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Electrospray ionization with ambient pressure ion mobility separation and mass analysis by orthogonal time-of-flight mass spectrometry

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Rapid screening and identification of drug and other mixtures are possible using a novel ambient pressure high-resolution ion mobility (APIMS) orthogonal reflector time-of-flight mass spectrometer (TOFMS). Departing ions from the APIMS drift tube traversed a pressure interface between the APIMS and TOFMS where they were subjected to numerous gas collisions that could produce selective fragmentation. By increasing the accelerating field in the pressure interface region, the ions generated using water-cooled electrospray ionization (ESI) underwent collision-induced dissociation (CID). Mixtures of ESI ions were separated by APIMS based on their respective size-to-charge (*s/z*) ratios while CID and analysis of mass-to-charge (*m/z*) ratios occurred in the pressure interface and TOFMS. Product ions that were formed in this pressure interface region could be readily assigned to precursor ions by matching the mobility drift times. This process was demonstrated by the examination of a mixture of amphetamines and the resulting fragmentation patterns of the mobility-separated precursor ion species $[M + H]^+$.

The coupling of IMS with MS[1] for chemical analysis has proven to be a powerful means for characterizing mixtures via a two-dimensional matrix of gas-phase ion mobilities and *m/z* ratios. Traditionally, the first IMS-MS instruments employed quadrupole mass spectrometers.[1–4] This arrangement was found to be relatively slow and insensitive because of the need to scan *m/z* values sequentially in the quadrupole mass filter. The ability of a time-of-flight (TOF) to acquire all ions without having to scan through the *m/z* makes this analyzer potentially much more sensitive for an IMS-MS hybrid instrument. Young *et al.* first coupled a low-pressure (2–10 Torr) low-temperature (~25 °C) IMS tube to an orthogonal TOF analyzer in order to measure the formation and decomposition rates of hydrates of the hydronium ion.[5] More recently, this approach has been used by several research groups in combination with ESI for the analytical separation and determination of various biochemical compounds.[3,6–8] Additionally, Clemmer *et al.* have coupled both an ion trap and a collision-induced dissociation (CID) cell to their low pressure IMS-TOF instrument to improve sensitivity and analyze fragmentation products.[9,10]

For the most part, IMS-TOF instruments have used low-pressure IMS drift tubes for the mobility separation step. However, because ESI is suited for operation at elevated pressures and higher IMS resolving power may be obtained under the same conditions, it is desirable to assess the IMS-TOF with an ambient pressure ion mobility spectrometer (APIMS). Furthermore, the time scale in which the ions drift through a typical APIMS tube (ms) is about three orders of magnitude larger than that of the ion flight times in the TOFMS (μs). This allows for simultaneous collection of both interleaved mobility and mass spectra. While Guevremont *et al.*[6] did use ambient pressure ion mobility separation prior to TOF analysis, the interface between the IMS and TOF was a capillary tube, which created band broadening of the mobility-separated ions, and the configuration of the IMS-TOF instrument was linear which limited mass spectral resolution and sensitivity.[6]

In this paper we describe our initial experience with an electrospray ionization high-resolution ambient pressure ion mobility spectrometer that was coupled to an orthogonal reflector time-of-flight mass spectrometer (ESI/APIMS-TOFMS). Utilizing the benefits afforded by this high-pressure system, a rapid two-dimensional separation of mixtures was possible. Initial evaluation of this instrumental design demonstrated the collision-induced dissociation (CID) of mobility separated amphetamine ions ($[M + H]^+$) in a high-pressure interfacial region. The precursor and product ions were then differentiated in the two-dimensional spectrum provided by this instrument. Recently, efforts have been made using low-pressure IMS with a quadrupole mass analyzer to explore interfacial CID.[11]

