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Rapid Separation and Quantitative Analysis of Peptides Using a New Nanoelectrospray-Differential Mobility Spectrometer−Mass Spectrometer System

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Differential mobility spectrometry (DMS) (see Buryakov, I. A.; Krylov, E. V.; Nazarov, E. G.; Rasulev, U. Kh. *Int. J. Mass Spectrom. Ion Processes* 1993, *128*, 143−148), also commonly referred to as high-field asymmetric waveform ion mobility spectrometry (FAIMS) (see Purves, R. W.; Guevremont, R.; Day, S.; Pipich, C. W.; Matyjaszczyk, M. S. *Rev. Sci. Instrum.* 1998, *69*, 4094−4105), is a rapidly advancing technology for gas-phase ion separation. The interfacing of DMS with mass spectrometry (MS) offers potential advantages over the use of mass spectrometry alone. Such advantages include improvements to mass spectral signal-to-noise, orthogonal/complementary ion separation to mass spectrometry, enhanced ion and complexation structural analysis, and the potential for rapid analyte quantitation. In this report, we investigate the use of our nanoESI-DMS-MS system to demonstrate differential mobility separation of peptides. The formation of higher order peptide aggregate ions (ion complexes) via electrospray ionization and the negative impact this has on DMS peptide separation are examined. The successful use of differential mobility drift gas modifiers (dopants) to reduce aggregate ion size and improve DMS peptide ion separation is presented. Following optimization of DMS peptide separation conditions, we examined next the feasibility of a new analytical platform which uses direct sample infusion with nanoESI-DMS-MS for ultrarapid analyte quantitation. Quantitation of a selected peptide from a semicomplex peptide mixture is presented. Initial feasibility results with this new approach demonstrate good accuracy and reproducibility, as well as an absolute mass sensitivity of 6.8 amol and a minimum dynamic range of 2500 for the peptide of interest. This report offers a first look at utilizing nanoESI-DMS-MS to create an ultrarapid (under 5 s) quantitative analysis platform and its potential in the high-throughput arena. Each ion separation technique, DMS and MS, offers orthogonal ion separation to one another, enhancing the overall specificity for this quantitative approach.

Differential mobility spectrometry,[1] is a rapidly advancing technology for gas-phase ion separation. DMS has the potential to be used as a stand alone analyte separation technique such as LC or GC. Many researchers have focused on interfacing DMS to mass spectrometry due to its continuous ion separation capabilities under atmospheric pressure gas-phase conditions and the detection specificity offered by mass spectrometry. Of particular interest has been the use of DMS combined with mass spectrometry for the enhancement of peptide analysis. Guevremont and others have demonstrated improvements in peptide analysis with an ESI-FAIMS-MS system over conventional ESI-MS by reducing chemical/background ion noise and consequently improving the signal-to-noise ratio for peptide ions.[4−9] Within the past 7 years, many researchers have identified the formation of multiply charged noncovalently bound peptide aggregate ions formed via the ESI process.[10−14] In this report, we investigate the

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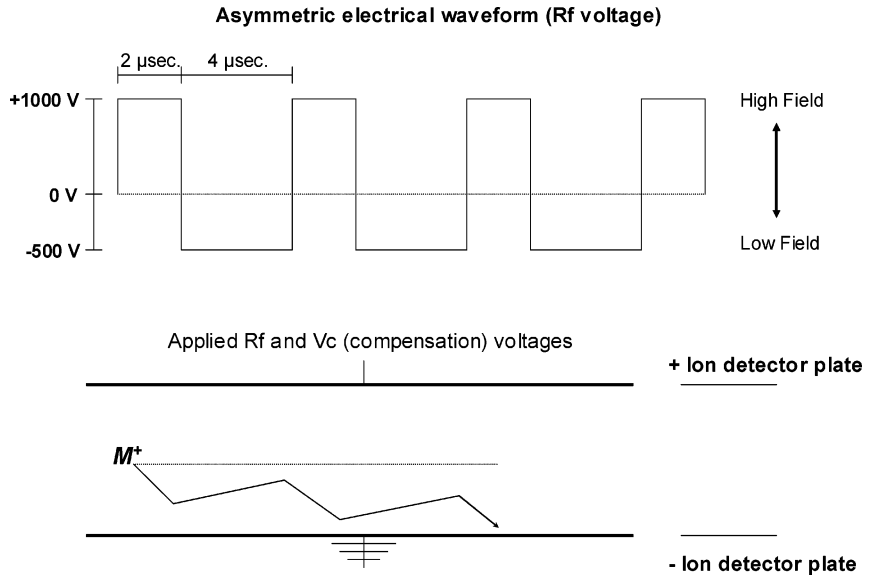

Figure 1. Ion trajectory between two parallel electrode plates as it experiences the asymmetric electrical waveform of +1000 V for 2 μs then −500 V for 4 μs.

role of peptide clustering or aggregation from the ESI process in DMS separation and the capability for selective enhancement of peptide ion separation in nanoESI-DMS via the introduction of drift gas modifiers/dopants. Following the discussion on differential mobility peptide separation, a new analytical platform for ultrarapid quantitation using direct sample infusion and nanoESI-DMS-MS is explored.

DMS is related to, but is fundamentally different from, conventional time-of-flight ion mobility spectrometry (IMS). In conventional IMS, ion identification is related to effective ion cross section, resulting in differences in flight times. In DMS, ion identification is related to changes in effective cross section based on the propensity of the ion to cluster/decluster, resulting in differences in applied compensation voltage values. In DMS an asymmetric electric field waveform is applied to two parallel electrode plates enabling the ions to pass through in a continuous, nonpulsed, manner. The electrical waveform consists of a high-field duration (up to 30 000 V/cm) of one polarity and then a low-field duration (less than 1000 V/cm) of opposite polarity, such that the integrated voltage−time being applied to the electrode is zero.[15] Figure 1 demonstrates the high and low voltages of opposite polarity applied to generate the asymmetric electrical waveform (identified as an rf voltage, correlating to the high-voltage value). As can be seen, after one cycle of the waveform the average voltage applied to the electrode is zero; however, the ion's mobility in this asymmetric electric field displays a net movement toward the bottom (grounded) electrode plate. Since an ion may experience a net mobility toward one of the electrode plates during its travel between the plates, a dc compensation voltage (Vc) is applied to maintain a safe trajectory through the plates without striking them. In this manner the DMS sensor acts as a tunable ion filter, where varying the applied waveform (rf) and compensation voltages (Vc), allows for selective ion transmission through the sensor. In DMS ions are separated at pressures sufficient for the occurrence of collisions between ions and the neutral gas molecules. The smaller the ion the fewer collisions it will experience as it is pulled through the drift gas. Because of this, ion cross-sectional area plays a significant role in its mobility through the drift gas.[15,16]

