

The Agilent Ion Mobility Q-TOF Mass Spectrometer System

Technical Overview

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The Agilent Ion Mobility Q-TOF Mass Spectrometer System

- Delivers an added dimension of separation
- Provides direct measurement of accurate collision cross sections
- Preserves structural characteristics of molecular conformations
- Expands coverage maps for complex samples.

Introduction

The Agilent 6560 Ion Mobility Quadrupole Time-of-Flight (IM-QTOF) LC/MS system enables high performance ion mobility and very precise and accurate collision cross section (CCS or Ω) measurements without class dependent calibration standards. The Agilent mobility device operates under uniform low field conditions, thus allowing the drift time information for ions to be directly converted to collision cross section information. The innovative ion funnel technology in this instrument dramatically increases the ion sampling into the mass spectrometer and results in higher quality MS/MS spectra at trace levels.

The Agilent IM-QTOF system is the first commercially available uniform field ion mobility system, which coupled with the Agilent 1290 UHPLC, provides the combined separation power and selectivity of LC, IM, and MS techniques. Laboratories involved in cutting edge research can speed up research programs and have greater confidence in compound identification with the additional dimension of separation as well as the structural information provided by ion mobility measurements. This instrument is the only commercially available drift tube ion mobility high resolution (both mobility and mass) LC/MS system that simultaneously provides high sensitivity and accurate collision cross section measurements.



Agilent Technologies

The Agilent ion mobility system was developed with the collaboration of scientists from a number of academic institutions and government laboratories. In multiple studies, the instrument has demonstrated the ability to reveal significantly greater analytical detail for complex samples compared to high resolution mass spectrometry technology alone.

Researchers have reported that while high resolution mass spectrometry has become the analytical cornerstone for proteomics, metabolomics, and other research applications requiring the analysis of highly complex samples, there has also been significant interest in the use of ultra-fast orthogonal techniques to provide added dimensions of separation. This new ion mobility system will provide researchers with greater analytical detail than ever before.

Principles of Ion Mobility Separation

In a classical uniform field drift tube, the electric field within the drift cell moves ions through the device while the drag force due to the collisions of these ions with the stationary buffer gas molecules acts against the electrical force that moves the ions. The drag force experienced by the ions depends on their collision cross sections (a function of size and shape), electrical charge, and mass. Multiply charged ions move through the buffer gas more effectively than singly charged ones since they experience a greater force due to the electric field. Ions with larger cross sections are retarded more easily by collisions with the buffer gas in the drift tube. The drag force resulting from collisions of ions with the buffer gas molecules acts against their acceleration imposed by the electric field. Thus an equilibrium state is quickly reached and the ions start moving with constant velocity (V_d) which is proportional to the applied electric field (E). The proportionality constant (K) is the gas phase mobility of an ion. The diffusion limited resolving power is dependent upon the length (L) of the drift cell, electric field (E), charge state of the analyte ions (Q), and the buffer gas temperature (T).

$$V_d = KE$$

Mobility is a function of the ion's interaction with the buffer gas, its mass and its electrical charge. Furthermore, the reduced mobility (K_0) depends on the gas temperature and the mass of the buffer gas molecules.

$$K_0 = \frac{L}{t_d E} \frac{P}{760} \frac{273.2}{T}$$

where L is the length of the drift cell, t_d is the corrected drift time, E is the electric field across the drift cell, P is the pressure of the drift cell, and T is the temperature of the buffer gas.

Agilent IM-QTOF System

The Agilent IM-QTOF system provides the following:

- Ion mobility resolving power of greater than 60
- Automated calculation of accurate collision cross sections (~ 1%) without the need for calibration standards
- High sensitivity for trace level compounds
- Sophisticated data browsing and mining tools

