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Desorption Electrospray Ionization (DESI) with Atmospheric Pressure Ion Mobility Spectrometry for Drug Detection

Kristyn M. Roscioli[1], Jessica A. Tufariello[2], Xing Zhang[2], Shelly X. Li[3], Gilles H. Goetz[3], Guilong Cheng[3], William F. Siems[2], and Herbert H. Hill Jr.[2]

[1] Pacific Northwest National Laboratory, Richland, WA, USA

[2] Department of Chemistry, Washington State University, Pullman, WA 99164

[3] Pfizer Global Research & Development, Groton Laboratories, Pfizer Inc., Groton, CT 06340

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Correspondence: Kristyn M. Roscioli (email: kristyn.roscioli@pnnl.gov; phone: 509-592-7749)

ABSTRACT

Desorption electrospray ionization (DESI) was coupled to an ambient pressure drift tube ion mobility time-of-flight mass spectrometer (IM-TOFMS) for the direct analysis of active ingredients in pharmaceutical samples. The DESI source was also coupled with a standalone IMS demonstrating potential of portable and inexpensive drug-quality testing platforms. The DESI-IMS required no sample pretreatment as ions were generated directly from tablets and cream formulations. The analysis of a range of over-the-counter and prescription tablet formations was demonstrated for amphetamine (methylphenidate), antidepressant (venlafaxine), barbiturate (barbituric acid), depressant (alprazolam), narcotic (3-methylmorphine) and sympatholytic (propranolol) drugs. Active ingredients from soft and liquid formulations, such as Icy Hot cream (methyl salicylate) and Nyquil cold medicine (acetaminophen, dextromethorphan, doxylamine) were also detected. Increased sensitivity for selective drug responses was demonstrated through the formation of sodiated adduct ions by introducing small quantities of NaCl into the DESI solvent. Of the drugs and pharmaceuticals tested in this study, 68% (22 total samples) provide a clear ion mobility response at characteristic mobilities either as $(M+H)^+$, $(M-H)^-$, or $(M+Na)^+$ ions.

INTRODUCTION

Desorption electrospray ionization (DESI), first introduced in 2004 by Cooks and coworkers [1], is an ambient pressure ionization method for the analysis of analytes on surfaces. In DESI, charged solvent droplets are sprayed towards and collide with the surface of interest, desorbing and ionizing the analyte. The coupling of DESI with mass spectrometry (MS) is useful for the direct analysis of many pharmaceutical products [2-12]. The earliest attempts by Cooks et al. described direct analysis on a number of different surface types including leather, nitrile gloves, a medicine tablet and a blood drop on a finger for a variety of compounds from small pharmaceutical molecules to large biopolymers [1]. Thin layer chromatography plates have been used as the DESI surface for the analysis of aspirin, acetaminophen and caffeine in an early reporting by Deibel et. al [4]. Direct characterization of the active ingredients in pharmaceutical samples formulated as tablets, ointments and liquids have been demonstrated by the use of DESI-MS [3, 5-12].

The mass spectra of pharmaceutical products such as liquids, tablets, ointments, capsules and creams can be rather complex due to the number of excipients and active ingredients formulated into the product. Excipients are added to formulations for many reasons including the prevention of bacterial growth in liquid or cream formulations, improved taste of an oral product, delay product absorption into the body or to help the drug disintegrate into particles small enough to reach the blood stream more quickly [13]. In addition to mass spectral investigations, DESI has been used as an ionization source for an ion mobility spectrometer coupled to a mass spectrometer (IMS-MS) for the rapid and direct analysis of over-the-counter and prescription drugs. Initial studies were conducted using a prototype low-pressure ion mobility-time-of-flight mass spectrometer [14]. DESI has also been coupled to a travelling-wave based ion mobility

time-of-flight mass spectrometer for the rapid and accurate mass analysis of multiple active ingredients formulated into a variety of pharmaceutical products [13]. Ion mobility cell separation of desorbed ions combined with mass analysis significantly enhances selectivity, as compared to MS analysis alone, and the generation of sample ions from tablets or cream formulations by DESI demonstrates the novel utility of this desorption method combined with IMS. The mobility separation of ions generated using DESI help provide a rapid and efficient means of separating active ingredients from excipients added to the final formulated product. An example of ion mobility separations to screen isobaric and chemically interfering species was demonstrated in 2010 by Fernandez and coworkers using a DESI coupled with a differential mobility (DM) ion separation cell [15].

Ion mobility separation approaches have matured into rugged, dependable field analytical techniques [16] and are commonly used for many pharmaceutical applications [17]. Advances in coupling ambient ionization with standalone IMS have given the technique potential for constituting the core of a portable drug-quality testing platform. Laser ablation/desorption electrospray ionization was demonstrated as a useful ionization technique for analyzing the active pharmaceutical ingredients for antimalarial drug quality [16]. Standalone IMS coupled with atmospheric ionization techniques, such as radioactive [18] or electrospray ionization (ESI), have been used to analyze pharmaceuticals used in over-the-counter tablets [19, 20] and dietary supplements [21].

Standalone IMS has also been used in the analysis of antimalarial drugs through the use of DESI [16]. The objective of the research presented in this paper was to expand on this initial work to more completely evaluate the use of DESI-IMS for the rapid and direct detection of active component of drug formulations. The hypothesis of this project was that the use of both

positive and negative modes of DESI operation along with the use of reactive DESI would expand the utility of DESI-IMS as a simple analytical method for monitoring drug formulations.

EXPERIMENTAL

Chemicals. Barbituric acid, sodium chloride and 3-methylmorphine were purchased from Sigma Aldrich Chemical Co. Inc. (Milwaukee, WI). Over the counter and prescription drugs were purchased from Rite Aid pharmacy and include Vicodin, Aspirin, Bayer Baby Aspirin, Nyquil, Zantac, Cetirizine, Icy Hot and Vitamin C. Methylphenidate, venlafaxine, desvenlafaxine, alprazolam and (R/S)-proranolol were kindly donated by Shelly Li at Pfizer Global Research & Development, Groton Laboratories, Pfizer Inc. Methanol solvent (HPLC gradient grade) was supplied by J.T. Baker Inc. (Phillipsburg, NJ).

Standalone Ion Mobility Spectrometer (IMS). The standalone IMS consisted of a desolvation and drift region (10.6 cm in length) constructed with stacked stainless steel rings and alternating ceramic insulating rings. The stainless steel rings (electrodes) were electrically connected by a series of 500-kΩ resistors in the desolvation region and one-MΩ resistors in the drift region (Caddock Electronics Incorporated, Riverside, California, ±1%) which created a uniform electric field within the tube. The desolvation and drift region were separated by a Bradbury-Neilsen style gate. Ions were pulsed into the drift region at a pulse width of 0.2 ms. The IMS tube, which was thermally insulated, was held at a constant temperature of 100^{o}C. The IMS was at atmospheric pressure which is 690-705 Torr for Pullman, Washington. A nitrogen drift gas was held a constant flow rate of ~ 1 L/min.

