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Elemental Speciation by Parallel Elemental and Molecular Mass Spectrometry and Peak Profile Matching

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Trace elemental speciation in complex, real-world matrixes is a daunting task because of the low concentration of metals/metalloids and the correspondingly high molecular chemical noise. We constructed a liquid chromatography parallel elemental and molecular mass spectrometry (PEMMS) system and evaluated the use of peak elution profiles to identify trace molecular species containing specific heteroatoms, using the case of Se in yeast. We demonstrate that it is possible to use the HPLC-inductively coupled plasma (ICP)MS peak profile (retention time, width) to identify candidate ions with matching peak profiles in the molecular MS data. Proof of principle was demonstrated by C18 separation of three Se-amino acid standards (0.005−15 ppm as Se). The molecular MS (atmospheric pressure chemical ionization time-of-flight, APCI-TOF-MS) data set was converted into selected ion chromatograms of 0.05 Th width. ICPMS and APCI-TOF-MS ion chromatograms were fit by the Haarhoff−Vander-Linde function using the following parameters: area, retention time, width, and skew. The ICPMS fit parameters were more reproducible than the APCI-TOF-MS fit parameters from run to run, and the APCI-TOF-MS signal was expected to limit correlation in most circumstances. Retention time and width were found to correlate well between the two MS systems for APCI-TOF-MS peaks with signal-to-fit-error (S/FE) of >25. Correction factors for differences in flow path length and peak broadening were required. The normalized correction factors were species and concentration independent and were stable from run to run. The skew parameter was found to be highly susceptible to noise and was not generally useful in matching ICPMS and APCI-TOF-MS peaks. An artificially noisy sample was generated by spiking 30 ppb Se-methionine (SeMet) and 5 ppb Se-methylselenocysteine (SeMSC) with unselenized yeast extract and run by PEMMS. The PEMMS software was able to detect four molecular MS peaks associated with SeMet and two for SeMSC, while filtering out >40 coeluting spectral peaks associated with chemical noise in each sample. In summary, we have demonstrated that correlated information in peak shape between parallel detectors can facilitate detection of trace elemental species in complex matrixes.

The complementary nature of molecular mass spectrometry and element-specific techniques for metal speciation analysis has been well documented in recent reviews.[1−3] In particular, coupling of HPLC to either inductively coupled plasma mass spectrometry (ICPMS) or atmospheric pressure ionization mass spectrometry (API-MS, e.g., atmospheric pressure chemical ionization MS (APCI-MS) or electrospray ionization MS (ESI-MS)) has been widely employed due to its versatility.[4] The high sensitivity and selectivity of HPLC-ICPMS has resulted in frequent reports of sub-ppb detection limits for some metal species, but these analyses come at the cost of destroying all molecular information. In the absence of other information, confirmation of a component's identity by HPLC-ICPMS requires retention index matching with an appropriate standard. Such an approach may yield ambiguous results and is incompatible with identification of unknown or unexpected species.[5] HPLC-API-MS can potentially identify unknowns based on fragmentation patterns, but the nonselective nature and lower sensitivity of most API-MS techniques compared to ICPMS can result in obfuscation of the analyte signal in complex mixtures.[3] In combination, ICPMS and API-MS can potentially provide low detection limits, exceptional selectivity, and the ability to analyze both known and unknown molecular species.

There is considerable variation in the literature in how HPLC, ICPMS, and API-MS are combined, but reports may be generally classified by whether API-MS is performed in an off-line or on-line fashion.[6] In off-line methods, the sample is run by HPLC-ICPMS, and the elution time of the metal-containing species is noted. The sample is rerun, fractions collected at appropriate time points, and the fractions infused into the molecular MS. On-line speciation methods couple the HPLC directly to the API-MS, usually with identical or similar chromatographic conditions. Ideally, the retention index of the species by HPLC-ICPMS should be similar to its retention index on HPLC-molecular MS, which

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would allow the analyst to search a narrow window for ions corresponding to unknown metal species. Alternatively, selected ion monitoring by single-stage MS or selected reaction monitoring by MS/MS has been used to confirm the presence of suspected metal species. While identification of all species in an unknown mixture by API-MS is possible in principle, the high levels of chemical noise and low levels of analytes usually result in many more peaks detectable by HPLC-ICPMS than can identified by molecular MS.

The recent literature on selenium speciation illustrates this problem. The speciation of Se is arguably the most often studied of any element in recent years,[7] owing to its rich diversity of covalently bound forms, its narrow range between essential and toxic levels, and its putative chemopreventative properties.[8] A thorough knowledge of Se speciation in foodstuffs and biological samples (tissue, plasma, urine) is necessary for understanding its intriguing nutritional role.[9] Recent reviews show at least 20 papers in which both molecular MS and element-specific techniques were used to speciate Se in biological matrixes, including yeast,[10−13] garlic,[14,15] Indian mustard,[16−18] Brazil nuts,[19,20] and urine.[5,21] While in a few studies API-MS has been used to postulate structures for some previously unseen species, e.g., *S*-(selenomethyl) -cysteine,[22] adenosyl containing selenocompounds,[11,12] and glutathione S-conjugates[23] in selenized yeast and (methyl)-*N*-acetylhexosamines as the major selenometabolites in urine,[21] this is far from routine and many selenocompounds detected by HPLC-ICPMS remain unidentified. For example, enzymatic extraction of Se-yeast followed by HPLC-ICPMS revealed the presence of at least 30 Se-species,[11] but less than 10 species could be identified by retention time matching or ESI-MS. As mentioned above, the high complexity of real matrixes and the low selectivity of API-MS make identification of ions associated with Se species difficult. One solution to this problem has been to use multidimensional chromatography, i.e., sequential separations on columns with orthogonal retention properties (i.e., size exclusion, ion exchange, reversed phase).[11,24,25] While this approach is often more successful than single-stage separations in reducing chemical noise, it is also more tedious, risks greater sample losses, and has not provided comprehensive speciation of real samples.

The key to elemental speciation is to reduce chemical noise such that the targeted heteroatom-containing ions can be efficiently and confidently identified in the molecular mass spectra. We hypothesized that using ICPMS and API-MS as parallel, simultaneous detectors for a single HPLC system would greatly reduce chemical noise associated with complex biological and environmental matrixes. The parallel combination of elemental and molecular MS with HPLC has been proposed previously by Houk,[26] but reports of its implementation have not appeared in the literature. The author suggested many advantages of such a system, including the potential to share a common vacuum system, the complementary nature of ICPMS and API-MS data, and the ability to acquire both data sets in a single run.

An unmentioned advantage of a parallel detection scheme is that the column is the primary determinant of not only the peak retention time but also the peak shape. Chromatographic peak profiles (retention index, peak width, peak skew) differ subtly due to specific analyte−stationary-phase interactions, and the peak profile of a single component detected by parallel API-MS and ICPMS should be well correlated. Chemical noise on the API-MS could then be reduced by rejecting ion chromatograms that have peak profiles dissimilar to species detected by ICPMS. Clearly, this approach is impossible with off-line API-MS as fraction collection destroys any information contained within the peak profile. Sequential HPLC-ICPMS and on-line HPLC-API-MS could preserve peak profile information, but this approach introduces greater uncertainty due to run-to-run variations in chromatographic conditions or injection size. Previous investigations of unknown metal species using on-line HPLC-API-MS have used HPLC-ICPMS retention time to narrow the search window on the API-MS data, but these approaches still result in large chemical noise backgrounds from coeluting compounds. True parallel speciation using GC coupled to electron impact-MS and either microwave-induced plasma-atomic emission spectroscopy[27] or ICPMS[28] has been reported, but the samples were of low complexity and all components of interest were baseline resolved chromatographically on the molecular MS. None of these reports has attempted correlating peak profiles between parallel elemental and molecular detection schemes. Peak profiles may also be affected by components downstream of the chromatographic separation, e.g., peak broadening in double-pass spray chambers for ICPMS,[29] but these effects should be largely compound independent.