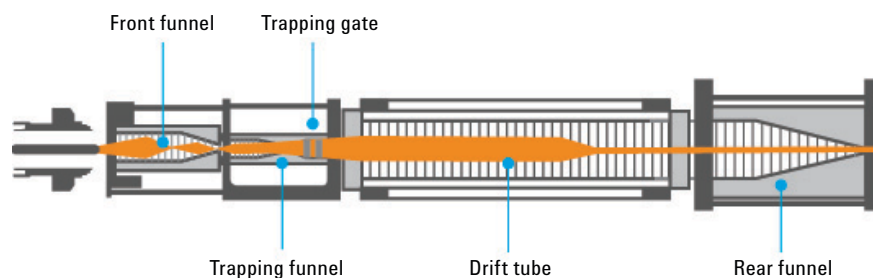


Figure 1. Schematic diagram of the ion mobility instrument. Ions generated in the source region are carried into the front ion funnel through a single bore capillary. The front ion funnel improves the sensitivity by efficiently transferring gas phase ions into the trapping funnel while pumping away excess gas and neutral molecules. The trapping funnel accumulates and releases ions into the drift tube. The drift cell is ~80 cm long and generally operated at 20 V/cm drift field. Ions exiting the drift tube enter the rear ion funnel that efficiently refocuses and transfers ions to the mass analyzer.

Operation Description

As shown in Figure 1, the Agilent ion mobility system consists of a front funnel, trapping funnel, trapping gate, drift tube, and a rear funnel that couples through a hexapole to the Q-TOF mass analyzer. The front funnel operates at high pressure where funnel DC and RF voltages propel ions toward the trapping funnel. The key function of the front ion funnel is to enrich the sample ions and remove excess gas.

The continuous ion beam from the electrospray process has to be converted into a pulsed ion beam prior to ion mobility separation. The trapping funnel operates by first storing and then releasing discrete packets of ions into the drift cell.

Ions are separated as they pass through the ion mobility cell based on their size and charge. Ions with larger collision cross sections undergo higher number of collisions with drift gas molecules compared to ions with smaller collision cross section. Therefore, larger ions travel through the drift cell slower than the smaller ions. Also, ions with higher charge states experience a higher electric force, and hence travel at a higher velocity, compared to ions with lower charge states. The drift cell is operated under low field limit conditions allowing the instrument to generate accurate structural information for compounds. Under the low electric field conditions the mobility is not dependent on the electric field but rather on the structure of the molecule and its interaction with the buffer gas.

Ions exiting the drift cell are refocused by the rear ion funnel before entering the hexapole ion guide.

Optimization of Performance - Drift Time Resolution

For ion mobility spectrometry, drift resolution depends on diffusional peak broadening, width of the initial ion packet, and space charge effects. The most important of these three factors is diffusional peak broadening. The diffusion limited resolving power is dependent upon the length (L) of the drift cell, electric field (E), charge state of the analyte ions and the buffer gas temperature. Longer drift tubes allow ions to drift for a longer period of time, which results in better ion separation and drift resolution.

$$R = \frac{t_d}{\Delta t} = \sqrt{\frac{LEQ}{16k_b T \ln 2}}$$

The Agilent ion mobility drift tube length is approximately 80 cm, and designed to optimize drift resolution and minimize signal loss. Use of a nitrogen buffer gas provides robust operation at higher drift tube voltages and provides drift resolutions of greater than 60 for small and large molecules.

Another factor contributing to higher drift resolution is the width of the initial ion packet. This instrument uses a double grid trapping funnel device to optimize for higher ion capacity and narrower ion packets. Setting appropriate ion gate pulse times will determine the number of ions contained in the ion packets which are subsequently injected into the ion mobility cell. The pulse times are typically in the range of 60 to 100 milliseconds.