Ion Mobility Time-of-Flight Mass Spectrometer (IM-TOFMS). The IM-TOFMS instrument used for these experiments has been previously described [22], with the only change being the replacement of the resistive glass IMS with the more conventional stacked ring IMS. The schematic for the overall instrument can be seen in **Figure 1**. There were four main sections of the IMMS: 1) desorption electrospray ionization source, 2) atmospheric pressure ion mobility spectrometer, 3) pressure-vacuum interface, 4) high resolution time-of-flight mass spectrometer. A fused silica transfer line of approximately 29.2 cm in length and 150 μm inner diameter was used to transfer an ionization solvent (50/50 MeOH/H_2O) from a KD Scientific syringe pump (Holliston, MA) to the sample slide of the desorption electrospray ionization set up. The IMS consisted of desolvation (8.5 cm in length) and drift regions (21.0 cm in length) constructed with stacked stainless steel conducting rings and alternating ceramic insulating rings. The stainless steel rings (electrodes) were electrically connected by a series of 500-kΩ resistors in the desolvation region and 1-MΩ resistors in the drift region (Caddock Electronics Inc., Riverside, CA, ±1%), which created a uniform electric field within the tube. The desolvation and drift regions were separated by a Bradbury-Neilsen style gate operated at 6.4 kV and made in house at Washington State University. Ions were pulsed into the drift region at a pulse width of 0.15 ms. The IMS tube, which was thermally insulated, was held at a constant temperature of 160^{o}C and operated at atmospheric pressure, which was 690-705 Torr for Pullman, WA. The typical drift gas used was nitrogen, held at a constant flow rate of ~ 2.5 L/min. After separation in the ion mobility spectrometer, ions entered the mass spectrometer through a 300 μm pinhole leak and were focused by a series of lenses and two segmented quadrupole ion guides. The first quadrupole had a RF of 2.07 MHz at 3.00 mbar and the second segmented quadruple had a RF of 1.60 MHz at 1.15 x 10^{-2} mbar. The DC ion optics was a series of ion lenses that operated at 1.20

x 10^{-5} mbar. A Tofwerk AG (Thun, Switzerland) high resolution TOF mass spectrometer with a multichannel plate (MCP) detector (Photonis USA, Sturbridge, MA) was used for experimentation. The extractor float voltage and the acceleration voltage were set at 40 V and 3.5 kV, respectively. The potential applied to the MCP was 2.0 kV. The data acquisition system consisted of a timing generator, preamp/discriminator, a time-to-digital converter (TDC) and a PC. The timing generator triggered the TOFMS extraction and the TDC. The IMS gate controller was made in house at Washington State University. The TDC was typically operated at 400 to 800 ps time resolution. The TOFMS extraction frequency was set to 16 kHz with an IMS frequency of 20 Hz; therefore, 800 mass spectra were generated for every IMS gate pulse. The instrument conditions are summarized in **Table 1**.

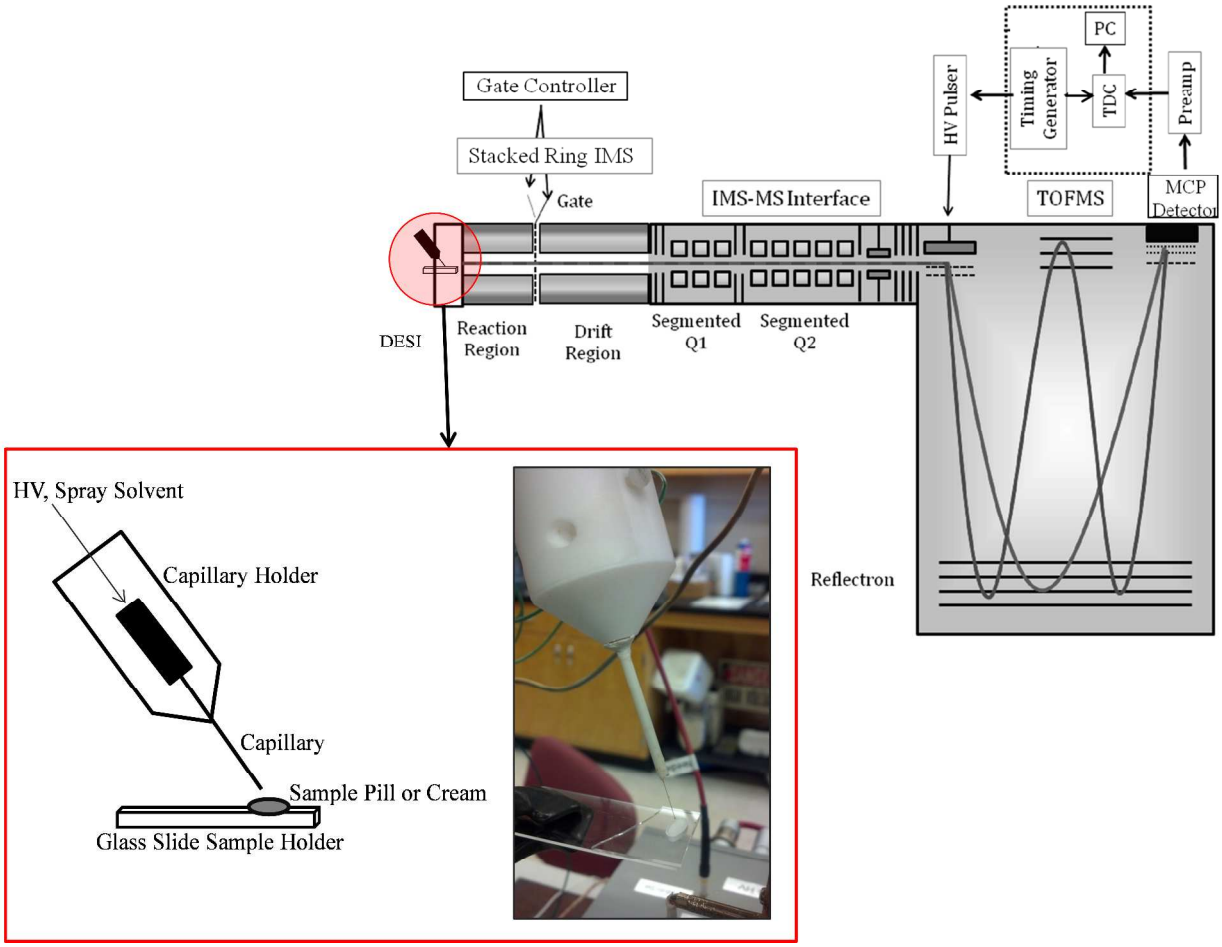


Figure 1. IM-TOFMS Instrument Schematic*. Pictured from left to right is the DESI source, ion mobility spectrometer, IMS-MS interface (segmented Q1, segmented Q2 and lens region), TOF mass spectrometer and MCP detector. Insert shows DESI schematic and photograph of set up.*