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The use of peak profiles to compare HPLC-API-MS ion traces to HPLC-ICPMS data superficially resembles deconvolution approaches frequently seen in the GC/MS literature. Deconvolution aims to extract "pure" mass spectra from complex GC/MS data that feature numerous overlapping components. Ion traces with similar peak profiles are assumed to have originated from a common component. The earliest deconvolution schemes searched for peaks within selected ion chromatograms with similar retention times.[30] Later approaches used "peak sharpness" in addition to retention index to parametrize ion traces.[31] An improvement on this scheme has been implemented in the widely used automated mass spectral deconvolution and identification system (AMDIS) algorithm.[32] As an alternative to peak parametrization methods, numerous matrix-based deconvolution algorithms have been reported in which the 2-D retention time−m/z matrix is assumed to be the linear sum of an unknown number of components with unique mass spectra.[33] These matrix-based approaches also rely upon the assumption that ion traces arising from the same component will share similar peak profiles. Despite some similarities, the use of peak profiles in identifying unknown metal species by parallel elemental and molecular mass spectrometry (PEMMS) is a distinct problem from these GC/MS deconvolution approaches outlined above. In PEMMS, the goal is to use the HPLC-ICPMS trace to identify target element-containing candidate ions on the HPLC-API-MS, while the goal of the aforementioned GC/MS deconvolution schemes is to extract pure spectra that can be compared to an MS library.

We report the construction of a PEMMS system. We have also developed PEMMS software on a LabVIEW platform to rapidly detect and parametrize peaks in ICPMS and API-MS (APCI-TOF-MS) ion chromatograms. We show that subtle differences in peak profile are due primarily to column interactions and are well correlated between the two detectors. Subsequent matching of peak profile data between the two detectors greatly reduces the chemical noise on the APCI-MS and facilitates species identification. We demonstrate these principles with pure Se-amino acid standards, and with the same standards spiked with a commercial unselenized yeast matrix. Although we focus in this report on selenium speciation by parallel HPLC-ICPMS and HPLC-APCI-TOF-MS, we perceive PEMMS as a general strategy that can be used with other separation (GC, CE) and detection (ESI-MS, ICP-AES) techniques for a range of problems in speciation analysis.

METHODS

Chemicals. L-Selenomethionine (SeMet) was purchased from Sigma-Aldrich (St. Louis, MO). L-Selenoethionine (SeEt) was purchased from Toronto Research Chemicals (North York, ON, Canada). Se-methylselenomethylcysteine (SeMSC) was purchased from Life Extension Vitamin Supplements (Albany, CA) in the form of 200 μg/tablet supplements. Deionized, 18-MΩ H_2O was produced on-site (Milli-Q, Millipore, Billerica, MA). Formic acid, ammonium hydroxide, perfluoroheptanoic acid (PFHA) and HPLC grade methanol were purchased from Sigma-Aldrich.

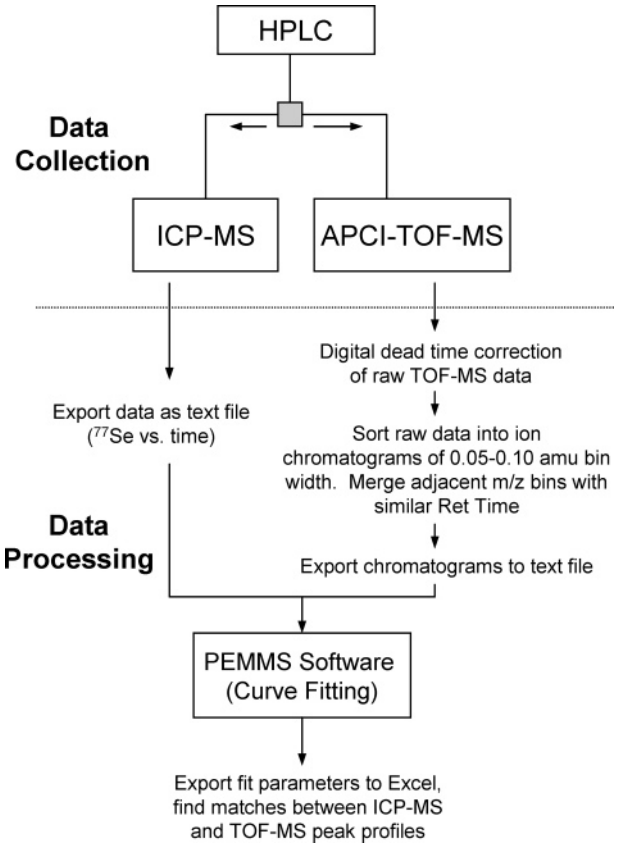

Figure 1. Schematic of the PEMMS system, including data flow. The top section ("Data Collection") shows the PEMMS hardware, including the postcolumn split to the parallel ICPMS and APCI-TOF-MS detectors. Following data acquisition, the data are exported ("Data Processing") in a format appropriate for the PEMMS software. The ICPMS data require minimal handling and are exported by the native software in ASCII format. The APCI-TOF-MS data set is corrected pointwise for digitizer pulse-pileup effects and then binned into 0.05−0.10 Th wide selected ion chromatograms before they are exported. The PEMMS software is then used to match peak shapes between the ICPMS and APCI-TOF-MS data sets.

Preparation of Clean and Yeast-Spiked Selenoamino Acid Standards. Stock solutions of SeEt, SeMet, and SeMSC at a nominal concentration of 50 μg of Se/g were prepared in 0.01 M HCl and stored in the dark at 4 °C. Serial dilutions from 0.03 to 10 (for SeMet), 0.06 to 15 (SeEt) and 0.005 to 1 ppm (SeMSC) were prepared monthly in deionized water. Nonselenized yeast (Hodgson Mill, Gainesville, MO) for use as an artificial complex matrix was purchased from a local supermarket. One gram was ground with a mortar and pestle and added to a 50-mL centrifuge tube along with 10 mL of 0.01 M HCl. The yeast mixture was stirred for 12 h at ambient temperature, centrifuged at 3000 rpm for 30 min, and passed through 0.45-μm syringe filter. The filtered extract was then diluted 50-fold with deionized water. The artificially spiked SeMet standard was prepared by addition of the diluted yeast extract with SeMet at a 2:1 ratio to yield a yeast-spiked standard with a Se concentration of 0.03 ppm.

Parallel Elemental and Molecular Mass Spectrometry System: HPLC, ICPMS, and APCI-(Qq)-TOF-MS Instrumentation. Figure 1 is a block schematic of the PEMMS hardware configuration. A Summit HPLC (Dionex, Sunnyvale, CA) with ASI-100 autosampler was used for all chromatographic

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Table 1. Experimental Conditions

	Chromatographic Conditions
HPLC system	Dionex Summit, binary high pressure
HPLC column	Supelco Discovery HS C18, 2 cm × 2.1 mm, 3-μm particle size.
mobile phase	A: 10 mM formic acid adj to pH 2.8, 1 mM perfluoroheptanoic acid, aqueous B: 10 mM formic acid adj to pH 2.8, 1 mM perfluoroheptanoic acid, 50% CH_3OH
HPLC conditions	isocratic, 50:50 A/B
flow rate	200 μL/min
postcolumn split	50 μL /min to ICPMS, 150 μL/min to QqTOF-MS
injection size	10 μL
	ICPMS Conditions
ICPMS system	ThermoFinnigan Element2
spray chamber	Apex cyclonic spray chamber (Elemental Scientific; Omaha, NE) at 100°C, Peltier at 2 °C
nebulizer	PFA-ST (Elemental Scientific)
sample nebulizer gas	Ar, 1.08 L/min + O_2, 0.040 L/min
auxiliary gas	Ar, 1.02 L/min
cool gas	Ar, 14 L/min
rf power	1150 W
isotopes monitored	^{77}Se and ^{78}Se
scan mode	low resolution, 10 ms sample time, 10 samples/peak, 3 passes/sample
detector	both modes, 2300 V
	APCI-(Qq) -TOF-MS Conditions
QqTOF system	ABI QSTAR Pulsar I (Applied Biosystems, Foster City, CA)
source	atmospheric pressure chemical ionization, positive ion mode
sample gas 1	N_2, 50
sample gas 2	N_2, 50
needle current	3.0
source temperature	450 °C
needle voltage	+5000 V
declustering potential 1	45 V
focusing potential	230 V
declustering potential 2	10 V
calibration	external, PPG
scan range	80−600 Th, 1 s accumulation time, full scan mode
detector	2200 V