In 1991 Meng and Fenn published the first findings for the formation of noncovalent amino acid cluster ions by electrospray ionization.[17] For proline, Nemes, et al., observed the ESI formation of cluster ions as large as Pro_{29}^{3+} with significant abundance.[18] Similar aggregate ion formations have also been observed for peptides. Zhan et al., identified that a substantial fraction of the ESI-generated leucine enkephalin peptide, singly charged, monomer ion signal was actually being generated from the doubly charged dimer ion.[14] In addition, work by Jurchen et al., identified that 17-Mer^{4+} and 19-Mer^{5+} aggregate ions of leucine enkephalin were formed with high abundance using a nanoESI-FT-MS system.[10] Counterman et al., utilized an electrospray-ion mobility/time-of-flight mass spectrometer to demonstrate the formation and separation of multiple peptide aggregates with the structure $(M_n + nH)^{n+}$ and the same m/z value as the singly charged monomer ion.[19] These studies clearly demonstrate the complexity of ions which can be generated by ESI for a single peptide analyte. To further convolute things, in addition to the ion complexity generated through homoaggregate peptide ion formation, researchers have identified the occurrence of heteroaggregate peptide ions from electrospray ionization of peptide mixtures.[11,13] The degree to which peptide aggregate ion formation has been observed has led to the idea that the combination of hetero- and homoaggregate ions may contribute to the background ion signal commonly observed in ESI-MS analysis of complex peptide mixtures.[11] While liquid chromatography coupled to ESI-MS can provide peptide separation prior to ESI resulting in a decrease of

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heteroaggregate ion formation, ion mobility and DMS are post-ESI separation techniques and would therefore not be expected to provide a decrease in aggregate formation.

Guevremont and Purves observed the formation of numerous leucine enkephalin aggregate ions by ESI, many with the same m/z, and utilized an ESI-FAIMS-MS system to separate various forms of the aggregate ions. In addition to the ESI-FAIMS-MS system, they utilized an electrometer-based ion detection with their FAIMS system and observed that a large fraction of the total electrospray ion current was likely the result of large cluster ions which exceeded the m/z limit of the mass spectrometer.[5] In a recent study we demonstrated the potential to decrease small molecule ion clustering during DM separation by introducing polar molecule vapors into the differential mobility drift gas.[20] In the present study we screen various polar drift gas modifiers (dopants), at different concentrations, to improve the peptide separation capabilities of our nanoESI-DMS-MS system. We hypothesize that the polar drift gas modifiers interact with the peptide clusters in the gas phase and compete with the H-bonding and van der Waals forces driving the clustering. In turn, introduction of the modifiers facilitates cluster dissociation and reduces ion cross sections, thereby increasing DM separation capabilities. Following optimization of DMS peptide separation conditions, we examined next the feasibility of using direct sample infusion and nanoESI-DMS-MS for ultrarapid quantitation of a selected peptide from a semicomplex peptide mixture.

EXPERIMENTAL SECTION

Instrumentation. The nanoESI-DMS-MS system encompasses a modified Sionex Corporation microDMx differential mobility sensor (Sionex SDP-1), a Waters ZQ single-quadrupole detector, and a custom-made nanospray source. The ESI source consists of a Valco microconnector union attached to a Newport micromovement plate with *XYZ* directional control. The ESI voltage is applied to the union where a liquid−liquid junction is created between PEEK tubing and a Proxeon nanobore steel emitter (part no. ES301). The small size of the DMS sensor unit, approximately 2 inches in length, 1 inch in height, and $^1/_4$ inch in width, simplified interfacing to the mass spectrometer inlet. Samples were directly infused into the nanospray source via a Harvard syringe pump at a flow rate of 500 nL/min. Sample analysis was performed in positive mode nanospray at a capillary voltage of 2.6 kV. For optimization of peptide separation, a cone voltage of 30 V was applied to the inlet cone, and the inlet source temperature in the mass spectrometer was set to 50 °C. For the quantitation studies a cone voltage of 40 V was applied to the inlet cone, and the inlet source temperature was set to 70 °C. Figure 2 shows a schematic of the ESI-DMS-MS interface design. As shown in Figure 2, a gas line is introduced into the DMS sensor opposite of the nanospray inlet. This provides an introduction site for the drift gas modifier vapors into the sensor as well as the curtain gas for the nanospray inlet. The two DMS electrometer detector plates immediately downstream from the separation electrodes provide an ion signal for both positive and negative ions and were used to generate total positive ion DMS dispersion plots and DMS spectra. In the present design, a hole was

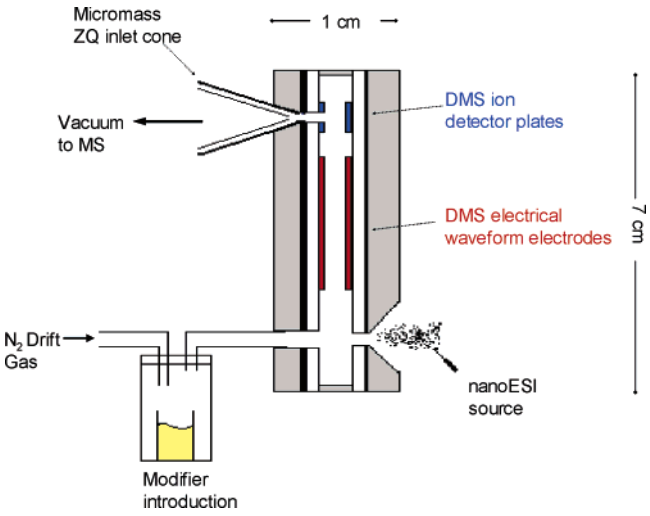

Figure 2. Schematic of the nanoESI-DMS-MS interface.

introduced into the positive ion detector plate to allow for ion transmission into the mass spectrometer. Despite the hole, an ion signal was still generated by the detector plate. The detector plates are biased +5 and −5 V depending on the ion signal polarity desired, with positive polarity assigned to the detector plate with the hole.

The vacuum generated by the mass spectrometer provided the gas flow (measured at approximately 1 L/min) through the sensor and into the mass spectrometer. The gas line opposite the sensor inlet had a constant flow of ultrahigh-purity nitrogen at approximately 0.7 L/min (with or without the addition of modifier). The drift gas modifiers (dopants) were introduced at an approximate concentration of either 150 or 8000 ppm of the total gas flow through the sensor. The design and operational details of the Sionex Corporation differential mobility sensors have been described previously.[15,21,22] For this study, the sensor was operated at ambient laboratory temperature.

Materials and Reagents. The following nine peptides were used throughout the study: angiotensinogen fragment 11-14 (MW 481.55), Glu-fibrinopeptide B (Glu-fib) (MW 1569.67), neurotensin (MW 1671.91), bradykinin (MW 1059.56), angiotensin I (MW 1295.68), angiotensin II (MW 1045.53), substance P (MW 1346.73), vasotocin (MW 1049.45), and bradykinin fragment 1-5 (MW 572.66), all purchased from Sigma-Aldrich Corp., St. Louis, MO. HPLC grade water (Sigma), HPLC grade methanol (Sigma), and formic acid (Sigma) were used for sample preparation. Samples used for optimization of separation were prepared in a solution of 80/20 water/methanol with 0.1% formic acid, while those used for quantitative analysis were prepared in a solution of 50/50 water/methanol with 0.1% formic acid. A sample matrix mixture of seven peptides at 10 nmol/mL each was prepared for the quantitation study and consisted of all of the above-mentioned peptides except for angiotensinogen fragment 11-14 and bradykinin fragment 1-5. The angiotensinogen fragment 11-14 was spiked into the mixture and quantified against a reference standard. Methanol, 2-propanol, and 2-butanol were tested as the various drift gas modifiers (Sigma).

