High Performance Ion Mobility Spectrometry (HPIMS) - Applications in Pharmaceutical Manufacturing and Development

Speed, sensitivity and selectivity for your workflows

Introduction to HPIMS

High Performance Ion Mobility Spectrometry (HPIMS) combines the speed, sensitivity, and robustness of ion mobility with laboratory-grade resolution, specificity and repeatability. It separates molecules by size and shape on a timescale of milliseconds, allowing for rapid compositional analysis and the differentiating between coeluting compounds and isomers.

In stand-alone mode, HPIMS offers a fast alternative to many chromatography-based workflows, including:

- Cleaning validation
- Dissolution studies
- Reaction monitoring

A typical HPIMS analysis takes only 20–30 seconds with excellent specificity and repeatability. Moreover, the straightforward measurement principle of HPIMS (see below) simplifies method development for new compounds, providing value for organizations with many or frequently changing analytes.

Combined with mass spectrometry, HPIMS offers the ability to differentiate between isomers, enabling:

- Screening for isomers
- 2D confirmation
- IMS-MS mapping

Applications for both IMS and IMS-MS are discussed in the following pages.

Measurement principle of HPIMS

The measurement principle of HPIMS is based on the separation of ions with different sizes and shapes through their collisions with a neutral drift gas (typically air). The larger a molecule's cross section, the longer it needs to pass through a gas-filled drift tube. As this separation happens on a time-scale of milliseconds, HPIMS separation is much faster than chromatography (see Fig. 1 for an example spectrum).

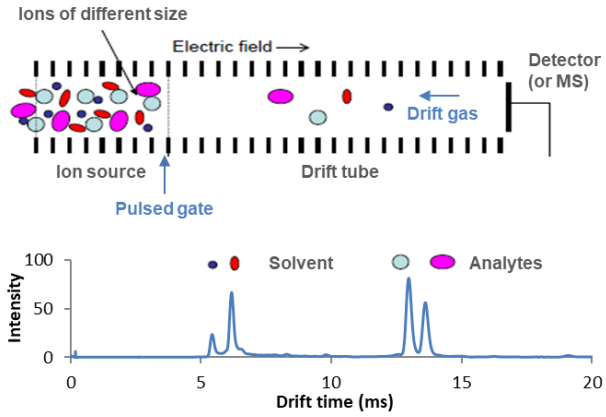


Fig. 1 Working principle of HPIMS

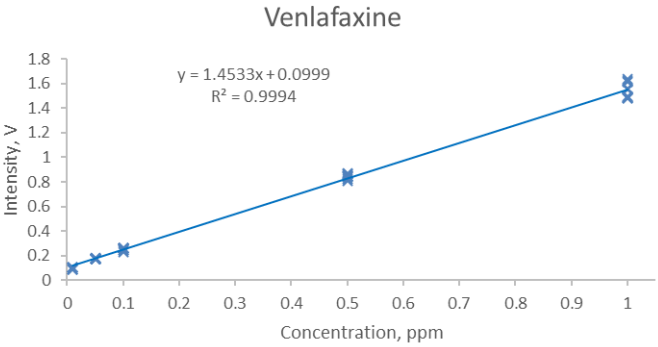
One of the characteristics of HPIMS is its resolving power (R > 70) which offers much higher specificity than regular field-based IMS. Another specialty is the coupling with electrospray ionization (ESI). The standard in LC-MS, ESI allows for the destruction-free introduction and ionization of a wide range of analytes from small molecules to proteins, e.g. sugars, peptides, lipids, antibiotics, detergents and many more. Using low field strength, drift times are directly correlated to a molecule's size (collisional cross section) and spectra can be easily interpreted, greatly simplifying method development. HPIMS is thus a powerful and fast technique where rapid distinction and identification of molecules is needed.

IMS for fast cleaning validation and screening

With the ability to identify small amounts of API or detergent in 30 seconds or less, HPIMS offers an excellent solution for cleaning validation.

Fig. 2 Stand-alone HPIMS analyzer GA2200

Swab extract or rinse samples are introduced in liquid form, either manually using a micro-syringe or in 2 mL vials from an autosampler (optional equipment). The analyst specifies the desired analysis method and starts the analysis. For each sample, drift time spectra are acquired and any peaks found compared to the library specified in the analysis method. If suitable calibration standards have been set up, the GA2200 will automatically calculated the concentration of the analyte. LODs in the ppb, two orders of linear range and single digit % repeatability are typical of this technique, as illustrated by the example in Fig. 3



Conc. [ppm]	Nr. of Samples	%RSD
0.01	9	2.4
0.05	11	2.1
0.1	12	2.9
0.5	9	2.3
1	11	3.9

Fig. 3 Linearity and repeatability for API in organic solvent (10–1,000 ppb, multiple repeats, 30s each)

1. Start with swab extract or rinse sample (typically in 80/20 MeOH:H_2O)
2. Introduce sample with micro-syringe or via autosampler
3. Specify analysis method and start acquisition (single or batch run)
4. Automatic comparison of peaks found & quantification (if curve defined)

Fig. 4 Sample analysis workflow with automatic quantification of API/detergents found

Using electrospray (ESI) for ionization, a wide range of molecules can be identified, including larger and thermally sensitive species as well as detergents / surfactants (see Fig. 5 for examples).

