefore critically important
 - Mobile phase is key
 - Consider particle size and column length
- ▶ New capabilities are available in chromatography to evaluate for speciation method improvements, including new columns and μ HPLC
- ▶ New ICP-MS capabilities are available for the detection step

- ▶ Selenium occurs in four valence states: selenates ($\text{Se}6+$), selenites ($\text{Se}4+$), selenides ($\text{Se}2-$), and elemental selenium ($\text{Se}0$) (Goyer, 1991) which include compounds formed with oxygen, sulfur, metals, and/or halogens. Selenium compounds are used in the glass industry as decolorizing agents and in the rubber industry as vulcanizing agents. Selenium compounds are also found in toning baths used in photography and xerography, and in insecticides and photoelectric cells. Selenious acid is a component of gun cleaning chemicals (Quadrani *et al.*, 2000). Selenium sulfide is used in shampoos as an anti-dandruff agent. The most widely used selenium compound in industry is selenium dioxide (SeO_2) which catalyzes reactions of organic compounds and is produced by the oxidation of selenium with nitric acid followed by evaporation or by burning selenium in oxygen (HSDB, 1995). The largest anthropogenic sources of atmospheric selenium are from the combustion of fossil fuels and the production/refining of copper; particulates are the primary expected form of the compound (National Academy of Sciences (NAS), 1976; U.S. EPA, 1984). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 12,417 pounds of selenium and 4846 pounds of selenium sulfide (CARB, 1999).
- ▶ Selenium is an essential trace element in humans and other species; selenium deficiency leads to cardiomyopathy in humans (Goyer, 1991). For dietary intake, the National Research Council has set a U.S. Recommended Daily Allowance (RDA) of 0.87 mg/kg (55-70 mg/person/day) (Subcommittee on the Tenth Edition of the RDAs, 1989). The average daily oral intake of selenium is 125 mg/person (U.S. EPA, 1991). Organic selenium compounds (e.g., dimethyl selenide) are known to occur as metabolites and as microbial degradation products in the environment. These compounds appear to have relatively low toxicity.

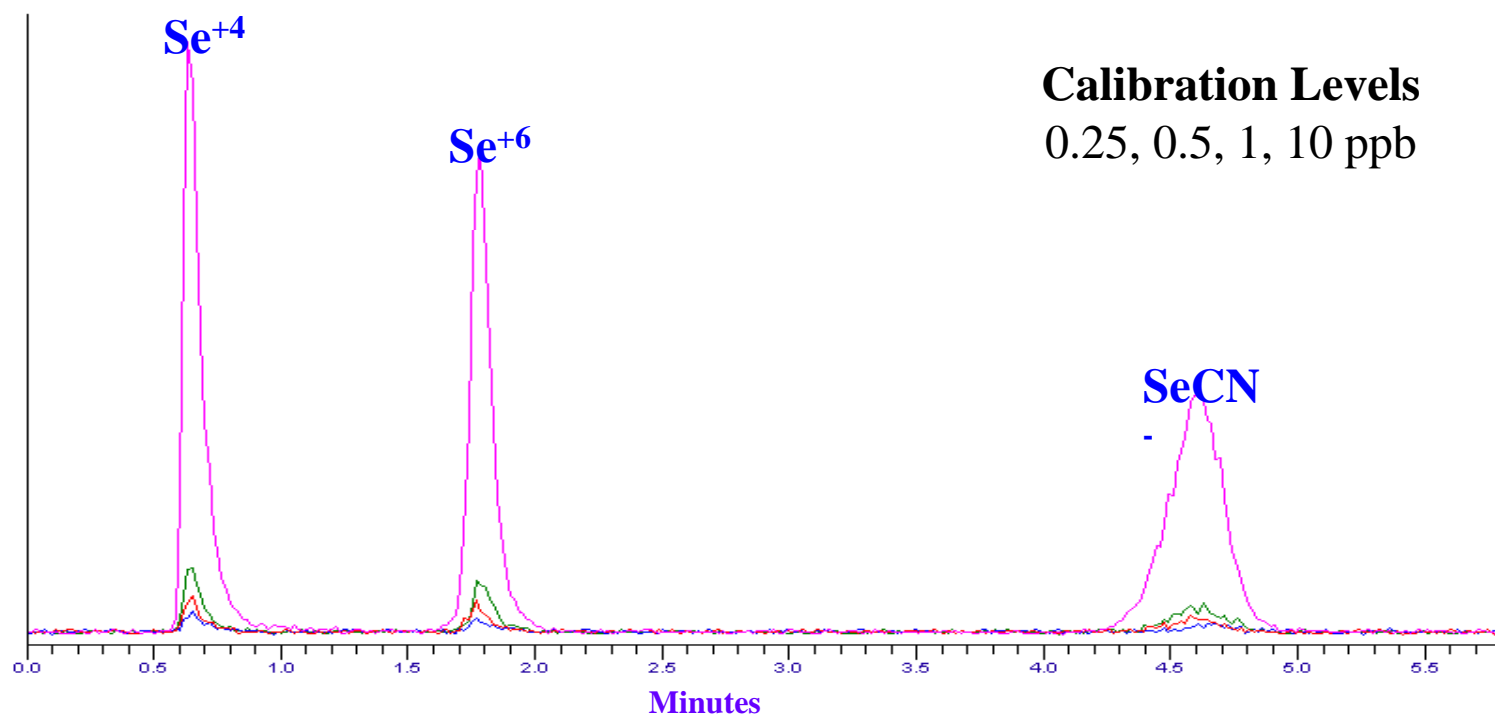
- ▶ Food is the primary source of exposure to selenium, with an estimated selenium intake for the U.S. population ranging from 0.071 to 0.152 milligrams per day (mg/d). (1)
- ▶ Humans are usually exposed to very low levels of selenium in air, with an average selenium concentration estimated to be below 10 nanograms per cubic meter (ng/m³). (1)
- ▶ Drinking water usually contains selenium at very low levels (usually less than 0.01 milligrams per liter [mg/L]). However, occasionally, higher levels of selenium may be found in drinking water, usually in areas where high levels of selenium in soil contribute to the selenium content of the water. (1)
- ▶ Occupational exposure to selenium in the air may occur in the metal industries, selenium-recovery processes, painting, and special trades. (1)

1. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Selenium* (Update). Public Health Service, Department of Health and Human Services, Atlanta, GA. 1996.

