

Selenium Speciation Analysis and its Role in Total Selenium Quantitation

Russell Gerads

russ@appliedspeciation.com



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AND CONSULTING, LLC

info@appliedspeciation.com

www.appliedspeciation.com

Total Selenium

- Total Selenium – Quantification of all forms of selenium present in the sample, regardless of speciation and phase
- Total Filtered Selenium – Quantification of all forms of selenium present in a sample which pass through a 0.45 μ m filter, regardless of speciation and phase

Very simple concept...until now.



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Current Promulgated Methods Associated with Selenium Analysis of Aqueous Matrices

EPA Approved Analytical Methods

6020

200.8

200.9

270.2

6010

270.3

1638

200.7

EPA Approved Digestion Methods

3005

3010

3015

3020

7740

7741A

7742

200

200.2

Standard Methods

3114

3113

3120

3125

3500-Se

3030



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Identification of Problem

- Filtered selenium concentrations greater than unfiltered selenium concentration
- Discrepancy of speciation results by IC-ICP-MS when compared to total selenium
- Interlaboratory comparisons identify different total selenium results between laboratories
- Temporally variable selenium concentrations



Identification of Problem

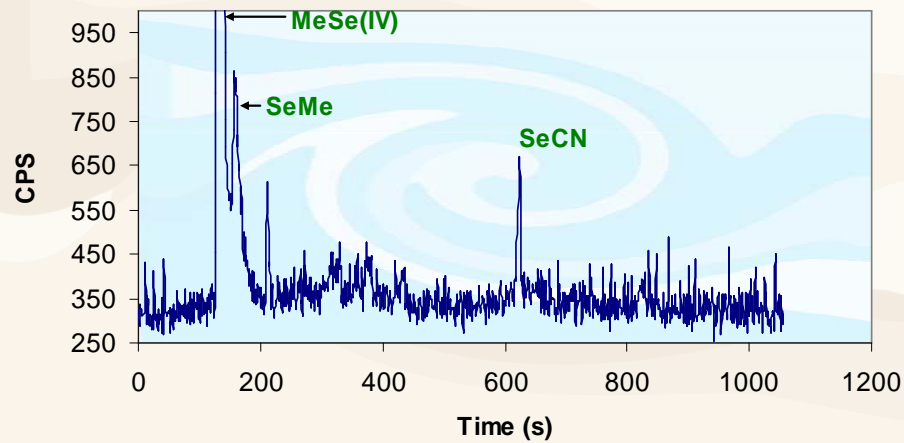
Results From Industrial Wastewater

Sample ID	Se(IV)	Se(VI)	SeCN	MSe(IV)	Total Se (1% HNO ₃)
Sample 1	19.6	4.4	1.82	2.3	49.5
Sample 2	12.0	5.1	ND (<0.44)	2.0	33.3
Sample 3	47.3	25.6	ND (<0.44)	2.6	140
Sample 4	46.1	21.5	ND (<0.44)	2.7	137
Sample 5	40.5	17.4	ND (<0.44)	1.9	101
Sample 6	31.1	10.5	ND (<0.44)	2.3	85.1
Sample 7	84.4	ND (4.3)	1629	ND (0.44)	23.1
Sample 8	3.41	ND (4.3)	6.84	ND (0.44)	10.6
Sample 9	75.9	8.2	ND (<0.44)	1.1	128
Sample 10	10.1	8.5	ND (<0.44)	1.6	50.8