separations. A 2 cm × 2.1 mm i.d., 3-μm particle size Discovery HS C18 guard column (Supelco, Bellefonte, PA) was used. The chromatographic conditions are described in Table 1. The postcolumn HPLC eluent was split by a PEEK T-piece (VICI, Houston, TX) and connected in parallel to an Element2 ICPMS and a hydrid quadrupole-TOF (QSTAR Pulsar i QqTOF-MS; Applied Biosystems, Foster City, CA). Connections to and from the split were made using PEEK tubing (VICI). The diameters and lengths of the tubing were chosen such that 50 μL/min of the HPLC eluant traveled to the ICPMS, while the remainder (150 μL/min) was directed to the APCI-(Qq)TOF-MS. The QSTAR was operated in TOF-MS (full scan) mode in all experiments. Data collection on the ICPMS and APCI-TOF-MS was synchronized through relay closure by the Dionex autosampler. The PEEK tubing was connected directly to the APCI source of the QqTOF-MS. The postsplit PEEK tubing was connected to the Apex cyclonic spray chamber (Elemental Scientific, Inc.) by 0.25-mm PTFE tubing. The Apex spray chamber was employed because it resulted in better desolvation and less peak tailing than the Scott double-pass spray chamber. A full list of experimental parameters for the APCI-TOF-MS and ICPMS is in Table 1.

PEMMS Approach. Our proposed scheme for PEMMS analyses is as follows:

(a) HPLC separation of sample was followed by a postcolumn T-split to an Element2 ICPMS and an ABI QSTAR (Qq)TOF-MS with APCI interface and simultaneous collection of HPLC-ICPMS and HPLC-APCI-TOF-MS data. The QqTOF-MS was used in TOF-MS (i.e., full scan) mode.

(b) The data sets are exported as a series of ion chromatograms.

(c) Using PEMMS software, all chromatographic peaks on ICPMS trace are parametrized by the HVL function.

(d) The PEMMS software is used to search for candidate ion peaks in the APCI-TOF-MS data set that have peak parameters similar to the ICPMS peaks.

(e) If necessary, the sample is run again, and MS/MS is used on candidate ions to determine or confirm the structure of species.

PEMMS Software. The PEMMS approach looks for similarities in the chromatographic peak profile between the ICPMS and molecular MS, which necessitates that the data from each system exist as a series of ion chromatograms. The ICPMS data were exported as a comma-delimited text file (^{77}Se and ^{78}Se vs time) using the native software and could be used by the PEMMS software without further treatment. However, the native Analyst QS 1.1 software does not offer a facile means to export all of the APCI-TOF-MS data as a series of ion chromatograms. A home-written Visual Basic macro was used to sort the raw data into bins of a user-defined mass window, typically 0.05 Th. Binning the APCI-TOF-MS data ensures that any peak will be distributed over a maximum of two bins, as the expected peak width at m/z = 100 is ~0.01 Th. Additionally, binning reduces the data file size by >10-fold. Following the binning step, m/z bins with total ion counts of <100 over the length of the run were discarded to further reduce the data file size, and the reduced data set would be written as a series of m/z chromatograms to a text file. As an example, data collected with a TOF-MS range of 80−600 Th and a bin spacing of 0.05 Th would initially be sorted into 10 400 m/z bins (m/z = 80.00, 80.05, 80.10,...). Removal of mostly zero ion chromatograms typically reduces data file size by 50%. Before the binning step, the Visual Basic macro performed a digital dead time correction on each data point. The TOF multichannel plate detector is known to be nonlinear due to pulse pileup at the high-speed digitizer, but this effect may be accounted for by transforming each point by the equation

$$A = A_0 e^{kA_0} \quad (1)$$

where A is the actual signal (in counts per second), A_0 is the observed signal, and k is the dead-time correction factor. k was determined experimentally by infusion of SeMet, such that the observed $[M + H]^+$ isotopic pattern of SeMet best matched its expected isotopic pattern. Following this correction, a linear range of 3 orders of magnitude was achievable with Se-amino acid standards and APCI-TOF-MS.

The PEMMS data analysis software was written in LabVIEW 6i (National Instruments, Austin, TX). ICPMS or APCI-TOF-MS data are imported, and an ion chromatogram is selected for display. Cursors are used to define a region of interest on the chromatogram, and the software attempts to curve fit the trace by the Levenberg−Marquardt algorithm. At least 90 empirical functions have been reported for modeling chromatographic

peaks.[34] We chose to use the Haarhoff−VanderLinde (HVL) function, as it is appropriate for modeling peaks that demonstrate fronting or tailing. Our group has had previous success in modeling chromatographic traces with the HVL function,[35] which has the form

$$f(x) = \left(\frac{a_0 a_2}{a_1 a_3 (2\pi)^{1/2}}\right) \left(\frac{\exp\left[-\frac{1}{2}\left(\frac{x - a_1}{a_2}\right)^2\right]}{\left[\exp\left(\frac{a_1 a_3}{a_2^2}\right) - 1\right]^{-1} + \frac{1}{2}\left[1 + \mathrm{erf}\left(\frac{((x - a_1)/a_2)}{2^{1/2}}\right)\right]}\right) \quad (2)$$

where a_0 is the peak area, a_1 is the retention time, a_2 is the peak width, and a_3 is the skew or asymmetry of the peak. Width as used in this report is equivalent to the standard deviation of the chromatographic peak. The skew parameter is unitless: Skew of >0 indicates a tailing peak, and skew of <0 indicates a fronting peak.

When analyzing APCI-TOF-MS data, the user has the option to "batch curve fit" all ion chromatograms sequentially within a specified time window. Upon completion, the PEMMS software prints a list of detected peaks and their corresponding areas, retention times, widths, and skews. It is not uncommon for a compound to fall between two m/z bins; e.g., $m/z = 166.97$ with peak width of 0.02. Th (fwhm) would be binned into both the 166.95 and 167.0 bins. If two sequential bins have a peak with similar retention time (within 5 s), the PEMMS software will merge the areas from the two bins into a new m/z bin and values of retention time, width, and skew determined as the weighted averages of the two bins.

RESULTS AND DISCUSSION

In principle, our PEMMS approach could work effectively with any molecular mass spectrometer that can be coupled on-line to an HPLC. In our studies, we used a hybrid QqTOF-MS, which provides several well-recognized advantages in speciation analyses over quadrupoles and ion traps.[12,17] The TOF detector has the ability to measure a wide mass range with low-duty cycle losses. Additionally, the high mass resolution ($\Delta M/M \sim 8000$) and mass accuracy of the TOF detector is advantageous for detecting metal-containing species, which will typically have a more negative mass defect than nonmetallic species with the same nominal mass. The high mass accuracy is also of assistance in determining the empirical formula of candidate ions. A disadvantage of the QqTOF-MS is that the TOF detector is poorly linear and has a lower dynamic range due to pulse pileup associated with the high-speed digitizer. The PEMMS principle demands that the detector behave linearly to prevent distortion of the peak profiles and requires the application of a pointwise digital dead-time correction to each raw data file using a home-written Visual Basic macro.

Because of the requisite for linear response, we chose APCI as our ionization method in these initial studies instead of the more commonly employed ESI. Compared to ESI, APCI usually provides better linearity and is less susceptible to suppression effects.[36] Furthermore, APCI-MS tends to be more robust and require less optimization for unknowns. A disadvantage of APCI is that the heated nebulizer can thermally degrade labile compounds and that the analyte must be stable in the gas phase. Therefore, some species may not be detected that would be detectable with ESI-MS. The Element2 ICPMS provides a linear dynamic range of 9 orders of magnitude according to the manufacturer, which should prevent any distortions of ICPMS peak shape. In general, ICPMS instruments provide excellent dynamic ranges and should be well-suited for the linear response demands of PEMMS.

