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Evaluation of capillary liquid chromatography–electrospray ionization ion mobility spectrometry with mass spectrometry detection

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Abstract

Due to the proteomics revolution, multi-dimensional separation and detection instruments are required to evaluate many peptides and proteins in single samples. In this study, electrospray ionization (ESI) ion mobility spectrometry (IMS) was evaluated as an additional separation after HPLC separations. Common HPLC mobile phase compositions (solvents, acid modifiers, and buffers) were assessed for the effect on ESI-IMS response. Up to 5 m*M* sodium phosphate, a non-volatile buffer, was able to be electrosprayed into the IMS without degradation of the instrumental performance. Due to the rapid separation times of IMS, multiple IMS spectra were obtained within a single HPLC peak. A five-peptide mixture was separated in a capillary HPLC column under isocratic conditions within 3 min. Coelution of two peaks due to non-optimal HPLC conditions occurred and these two peaks could not be distinguished by HPLC with UV detection. In contrast, the single ion mobility chromatograms provided separation of each peptide as well as providing a second degree of analyte identification (HPLC retention time and IMS mobility). Furthermore, IMS–MS analysis of the five peptides and comparison with HPLC retention times showed that each peptide had a unique retention time–ion mobility–mass to charge value. This work showed that IMS could be employed for direct separation and detection of HPLC eluents and also could be combined with HPLC–MS for three unique dimensions of separation.

Keywords: Ion mobility spectrometry; Detection, LC; Peptides; Methionine enkephalin; Cocaine

1. Introduction

Ion mobility spectrometry (IMS) has been employed as a selective detector for many separation techniques including gas chromatography (GC) and supercritical fluid chromatography (SFC) [1–9]. With the introduction of coronaspray ionization and electrospray ionization (ESI) as ionization techniques for IMS, the coupling of LC systems was achieved due to their ability to directly ionize liquid phase samples [9–11]. However, the incompatibility between typical macro high-performance liquid chromatography (HPLC) flow-rates (ml/min) and the low flow-rates employed in ESI-IMS (μl/min) required splitting of the eluent flow and a reduction in the available analyte. Initial studies employed corona discharge as the ionization source and only volatile analytes could be detected from the HPLC eluent

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[9,10]. In 1998, Lee et al. showed the first coupling of micro-bore HPLC with ESI-IMS, although splitting of the HPLC eluent still had to be performed [11]. This study is the first example of direct coupling between capillary HPLC with ESI-IMS and mass spectrometry (MS) detection without splitting the HPLC eluent.

IMS is a gas-phase separation technique that separates ions based on size and charge [12]. The mobility of an ion (measured from the ion drift time) can be utilized to identify the ion and offers different analyte information than HPLC retention times or MS m/z values. IMS separation times are typically 10–100 ms and this rapid analysis time enables multiple IMS spectra to be obtained within one capillary HPLC peak [11]. Within the past 10 years, several groups have expanded on the resolution of ion mobility (defined as the drift time/peak width at half height) spectrometers by increasing the voltage and interfacing IMS with a mass spectrometer [13–17]. Due to the higher resolving power and coupling with ESI, IMS has been shown to separate a wide range of analytes including carbohydrates [11], sugars [11], and proteins [18,19]. Many HPLC detectors do not provide additional analyte information besides the HPLC retention times and can only analyze ions with certain properties (i.e., for UV detection the analyte should have a chromophore). The coupling of HPLC with ESI-IMS would provide a selective and sensitive detector without the expensive and extensive requirements of MS.

In the past decade, the field of HPLC has been significantly improved and the coupling of HPLC with ESI-MS has provided one of the most powerful analytical techniques to date [20]. LC–MS–MS systems are now commonly employed for routine sequencing and have been essential in the proteomics revolution. However, these instruments are expensive and not available to all laboratories. The advancement of other instruments that can be coupled with HPLC can only improve the overall study of biological systems. A second advantage of employing HPLC–IMS would be its coupling with MS for three dimensions of separation. The three dimensions of separation could aid in the reduction of analysis times and provide the ability to analyze more complex mixtures.

Due to the significant rise in popularity of LC–ESI-MS methodology, many studies have documented the effect of common HPLC mobile phases on ESI ionization efficiency [21]. There is a balance that the analyst must achieve between optimal LC mobile phase conditions for good separation efficiencies and good ESI conditions for optimal analyte sensitivity in the MS. Low organic content (high aqueous) mobile phases typically produce decreased ESI intensities [22]. Although volatile acid modifiers (acetic acid and formic acid) are optimal for ESI, the commonly used HPLC modifier, trifluoroacetic acid (TFA), causes significant ion suppression in ESI [23]. Non-volatile buffers have a similar effect on ESI sensitivity [24].

The work performed in this study had two main objectives: (1) to determine the optimal ESI parameters (based on analyte sensitivity) with respect to modifiers and solvents commonly used in HPLC and (2) to evaluate the coupling of capillary HPLC with ESI-IMS for two-dimensional separations.

