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Project Title: Separation and Identification of Drugs by Electrospray Ionization-Ion Mobility Spectrometry-Mass Spectrometry (ESI-IMS-MS)

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Introduction

Ion mobility spectrometry (IMS) has been described throughout the scientific literature as both a stand-alone separation technique and as a hyphenated technique used to enhance other analytical determinations [1]. Despite this flexibility and versatility, the applications of IMS have not grown as quickly as that of gas chromatography (GC) or liquid chromatography (LC) even though IMS has similar, if not greater resolving capabilities than that of the aforementioned techniques [2]. This research describes the use of IMS as a lab-based analytical technique able to perform separations on par with GC and LC separations. The speed of IMS has driven those applications that take advantage of both the speed of analysis and the potential lower cost of analysis as well as the portability and miniaturization of the technique. Although IMS has been commonly found in military and security applications, additional uses have also found in pharmaceutical, aeronautical, agricultural and petrochemical industries [3, 4]. The current research project described here was motivated from the necessity to perform rapid and inexpensive analyses on substances (seized drugs) commonly encountered by law enforcement, in particular, analysis of controlled amphetamine type substances and the emerging designer drugs, some of which are very similar in structure.

Utilization of Ion Mobility Spectrometer for Chiral Separation

The ability to perform separations of chiral compounds has been demonstrated utilizing both an off-the-shelf (OTS) ion mobility unit, the Barringer 400B, and an electrospray ionization ion mobility mass spectrometer (ESI-IMS-MS), called the Excellims RA4100. Previously, the gas-phase chiral separation performed using ion mobility was reported by Dwivedi *et. al.* [5]. In their study, a chiral compound, S-2-Butanol, was used within the drift gas in order to

preferentially interact with chiral analytes for the separation. To begin with, experiments for the chiral separation were performed using the Barringer IMS equipped with a 10 milliCurrie ^{63}Ni ionization source. Significant modifications were required on this commercial IMS unit because this instrument was not originally designed for the external delivery of the chiral compound. In order to introduce the modifier (S-2-Butanol), the dopant was removed and an independent infusion line was added. Since the dopant (nicotinamide) was removed and no reactant ion peak (RIP) was available, the reduced mobility value of RIP (1.86 $cm^2V^{-1}s^{-1}$) was replaced to the new reduced mobility of air (2.31 $cm^2V^{-1}s^{-1}$) for the calculation of a reduced mobility (K_0) of analyte in the software [6, 7]. Amphetamine type substances (ATS) and their enantiomers, including methamphetamine, cathinone, ephedrine, and pseudoephedrine, were analyzed to investigate the potential of chiral separation using this IMS in the positive ion mode. It was shown that the modifier (S-2-Butanol) is effective in separating D and L Methionine as well as ATS, (S,R)- and (R,S)-ephedrine from (R,R)- and (S,S)- pseudoephedrine. However, modifier introduction is required at relatively high infusion rates to cause separation, but with the changes made to the OTS Ionscan 400B (Table 1), the infusion rates had to be maintained below 300µL per hour to prevent condensation and accumulation of modifier within transfer lines. On the basis of these conclusions the next phase of experiments would require a more efficient mechanism of modifier introduction into the IMS drift tube. A commercially available instrument (Excellims RA4100) with high resolving capabilities (R>90) was selected with an aim to improve the overall separation between analytes. This instrument was also equipped with a mass spectrometer detector in order to determine the type of species being formed within the drift tube during introduction of the chiral modifier.

Table 1. Effect of modifier on drift time of analytes in the Barring IMS.

