# HPLC-HPIMS: An Integrated Tool for 2D Separation

Carol Moraff, Clinton Krueger, Anthony Midey, Mark Osgood, Steven Pomerantz, and Ching Wu, Excellims Corporation

A novel separation technique combining high performance ion mobility spectrometry (HPIMS) and high performance liquid chromatography (HPLC) is an exciting new tool for separation scientists. The electrospray ionisation (ESI)-HPIMS simultaneously separates and detects complex mixtures in a single step; it is an ideal detector for modern HPLC systems. This new technology is designed for use in the pharmaceutical, chemical and food industries. Applications include isomer separation, reaction monitoring, cleaning verification, contaminant screening, and counterfeit drug detection.

The new HPLC-HPIMS -- IA3100 (Excellims Corporation, Acton, Massachusetts, USA) separates by two entirely different mechanisms. HPIMS separation is based on the size and shape of a molecular ion, while HPLC separation is based on polarity. Combining these two orthogonal methods into a single run allows for full separation of compounds that are not easily analysed by one method or the other.

## A new Generation of Ion Mobility Spectrometers

Ion mobility spectrometry involves introducing molecular ions into a drift tube with an applied electric field. This field pulls the ions through the drift tube to a Faraday plate detector, where they create an electrical signal that is sent to a computer and plotted as an ion mobility spectrum. The ion mobility spectrum shows how long it takes each group of ions to travel the length of the drift tube. As the ions travel through the tube, a gas (usually air) is pumped through in the opposing direction. The collisions between this drift gas and the travelling ions cause separation. The smaller and more compact an ion is, the fewer collisions it will undergo and the faster it will reach the end of the drift tube as compared to larger, bulkier ions. Because of its speed, robustness, and the fact that no vacuum pumps are required, traditional IMS has been widely used in the security industry especially for explosive detection. The new generation of IMS instruments – high performance ion mobility spectrometers (HPIMS) – have more than twice the resolving power of traditional IMS. This opened up the method to various applications in the pharmaceutical and other industries. These HPIMS based instruments reach resolving power of 60+ and have improved precision and linearity for optimal quantitation.

## Multidimensional Separation

IMS is an ideal technique to pair with a complimentary method, because it works so quickly that its addition to any method will not add significant analysis time. IMS-MS systems are currently in use in many analytical laboratories. This pairing is intuitive because of the correlation between drift time and mass-to-charge ratio (generally, lighter molecules have shorter drift times). However, the two techniques have some important differences. Shape is a factor in IMS drift time; a very compact but heavy molecule could have a shorter drift time than a very bulky, slightly lighter molecule. IMS and MS benefit each other when used in combination, because IMS separates molecules of equal mass by shape while MS correlates drift time to molecular weight for compound identification. The success of this combination is demonstrated by the fact that there are now a variety of commercially available IMS-MS instruments.

Building on the success of IMS-MS, HPIMS is now being paired with liquid chromatography. There is no direct relationship between chemical polarity and ion mobility, which adds to the strength of this combination. There are often compounds that only partially separate by either method alone that can be fully resolved when the two come together. A two-dimensional plot of retention and drift time ('retention and drift time plot', RDTP) clearly illustrates this performance, where HPLC retention time is plotted on the Y axis and HPIMS drift time on the X axis as shown in the following section.

## HPLC-HPIMS

The HPLC-HPIMS system is designed to carry a sample thought both separations in a single run. First, an autosampler and pump carry the sample to an HPLC column. There, HPLC separation by polarity takes place according to established HPLC methods. Each compound or group of compounds that elutes from the HPLC column can be detected with UC/Vis spectroscopy (optional) before being pumped through PEEK tubing to an electrospray ionisation source. The compound ionises by ESI and enters the desolvation region of the HPIMS where the excess solvent evaporates, and then an ion gate allows pulses of ions to enter the drift tube. Those ions travel through the tube in a matter of milliseconds, separating as they collide with the drift gas with a frequency corresponding to molecular size and shape. Finally they hit the Faraday plate detector at the end of the drift tube, and the detector creates a signal that can be plotted as a two-dimensional RDTP.


*Figure 1. The IA3100 HPLC-HPIMS system from Excellims Corporation. The HPLC module on the left (courtesy of Shimadzu Corporation) includes autosampler, column, and pumps. The HPIMS on the right includes an ESI source, drift tube, and Faraday plate detector.*

The major advantage of using an integrated HPLC-HPIMS system is that it allows separation of complex mixtures that cannot be fully resolved by either method alone. HPIMS and HPLC are entirely orthogonal techniques. If a set of compounds coelutes by HPLC, they still have a good chance of separating by HPIMS. If a set of compounds have very similar drift times when run by HPIMS, they could easily separate by HPLC.