2. Experimental

2.1. Instrumentation

A Micro-Tech Scientific (Sunnyvale, CA, USA) Ultra-Plus II micro-HPLC system was utilized for all HPLC experiments. The capillary column (purchased from Micro-Tech Scientific) employed for all LC experiments was a 15 cm×0.32 mm, 5 μm Zorbax SB-C_{18}W column for peptide mixtures and was operated at a flow-rate of 10 μl/min. All injections were performed for 0.05 min with an injection volume of 0.5 μl. A diagram of the instrument employed for all experiments is shown in Fig. 1. The column was connected to the UV detector via a 2 ft.×50 μm I.D. fused-silica transfer line (1 ft.=30.48 cm). As seen in Fig. 1, the fused-silica transfer line was directly connected from the UV detector to the ESI source. The ESI source was placed in conjunction with the IMS system as shown in Fig. 1. The UV detector was operated at 214 nm to monitor all peptides analyzed in this study. Signal output from the UV detector was collected with the IMS Labview software.

The ESI source employed was a laboratory-built water-cooled system [12]. The ESI system was

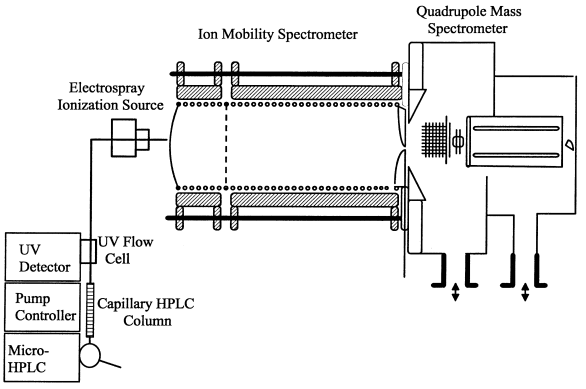

Fig. 1. Diagram of capillary HPLC–ESI-IMS–MS instrument.

interfaced to a laboratory-built IMS–MS system that has been described previously [13], modifications to the original design can be found in Ref. [25]. The IMS system was a stacked ring design with alternating stainless steel rings and insulating alumina spacers. The atmospheric pressure IMS was interfaced to the quadrupole mass analyzer via a 40 μm pinhole (10^{-4} Torr in the first chamber and 10^{-5} Torr in the second chamber; 1 Torr=133.322 Pa). A series of six ion lenses were employed to transmit ions from the IMS system to the quadrupole. The MS system utilized for both IMS and MS detection was a 150-QC ABB Extrel (Pittsburgh, PA, USA) quadrupole (0–4000 u mass range). All voltages and other instrumental parameters employed for the IMS–MS system were as follows; (1) ESI voltage +14 kV, (2) IMS voltage +10 kV (field of 385 V/cm), (3) nitrogen was the drift and nebulization gas, 800 and 100 ml/min, respectively, (4) multiplier and dynode voltages, −1700 and 5000 V, respectively, and (5) desolvation and drift region temperature, 250 °C. Mobile phase pumping for the initial ESI evaluations was performed with a Brownlee Labs (Santa Clara, CA, USA) dual-piston syringe pump.

2.2. Reagents and chemicals

Cocaine was obtained from Supelco (Bellefonte, PA, USA) as 1 ml standards (1 mg/ml) prepared in acetonitrile. All solvents employed in the ESI and HPLC studies were HPLC grade and were purchased from J.T. Baker (Phillipsburg, NJ, USA). The peptide mixture was obtained from Sigma–Aldrich (Milwaukee, WI, USA) and diluted with the specified ESI solvents.

2.3. Data collection

The data collection and IMS gating electronics have been described in detail previously [12]. All data collection for both UV detection and IMS detection was performed with a laboratory-written Labview program [26]. When the UV detector was used, the Labview software was set to obtain a signal every 25 ms. For the ion mobility spectra, the mass spectrometer was always employed as the detector. Two types of ion mobility data were obtained in these experiments; non-selective ion mobility (NSIM) and mass-selective ion monitoring (SIM). For NSIM, the quadrupole direct current (d.c.) voltage [radio frequency (rf)-only mode] was turned off while continually gating the ion mobility instrument. In this mode, ion mobility spectra were detected for all ions and the quadrupole simply transmitted the ions to be detected by the electron multiplier. In SIM mode, the ion mobility experiment was continually gated but only one m/z value was transmitted through the quadrupole and detected. In this mode, ion mobility spectra were obtained for ions with the designated m/z value.

