Technical Overview

HRdm 2.0: Maximize Your IM Resolution Without Sacrificing Drift Range, Mass Range, or Data Acquisition Time

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Introduction

Ion mobility mass spectrometry (IM-MS) has gained widespread popularity in the past few years as researchers attempt to tease apart more and more complex samples and dig deeper to better understand biological and chemical processes. Drift tube IM-MS instruments serve as the standard for the determination of collision cross section (CCS) values[1], which is often an end goal of IM-MS measurements, but their drift resolution is limited by the length of their drift tube. In the race to achieve higher and higher mobility resolution, many scientists have instead turned to IM-MS systems with cyclic paths or those that use trapped ions. Although IM-MS instruments using trapped ions or cyclic paths can provide higher native resolutions than drift tube IM-MS instruments, the downside is that independent determination of CCS values is not possible, and the time required to achieve higher resolving power is significantly extended, limiting their utility when combined with chromatography. Because these instruments cannot provide both fast sampling and high resolution across a broad drift range, they are not ideal for untargeted experiments.

Since the launch of the Agilent 6560 IM-MS instrument in 2014, Agilent has been working with numerous visionaries in the field of ion mobility. In 2018, Agilent began a collaboration with RKResearch to investigate how to achieve high resolving power ion mobility separations without sacrificing the speed of the analytical measurement. The first commercial result came in 2020 with the release of HRdm 1.0.[2] Expanding upon that work, in 2021 Agilent announced HRdm 2.0 enabling researchers to obtain drift resolutions as high as 250 on an LC time scale, while continuing to obtain accurate, precise, and reproducible CCS values. HRdm 2.0, combines demultiplexing and deconvolution into an easy-to-use tool yielding previously unattainable performance on a commercial drift tube instrument.

Simply described, drift tube IM-MS instruments operate by pulsing a discrete packet of ions into a drift tube where they are separated based on the ion's charge and three-dimensional size and shape. When coupled to a continuous source of ions such as an electrospray source, IM-MS instruments optimize their sensitivity by varying the accumulation time for the trapping gate. Typical trap accumulation times are often tens of milliseconds, while higher abundance analytes or samples with a high matrix optimize with shorter trapping times.[3]

Figure 1 shows a timing diagram. In Figure 1A, ions are trapped for 20 ms, then quickly released into the drift cell. For the next 40 ms, however, no ions are trapped or pulsed into the drift cell, resulting in only one third of the ions being analyzed. This mode of operation is referred to as single pulse mode.

One way of increasing the duty cycle, and thus the sensitivity, is to use multiplexing. In multiplexing mode, multiple packets of ions are pulsed into the drift cell during the IM separation. With multiplexing, instead of analyzing a single pulse of ions, waiting for all the ions to traverse the drift cell, and then applying a second pulse, multiple sequential pulses of ions are introduced into the drift tube during each frame as illustrated in Figure 1B. Using multiplexing, one can improve the sensitivity over single pulse systems because of the increased instrument duty cycle. In Figure 1B, the duty cycle has increased to 50%.

Since its launch, the Agilent 6560 IM-MS instrument has supported both 3-bit and 4-bit multiplexing capabilities providing sensitivity and dynamic range gains. HRdm 2.0 expands upon these gains and adds an increase in drift resolution as well.

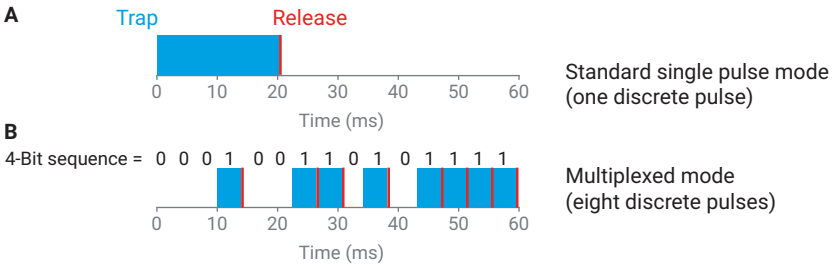

Figure 1. Timing diagram for ion mobility instruments. (A) shows the timing diagram for an instrument in single pulse mode while (B) illustrates an instrument operating in multiplexed mode.

