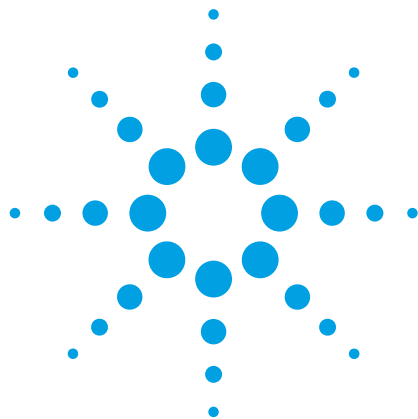
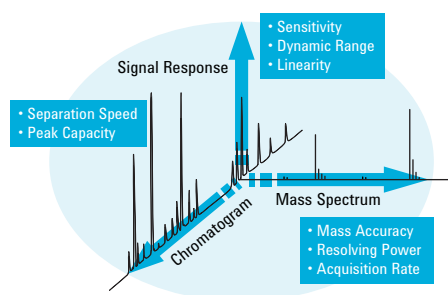




Matching UHPLC Resolution and Speed with Agilent Ultra High Definition Q-TOF Technology

Superior Results for Complex Sample Analyses

Technical Overview



Ultra High Definition: Optimizing all analytical dimensions

In fields such as metabolomics, proteomics, and food safety, a combination of sample complexity and the need to increase analytical throughput has many labs adopting ultra high performance liquid chromatography (UHPLC). As productivity demands continue to escalate, UHPLC becomes increasingly attractive because the technique enables better compound separations, which helps to reduce ion suppression; and permits shorter LC analysis times and increases sample throughput.

With high-resolution UHPLC separations, peak widths can be as small as 0.5 sec, which places new demands for mass scan speed on mass spectrometers. Mass spectrometry (MS) systems that fully exploit UHPLC must be able to acquire spectra rapidly without loss of resolving power, mass accuracy, sensitivity, dynamic range, or isotopic fidelity. Unlike other Q-TOF and Orbitrap systems, Agilent's Accurate Mass Q-TOF instruments deliver excellent performance in all of these dimensions simultaneously, routinely, and without compromise.

Ultra High Definition means you extract the greatest amount of information about every sample—in the shortest possible time. Ultra High Definition performance comes from the combined analytical power of:

- High resolution UHPLC separations that reveal more sample components
- Exceptional accurate mass MS that keeps pace with the sub-second peak widths of fast UHPLC analyses, with *no compromise in performance*
- Outstanding sensitivity and dynamic range for detection of trace level compounds
- Data mining tools that extract the maximum information from accurate mass MS and MS/MS analyses

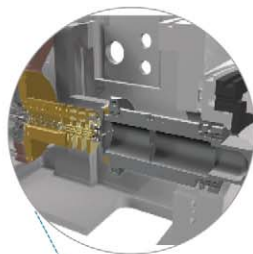


Agilent Technologies

Dual-stage ion mirror improves second order time focusing for **high mass resolution**



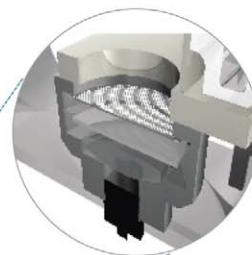
Ion Beam Compression (IBC) Technology (patent pending) compresses and cools the ion beam up to 10-fold, resulting in fewer ion losses and more precise mass assignment. **Mass resolution and mass accuracy are improved up to 200 percent.**



Ion source innovations such as proprietary orthogonal spray **maximize ion generation and reduce noise, while maintaining excellent accurate mass** with automated introduction of an internal reference mass.