- ▶ 91 articles on selenium speciation in last 2 years
- ▶ 9 book chapters

<p>Microwave-assisted Extraction and Ion Chromatography Dynamic Reaction Cell Inductively Coupled Plasma Mass Spectrometry for the Speciation Analysis of Arsenic and Selenium in Cereals</p> <p>Chia-Ying TSAI and Shiu-Jen JIANG Analytical Sciences, March 2011, Vol 27.</p>	<p>Hamilton PRP-X100, 10 µm diam</p>	<p>(As(III)), arsenate(As(V)), Monomethylarsonic acid (MMA), dimethylarsinic acid(DMA), selenite (Se(IV)), selenate (Se(VI)), Se-Methylselenocysteine (Se-MeSeCys), selenocystine (SeCys)₂ and selenomethione (SeMet)</p>	<p>10 min</p>
<p>Selenium speciation in different organs of African catfish (<i>Clarias gariepinus</i>) enriched through a selenium-enriched garlic based diet</p> <p>Zoyne Pedrero, Sara Murillo, Carmen Camara, E. Schram, J. B. Luten, Ingo Feldmann, Nobert Jakubowski and Yolanda Madrid, <i>J. Anal. At. Spectrom.</i>, 2011, 26, 116–125</p>	<p>Hamilton PRP X-100 (250 4.1 mm)</p>	<p>Selenomethionine (SeMet), melenomethylselenocysteine (MetSeCys) and selenocysteine (SeCys)₂</p>	<p>12-20 min</p>
<p>Determination of the selenium isotopic compositions in Se-rich yeast by hydride generation-inductively coupled plasma multicollector mass spectrometry</p> <p>Johann Far, Sylvain Berail, Hugues Preud'homme and Ryszard Lobinski <i>J. Anal. At. Spectrom.</i>, 2010, 25, 1695–1703</p>	<p>Hamilton PRP-X100 (250 mm 4.1 mm 10 mm)</p>	<p>seleninic acid, sodium selenite and sodium selenate</p>	<p>15 minutes</p>
<p>Investigation of selenium metabolites in Se-enriched kale, <i>Brassica oleracea A</i>, via HPLC-ICPMS and nanoESI-ITMS</p> <p>Qilin Chan, Scott E. Afton and Joseph A. Caruso, <i>J. Anal. At. Spectrom.</i>, 2010, 25, 186–192</p>	<p>Zorbax SB-C18</p>	<p>Sodium selenite (Se(IV)), sodium selenate (Se(VI)), Se-Methylseleno-L-cysteine, (MeSeCys), selenocystine (SeCys)₂ L-Selenomethionine, (SeMet)</p>	<p>30 min</p>
<p>How Safe are Antioxidant Food Supplements Containing Selenium?</p> <p>Petra Cuderman and Vekoslava Stibilj, <i>Acta Chim. Slov.</i> 2010, 57, 668–676</p>	<p>Hamilton PRP-X 100 column</p>	<p>Se(IV), Se(VI), Se-cystine (SeCys)₂, selenomethylselenocysteine (SeMe-SeCys)</p>	<p>30 min</p>
<p>Simultaneous determination of seven elemental species in estuarine waters by LC-ICP-DRC-MS</p> <p>Yeuk-Ki Tsoi and Kelvin Sze-Yin Leung, <i>J. Anal. At. Spectrom.</i>, 2010, 25, 880–885</p>	<p>Hamilton PRP-X100</p>	<p>As(III), MMA Se(IV), As(V), Cr(III), Se(VI), Cr(VI)</p>	<p>10 min</p>

- ▶ Separate inorganic selenium species by reversed phase ion pairing
 - Se⁴, Se⁶, SeCN



- ▶ Goal: Add organo selenium species to the method
 - Start with Selenocystine and Selenomethionine
 - Most common

- ▶ This work will expand the work done previously with three species
 - Good separation with added selenium compounds
 - Short analysis time to match detector
 - Describe method development process

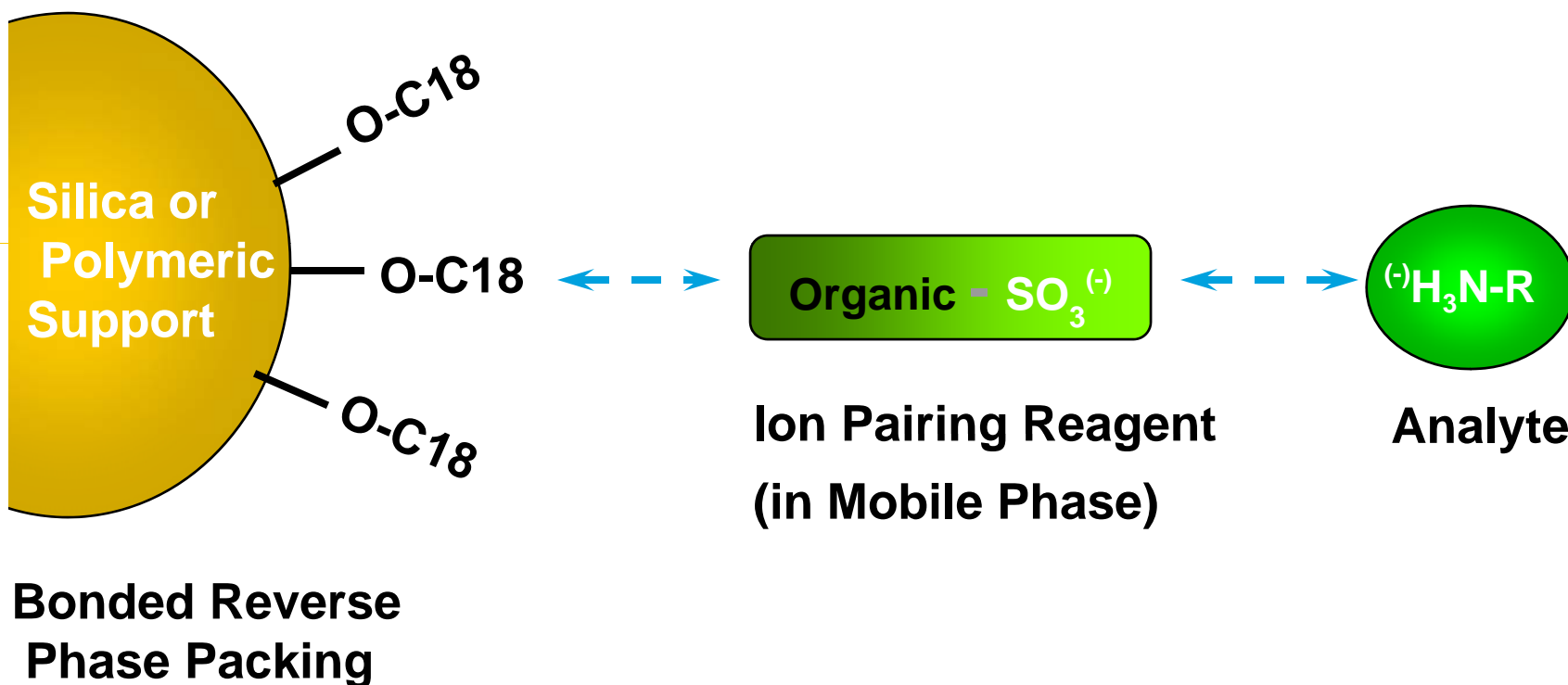
- ▶ Selenium monitored at 78 with oxygen
 - Remove argon dimer interference