	Drift Time/ms					
Modifier Flow Rate ul/HR	**RS Ephedrine**	**SR Ephedrine**	**RR Psuedoephedrine**	**SS Pseudoephrine**	**Cocaine**	**Diazepam**
0	12.489	12.491	12.501	12.498	16.849	16.179
50	12.869	12.731	12.81	12.79	16.812	16.287
100	12.945	12.977	13.061	13.078	16.835	16.518
200	13.391	13.378	13.512	13.576	16.862	16.673
300	13.454	13.403	13.536	13.636	16.871	16.812

Improvements in separation were obtained with the use of the RA4100 ESI-IMS-MS. This ESI-IMS-MS was designed with features that allowed introduction of a variety of modifiers, which

resulted in separations with greater efficiency than that observed with the Barringer IMS. This improved modifier introduction efficiency produced more reliable separations of chiral compounds such as D and L Methionine as well as other compounds (S,S)-pseudoephedrines and (R,S)-ephedrines. The developed modifier introduction also allowed the exploration of other modifier compounds (straight-chain alcohols). Surprisingly, these achiral modifiers produced similar separations to the chiral modifiers which prompted further investigation as to the exact mechanism of separations occurring. The hybrid instrument, Excellims ESI-IMS-MS, allowed for the identification of ions formed within the drift tube during the introduction of modifier for chiral separations providing information on the interactions that occur between modifier and chiral analyte. Numerous modifier molecules were observed to interact with each chiral analyte resulting in a molecular ion cluster (water) that possessed a greater effective collision cross sectional area (Ω) than the molecules formed without modifier. This was found to be in contradiction to the previously believed Pirkle interaction that dictated interaction of one modifier to one analyte and that the modifier itself had to be chiral in nature [8]. Computational analysis at the B3LYP/6-31g level of theory showed that each chiral molecule, though possessing the same cluster of modifier molecules, had different cross sectional areas. The differences observed in effective collision cross sectional area for each cluster was as a result of the position of specific functional groups on the chiral analyte itself. For pseudoephedrine and ephedrine, these functional groups were an amine and a hydroxyl group that were on different chiral carbon atoms adjacent to one another on the molecule. The close proximity of these functional groups on adjacent chiral atoms created internal hydrogen bonds that were either more accessible to interact with hydroxyl groups on the modifier molecules or were less accessible depending upon the chirality of the analyte. The position of these functional groups around the chiral carbon atoms resulted in modifier molecules having different special arrangements around the chiral analyte inside the IMS drift tube. Compounds with an internal or “bridged” structure arranged modifiers more tightly than compounds without the internal or “independent” structure. This provided bridged compounds a slightly smaller collision cross sectional area than the independent structure and allowed bridged compounds to interact less with the surrounding drift gas and, so, migrate at a faster rate down the drift tube. The independent structure resulted in slightly larger collision cross sectional

area, resulting in more interactions with the drift gas and a subsequently slower migration time down the drift tube. These differences were sufficient to cause separations of isomeric chiral compounds within the IMS even when the modifiers being used were achiral. The separation mechanism revealed that achiral modifiers were sufficient in causing differences in effective collision cross sectional areas as the chiral analytes themselves were responsible for the arrangement of the cluster ions being formed. Larger modifier molecules resulted in greater separation power and hence the most effective modifier used in the study was n-octanol (Table 2). The separation of chiral molecules in the gas phase of an IMS using achiral modifiers was reported for the first time in peer reviewed literature as a result of this research effort [9].

Table 2. Showing effect of modifier on resolution between (R,S)-ephedrine and (S,S)-pseudoephedrine.

	(R,S)-ephedrine drift time (ms)	(S,S)-pseudoephedrine drift time (ms)	Resolution between analytes	% Difference in Drift Time
No Modifier	9.012	9.029	0.09±0.20%	0.19%
Methanol	9.162	9.21	0.31±0.30%	0.52%
Ethanol	9.301	9.502	1.27±0.30%	2.16%
n-Propanol	9.465	9.668	1.19±0.10%	2.14%
n-Butanol	9.854	10.067	1.05±41%	2.16%
n-Pentanol	9.984	10.212	0.84±0.48%	2.28%
n-Hexanol	10.531	10.699	0.67±0.44%	1.60%
n-Heptanol	10.867	11.171	1.01±0.24%	2.80%
n-Octanol	10.691	11.186	1.34±0.14%	4.63%

