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Electrospray Ionization – High Performance Ion Mobility Spectrometry for Rapid On-site Cleaning Validation in Pharmaceutical Manufacturing

Abstract

A novel high performance ion mobility spectrometer (HPIMS) equipped with an electrospray ionization (ESI) source was used to analyze active pharmaceutical ingredients from top selling drugs to demonstrate its performance in cleaning validation of pharmaceutical manufacturing equipment. The ESI-HPIMS system allowed rapid analysis of pharmaceutical samples that were difficult to detect using conventional IMS systems, while providing resolving power *R* greater than 60 and good sensitivity over a wider linear dynamic range. This research demonstrated analysis of high molecular weight and thermally labile compounds, which were detected as intact molecular ions. High performance IMS thus provides a rapid, effective analytical tool that offers improved performance for cleaning validation.

1. Introduction

In pharmaceutical manufacturing, equipment can be contaminated by any of the materials that have been in contact with the surfaces, including active pharmaceutical ingredients (APIs) from previous runs and cleaning agents. Cleaning validation (CV) is a critical control step, and the cleaning procedure must be in accordance with good manufacturing practices (GMPs).[1,2] Companies spend significant resources developing and validating the analytical methods required for cleaning verification. The analytical method must be specific, sensitive, accurate, and precise. To be cost-effective, it must also be fast and should be easy-to-use.

Companies have several options for sampling and analyzing the residues present on manufacturing equipment, such as high performance liquid chromatography (HPLC) and measurement of total organic carbon (TOC). Under current FDA guidelines for validating cleaning processes, HPLC analysis of swipe samples collected from the production area is the most commonly used assay. One drawback of HPLC is the comparatively long run-time for each sample. Until the production area is proven clean, the manufacturing process is halted, awaiting validation results from the analytical laboratory.[3,4] This manufacturing downtime results in significant losses.

Other analytical methods are becoming more accepted due to the Process Analytical Technology (PAT) initiative, including Ion Mobility Spectrometry (IMS). A particular advantage of IMS is its short run time (seconds to a minute), combined with high versatility and robustness, allowing the analyzer to be deployed at-line, outside the laboratory. [5,6,7]

The first commercial IMS systems used for cleaning validation were designed in the early 1990s for explosives detection. Due to this original purpose, these systems have several drawbacks which limit their use as rapid process analytical technology devices. First, traditional IMS systems are limited in their quantitation capabilities. Second, using thermal desorption (or GC injector) for sample introduction, only thermally stable volatile or semi-volatile compounds can be introduced. As molecular weight increases, samples are not volatile enough to be introduced into traditional IMS systems. Out of the small molecule drugs shown in **Table 1**, only 5 out of 14 (36%) could be analyzed using traditional IMS systems.

Table 1. API Samples Evaluated for this Study

Trade Name	Generic Name	M.W.	Ion. Mode	Tested on trad. IMS
Effexor	Venlafaxine	277	+	
Zoloft	Sertraline	306	+	Yes
Plavix	Clopidogrel	321	+	
Nexium	Esomeprazole	345	+	Yes
Prevacid	Lansoprazole	369	+/-	
Norvasc	Amlodipine	408	+	Yes
Risperdal	Risperidone	410	+	
Zocor	Simvastatin	418	+	Yes
Advair	Fluticasone	500	+	Yes
Camptosar	Irinotecan	587	+	Yes
EES	Erythromycin	734	+	
TAXOL	Paclitaxel	854	+	Yes
Vancocin	Vancomycin	1485	+	
	Insulin	5808	+	

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Electrospray ionization (ESI), on the other hand, has been shown to allow thermally labile molecules to be ionized and analyzed as intact ions using Excellims' ion mobility spectrometers.[8,9] Consequently, Excellims ESI-HPIMS has been used to study a variety of the APIs comprising top selling drugs, including many of high molecular weight as well as thermally labile compounds. The system has demonstrated improved resolving power, high sensitivity and greater linear dynamic range than traditional IMS systems for CV which rely entirely on thermal desorption.

2. Materials and methods

This research was conducted using a commercially available ESI-HPIMS from Excellims Corporation (Acton, MA), as shown in **Fig. 1**. Analyte ions were generated by ESI from liquid samples continuously infused into the ion source via a 100 µm ID fused silica capillary tube and using a Chemyx Fusion 100 syringe pump (Stafford, TX). The sample flow rate was 5 µl min^{-1}. The electrospray needle was held at a potential of 13.4 kV with a current limitation of 1 µA. The ionized droplets underwent desolvation in the desolvation region and were subsequently introduced into the drift tube via a Bradbury-Neilson ion gate with gate pulse widths from 40 to 100 µs (nominal gate voltage 120 V).