Nebulizer	Concentric
Spray Chamber	Cyclonic
RF Power	1600 W
Neb Flow	1.04-1.06 L/min
Analyte	Se 78
Cell Gas	Oxygen = 0.5 mL/min
RPq	0.6



- ▶ Try with Reversed-Phase Ion Pairing
 - Columns are less expensive
 - Mobile phase generally has lower levels of dissolved solids
- ▶ Inject Individual Species Instead of Mixed Standard
 - Easier to focus on each species and see the effects of changes to the method
 - Overlay chromatograms of individual species
- ▶ Start with 3-cm columns
 - Fast LC
 - Shorter chromatograms
 - Shorter equilibration and wash times as change conditions
 - Perform method development with short columns, then move conditions to longer columns

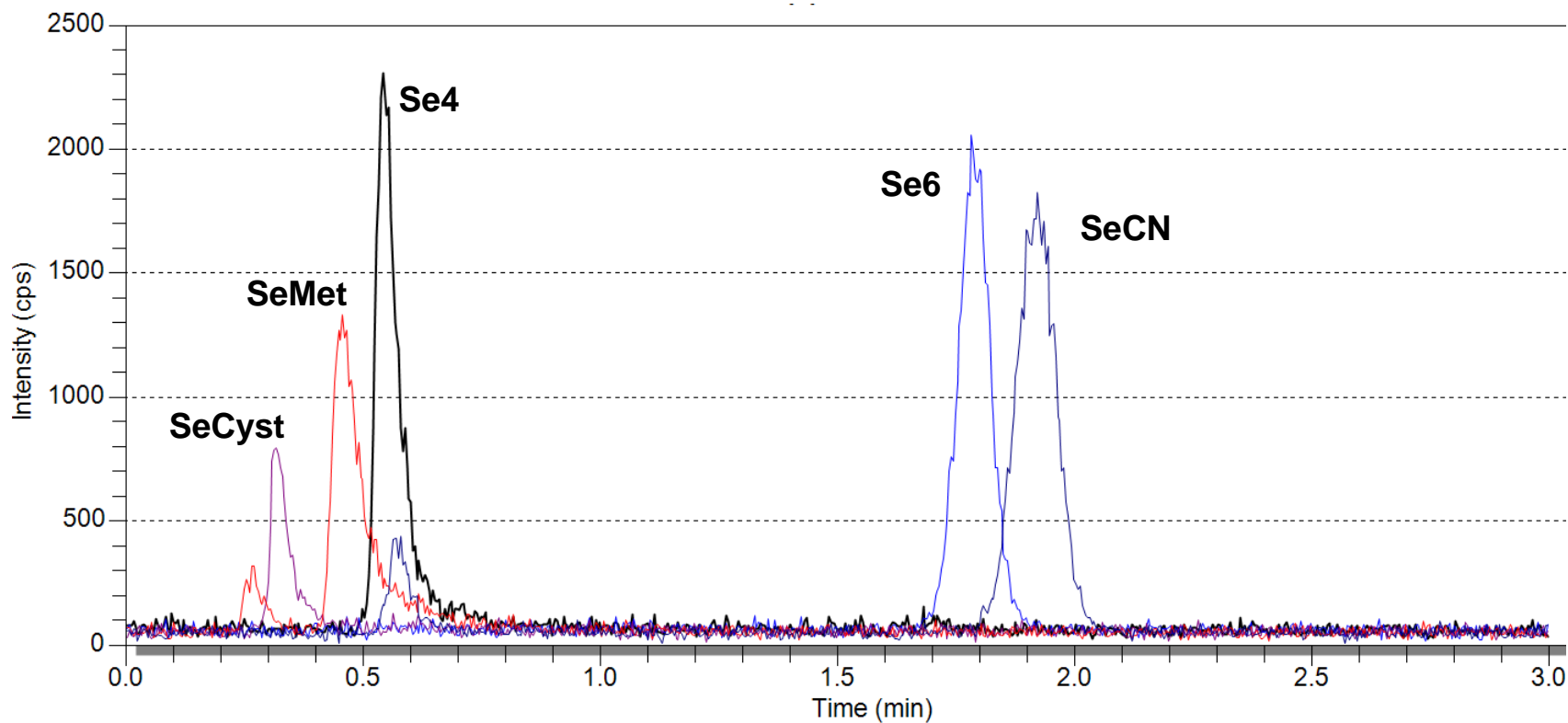
Mechanism of Reverse Phase-Ion Pairing



- ▶ UHPLC Columns
 - $\leq 2 \mu\text{m}$ particles
 - Available for reversed-phase work
- ▶ Advantages
 - Fast LC
 - Shorter columns have more active sites, so peaks elute faster
- ▶ Disadvantages
 - Special pumps are required which are capable of handling the higher pressures
 - $> 10,000$ psi
 - Mobile phase and all samples must be filtered through $0.25 \mu\text{m}$ filters
 - System clogs easily
 - Column lifetime may be shorter
 - Longer equilibration and wash times
 - More active sites