2.4. Calculations

The mobility (K) of an ion is typically employed to identify the analyte ion and is defined as the following:

$$K = \frac{v_d}{E} = \frac{l^2}{Vt_d} \quad (1)$$

where v_d is the drift velocity of the ion, E is the electric field, l is the length of the drift region (22.5 cm), t_d is the drift time (defined as the measured time it takes the ion to drift through the drift tube), and V is the voltage applied to the drift tube (8680 V). Due to the variations in operating conditions (different pressures and temperature), the mobilities are standardized for instrumental comparison. The standardized or reduced mobility constants (K_0) were calculated according to the following equation:

$$K_0 = \left(\frac{L^2}{Vt_d}\right) \cdot \left(\frac{273}{T}\right) \cdot \left(\frac{P}{760}\right) \quad (2)$$

where T was the effective temperature in the drift region (523 K), and P was the pressure in the drift region (695–700 Torr). The reduced mobility values are typically the ion identification information discussed in the literature and a standard way of identifying an unknown compound.

2.5. Experimental protocol

In order to understand the HPLC conditions that may affect the ESI response, different ESI solution conditions were initially investigated (Figs. 2–5). For all ESI evaluation experiments (Figs. 2–5), the mass-selected ion mobility intensities were measured for the protonated and sodiated ions of methionine enkephalin and the protonated ion of cocaine. For each study, at least three consecutive IMS spectra (due to 1000 individual IMS spectra averaged together) were measured. The maximum intensity for each ion was extracted from the IMS spectra and employed as the measurement of the ion response for the specific ESI conditions. The error bars on Figs. 2–5 represent the standard deviation of the intensity values measured from the three consecutive spectra and the bar graph intensity represents the average intensity value. For the HPLC–IMS experiments, consecutive IMS (30 averages per spectra) spectra were obtained for the given chromatographic run. After data collection, a 0.6 ms wide window centered around the drift time for each peptide was extracted from the IMS spectra. Within the 0.6 ms window, 10 data points for each IMS spectrum were extracted and averaged together to produce the selected ion

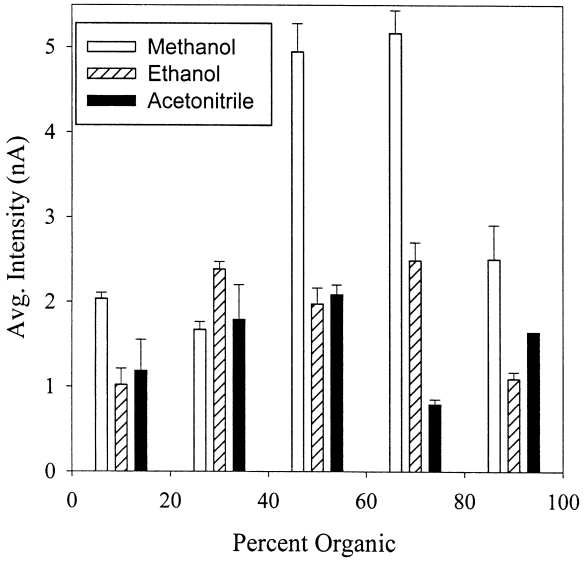

Fig. 2. Graphs show effect of organic solvent percentage and solvent type on cocaine signal intensity. IMS conditions: 1000 averages per spectra, 0.2 ms pulse width, scan time 50 ms. ESI conditions: flow-rate 10 μl/min. Points on the graph represent the average of three IMS spectra. Signal was reported for the drift time where the greatest intensity was observed for the protonated cocaine ion. The cocaine concentration was 10 ppm and the total amount injected was 100 ng (0.33 nmol).

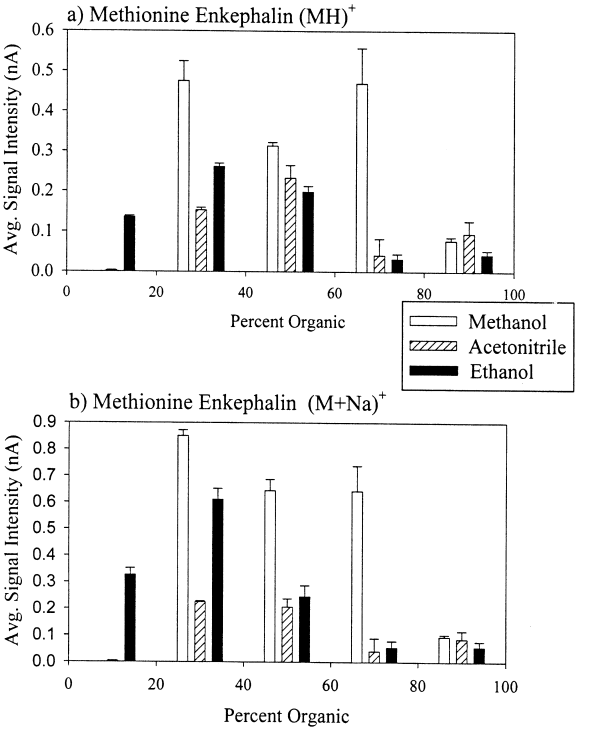

Fig. 3. Graph shows effect of three solvents on methionine enkephalin ESI-IMS response for (a) MH^+ and (b) $(M+Na)^+$. IMS conditions: 1000 averages per spectra, 0.2 ms pulse width, scan time 50 ms. ESI conditions: flow-rate 10 μl/min, each solution was flushed for 1 h prior to analysis. Points on the graph represent the average of three IMS spectra at the greatest drift time intensity observed for each specific ion. The concentration of the methionine enkephalin standard was 100 ppm and the total amount injected was 1000 ng (1.74 nmol).

