Precision, Linearity, and Limit of Detection of an Electrospray Ionization High Performance Ion Mobility Spectrometer

Abstract

This study investigates the analytical performance of an electrospray ionization high performance ion mobility spectrometer (ESI-HPIMS) for pharmaceutical drug and forensically relevant compounds. Erythromycin, ibuprofen, phencyclidine, and 3,4-methylene-dioxy-methamphetamine were chosen as examples to determine sensitivity, linearity and precision of the method. The observed limits of detection ranged from 500 ng/mL to as little as 10 ng/mL, with a linear range of 2–3 orders of magnitude. The standard deviation for repeat measurements was below 12% for all data sets. Combining a short analysis time of typically 30 seconds, high analytical resolution and in-situ capabilities with low RSD and limit of detection, ESI-HPIMS becomes an attractive alternative to HPLC-based methods, offering both higher throughput and lower operational cost by eliminating the need for large quantities of solvents.

Introduction

Electrospray Ionization High-Performance Ion Mobility Spectrometry (ESI-HPIMS) is an analytical technique that separates molecules based on their size and shape. Molecules in liquid solution are brought into the gas phase and ionized using electrospray ionization (ESI). They are then introduced into an atmospheric drift tube. An applied voltage of 8-10 kV pulls the ions through the drift tube while collisions with the neutral drift gas molecules slow them down, leading to a separation in time determined by the ions' collisional cross section. Ions are detected by a Faraday cup at the end of the drift tube, and an ion mobility spectrum is generated that allows for the detection and quantification of compounds. Individual spectra are completed in a milliseconds time frame, leading to a total analysis time of typically 30 seconds.

ESI-HPIMS allows for the analysis of molecules which are not easily separated by HPLC, is applicable to polar and non-polar compounds and those lacking a UV chromophore. At the same time, ESI eliminates the serious restrictions imposed by the use of thermal desorption in traditional IMS, allowing for the analysis of larger, non-volatile and thermally labile compounds. Combined with a typical resolving power of 70–120, the ESI-HPIMS demonstrates superior performance to both HPLC and traditional IMS-based methods.

Beyond the traditional IMS domain of explosives detection[1,2], ESI-HPIMS has been used successfully to detect illicit drugs[3,4] in forensic applications, as well as additives and contamination in food and beverage samples[5]. It can dramatically increase the throughput for cleaning validation / verification[6] in a pharmaceutical setting by providing fast analysis results with high accuracy and low limits of detection, as demonstrated in this application note. The same characteristics make ESI-HPIMS a promising alternative to HPLC-based methods in a wide range of applications like process monitoring, degradation and dissolution studies.

Experimental

This research was conducted using Excellims Corporation's ESI-HPIMS system (Acton, MA), as shown in **Figure 1**. The system allows liquid phase samples to be continuously infused into the HPIMS using an ambient pressure electrospray ionization source, with minimal or no sample preparation. Methanol, water, and acetic acid (purchased from Sigma-Aldrich, Haverhill MA) were HPLC grade solvents. The drift gas supply used in these experiments was air, which was cleaned of contaminants by passing through a 13X molecular sieve (Fluka) trap before entering the IMS drift tube. The flow rate of the drift gas was approximately 3 L/min.

Fig. 1 Excellims GA2100 ESI-HPIMS (shown here with cart mount).

Solutions of ibuprofen (Sigma Aldrich, St. Louis MO), erythromycin (Sigma Aldrich, St. Louis MO), phencyclidine (Cerilliant, Round Rock TX), and 3,4-methylene-dioxy-methamphetamine (Cerilliant, Round Rock TX) were prepared in a solvent consisting of 80:20 MeOH:H_2O and 0.1% acetic acid. Serial dilutions were carried out to create calibration curves over several orders of magnitude, with approximately 5-7 solutions making up each calibration curve. Each solution was analyzed in the ESI-HPIMS instrument five consecutive times, with the 80:20 MeOH:H_2O solvent injected into the instrument between every run. The measurements were taken over 30 seconds as the sample was injected at a rate of 3 µl/min.

Results and Discussion

The ESI-HPIMS was used to analyze various pharmaceutical compounds and drugs of abuse which differed in size, shape, and functional group in order to determine the sensitivity and precision of the method. The structures of these molecules are shown in **Figure 2**.