Occurrence of SeCN

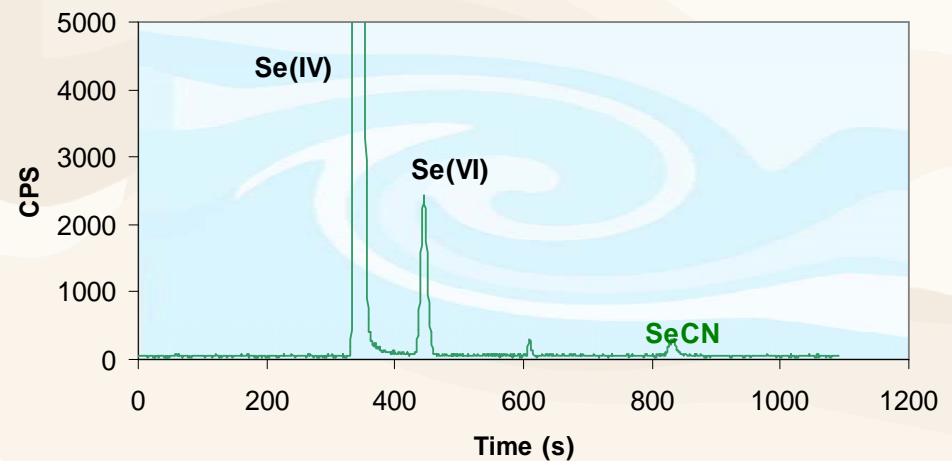
Selenium Speciation of Human Urine



- Municipal wastewater treatment plants

- Naturally occurring selenium reducing bacteria (river sediment)
- Industrial wastewater treatment plants incorporating biological treatment

Selenium Speciation of *Pseudomonas fluorescens* K27 in Sodium Nitrate



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Fate of SeCN

SeCN reaction with 1% HNO₃ in glass

1ppm SeCN Solution



1ppm SeCN Solution
2 min after HNO₃ addition



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Fate of Elemental Selenium

Vessel	Preservation	Ovened?	Mass of Se ⁰	% Recovery
125mL Amber HDPE	1% HNO3	yes	10mg	0.04
125mL Amber HDPE	10% HNO3	yes	10mg	1.4
125mL Amber HDPE	1% HNO3	no	10mg	0.02
125mL Amber HDPE	10% HNO3	no	10mg	0.02
60mL Clear HDPE	1% HNO3	yes	10mg	0.05
60mL Clear HDPE	10% HNO3	yes	10mg	4.0
60mL Clear HDPE	1% HNO3	no	10mg	0.01
60mL Clear HDPE	10% HNO3	no	10mg	0.01
50mL Polypropylene	1% HNO3	yes	10mg	0.2
50mL Polypropylene	10% HNO3	yes	10mg	79
50mL Polypropylene	1% HNO3	no	10mg	0.04
50mL Polypropylene	10% HNO3	no	10mg	0.05
40mL Amber Glass	1% HNO3	yes	10mg	1.6
40mL Amber Glass	10% HNO3	yes	10mg	96.0
40mL Amber Glass	1% HNO3	no	10mg	0.18
40mL Amber Glass	10% HNO3	no	10mg	0.30



Fate of Elemental Selenium

Comparison of different containers

Red Se(0) in glass



Red Se(0) in polyethylene



Black Se(0) in polyethylene



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Elemental Selenium

- Elemental selenium occurs in two forms
 - Amorphous red elemental selenium – Insoluble in water but can be suspended in solution as a colloid or particulate
 - Crystalline black elemental selenium – Insoluble in water but depending on the size of the crystals it may float on water due to surface tension
- Depending on the conditions of the sample matrix (salts, pH, ORP, etc.) interconversion between forms may occur resulting in further loss and an increased energy demand for digestion



Elemental Selenium

- Why is element selenium important if it is insoluble?
 - Chemical and biological (microbial) processes convert $\text{Se}(0)$ to SeO_3 and SeO_4
 - Up to 10% of red, amorphous, elemental selenium was found to be converted to SeO_3 after 125days¹
 - $\text{Se}(0)$ found to have similar induction into glutathione peroxidase, phospholipid hydroperoxide glutathione peroxidase (PHGPx), thioredoxin reductase-1, and protein integration

¹Losi, M, Frankenberger, W. 1998. *Microbial Oxidation and Solubilization of Precipitated Elemental Selenium in Soil*. Journal of Environmental Quality. 27: 836-843.