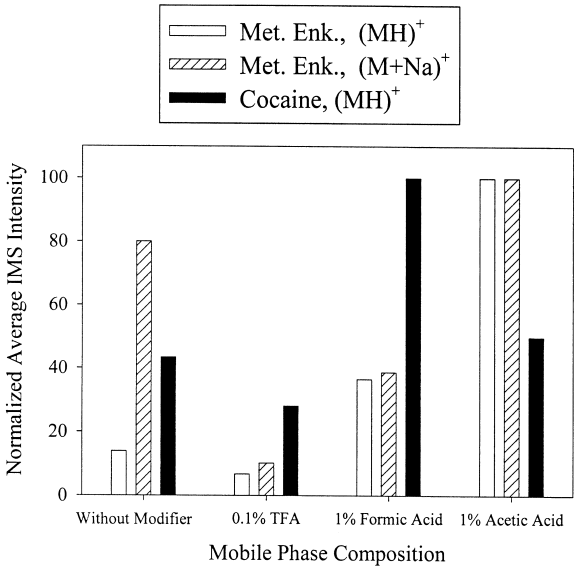

Fig. 4. Effect of modifiers on methionine enkephalin and cocaine signal intensity. Values were normalized to highest intensity observed for four different ESI mobile phase compositions. A value of 100 indicates the maximum intensity observed for that specific ion under the four conditions. IMS conditions: 1000 averages per spectra, 0.2 ms pulse width, scan time 50 ms. ESI conditions: flow-rate 10 μl/min, each solution was flushed for 1 h prior to analysis. All solutions were prepared in water–acetonitrile (50:50). Points on the graph represent the average of three IMS spectra at the greatest drift time intensity observed for each specific ion. The cocaine concentration was 10 ppm and the total amount injected was 100 ng (0.33 nmol). The concentration of the methionine enkephalin standard was 100 ppm and the total amount injected was 1000 ng (1.74 nmol).

mobility chromatograms shown in Fig. 6 and Fig. 8. (In Fig. 6, the window was 1 ms wide instead of 0.6 ms). In the HPLC–IMS experiments, MS was employed in the non-selective mode (rf only) in which it was operated to transmit all ions greater than a m/z of 200.

3. Results and discussion

3.1. ESI response for typical HPLC solvents

Early in ESI development, the dependence of analyte sensitivity on ESI solution conditions was realized (solvents, buffer, acidic additives) [24]. Often the optimal ESI and HPLC conditions do not coincide and compromises between the optimum separation and detection are required. The first portion of this study evaluated effects of HPLC mobile phase compositions on ESI analyte response. It has been documented that coronaspray can occur simultaneously with ESI [12]. Coronaspray ionization is a gas-phase ionization process capable of ionizing volatile compounds. In contrast, ESI is a liquid-phase ionization process that ionizes both volatile and non-volatile compounds. For this reason, cocaine (a volatile compound) and methionine enkephalin (a five-amino acid peptide) were both employed as test compounds to show any differences in the relative ESI responses. The actual intensities measured and relative intensities of the ions (protonated/sodiated ions) will be a complex function of the transmission efficiency, interfacial lenses, and overall instrumental set-up. However, it is expected that the general relationships observed in these studies will be similar to those observed for other systems.

In general, increasing the organic content of the ESI solvent composition has been shown to increase the ESI response and some organic solvents provide greater ESI responses than others (i.e., methanol > acetonitrile) [20]. In this study, three organic solvents (methanol, ethanol, and acetonitrile) with varied percentages of water were evaluated for ESI performance based on the measured IMS response.

In Fig. 2, the effect of solvent on cocaine intensity was shown. Methanol provided the greatest signal intensities for cocaine. Similar to the ESI-MS literature, the percent of organic content had a beneficial effect on the signal intensity of the analyte. For the methanol results, 50 and 70% methanol provided the optimal sensitivities with ~2.5 times the intensity of the other percentages. Based on the cocaine results (Fig. 2), methanol provided the optimal sensitivity while ethanol and acetonitrile provided very similar intensities.