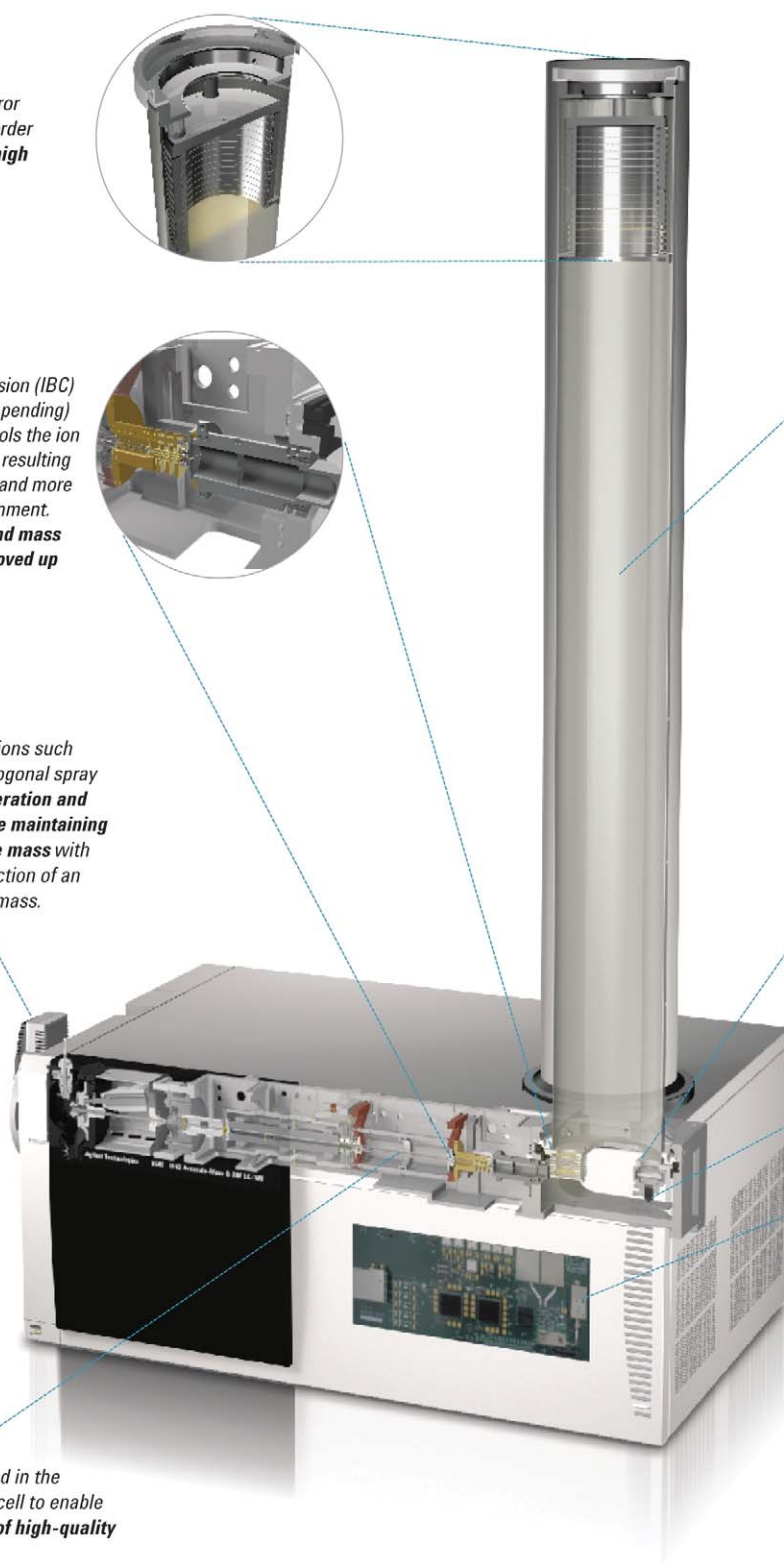
Proprietary INVAR flight tube sealed in a vacuum-insulated shell eliminates thermal mass drift due to temperature changes to **maintain excellent mass accuracy, 24/7**. Added length **improves mass resolution**.

Unlike time-to-digital (TDC) detectors which record single ion events, analog-to-digital (ADC) detection records multiple ion events, allowing **very accurate mass assignments over a wide mass range and dynamic range of concentrations**.



4GHz ADC electronics enable a high sampling rate (32 Gbit/s) which improves the resolution, mass accuracy, and sensitivity for low-abundance samples. Dual gain amplifiers simultaneously process detector signals through both low-gain and high-gain channels, **extending the dynamic range to 10⁵**.