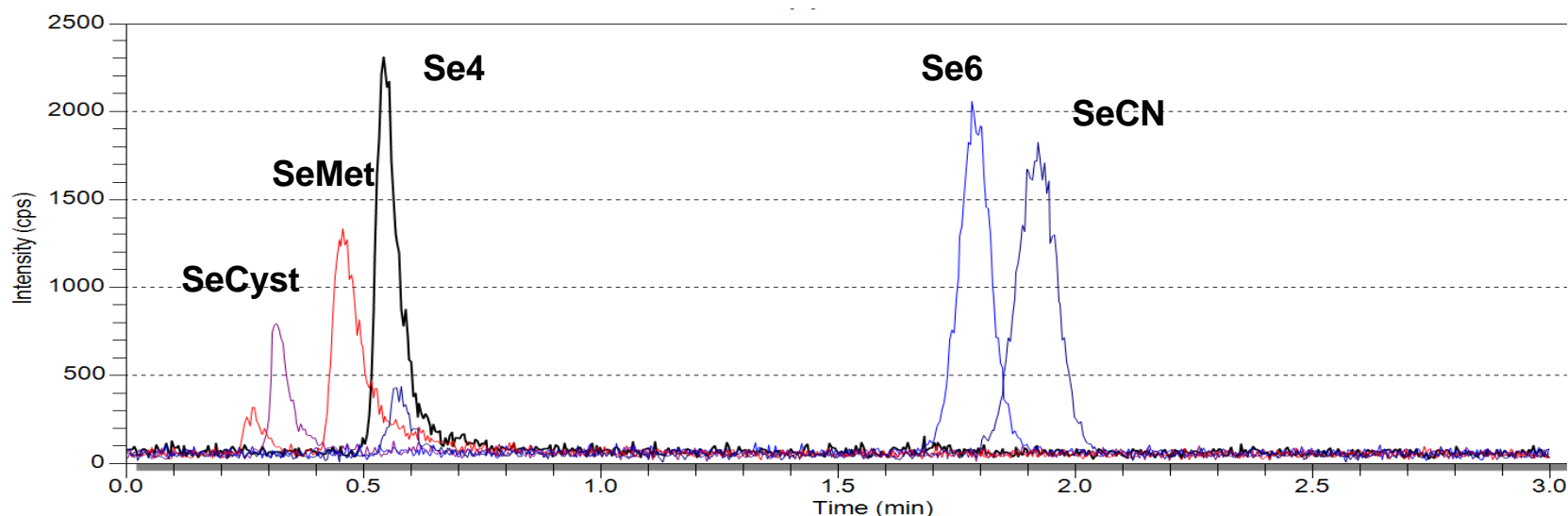
- ▶ **Column**
 - Pecosphere C8: 3 μm x 3 cm x 4.6 mm
- ▶ **Mobile Phase**
 - TBAOH = 0.1 mM
 - Allows the ionic species to interact with / be retained on the column
 - EDTA (dipotassium salt) = 0.07 mM
 - Complexes the inorganic species in solution
 - Without it, Se6 never elutes; Se4 and SeCN have significant tailing
 - Ammonium Acetate = 0.15 mM
 - Decreases tailing and retention time of SeCN
 - Methanol = 5%
 - Decreases retention time and tailing of all components
 - Biggest effect seen on later eluting peaks
 - pH = 7.0
 - Hope to minimize interconversion of inorganic species
- ▶ **Temperature**
 - 30°C

- ▶ Column Temp = 50°C
 - Faster retention times



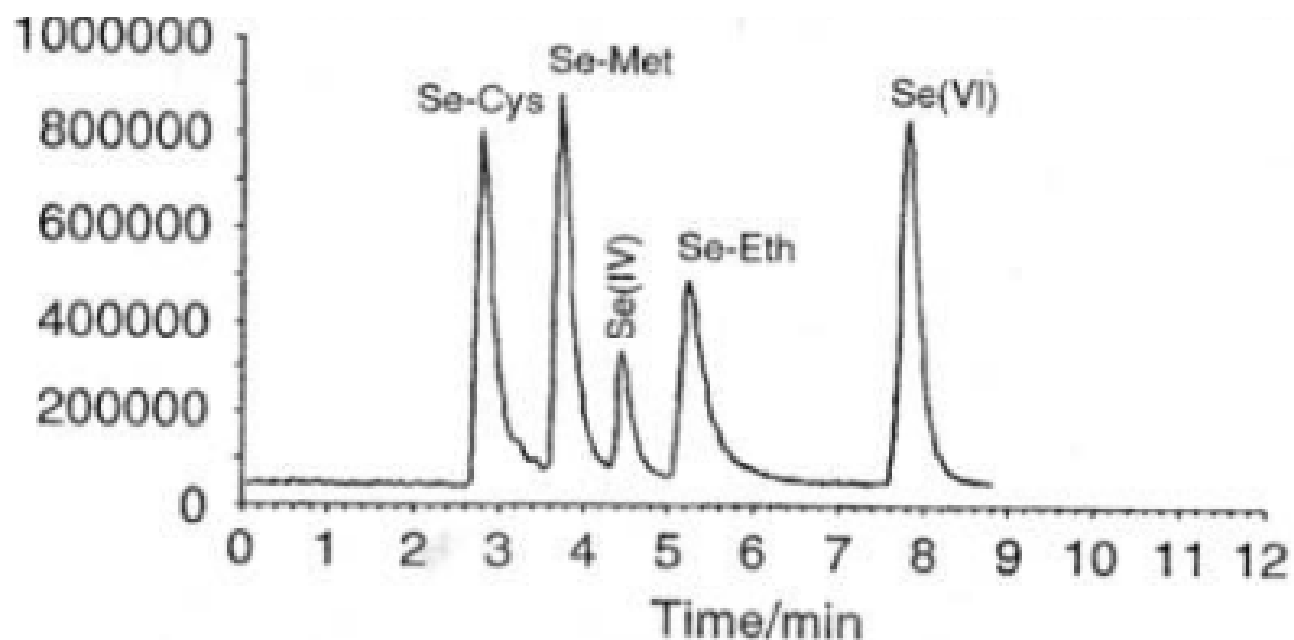
- ▶ Problems
 - SeCys, SeMet, and Se4 peaks are not baseline resolved
 - Se 6 and SeCN peaks are not resolved