Current issues with accepted methodologies

- SeCN is known to convert to elemental selenium when 1% HNO₃ (v/v) preservation is applied
- Elemental selenium is known to adsorb onto plastic bottle walls
- Oxidation of elemental selenium is an endothermic reaction which is facilitated by heating and is not efficiently converted with 1% HNO₃
- Some EPA methods require an aliquot of sample be transferred from the original sample collection vessel prior to digestion, regardless of material (plastic or glass)



Possible Corrections

- Do not preserve sample with HNO_3
- Limit sample collection to borosilicate glass bottles
- Target the sample digestion scheme to focus on elemental selenium and other suspended particulate forms of selenium (SeS_2 ?)
- Solubilization and stabilization of selenium species (especially colloidal elemental selenium) requires the sample to be digested. What are the options and issues associated with external and in-bottle digestion?



Sample Preservation

- Do not preserve sample with HNO₃
 - Will result in low recoveries for other metals (Pb, Zn, Cu, etc.) due to adsorption onto bottle walls
 - Inconsequential if colloidal elemental selenium is present in sample
 - Field preservation increases the possible sources of contamination; therefore, there is no advantage of field preservation if preserved in the laboratory

Samples should not be preserved prior to digestion



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Sampling Vessels

- Do not preserve sample with HNO_3
- Limit sample collection to borosilicate glass bottles
 - Lowest adsorption capacity of all tested sample vessel materials for elemental selenium
 - Colloidal elemental selenium may aggregate and precipitate out of solution necessitating vigorous shaking prior to subsampling

Sampling Vessels should be limited to borosilicate glass to minimize adsorption of elemental selenium.



Sample Digestion

- Target the sample digestion scheme to focus on elemental selenium and other suspended particulate forms of selenium (SeS_2)
 - The oxidation potential of the sample must be increased
 - The volume of chemical(s) used for in-bottle digestions must not alter the final volume of the sample which may result in an increased variability due to unknown dilution. Addition of solid chemical is optimal. Sodium sulfite has been applied extensively in academia to quantitate elemental selenium in soil and sediment matrices – does it show promise for aqueous matrices as well?
 - Current data shows external digestion of elemental selenium (red and black) with 10% concentrated HNO_3 (v/v) yields acceptable recoveries.



Sample Digestion

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 - While the SeSO_3^{2-} complex can be stabilized the performance is operationally defined by the sample matrix (sulfite consumption)



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Currently concentrated HNO_3 shows the most promise for conversion of elemental selenium to selenite in some of the most difficult matrices.



Sample Digestion

- Do not preserve sample with HNO_3
- Limit sample collection to borosilicate glass bottles
- Concentrated HNO_3 should be applied for conversion of elemental selenium to selenite
- Solubilization and stabilization of selenium species (especially colloidal elemental selenium) requires the sample to be digested. What are the options and issues associated with external and in-bottle digestion?
 - A minimum of 10% HNO_3 (v/v) was required to recover 96% of 10mg Se^0 in a highly complex industrial waste influent sample. Application of an in-bottle digestion necessitates a known volume of sample which is typically not available.

External Digestion is required for total selenium quantification on a reproducible basis.



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Sample Digestion

- Do not preserve sample with HNO_3
- Limit sample collection to borosilicate glass bottles
- Concentrated HNO_3 should be applied for conversion of elemental selenium to selenite
- Pipette 4mL concentrated HNO_3 into a 40mL I-Chem vial. Shake the borosilicate glass sample vial vigorously then bring the 40mL vial up to volume. Cap the 40ml vial and heat at 90°C for a minimum of 4 hours. Let the sample equilibrate to room temperature and analyze.
- The final volumes can change according to the available glass digestion vessels; however, the ratio and procedure should remain the same.
- The sample should NEVER be pipetted from the original sample bottle.



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Now how do we analyze the digest?