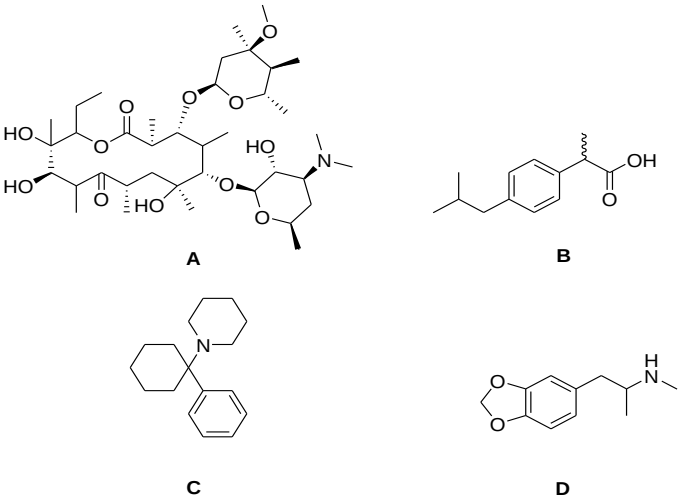


Fig. 2 Structures of pharmaceutical drugs tested. A) erythromycin, B) Ibuprofen, C) Phencyclidine, D) 3,4-methylene-dioxy-methamphetamine.

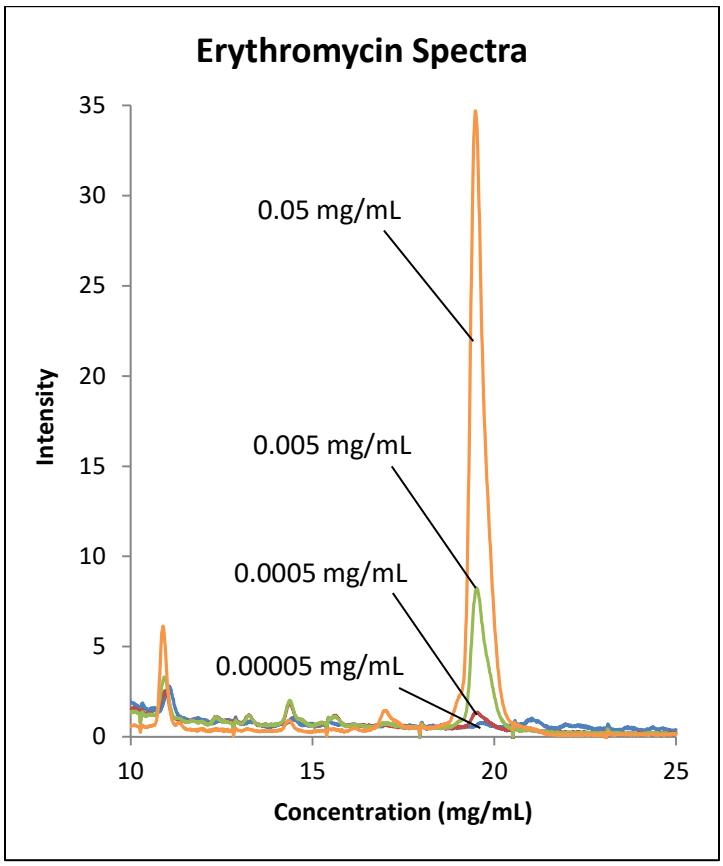
Erythromycin (**A**) is a macrocyclic compound that contains a 14-membered lactone ring with ten asymmetric centers and two sugars. Erythromycin is a macrolide antibiotic that has an antimicrobial spectrum similar to or slightly wider than that of penicillin. Since the molecular weight is 733.93 this compound is not volatile and cannot be analyzed on other commercial ion mobility spectrometry (IMS) systems which use ^{63}Ni ionization source and thermal desorption. Ibuprofen (**B**) is a nonsteroidal anti-inflammatory drug with a molecular weight of 206.29 having an acid functionality.

Phencyclidine (**C**) was formerly used as an anesthetic agent, and is now better known as the recreational drug PCP. It has a tertiary amine functional group and a branched structure with a molecular weight of 243.39. 3,4-Methylene-dioxymethamphetamine (**D**), commonly known as “ecstasy”, has a secondary amine functional group and a molecular weight of 193.25.

Response curves were constructed for the compounds by calculating the average peak area at each concentration and plotting it as a function of analyte concentration. The ion mobility spectra for erythromycin are shown as an example in **Figure 3** (top). The linear response range was 2 orders of magnitude for erythromycin **Figure 3** (bottom), 2 orders of magnitude for ibuprofen, 2.5 orders of magnitude for phencyclidine, and 3 orders of magnitude for 3,4-methylene-dioxy-methamphetamine. The linear response curves are shown in **Figure 4**.