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Procedures. For each sample condition tested, a DMS dispersion plot was generated from the sensor's positive ion detector plate signal and by scanning compensation voltages (Vc) from −20 to +5 V for each rf voltage ranging from +500 to +1500 V, in 10 V increments. In most cases, as the rf voltage is increased, the mobility/velocity of positive ions away from the rf electrode toward the ground electrode is increased, requiring a larger Vc of opposite polarity to be applied to the rf electrode for safe travel to the detector plate. A scan rate of 1.03 s/scan was used for each Vc scan, consisting of 100 steps between −20 and +5 V, enabling an entire dispersion plot to be generated in approximately 100 s. From the dispersion plot data a DMS spectrum (Vc vs ion signal) can be generated for a given rf voltage. For each DMS spectrum, mass spectra were collected at selected Vc values across the DMS spectral range. Each Vc-selected mass spectrum consisted of an averaged 30 s worth of 0.4 s mass scans for the m/z range of 0−2000. The DMS spectra provided are generated from the DMS detector plate ion signal and may contain cluster/aggregate ions of high mass which are outside the mass spectrometer's detection range. Mass spectra were also collected for each sample condition with the DMS turned off, allowing all the ions to enter the mass spectrometer.

The generation of the 10 s Vc scans used for the quantitative analysis required the use of faster MS scan times. The MS m/z scan window was limited to m/z 481−574, enabling a scan time of 0.02 s. The Vc scan range was from −15 to 0 and consisted of 100 steps of 0.15 V each , at 0.1 s each. Six replicate Vc scans were collected for each sample. The selected ion signals of interest, m/z 482 for the angiotensin fragment or m/z 573 for the bradykinin fragment, were extracted from the collected data to generate the selected ion DMS spectral peaks. The six replicate peaks were integrated for total peak area. The average peak area and percent residual standard deviation values were calculated.

RESULTS/DISCUSSION

An initial investigation to determine whether the use of a selected drift gas modifier at the appropriate concentration could improve DMS separation of peptide ions is presented. Upon selection of an ideal drift gas modifier, the capability to separate individual MH^+ peptide ions from a mixture of three peptides is demonstrated. Finally, we present the use of our nanoESI-DMS-MS system for creating an ultrarapid analyte quantitation platform, by combining direct sample infusion and rapid Vc scanning for accurate peptide quantitation from a semicomplex synthetic mixture of peptides.

Separation Optimization. The angiotensinogen fragment 11-14 peptide was selected as a test compound to establish the optimum parameters for peptide ion "selection" in the DMS sensor. Three different drift gas modifiers at two different concentrations were screened for their effects on the DMS ion selection of the ang fragment peptide. DMS dispersion plots and selected Vc point mass spectra were collected to determine the ideal Vc point corresponding to the maximum MH^+ ion signal of the ang fragment. The extent to which the angiotensin singly charged monomer ion, m/z 482, was shifted to a larger negative Vc for a given rf voltage was monitored for each condition. It is presumed that the greater the shift away from a Vc of zero to a larger negative Vc value, the greater the decrease in clustering of the peptide aggregate ions, resulting ultimately in an improved drift gas medium for separation between individual peptides.

Figure 3A−D provides the DMS dispersion plots, DMS spectra at rf = 1200 V, and selected Vc point mass spectra for the maximum angiotensinogen fragment monomer ion signal of a 0.05 mg/mL sample, without (Figure 3A) and in the presence of approximately 150 ppm each of three different drift gas modifiers, methanol (Figure 3B), 2-propanol (Figure 3C), and 2-butanol (Figure 3D). The dispersion plots on the left side of Figure 3 are plotted as rf voltage in the y-axis (500−1500 V), compensation voltage in the x-axis (−20 to +5 V), and ion intensity is reflected in the plot color (red equaling no signal and blue the most intense signal). The dispersion plots provide a comprehensive view of DMS ion separation for a given sample. The observed bands (tracts) in the dispersion plots demonstrate unique differences in DMS ion separation for the different sample conditions presented in Figure 3. As demonstrated in Figure 3A, without any modifier the dispersion plot contains only one low-resolved tract, reflecting limited ion separation. The maximum ang fragment 482 m/z ion signal for the DMS spectrum of Figure 3A is found at a Vc of −0.5 V. Parts B−D of Figure 3 demonstrate that the introduction of the drift gas modifiers at 150 ppm produce new tracks in the dispersion plots. Each of the drift gas modifiers induced an analyte peak shift toward a greater negative Vc value, with 2-propanol providing the greatest shift to −3.5 V, while both methanol and 2-butanol provide a shift to −2.8 V. Of significant interest in Figure 3 is the reduction of the mass spectral ion signal, in the m/z range of 1400−2000, as the optimum Vc point of the protonated angiotensin monomer ion is shifted to larger negative values upon addition of the drift gas modifiers (compare spectra in Figure 3B−D to that in Figure 3A). We hypothesize that the ion signal around Vc = 0, observed for all of the DMS spectra in Figure 3, is a result of higher order peptide aggregate ions, resulting in what mass spectrally appears as high m/z background ion noise. Much of the ion signal observed in this Vc region of the DMS plots may be generated from ions above the m/z detection limit of our mass spectrometer, m/z 2000. The use of the drift gas modifiers does not eliminate the ion signal in the Vc = 0 range of the DMS spectra, indicating that dissociation of the peptide aggregates into monomer ion units is not complete. We believe that the drift gas modifiers interact with the peptide units at the surface of the aggregate ions and, in doing so, compete with the forces that hold the clusters together. In this manner the drift gas modifier facilitates the release of the more loosely bound peptides from the outer layer of the peptide aggregate, as either smaller aggregate or monomer ions. Upon release of the smaller aggregate or monomer units, the drift gas modifier molecules would solvate the H-bonding sites on the aggregate or monomer ions reducing the chance for reclustering of the peptides, thereby enabling enhanced DM separation via reduction in ion cross section.