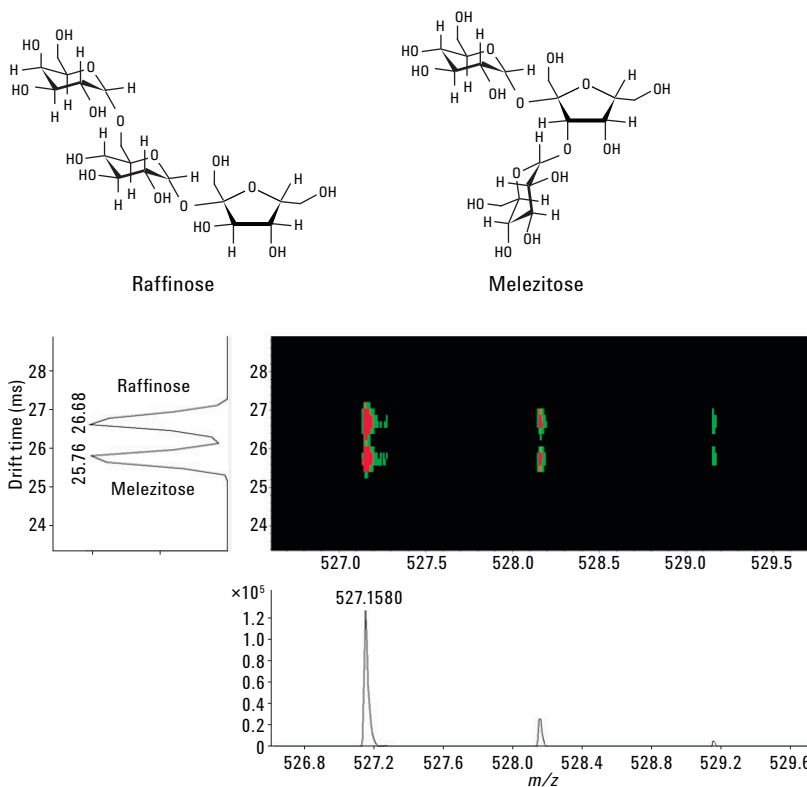


Figure 2. Separation of isobaric tri-saccharides using the IM-QTOF. A 1:1 mixture of melezitose and raffinose was infused using a syringe pump. These two carbohydrates can be baseline separated using the ion mobility drift cell and detected using the Q-TOF mass analyzer as sodium adducts. The ion mobility resolving power for this separation is 60.

Lastly, a tapered section at the exit region of the trapping funnel is designed to focus the ion packets into the drift cell to avoid ion losses and improve resolution and sensitivity.

The net result is a high abundance, well confined packet of ions entering the drift region resulting in high drift resolution and high sensitivity.

Optimization of Sensitivity

Uniform field ion mobility has existed for many years with numerous research built designs. These uniform field designs show very high ion losses (> 99.9%) in the ion gating region before the drift tube. Additionally, considerable number of ions can be lost at the exit of the drift tube because of the small exit aperture.

The emergence of modern electrodynamic ion funnel technology pioneered by the Richard Smith group at PNNL has enabled greater sensitivity gains for uniform field drift tube designs. The PNNL trapping ion funnel was the first device that traps ions at high pressure. System sensitivity performance is a function of ionization efficiency, efficient ion transfer into and out of the drift tube region and minimization of transmission losses between the ion source and mass spectrometer.

The Agilent IM-QTOF is shown schematically in Figure 3. The Agilent Jet Stream ionization source provides very high ionization efficiency, with a 5-fold sensitivity advantage compared with standard electrospray designs. The first stage ion funnel efficiently removes excess gas while concentrating the ion beam for the second stage trapping funnel.

At the exit of the drift tube, a rear funnel is employed to refocus the ion packets into a narrower beam before entering the optics of the mass spectrometer. The high efficiency ion trapping and rear funnel together result in a 1,000-fold sensitivity improvement over previous non-funnel designs.