Variables Tried with Same Column



- Pecosphere C8: 3 μ m x 3 cm x 4.6 mm
- Want to Maintain pH = 7.0
 - Minimize conversion of species
- Vary Concentrations of Mobile Phase Components
 - Se-Cystine & Se-Methionine behave similarly
 - Se4 and Se6 behave similarly
 - SeCN behaves differently
 - But all 3 sets of species behave differently from each other
- Tried Different Mobile Phase Components
 - Example: Different ion-pair reagents, different counter-ions
 - Nothing helped
- No Set of Conditions Gave Baseline Separation of All Species at pH=7.0

- ▶ JAAS, 2000, 15, 1093-1102 Marchante-Gayón, et. al.
- ▶ Separation of Se-cystine, Se-methionine, Se⁴, and Se⁴
 - Nucleosil C18: 5 μ m x 25 cm x 4.0 mm



- ▶ Mobile Phase
 - 30mM Ammonium Formate + 10 mM TBAA + 5% MeOH, pH 5.0

► Issues

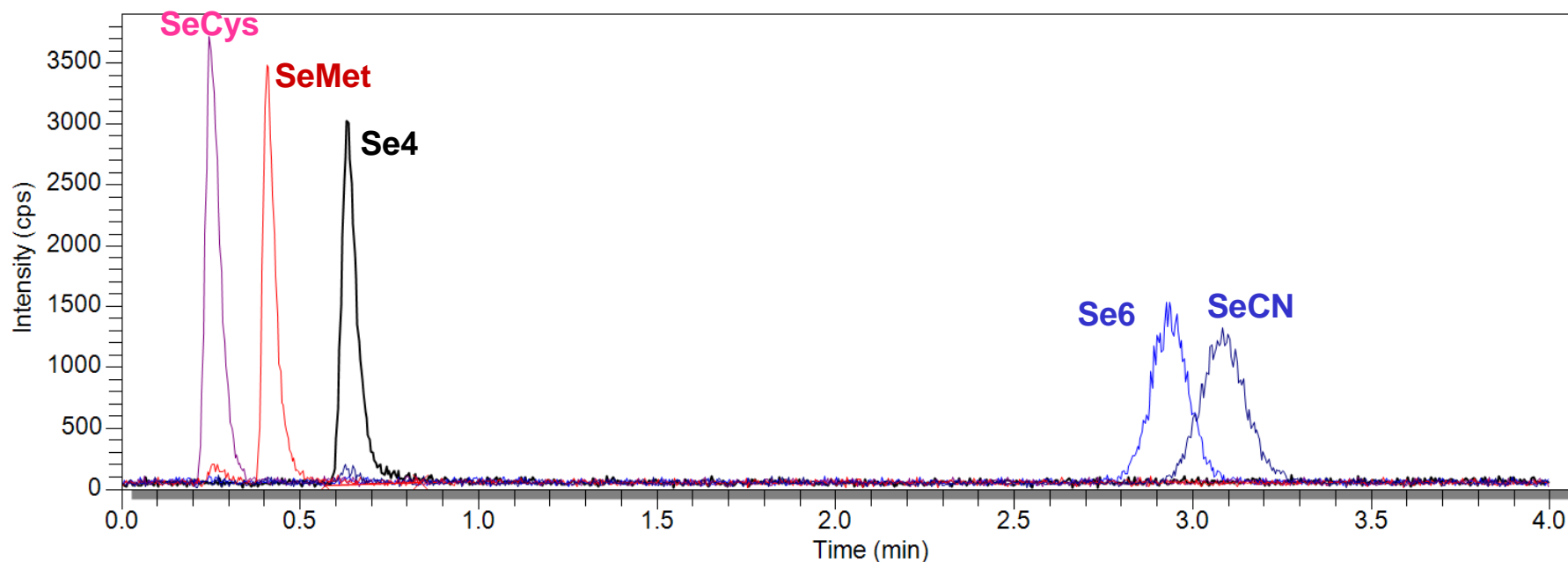
- Not baseline resolved
- High level of dissolved solids in the mobile phase
 - 4900 mg/L total dissolved solids
 - Increased instrument maintenance
 - Cones, ion optics, pump oil, etc.
- Acidic pH
 - Stability of species not addressed in paper