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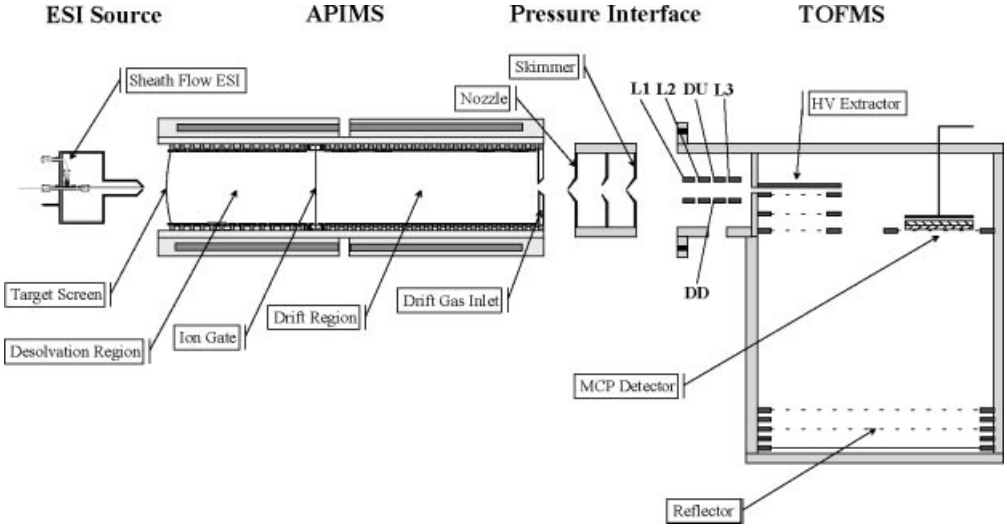

Figure 1. Schematic diagram of the electrospray ionization high-resolution atmospheric pressure ion mobility orthogonal reflector time-of-flight mass spectrometer (ESI/APIMS-TOFMS).

However, care must be taken to avoid incorrect assignment of ions to their respective precursors using IMS-MS pinhole interfaces.[12]

EXPERIMENTAL

The basic components of the ESI/APIMS-TOFMS instrument, as shown in Fig. 1, are: (1) ESI source; (2) APIMS drift tube; (3) high-pressure interface; (4) TOF *m/z* analyzer; and (5) data acquisition system. Each module is described in detail below. Since the fundamental operating principles and experimental procedures of ion mobility separation have been presented in detail previously,[13,14] a short outline of the typical experimental sequence is presented here. Desolvated ions from the electrospray process drift through an inert APIMS tube under a weak uniform electric field, facilitating separation based on differing mobilities. Ions exiting the APIMS drift tube enter a pressure interface where precursor ion fragmentation may be induced. Precursor and product ions are then transported through a series of lenses into the TOFMS. This configuration allows for MS/MS-like performance without the need to scan the first MS.

ESI

Solvents (methanol, water, and acetic acid) were obtained from J.T. Baker (Phillipsburg, NJ, USA). Amphetamine derivatives (methamphetamine (MA), ethylamphetamine (EA), 3,4-methylenedioxy methamphetamine (MDMA) and 3,4-methylenedioxy ethamphetamine (MDEA)), at 1 mg/mL in methanol, were purchased from Radian (Austin, TX, USA). Positively charged ions were formed by electrospraying solutions containing 10 ppm amphetamine derivatives in 47.5% water/47.5% methanol/5% acetic acid. For all experiments, the electrospray needle was held at +13 kV, resulting in a +3.5 kV difference between the ESI and the target screen (first ring in the APIMS tube). A KD Scientific (New Hope, PA, USA) 210 syringe pump was employed to maintain solution flow rates of 8 μL/min for each 25-min run.