While cocaine is volatile and could be ionized by either electrospray or corona discharge, methionine enkephalin (ME) is a five-amino acid peptide and could only be ionized by ESI. The relative intensities of ME were evaluated in Fig. 3 [(a) MH^+ and (b) $(M+Na)^+$]. Both the protonated and sodiated ions were predominant ions in the mass spectrum and consequently both ions' intensities were monitored as a function of solvent identity and percent organic

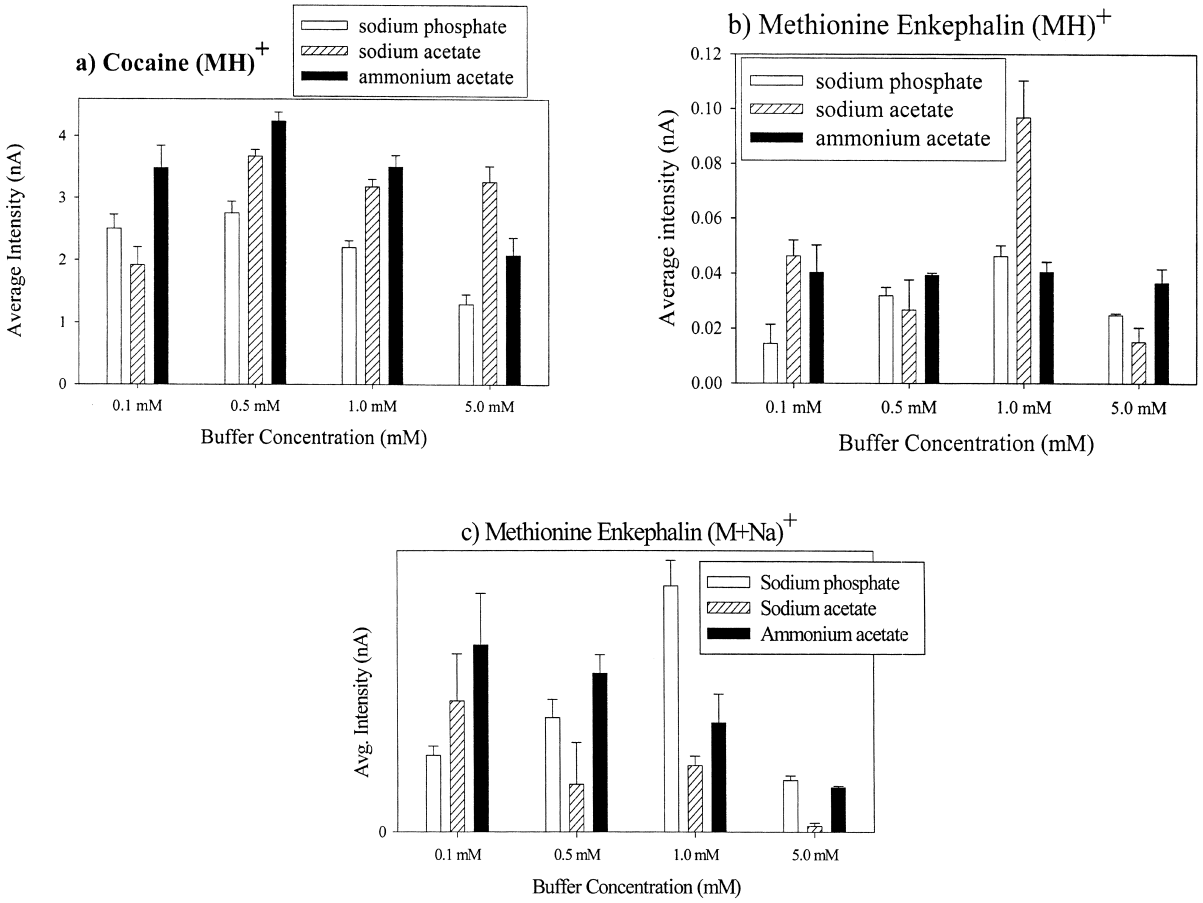

Fig. 5. Graphs show the effect of buffer concentrations on methionine enkephalin and cocaine signal intensities. (a) Cocaine, (b) methionine enkephalin (MH^+), and (c) methionine enkephalin $(M+Na)^+$. IMS conditions: 1000 averages per spectra, 0.2 ms pulse width, scan time 50 ms. ESI conditions: flow-rate 10 μl/min, each solution was flushed for 1 h prior to analysis. All solutions were prepared in water–acetonitrile (50:50). Points on the graph represent the average of three IMS spectra at the greatest drift time intensity observed for each specific ion. The cocaine concentration was 10 ppm and the total amount injected was 100 ng (0.33 nmol). The concentration of the methionine enkephalin standard was 100 ppm and the total amount injected was 1000 ng (1.74 nmol).

content. Similar to the previous results with cocaine (30 to 70%), methanol provided the best response for both ions. At either of the two extremes (10 and 90%), signal intensities were significantly less. For the higher organic content percentages (50–90%), ethanol and acetonitrile produced analyte responses significantly less than that of methanol.