Nominally, multiplexing itself has no impact on the drift resolution of the instrument. The novel approach using in HRdm 2.0, high-resolution demultiplexing, came through the realization that peak deconvolution could be simultaneously applied with demultiplexing. By thoroughly investigating and modeling the properties of the instrument, Agilent was able to determine what the exact drift peak shape under various operating conditions. HRdm 2.0 then applies this drift peak shape in the deconvolution process. The high precision of the peak shape model comes from the fact that the ion mobility peaks are Gaussian and governed by diffusion, without the nonideal contributions of partitioning between mobile and stationary phases associated with deconvolution technics applied to chromatographic separations. By applying deconvolution algorithms to statistically rich multiplexed data, HRdm 2.0 can effectively increase the drift resolution from 50 up to as high as 250. To better understand this software, multiplexing, demultiplexing, and deconvolution processes are explored in more detail below.

Multiplexing

By multiplexing or pulsing multiple packets of ions into the drift cell during any given IM measurement, the Agilent 6560 IM-MS instrument with HRdm 2.0 achieves a duty cycle of 50% while also keeping the maximum trapping time short. Short trapping times reduce space charge effects and the onset of detection saturation, thereby increasing dynamic range.

The combination of ion accumulation and pulsing based on a pseudo-random sequence creates what is referred to as an extended Hadamard transform sequence.[4] This is a pseudo-random binary sequence of 0s and 1s. Zero corresponds to off and no ions are pulsed down the drift cell. One corresponds to on, and a packet of ions is pulsed down the drift cell. With the recent release of MassHunter Acquisition SW 11.0, the 6560 IM-MS can make use of either a 3-bit, 4-bit, or 5-bit multiplexing sequences. 3-bit multiplexing consists of four on pulses and three off pulses where ions are released to the drift tube. For 4-bit multiplexing, there are eight on pulses and seven off pulses whereas in 5-bit multiplexing, there are 16 on and 15 off pulses. The example shown in Figure 1B uses 4-bit multiplexing.

Figures 2A through 2D provide more detail on how multiplexing functions in the 6560 IM-MS instrument. Figure 2A illustrates what happens when the gate opens, and ions are pulsed down the drift tube. On the left, is the binary sequence with a box around the first 1 in the sequence. This 1 means that the trapping gate releases ions into the drift cell. As the ions traverse the drift cell, they separate based on their size and shape. In Figure 2B, there is a box around the 0 in the sequence, so the gate remains closed, and no further ions are allowed into the drift cell. In this figure, ions from the first pulse have reached the TOF detector and are measured.

The magnifying glass gives a perspective on how the TOF analyzer samples ions arriving from the drift tube. In this example, the instrument is operated in 1,700 *m/z* mode with 8,000 TOF transients per second being recorded.

In Figure 2C, the gate opens again, pulsing additional ions down the drift cell and into the TOF flight tube where they are detected. Figure 2D shows more clearly the effect of the pseudo-random nature of the gating sequence on the spacing of the data points. The process described in Figure 2 repeats continually and is what is referred to as "multiplexing" in IM-MS.

Some definitions

TOF transient: A single time-of-flight spectrum acquired at very high rates

IM transient: The collection of TOF transients sampling the drift separation creating a 2D spectrum

Frame: The sum of several IM transients which increase the signal level prior to data storage