Investigation of Solvent System for Chiral Analysis

The utilization of an electrospray ionization (ESI) source on the RA4100 offered greater analytical flexibility than that observed with the conventional IMS with the ^{63}Ni ion source. As the ionization of molecules occurs under ambient conditions in the presence of air in IMS, charge competition was a constant inhibitor in ESI-IMS-MS. However, this was alleviated through the use of selective solvent chemistry, allowing simultaneous ionizations of analytes with different proton affinities. Consequently, this allowed the simultaneous analysis of compounds by ESI-IMS-MS that would typically be difficult utilizing the typical IMS with the ^{63}Ni ion source. The use of selective solvent chemistries also resulted in improvements in the dynamic response range of analytes, as the solvent promoted increased ionization efficiency resulting in increased ion current generation and hence increased ion cluster detection by the faraday detector. However, it was also noted that the use of very high acid levels within the

solvent chemistry for positive ions created wider, more diffused peaks, resulting in a reduction in the overall resolving power of the technique. The use of selective solvents therefore must be tempered to balance the need for identifying a complex mixture of analytes without jeopardizing instrument resolution. Though electrospray ionization is heavily dependent on concentration, which has been reported to provide better sensitivity for low volume samples [10], the addition of acid modifiers in the positive mode and methyl halides in the negative mode proved useful in allowing simultaneous ionization of analytes and increasing overall signal intensity of analytes. As a result, formic acid at a concentration of at least 2.5% (*v/v*) was found to show enhanced ionization efficiency in the positive ion mode when compared to solvents with less or no formic acid added (Figure 1).

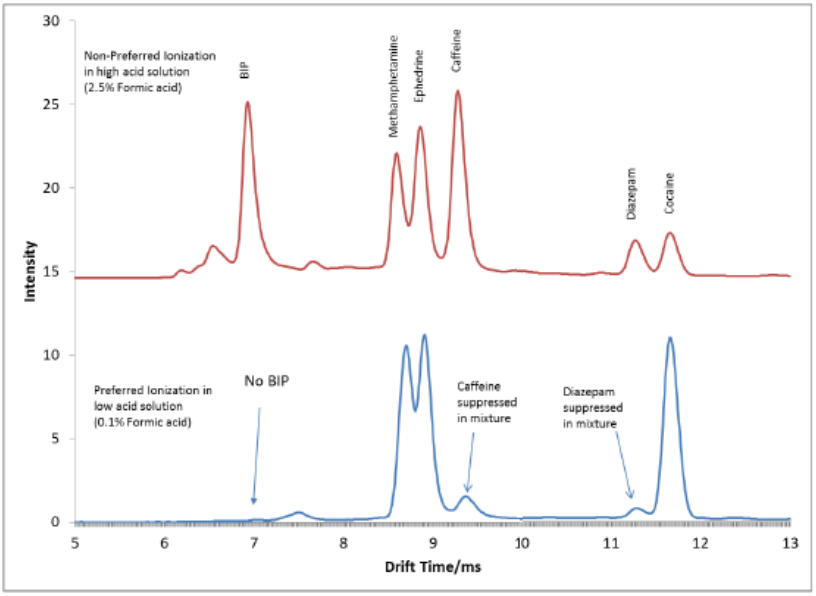

Figure 1. Analysis of 50ppm caffeine, (R)-methamphetamine, (S,R)-ephedrine, diazepam and cocaine using 2.5% formic acid (top) and 0.1% formic acid (bottom) of acid modifier using the RA4100.