There are many reasons to use HPLC-HPIMS beyond separation of complex mixtures. HPLC-HPIMS can eliminate the need for extensive HPLC method development. In addition, the Faraday plate detector permits analysis of compounds without UV chromophores.

## Applications of HPLC-HPIMS

The applications of HPLC-HPIMS fall into several categories. The first is the analysis of complex mixtures. Another is as a replacement for developing new HPLC methods. In addition, HPLC-HPIMS is useful for mixtures in complex matrices. The HPLC can separate the desired analyte targets from troublesome components in the matrix, and then the HPIMS can detect the analyte without excessive matrix interference.

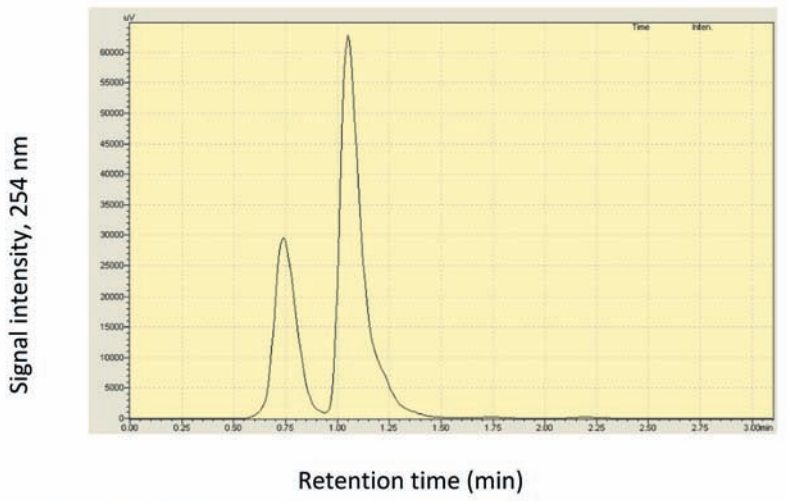


*Figure 2. UV spectrum*

An HPLC-HPIMS system was used to analyse a mixture of 4 compounds: MDPV (methylenedioxypyrovalerone), butylone, 4-FMC (fluoromethcathinone), and 3-benzoylpyridine. When analysed alone by a standard HPLC method (isocratic reverse-phase, C18 column, mobile phase composed of 75% MeOH and 25% H2O), this mixture of 4 compounds produced only two peaks in a UV spectrum:

This same mixture of 4 compounds showed 3 peaks when analysed by HPIMS alone.

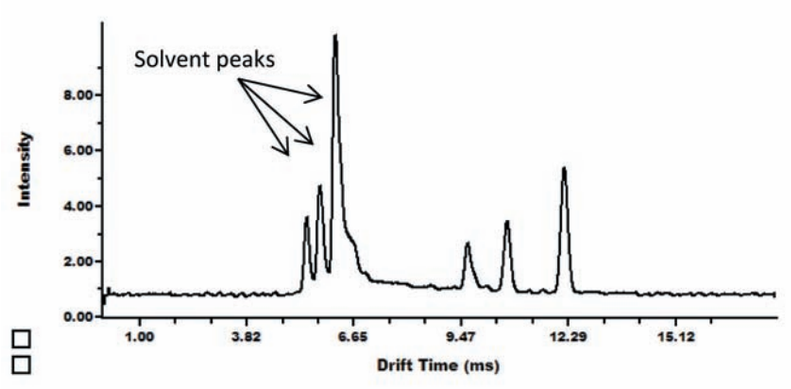


*Figure 3. HPIMS spectrum*

When these identical HPLC and HPIMS methods were run together, full separation was achieved:

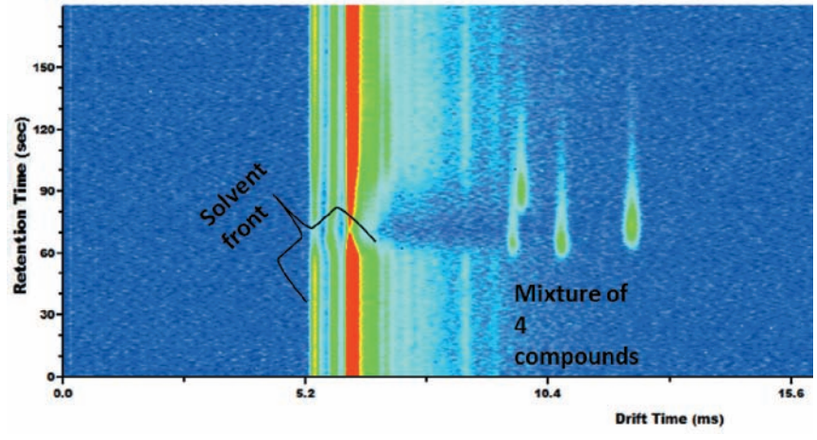


*Figure 4. 2D spectrum of a complex mixture*

The above two-dimensional RDTP clearly demonstrates the power of HPLC-HPIMS. Although three compounds co-eluted by HPLC and two had nearly identical drift times, the hyphenated system separated all four compounds with minimal HPLC method development. While an HPLC method could eventually have been developed to separate these compounds, the HPLC-HPIMS method separated them in only three minutes.

The analysis of amino acids is another example of the benefits derived from an HPLC-HPIMS. This set of compounds is notoriously difficult to separate by HPLC. Specialised methods have been developed that include specialty columns and procedures for derivatisation of the compounds. Many of these amino acids can be separated by adding HPIMS to a more common HPLC method saving time and equipment.

A pair of coeluting amino acids, arginine and histidine, was run on the HPLC-HPIMS system, and produced the two-dimensional RDTP shown below:

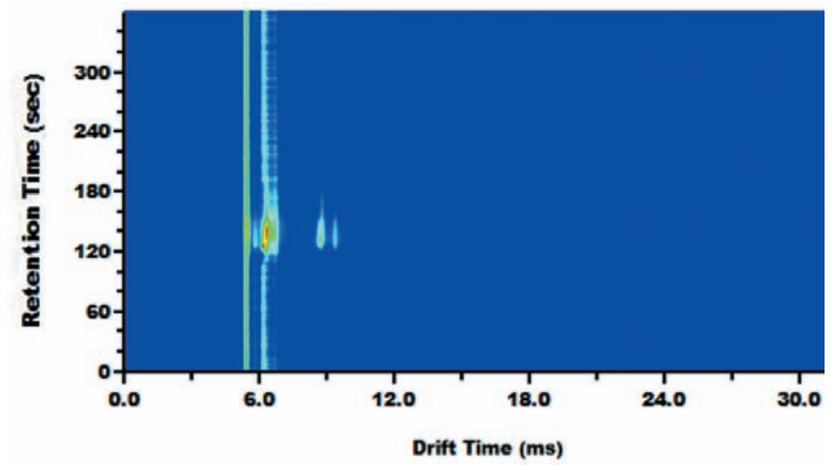


*Figure 5. HPIMS separation of coeluting amino acids*

These two amino acids have equal retention times; they did not separate by HPLC. Moreover, these amino acids cannot be detected with 254nm UV/Vis and produce one unresolved peak using 214nm. There are several sets of common amino acids for which this is true, and the addition of HPIMS would allow for separation of those combinations as well as of the ones that separate in the HPLC column.

It is clear from the examples above that there are cases in which the combined HPLC-HPIMS is a great advantage to an analytical chemist. It saves time and energy on the part of the scientists who need to develop methods and run through groups of samples. It greatly increases the versatility of both HPLC and HPIMS by adding the best features of the other method.

HPLC and HPIMS are useful on their own, but each has challenges. HPLC generally has long analysis times. Waiting 20 minutes or more for results is not always practical in applications such as reaction monitoring (where up-to-the-minute data are valuable) or cleaning validation (where high throughput analysis is important). Adding HPIMS to HPLC resolves these issues by allowing for a faster, cruder LC separation and then completing the separation in the HPIMS. The LC needs only to separate the compounds in the mixture enough so that they do not interfere with one another in the HPIMS, and a less complete HPLC separation can be achieved in a few minutes as evidenced by the examples above. In turn, HPLC enhances the abilities of HPIMS by limiting charge competition between molecular ions with different electron or proton affinities. If a mixture has been somewhat separated before it enters the HPIMS drift tube, there will be minimal charge competition between compounds and matrix effects can be greatly reduced. HPLC-HPIMS analysis thus provides this separation in a single step.

A commercial HPLC-HPIMS instrument is demonstrated to be useful in pharmaceutical and food industries. Its versatility, speed, and power to separate complex mixtures make it a tool of choice for analytical chemists – especially those working with large sample volumes. HPLC-HPIMS shows the power of combining two orthogonal methods enabling the most complete separation possible.