► Key Point

- Lower pH improves the separation

Lower the pH

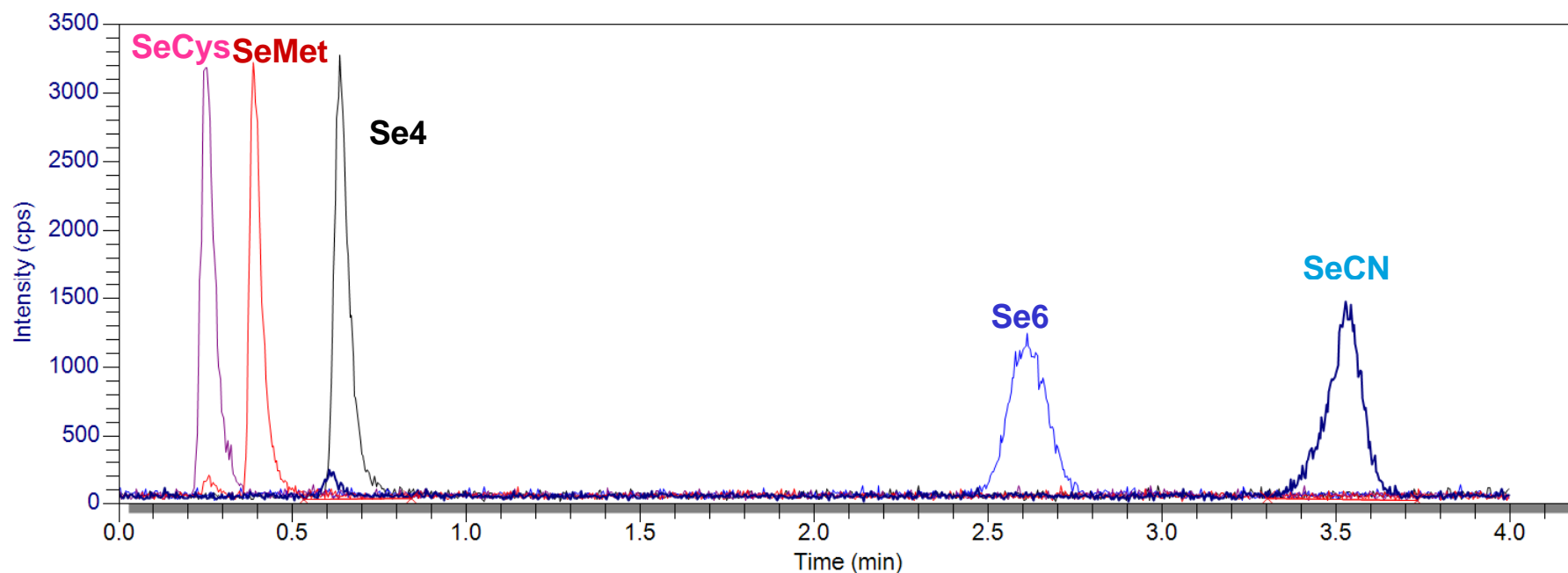
0.2 mM TBAOH + 0.15 mM NH₄Ac + 0.5 mM EDTA; **pH = 5.0** ; 5% MeOH; T = 50°C



- ▶ Good Separation of 1st 3 Peaks
 - Easy to resolve Se6 & SeCN peaks by varying conditions
- ▶ Separation Can Be Improved with Lower pH

Adjust the pH; Adjust Conditions

0.5 mM TBAOH + 0.5 mM NH₄Ac + 0.5 mM EDTA; **pH = 5.5** ; 7% MeOH; T = 50°C



- Better Separation with Modified Conditions
 - But mobile phase is still too acidic
- As pH Increases
 - Separation of 1st 3 peaks becomes worse

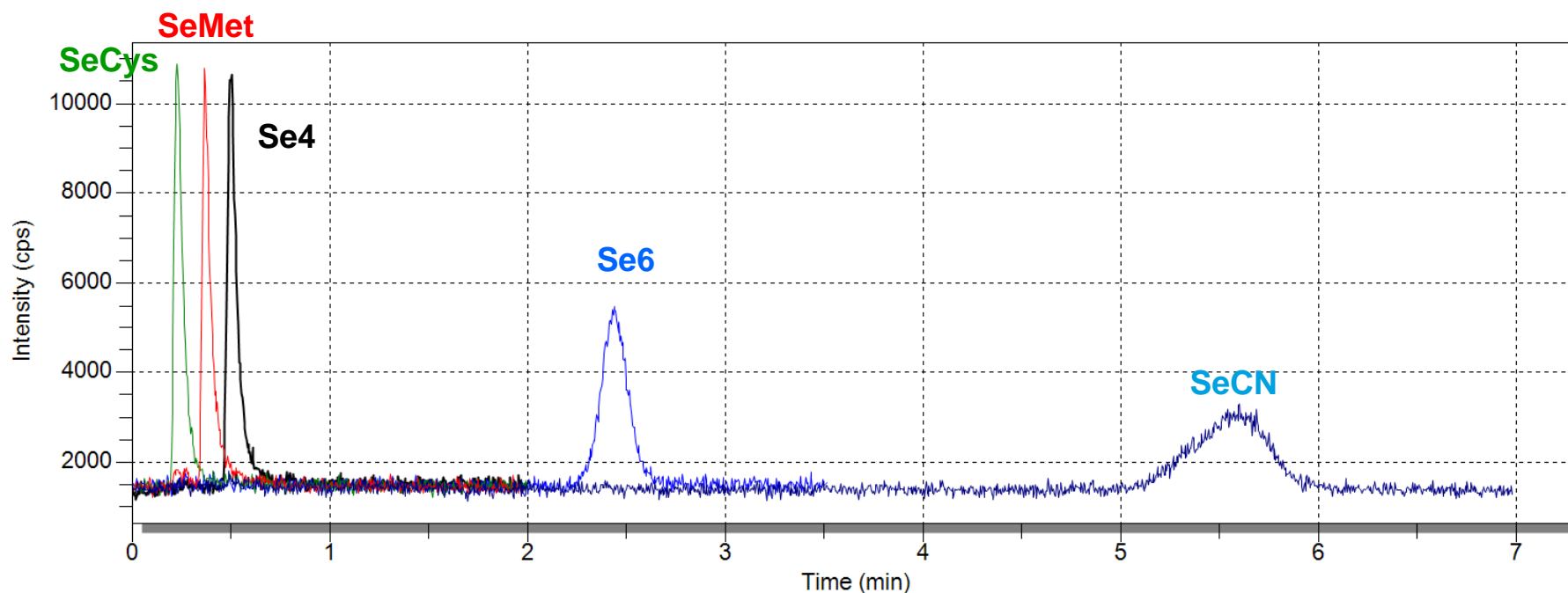
- ▶ Various Chemistries Occurring
 - SeCys and SeMet behave similarly
 - Se4 and Se6 behave similarly
 - SeCN has it's own chemistry
- ▶ pH is Important
 - Especially for the 1st 3 peaks
- ▶ Effects of Various Mobile Phase Components
 - EDTA is necessary to complex Se4 and Se6
 - Without EDTA
 - Se6 doesn't come off the column
 - Se4 peak is ugly
 - Retention times are shortened significantly by increasing the column temperature and the MeOH content

Next Step: Try a C18 Column

- ▶ Pecosphere C18: 3 μm x 3 cm x 4.6 mm
 - Same column material as before, just change carbon chain length on packing material
- ▶ Why C18?
 - Se-cystine and Se-methionine are primarily organic molecules
 - Maybe they will have a stronger affinity for the longer carbon chains