In addition, changes in solvent chemistry were able to produce changes in the analyte ion species being formed in the negative mode.[11] Methyl halides specifically, chloroform at a concentration of 0.1% (*v/v*) was found to promote improved ionization efficiency in the negative ion mode. Other solvents such as ammonium nitrate proved useful in altering the ionic species being obtained for some analytes. For the positive ion mode, such solvent chemistries produced ammonium adducts $[M+NH_4]^+$ versus the typical protonated $[M+H]^+$ adducts. In the negative ion mode, this same solvent system produced $[M+NO_3]^-$ adducts versus the typical $[M-H]^-$ ions. However, it was also observed that the ionization of nitro-aromatics was suppressed in

the ammonium nitrate solvents. In summary, the ability to alter ion response by changing solution chemistry has allowed for a better understanding of gas phase reactions that occur under atmospheric conditions. Rapid and sensitive analysis is now possible of more complicated mixtures than were previously analyzed using conventional ^{63}Ni- IMS devices. These findings were also published in peer reviewed literature [12].

Implementation of Ion Mobility Spectrometry for Analysis of Designer Drugs

Rapid and inexpensive analysis has been also investigated for emerging designer drugs utilizing the Barringer IMS and the Excellims ESI-IMS-MS. First, the rapid detection and characterization of designer drugs were evaluated using the Barringer IMS. The advantages of this instrument includes ease of operation and maintenance, high sensitivity, and relatively inexpensive instrument, while the small dynamic range, lack of identification capability, and potential for false positive alarms are the drawbacks. Figure 2 shows that those representative designer drugs were successfully detected with their characteristic reduced mobilities. These results proved that this technique can be used as an alternative rapid screening of designer drugs with a sub-nanogram detection capability. The rapid screening capability of the IMS for designer drugs was reported and published in peer-reviewed literature [13].

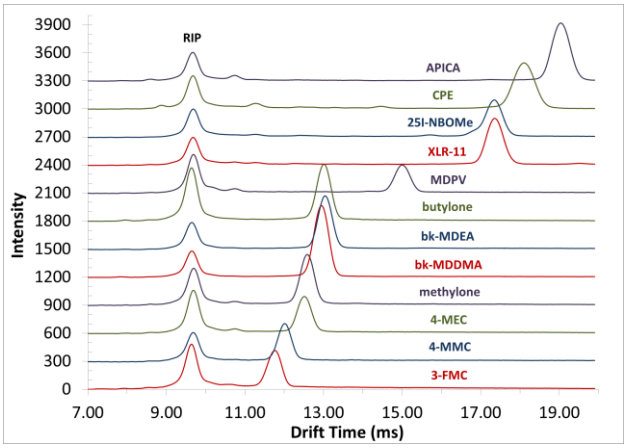

Figure 2. Overlaid ion mobility spectra of the representative designer drugs by the Barringer IMS.

The rapid detection (20 ms) and identification of 6 synthetic cathinones was also successfully accomplished with the acquisition of both ion mobility spectra and mass spectra using the Excellims ESI-IMS-MS (Table 3). The optimal solvent system was found such that the ratio of methanol to water is 80 to 20 (*v/v*) with 2.5% of formic acid for the efficient protonation of all analytes. This result was consistent with the solvent system used for the analysis of ATS. The

ability to analyze the mixtures was also investigated utilizing ESI-IMS-MS. In contrast to the results obtained from the previous solvent system study using the mixture of multiple compounds (Figure 1), the preferential ionization of MDPV versus other synthetic cathinones was observed because of the charge competition. In order to ionize other compounds in the mixtures, the concentration of the analytes with higher proton affinity (e.g. MDPV) was reduced from 100 to 20 µg/mL while others were successfully analyzed at 100 µg/mL. The results showed that the baseline separation of two-analyte mixture was achieved for MDPV with 5 other synthetic cathinones. The presence of different analytes were also confirmed in other mixtures with the observed protonated molecular ions in their mass spectra.

Table 3. Summary of results for 6 synthetic cathinones analyzed by the Exellims ESI-IMS-MS.