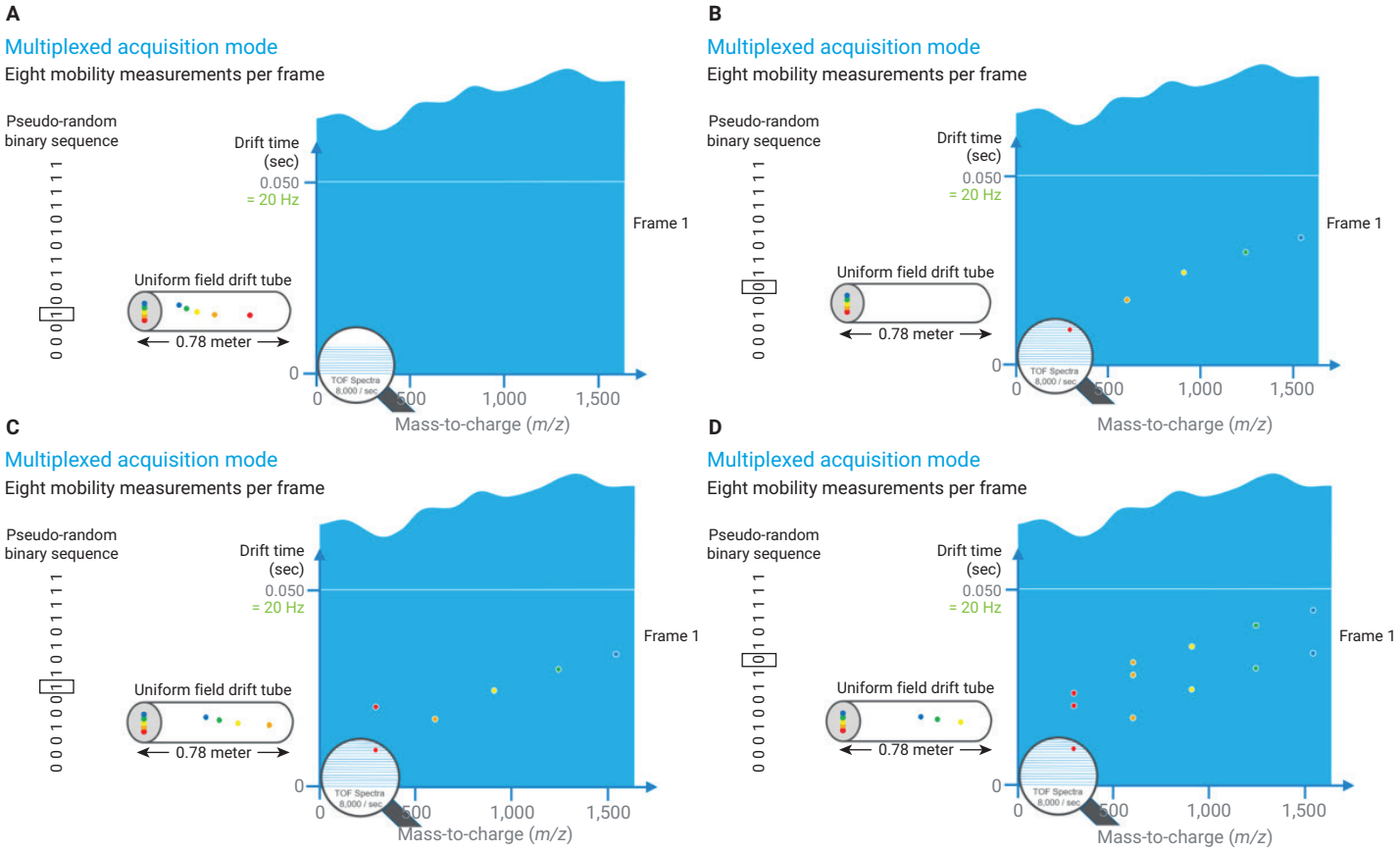

Figure 2. Illustration of multiplexing. In (A), ions are gated into the drift tube and begin to separate based on collision cross section. When there is a 0 in the pseudo-random sequence that controls the gating, no ions are allowed into the drift cell but ions from the first pulse are detected by the TOF (B). The next groups of ions are gated into the drift cell (C) and (D) and detected in the TOF where to spacing between sets of data points shows the effect of the on/off gating sequence.

Demultiplexing and deconvolution

Demultiplexing is the process by which the multiplexed data are converted back into a spectrum with each species appearing at a specific drift time.

Figure 3A below shows the multiplexed signal for PE(28:2) by plotting the response of the $[M+H]^+$ ion at *m/z* 632.4286 over 60 msec, which is equivalent to one frame (see the definitions section). Clearly visible are eight peaks spaced according the the pseudo-random sequence used during the multiplexed data acquistion. Also apparent is the indication that two species are present, but with insuffience resolving power to separate them.