To improve peptide−modifier interaction and enhance declustering of peptide aggregates, the concentration of the drift gas modifiers was increased from 150 to 8000 ppm. The resulting DMS dispersion plots, DMS spectra (rf = 1000 V), and selected Vc point mass spectra for the maximum protonated angiotensin monomer ion signal of the same sample as in Figure 3, are shown in Figure 4A−C. An increase in drift gas modifier concentration resulted in

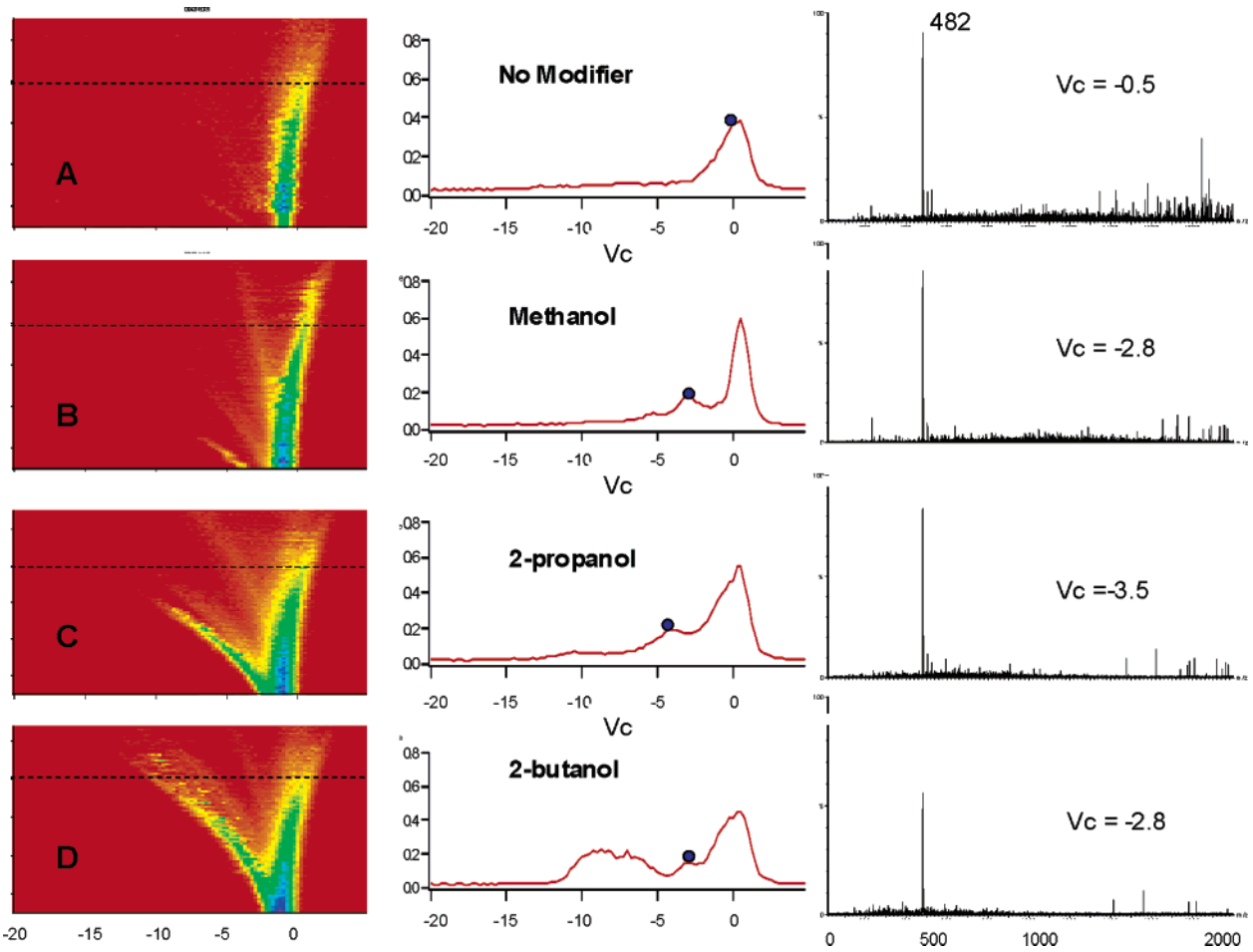


Figure 3. Examples of a 0.05 mg/mL angiotensin fragment 11-14 sample DMS dispersion plot, DMS spectra (rf = 1200 V), and selected Vc point mass spectra (normalized to the same ion signal scale) for the maximum MH^+ *m/z* 482 ion signal without any drift gas modifier (A), 150 ppm methanol modifier (B), 150 ppm 2-propanol modifier (C), and 150 ppm 2-butanol modifier (D).

significant shifts of the *m/z* 482 monomer ion to greater negative Vc values and, perhaps most significantly, at a lower rf setting than that obtained with drift gas modifier concentrations of 150 ppm. Since the use of 8000 ppm of 2-butanol provided the greatest shift to a more negative Vc (−6.8 V), these conditions were selected for all subsequent DMS separation experiments. Figure 5 demonstrates that the signal observed in the low Vc range of the DMS spectra is a result of high *m/z* background ions and why the mass spectra shown in Figure 4 represent analyte separation away from this high *m/z* background signal. Parts A and B of Figure 5 show additional selected Vc point mass spectra collected for the analysis shown in Figure 4A (8000 ppm methanol, rf = 1000). The ion signals for the higher *m/z* ions (1400−2000) are greater at the lower Vc of −1.3 (Figure 5B), than the Vc of −3.3 (Figure 5A). Following the optimization of drift gas modifier conditions for peptide separation, we investigated next the potential to separate individual peptide ions by DMS from a mixture of three peptides.

Analysis of Peptide Mixture. The drift gas modifier 2-butanol, at a concentration of 8000 ppm, was used to enhance the DM separation of a simple mixture of three peptides, ang fragment, Glu-fib, and neurotensin, at 0.05 mg/mL each. Figure 6A shows the DMS dispersion plot, demonstrating one main tract, for this sample without the use of any drift gas modifier. Figure 6B shows the collected mass spectra for the same sample with the DMS turned off and all ions allowed to pass through to the mass spectrometer. The most abundant ions in Figure 6B represent the ang fragment $(M + H)^+$ ion at *m/z* 482, the Glu-fib B $(M + 2H)^{2+}$ at *m/z* 786, and the neurotensin $(M + 2H)^{2+}$ at *m/z* 837. Parts C and D of Figure 6 show the DMS dispersion plot (multiple tracts) and DMS spectra at rf = 1200 V of the same sample but with the use of 8000 ppm 2-butanol. The Vc points used for the generation of the mass spectra in Figure 6, parts E and F, were selected based on Vc values determined to achieve optimum DM separation between the three peptide ions of interest. Accordingly, Figure 6E shows the mass spectrum at the selected Vc point of −12.8 V, optimized for the separation of the *m/z* 482 ang fragment ion, and parts F and G of Figure 6 show the selected Vc point mass spectra at the optimized setting of −9.2 V for the *m/z* 786 Glu-fib B ion (Figure 6F) and −8.2 V for the *m/z* 837 neurotensin ion (Figure 6G). The results shown in Figure 6 demonstrate that under optimized drift gas modifier conditions, DMS is capable of selectively separating peptide ions of interest. Again, as demonstrated previously, an increase in DMS separation of the peptide ions of interest away from the low Vc range (−2 to 0 in Figure 6D) provides a significant reduction in the high *m/z* (1500−2000) background ion signal present in Figure 6B. The selected Vc point mass spectra (ranging from −8.2 to −12.8 Vc) in parts E and F of Figure 6 all demonstrate a reduction in this background ion signal. As a result of the high *m/z* background ion reduction, the