The chromatographic demands of APCI-MS and ICPMS are somewhat orthogonal. The ICP plasma does not tolerate high levels of organic solvent, while higher levels of organic modifier in APCI result in better desolvation of the analyte.[37] We achieved the best compromise of APCI signal and ICP plasma stability when using 75:25 H_2O/CH_3OH with 10 mM formic acid (adjusted to pH 2.8) and 1 mM perfluoroheptanoic acid as an ion-pairing agent. Because consistent detector response is essential to our PEMMS technique, and because the sensitivity of the ICPMS and APCI-MS are both matrix-dependent, we chose to use isocratic HPLC conditions rather than a gradient. A Supelco Discovery HS C18 column was employed throughout the study. In these proof-of-principle experiments we used a short, narrow-bore (2 cm × 2.1 mm) column for the sake of expediency.

PEMMS of Selenoamino Acid Standards. The HVL function relies on four parameters: area, retention time, width, and skew. Area is not an effective parameter for matching ICPMS peaks with TOF-MS peaks, as the response of the ICPMS and APCI-TOF-MS to a particular component will be mostly uncorrelated. Retention time, width, and skew are all dependent on the chromatography and, thus, should be correlated between the ICPMS and APCI-TOF-MS.

We ran standards of SeEt, SeMet, and SeMSC by PEMMS on a short (2 cm × 2.1 mm) C18 column with a flow rate of 200 μL/min. The flow was split such that 50 μL/min went to the ICPMS and 150 μL/min went to the QqTOF-MS. TOF-MS data were collected in full scan, enhanced ion mode. Structures and APCI-TOF-MS spectra of these selenoamino acids are shown in Figure 2. The observed spectra are similar to those in previous ESI-MS and ESI-MS/MS reports,[16,38] although the degree of in-source fragmentation is higher due to the heated APCI nebulizer. Also, the most prominent peak was typically not the pseudomolecular ion, $[M + H]^+$, but that associated with loss of an amine group, $[M + H - NH_3]^+$. Representative parallel ICPMS and APCI-TOF-MS traces are shown in Figure 3. Even though both ICPMS and APCI-MS are mass-dependent detectors, the traces show different relative peak heights for the same Se compounds because the ICPMS response is also dependent on the transfer efficiency of the analyte through the spray chamber, while the APCI-MS response is dependent on the gas-phase proton affinity of the analyte. Visual inspection clearly shows that the ICPMS peaks elute later and are broader than their corresponding TOF-MS peaks. The Apex spray chamber for the ICPMS has an internal volume of ~150 mL and is thus the likely cause of peak

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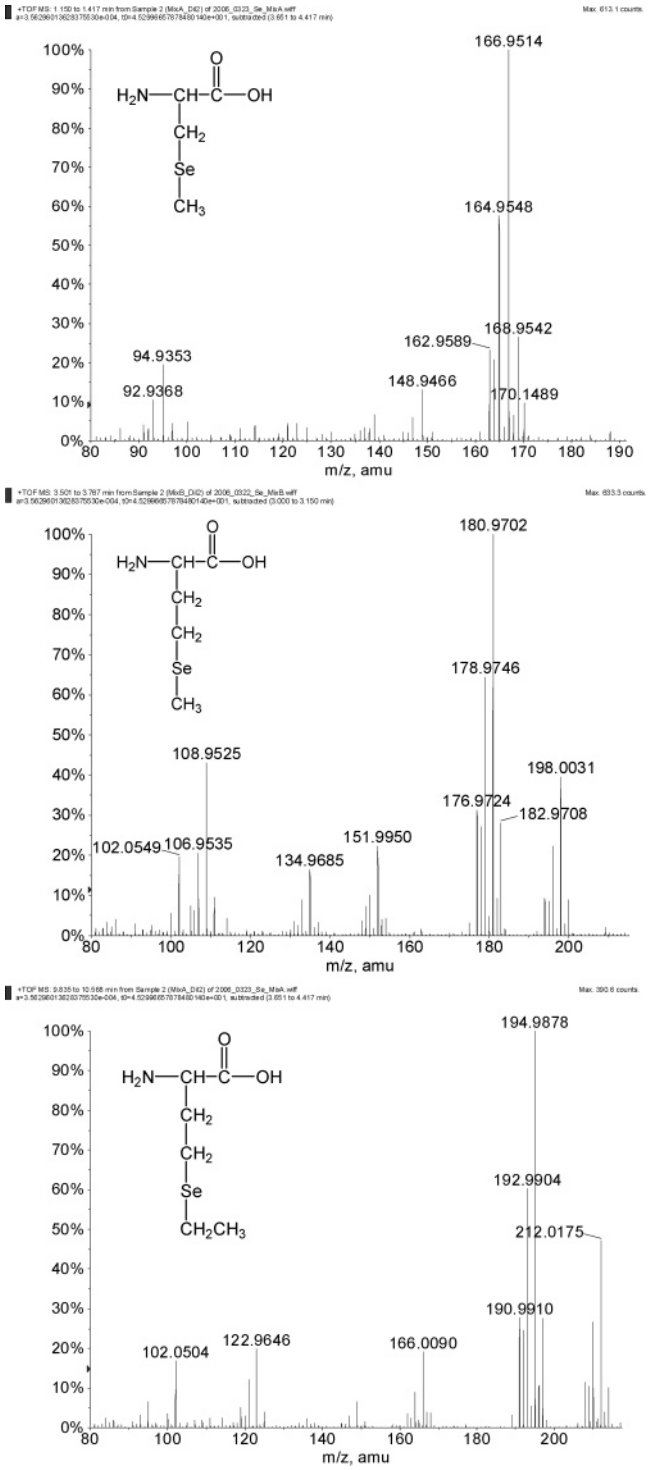

Figure 2. Structures and APCI-TOF-MS spectra of the three Se-amino acid standards: SeMSC (top), SeMet (middle), and SeEt (bottom).

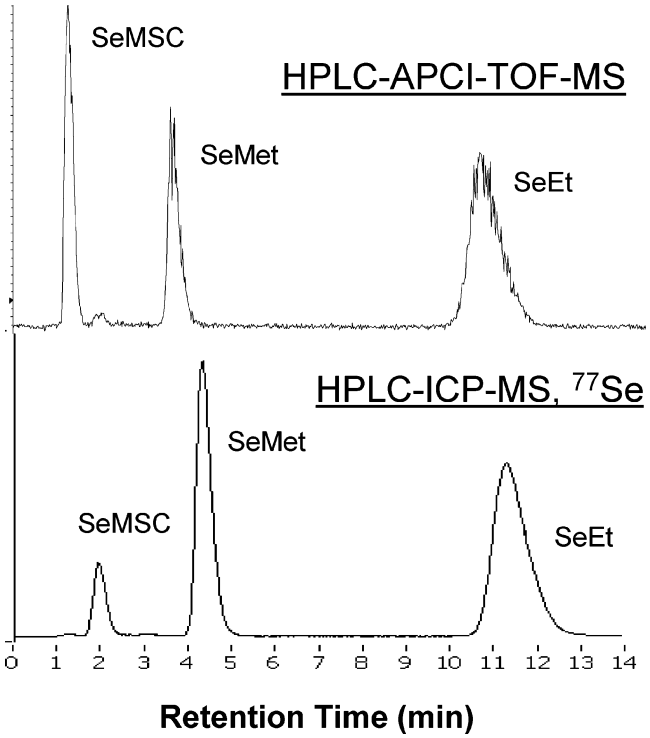

Figure 3. Parallel ICPMS and APCI-TOF-MS traces for the Se-amino acid standards, demonstrating that ICPMS peaks are slightly broader and elute later than their corresponding APCI-TOF-MS peaks. Both traces are on the same time axis. The ^{77}Se trace is shown for the ICPMS, and selected ion chromatograms for $m/z = 167$ (^{80}SeMSC, $[M + H - NH_3]^+$), $m/z = 198$ (^{80}SeMet, $[M + H]^+$), and $m/z = 212$ (^{80}SeEt, $[M + H]^+$) are shown for the TOF-MS traces.