NPS	Molecular Weight (amu)	Drift Time (ms)	K_0 (cm^2/Vsec)	Identified $[M+H]^+$
4-MMC	177.24	9.68	1.45	178.7
4-MEC	191.70	10.01	1.41	192.7
3-FMC	181.21	9.75	1.44	182.7
Methedrone	193.24	9.93	1.42	194.7
Methylone	207.23	10.13	1.39	208.6
MDPV	275.34	11.65	1.21	276.8

The applicability of the proposed rapid and inexpensive analysis for designer drugs was evaluated by analyzing actual seized drug samples provided by a local forensic laboratory. The Barringer IMS and the Excellims ESI-IMS-MS were used for the analysis of two different sets of four seized drug samples. Figure 3 shows the successful detection of four seized samples by the Barringer IMS. It was found that three samples contained a single compound, while the other seized sample contained a mixture of at least two compounds. The disadvantage of this instrument, however, is the potential for false positive alarms, as have been observed during our analysis when only the IMS mode was used. For example, there were four positive alarms obtained for the mixture sample although there were only two peaks present. This was because of the similar mobilities between 4-MePBP (K_0 : 1.3024 $cm^2V^{-1}sec^{-1}$) and α-PVP (1.3094) as well as bk-MDEA (1.3790) and butylone (1.3808). Therefore, the use of the Barringer IMS may be limited for screening purposes only.

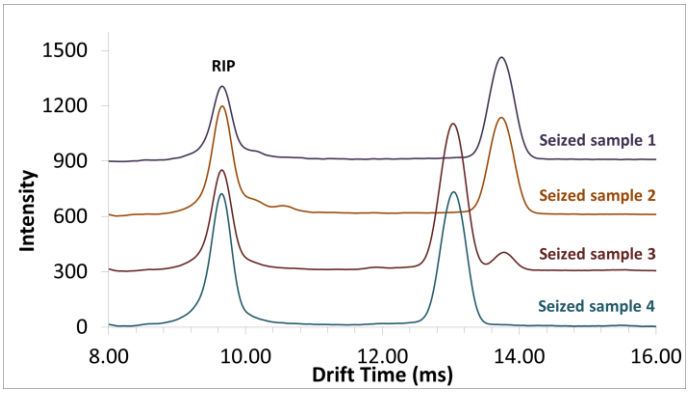

Figure 3. Overlaid ion mobility spectra of four seized samples analyzed by the Barringer IMS.

The results from the other set of four seized drug samples showed that the seized samples can be detected and identified utilizing the Excellims ESI-IMS-MS. Figure 4 is an example of those four seized drugs, identified as methylone. These findings demonstrate that this approach can be readily used as a confirmatory test in the qualitative analysis of synthetic cathinones with rapid analysis in less than five minutes. This rapid analysis capability of seized drugs is the biggest advantage over the conventional chromatographic analysis.

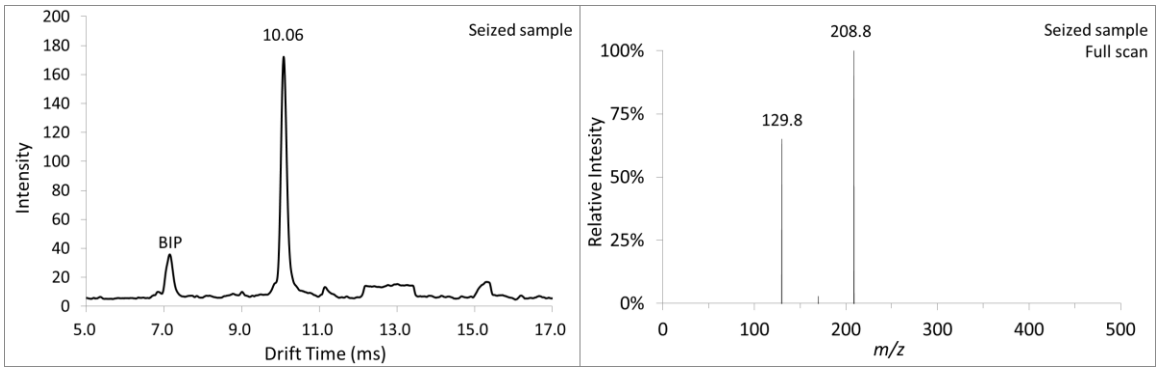

Figure 4. Ion mobility spectrum (left) and corresponding mass spectrum (right) of the seized drug sample by the Excellims ESI-IMS-MS.