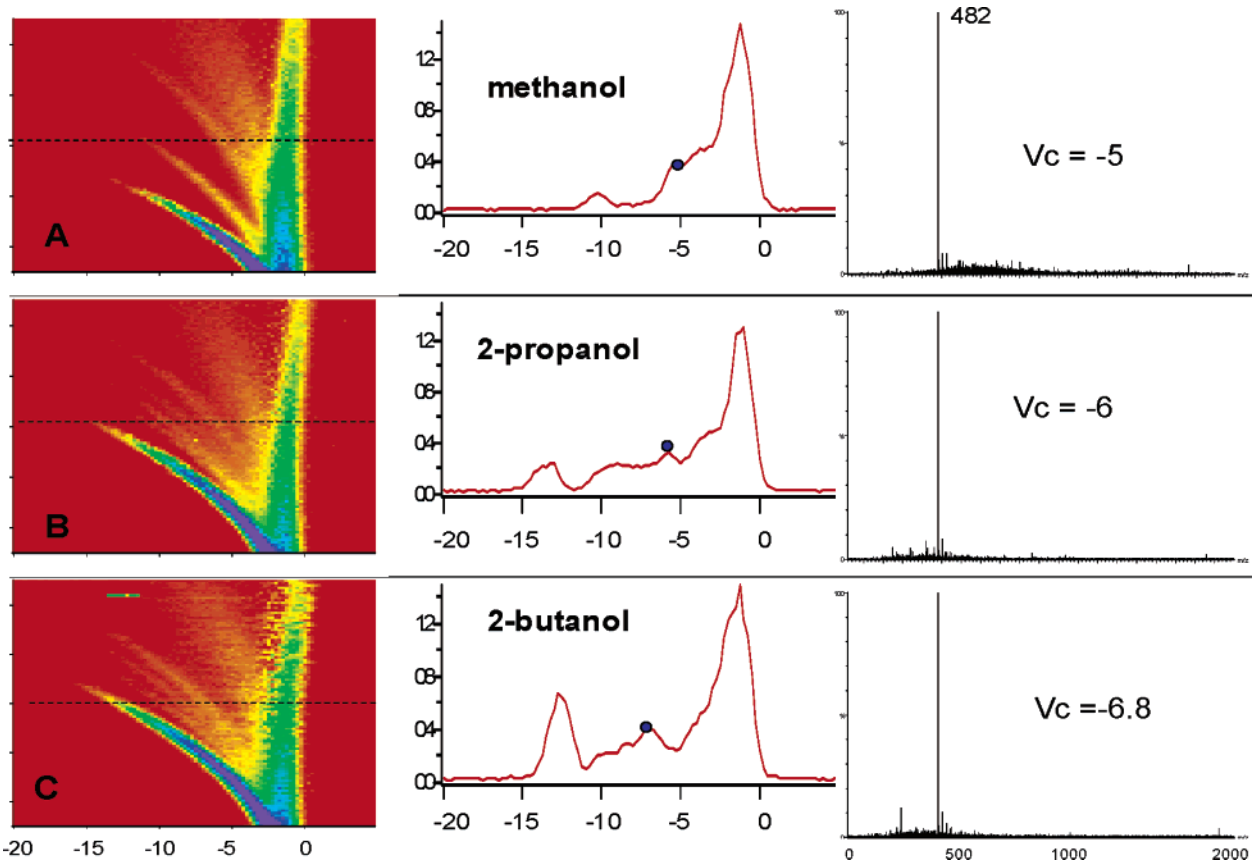


Figure 4. Examples of a 0.05 mg/mL angiotensin fragment 11-14 sample DMS dispersion plot, DMS spectra (rf = 1000 V), and selected Vc point mass spectra for the maximum MH^+ *m*/*z* 482 ion signal with 8000 ppm methanol modifier (A), 8000 ppm 2-propanol modifier (B), and 8000 ppm 2-butanol modifier (C).

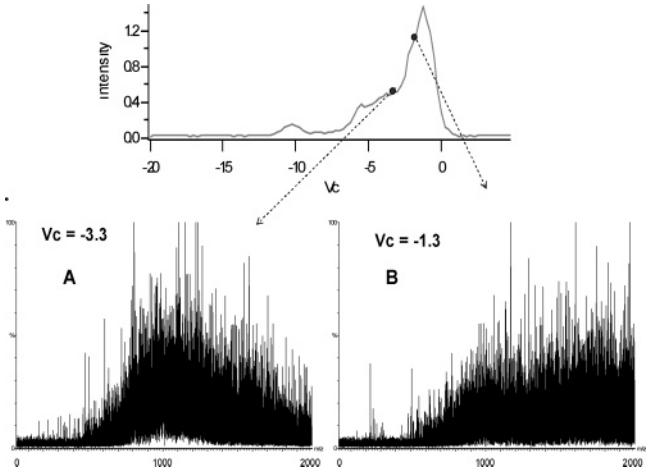


Figure 5. DMS spectrum and selected Vc point mass spectra (normalized to the same ion signal scale), −3.3 V (A) and −1.3 V (B), of 0.05 mg/mL angiotensin fragment 11-14 with 8000 ppm methanol drift gas modifier (rf = 1000 V).

Glu-fib $(M + H)^+$ *m*/*z* 1571 ion can be observed as one of the most abundant ions in Figure 6, parts F and G, whereas in Figure 6B it is not discernible because of the background ions present. The demonstrated capabilities to separate individual peptide ions led next to the investigation of using our nanoESI-DMS-MS system to create an analytical platform capable of rapid peptide quantitation.

Rapid Peptide Quantitation. With the optimization of the drift gas modifier conditions completed, the use of the nanoESI-DMS-MS system for rapid peptide quantitation of directly infused samples was investigated, utilizing the 8000 ppm 2-butanol drift gas modifier condition for all analyses. The angiotensin fragment peptide was selected as the analyte of interest to be quantified. The analyte MS signal sensitivity was further improved for the quantitation work by optimizing the sample solvent, nanospray capillary tip position, and mass spectrometer conditions. The sample solvent was changed to 50/50 methanol/water with 0.1% formic acid, the MS cone voltage was increased to 40 V, and the MS inlet source temperature increased to 70 °C. Parts A−D of Figure 7 present the DMS dispersion plot (Figure 7A), DMS spectra, rf = 900 V (Figure 7B), and the selected Vc point mass spectra (Figure 7, parts C and D), for a 25 μg/mL ang fragment reference sample. Figure 7C demonstrates the results of the combined MS signal optimization with the DMS separation optimization presented previously. In support of our hypothesis presented in the previous sections, that the drift gas modifier facilitates the declustering of higher order peptide aggregate ions, parts C and D of Figure 7 reveal the DM separation of the *m*/*z* 482 $(M + H)^+$ monomer ion at a compensation voltage of −5.3 V from the *m*/*z* 963 $(2M + H)^+$ dimer and 1444 $(3M + H)^+$ trimer ions at a lower Vc of −2.5 V.