C18 Column – Same Conditions as C8

0.5 mM TBAOH + 0.5 mM NH₄Ac + 0.5 mM EDTA; **pH = 6.0**; 5% MeOH; T = 60°C



➤ Positives

- pH closer to neutral
- Decent separation of first 3 peaks

➤ Negatives

- pH still acidic
- First 3 peaks are not baseline resolved

- ▶ Varying Concentration of Various Components
- ▶ Different Ion Pairing Reagents
- ▶ Different “other” components

- ▶ Could Not Attain Baseline Separation of SeCys, SeMet, Se4

- ▶ C18 Gave Better Separation than C8 at a Higher pH
 - Moving in the right direction

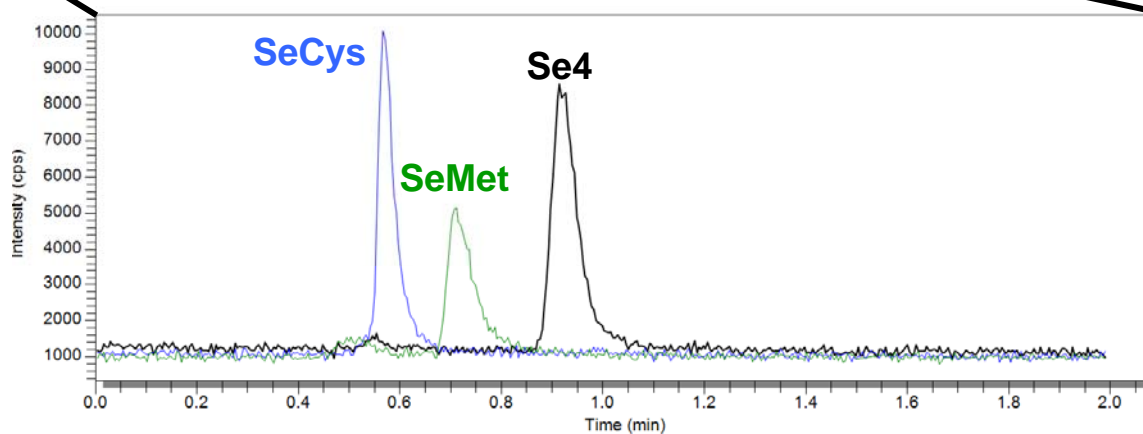
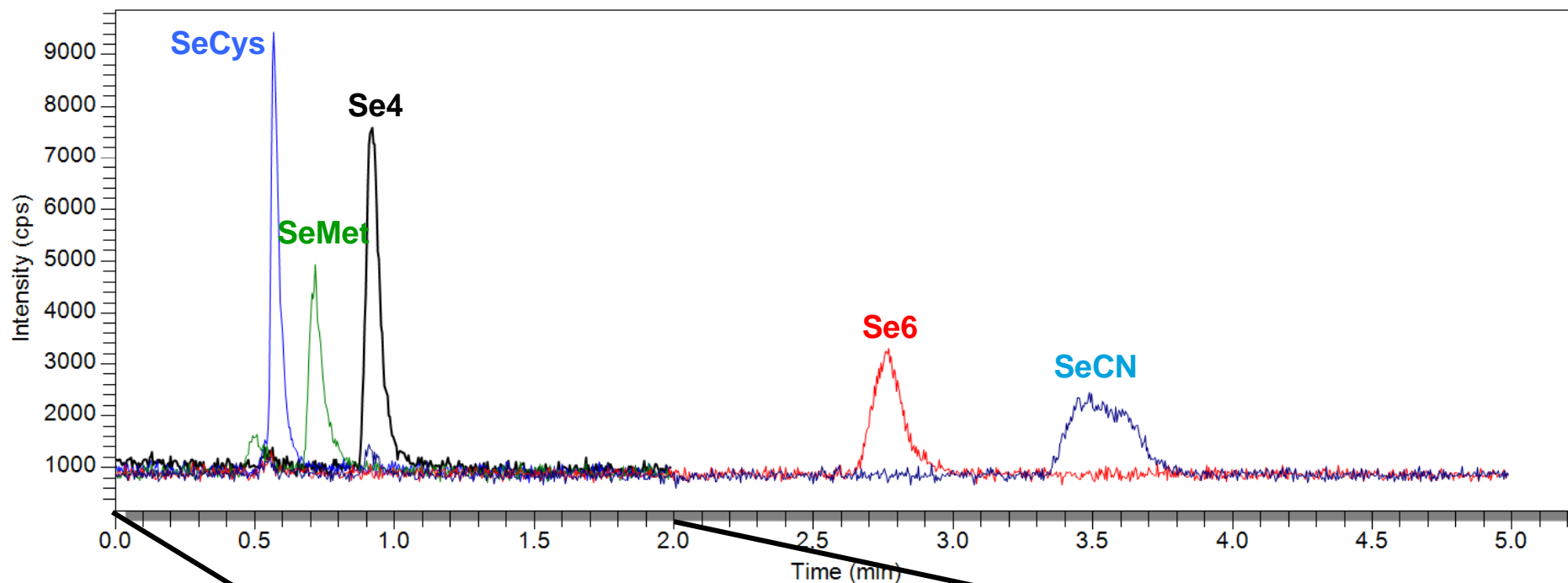
- ▶ Try a Longer C18 Column
 - Pecosphere C18: 3 μm x 8 cm x 4.6 mm

- ▶ Benefit
 - More active sites should give a better separation

- ▶ Drawbacks
 - Longer column means longer chromatograms
 - More active sites mean longer equilibration times
 - Slower method development

Success!