broadening and some of the retention time lag. Also, although the postcolumn flow path volumes to the two mass spectrometers were approximately equal, the flow rate to the ICPMS was 1/3 of the flow rate to the APCI-TOF-MS. Because the Apex spray chamber does not have any unswept dead volumes, we did not observe any statistically significant changes in peak skew, as has been seen in studies with double-pass type spray chambers.[29]

PEMMS of Se Standards: Calculating Correction Factors. To investigate whether the observed retention time shifts and broadening phenomena were reproducible, we ran a 130 ppb standard of SeMSC in triplicate by PEMMS. In the APCI-TOF-MS data set, the $[M + H]^+$ ^{80}Se pseudomolecular ion at $m/z = 184$ was barely detectable, but the isotopomeric cluster associated with the $[M + H - NH_3]^+$ ion and centered on $m/z = 166.95$ (^{80}Se) was prominent. Se-80 fragments at $m/z = 148.95$, 138.95, 122.95, and 94.90 were also observed. There were 22 TOF-MS ions present in each run that were of sufficient intensity for acceptable curve fitting. Average fit parameters for these APCI-TOF-MS ion traces and the parallel ^{77}Se and ^{78}Se ICPMS traces are shown in Table 1. The ICPMS fit parameters of retention time, width, and skew were indeed larger than the corresponding APCI-TOF-MS parameters.

We also noted that the errors associated with curve fits of APCI-TOF-MS peaks were on average much larger than for ICPMS peaks. For example, the average width error (1 SD) was 0.34 s on the APCI-TOF-MS and 0.03 s on the ICPMS. The better reproducibility associated with ICPMS curve fitting is assumed to be a consequence of its higher ionization efficiency, selectivity, and signal-to-noise ratio. This result indicates that the noisier APCI-TOF-MS signal limits the correlation between the two systems in most circumstances.

We attempted to normalize for differences between the two mass spectrometers by introducing correction factors. For the retention time parameter, the time shift between the APCI-TOF-MS and the ICPMS should be dependent only on the additional flow path length and the volume of the Apex spray chamber and,

Table 2. ^{80}Se-Containing TOF-MS Ions for Each Se-Amino Acid Standard Used in Determining the Relationship of S/N to Peak Parametrization Error[a]

compound	maximum no. of TOF-MS ions used	^{80}Se TOF-MS ions
SeMSC	42	184, 167, 149, 139, 122, 108, 95
SeMet	36	198, 181, 152, 135, 109, 95
SeEt	36	212, 195, 166, 149, 123, 109

[a] ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, and ^{82}Se isotopomers were also used.

thus, should be constant. The time shift, Δt, is calculated as

$$\Delta t = R_t^{ICP} - R_t^{TOF} \quad (3)$$

where R_t is the retention time of the ICPMS peaks or mean value of the APCI-TOF-MS peaks for a given component. Δt was calculated to be 43.0 ± 0.1 s for the three replicate SeMSC runs listed in Table 1.

For the width parameter, the increase in peak broadening on the ICPMS trace is likely due to the large diameter of the Apex spray chamber. The width parameter, defined as a_2 in eq 2, is a measure of the standard deviation of the best-fit curve, and the square of the width parameter is its variance. Because the extracolumn broadening is likely also Gaussian in nature, a broadening factor, σ_b, can be calculated from the difference in variances between the ICPMS and APCI-TOF-MS peaks:

$$\sigma_b^2 = W_{ICP}^2 - W_{TOF}^2 \quad (4)$$

where W_{ICP} is the width of the ICPMS peak and W_{TOF} is the mean width of the corresponding APCI-TOF-MS peaks. The mean value of σ_b was 6.19 ± 0.16 s for the three SeMSC runs.

The skew (S) parameter, defined in eq 2 as a_3, was slightly more positive for the ICPMS peak than its corresponding APCI-TOF-MS peaks; i.e., the ICPMS peak had slightly more tailing. The skew correction factor, Δs, was calculated in a fashion similarto Δt.

$$\Delta s = S_{ICP} - S_{TOF,av} \quad (5)$$

The mean value of Δs was 0.26 ± 0.11 for the three SeMSC runs. This small increase in tailing is difficult to perceive by visual inspection.

PEMMS of Se Standards: Robustness of Correction Factors. To test the robustness of calculated correction factors, serial dilutions of SeMSC (0.005−1 ppm as Se), SeMet (0.03−10 ppm), and SeEt (0.06−15 ppm) were run by PEMMS. The PEMMS software was used to determine peak parameters on the ^{77}Se ICPMS trace and for the APCI-TOF-MS peaks listed in Table 2. APCI-TOF-MS peaks with areas less than 200 counts were not used in this experiment. The ICPMS peak parameters (retention time, width, skew) for each run were corrected using their respective calculated correction factors (Δt, σ_b, Δs) and were compared with the mean TOF-MS peak parameters. Results of this experiment for each PEMMS run along with mean absolute peak parameters for each seleno compound are shown in Figure 4.

Table 3. Average Fit Parameters for TOF-MS and ICPMS Ion Chromatograms for Three Replicate Injections of 130 ppb SeMSC[a]

m/z	area (cts)	width (s)	skew (unitless)	ret time (s)
		TOF-MS Data		
90.95	323	4.18 (0.50)	0.72 (0.65)	74.7 (1.2)
91.95	233	4.01 (0.16)	0.55 (0.24)	74.7 (0.6)
92.94	1208	4.20 (0.38)	0.54 (0.15)	74.7 (0.6)
94.94	1979	4.42 (0.13)	0.90 (0.05)	74.8 (0.1)
96.95	477	4.09 (0.53)	1.16 (0.49)	74.0 (1.0)
118.95	243	4.29 (0.59)	0.89 (0.58)	74.3 (1.2)
120.95	701	4.54 (0.39)	1.10 (0.41)	74.3 (0.6)
122.94	666	4.19 (0.07)	0.78 (0.57)	74.1 (1.0)
124.95	446	4.40 (0.41)	0.67 (0.35)	75.0 (1.0)
134.95	251	4.36 (0.74)	1.39 (0.60)	73.7 (1.2)
135.95	450	4.80 (0.47)	0.01 (0.33)	76.0 (0.0)
136.95	900	4.80 (0.40)	0.99 (0.21)	74.3 (0.6)
138.95	560	4.31 (0.43)	0.69 (0.25)	74.7 (0.6)
144.95	269	3.97 (0.40)	0.86 (0.62)	74.0 (1.0)
145.95	711	4.60 (0.40)	0.85 (0.14)	74.7 (0.6)
148.94	2065	4.77 (0.03)	0.90 (0.22)	74.8 (0.4)
150.95	370	4.90 (0.57)	0.56 (0.66)	75.7 (0.6)
162.95	3948	4.77 (0.09)	1.02 (0.07)	74.7 (0.6)
163.95	3480	4.88 (0.16)	0.93 (0.14)	75.0 (0.0)
164.95	12603	4.73 (0.08)	0.96 (0.21)	74.3 (0.5)
166.95	28712	4.69 (0.07)	0.89 (0.18)	74.4 (0.5)
168.95	5007	4.76 (0.07)	0.98 (0.10)	74.3 (0.6)
av SD		0.32	0.33	0.60
		ICPMS Data		
Se-77	8.6×10^6	7.74 (0.02)	1.11 (0.03)	117.6 (0.0)
Se-78	2.7×10^7	7.76 (0.05)	1.09 (0.10)	117.6 (0.0)
av SD		0.03	0.07	0.0

[a] Standard deviations for the fit parameters are reported in parentheses.

The mean difference between the corrected ICPMS retention time and APCI-TOF-MS retention time was within ±2 s in 13 of the 15 PEMMS runs and within ±4 s for all of the runs. The 95% CI was within ±6 s for all runs. There was no dependency of the corrected difference in retention time on injection size or any variation between Se compounds. Thus, a single correction factor, Δt, is appropriate for correcting retention time shifts for all compounds regardless of injection size.