The 25 μg/mL angiotensin reference sample shown in Figure 7 was utilized to investigate the appropriate DMS sensor and MS conditions necessary for rapid and accurate quantitative analysis of directly infused nanoESI samples. The procedure utilized for rapid peptide quantitation is as follows. The rf voltage is set to

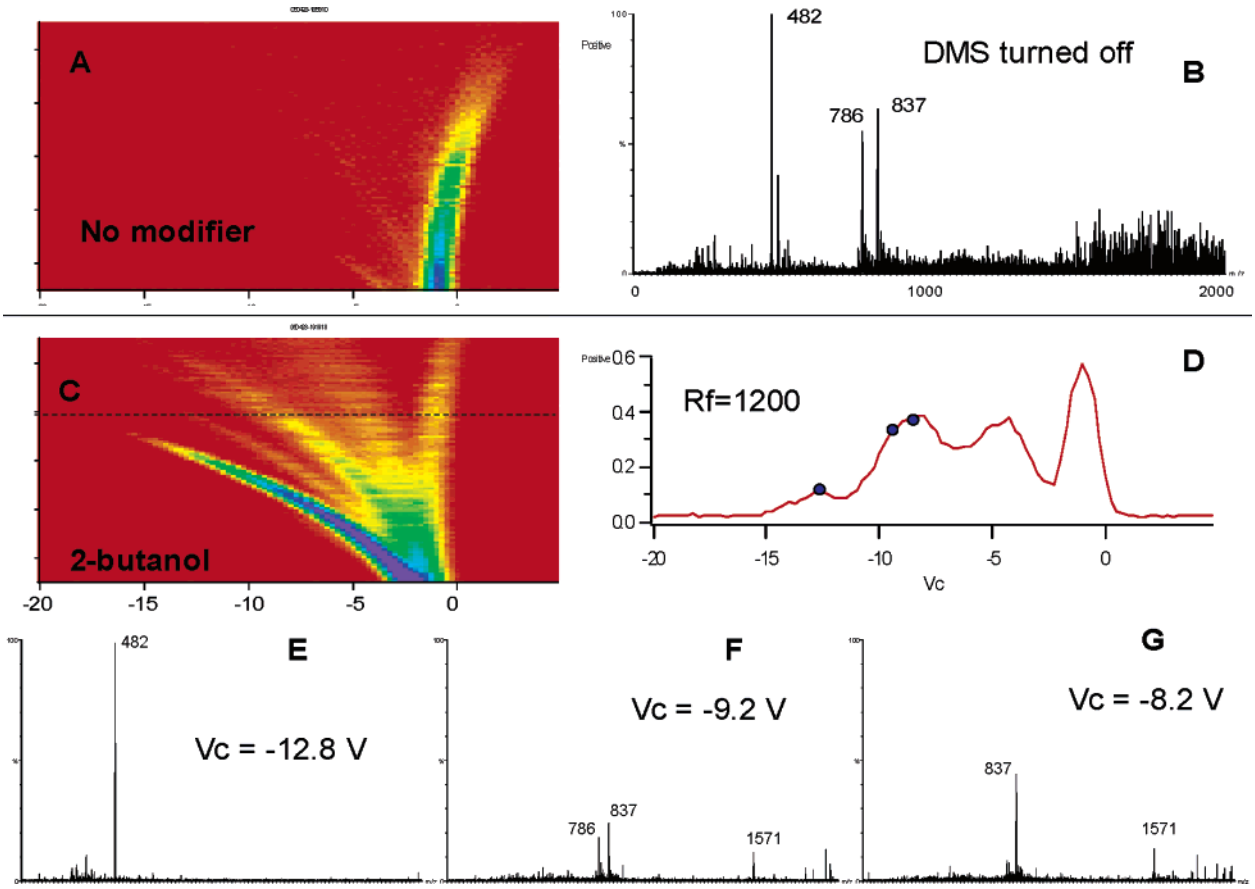


Figure 6. Examples of a 0.05 mg/mL each angiotensin fragment 11-14, Glu-fib B, and neurotensin sample DMS dispersion plot and collected mass spectra (DMS off) with no drift gas modifier (A and B). Parts C−G are for the same sample but with 8000 ppm 2-butanol, the DMS dispersion plot (C), DMS spectra, rf = 1200 (D), and selected Vc point mass spectra (normalized to the same ion signal scale) for optimized separation of the three MH^+ ions, *m/z* 482 (E), *m/z* 786 (F), and *m/z* 837 (G).

900 V, and the Vc voltage then scanned from −15 to 0 V in 10 s. During the Vc scan, the MS signal is collected over time in selected ion mode for the ion of interest. As the Vc range is scanned, and the selected *m/z* ion signal monitored, a peak specific to the analyte of interest is created when the Vc corresponds to the transmission of the analyte ion into the mass spectrometer. This peak is then integrated for peak area, enabling analyte quantitation. The entire sample analysis time is only limited by the selected Vc scan time, resulting in a sample analysis time of 10 s. For this study, each sample analysis consisted of performing six replicate Vc scans to provide a measure of repeatability for this technique.

To create a peak area reference value reflective of 25 μg/mL angiotensin, six replicate *m/z* 482 selected ion Vc scans were collected and their peak areas integrated. Table 1 summarizes the average peak area for 25 μg/mL reference sample and the percent residual standard deviation of the six peak area measurements. The RSD of 4.6% demonstrates the repeatability of peak generation with this selected ion−rapid Vc scanning platform.

Following optimization of the DMS and MS conditions for the detection and quantitation of angiotensin, a sample was prepared which contained a mixture of seven different peptides (10 nmol/mL each), ranging in mass from 1045 to 1672 Da to create a semicomplex peptide mixture, without the angiotensin fragment (matrix sample). Two additional samples were prepared by adding varying amounts of the angiotensin fragment to this peptide mixture, one with an angiotensin concentration of 2 μg/mL and the second 10 μg/mL. The identical settings used for the rapid Vc scanning of the 25 μg/mL reference sample were used for the three samples, the two angiotensin-spiked and the blank peptide mixture used as matrix. Six replicate Vc scans were collected for each sample in a continuous fashion, resulting in what visually appears like a single DMS spectrum with six replicate peaks. Figure 8 demonstrates the mass spectra (DMS off) for the semicomplex peptide mixture sample with 2 μg/mL angiotensin (Figure 8A), the six replicate *m/z* 482 selected ion−rapid Vc scanning peaks for the blank peptide mixture matrix sample (Figure 8B), the 2 μg/mL angiotensin-spiked peptide mixture sample (Figure 8C), the 10 μg/mL angiotensin-spiked peptide mixture sample (Figure 8D), and the 25 μg/mL angiotensin reference sample (Figure 8E). The tables in Figure 8 summarize the individual scan peak area values, peak area average, % RSD, and the angiotensin percent quantitative recovery for the spiked 2 μg/mL and 10 μg/mL peptide mixture samples. The amount quantified for each of the spiked samples is based on peak area quantitation against the 25 μg/mL reference sample peak area assuming a linear response ($y = mx$). The percent recovery values are 90% and 91% for the 2 μg/mL and 10 μg/mL samples, respectively. The Vc scan peak area % RSD and recovery data provide a first look at the feasibility for rapid quantitation of directly infused samples with the nanoESI-DMS-MS system, utilizing the selected ion−rapid Vc scanning platform.