Table 1. *Typical DESI IM-TOFMS Instrument Conditions.*

Instrument Parameter	***Value***
IMS:	
Ionization Source	DESI
Source Bias	± 3 kV
Desolvation Region Length	8.5 cm
Drift Region Length	21.0 cm
Gate Pulse Width	0.15 ms
Temperature	160^{o}C
Drift Gas	N_2
Interface Region:	
Pinhole Size	300 µm
First Quadrupole RF/Pressure	2.07 MHz/ 3.00 mbar
Second Quadrupole RF/Pressure	1.60 MHz/ 1.15 x 10^{-2} mbar
Ion Optic Pressure	1.20 x 10^{-5} mbar
TOF MS:	
Extractor Float Voltage	± 40 V
Acceleration Voltage	± 3.5 kV
MCP Potential	± 2.0 kV
TDC Time Resolution	400-800 ps

Data Acquisition. TofDAQ Recorder Version 1.2.92 (Tofwerk AG, Thun, Switzerland) was used to run the IMS-TOFMS instrument and used to collect all data files. TofDAQ Viewer Version 1.2.92 (Tofwerk AG, Thun, Switzerland) had the capability of selecting a single mass from the total mass spectrum and plotting its mobility in a separate window and was utilized to analyze the positive mode data presented. IGOR Pro 6.22A Tofwerk IMS-MS Viewer v1.5 beta software (Tofwerk AG, Thun, Switzerland) was utilized for the processing of the negative mode data.

Sample Introduction and Ionization. Seven drug standards that did not contain excipients were chosen to represent different drug classifications including amphetamines, sympatholytics, barbiturates, depressants, narcotics and antidepressants. Standard drug samples were diluted to 3mM and 200 μL of the solution were placed on a glass microscope slide and analyzed on a standalone IMS system. The solvent used for desorption electrospray ionization (DESI) experiments was a 50/50 MeOH/H_2O mixture with 0.1% (v/v) acetic acid added for the positive mode experiments. The flow rate of the solvent was 5 μL/min and the needle was held at a ± 3 kV bias over the IMS entrance voltage of ± 9 kV. Nitrogen was used as the nebulizer gas and held at a constant flow rate of 0.5 L/min. The standard drugs were investigated by diluting the solid samples in DESI solvent at a concentration of 3 mM and placing 200 μL of the solution on a glass slide and allowing sufficient time for the solvent to evaporate. Creams and solid pills were investigated by placing them on a glass microscope slide mounted on a ring stand set up and held the slide in the center of the IMS entrance. The samples were placed so that the top surface was 1 cm below the DESI spray emitter tip and 1 cm away from the entrance of the drift tube. The DESI needle was angled at 45^o in reference to the glass slide.

RESULTS AND DISCUSSION

Positive Mode DESI:

Figure 2 demonstrates the mobility data for Codeine, Ritalin, Effexor, Barbituric Acid and Xanax drug standards. These standards were the pure active ingredients and did not contain excipients. The solid green trace (**2A**) is a spectrum of the DESI background showing only the reactant ion peaks (RIP); those peaks that are present when the glass DESI slide is blank. Positive ion peaks for Codeine (**2B**), Ritalin (**2C**) and Effexor (**2D**) were observed with reduced mobility (K_o) values of 1.27, 1.43 and 1.26 cm^2/Vs (± 0.01cm^2/Vs), respectively. Barbituric acid (**2E**) and Xanax (**2F**), however, were not detected in the positive mode on the standalone IMS system. At this point a general discussion of the ionization mechanism of DESI is warranted. In DESI, an electrospray stream is directed toward the sample where charged droplets collide with the analytes on the surface. The analytes are desorbed and ionization is thought to occur by the charge from the electrospray being transferred to the analyte during the desorption process. It has been shown in previous work that the formation of positive ions in an IMS depends on the functional groups that are present in the structures of analytes [28], where the analytes with the higher proton affinities will compete for the positive charge in the gas phase. Codeine, Effexor and Ritalin all contain ketone and hydroxyl functional groups which have sufficient proton affinity to produce a positive ion for detection. Xanax and Barbituric Acid do not contain ketone and hydroxyl functional groups; instead these drugs contain amines which tend to favor a negative ion formation pathway because of a low proton affinity.

To determine the identity of the response ions of those samples that responded in the positive mode, the experiment was repeated on an instrument in which an ion mobility spectrometer was interfaced to a time-of-flight mass spectrometer to allow for mass

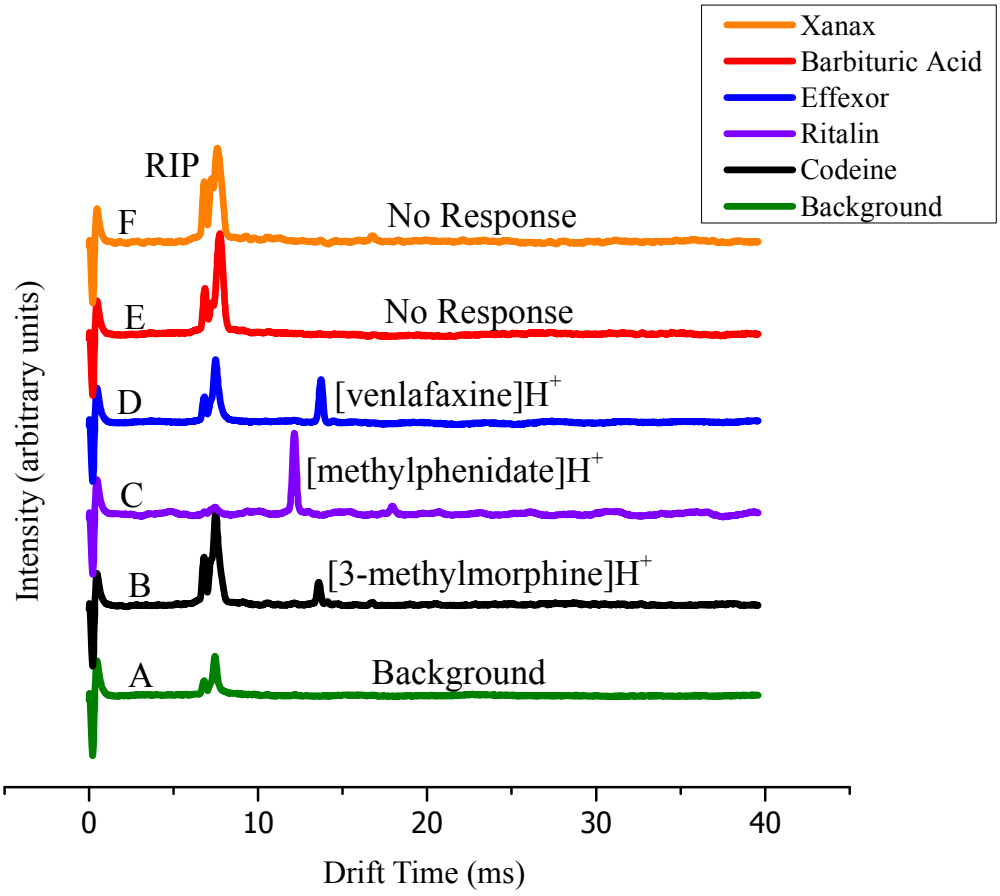


Figure 2. Positive Mode Standalone DESI-IMS Drug Standard Data *The calculated K_o values for (**B**) Codeine ([3-methylmorphine]H^+), (**C**) Ritalin ([methylphenidate]H^+) and (**D**) Effexor ([venlafaxine]H^+) were* 1.27, 1.43 and 1.26 *cm^2/Vs ($\pm$ 0.01cm^2/Vs), respectively. (**E**) Barbituric acid and (**F**) Xanax were not detected in the positive mode.*