The precision of the peak area data obtained in these experiments was determined by the relative standard deviation (RSD), and these values are shown alongside the raw data in **Tables 1-4**. Acceptable RSD values for cleaning validation methods are typically less than or equal to 10 or 15%. The RSD of the data obtained in this experiment falls within this range.

The limit of detection for each compound was calculated experimentally, by diluting the samples until a peak was no longer visible. The limit of detection for erythromycin was 0.05 µg/mL (0.00075 mg), LOD for ibuprofen was 0.5 µg/mL (0.00075 mg), LOD for phencyclidine was 0.01 µg/mL (0.000015 mg), and LOD for 3,4-methylene-dioxy-methamphetamine was 0.025 µg/mL (0.0000375 mg).



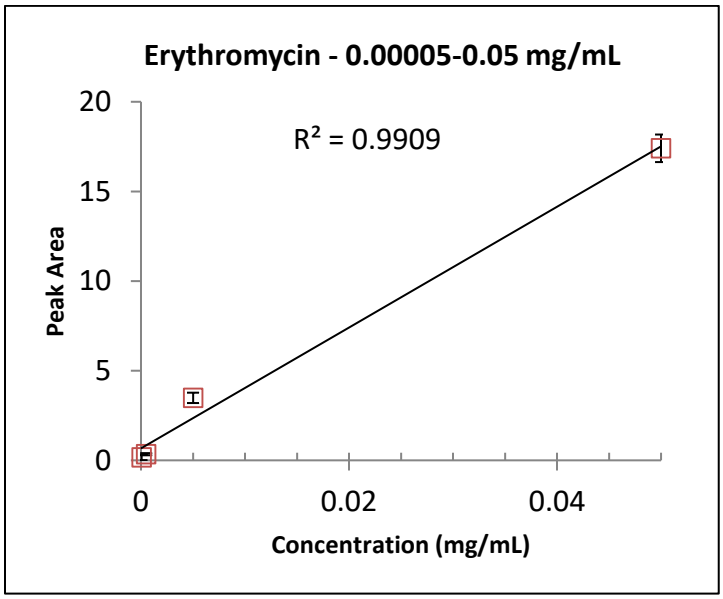
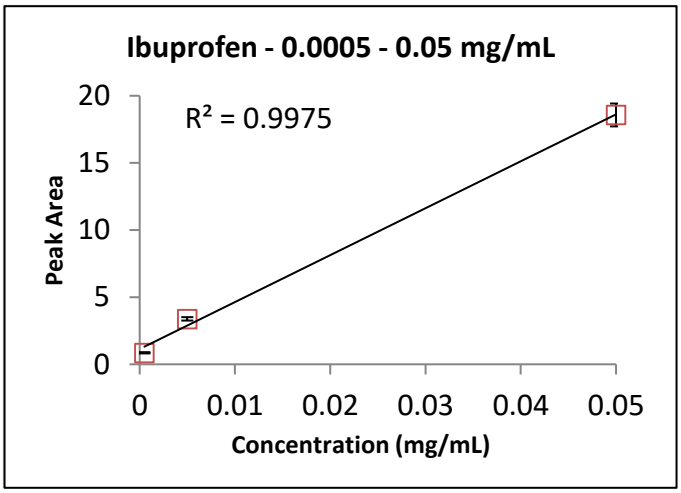
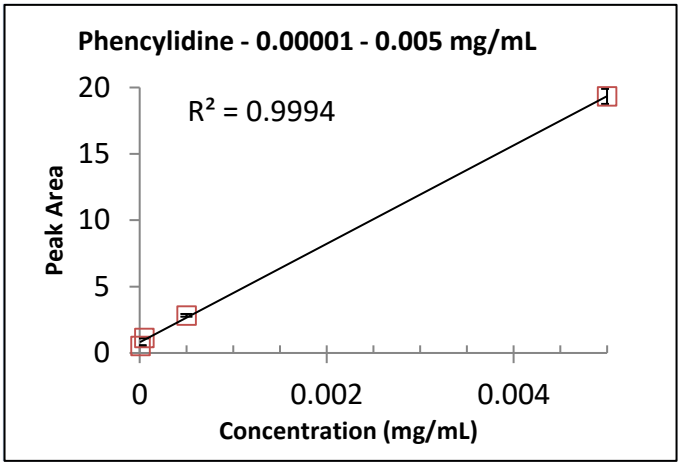


Fig. 3 (top) Ion Mobility Spectra of Erythromycin in a series of tested concentrations; (bottom) linear response curve over 3 orders of magnitude range.