The upper potential of the desolvation region of the IMS was set from 8 to 10 kV, producing drift fields as high as 850 V cm^{-1} over the 10.85 cm long drift tube. The drift tube was held at a constant temperature of 150°C. Ions were separated according to their mass-to-charge ratio, size and structure as they moved under the influence of the drift field through 0.8 L/min of counter-flowing drift gas in the drift region as discussed below. Each mobility spectrum represented the sum of 10 spectra ranging in length from 10 to 25 ms depending on the drift time of the analyte ions, sampled at a Faraday plate detector. Data were acquired using Excellims VisIon™ control and acquisition software and were exported for post-processing to Microsoft Excel. Atmospheric pressure in the laboratory was recorded for all experiments to properly correct the drift spectra shown.

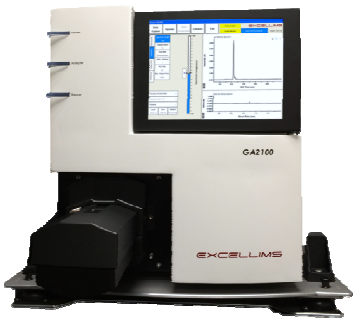

Fig. 1 GA2100 Standalone ESI-HPIMS System

The experiments were performed using high performance liquid chromatography (HPLC) grade solvents, including methanol, water, acetylnitrile and acetic acid, (purchased from Sigma-Aldrich) to dissolve pure chemical standards. Pharmaceutical drugs shown in **Table 1** were purchased from Sigma-Aldrich. The drift gas supply used in this project was pure nitrogen (99.999%). Water vapor and other contaminants were removed by passing the drift gas through a 13X molecular sieve (Fluka) trap before entering the IMS tube.

3. Results and discussion

The compounds listed in **Table 1** were used as representative APIs to demonstrate detection, determine system sensitivity and measure the dynamic response range of the ESI-IMS. Known solution concentrations, infusion rate and time were used to calculate instrument sensitivity. For a comparative study, the same samples were loaded on a sample swab of a current commercial IMS cleaning validation system and then placed into the thermal desorber and analyzed according to the instruments' standard procedure. Small molecule drugs and related compounds were evaluated using both ESI-IMS and the commercial IMS system, while large organics and biopharmaceuticals were only studied using the ESI-IMS method.

In the traditional IMS systems designated for CV use, samples are placed into a high temperature thermal desorber at 200-250ºC and the evaporated samples are introduced into a radioactive ionization source. Similar to a GC injector, the thermal desorption method only allows the instrument to analyze samples with low boiling points. For the top selling drugs, only a small percentage of the drug molecules could be analyzed with these instruments. Also, thermal decomposition of labile analyte molecules is commonly observed with current cleaning validation systems. In a conventional thermal desorber, the sample evaporation accompanied with decomposition could generate complex ion mobility spectra, reduce system sensitivity and limit capability for quantitation. In comparison, ESI is cited as a "soft" ionization and sample introduction method; direct liquid sample introduction could result in greater system performance, and in this study, thermally labile compounds did not decompose using ESI. As an example, **Fig. 2** shows the test result of Irinotecan from the IMS with a thermal desorber. As expected, both molecular ion and thermal decomposition products are observed, distributed across the 4-9 millisecond time frame.

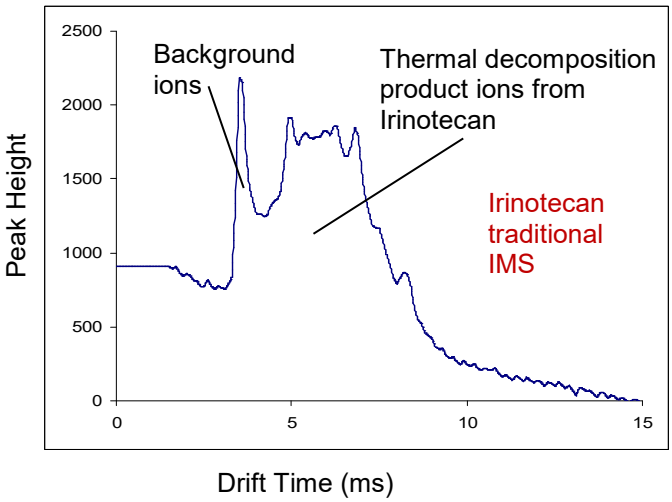

Fig. 2 IMS spectrum of Irinotecan produced by conventional IMS with thermal desorption and Ni63 ionization

Fig. 2 shows the drawback of the thermal desorption sample introduction method for the application, i.e. high temperature leading to sample decomposition. With this complex fragmentation pattern and poor resolution, identification of Irinotecan is almost impossible.