In LC and GC, deconvolution algorithms based on peak modeling are commonly applied to help better separate coeluting compounds. This concept is applicable to the drift separation of the IM-MS instrument. Due to extensive mathematical modeling of the 6560 instrument and after running many standards at a wide variety of conditions, it is possible to predict the exact Gaussian peak shape expected from the instrument. This is shown in Figure 3B.

When applying standard demultiplexing techniques, the timing sequences for gating ions into the drift cell are overlayed onto the raw data. Where coincidence occurs, the resulting data are displayed as the output spectrum.

HRdm 2.0 does not simply correlate the pseudo-random sequence to the raw data. Instead, HRdm 2.0 overlays the predicted drift peak profile for each ion (the red peaks shown in Figure 3C) with the raw multiplexed data (Shown in black in Figure 3C). It then uses advanced maximum likelihood deconvolution algorithms to capture the coincidence of the modeled peak profile and the raw data, yielding, in this case, the two peaks now well resolved (Figure 3D, blue plots). As the total signal is preserved, the reduction in peak width results in an increase in peak intensity, giving an additional increase in signal-to-noise.

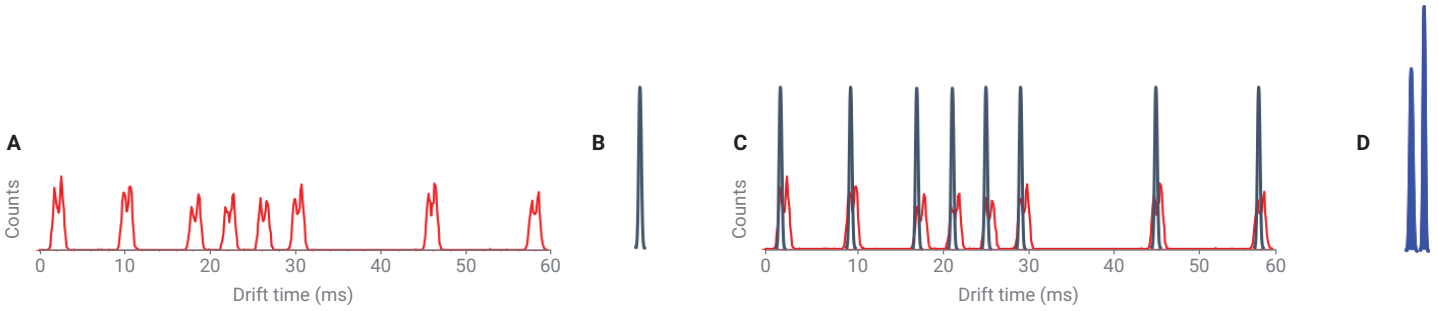

Figure 3. Illustration of demutiplexing and deconvolution process. (A) is the raw data prior to processing. The expected peak shape based on extensive system modeling is shown in (B). In (C) the theoretical peak is overlaid on the experimental data with the resulting deconvoluted and demultiplexed data shown in (D).

Figure 4A shows four leucine isomer standards infused separately and processed using the previous HRdm 1.0 release and standard TOF transient sampling rates. One can easily see the stair-step shape of the peaks, showing how the drift separation is under-sample by the TOF analyzer.

To increase the TOF sampling rate, a creative approach was used. By examing the spectral signals of adjacent TOF transients, a user-specified number of new transients was numerically inserted in between acquired transients. The ion intensities in the new transients are interpolated from the original.

Figure 4B displays the same data as in Figure 4A after processing the data through the HRdm 2.0 process. The first step of this process is accomplished with the PNNL PreProcessor.[5] This freely available tool performs the TOF transient interpolation as well as the first pass demultiplexing. In Figure 4B, the efffective TOF sampling rate was increased to 24,000 by 3-fold TOF transient interpolation. One can now see smoother Gaussian peaks resulting from the insertion of the TOF transients.

Figure 4C shows the power of HRdm 2.0. In this figure, the same four leucine isomers were analyzed but this time, they were mixed together prior to infusion. One can see nice separation of the isomer mixture illustrating the increased resolving power achievable, in this case of approximately 200.