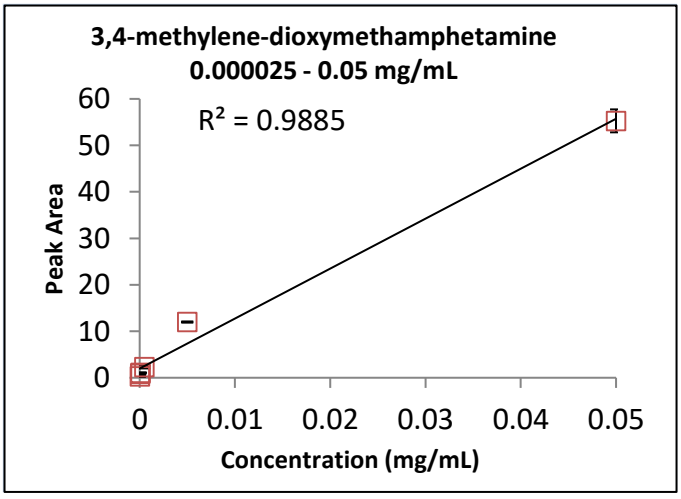


Fig. 4 Linear response of a series of pharmaceutical compounds over 2 to 3 orders of magnitude range.

Table 1 Raw data and % RSD for Erythromycin

Conc. (mg/mL)	*0.0005*	*0.005*	*0.05*	*0.5*
Area 1	0.318	3.72	16.6	24.6
Area 2	0.418	3.80	17.0	23.9
Area 3	0.352	3.16	18.4	25.1
Area 4	N/A	3.22	17.1	25.8
Area 5	0.370	3.52	18.0	25.1
Average	0.365	3.48	17.4	24.9
St. Dev.	0.041	0.288	0.768	0.702
RSD (%)	**11.4**	**8.27**	**4.41**	**2.82**

Table 2 Raw data and % RSD for ibuprofen

Conc, (mg/mL)	*0.0005*	*0.005*	*0.05*	*0.5*
Area 1	0.901	3.24	17.4	34.5
Area 2	0.789	3.58	18.1	34.2
Area 3	0.841	3.42	18.7	29.2
Area 4	0.859	3.38	19.6	38.5
Area 5	0.898	3.30	19.0	N/A
Average	0.858	3.38	18.6	34.1
St. Dev.	0.046	0.127	0.852	3.80
RSD (%)	**5.39**	**3.76**	**4.59**	**11.1**

Table 3 Raw data and % RSD for 3,4-**methylene-dioxy-methamphetamine**

Conc. (mg/mL)	*0.00005*	*0.0001*	*0.0005*	*0.005*	*0.05*	*0.1*
Area 1	0.786	1.24	2.73	12.0	53.4	71.2
Area 2	0.808	1.02	2.03	12.1	54.2	71.0
Area 3	0.941	1.06	2.14	11.8	53.4	71.0
Area 4	0.926	1.08	2.18	12.1	59.1	73.7
Area 5	0.944	0.974	2.27	11.9	56.3	71.9
Average	0.881	1.07	2.27	12.0	55.3	71.7
St. Dev	0.077	0.098	0.269	0.127	2.46	1.15
RSD (%)	**8.79**	**9.12**	**11.8**	**1.05**	**4.46**	**1.60**

Table 4 Raw data and % RSD for phencyclidine

Conc. (mg/mL)	*0.00005*	*0.0005*	*0.005*	*0.05*	*0.1*
Area 1	1.15	2.88	20.0	66.6	79.9
Area 2	1.09	2.76	19.6	67.5	81.6
Area 3	1.22	2.85	19.9	69.8	80.8
Area 4	1.15	2.92	19.2	69.8	80.8
Area 5	1.18	2.91	18.3	68.3	78.8
Area 6	1.10	2.78	19.4	66.6	80.7
Area 7	1.11	2.93	18.7	68.2	79.5
Area 8	1.14	2.64	19.5	68.8	80.1
Average	1.14	2.83	19.3	68.2	80.3
St. Dev.	0.043	0.102	0.567	1.25	0.909
RSD	**3.76**	**3.60**	**2.93**	**1.84**	**1.13**

Conclusions

This study demonstrates the GA2100's ability to combine speed of analysis with high sensitivity and accuracy. Using only 30 seconds of analysis per sample, the instrument demonstrated ppb-range detection limits and RSD values of 12% or better, without any of the limitations on molecular size or polarity imposed by HPLC and traditional IMS. Its speed, versatility and ease-of-use make ESI-HPIMS highly suitable for a wide range of applications, including cleaning validation, reaction monitoring, content uniformity and dissolution testing.

References

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[6] See also: *Excellims Application Note: GA02-Cleaning Validation* for more examples of pharmaceutical compounds