APIMS

The APIMS used in these studies was built at Washington State University. The basic stacked-ring design has been described previously[15] and modifications to the original design were reported.[16] The APIMS was divided into two regions, the desolvation region (11 cm in length) and the drift (16 cm in length), which were separated by a Bradbury-Nielsen style ion gate.[17] Both regions consisted of alternating high-purity alumina spacers (Coors Ceramics, Golden, CO, USA) and stainless steel rings that were connected via 500 kΩ and 1 MΩ high-temperature resistors (Caddock Electronics Inc., ±1%) for the desolvation and drift regions, respectively. Nitrogen drift gas was employed counter-flow to ion migration at a rate of 1 L/min. The temperature in both the drift and desolvation regions was maintained at 220 °C.

Pressure interface

As ions exited the drift tube, they entered the pressure interface through a 300-μm pinhole nozzle (+200 V) and underwent a pressure drop from 690–705 Torr to 1.7 Torr (600 L/min DS602 rotary vane pump, Varian). A focusing lens in the interface allowed control of the collisional energy of the ions with residual gas particles. Thus, the electrosprayed ions could be fragmented by CID or passed through the pressure interface intact, depending on the potentials applied to the focusing lens. The precursor ions and/or the fragment ions exited the pressure interface though a second 300-μm pinhole skimmer (+100 V).

TOFMS

The compact orthogonal reflector time-of-flight mass spectrometer has been described previously.[18] Ions exited from the pressure interface into the first chamber, the primary beam chamber, which was at roughly 2×10^{-4} Torr, pumped by a 46 L/s V70 turbo pump (Varian, Lexington, MA, USA). A series of lenses and a deflector (labeled L1-L3, DU, DD in Fig. 1) were used to focus the

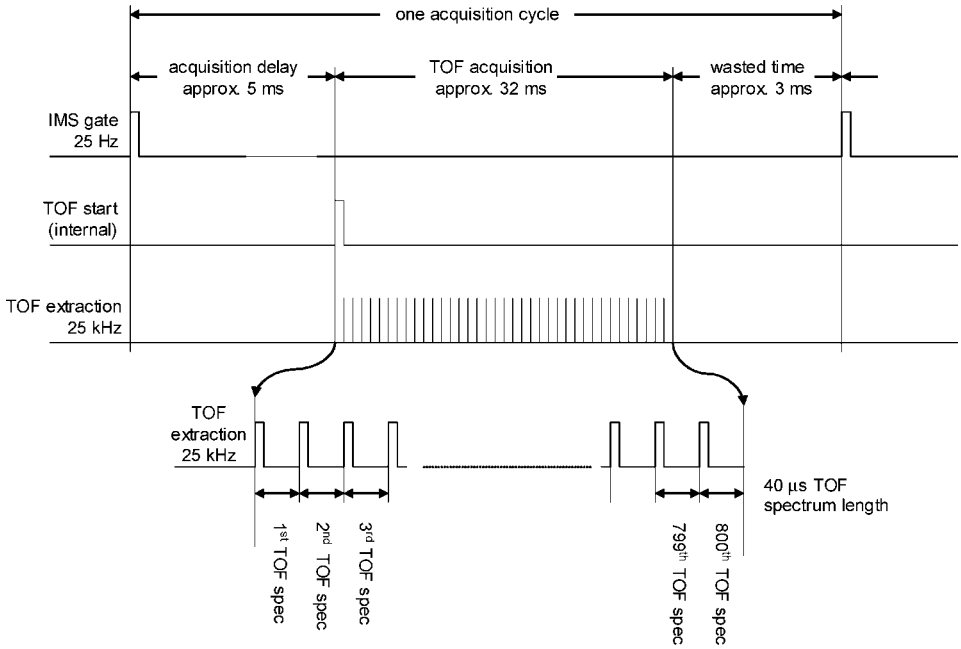

Figure 2. Example of a typical acquisition timing sequence. Ions are gated for 200 μs into the drift region at a frequency of 25 Hz. This provides 40 ms for each APIMS mobility spectrum. Allowing a 5 ms acquisition delay in order to wait for the fastest mobility ions, the APIMS is then sampled every 40 μs by the TOFMS for roughly 32 ms. This provides sampling of the APIMS mobility spectrum with 800 TOFMS extraction spectra.