3.2. ESI response for acidic modifiers

HPLC methods have typically incorporated an acidic modifier to attain optimal separation efficiencies with the most common additive being 0.1% TFA. However, TFA has been found to cause ion suppression within the ESI process. Consequently, other acidic modifiers (formic acid and acetic acid) have been employed to achieve good HPLC efficiencies while maintaining adequate ESI efficiencies for the analytes of interest [23]. Fig. 4 shows the effect of acid modifiers on cocaine and ME responses. In Fig. 4, the intensities measured for cocaine and ME (both protonated and sodiated ions) have been normalized. A value of 100% represented the greatest observed signal intensity for the four ESI solvent conditions; water–acetonitrile (50:50), 0.1% TFA, 1% formic acid, and 5% acetic acid (all acid modi-

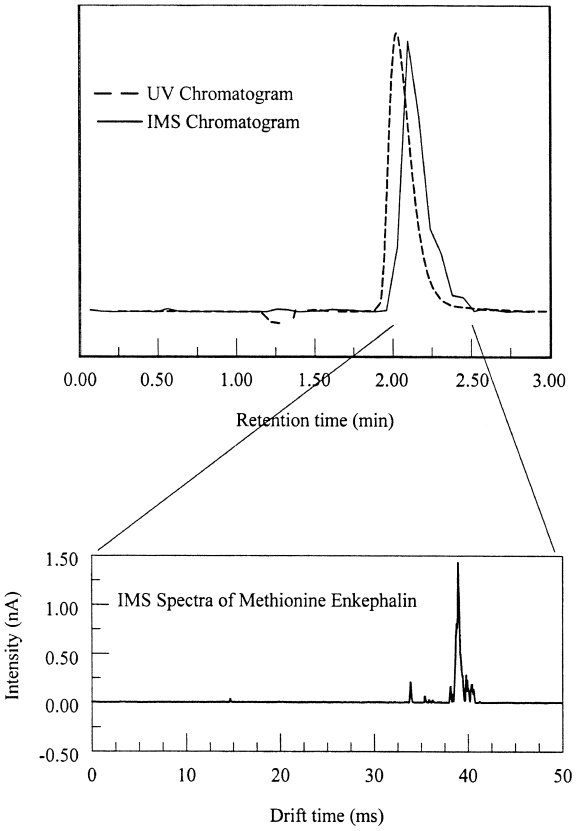

Fig. 6. Graph shows comparison of chromatogram of methionine enkephalin (50 ng injected) detected by the UV detector and the mass spectrometer. IMS chromatogram was reconstructed from a 1 ms wide drift time window centered at 39.0 ms. Each spectra was the result of 20 averages and an IMS pulse width of 0.2 ms.

fiers were added to water–acetonitrile, 50:50, solution). For all three ions, the lowest response was measured with the solution containing 0.1% TFA. For cocaine, the greatest intensity was obtained with 1% formic acid added to the ESI solution and 1% acetic acid provided the optimal response for ME (both ions). However, either acidic modifier (acetic acid or formic acid) provided better ESI responses than with the use of TFA and would be a viable replacement modifier for TFA in the HPLC mobile phase.

3.3. ESI response for HPLC buffers

In many HPLC separations, buffers are employed to stabilize pH. However, typical buffers are non-volatile (i.e., sodium phosphate) and have been found to cause reductions in ESI responses [24]. Three buffer solutions were studied: sodium phosphate, sodium acetate and ammonium acetate. The concentration ranges employed were reflective of typical buffer concentrations employed in HPLC mobile phases. In Fig. 5a, the effect of three buffers on cocaine response was shown. The presence of both volatile buffers (sodium acetate and ammonium acetate) caused the cocaine response to increase as the buffer concentration was increased. While increasing sodium phosphate concentrations did not improve the cocaine intensity, the response did not decrease until 5.0 m*M* where a ~50% intensity decrease was observed. Although the buffers did form more low-molecular-mass ions in the IMS and MS spectrum, there were no deleterious effects observed in the instrumental performance. In our IMS–MS instrument, there is a high counterflow of drift gas molecules which serves to aid in ion desolvation, removal of neutrals from the desolvation region and prevention of drift region contamination. In ESI-MS, the HPLC mobile phase is typically sprayed into a small orifice that is easily plugged or contaminated by non-volatile components, causing severe effects on instrumental performance. One benefit of employing IMS prior to MS (either following an LC separation or directly) is that the IMS drift gas serves to clean up the sample and prevent plugging of the MS entry orifice.

In Fig. 5b and c, the effect of the three buffers on methionine enkephalin ion intensities are shown, MH^+ and $(M+Na)^+$, respectively. For ammonium acetate, the buffer had little effect on the MH^+ ion intensity over the concentration range studied and caused the sodiated ion to decreased with increasing buffer concentration. At 5.0 m*M*, the protonated ion was actually higher in intensity than the sodiated ion. In general, all three buffers had little effect on the protonated ion intensity. However, both sodium acetate and ammonium acetate caused a decrease in the sodiated ion intensity. In contrast, sodium phosphate actually increased the intensity of the sodiated ion (up to 1 m*M*).