identification of the detected ions. In addition to the standards detected with the stand alone instrument (Codeine, Ritalin, Effexor, Barbituric Acid, and Xanax) Pristiq and Deralin (a mixture of R and S-propranolol) were added compounds tested. For those pharmaceuticals responding in the positive mode, the protonated molecular ions $(M+H)^+$ were the predominant response ions, verified by mass identification with the mass spectrometer. **Figure 3** shows mass selected mobility plots for the $[M+H]^+$ ion species of Codeine (**3A**), Ritalin (**3B**), Effexor (**3C**), Pristiq (**3D**) and Deralin (**3E**). These $[M+H]^+$ ions had mobility values of 1.30, 1.46, 1.22, 1.17 and 1.26 cm^2/Vs (± 0.01 cm^2/Vs), respectively. Once again, all ions detected on the IMS-MS instrument could be mass identified to verify the mobilities that were measured in the standalone IMS. Additionally, these analytes all contained hydroxyl and ketone functional groups which allowed for a positive mode ionization pathway, producing the H^+ ions that were detected.

In addition to the standards, seven drug samples were used to demonstrate the potential of ion mobility mass spectrometry for detection of active ingredients in real samples such as liquids, tablets and creams where there are a large number of excipients formulated into the product. Vicodin, Nyquil, Zantac, Cetirizine, Icy Hot and Vitamin C were chosen as test drug samples. The active ingredients in each of these samples can be seen in **Table 2**. Also presented in **Table 2** are the K_o values and ions that were detected in the IM-TOFMS instrument for each sample. The mass selected mobility spectra for the drug samples can be seen in **Figure 4**. The K_o value for Zantac (**4B**) was 1.07 cm^2/Vs. Protonated ions were detected for both active ingredients in Vicodin (**4C**), $[hydrocodone]H^+$ and $[acetaminophen]H^+$, and the K_o values were 1.02 and 1.41 cm^2/Vs, respectively. Protonated ions were detected for the active ingredients in Nyquil (**4D**), $[doxylamine]H^+$, $[dextromethorphan]H^+$ and $[acetaminophen]H^+$, and the K_o values were

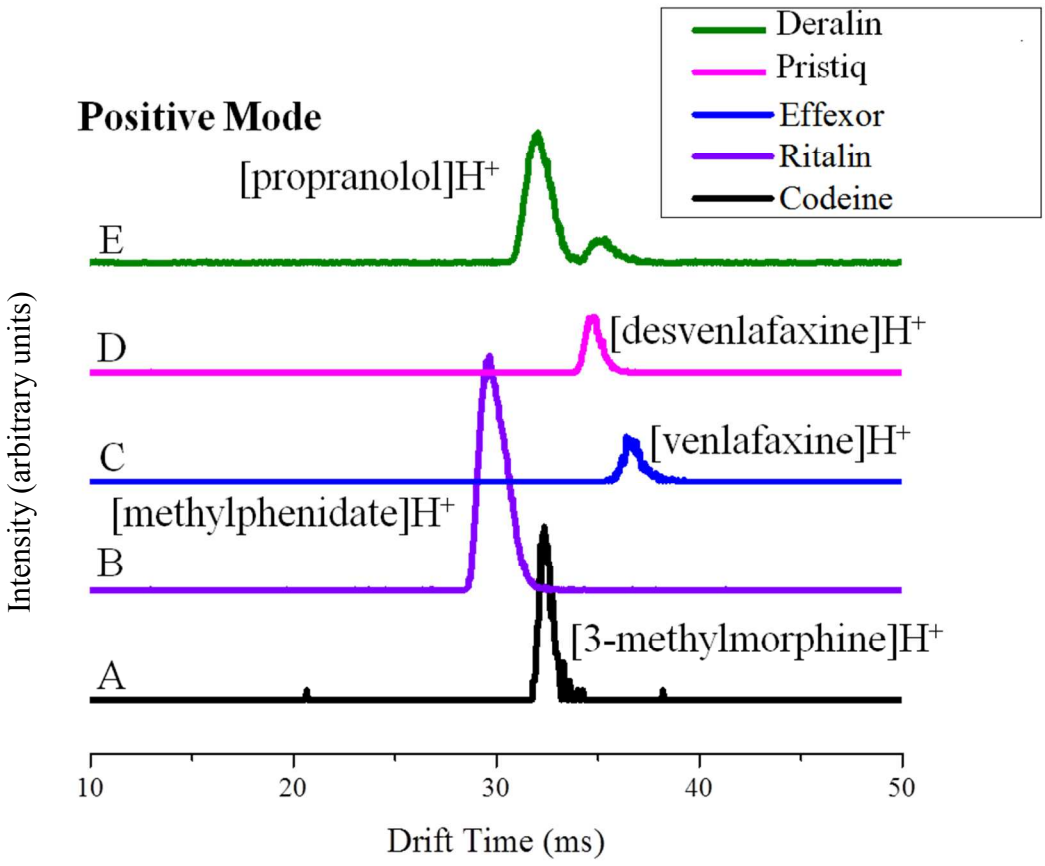


Figure 3. ***Positive DESI-IMS-TOFMS Drug Standard Data***. *The calculated K_o values for* (***A***) *Codeine* (*[3-methylmorphine]H^+*), (***B***) *Ritalin* (*[methylphenidate]H^+*), (***C***) *Effexor* (*[venlafaxine]H^+*), (***D***) *Pristiq* (*[desvenlafaxine]H^+*) *and* (***E***) *Deralin* (*[R/S-propranolol]H^+*) *were* 1.30, 1.46, 1.22, 1.17 and 1.26 *cm^2/Vs, respectively.*

Table 2. Drug Samples and Active Ingredients. *Also included in table is K_o values and ion identification determined on IMS-TOFMS.*

Drug	**Active Ingredients**	**m/z (Da)**	**K_o (cm^2/Vs) on IMS-TOFMS [ion idenification]**
Vicodin	Hydrocodone Acetaminophen	300.15 152.06	1.02 [Hydrocodone]H^+ 1.41 [Acetaminophen]H^+
Aspirin	Aspirin	-	No Response
Nyquil	Acetaminophen Dextromethorphan Doxylamine	152.06 272.19 271.17	1.41 [Acetaminophen]H^+ 1.12 [Dextromethorphan]H^+ 1.15 [Doxylamine]H^+
Zantac	Ranitidine	315.14	1.07 [Ranitidine]H^+
Icy Hot	Methyl Salicylate	151.05	1.49 [Methyl Salicylate-H]$^-$
Vitamin C	Vitamin C	175.03	1.36 [Vitamin C-H]$^-$