ions into a parallel beam. Ions then entered the TOF chamber through a 2×10 mm slit. The TOF chamber was at 4×10^{-6} Torr (250 L/s V250 turbo pump, both turbo pumps were backed by a 100 L/min DS102 rotary vane pump, Varian, Lexington, MA, USA). Segments of the primary beam of ions were orthogonally extracted by a bipolar extractor pulsed at 20–80 kHz (Ionwerks pulser model HVPS, Houston, TX, USA). The ions were then further accelerated into the −2000 V drift region. After passing the reflector they were detected by a chevron microchannel plate (MCP) detector.

Data acquisition

A typical acquisition timing sequence, comprised of a real-time two-dimensional matrix of simultaneous drift times and flight times, is shown in Fig. 2. Ions were gated for 200 μs into the drift region at a frequency of 25 Hz; this provided a maximum of 40 ms for the APIMS spectrum. The TOFMS provided 800 TOF spectra with a maximum length of 40 μs when operated at a frequency of 25 kHz. The APIMS gate and the TOFMS extractor were both triggered by a personal computer (PC)-based timing controller (Ionwerks model 307-001, Houston, TX, USA). Data acquisition could be delayed with respect to both APIMS gate and TOFMS extraction, in order to reduce the size of the data array. Experimental data acquisitions were typically run for 25 min to provide satisfactory ion statistics. This ensured that the effects of ionization efficiency and ion transmission were not a limiting factor. Flight times were recorded by a fast time-to-digital converter (TDC, Ionwerks model TDCX4) activated by the same timing controller. Synchronization of this electronic hardware was facilitated by the use of a dual Pentium III workstation running Ionwerks two-dimensional acquisition software. Spectral compilations of data once acquired were then exported into both 2D Transform[19] and 3D NoeSYS[20] software.

APIMS-TOFMS calculations

A practical measure, and often the most useful definition of resolving power, R_{IMS} for IMS[21] is given by:

$$R_{IMS} = t_d/\Delta t_{FWHM} \quad (1)$$

where t_d is the drift time of ions of interest and Δt_{FWHM} is the temporal peak width measured at half-height. The drift time, t_d, of an ion in the APIMS drift tube is related to the length of the drift cell space, L, in centimeters, the voltage drop across the drift space, V, and the mobility of the ion K in cm^2/Vs as shown by:

$$t_d = L^2/KV \quad (2)$$

However, to take into account varying environmental and experimental conditions, it is more prudent to discuss ion drift times in terms of reduced mobility constants (K_o)[15] which are defined by:

$$K_o = (L^2/V^* t_d)(273.5/T)(P/760) \quad (3)$$

where L is the drift region length (15.0 cm), V is the drift voltage (7400 V), T is the effective temperature in the drift region (220 °C), and P is the atmospheric pressure.