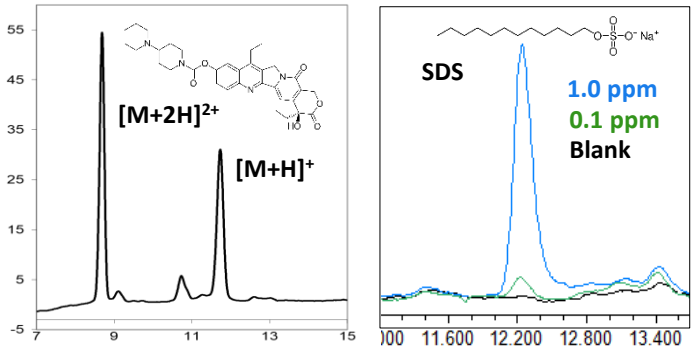


Fig. 5 HPIMS spectra of Irinotecan (left) and sodium dodecyl sulfate SDS (right)

The same fast approach can potentially be applied for other screening applications, as long as the majors are separated out first, e.g. by extraction.

Method development for new compounds is easy as the built-in Faraday detector will register any charged analyte molecules regardless of distinctions like polar/non-polar, with or without chromophore.

Thus, one drift tube can by used for many different species and method development is reduced to optimizing spray parameters upon initial method setup, greatly lowering the burden if a large number of APIs or other analytes need to be set up. For more on method setup, see the section on 21 CFR part 11 compliance below.

Dimension	HPLC	HPIMS
Specificity	Specific	Specific
Time-scale	Minutes	Seconds
Throughput	50 / day	> 200 / day
Chromophore	Required / MS	Not required
Column-based	Yes	No
Method dev.	2 - 4 days	1 - 2 days
Solvent waste	Mobile phase	mL sample only

Tab. 1 Comparison table of HPLC and HPIMS

Tab. 1 shows a comparison of HPLC and HPIMS: while both methods are highly specific, HPIMS operates on a much fast time scale without sacrificing sensitivity.

Other techniques like TOC lack specificity or are sensitive to surface effects. HPIMS is universally applicable as no chromophore is needed; one drift tube fits all, thus no column-matching is required, greatly simplifying method development. Finally, solvent waste in HPIMS is negligible, saving operating cost in addition to increasing throughput and drastically cutting down-time in production.

Dissolution studies

The same characteristics – fast determination of low-level concentrations of drug molecules in solution – also lend themselves to dissolution studies.

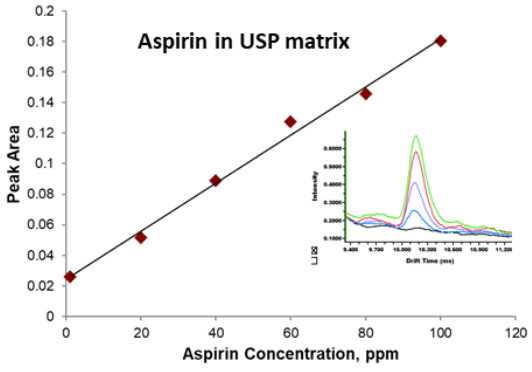


Fig. 6 Aspirin in USP matrix with HPIMS (typical HPIMS measurement time: 30 s per sample)

As for cleaning validation, the principal benefits consist in the speed of analysis combined with easy method development for new compounds in a small-footprint, easy-to-use benchtop instrument.

Rapid reaction monitoring

Another interesting routine analytical application is reaction progress / end point monitoring. With typical analysis times of 20–30 seconds per sample, reactions can be monitored in near real time as long as starting materials, intermediates and products can be differentiated by their size and shape.