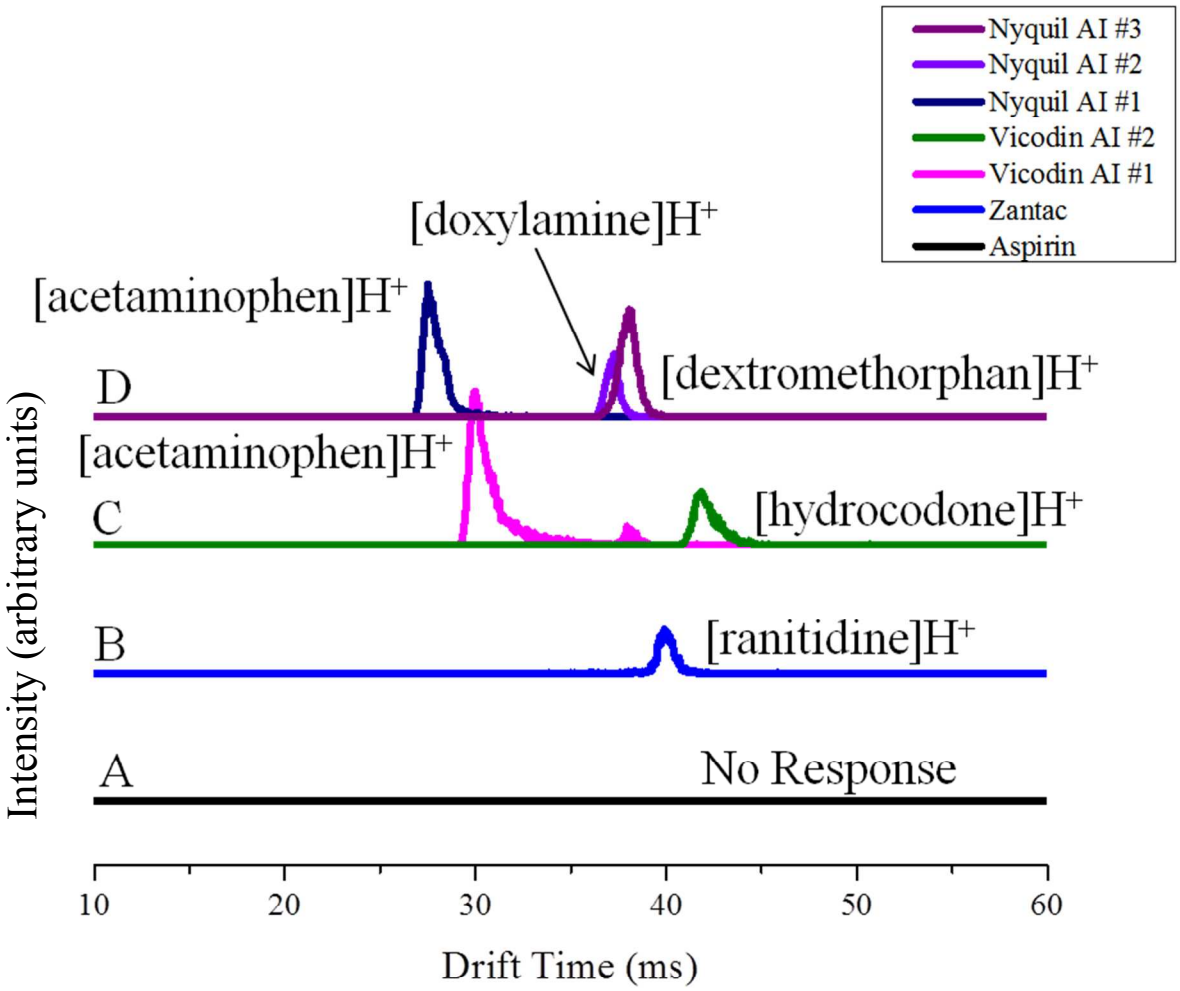


***Figure 4*. Positive Mode DESI-IMS-TOFMS Sample Drug Data.** *The calculated K_o values for (**B**) Zantac ([ranitidine]H^+), (**C**) Vicodin active ingredient #1 ([acetaminophen]H^+), (**D**) Vicodin active ingredient #2 ([hydrocodone]H^+), Nyquil active ingredient #1 ([acetaminophen]H^+), Nyquil active ingredient #2 ([doxylamine]H^+) and Nyquil active ingredient #3([dextromethorphan]H^+) were 1.07, 1.41, 1.02, 1.41, 1.15 and 1.12 cm^2/Vs, respectively.*

1.15, 1.12 and 1.41 cm^2/Vs, respectively. No response ion was detected for the Aspirin tablet (**2A**) in the positive mode.

A general comment that can be made this point is that matrix effects are likely in DESI in the analysis of trace analytes present in complex matrices [5]. This case can be made for the aspirin tablet used in this study where the excipients in the drug formulation created high mass ions that had a greater affinity for the proton charge. The inactive ingredients in this particular tablet included carnauba wax, corn starch, hypromellose, powered cellulose and triacetin. These ingredients are present for several reasons including holding the pill together in a solid form and allowing for quick absorbance of the drug into the body. Strong signals were detected for the inactive ingredients in the aspirin drug formulation, masking the signal for the $[M+H]^+$ of aspirin.

Negative Mode DESI:

The drug standards and samples (described in the previous section) were also analyzed in the negative mode; however there was no analyte signals detected and it was determined that these samples were only positive mode active. Because Barbituric Acid and Xanax were not detected in the positive mode, a focus was placed on the detection of these compounds in the negative mode. Barbituric Acid generated a negative mode ion, $[Barbituric\ Acid\text{-}H]^-$, that had a K_o value of 1.71 cm^2/Vs; however no ions were detected for the $[Xanax\text{-}H]^-$ in the negative mode most likely due to matrix effects from the drug excipient ingredients (i.e. inactive ingredients such as cellulose, starches, silicon dioxide and coloring dyes) that suppressed the ionization both in positive and negative mode. Icy Hot and Vitamin C were negative mode active and the mass selected mobility spectra can be seen in **Figure 5**. **Figure 5a** shows the mass selected mobility

spectrum for the Icy Hot sample. The peak had a mobility of 1.49 cm^2/Vs for the active ingredient ion, $[methyl\ salicylate\text{-}H]^-$ with an m/z = 151. **Figure 5b** shows the mass selected mobility spectrum for the Vitamin C sample. The peak had a mobility of 1.36 cm^2/Vs for the active ingredient ion, $[vitamin\ C\text{-}H]^-$ with an m/z = 175. **Figure 5c** is the mass selected mobility plot for the $[aspirin\text{-}H]^-$ (m/z = 179) ion showing that no response ion was detected for the Aspirin sample in the negative mode. **Figure 5d** is the mass selected mobility plot for the $[xanax\text{-}H]^-$ (m/z = 307) ion showing that no response ion was detected for the Xanax sample in the negative mode.

The use of DESI in the negative ion mode is motivated by the fact that negative ionization is more likely to yield selective and sensitive detection of those compounds that are easily deprotonated or have high electron affinities such as phenols and quinones [5]. Both Xanax and Aspirin have no phenol groups, but do have conjugated structures that make ionization in the negative mode seem plausible. However, as mentioned previously, DESI is sensitive to matrix effects; so samples examined with active ingredients that are at a high concentration will be successful, but samples where the active ingredients are not present at a high concentration will not be detected. This is the case with Xanax and Aspirin; however forms of reactive DESI can be used to mitigate the complex matrix ionization problems associated with DESI (discussed further in the next section).