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Analytical Techniques

- Reactionary Based Analyses
 - Hydride generation atomic absorption spectrophotometry (HG-AAS)
- Direct Analysis (Plasma)
 - Inductively coupled plasma atomic emission spectroscopy (ICP-AES)
 - Inductively coupled plasma mass spectrometry (ICP-MS)



Hydride Generation

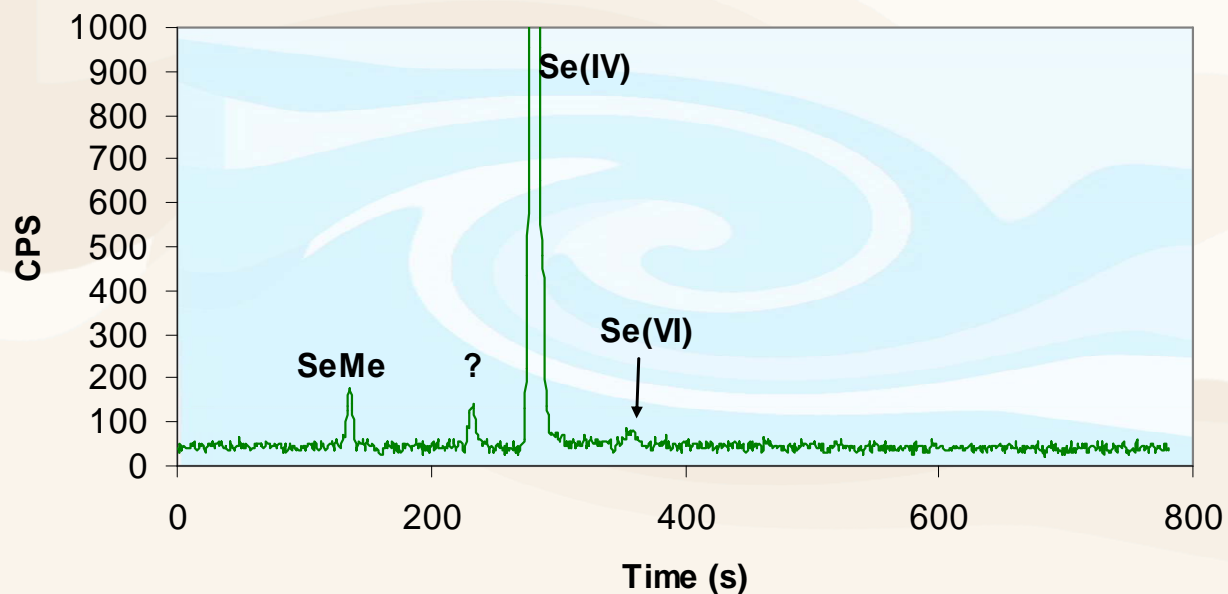
- Only Se(IV) can form the volatile hydrides for detection
- Analytical method requires conversion of all selenium species to Se(IV) prior to analysis
 - Multiple sample digestion techniques have been promulgated for analysis via hydride generation (7741A and 7742)
 - Current promulgated digestion techniques do not require incorporation of different selenium species to monitor digestion efficiency
 - Some laboratories have generated their own digestion techniques similar to Standard Method 3500 using persulfate and hydrochloric acid



Performance of Standard Method 3500-Se

- Digestion facilitates reactions with HCl, $K_2S_2O_8$, and heat (specifics of digestion method can be acquired from Standard Methods)