For quantitative purposes, it is important to understand how the presence of buffers can affect the relative intensities of the protonated and sodiated ion. The optimal buffer was found to be both

compound and concentration dependent. For instance at the 1.0 m*M* level, the buffer of choice for the separation and detection of cocaine was sodium acetate or ammonium acetate. However, the buffer of choice for ME (sodium ion) would be either sodium acetate or sodium phosphate Increasing the buffer concentration to 5.0 m*M* changed the optimum buffer for cocaine to sodium acetate only and the optimum for ME was the ammonium acetate buffer with the predominant ion then becoming the protonated ion.

3.4. Evaluation of LC–IMS

Previous evaluation of our high-resolution IMS–MS system (with a 40 μm pinhole interface) showed that several IMS spectra need to be averaged in order to attain a good representation of the ion population [13]. However, the speed of micro-HPLC separations and small peak widths dictated that the number of IMS averages be minimized. When the micro-HPLC column was interfaced to the ESI-IMS–MS system, IMS spectra were continually obtained. Therefore, both the ion mobility spectra and single ion mobility chromatogram can be evaluated for a given analyte. For these preliminary evaluations, the HPLC eluent was first introduced into the UV detector flow cell (see Fig. 1) and then directly routed into the ESI source. Any band broadening due to the IMS could be realized based on the comparison between the two chromatograms. For the initial experiments, 40% acetonitrile with 0.1% TFA was employed as the HPLC mobile phase in order to follow the methods described by the manufacturer even though this mobile phase containing TFA does not provide the optimal response. In Fig. 6, both the IMS and UV chromatograms for the analysis of ME were shown (top) and the IMS spectrum was shown (bottom). Based on the comparison of the two chromatograms, little band broadening occurred but a shift in retention time was observed due to the additional fused-silica transfer line between the UV detector outlet and the end of the ESI source.

There have been a number of studies published in the 1990s that showed the two-dimensional information obtained with IMS–MS and HPLC–MS. The coupling of the three techniques together would enable three unique dimensions of separation and enable complex mixtures to be resolved in a more rapid fashion. Typically in HPLC, solvent gradients are employed to optimize the resolution between two components, adding time to the analysis due to the time required to flush the column and equilibrate to the initial solvent conditions. The ability to perform isocratic HPLC separations in more cases would improve analysis times. In Figs. 7 and 8, the IMS–MS separations and HPLC–IMS separations of a peptide mix are shown, respectively. In this case, the IMS–MS separation was used as a secondary identification for each peptide since all of the ions had a

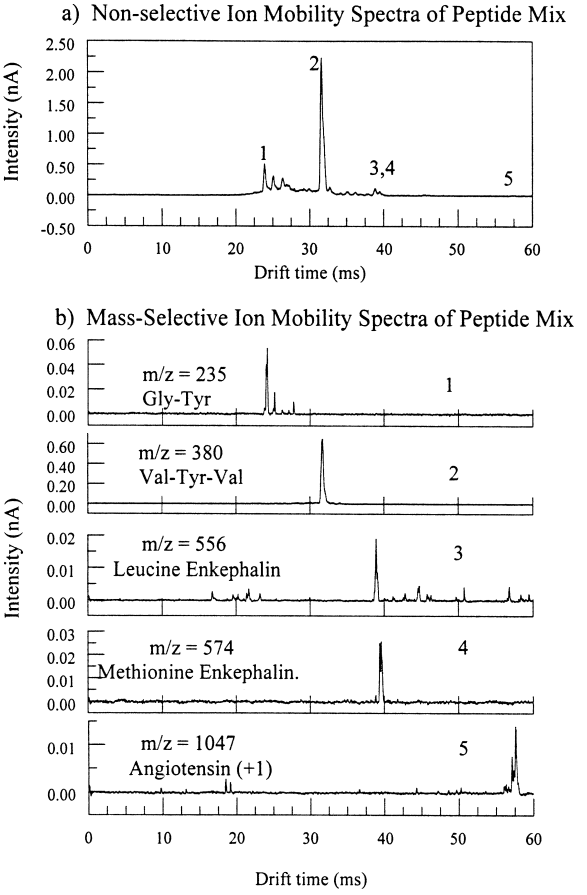

Fig. 7. (a) Non-selective and (b) mass-selected ion mobility spectra of peptide mix. Graph shows two-dimensional information which can be achieved by IMS–MS (drift times and *m*/*z* values). IMS Conditions: 0.2 ms pulse width, 1000 averages, scan time 60 ms. the peptide mixture was diluted to a concentration of 100 ppm and 1000 ng of each peptide was injected for each ion mobility spectrum.