A summary of the drug standards and reduced mobility values calculated on both the standalone IMS and IM-TOFMS systems in both positive and negative mode can be seen in **Table 3**.

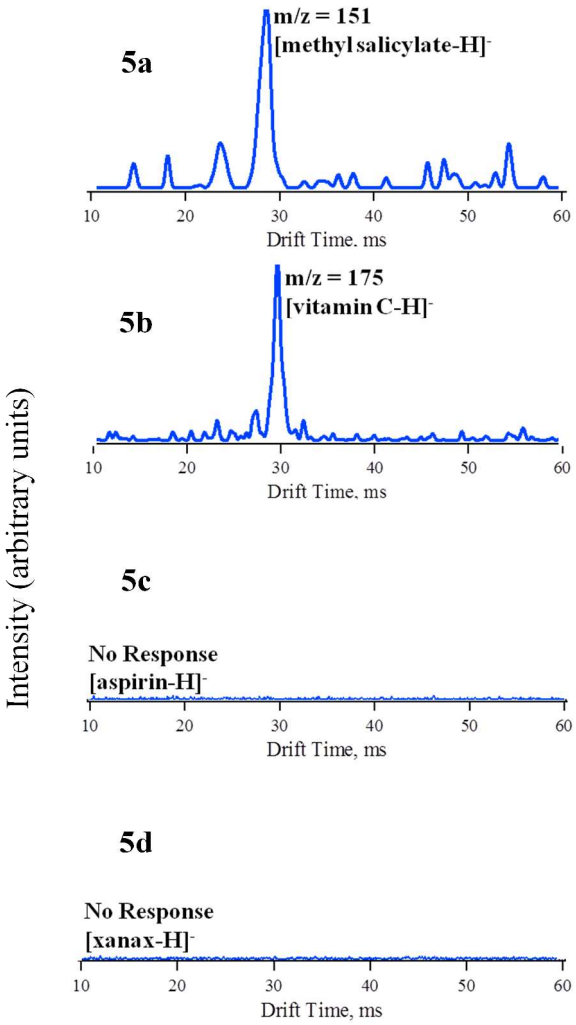
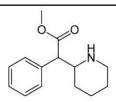
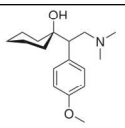
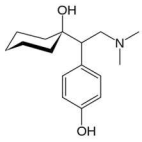
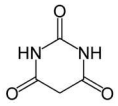
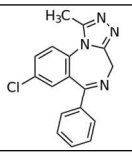
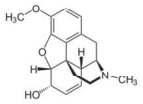
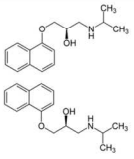


Figure 5. Negative Mode DESI-IMS-TOFMS Sample Drug Data. *Spectrum* ***5a*** *is the mass selected mobility spectrum for Icy Hot. The active ingredient, methyl salicylate, produced the [methyl salicylate-H]- ion and had a K_o value of 1.49 cm^2/Vs. Spectrum* ***5b*** *is the mass selected mobility spectrum for Vitamin C. Vitamin C produced a [vitamin C-H]- ion at a mobility of 1.36 cm^2/Vs. Spectrum* ***5c*** *is the mass selected mobility spectrum for Aspirin showing that this sample had no response in the negative mode. Spectrum* ***5d*** *is the mass selected mobility spectrum for Xanax showing that this sample had no response in the negative mode.*

Table 3*. Standard Drug Classification and Uses.** *Also included in table are structure, K_o values determined on Standalone IMS, K_o values and ion identification determined on IMS-TOFMS.* ***Note*: there is no Standalone K_o value listed for Pristiq and Deraline because these standards were only analyzed on the TOFMS instrument.*

Drug Classification	Uses	Common Name	Standard	Structure	Molar Mass (g/mol)	K_o (cm^2/Vs) on Standalone IMS	K_o (cm^2/Vs) on IMS-TOFMS [ion idenification]
amphetamines	mimic adrenalin	Ritalin	Methylphenidate		233.14	1.43	1.46 [Methylphenidate]H^+
antidepressant	treat depression	Effexor XR	Venlafaxine		277.20	1.26	1.22 [Venlafaxine]H^+
antidepressant	maintain mental balance	Pristiq	Desvenlafaxine		263.19	-	1.17 [Desvenlafaxine]H^+
barbiturates	depress central nervous system	-	Barbituric acid		128.02	Not detected in positive mode	1.71 [Barbituric Acid-H]$^-$ *negative mode
depressants	reduce function of central nervous system	Xanax	Alprazolam		308.08	Not detected in positive mode	1.15 [Xanax]Na^+ *doped with Na^+
narcotics	treat pain, anxiety, aggression	Codeine	3-Methylmorphine		299.15	1.27	1.30 [Codeine]H^+
sympatholytic	treat hypertension, anxiety and panic	Deralin	(R/S)-Propranolol		259.16	-	1.26 [Propranolol]H^+

Reactive DESI:

Both the Aspirin and Xanax active ingredient ions were unresponsive in the positive and negative mode most likely due to matrix effects from the drug excipient ingredients that suppressed the ionization. Increased selectivity can be obtained by DESI using a variant of DESI called reactive-DESI when coupled with mass spectrometry techniques [11, 23-27]. To increase the sensitivity of the atmospheric pressure DESI-IM-TOFMS in positive mode, a doping agent (sodium chloride, 2 μM) was added to the DESI solvent (50/50 MeOH/H_2O + 0.1% Acetic Acid). The resulting spectra can be seen in **Figure 6**. With the addition of the doping agent, Aspirin, Xanax and Cetirizine produced [Aspirin]Na^+, [Xanax]Na^+ and [Cetirizine]Na^+ ions with K_o values of 1.21, 1.15 and 0.90 cm^2/Vs, respectively. **Figure 6a** shows the selected mass mobility spectrum for the [Aspirin]Na^+ ion with the doping agent and **Figure 6b** shows the mass selected mobility spectrum of the [Aspirin]Na^+ (m/z = 203) ion without the addition of a doping agent. It is clear from this spectrum that the ion was not detected without the addition of Na^+ into the DESI solvent demonstrating for the first time the used of reactive-DESI with a ion mobility mass spectrometer instrument. This agrees with previous work where the formation of the $[M+Na]^+$ ion is favored over the protonated molecules in matrixes which contain sodium salts [5]. This phenomenon occurs because the presence of Na^+ creates more balanced ionization efficiency across all of the analytes present in the sample by eliminating discrimination against low proton affinity species. **Figure 6c** shows the selected mass mobility spectrum for the [Xanax]Na^+ ion with the doping agent and **Figure 6d** shows the mass selected mobility spectrum of the [Xanax]Na^+ (m/z = 331) ion without the addition of a doping agent. **Figure 6e** shows the selected mass mobility spectrum for the [Cetirizine]Na^+ ion with a doping agent and **Figure 6f** shows the mass selected mobility spectrum of [Cetirizine]Na^+ (m/z = 411) ion without

