The separation of opiates and the primary metabolites was evaluated with ESI-IMS/MS. Seven opiate molecules were analyzed, and spectra were shown for each compound. The IMS separation of two isomers (morphine and norcodeine) was shown with baseline separation. Differences in the mobilities were found for the nonacetylated, monoacetylated, and biacetylated compounds. In this study, two primary findings are reported. First, IMS can easily separate metabolic isomers, and second, the two-dimensional separation capability of IMS/MS can be employed to confidently identify and separate both the opiates and metabolites. Although previous IMS studies have shown the separation of isomers, this is the first example to show the capability of IMS to separate metabolic isomers (within 70 s), a significant advantage in high-throughput screening for pharmacokinetic studies. Second, the monoacetylated and biacetylated compounds were found to form more compact ions for the sodium adducts in comparison to the protonated molecular ions. On the basis of the mobilities, information on structures and conformation can be deduced when sodium and protonated ions are compared.

The high incidence of drug abuse necessitates sensitive techniques for drug detection and identification.1 Heroin, morphine, and codeine are the primarily abused opiate compounds, although their metabolites are evaluated as well for characteristic biological markers present hours after intoxication.2 For this reason, opiate separations address the analysis of both the opiates and metabolites.2,3 While many different analytical techniques have been employed for opiate detection,4-6 liquid chromatography/mass spectrometry (LC/MS) is currently the most commonly used method due to its ability to separate metabolic isomers while achieving the required sensitivity with MS detection.2,3

Since its inception, ion mobility spectrometry (IMS) has been utilized as a technique for detecting drugs of abuse.7 Until recently, IMS research for drug detection focused on field portable devices.8,9 Both the coupling of electrospray ionization (ESI) to IMS10 and the introduction of high-resolution IMS instruments11-13 have extended IMS applications to include more complex mixtures (drugs of abuse,14 chemical warfare agents,15 and tryptic digests16,17). Current state-of-the-art IMS/M S systems have achieved separation efficiencies comparable to common chromatographic techniques and, therefore, are being evaluated as a high-resolution separation technique.18

In IMS, ions are identified by measuring the time it takes the ion to travel through a drift tube. An ion’s mobility can be determined from the drift time and is related to an ion’s size-to-charge ratio. Evaluations of high-resolution ESI-IMS/M S systems have elucidated different gas-phase mobilities for many peptide isomers19 and amino acid isomers (leucine and isoleucine).18 Although the relationship between solution-phase geometry and gas-phase geometries remains undertermined,20 ion mobilities provide structural information that can be utilized to consider

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Evaluation of Opiate Separation by High-Resolution Electrospray Ionization-Ion Mobility Spectrometry/Mass Spectrometry

The separation of opiates and the primary metabolites was evaluated with ESI-IMS/MS. Seven opiate molecules were analyzed, and spectra were shown for each compound. The IMS separation of two isomers (morphine and norcodeine) was shown with baseline separation. Differences in the mobilities were found for the nonacetylated, monoacetylated, and biacetylated compounds. In this study, two primary findings are reported. First, IMS can easily separate metabolic isomers, and second, the two-dimensional separation capability of IMS/MS can be employed to confidently identify and separate both the opiates and metabolites. Although previous IMS studies have shown the separation of isomers, this is the first example to show the capability of IMS to separate metabolic isomers (within 70 s), a significant advantage in high-throughput screening for pharmacokinetic studies. Second, the monoacetylated and biacetylated compounds were found to form more compact ions for the sodium adducts in comparison to the protonated molecular ions. On the basis of the mobilities, information on structures and conformation can be deduced when sodium and protonated ions are compared.

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possible structures. Additional peptide structural information has been elucidated from sodium adduct formations in which it was found that doubly charged bradykinin ions that wrapped around a sodium ion formed a more compact structure than the \((M + 2H)^{2+}\) ion.

Understanding the relationship between conformational geometries and biological activity is important for not only proteins (i.e., enzymes) but drug molecules as well. As described above, the utility of IMS/MS for discerning peptide and protein conformations has been shown. The objectives of this study were to develop IMS/MS as an alternative (faster) separation technique to LC/MS. Because IMS provides ion size information, structural features of the opiates and metabolites were also investigated.