For the TOFMS,[18] the mass resolving power, R_{TOF}, is

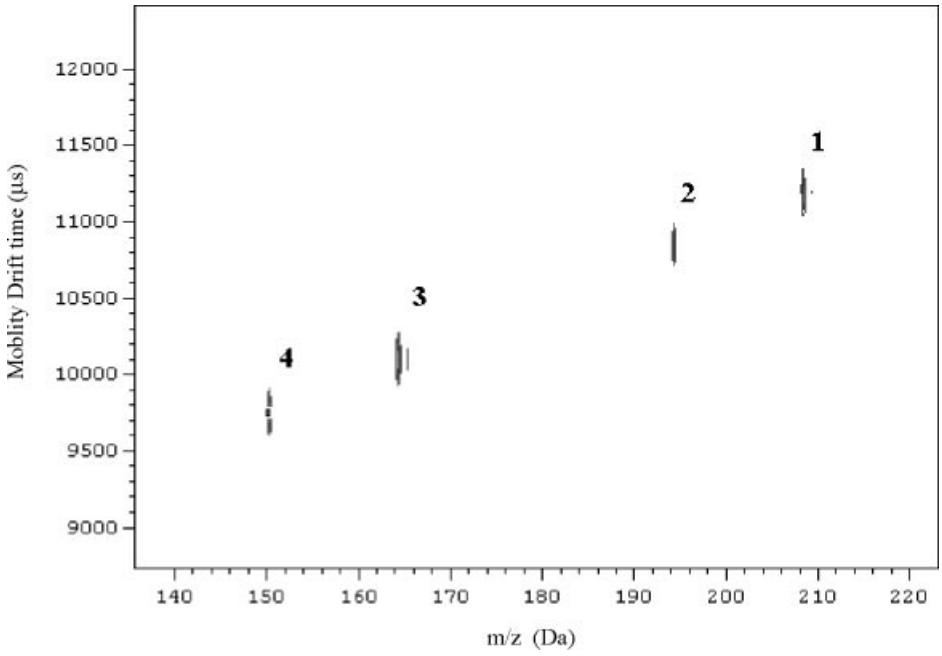

Figure 3. Two-dimensional contour plot of four amphetamines; (1) 3,4-methylenedioxy ethamphetamine (MDEA), (2) 3,4-methylenedioxy methamphetamine (MDMA), (3) ethylamphetamine (EA), and (4) methamphetamine (MA). The voltages of the pressure interface were +175 V nozzle, +130 V focus, and +100 V skimmer, which minimized CID. The mass resolving power, R_{TOF}, was found to be around 660, whereas the resolving power along the drift time axis, R_{IMS}, was typically around 62.

defined by:

$$R_{TOF} = m/\Delta m = \tau_f/2\Delta\tau_{FWHM} \quad (4)$$

where, τ_f denotes the *flight time* of an ion species in the TOFMS and $\Delta\tau_{FWHM}$ denotes the width of the distribution of the flight times. The ion flight time has a square root relationship with the ion mass/charge ratio, *m/z*:

$$\tau_f = k(m/z)^{1/2} \quad (5)$$

where the proportionality factor, *k*, is the TOFMS instrumental constant.

RESULTS AND DISCUSSION

Four common amphetamine derivatives were used to evaluate this instrumental method (MA, EA, MDMA, and MDEA). Although the compounds themselves can be divided into two classes, simple amphetamines (MA and EA) and 3,4-methylenedioxy amphetamine derivatives (MDMA and MDEA), they all contain just one protonation site.[22] Thus, the ions produced from both low and high fragmentation voltages on the pressure interface were only singly charged. Figure 3 shows a two-dimensional APIMS-TOFMS contour plot of the $[M + H]^+$ ions for these four amphetamines. The voltages on the pressure interface were low enough (+175 V nozzle, +130 V focus, and +100 V skimmer) to limit CID. The overall resolving power along the TOFMS flight time axis, R_{TOF}, is 500 to 1000. For the data presented here, the resolving power was around 660. The resolving power along the drift time axis, R_{IMS}, can reach up to 100 for singly charged ions.[2] For the data in Fig. 3, the resolving power was typically around 62, most likely due to the field heterogeneities at the nozzle, which have not yet been completely optimized. Table 1 presents the tabulated values of R_{IMS}, R_{TOF}, and K_o, which are calculated based on Eqns (1), (4), and (3), respectively. Values for K_o for all four

Table 1. Reduced mobility values (K_o) and literature K_o[22] values for the four amphetamines employed in this study. Resolving power for both APIMS and TOFMS is also shown

Amphetamine	MW (u)	Drift time (ms)	K_o($cm^2V^{-1}s^{-1}$)	Literature K_o[22] ($cm^2V^{-1}s^{-1}$)	R_{IMS}	R_{TOF}
MA	149	9.750	1.57	1.585	57	625
EA	163	10.10	1.51	1.525	59	631
MDMA	193	10.85	1.41	1.420	64	692
MDEA	207	11.20	1.36	1.374	66	693