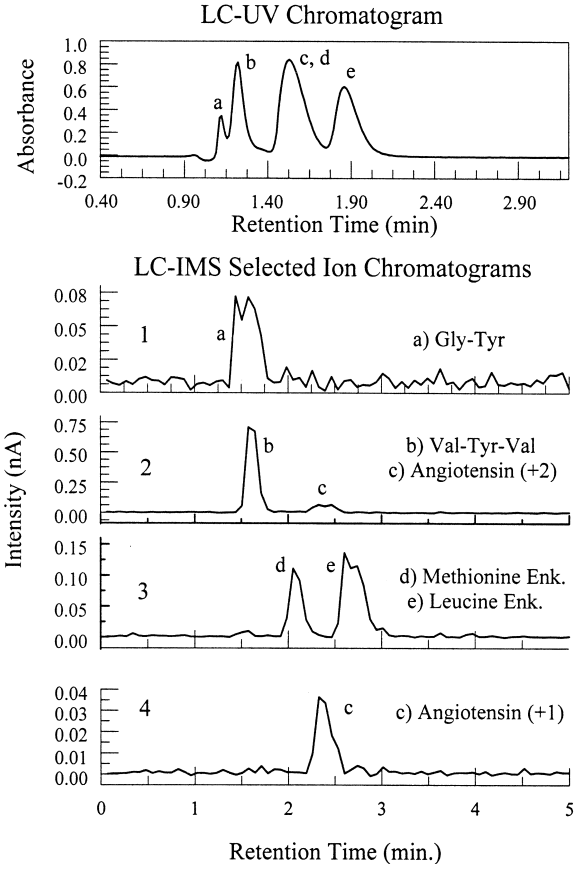

Fig. 8. LC–UV chromatogram and LC–IMS selected ion mobility chromatograms. HPLC conditions: isocratic acetonitrile–water (40:60) with 0.1% TFA, flow-rate 10 μl/min. IMS conditions: 0.5 ms pulse width, 30 averages, 60 ms scan time. Ion mobility windows were as follows (all with 0.6 ms window) 1—24.2 ms, 2—31.7 ms, 3—39.0 ms, 4—57.6 ms. A 50-ng amount of each peptide was injected.

different mobility and could be easily distinguished. Fig. 7 shows the two dimensions of information that can be obtained with IMS and MS (the drift time and m/z value for each compound). For these experiments, the protonated ions were monitored and evaluated. The drift times, reduced mobility values, and corresponding m/z values are reported in Table 1.

In Fig. 8, the HPLC–UV chromatogram (top) and the HPLC–IMS selected ion mobility chromatograms (bottom) are shown. The HPLC conditions employed in Fig. 8 were isocratic (40% acetonitrile with 0.1% TFA) and the separation of the five compounds occurred in 3 min. The isocratic conditions did not enable baseline resolution of angiotensin and methionine enkephalin and the two peaks co-eluted as seen in the HPLC–UV chromatogram (Fig. 8a). However, all five compounds were easily distinguished in the selected ion mobility chromatograms (Fig. 8b). This is a prime example of the additional selectivity and information that IMS can add to HPLC. Fig. 8b showed that the selected ion mobility chromatograms were able to identify all five components where the UV detector could not.

4. Conclusion

Based on this work, there were three main advantages that IMS could provide HPLC separations.

The ESI-IMS analysis of non-volatile buffers (for example sodium phosphate) did not cause deterioration of the instrumental performance. Therefore, IMS could be utilized as an interface between HPLC and

Table 1
K_0 values for five peptides studied in the evaluation of the HPLC–IMS separation

Peptide	m/z	Ion identity	Drift time (ms)	K_0* ($cm^2\ V^{-1}\ s^{-1}$)
Gly–Tyr	235	MH^+	24.15±0.09	1.211±0.044
Val–Tyr–Val	380	MH^+	31.68±0.06	0.922±0.017
Leucine enkephalin	556	MH^+	38.94±0.06	0.750±0.011
Methionine enkephalin	574	MH^+	39.48±0.06	0.739±0.011
Angiotensin	1047	MH^+	57.60±0.06	0.506±0.011
Angiotensin	524	$(M+2H)^{2+}$	39.00±0.06	0.748±0.011

*K_0 values deviate by 3% on a day to day basis. For these experiments, the K_0 values were checked and verified on 5 separate days and found to be within the 3% error.

MS, to clean the sample or HPLC mobile phase prior to introduction into the MS interface.

Second, many HPLC detectors require analytes to have specific chemical properties (i.e., UV detectors require analytes to be chromophores) and do not offer additional component information besides HPLC retention times. Unlike many HPLC detection methods, IMS does not require specific analyte properties and provides a second dimension of information (ion mobilities). Third, IMS offers a third dimension of analyte information and separation for HPLC–MS. With our ESI-IMS–MS instrument, the quadrupole cannot scan fast enough to attain mass spectra for a given ion mobility peak but a time-of-flight MS is able to obtain mass spectra in time with the ion mobility. This three-dimensional approach would offer several advantages; increases in sample throughput, more confident analyte identification, ability to use more complex HPLC mobile phases, and ability to analyze more complex samples without the use of a second column. While this preliminary study has not addressed all the issues required to make IMS a routine HPLC detection method and HPLC–MS interface, it has shown the possibility of the significant improvements that could be made with further instrumental development.

Acknowledgements

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