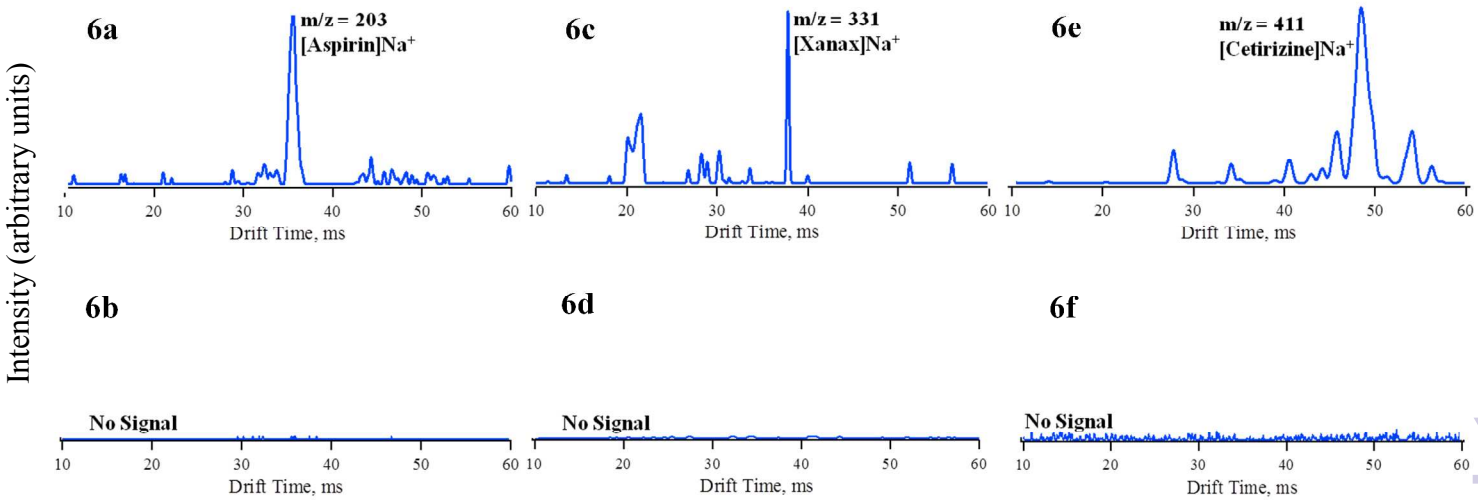


Figure 6. Positive Mode DESI-IMS-TOFMS Sample Drug Data with Addition of Na^+. 6a) *The mass selected mobility plot of the Na^+ adduct ion, [aspirin]Na^+, with a K_o value of 1.21 cm^2/Vs.* ***6b)*** *The mass selected mobility plot of the Na^+ adduct ion, [aspirin]Na^+, without the addition of Na^+. No response is detected.* ***6c)*** *The mass selected mobility plot of the Na^+ adduct ion, [Xanax]Na^+, with a K_o value of 1.15 cm^2/Vs.* ***6d)*** *The mass selected mobility plot of the Na^+ adduct ion, [xanax]Na^+, without the addition of Na^+. No response is detected.* ***6e)*** *The mass selected mobility plot of the Na^+ adduct ion, [Cetirizine]Na^+, with a K_o value of 0.90 cm^2/Vs.* ***6f)*** *The mass selected mobility plot of the Na^+ adduct ion, [cetirizine]Na^+, without the addition of Na^+. No response is detected.*

the doping agent. **Figure 6** demonstrates a sensitivity increase for the sodiated ions of the drug compounds with the addition of a doping agent, Na^+, into the solvent used in the DESI source. In each of these examples the addition of a charged sodium ion to the electrospray plume results in a more uniform ionization and eliminates discrimination against low proton affinity species, in this case the active ingredients in the complex drug formulations.

CONCLUSIONS

This work demonstrates direct analysis of pharmaceutical drug formulations using both standalone atmospheric pressure IMS and hyphenated atmospheric pressure ion mobility/time-of-flight mass spectrometry combined with desorption electrospray ionization that requires no sample pretreatment. **Figure 7** shows a pie chart demonstrating a 68% success detection rate of either a $[M+H]^+$, $[M-H]^-$ or $[M+Na]^+$ ion for the 22 samples that were analyzed (summarized in **Table 4**). It is important to note that each of the 22 samples were analyzed in both the positive and negative mode both with and without reactive DESI to verify the results shown in the pie chart. Optimization of the current technique could lead to a decreased non-detect percentage by changing the doping agent in reactive-DESI. The standalone IMS data presented demonstrates promising data in comparison to IMMS data for a subset of drug standards in positive mode operation. The use of standalone IMS techniques in the detection of drug standards without excipients such as Codeine, Ritalin and Effexor demonstrates the applicability of a portable and affordable drug-testing platform; however further studies need to be completed in order to clearly articulate the value of standalone IMS for pharmaceutical analyses. The fact that DESI generates ions in the gas phase at atmospheric pressure makes DESI compatible with ion-mobility measurements. The simplicity of the DESI source also matches well with the simple IMS instrument. Both operate in air at atmospheric pressure, have hihn sensitivity, and are rapid

experiments. For these reasons, combinations of DESI with ion mobility are beginning to be realized. The active ingredients of several hard and soft drug formulations including Vicodin, Zantac, Nyquil and Icy Hot can be detected in less than 1 minute with IM-TOFMS instrumentation presenting the potential applicability of this novel method to pharmaceutical screening and development of drug formulations. Due to the broadband capabilities of DESI-IMMS, fake active ingredients present in fake medicines can also be detected by this approach. Reactive-DESI significantly enhances selectivity for target active ingredients in drug formulations such as Xanax, Aspirin and Cetirizine. This assay should prove useful for verifying the quality of hard to detect active ingredients in drug tablets distributed worldwide by many pharmaceutical companies.