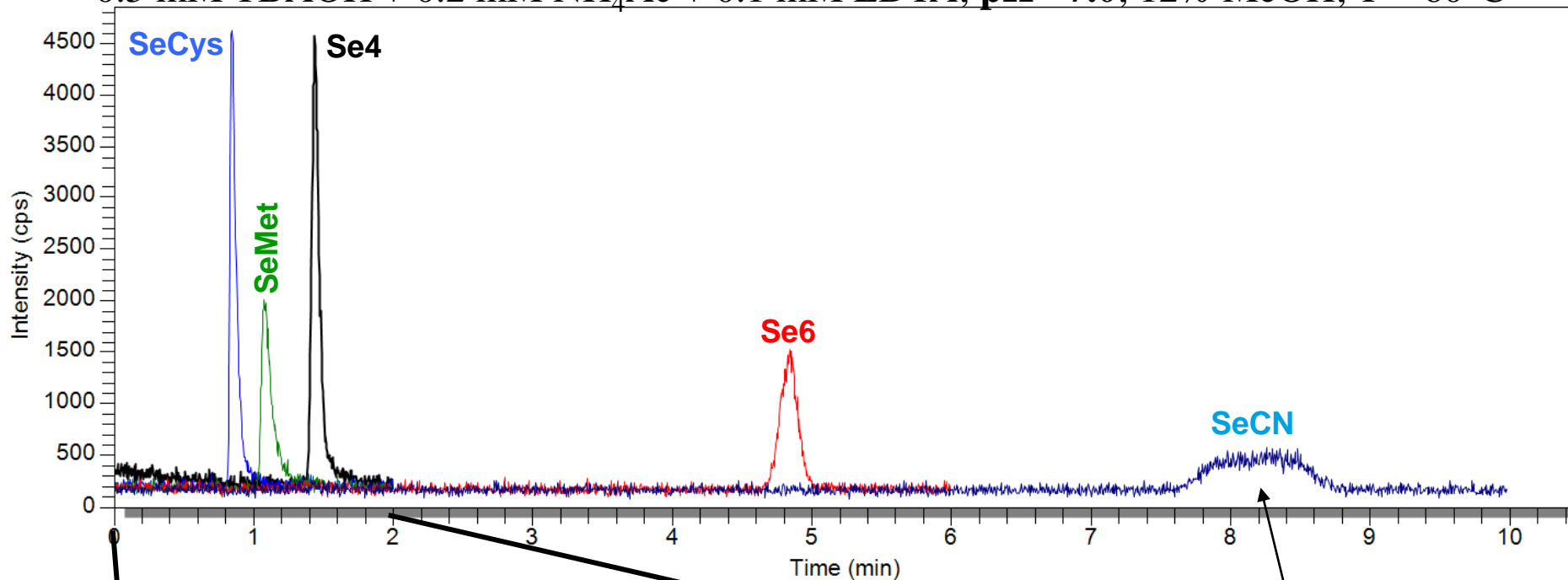
0.5 mM TBAOH + 0.2 mM NH₄Ac + 0.05 mM EDTA; **pH = 7.0**; 12% MeOH; T = 60°C



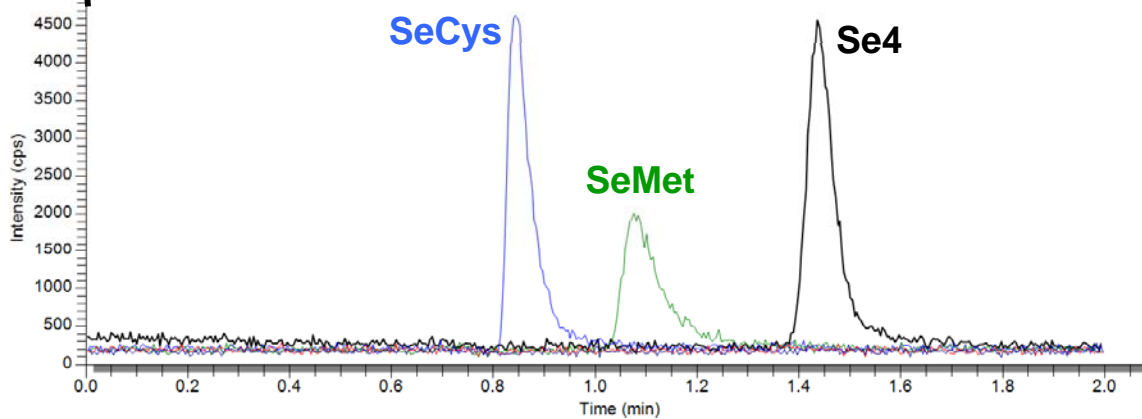
- ▶ Yes
 - Baseline resolution of SeCys, SeMet, and Se4 at pH = 7.0
 - 5 minute chromatograms
- ▶ No
 - Ideally, would like to get more space between SeCys, SeMet, and Se4
 - SeCN peak is ugly (according to real chromatographers)
- ▶ Maybe
 - Should there be more time between the Se4/Se6 and Se6/SeCN peaks?
 - With so many Se compounds, maybe more time would be good
 - Run 2 columns in series
 - 3 μ m 3 cm C18 column first
 - Acts like a guard column
 - 3 μ m 8 cm C18 column second

Two Columns

0.5 mM TBAOH + 0.2 mM NH₄Ac + 0.1 mM EDTA; pH = 7.0; 12% MeOH; T = 60°C



Need to sharpen
SeCN peak



- ▶ **Species Stability**
 - Multiple injections from same vials over time
 - Do species convert?
- ▶ **Reproducibility**
 - Repeat separation on the same column from a different lot
 - Did we get lucky?
- ▶ **Matrix Effects**
 - How will salts affect the separation?
 - How will other components in a potential sample affect the separation?
 - Need to adjust mobile phase again?
- ▶ **Quantification**
 - Calibration
 - Spike Recoveries
 - In a matrix

- ▶ LC method development is a slow, painful process
 - Many variables
 - Must wait for column to equilibrate each time conditions are changed
- ▶ Different Se species have very different chemistries
- ▶ When looking at organo-Se compounds, C18 columns are better than C8 columns
 - Is this true for other organo-metallic compounds too?
- ▶ Increasing column temperature and MeOH content dramatically shortens retention times
- ▶ Lots of work left to validate and characterize the method

- ▶ Optimize Detector Parameters
 - Focused only on the LC parameters
- ▶ Other Organo-Se Species
 - Will this method work for other species?
 - Many species to choose from
- ▶ What Matrices are Important?
 - How will matrices affect the separation
- ▶ Examine water samples
- ▶ Extend to extracted food samples