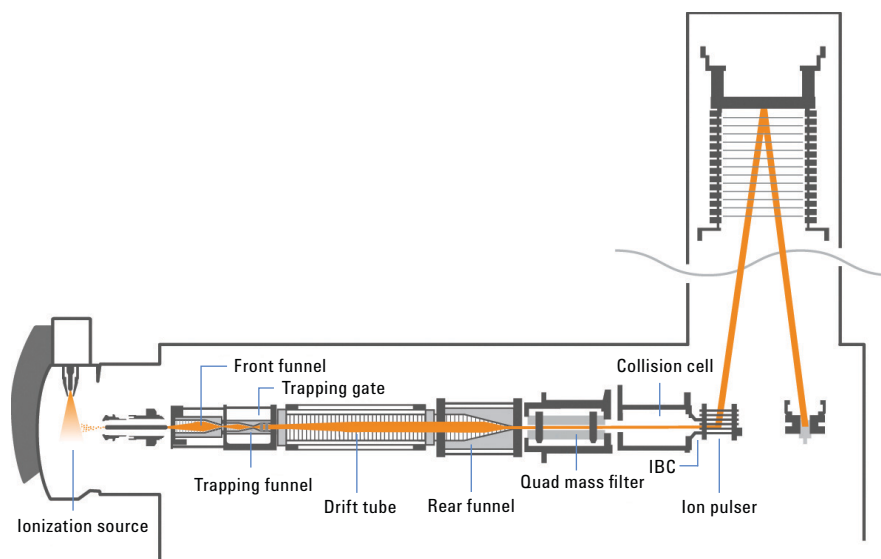


Figure 3. Schematic of the Agilent IM-QTOF instrument. The ion mobility spectrometer is coupled to a quadrupole time-of-flight mass spectrometer using a hexapole ion guide.

Development of Accurate Collision Cross Section Measurements

Collision cross section values are derived from ion mobility measurements. All of the first order equations governing ion mobility apply at low electric fields. Uniform field drift tube designs typically operate at low electric field resulting in very predictable and accurate mobility measurements.

Conventional uniform field drift tube ion mobility provides a direct method to calculate collision cross sections (Ω) using the Mason-Schamp equation given below:

$$\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_b T)^{1/2}} \left[\frac{1}{m_i} + \frac{1}{m_B} \right]^{1/2} \frac{t_d E}{L} \frac{760}{P} \frac{T}{273.2} \frac{1}{N}$$

where Ω is the rotationally averaged collision cross section, k_b is the Boltzman constant, T is the temperature of the buffer gas, m_i is the mass of analyte ion, m_B is the mass of buffer gas molecules, t_d is the corrected drift time, ze is the charge state of the analyte ion, E is the electric field, L is the length of the drift cell, P is the pressure in drift cell, and N is the number density in the drift cell. It is important to note that t_d can be determined from the total ion drift time in the IMS QTOF system. Once t_d values are calculated they can be used to directly generate CCS measurements.

The accuracy to which the collision cross section can be calculated is determined by the extent to which experimental parameters (pressure, temperature and electric field) are maintained during the mobility experiment. Any time the ion spends outside of the defined drift region produces "end effects," which cause loss of measurement accuracy. Measurements of CCS within 2% accuracy or less can be routinely achieved using uniform field drift tubes.

Table 4. Collision cross section data for a series of tetra alkyl ammonium (TAA) salts. A mixture of TAA salts were directly infused into the Agilent IM-MS instrument at seven different drift fields. The t_0 values for each salt was obtained by plotting drift time versus 1/voltage graphs. The corrected drift times were then used to calculate the CCS values.

| Analyte | Mass (Da) | CCS literature* (Å ²) | CCS Agilent IM-MS (Å ²) | % Deviation from literature |
|---------|-----------|-----------------------------------|-------------------------------------|-----------------------------|
| TAA-3 | 186.22 | 143.8 ± 0.1 | 146.1 ± 1.0 | 1.54 |
| TAA-4 | 242.28 | 166.0 ± 0.3 | 167.3 ± 0.9 | 0.77 |
| TAA-5 | 298.35 | 190 ± 0.1 | 190.3 ± 1.1 | 0.10 |
| TAA-6 | 354.41 | 214.0 ± 0.3 | 213.9 ± 0.6 | 0.04 |
| TAA-7 | 410.47 | 236.8 ± 0.2 | 237.7 ± 0.4 | 0.37 |
| TAA-8 | 466.54 | 258.0 ± 0.4 | 258.7 ± 0.4 | 0.14 |
| TAA-10 | 578.66 | – | 296.2 ± 0.4 | – |
| TAA-12 | 690.79 | – | 325.5 ± 0.5 | – |
| TAA-16 | 915.04 | – | 365.6 ± 0.7 | – |

*Campuzano, I., Bush, M. F., Robinson, C. V., Beaumont, C., Richardson, K., Kim, H., Kim, H. I. Anal Chem 2012, 84(2) 1026-33. Structural Characterization of Drug-like Compounds by Ion Mobility Mass Spectrometry.