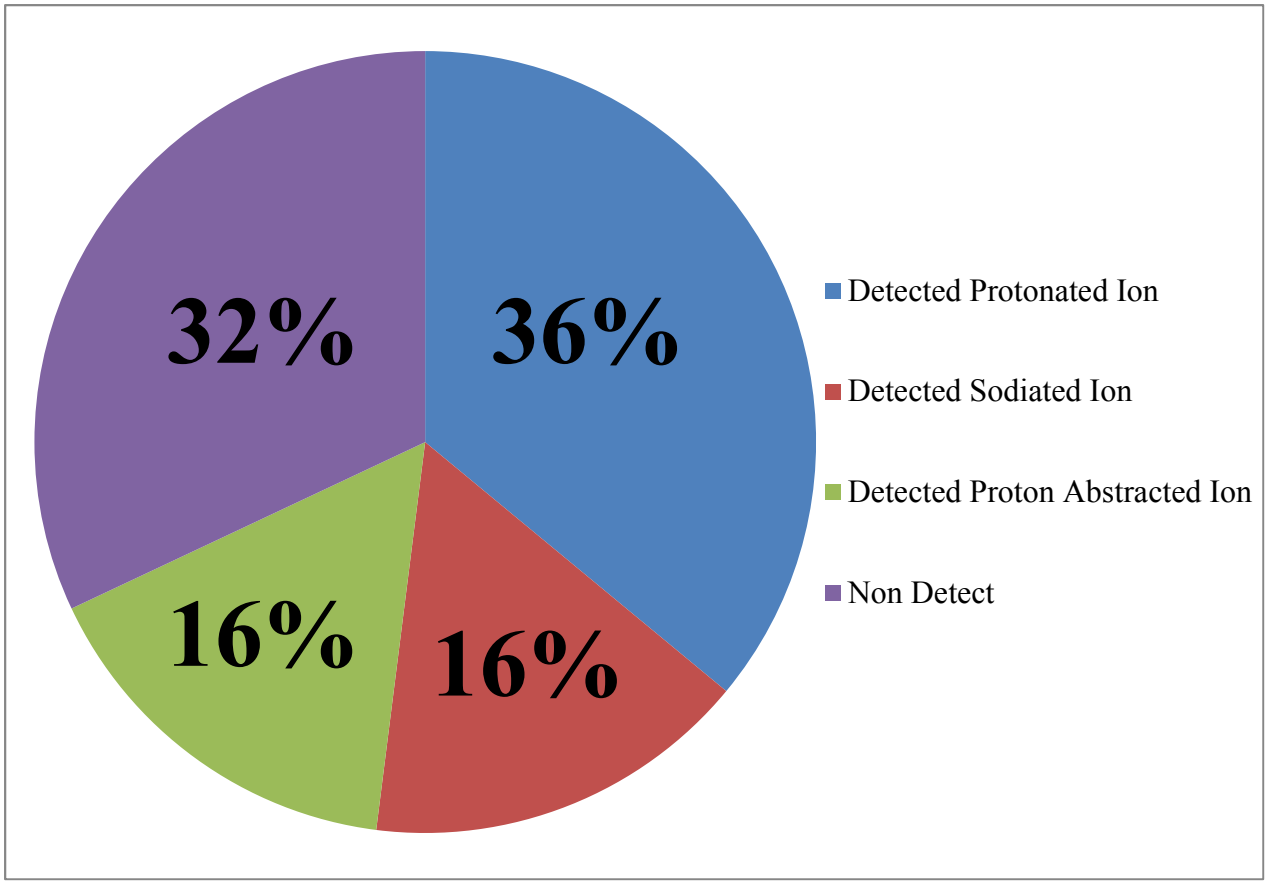
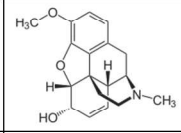
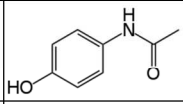
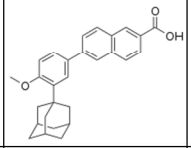
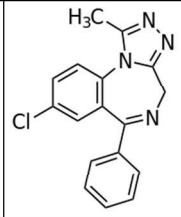
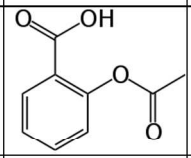
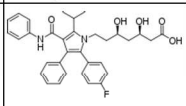
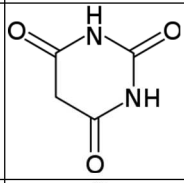
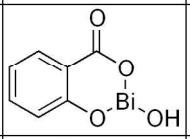
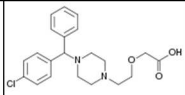
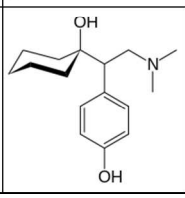
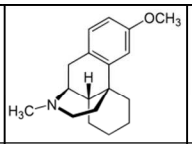
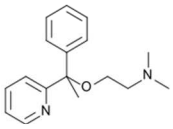
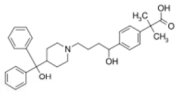
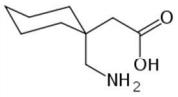
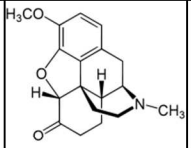
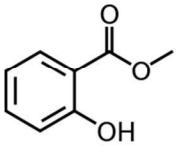
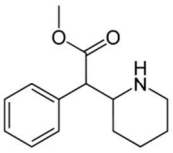
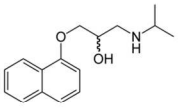
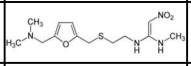
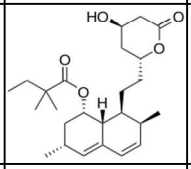
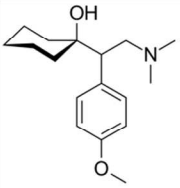
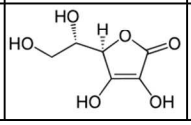


Figure 7. Success Rates of DESI-IMS Method. *68% of all samples analyzed (22 total) were successfully detected. 36% were detected in the positive mode as* $[M+H]^+$ *ions, 16% were detected using reactive-DESI as* $[M+Na]^+$ *ions and 16% were detected in the negative mode as* $[M-H]^-$ *ions. 32% of the samples analyzed were not detected; however optimization of the current techniques could allow for a decreased non-detect percentage.*

Table 4. Summary of all samples analyzed. *Also included in table are structure, detection mode, dopant use and ion detected.* (Please note that "*n/a*" signifies that the use of reactive DESI did not improve the detection of the product ion, therefore the ion remained undetected, "*no*" signifies that reactive DESI was not needed and that the product ion was detected as an H^+ ion species)

Compound Name or Active Ingredient (Common Name)	*Structure*	*Detection Mode*	*NaCl Dopant Use*	*Ion Detected*
3-Methylmorphine (Codeine)		positive	no	[3-Methylmorphine]H^+
Acetaminophen (Nyquil)		positive	no	[Acetaminphen]H^+
Adapalene (Differin Gel)		not detected	n/a	n/a
Alprazolam (Xanax)		positive	yes	[Alprazolam]Na^+
Aspirin		positive	yes	[Aspirin]Na^+
Atorvastatin (Lipitor)		not detected	n/a	n/a
Barbituric Acid		negative	no	[Barbituric Acid-H]$^-$
Bismuth Subsalicylate (Pepto-Bismol)		not detected	n/a	n/a
Cetirizine		positive	yes	[Cetirizine]Na^+
Desvenlafaxine (Pristiq)		positive	no	[Desvenlafaxine]H^+

Dextromethorphan (Nyquil)		positive	no	[Dextromethorphan]H^+
Doxylamine (Nyquil)		positive	no	[Doxylamine]H^+
Fexofenadine (Allegra)		not detected	n/a	n/a
Gabapentin (Neurotin)		not detected	n/a	n/a
Hydrocodone (Vicodin)		positive	no	[Hydrocodone]H^+
Methyl Salicylate (Icy Hot)		negative	no	[Methyl Salicylate-H]$^-$
Methylphenidate (Ritalin)		positive	no	[Methylphenidate]H^+
Propranolol (Deralin)		positive	no	[Propranolol]H^+
Ranitidine (Zantac)		positive	no	[Ranitidine]H^+
Simvastatin (Zocor)		not detected	n/a	n/a
Venlafaxine (Effexor)		positive	no	[Vanlafaxine]H^+
Vitamin C		negative	no	[Vitamin C-H]$^-$

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