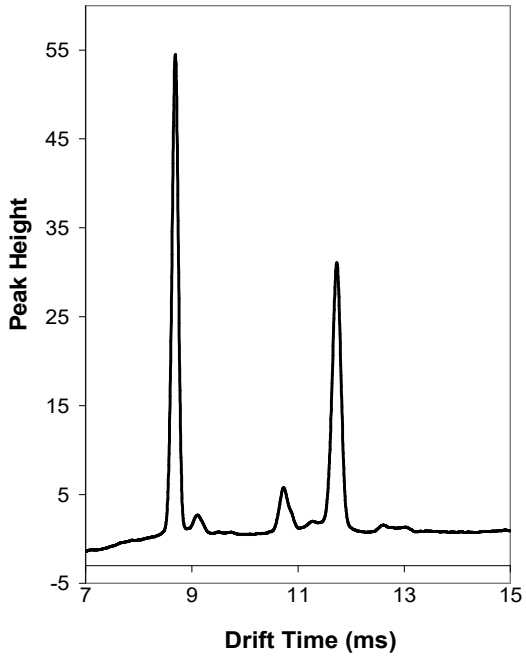

Fig. 3. Positive ion mobility spectrum of 0.1µg/µl irinotecan HCl in 80/20 MeOH/H_2O and 0.5% acetic acid (under 150°C drift tube condition)

When Irinotecan is introduced into Excellims' system via electrospray as shown in **Fig. 3**, two distinguishable peaks were observed. These peaks represent $[M+H]^+$ and $[M+2H]^{2+}$ and resemble the results seen in an electrospray ionization mass spectrometer (ESI-MS) experiment.

Quantitative measurement of trace amounts of chemicals is one of the critical requirements for cleaning validation systems. **Fig. 4** shows the response curve for the Excellims ESI-IMS system for risperidone. In a comparative study, the ESI-IMS system demonstrated a linear response range of two orders of magnitude (from 0.001-0.1 ug/uL), compared to the only one order of magnitude linear range on the conventional IMS.

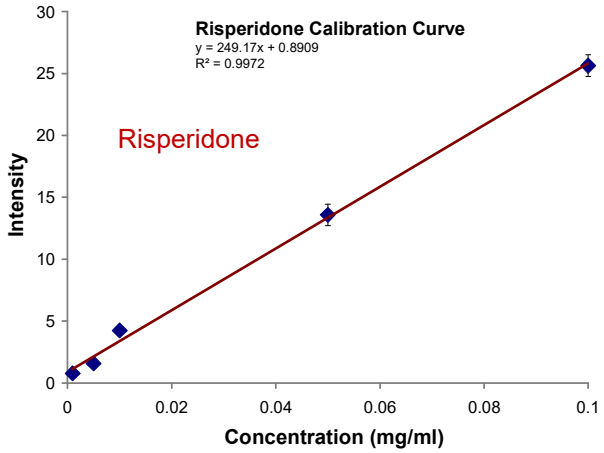

Fig. 4 Response/calibration curve for risperidone using Excellims ESI-IMS.

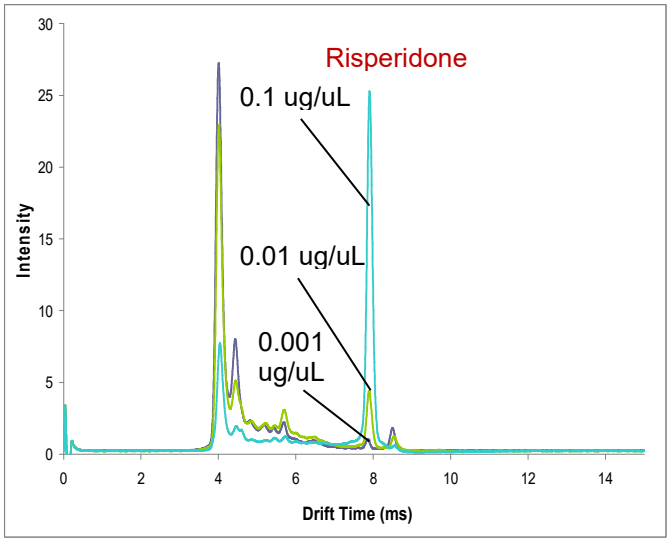

Fig. 5 Ion mobility spectra of risperidone analyzed using Excellims' ESI-IMS at varying concentration.