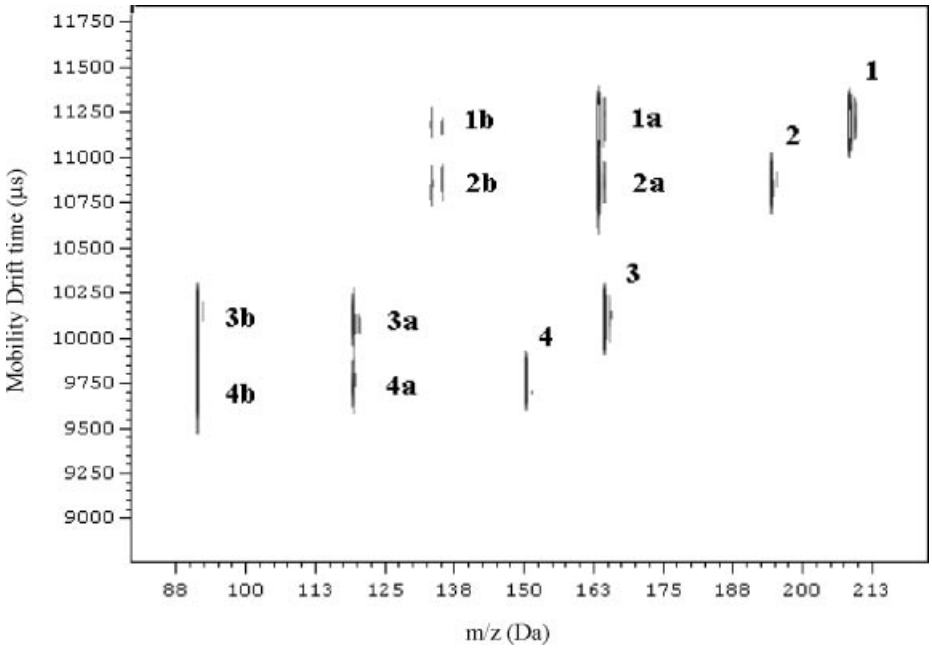

Figure 4. Two-dimensional contour plot of the four amphetamines precursor ions shown in Fig. 3 and their fragment ions. For example, (1a) corresponds to the loss of neutral ethylamine, while (1b) indicates the loss of both the neutral ethylamine, but an ethylene group as well (see Fig. 5). All fragment ions formed in the pressure interface (+175 V nozzle, +170 V focus, and +100 V skimmer) have APIMS drift times that are comparable to their precursor ions. All other experimental conditions were the same as in Fig. 3.

amphetamines were found to be consistent with literature values.[22]

Figure 4 shows the two-dimensional contour plot of a mixture of these four amphetamines with CID. The voltage on the pressure interface was increased (+175 V nozzle, +170 V focus, and +100 V skimmer) to produce CID