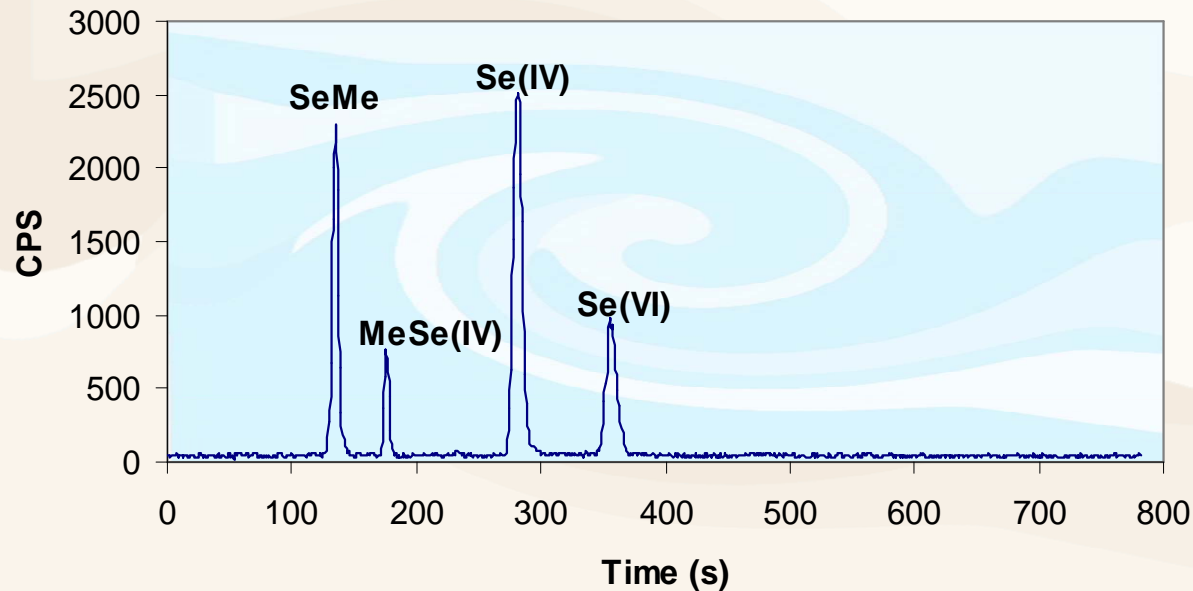
SM 3500 Digestion of Groundwater Spiked with 5 Different Selenium Species



Performance of EPA Method 7741A

- Digestion facilitates reactions with HNO_3 , H_2SO_4 , and heat (specifics of digestion method can be acquired from Standard Methods)

EPA Method 7741A Digestion of Groudwater Spiked with 5 different Selenium Species



Hydride Generation

- Confirmation that not all selenium species are converted to Se(IV) during digestion which can result in low biased results for all sample matrices

Hydride generation, with any detection scheme, for total selenium quantification is not recommended due to its propensity to produce low biased results for any sample matrix



Inductively Coupled Plasma

- Sample is atomized and is transferred (Ar carrier gas) to a radio frequency induced plasma
 - High TDS may alter nebulization efficiency resulting in a low biased result.
 - Plasma ionization efficiency can be quenched by excessive TDS
- Atomized sample is ionized by the plasma, regardless of species or valence state.
- The ionized selenium atoms are then detected using mass spectrometry or atomic emission spectroscopy.

Inductively coupled plasma is species independent; therefore, is the better choice for total selenium quantification.



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Inductively Coupled Plasma

- Due to the concentration of HNO_3 in the digestion, a minimum of 10x dilution would be recommended prior to analysis by ICP-MS or ICP-AES.
- The detection limit of the analytical method increases linearly with the applied dilution factor. The adjusted reporting limit (5-500 $\mu\text{g/L}$), depending on analytical platform, may not be acceptable to meet regulatory limits which will require the application of more performance based analytical methods. Current technology facilitating collision cell technology (detection limits 5-50 ng/L) can support all regulatory requirements.

Regulatory offices should be more open to performance based methods, especially collision cell technologies, in order to meet regulatory requirements



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Overview of Method for Total Selenium Quantification in Aqueous Matrices

- 1) Do not preserve sample with HNO_3
- 2) Limit sample collection to borosilicate glass bottles
- 3) Pipette 4mL concentrated HNO_3 into a 40mL I-Chem vial. Shake the borosilicate glass sample vial vigorously then bring the 40mL vial up to volume with sample. Cap the 40ml vial and heat at 90°C for a minimum of 4 hours. Let the sample equilibrate to room temperature and analyzed by ICP-AES or ICP-MS.
- 4) The final volumes can change according to the available glass digestion vessels; however, the ratio and procedure should remain the same.
- 5) The sample should NEVER be pipetted from the original sample bottle for quantitation purposes.



Final Thoughts

- Elemental selenium is currently being discharged into US rivers and lakes from industrial treatment facilities.
- Current research has identified a known issue with accepted methodologies for sample collection and digestion prior to analysis.
- Current research has identified that hydride generation may produce unrepresentative results without the performance of method development on each sample type and each selenium species of interest. All selenium species of interest **MUST** be applied in matrix spikes to generate a true representation of the method's performance.