The mean difference between corrected ICPMS width and the APCI-TOF-MS width was not significantly different from zero in any of the PEMMS runs and was not dependent on injection size. However, there was a discernible bias in the mean value of σ_b for different compounds, where SeMet and SeEt had APCI-TOF-MS peaks that were narrower than were predicted by the σ_b correction factor. The origin of this phenomenon is not known; two possibilities are that the treatment of the broadening as Gaussian is inaccurate or that this is an artifact of the HVL curve fit model. However, the magnitude of this effect is relatively small compared to the differences in width. For example, SeMSC has mean width(TOF) of 4.53 s and mean width(TOF) − width(ICP, corrected) of 0.19 s, while the corresponding values for SeEt are 18.31 and −1.53 s. The observed bias is only ~10% of the much larger absolute difference between the widths of SeEt and SeMSC. Therefore, width should be a useful parameter for correlating ICPMS peaks with APCI-TOF-MS peaks.

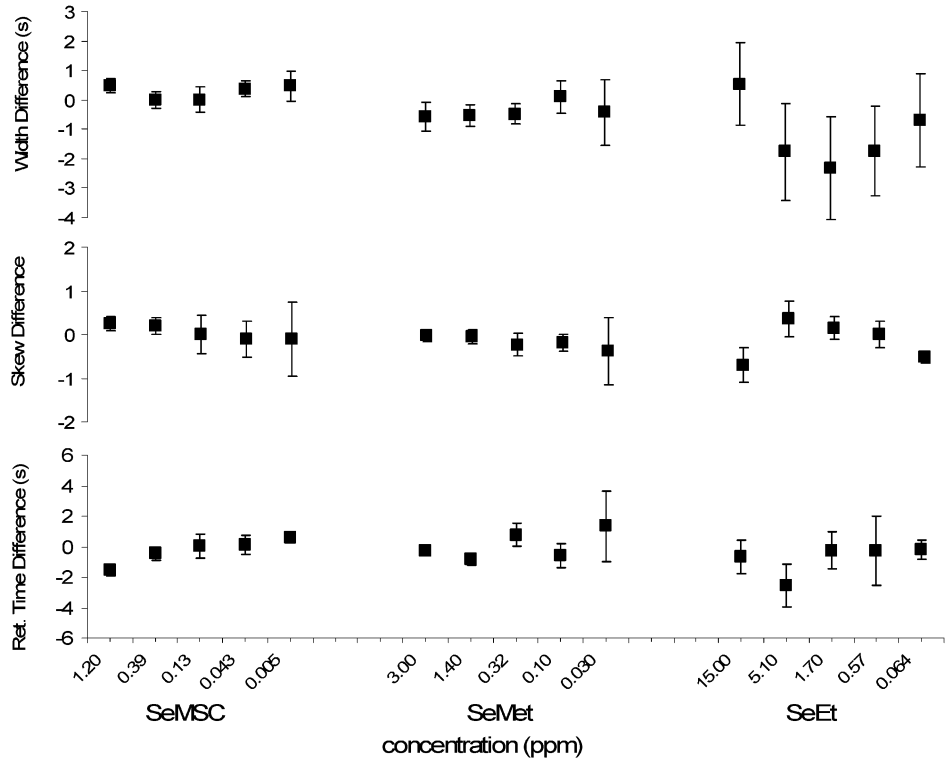

Figure 4. Mean difference between the corrected ICPMS and APCI-TOF-MS best-fit parameters for width (top), skew (middle), and retention time (bottom) for PEMMS injections of Se-amino acid standards over a range of concentrations. The ICPMS parameters were corrected using the factors (σ_b, Δs, Δt) described in the text. The error bars represent one standard deviation. The figure demonstrates that the correction factors between the two MS detectors are largely reproducible and are independent of injection size.

The skew parameter was determined to be too variable to be useful in distinguishing peaks. The range of average values for skew(ICP, corrected) − skew(TOF) was within ±0.5 for 14 of the 15 runs, and the 95% CI ranged over ±2.0. This range for the 95% CI is nearly as large as the range of absolute skew values observed in all of the PEMMS runs (−3 to +4). Furthermore, we observed a decrease in skew at lower injection sizes. Simulations of chromatographic peaks convinced us that skew values are strongly influenced by random noise or coeluting peaks, while retention time and width are much more robust (data not shown). Therefore, we did not use skew as a parameter for correlating ICPMS and APCI-TOF-MS peaks in further experiments.

PEMMS of Se Standards: Normalizing Errors. The previous experiment demonstrated that retention time and width are useful parameters for correlating PEMMS peaks. However, determining the error tolerances for peak profile matching is not straightforward from the data presented in Figure 4. There is a larger standard deviation associated with both retention time and peak width for later eluting, broader peaks (i.e., SeEt) than for earlier eluting, narrower peaks, because broader peaks will have more uncertainty associated with their fit parameters on an absolute scale. Therefore, normalization of the errors associated with peak fit parameters is necessary.

Normalization of the width parameter was accomplished by calculating the percentage difference of the mean APCI-TOF-MS width and the corrected ICP width, width(norm):

$$\text{width(norm)} = \frac{\text{width}_{\text{ICP,corrected}} - \text{width}_{\text{TOF, mean}}}{\text{width}_{\text{TOF,mean}}} \times 100\% \quad (6)$$

The error associated with the retention time parameter was expected to scale with the square root of the retention time. The normalized retention time, RT(norm), was calculated as

$$\text{RT(norm)} = \frac{\text{RT}_{\text{ICP,corrected}} - \text{RT}_{\text{TOF, mean}}}{(\text{RT}_{\text{TOF,mean}})^{1/2}} \quad (7)$$

Width(norm) and RT(norm) and their respective errors for each Se-amino acid injection are shown in Figure 5. The mean values of width(norm) were within ±10% and for RT(norm) were within ±0.1 $s^{1/2}$ for all but one of the runs. The ranges of the 95% CI for both width and RT(norm) are very similar for all three standards, which indicates that the normalization scheme was appropriate. The 95% CI fell within ±30% for width(norm) and ±0.3 $s^{1/2}$ for RT(norm) for all but the most dilute SeMet and SeMSC standard.

In summary, our data indicate that determining retention time and width correction factors, (Δt and σ_b) from a single standard run is appropriate and that the normalized errors for these parameters are constant. We have also demonstrated that Δt and σ_b are independent of injection size and compound.

PEMMS of Se Standards: Signal-to-Fit Error (S/FE) Ratio as a Filter. We wished to identify an appropriate metric for determining whether a proposed match in peak parameters was reliable. We noticed that small APCI-TOF-MS peaks as measured by height or area were usually poorer matches than large peaks. However, peak area or height by itself is an inappropriate metric, because large peaks on noisy backgrounds or with coeluting

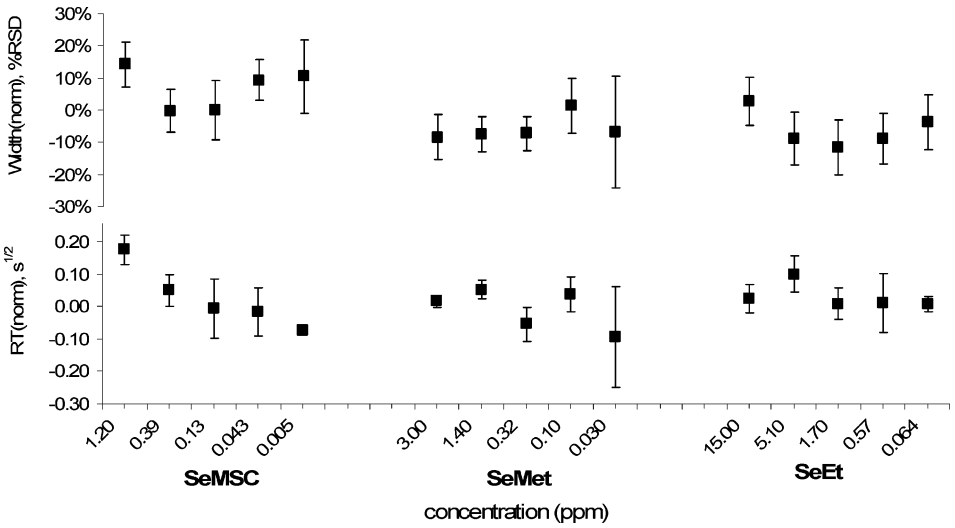

Figure 5. Normalized differences between the corrected ICPMS and TOF-MS best fit parameters for width (top) and retention time (bottom) for PEMMS injections of Se-amino acid standards over a range of concentrations. The equations for calculating width(norm) and RT(norm) are described in the text. The error bars represent one standard deviation. The figure demonstrates that the errors associated with normalized correction factors are compound-independent.