Fig. 5 shows the ion mobility spectra of risperidone on the Excellims GA2100 ion mobility spectrometer. The zoomed in spectrum in **Fig. 6** shows the signal-to-noise level for 7.5 picograms (0.001 ug/uL at flow rate of 1 uL/min and signal summing of 450 ms) of risperidone. The noise level in the spectrum is still mainly chemical noise. The true instrument sensitivity could approach sub-picogram level.

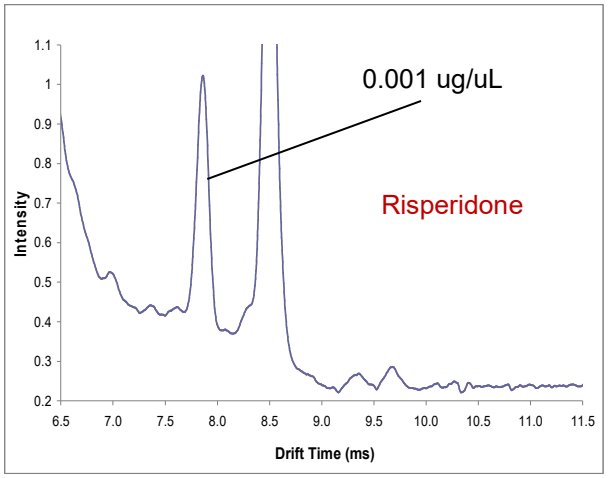

Fig. 6 Zoomed-in ion mobility spectrum of risperidone analyzed using Excellims' ESI-IMS at 0.001 µg/ µL

As expected, the thermal desorption based IMS was effective at detecting small molecules. It is worth noting, however, that most commercially available IMS systems used in cleaning validation have a resolving power of 10-30, where resolving power is commonly defined as $R = t_d/t_{1/2}$ where t_d is drift time and $t_{1/2}$ is peak width at half height. With its proprietary design, Excellims' IMS systems offer R > 60.

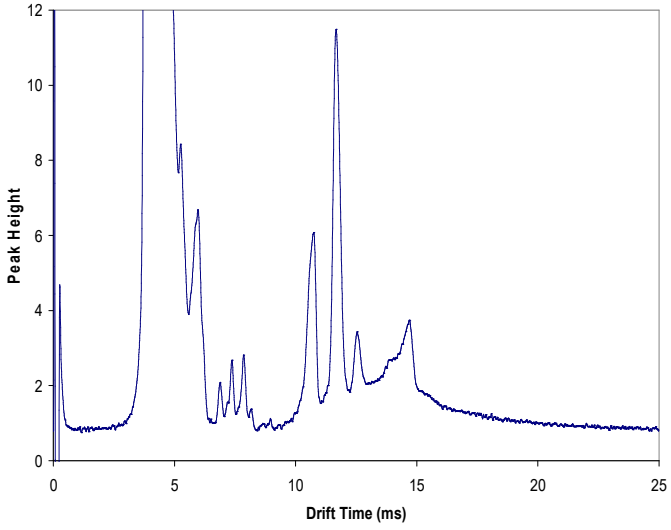

Fig. 7 Ion mobility spectra of Insulin analyzed using Excellims ESI-IMS.

Fig. 7 shows an ion mobility spectrum of multiply charged insulin ions. According to previous research under similar electrospray conditions, the predominant ions for the insulin B-chain have 3-5 charges. Intact insulin is known to form dimers and large multimers under broad ranges of solution conditions. The MS/MS studies of the suspected dimer species yield the expected monomers of higher and lower m/z. It is highly unlikely that dimers are formed in the gas phase because of the considerable Coulombic barrier. It is now apparent that such dimeric species were unsuspected contributors to some of the initial MS/MS studies of proteins. In the cleaning validation application, specific peaks are not identified, but the predominant charge state peaks can be used to quantify residual active ingredients.

4. Conclusions

In this study, fourteen active pharmaceutical ingredients found in several of the top selling drugs have been analyzed using commercial ESI-HPIMS. The observed ion mobility spectra demonstrated that ESI provides a robust way to analyze drug molecules, particularly those with high molecular weight that cannot be volatilized in conventional IMS systems that employ thermal desorption for sample introduction. ESI also allows thermally labile drug compounds to be ionized in their intact molecular form. IMS with high resolution (R > 60) can provide significantly more detail about sample composition, where R is at least a factor of two higher than in the conventional IMS. A linear dynamic response range over two orders of magnitude in concentration has been demonstrated, compared to the one order of magnitude in the current IMS CV instruments. Consequently, an ESI-HPIMS can be used as an effective tool for rapid analysis in cleaning validation applications for a wider range of target pharmaceutical compounds.

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