Ions are accelerated in the hexapole collision cell to enable **faster generation of high-quality MS/MS spectra**.



Ultra High Definition Q-TOF technology in the Agilent 6500 Series Accurate-Mass Q-TOF LC/MS System brings together complementary technologies and proprietary engineering innovations to achieve Q-TOF performance that measurably surpasses any other Q-TOF system. Many of the same technologies are available on the Agilent 6200 Series Accurate-Mass TOF LC/MS Systems.

Agilent's Accurate-Mass Q-TOF instruments are the only commercially available benchtop Q-TOF systems that deliver uncompromising data quality at the speeds needed to keep pace with UHPLC. These Q-TOF systems are the culmination of years of relentless innovation. They incorporate Ion Beam Compression (IBC) and Enhanced Mirror Technology (EMT)—exciting new technologies that deliver even better mass accuracy and resolution, for more confidence in results. And the Accurate-Mass Q-TOF systems are available with Agilent Jet Stream technology, which increases sensitivity by up to 10-fold.

Agilent's 6500 Series Accurate-Mass Q-TOF LC/MS Systems uniquely deliver the speed and certainty of Ultra High Definition Q-TOF performance:

- **Wide dynamic range** up to five orders of magnitude
- **Exceptional, high-femtogram level sensitivity** to find low abundance components in complex mixtures
- **Excellent mass accuracy** – to 250 ppb for MS and < 700 ppb for MS/MS spectra
- **Mass resolving power** of 40,000 to enhance identification of nearly isobaric compounds
- **Fast data acquisition** of up to 20 MS spectra per second and 10 MS/MS spectra per second, to identify and accurately quantitate very narrow chromatographic peaks from ultra-fast separations
- Near universal detection with a **broad mass range** to analyze a wide range of molecular species
- **Powerful software tools to process complex data** and produce statistically meaningful answers

Unsurpassed dynamic range and sensitivity for global sample characterization

The goal of many analyses is global characterization: to identify every important compound present in a complex sample. While this is an impossible task, Agilent Ultra High Definition Q-TOF technology allows you to come remarkably close. Coupled with fast, high-resolution chromatography, Agilent's Accurate-Mass Q-TOF systems can routinely screen complex samples for the presence of target compounds or low-level contaminants or impurities. With more thorough fractionation, they can perform global differential analysis of proteins or metabolites.

In each of these applications, the concentrations of sample components vary by many orders of magnitude, and the compounds present at the lowest levels are often of greatest interest. Ultra High Definition Q-TOF technology delivers the unique combination

The Agilent 6200 Series Accurate-Mass TOF LC/MS Systems share many of the innovations of the Agilent 6500 Series Accurate-Mass Q-TOF LC/MS Systems. The TOF systems currently do not contain the latest UHD technology (Ion Beam Compression, Enhanced Mirror Technology, and the Fast Bipolar Detector), but they may incorporate this technology in the future.

of wide dynamic range and high-femtogram level sensitivity, making it possible to perform these applications quickly and easily (**Figures 1 and 2**). Compound-centric data reduction makes post-acquisition data analysis effective and efficient.