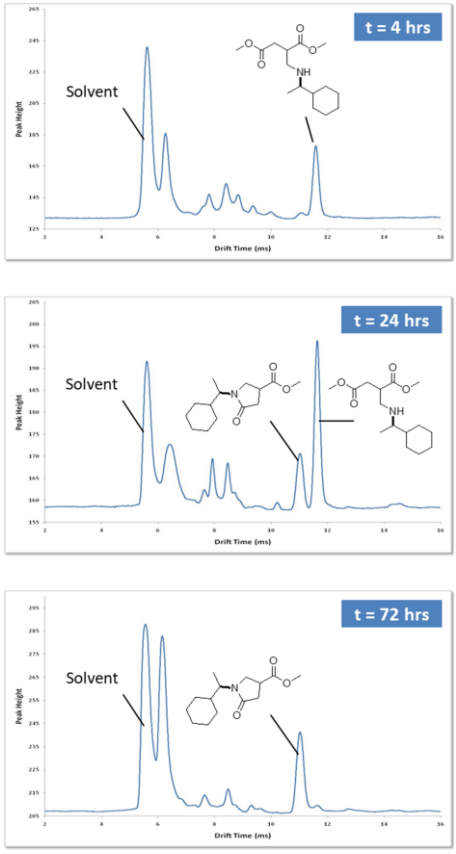


Fig. 7 Example for reaction monitoring by HPIMS, showing intermediary and product formation

This includes monitoring for unwanted pathways as well as tracking for isomers (see examples of isomer separation further down with the MA3100).

Full 21 CRR part 11 software compliance

VisionControl for the GA2200 is fully 21 CFR part 11 compliant. It offers several hierarchical user levels with individual log-ins, full tamper protection for methods and data, electronic signatures and a comprehensive, searchable audit trail. Lab managers can create an unlimited number of analytical methods, finalize them and make them available for use by analysts in a QC or production environment. This also includes operation of a CTC autosampler.

IMS-MS – Introduction to the MA3100

HPIMS can also be hyphenated with other analytical techniques. The MA3100 is designed to be added within seconds to a mass spectrometer in place of the regular ion source, providing ion mobility analysis whenever needed, e.g. for the distinction between isomers or to separate complex mixtures into classes of differently sized molecules. Fig. 8 shows the MA3100 add-on mounted on a Thermo Fisher Scientific Orbitrap (other couplings are possible).

Fig. 8 MA3100 mounted on a Thermo Orbitrap

The MA3100 consists of the same ESI source and high-performance drift tube as in the GA2200, separating molecules by their shape and size with a resolving power of 70 or higher and using a Faraday detector for visualization. A second ion gate then allows selective introduction of individual ion species or ion regions into the MS based on their drift time.

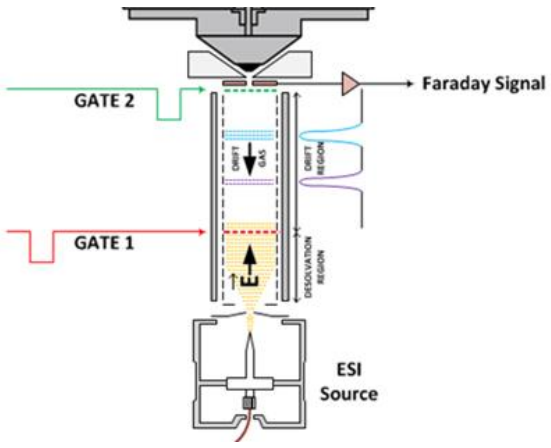


Fig. 9 Diagram showing the dual gates of the MA3100 (for total flexibility in IMS-MS experiments)

Isomer differentiation and 2D confirmation

A principal benefit of adding the MA3100 to your mass spectrometer is the ability to easily distinguish between isomers that may be hard to separate by MS/MS or by chromatography. Fig. 10–12 show several examples including disaccharides, metabolites and distinction of a vitamin from its epimer.

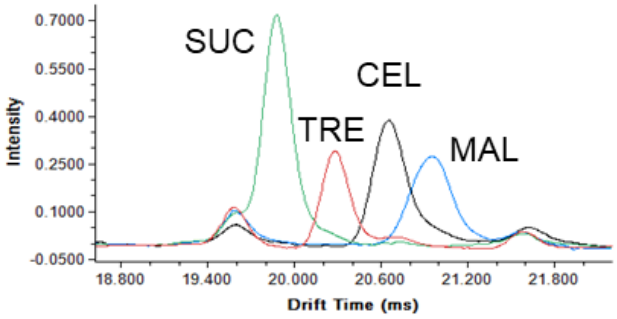


Fig. 10 Disaccharide isomers (sucrose, trehalose, cellobiose and maltose) separated by HPIMS