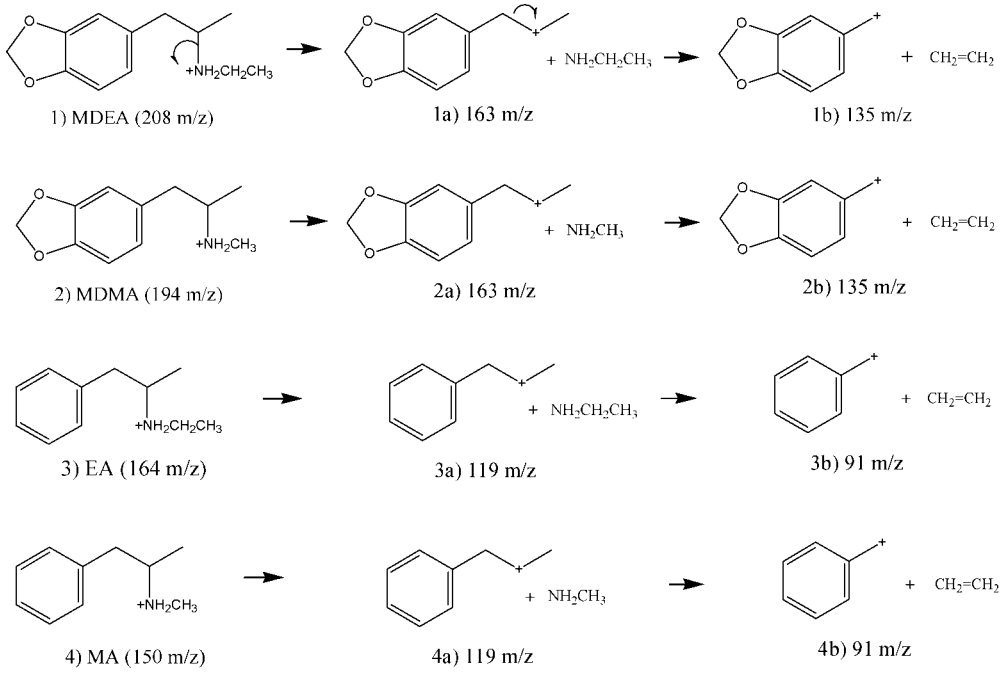

Figure 5. Expected molecular fragments of the four amphetamine precursor ions. In the case of (1) MDEA and (3) EA dissociation occurs with the loss of an ethylamine group or as a loss of both the ethylamine and ethylene group. This type of dissociation process is also indicative of the (2) MDMA and (4) MA precursor ions.

fragmentation. Examination of Fig. 4 shows that many additional peaks were present (compared to Fig. 3). Analysis of both the APIMS mobility times and TOFMS mass dissociation spectrum for these peaks showed that the new peaks could be assigned to expected fragments of the four precursor ion amphetamines. CID fragmentation pathways for the four amphetamines are shown in Fig. 5. For example, at a mobility drift time of 11.20 ms, the 208 MDEA precursor ion was observed to be clearly resolved from two fragment peaks (1a) and (1b) with *m/z* ratios of 163, and 135, respectively. The *m/z* 163 fragment corresponded to the loss of a neutral ethylamine group, while the *m/z* 135 fragment corresponded to the loss of both neutral ethylamine and ethylene groups. Every peak in Fig. 4 was either a precursor ion that did not undergo CID in the pressure interface, or a CID product that was linked to its precursor ion by identical mobility drift times. These data effectively illustrated the use of ESI/APIMS-TOFMS as a method for simultaneous structural analysis of ion mixtures using CID analysis. Without APIMS separation of the precursor ions, the mass spectrum of the precursor and product ions would be complex and difficult to interpret.

CONCLUSIONS

These two-dimensional data demonstrate that it is possible to successfully interface an ambient pressure ion mobility spectrometer to a time-of-flight mass spectrometer. In addition to two-dimensional separation data, this instrumental configuration provides the option of operating the APIMS-TOFMS in a collision-induced dissociation mode. By adjusting the ion focusing voltages of the ions lens in the pressure interface, mobility-separated precursor ions can be fragmented to product ions or they can be passed into the mass spectrometer without fragmentation. With mobility separation of precursor ions before CID fragmentation, the APIMS-TOFMS becomes a powerful instrument for the rapid identification of unknowns in complex mixtures. While the example mixture used in this initial evaluation of the instrument was limited to amphetamines, demonstrating its potential as a rapid screening method for drugs, two-dimensional IMS-MS analysis has been successfully employed for a variety of sample mixtures including amino acids, peptides, proteins, chemical warfare agents, and pesticides. From initial resolution, sensitivity, and fragmentation data provided in these experiments, it appears that the APIMS-TOFMS will be applicable for the rapid analysis of a wide variety of environmental, industrial, and biological samples.

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