**EXPERIMENTAL SECTION**

**Reagents and Chemicals.** The seven opiate derivatives employed in this study were purchased from Alltech Associates (Bellefonte, PA) as 1.0 mg/mL standards in methanol (heroin concentration was 0.10 mg/mL) and included norcodeine, codeine, morphine, 6-monoacetyl codeine (MAC), 6-monoacetyl morphine (MAM), and heroin. All standard solutions were diluted to concentrations of ~100 ppm (~ 300 \(\mu\)M) (heroin was diluted to 50 ppm concentration). All solvents (water, methanol, and acetic acid) were obtained from J. T. Baker (Phillipsburg, N. J.) and were HPLC grade.

**Instrumentation.** A high-resolution ESI-IMS/MS instrument was employed for all experiments and has been described previously (water-cooled ESI source, high-resolution IMS/MS, and recent modification to the IMS/MS 18). The ESI source was employed for all experiments and has been described. A high-resolution ESI-IMS/MS instrument was developed by IMS/MS as an alternative (faster) separation technique to LC/MS. Because IMS provides ion size information, structural features of the opiates and metabolites were also investigated.

The IMS was interfaced to a 150-QC ABB-Extrel (Pittsburgh, PA) quadrupole mass spectrometer via a 40-pinhole, 40-pinhole, 40-pinhole, 40-pinhole, 40-pinhole, 40-pinhole, 40-pinhole, 40-pinhole. A series of six Einzel lenses were placed after the drift tube and were operated at the following voltages (listed in order): +8.0 (pinhole), −12.6 (screen), −27.3 (first element of einzel), −14.6 (second element of einzel), −126.7 (third element of einzel), and +31.2 V (ELFS plate). The dynode and electron multiplier were operated at −5.0 and 1.7 kV, respectively, and the quadrupole mass filter was biased at −15.2 V.

The current signal was collected with a Keithley model 427 amplifier (Keithley Instruments, Cleveland, OH) and amplified (10⁶ gain), and the signal was sent to a Labview (National Instruments, Houston, TX) data acquisition system. All spectra were the result of 1000 averages obtained with a gate pulse width of 0.2 ms and total scan time of 50.0 ms. Due to overhead from Labview data processing, each spectrum took 70 s to obtain. All IMS spectra presented were obtained in one of two instrumental operation modes: non-mass-selective and mass-selective ion monitoring (SIM). For the first mode (rf only), the quadrupole served to transmit ions from the IMS drift tube to the electron multiplier. For SIM, the quadrupole selectively transmitted one m/z value from the IMS tube.

**Calculations.** All reduced mobility constants \((K_o)\) and collision cross-section \((\Omega)\) values were calculated from experimentally determined drift times \(t_d\). The reduced mobility constants were calculated according to the following equation:

\[
K_o = \left(\frac{L^2}{Vt_d}\right)\left(\frac{273}{T}\right)\left(\frac{P}{760}\right)
\]

where \(L\) was the drift region length (22.5 cm), \(V\) was the drift voltage (8680 V), \(T\) was the effective temperature in the drift region (523 K), and \(P\) was the pressure in the drift region (695–700 Torr).

The average ion collision cross section \((\Omega)\) was calculated from the following equation:

\[
\Omega = \left(\frac{3}{16N}\right)\left(\frac{2\pi}{\mu kT}\right)^{1/2}\left(\frac{z e V t_d}{L^2}\right)
\]

where \(z\) is the number of the charges on the ion, \(e\) is the charge of one proton, \(N\) is the number density of the drift gas, \(\mu\) is the reduced mass of an ion \((m + M)\), and \(k\) is Boltzmann’s constant.

**RESULTS AND DISCUSSION**

In Figure 1, the structures for the seven opiates compounds studied are shown. The molecular weight for each compound is also presented and listed in the order of increasing mass. Three abused drugs (heroin, codeine, and morphine) and four primary metabolites were evaluated. The differences in the structures were due to functional group placement at one or more of the three sites, \(R_1\), \(R_2\), or \(R_3\). Norcodeine and normorphine are the demethylated derivatives of codeine and morphine, respectively. MAC and MAM are the acetylated derivatives of codeine and morphine, respectively.