Preservation of Structural Characteristics of Molecular Conformation

Collision cross section measurements are dependent on preserving molecular structure in the gas phase and studies have shown that structural conformation can be preserved with reduced ion heating. The Agilent ion mobility design imparts minimal ion heating since direct current (DC) is used to propel ions forward in the drift cell.

The Agilent IM-QTOF system also allows use of tunable trap voltage settings to accommodate preservation of structural forms for a wide range of labile to very stable compounds. Ion heating is minimized by having tunable RF voltages on the ion funnels that can be optimized for specific studies. Fragmentation along the ion path is minimized by optimizing electric field strengths and pressure in the drift cell and the Q-TOF interface.

Figure 4 shows IMS-TOF separation of different ubiquitin charge states by means of tunable trapping voltages to preserve the low energy structures.

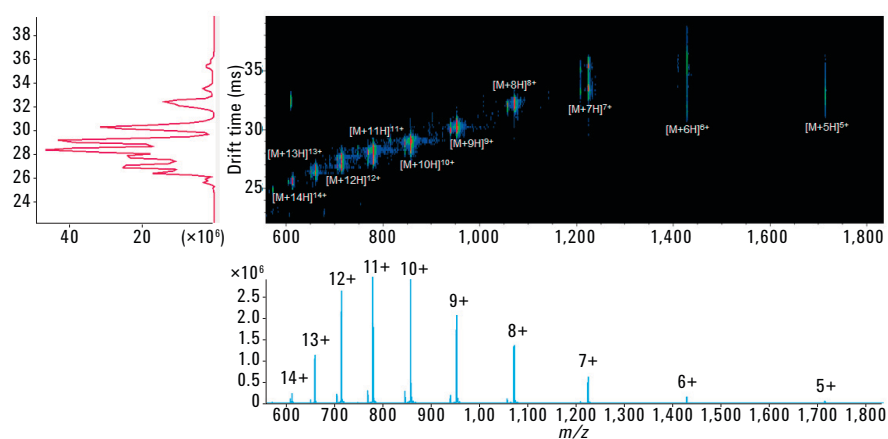


Figure 4. IM-QTOF separation of ubiquitin charge states. Ubiquitin was dissolved in 50:50 water:MeOH solution with 0.1% acetic acid and infused into IM-QTOF instrument. The 3D plot of drift time versus m/z shows the distribution of charge states in the drift and m/z space. The drift time distribution plot shown to the left side is generated by summing all the drift distributions for different charge states. Some charge states show multiple drift peaks corresponding to multiple conformers.

Enhanced Separation and Peak Capacity

Separation power is especially important for complex mixture analysis containing multiple compound types. For in depth analysis, chromatographic separation alone is not sufficient to allow characterization of several compounds that may elute within a single LC peak. Additionally, high resolution mass spectrometers may be limited by how fast they can perform MS scans and are unable to generate data for all compounds in a sample without efficient front end separation. Typically, data dependent MS/MS experiments often miss low abundance peaks.

Ion mobility separation speed (milliseconds) fits between the HPLC (seconds) time scale and TOF analyzer time scale (microseconds). Ion mobility provides the ability to separate trace level compounds that are not resolved chromatographically. Under traditional MS/MS conditions, a quadrupole is used to select precursor ions for fragmentation. In All Ions MS/MS experiments, no precursor ion selection is done and all ions are sent to the collision cell for fragmentation. The advantage of All Ions MS/MS over traditional MS/MS is that one can collect structural information on all compounds present. The use of All Ions MS/MS is made more powerful in combination with ion mobility since the drift time separation provides additional separation to help understand sample complexity and more easily associate fragments with precursors. The benefit of this is less ambiguity in identifying compounds when using All Ions MS/MS with the better detection limits for trace level compounds.

As an example, a recent study by Vanderbilt University using the Agilent IM-QTOF system showed an increase in the overall coverage of lipids by 5-fold. Similarly, in a recent proteomics study by Pacific Northwest National Laboratory, a 3-fold improvement in the number of peptides and proteins identified was achieved using IM-QTOF compared with LC/MS analysis alone, as illustrated in Figure 5.