Analysis of Designer Drugs by Other Analytical Techniques

Detection and characterization of designer drugs were also evaluated using other analytical techniques including direct analysis in real time quadrupole time-of-flight mass spectrometry (DART-QTOF-MS), gas chromatography tandem mass spectrometry (GC-MS/MS), and gas chromatography quadrupole time-of-flight mass spectrometry (GC-QTOF-MS). The novelty of DART-QTOF-MS is the rapid identification of compounds with less than two minutes of total analysis time. The soft ionization capability of the DART source enables the presence of

protonated molecular ion in the obtained mass spectrum. The coupling of DART with QTOF-MS also provides profiles of produced ions with high mass accuracy.

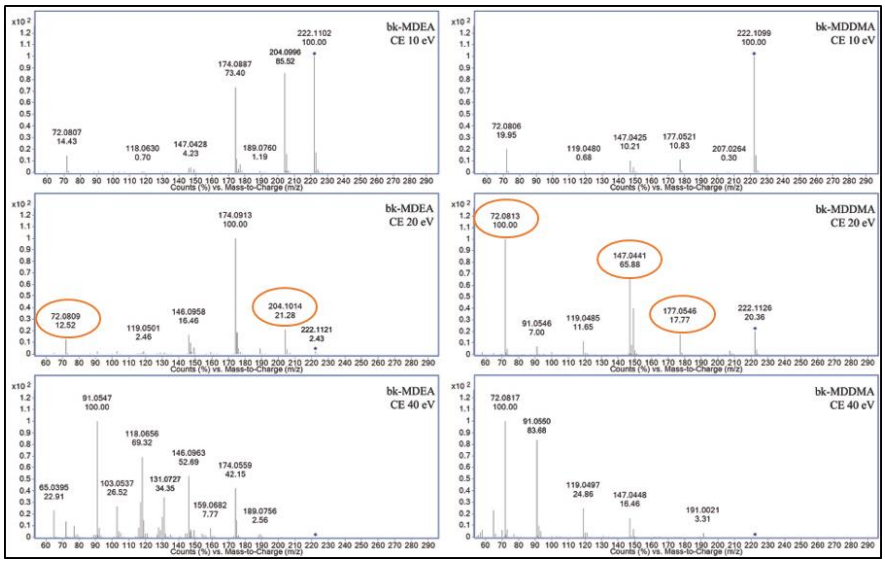

Figure 5. Examples of product ion mass spectra for bk-MDEA (left) and bk-MDDMA (right) at different collision energies of 10, 20, and 40 eV.

Figure 5 shows examples of product ion scan mass spectra for two constitutional isomers, bk-MDEA and bk-MDDMA. These two compounds are not distinguished in full scan mass spectra because of having the same exact mass each other using DART-QTOF-MS. In this case, the differentiation of these compounds was possible with the product ion scan mode providing different fragmentation patterns. In addition, it is found that product ion scan spectra between the DART ion source and ESI source produce no significant differences, which enables the use of spectral libraries for these designer drugs generated by ESI-QTOF. Therefore, DART-QTOF-MS can facilitate the analysis of these emerging compounds by providing minimal to no sample preparation and the rapid identification is less than one minute per sample. The results from the rapid screening of designer drugs was published in the peer reviewed journal [13].