For all seven compounds, both nonselective IMS spectra and SIM spectra were obtained to mass identify each ion mobility peak that was observed. The reduced mobility constants \((K_o)\) and corresponding m/z values for the seven compounds are listed in Table 1. Several trends in ion formation can be elucidated from Table 1. First, all seven compounds formed a protonated molecular
Table 1. Reduced Mobility Values and Mass Identities for Ions Formed from Seven Opiate Compounds

<table>
<thead>
<tr>
<th>compound</th>
<th>ion</th>
<th>m/z</th>
<th>m/z</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M - 18 (M - H2O)+</td>
<td>1.16</td>
<td>1.12</td>
<td>1.10</td>
</tr>
<tr>
<td>normorphine</td>
<td>M + 1 (MH)+</td>
<td>253</td>
<td>272</td>
<td>294</td>
</tr>
<tr>
<td>norcodeine</td>
<td>M + 23 (M + Na)+</td>
<td>267</td>
<td>286</td>
<td>308</td>
</tr>
<tr>
<td>codeine</td>
<td>M (M + Na)+</td>
<td>267</td>
<td>286</td>
<td>308</td>
</tr>
<tr>
<td>6-monoacetyl morphine</td>
<td>M - 59 (M - COOCH3)+</td>
<td>1.14</td>
<td>1.07</td>
<td>1.04</td>
</tr>
<tr>
<td>6-monoacetyl codeine</td>
<td>M - 59 (M - COOCH3)+</td>
<td>281</td>
<td>300</td>
<td>322</td>
</tr>
<tr>
<td>heroin</td>
<td>M - 59 (M - COOCH3)+</td>
<td>1.10</td>
<td>1.07</td>
<td>1.04</td>
</tr>
</tbody>
</table>

*a* Identified as M - 43 ion for heroin.

![Figure 1](image-url)  
**Figure 1.** Structure of seven opiate compounds evaluated in this study.

![Figure 2](image-url)  
**Figure 2.** Nonselective ESI-IMS/MS spectra of four nonacetylated compounds: (a) normorphine, (b) morphine, (c) norcodeine, and (d) codeine. Products ion for each compound were mass identified to be (1) (M - H2O)+, (2) MH+, and (3) (M + Na)+.

Although similar fragmentation has been seen by increased skimmer voltages in ESI-MS, the unique mobilities for the decomposition products indicated that the fragmentation occurred prior to beginning the ion mobility experiment. The difference in the ionization process is not obvious, although a fundamental understanding of the difference would enable manipulation of experimental parameters to gain molecular information. Based on these structural observations and distinguishing functional group losses, the following results are broken into two groupings: nonacetylated opiates and acetylated opiates.


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IMS Spectra for Nonacetylated Opiates. Nonselective ESI-IMS/MS spectra for the four nonacetylated opiates are shown in Figure 2. Although not presented in Figure 2, each observed mobility peak was also mass identified to determine the ion identity and each is labeled as (1) \((M - H_2O)^+\), (2) \(MH^+\), and (3) \((M + Na)^+\) for the four compounds: (a) normorphine, (b) morphine, (c) norcodeine, and (d) codeine. Although the ion formation for the four opiates is similar, there are differences in the relative responses that allude to structural dependences on ion formation in ESI. First, the overall signal intensities for normorphine and morphine \(MH^+\) ions were \(60\%\) less than those observed for codeine and norcodeine. The structural difference in the two groups is a methyl group on the \(R_1\) oxygen group (refer to Figure 1). Signal intensity ratios \((M - 18: M + 1, M + Na)\) were found to be greater for codeine and norcodeine, as well. The addition of the methyl group on the \(R_1\) group seems to have an affect on ESI ion signal intensity. The formation of all three ions (loss of water, protonated ion, and sodium adduct) was increased by the methyl addition and can be observed by speculation in Figure 2 (a and b compared to c and d).

As seen in Figure 2, increasing ion masses correlated with longer drift times and, hence, lower \(K_o\) values. Although this general relationship was expected and observed, the relative increase for each compound was dependent on the molecular structure. Both the collision cross sections and percent increase in cross section are listed in Table 2 for the four compounds. For normorphine, addition of a sodium to the molecule increased the collision cross section by \(1.4\%\). In comparison, both morphine and codeine collision cross sections increased (addition of a sodium) by \(2.7\%\). This difference in collision cross section due to the sodium addition indicate that there could be differences in the placement of sodium on the molecule.