chemical noise should result in larger errors in fit parameters than small peaks with low backgrounds. We defined a metric, S/FE ratio, as the peak area divided by the root-summed mean-squared error of the curve fit, or

$$\mathrm{S/FE} = \frac{\text{area}}{\left(\sum_j (I_{\mathrm{obs},j} - I_{\mathrm{fit},j})^2\right)^{1/2}} \qquad (8)$$

where area is determined by the HVL curve fit, $I_{\mathrm{obs},j}$ is the measured signal intensity at time j for the APCI-TOF-MS ion chromatogram, and $I_{\mathrm{fit},j}$ is the intensity of the best-fit curve at time j. The S/FE metric is not the same as a signal-to-noise metric as it measures not only random noise but also the appropriateness of the HVL model. However, a poor fit with the HVL model would likely indicate the presence of a peak shoulder or signal enhancement/suppression and, thus, is grounds for rejecting the peak.

SeEt, SeMet, and SeMSC were run in triplicate by PEMMS. S/FE, RT(norm), and width(norm) were determined for 247 APCI-TOF-MS ion chromatograms associated with the Se standards. RT(norm) and width(norm) are plotted versus S/FE for each TOF-MS ion chromatogram in Figure 6. The plot of width(norm) versus S/FE shows an increase in error around S/FE < 25. There is also degradation in the accuracy of the width parameter at low S/FE, and the mean value of width(norm) for peaks of S/FE < 20 is $-11.9 \pm 15.9\%$. The retention time fit parameter is much more robust at low S/FE. A small increase in error at S/FE < 25 is seen in the plot of RT(norm) versus S/FE, and corresponding degradation in accuracy of the retention time parameter is not observed. Because the errors in determining width and retention time were relatively constant at S/FE > 25, this S/FE level was chosen as the benchmark for accepting a match of an APCI-TOF-MS ion chromatogram with an ICPMS peak. The standard deviations of RT(norm) and width(norm), $\sigma_{\mathrm{rtn}} = 0.075\ \mathrm{s}^{1/2}$ and $\sigma_{\mathrm{wn}} = 12.5\%$, respectively, were calculated from APCI-TOF-MS data points with S/FE > 25. These errors were used as tolerances in the next PEMMS experiment.

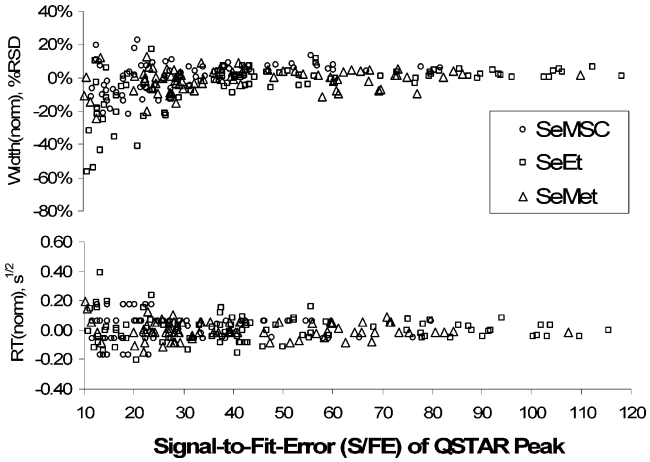

Figure 6. Plots of the RT(norm), and width(norm) versus S/FE ratios for 247 APCI-TOF-MS ion chromatograms detected during three replicate runs of SeEt, SeMet, and SeMSC by PEMMS. The errors associated with RT(norm) and width(norm) are constant for S/FE > 25.

PEMMS with Yeast-Spiked Se Standards: Finding the "Needle-in-the-Haystack". To test the ability of our PEMMS technique to detect metal species in a complex background, a 0.01 M HCl extract of unselenized yeast was prepared. The yeast extract was added to SeMet and SeMSC standards to generate Se standards in an artificially complex matrix. The final concentrations of the SeMet and SeMSC spiked standards were 30 and 5 ppb as Se, respectively. Selenized yeast is a common subject of Se speciation reports,[2] and low ppb levels of Se species in an unselenized yeast matrix is a realistic simulation of a difficult speciation analysis if the species were unknown. The PEMMS protocol was followed; the ICP retention time and width of the SeMet and SeMSC peaks were determined by curve fitting and were corrected by using the Δt and σ_{b} correction factors described earlier. Peak detection and curve fitting was attempted on every APCI-TOF-MS m/z trace within a 40-s window defined by the expected retention time. APCI-TOF-MS ion chromatograms with S/FE < 25 were rejected. Tolerances on the corrected retention

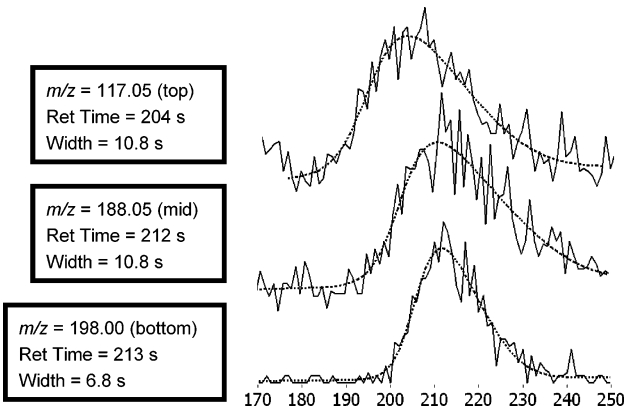

Figure 7. Three APCI-TOF-MS selected ion chromatograms for PEMMS runs of SeMet in a yeast matrix, demonstrating that subtle differences between selected ion chromatograms are quantifiable by peak fitting. The solid lines represent the raw traces and the smooth dashed lines represent the least-square curve fits. The target APCI-TOF-MS parameters derived from the HPLC-ICPMS trace are width = 7.2 ± 0.8 s and retention time = 211.8 ± 2.1 s. m/z = 198 (bottom) corresponds to the $[M + H, {}^{80}Se]^+$ peak and also matches the target APCI-TOF-MS parameters. Yeast matrix components m/z = 117.05 (top) and 188.05 (middle) coelute with SeMet but do not have matching parameters and thus are rejected by PEMMS. The subtle differences in retention time and peak width are sufficient to distinguish the target 198 ion chromatogram from the chemical noise.

time and width were calculated using the previously described error normalization methods.

The HPLC-ICPMS of SeMet in the spiked standard had an ICP retention time of 254.8 and width of 9.52, which leads to target APCI-TOF-MS parameters of 211.8 ± 1.1 s for retention time and 7.23 ± 0.8 s for width, where the errors (one SD) were calculated using eqs 6 and 7. Peaks were detected and fit in 49 APCI-TOF-MS m/z traces. Selected ion chromatograms for three of these ions are shown in Figure 7 along with their retention time and width parameters. The three ions at m/z = 117.05, 188.05, and 198.00 all coelute within 10 s of each other. Standard speciation methods would have difficulty determining which of these ions contained Se and corresponded to the HPLC-ICPMS peak. The ions are not sufficiently resolved to collect them in separate fractions, and the peak retention times are difficult to determine by visual inspection. However, precise determination of the peak retention times and widths by curve fitting (Figure 7) reveals that the parameters associated with m/z = 198.00 (RT = 213 s, W = 6.8 s) closely match the target parameters, while the other two ion traces are poorer matches.