Gas chromatography mass spectrometry (GC-MS) is the gold standard technique that is widely utilized in the forensic laboratories for the various applications. While GC-MS equipped with a single quadrupole mass spectrometer and an electron ionization (EI) source is the most commonly utilized setting, the GC system coupled with a triple quadrupole mass spectrometer (MS/MS or QQQ) or a quadrupole time-of-flight mass spectrometer (QTOF-MS) was utilized in

the qualitative analysis of 244 designer drugs proposed as a new confirmatory test. First, the novelty of this GC-MS/MS is the capability of multiple transitions scan known as multiple reaction monitoring (MRM) mode. With this acquisition mode, only specific transitions can be monitored, eliminating noises, background peaks, or other co-eluting analyte peaks. In addition, it is shown that additional spectral information from product ion scan and MRM can be used to differentiate isomers, which is one of the major challenges in the analysis of NPS. Most importantly, the unambiguous identification of NPS were successfully achieved with the implementation of chemical ionization (CI) source for those substances that are extensively fragmented in EI mass spectra. These results also have been reported and published in peer reviewed literature [14]. The GC system coupled to a high resolution QTOF mass spectrometer also has been proposed for an alternative confirmatory technique in the analysis of NPS. This hyphenated analytical technique was developed to provide advantages from both GC-MS/MS and LC-QTOF-MS. The MS/MS capability from GC-MS/MS was enhanced by switching the third quadrupole with a TOF mass analyzer. As a result, high resolution full scan and MS/MS scan with a high scan rate was possible with enhanced resolving power in GC-QTOF-MS. To investigate the potential of GC-QTOF-MS as a confirmatory method, various designer drugs have been analyzed with both EI and CI sources. From these results, it is expected that the creation of a database using the acquired high mass accuracy full scan and MS/MS scan mass spectra will be beneficial in the various aspects.

Conclusions

Experiments were carried out utilizing ion mobility spectrometry and other analytical techniques throughout this research project. The hybrid instrument, Excellims ESI-IMS-MS provided higher resolving powers of R~80 versus R~35, when compared to the Barringer IMS. In addition, the acquisition of MS information enabled the identification of the compounds. The results from this research suggest that chiral separation by an achiral modifier in the gas phase and the detection and identification of designer drugs are possible using the ESI-IMS-MS with an optimal solvent system. A total of four peer-reviewed manuscripts were published and more than 20 oral and poster presentations were presented at national and international scientific conferences.

List of Publications

1. Holness H, Jamal A, Mebel A, Almirall J. Separation mechanism of chiral impurities, ephedrine and pseudoephedrine, found in amphetamine-type substances using achiral modifiers in the gas phase. *Analytical and Bioanalytical Chemistry*, **2012**;404:2407-16.
2. Holness H, Almirall J. Speciation effects of solvent chemistry on the analysis of drugs and explosives by electrospray ion mobility mass spectrometry. *International Journal for Ion Mobility Spectrometry,* **2013**;16:237-46.
3. Gwak S, Arroyo-Mora LE, Almirall JR. Qualitative analysis of seized synthetic cannabinoids and synthetic cathinones by gas chromatography triple quadrupole tandem mass spectrometry. *Drug Testing and Analysis*, **2015**;7:121-30.
4. Gwak S, Almirall JR. Rapid screening of 35 new psychoactive substances by ion mobility spectrometry (IMS) and direct analysis in real time (DART) coupled to quadrupole time-of-flight mass spectrometry (QTOF-MS). *Drug Testing and Analysis*, **2015**: DOI:10.1002/dta.1783.

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[13] Gwak S, Almirall JR. Rapid screening of 35 new psychoactive substances by ion mobility spectrometry (IMS) and direct analysis in real time (DART) coupled to quadrupole time-of-flight mass spectrometry (QTOF-MS). *Drug Testing and Analysis*, **2015**: DOI:10.1002/dta.1783.
[14] Gwak S, Arroyo-Mora LE, Almirall JR. Qualitative analysis of seized synthetic cannabinoids and synthetic cathinones by gas chromatography triple quadrupole tandem mass spectrometry. *Drug Testing and Analysis*, **2015**;7:121-30.