Because high resolution demultiplexing is applied as a postacquisition method, there is no change to the basic data acquisition method, other than to ensure that the data was acquired in multiplexed mode. When run on commonly available high-performance PCs, HRdm 2.0 can process thousands of peaks in LC/MS runs across both the mass and drift dimensions forming the bases for achieving high resolution results in untargeted or targeted experiments.

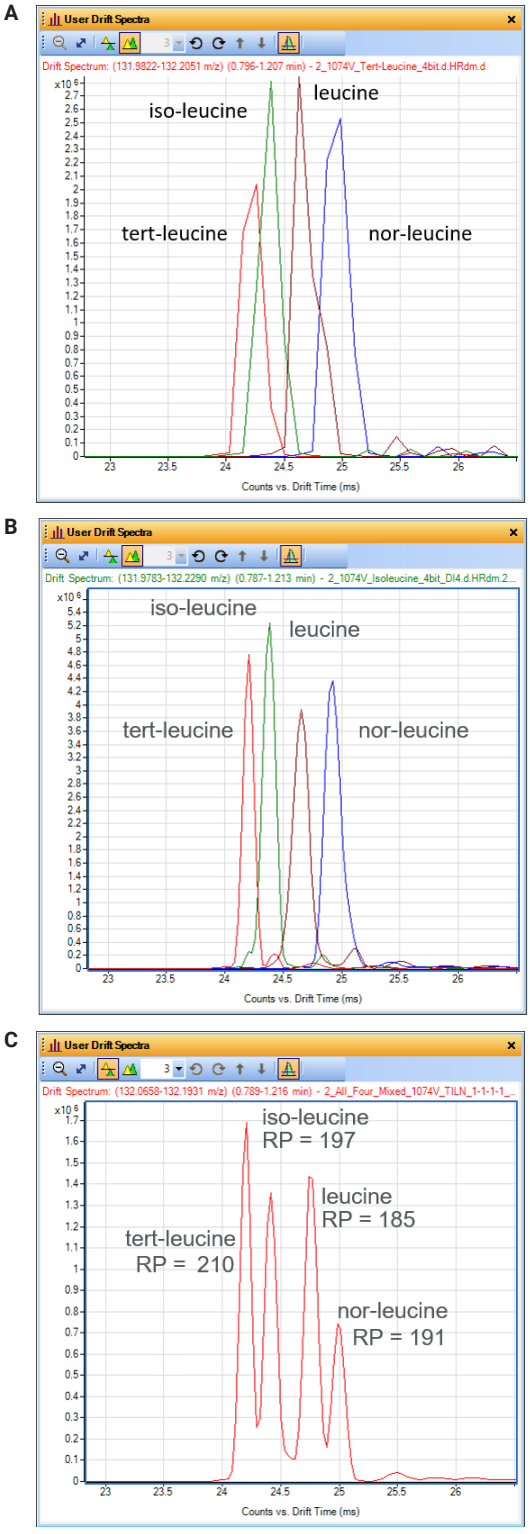

Figure 4. Four isomeric lipid standards with very similar drift times were infused (A) without using HRdm 2.0 functionality and (B) when the HRdm 2.0 functionality is used. The power of HRdm 2.0 is shown in (C) where those same four lipid isomers are mixed, infused simultaneously, and can be detected, resolved, and identified with resolving powers of up to 200.

Conclusion

The Agilent 6560 Ion Mobility LC/Q-TOF instrument with HRdm 2.0 is unique among ion mobility instrumentation. This very powerful instrument can achieve resolving powers up to 250 while providing both fast sampling and full spectrum ion mobility data across broad mass ranges and drift times. This truly is the go-to instrument for untargeted, fast, UHPLC based workflows. When combined with the powerful software suite including IMMS Browser, Mass Profiler, compound libraries containing molecular weight and ion drift times, and third-party software such as Skyline, one needs to look no further than the Agilent 6560 IM-MS with HRdm 2.0 for their next discovery mass spectrometer.

References

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