A common metric for separation power is peak capacity, which can be defined as the maximum number of peaks that can fit in any multidimensional method. A high peak capacity can be achieved if the resolution of each method (LC, IMS, MS) is high and the difference in their separation mechanism (orthogonality) is significant. Peak capacity is a better indicator of separation power when compared to mass resolution alone. In an ideal situation, peak capacity of the multidimensional method is a multiplicative product of each dimension:

$$\text{Peak capacity} = \text{UHPLC resolving power} \times \text{IM resolving power} \times \text{MS resolving power} \times \text{fraction orthogonality}$$

A recent Vanderbilt University study has shown a 5-fold increase in peak capacity for singly charged compounds compared to previous LC/MS methods.

The Agilent IM-QTOF system achieves a very high peak capacity since it can simultaneously combine the resolving power of UHPLC, IM, and mass resolution without compromising sensitivity performance. As shown in Figure 6, a separation of near isobaric pesticides is achieved for mass differences of less than 0.2 mDa, which would require a mass resolving power of 2,000,000 to effectively resolve.

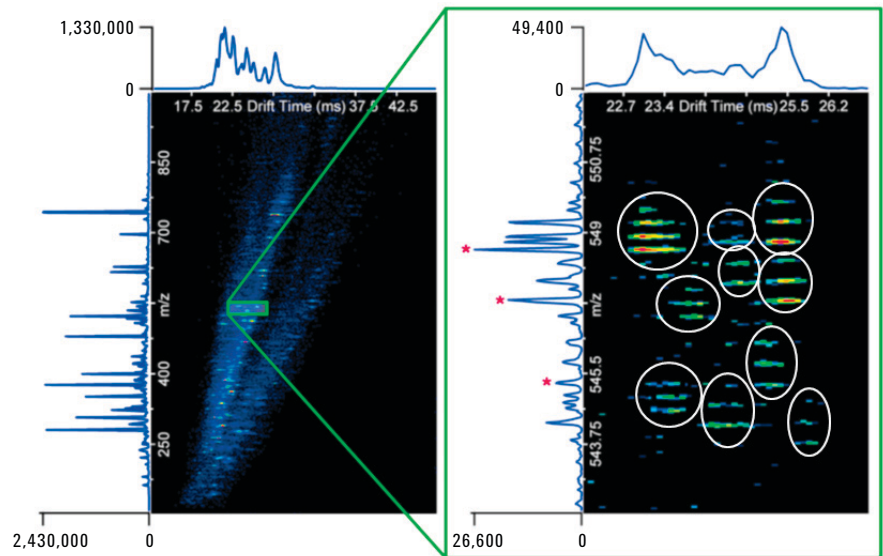
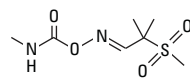


Figure 5. Increased selectivity obtained using the Agilent IM-QTOF instrument. The m/z versus drift time plot shows the separation of tryptic peptides derived from mouse blood plasma sample spiked with 20 reference peptides. The sample was subjected to 15 minutes LC separation before IM-QTOF analysis. The inset shows a zoomed in region of the 3D plot where 10 peptides were identified for the LC-IM-QTOF experiment. The same sample was run with a 100-minute LC gradient using LTO-FT-MS instrument that yielded only three identifications, as indicated by asterisks.

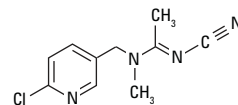
Aldicarb-sulfone ($C_7H_{14}N_2O_3S$)

$[M+Na]^+ = 245.056649$



Acetamidrid ($C_{10}H_{11}ClN_4$)