A plot of retention time versus width for each detected APCI-TOF-MS peak is shown in Figure 8 along with error ovals for the target peak parameters based on the ICP peak. Four of these ions (m/z = 198, 181, 179, and 109) are from SeMet, while the other 45 ions were assumed to be chemical noise from the yeast. All four APCI-TOF-MS m/z traces had parameters that fell within ±2 SD of the target parameter, and 44 of the 45 m/z traces associated with chemical noise had parameters that fell outside of the target region.

The HPLC-ICPMS of SeMSC in the spiked standard had an ICP retention time of 115.7 s and width of 7.3 s. The target APCI-TOF-MS parameters were calculated as 72.7 ± 0.6 s for retention time and 3.9 ± 0.5 s for width (target ± 1 SD). Peaks were detected and fit in 54 APCI-TOF-MS m/z traces. Two of these

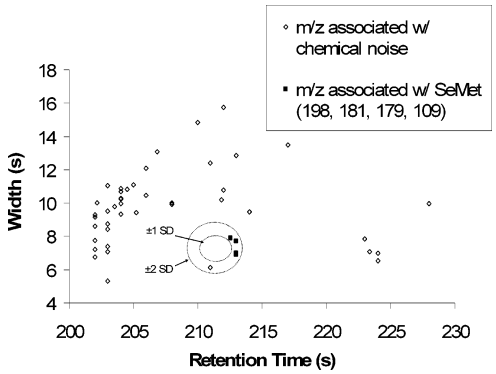

Figure 8. Plot of width versus retention time of APCI-TOF-MS ion chromatograms for PEMMS run of 30 ppb SeMet in yeast matrix. The target APCI-TOF-MS parameters (width = 7.2 ± 0.8 s, retention time = 211.8 ± 2.1 s) were determined from the HPLC-ICPMS parameters. The ovals define regions one and two standard deviations from the target parameters. Of the five ion chromatograms that fall within two standard deviations of the target, four are from SeMet (m/z = 198.0, 181.0, 179.0, 109.95)

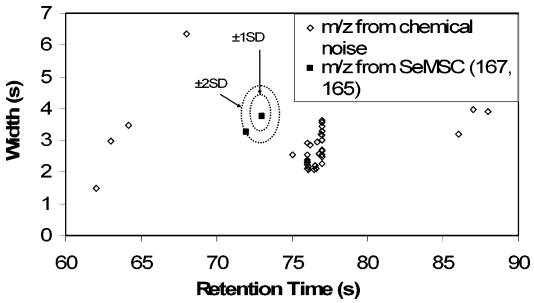

Figure 9. Plot of width versus retention time of APCI-TOF-MS ion chromatograms for PEMMS run of 5 ppb SeMSC in yeast matrix, where the ovals define regions one and two standard deviations from the target parameters. The two ion chromatograms that fall within two standard deviations of the target are associated with SeMSC (m/z = 166.95, 164.95). The target APCI-TOF-MS parameters were width = 3.9 ± 0.5 s and retention time = 72.7 ± 0.6 s.

ions, m/z = 165 and m/z = 167, were known to come from SeMSC, while the other 45 ions were assumed to be chemical noise from the yeast. A plot of retention time versus width for each detected APCI-TOF-MS peak is shown in Figure 9 along with error ovals for the target peak parameters based on the ICP peak. The two target SeMSC ion traces had parameters that fell within ±2 SD of the target parameter, and all of the ion traces associated with chemical noise had parameters that fell outside of the target region. This demonstrates that the retention time and width parameters from the ICPMS data can be used as an effective filter of the APCI-TOF-MS data when the two are run in parallel.

Following detection of the APCI-TOF-MS hits by PEMMS, the Analyst QS software was used to inspect the spectrum at the appropriate retention time to confirm the presence of the candidate ions and to determine their exact mass. Figure 10 shows the background subtracted APCI-TOF-MS spectrum at 71−75 s for SeMSC in the yeast matrix. The two APCI-TOF-MS ions detected by the PEMMS algorithm are observed at masses 166.9549 and 164.9561 u, within ±35 ppm of the exact mass. The signal intensity of these peaks was <1% of the base peak height, and associating these peaks with the ICPMS signal without the information from PEMMS or knowledge of the sample would have been very difficult.

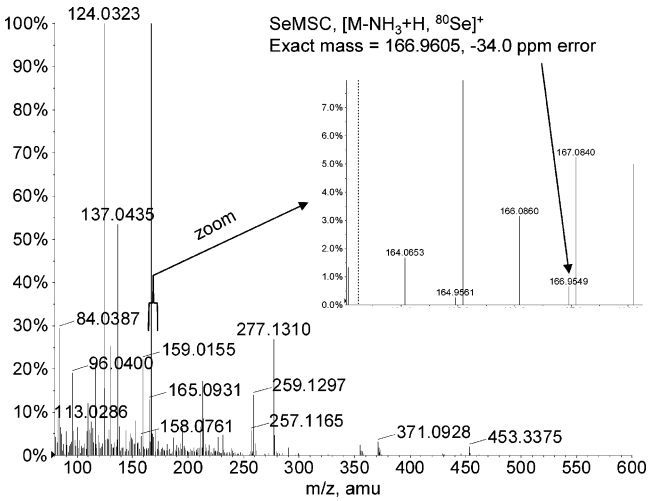

Figure 10. Background-subtracted APCI-TOF-MS spectrum of 5 ppb SeMSC in yeast matrix. The two peaks of interest, $[M - NH_3^+H, {}^{80}Se]^+$ at $m/z = 166.95$ and $[M - NH_3^+H, {}^{78}Se]^+$ at $m/z = 164.95$, are at less than 1% of the base peak height but could be clearly detected in Figure 9 by the PEMMS technique.

Caveats to the PEMMS Approach. PEMMS relies on the premise that changes in peak profile on either mass spectrometer should be dependent primarily on column interactions. This approach will be compromised if the measured analyte response is altered on either system due to signal overlaps, suppression, or enhancement. For example, coeluting ICPMS peaks cannot be readily fit by the single HVL function in eq 2 (data not shown). However, a potential solution to this would be to use a double HVL function to fit the two peaks simultaneously. We have demonstrated previously that coeluting gas chromatography-combustion isotope ratio mass spectrometry (GC-C-IRMS) peaks with less than 40% overlap can be fit with excellent precision.[39] Because the precision demands for GC-C-IRMS are more exacting than for PEMMS, we expect that a similar approach should be successful for PEMMS. A more difficult problem would be if the degree of overlap on the ICPMS was so severe that the peak could not be recognized as a double peak. In this case, the fit parameters for this composite peak will not match APCI-TOF-MS ion chromatograms. However, if an initial hit does not occur, inspection of the APCI-TOF-MS peaks eluting on either side of the target retention time may still assist in the PEMMS match. A final pitfall with the PEMMS approach is that coeluting interferences can suppress or enhance the target analyte ion signal, altering its peak parameters. If interferences are suspected, an infusion experiment with a model standard should be conducted via a T-connection to detected regions where suppression is occurring.[40]

CONCLUSIONS

We have demonstrated that chromatographic peak profiles on a PEMMS are well correlated for three Se-amino acid standards over a range of concentrations. We have also demonstrated with Se-amino acids in a yeast matrix that the HPLC-ICPMS peak profiles can be used to dramatically reduce chemical noise in the HPLC-APCI-TOF-MS data set. We can envision many permutations and improvements on this prototype PEMMS system. A direct injection nebulizer for coupling the ICPMS would result in less extracolumn broadening of the ICPMS peaks, which would improve resolution and likely mitigate the need for a width correction factor.[41] An ESI source will be more appropriate for speciation of thermally labile compounds such as metal−ligand complexes, although care must be taken to prevent distortion of peak shape by ionization suppression. The use of a nanosplitter to direct submicroliter flow rates to the ESI is an attractive option as low flow rates typically result in less ion suppression.[42] Alternatively, the HPLC system could be replaced by another separation technique such as GC or CE. We are currently exploring many of these possibilities in our laboratory. Finally, we have recently presented results that show the application of PEMMS to speciation of a Se-enriched broccoli supplement and the tentative identification of at least three novel low molecular weight Se species.[43]

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