Although morphine (Figure 2b) and norcodeine (Figure 2c) were isomers, differing in the placement of a methyl group (\(R_3\) on morphine, \(R_1\) on norcodeine), inspection of the IMS spectra for the two compounds shows that the drift times were different for the two ions. This difference was further elucidated by preparing the two isomers in a mixture and evaluating their separation. Figure 3 shows the SIM spectra for each ion formed.
for the two isomeric compounds: (1) morphine and (2) norcodeine. It can be seen that, for both the (M-H2O)+ and MH+ ions, the morphine ions drifted faster than the norcodeine ions. Baseline separation was easily obtainable for the two isomers, demonstrating a significant advantage of IMS separation.

**IMS Spectra for Acetylated Opiates.** In Table 3, the collision cross sections for the three acetylated molecules are shown. As expected, the ions that lost a COOCH3 (59) group had a lower collision cross section than the MH+ ion which differed by approximately 12–15%.

Acetylation of either oxygen atom (R1 for MAM and MAC, R1 and R2 for heroin) altered the relative mobilities for the MH+ ion and sodium adduct. In Figure 4, both the nonselective (a) and SIM (b) IMS/MS spectra for MAC are shown. In the top nonselective IMS spectra, only two peaks are observed. Upon mass identifying each peak (b), it was determined that the peak at ~25 ms correlated to the M-59 ion and the larger peak at 28 ms is due to both the protonated molecular ion (28.2 ms) and sodium adduct (28.4 ms). The two ion collision cross sections (161 and 162 Å², respectively) differ by 0.6% compared to 2.7% for the nonacetylated counterpart, codeine.

The affect of the sodium on ion conformation had an even greater impact on the ion mobility spectrum of MAM and heroin. In Figure 5, a similar ion mobility representation is shown for MAM (a, nonselective mode; b, SIM mode). While the deacetylated ion drifts for 24 ms, both the protonated and sodiated ions have drift times of 27 ms. The interaction with the sodium atom appears to be even stronger for the MAM molecule, and the two ions (protonated and sodiated ions) are approximately the same size.

The replacement of the methyl group on MAC with a COCH3 results in the formation of heroin. In Figure 6, both the nonselective and SIM IMS/MS spectra are shown. Comparison of the lower two SIM spectra (m/z 370 (MH+)+ and 392 (M+Na)+) shows that the sodium adduct drifted faster in the ion mobility tube than the protonated ion. This implies that the sodiated ion actually formed a smaller structure (lower collision cross section) than the molecular ion. Upon inspection of the structures (see Figure 1) and the mobility results, it suggests that the electronegative oxygen groups due to acetylation may interact with the sodium to form a compacted ion.

**Separation of Opiates by ESI-IMS/MS.** Although IMS mobilities and m/z values are not completely independent, the higher resolution of our instrument enables small differences in mobilities to be measured. In Figure 7, the measured K₀ values...
for the opiate ions were graphed with each ion’s corresponding m/z value. For all but two sets of ions, each ion had a unique m/z and/or K_o value. Therefore, the two-dimensional separation capability of IMS/MS was realized.

Figure 8 shows an ESI-IMS/MS separation of morphine and its two primary metabolites, normorphine and acetyl morphine. Although, some mobility peaks overlap in the nonselective IMS spectra, each SIM spectra (for the protonated molecular ions) are shown in Figure 8b and can be identified by each ion’s unique K_o:m/z relationship. The complementary separation capabilities of IMS and MS enable opiates as well as other small drug molecules to be efficiently separated.

CONCLUSIONS

The results of this study show two beneficial features of ESI-IMS/MS as a tool for opiate separation. First, with our high-resolution instrument, the two-dimensional separation of the three opiates and metabolites was realized. Because IMS separates ions on the basis of size/charge density, it was found that the predominant ions had a unique K_o:m/z value. Not only was the separation possible, but the separation was performed in 70 s. This is a significant enhancement in analysis times and could produce higher sample throughput. Metabolic isomers were found to be easily separated and had substantially different mobilities. In many pharmacokinetic studies, the capability of identifying isomers is important and this work shows that the unique separation of IMS could contribute to these type of studies.

The findings of this study also alluded to more fundamental contributions of IMS. The difference in molecular ion and sodiated ion mobilities provided insights into the underlying conformations of the ions and, subsequently, the molecules themselves. Heroin was found to form a more compact structure when associated with a sodium ion than the protonated ion. This study provides an example of the size and conformation information that can be determined by determining ion mobilities.

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