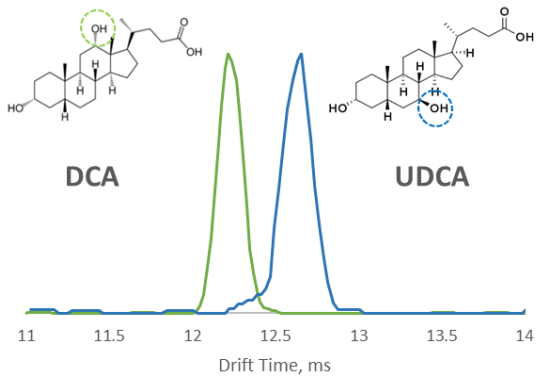


Fig. 11 Separation of bile acids isomers on the MA3100 + Orbitrap

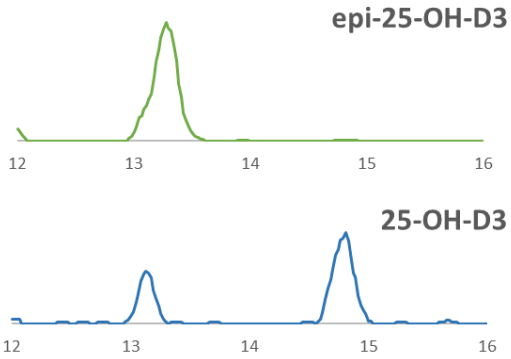


Fig. 12 Differentiation of Hydroxy-Vitamin D3 from its epimer (using a diagnostic secondary conformer)

The same capability to differentiate conformers and other isomers can also be applied to larger molecules, as shown in Fig. 13 and 14.

The MA3100's ability to easily distinguish between isomers based on their size and shape can be used for both initial characterization and screening. For new development candidates, the target compound can be presented with direct injection and analyzed for the presence of isomers by scanning the drift time and recording an IMS-MS map. In targeted workflows, the ion mobility window can be set to the drift times of known isomers to check for the presence of each.

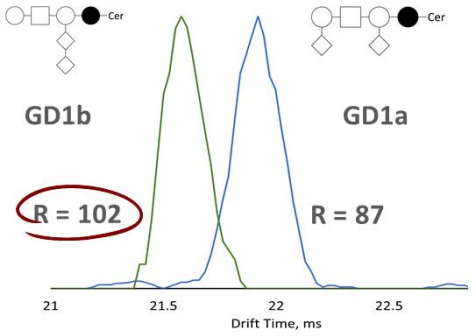


Fig. 13 Separation of two gangliosides by HPIMS on the MA3100 with resolving power 80 – 100

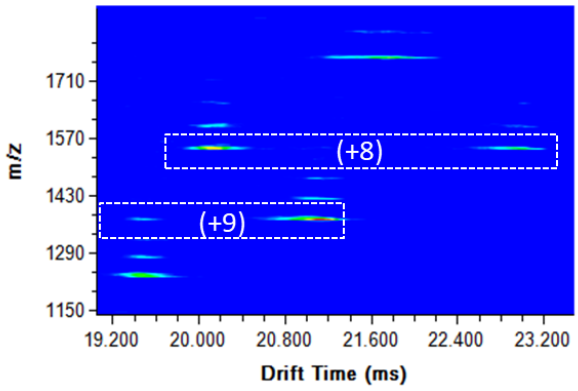


Fig. 14 Charge states of ubiquitin with multiple conformations visible (2D IMS-MS map on Orbitrap)

HPIMS-MS can also be used for 2D identification of APIs and other molecules in a drug formulation. Obtaining both drift time (collisional cross section) and m/z of a target molecule significantly increases the confidence in the identification in cases were 2D confirmation is not easily obtainable by HPLC/UPLC or were the related workflows are too laborious.

Summary

HPIMS offers a wealth of solutions in pharmaceutical research, development and production control. Providing rapid separation of molecules by shape and size, HPIMS provides a high-speed alternative to chromatographic workflows in cleaning validation, dissolution studies and reaction monitoring.

Combined with mass spectrometry, HPIMS offers complementary information to distinguish isomers, allow for 2D identification, confirm assumptions about molecular shape or charge location or to simplify/ replace chromatography in traditionally challenging tasks like sugar isomer separation. To learn more about HPIMS, to discuss your application or to schedule a demonstration on your samples, please contact us at sales@excellims.com. We look forward to speaking with you!

Additional Literature

- For more examples for APIs in cleaning validation see Excellims Application Notes GA02 (*ESI–HPIMS for Rapid On-site Cleaning Validation in Pharmaceutical Manufacturing*) and GA03 (*Precision, Linearity, and Limit of Detection of an ESI High Performance Ion Mobility Spectrometer*)
- For reaction monitoring: *Rapid Synthetic Organic Reaction Analysis Using High Performance,* Excellims Application note GA05
- Dissolution studies: Moraff et al., *Electrospray Ionization High Performance Ion Mobility Spectrometry for Dissolution Studies*, Pittcon 2013
- Metabolites and isomers: Kaszycki et al., *Separation of biologically relevant isomers on an Orbitrap mass spectrometer using high-resolution drift tube ion mobility and varied drift gas mixtures*, Rapid Commun. Mass Spectrom. 2019; 1– 8 and Oranzi et al., *Measuring the Integrity of Gas-Phase Conformers of Sodiated 25-Hydroxy-vitamin D3 by Drift Tube, Traveling Wave, Trapped, and High-Field Asymmetric Ion Mobility*., Anal. Chem. 2019 Mar 19;91(6):4092-4099

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