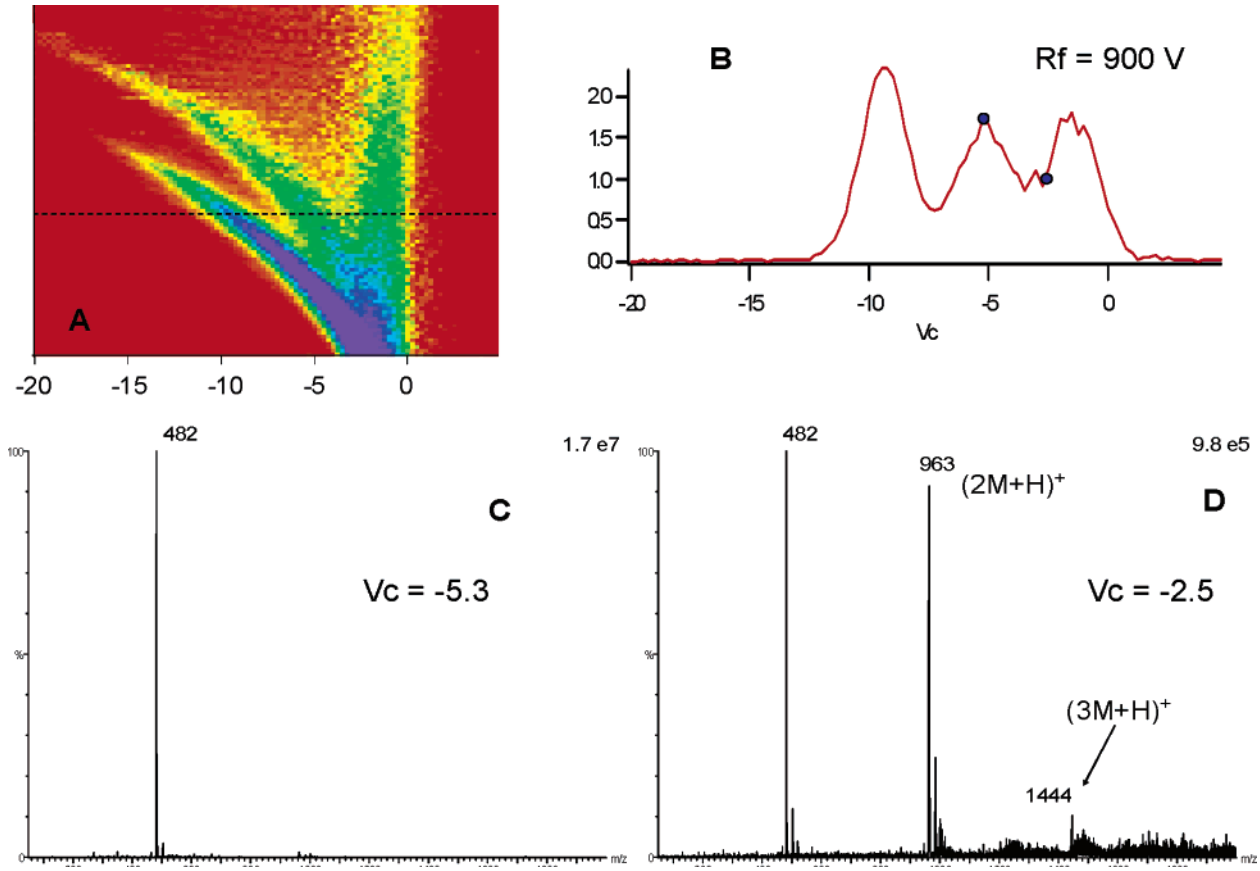
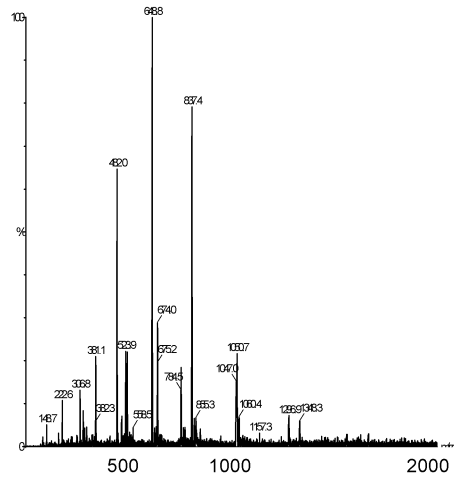


Figure 7. Examples of a 25 μg/mL angiotensin fragment 11-14 sample DMS dispersion plot (A), DMS spectra, rf = 900 (B), and selected Vc point mass spectra at Vc = −5.3 (C) and Vc = −2.5 (D).

Ultrarapid (0.5−5 s range) sample analysis times appear reasonable to achieve with the fast Vc scanning capabilities of the DMS sensor and a fast scanning mass spectrometer, making this quantitative analysis platform very desirable for numerous applications in the high-throughput arena. As this platform is further developed, software improvements, providing linked scans of the DMS and MS data, will enable the generation of DMS spectra where the Vc value corresponding to the analyte peak apex can be accurately identified. The use of peak apex Vc will be used to provide an increased measure of specificity in the same manner that retention time is used for chromatography. To further evaluate this platform for quantitative analysis, we investigated next the linear dynamic range for peak area generation by selected ion−rapid Vc scanning.

Linear Dynamic Range of the Selected Ion−Rapid Vc Scanning Quantitative Platform. Any quantitative analytical technique is expected to provide a reasonable dynamic range for analyte response as a function of concentration. The linear dynamic range for peak area generation by our quantitative, selected ion−rapid Vc scanning, platform was thus investigated next. With the present configuration of our system, it is difficult to maintain an identical nanospray tip position between samples. This leads to potential differences in analyte ion abundance based on tip position alone and not analyte concentration. To overcome this, an internal standard was utilized for all of our dynamic range samples, enabling our dynamic range to be based on the peak area ratio of analyte/internal standard. The angiotensin fragment 11-14 (MW 481) peptide was selected as the analyte and the bradykinin fragment 1-5 (MW 572) as the internal standard. All samples were prepared in a solution containing 50/50 methanol/water, 0.1% formic acid, and 10 μg/mL of the bradykinin fragment. Eight different angiotensin samples were prepared, ranging in concentration from 0.008 to 50 μg/mL. Each sample, including the blank solution (no angiotensin), was analyzed using the selected ion−rapid Vc scanning approach described in the previous section, resulting in the generation of six replicate, 10 s Vc scans, at an rf = 900 V. For each sample, the selected ion DMS peaks of m/z 482 and 573 were extracted and integrated for peak area. The average peak area from the m/z 482 peaks was divided by the average peak area from the m/z 573 peaks, providing a normalized response for angiotensin. The analysis of the blank solution resulted in the generation of an extremely small m/z 482 peak from the background/chemical ion noise.

Figure 9 provides the linear regression for angiotensin concentration versus response (peak area ratio). The 0.008 μg/mL sample was not included in the regression due to its lack of response over the blank solution. The R^2 value for the regression is 0.9997, demonstrating a minimum linear dynamic range of 2500 for the rapid DMS peak area generation of the angiotensin fragment. On the basis of the linear detection of angiotensin fragment in the 0.02 μg/mL (41.5 pmol/mL) sample, the absolute mass sensitivity for this analysis is 6.8 amol, calculated based on the MS scan time of 20 ms and a sample flow of 500 nL/min.