$[M+Na]^+ = 245.056445$



IMS drift separation

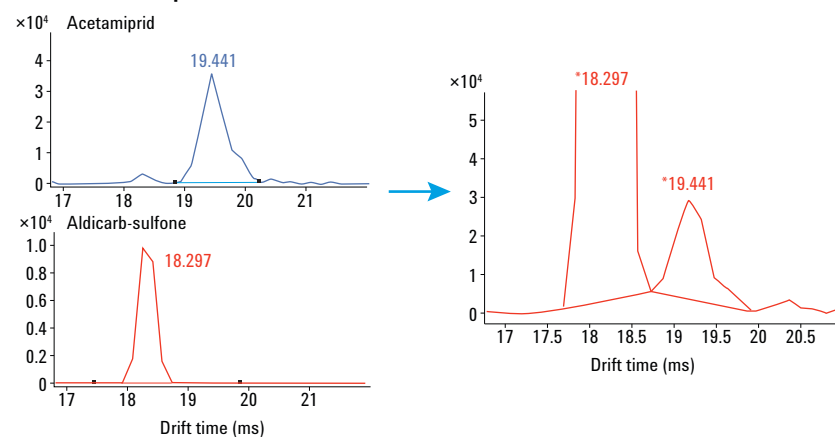


Figure 6. IM-QTOF separation of pesticides aldicarb-sulfone and acetamidrid, which had a 0.2 mDa mass difference. A mass resolution of about 2 million is required to separate these two compounds in an m/z domain. Due to the structural differences between these compounds, they can be easily separated in the drift dimension.

Beyond peak capacity - selectivity matters!

High resolving power or peak capacity is useful in situations where there are differences in mass or other physical properties. In situations where isobaric compounds are analyzed, mass resolution $> 1,000,000$ may be insufficient to separate and identify these isomers. The ability to determine CCS values can be used to confirm the identity of structural isomers as shown below in Figure 7 for permethylated oligosaccharides.

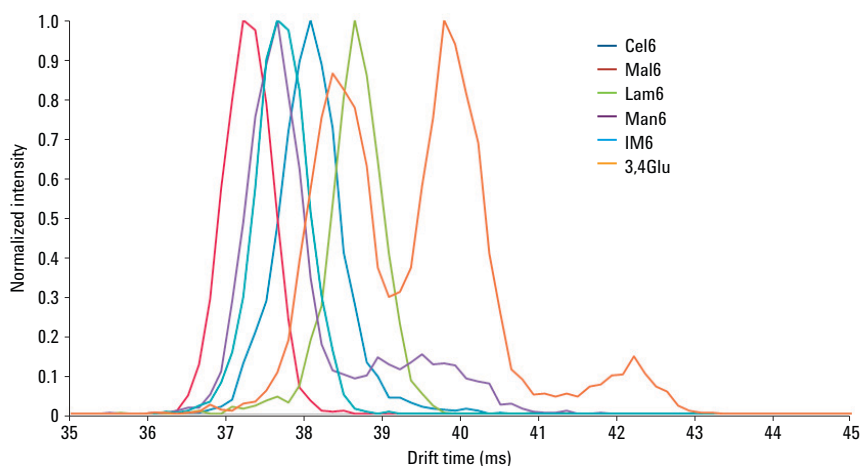


Figure 7. IM-QTOF separation of permethylated oligosaccharides. These oligosaccharide samples were infused separately and analyzed using IM-QTOF as sodium adducts. These isobaric hexoses show different drift distributions, indicating their structural differences. Ion mobility separation is a valuable technique that can be used to separate isobaric compounds with different structures.

Conclusions

The Agilent IM-QTOF LC/MS system is a major advance in the commercial development of analytical ion mobility-mass spectrometry. Optimized development of a uniform drift field mobility cell and interface to a high resolution Q-TOF instrument gives a significant gain in ion mobility performance. The use of ion funnel technology pioneered by Agilent for both triple quadrupole and Q-TOF instruments over the past three years has been incorporated into the new IM-QTOF system. This has resulted in combined ion mobility separation and mass resolution with high sensitivity.

Recent work with several collaborators has confirmed that the instrument delivers:

- Greater separation of lipids and glycopeptides
- More accurate collision cross section measurements enabling more confident characterization of structural conformations and isomeric compounds
- Greater numbers of trace level peptides in complex matrices
- Preservation of structural fidelity of metallo-proteins in liquid phase solutions

In order to maximize the analytical utility of this system, Agilent has also developed software tools for data visualization of ion mobility data. This software is designed to allow researchers to interrogate mobility/mass domain data and easily determine collisional cross section values with high precision and accuracy.

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Factors influencing drift resolution

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