2 ug/ml Ang. Spiked into mixture of 7 peptides (10 nmol/ml)	
scan #	peak area
1	202817
2	220863
3	213123
4	201225
5	203341
6	208827
average	**208366.0**
std dev	7563.8
%RSD	3.6
Amount Quantified	1.8 ug/ml
% recovery	**90%**

10 ug/ml Ang. Spiked into mixture of 7 peptides (10 nmol/ml)	
scan #	peak area
1	1099078
2	1040424
3	1053177
4	1019238
5	1055779
6	1047123
average	**1052469.8**
std dev	26312.8
%RSD	2.5
Amount Quantified	9.1 ug/ml
% recovery	**91%**

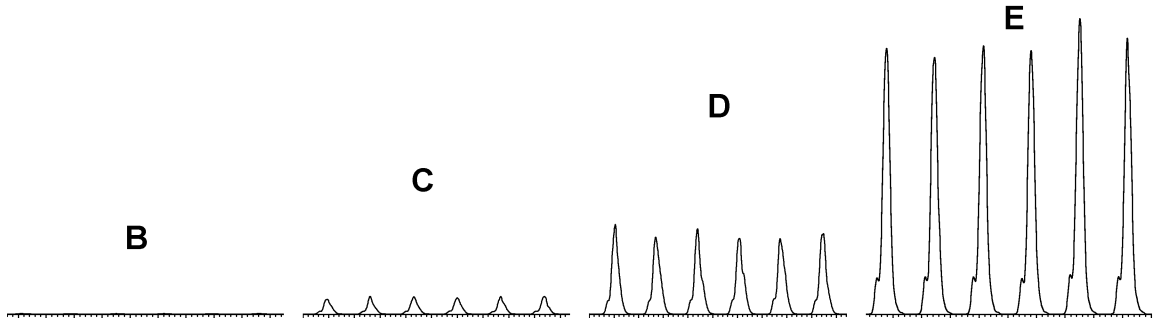


Figure 8. Mass spectra (DMS off) for the semicomplex peptide mixture sample with 2 μg/mL angiotensin (A). The six peaks (B−D) are normalized to the same peak height scale from the replicate 10 s rapid Vc scans at selected *m*/*z* 482 for the peptide mixture without angiotensin (B), the 2 μg/mL angiotensin-spiked peptide mixture sample (C), the 10 μg/mL angiotensin-spiked peptide mixture sample (D), and the 25 μg/mL angiotensin reference sample (E). The tables show individual peak area values, peak area average, % RSD, and percent recovery for the 2 μg/mL and 10 μg/mL samples, respectively.

Table 1. Six Replicate Peak Area Values, Peak Area Average, and Peak Area Percent Residual Standard Deviation for the *m*/*z* 482 Selected Ion−Rapid Vc Scanning of a 25 μg/mL Reference Sample

25 μg/mL angiotensin reference	
scan no.	peak area
1	2899431
2	2762516
3	2869688
4	2802962
5	3147256
6	2922368
ave	**2900704**
std dev	134811
% RSD	**4.6**

CONCLUSION

Mass spectrometry is a principal analytical platform for the analysis of peptides, particularly in proteomic and biomarker applications. The interfacing of DMS with mass spectrometry offers advantages over the use of mass spectrometry alone. Such advantages include the capability of orthogonal/complementary ion separation to mass spectrometry and improvements in analyte mass spectral signal-to-noise. We have established that DMS can be used to separate individual peptides and improve peptide analysis by mass spectrometry. A major obstacle to providing DMS peptide separation is the formation of peptide aggregate ions during the ESI process, but as described here, the problem can be overcome through the use of polar drift gas modifiers. We hypothesize that the drift gas modifier interacts with the peptide aggregate ions, competes with the H-bonding and van der Waals forces driving the clustering, and, in turn, facilitates cluster dissociation and reduces ion cross sections. This reduction in clustering and peptide ion cross section results in improved DM separation of peptide ions away from the DM spectral region of high *m*/*z* background ion signal, improving the overall signal-to-noise ratio for the peptide ion of interest and thus significantly enhancing the analytical power of the Waters ZQ mass spectrometer used in these studies.

Upon successful demonstration of DMS peptide separation, we present a first look at utilizing our nanoESI-DMS-MS system to create an ultrarapid quantitative analysis platform with good accuracy and reproducibility. The use of nanospray in place of conventional ESI increases ionization efficiency and reduces ion suppression, improving the capability for direct infusion analysis of complex or "dirty" samples.[23,24] As such, differential mobility analyte separation with our nanoESI-DMS-MS system, which occurs post-ionization, is advantageous for rapid analysis of directly infused samples. Ultrarapid (0.5−5 s range) sample analysis times appear reasonable to achieve with the fast Vc scanning capabilities of the DMS sensor and a fast scanning mass spectrometer, making this quantitative analytical platform very desirable for numerous

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angiotensin fragment concentration (ug/ml)	peak area ratio (angiotensin / internal std.)
0	0.0040
0.02	0.0086
0.1	0.0248
0.2	0.0417
0.4	0.0906
2	0.4510
10	2.0944
50	11.5238

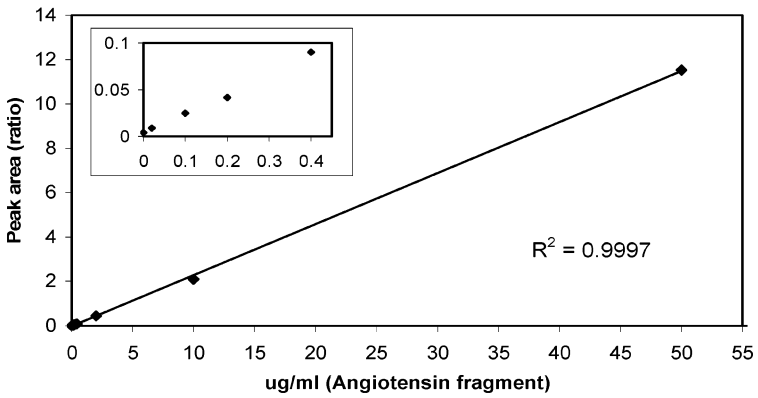

Figure 9. Linear regression of angiotensin concentration (μg/mL) vs response (peak area ratio of angiotensin/internal standard). The inset plot provides a zoomed-in view of the four low concentration and blank solution data points.

high-throughput applications and for a broad range of analytes and matrixes. Each ion separation technique, DMS and MS, offers orthogonal ion separation to one another, enhancing the overall specificity for this quantitative approach. The combination of a fully automated sample handling/nanoelectrospray system with DMS-MS would significantly improve issues commonly associated with the use of nanoelectrospray for quantitative work. Such improvements are stable nanospray, no carryover, and consistent spray position. In addition, such automation would provide increased sample throughput times and low sample consumption. Future research will continue the instrumentation and linked scan development of nanoESI-DMS-MS and investigate the use of the presented ultrarapid quantitative analysis platform for various applications with a fully automated sample handling/nanospray device.

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