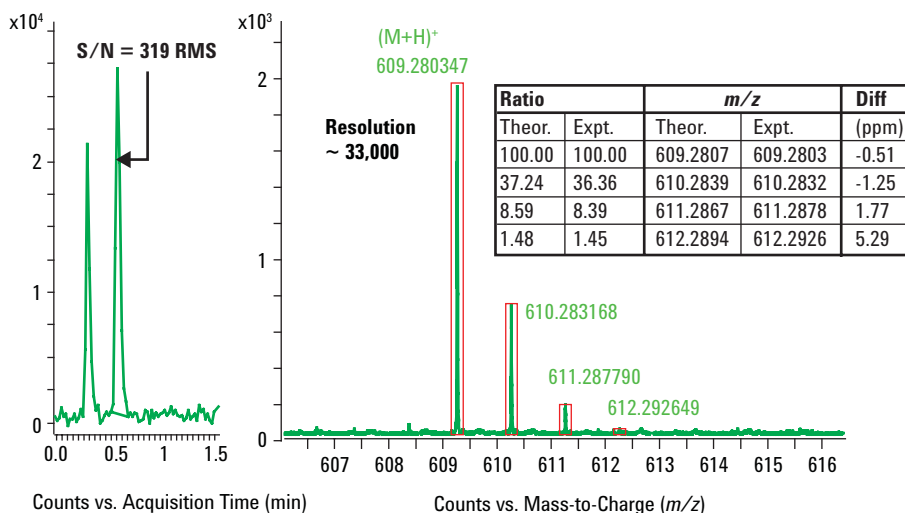


Figure 1. The Agilent 6540 Accurate-Mass Q-TOF shows excellent sensitivity, mass accuracy, isotopic fidelity, and resolution for 1 pg reserpine. This analysis shows typical performance in full-scan MS mode for this low level concentration. UHD Q-TOF technology produces excellent results in all dimensions, without compromise.

High resolution and mass accuracy for high confidence compound characterization

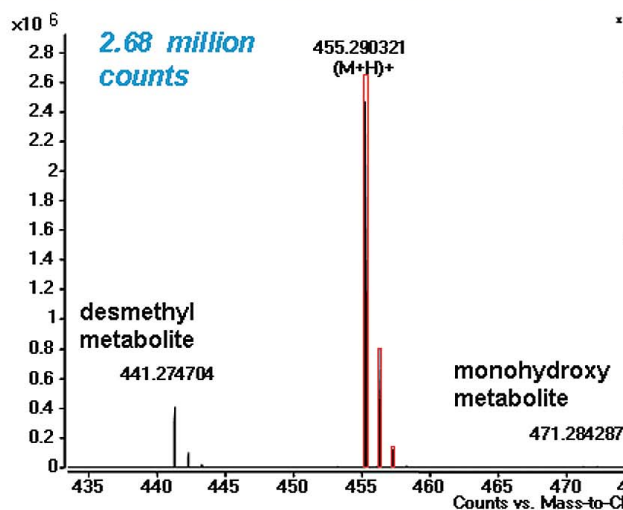
Agilent Ultra High Definition Q-TOF technology delivers data of exemplary quality, with high resolution and exceptional, stable mass accuracy. See **Figures 3** and **4** for sample data. Routine 250 ppb mass accuracy allows you to quickly narrow search results to fewer compounds, and accurate MS/MS information enables precise structural assignment and increases confidence in results.

- **Rapidly confirm intact protein structure** – Determine glycoforms and identify modifications such as oxidation and deamidation. More extensive characterization can easily be done with digestion and analysis by HPLC-Chip/MS.
- **Accurate protein identification** – Accurate MS and MS/MS data reduce false positive identifications in protein database searches.
- **Analysis of unknowns** – Detect very low level contaminants and identify their structures. This is especially important for food and water safety testing.
- **Screening** – Excellent MS and MS/MS mass accuracy enables confident, simultaneous screening for hundreds of compounds using confirmatory MS/MS data.
- **Accurate peptide quantitation** – Fast acquisition of accurate-mass MS/MS spectra gives excellent quantitation of iTRAQ reporter ions. Superior resolution separates reporter ions signals from matrix background, for better quantitation than on Orbitrap systems.

Agilent 6540 Accurate-Mass Q-TOF Five decades of response in a single scan

verapamil

Best	Formula (M)	Ion Formula	Score
☒	C ₂₇ H ₃₈ N ₂ O ₄	C ₂₇ H ₃₉ N ₂ O ₄	99.43
Calc m/z	Diff (ppm)	Mass Match	Abund Match
455.290434	0.25	99.94	98.27
			Spacing Match
			99.83



dihydroxy metabolite of verapamil

Best	Formula (M)	Ion Formula	Score
☒	C ₂₇ H ₃₈ N ₂ O ₆	C ₂₇ H ₃₉ N ₂ O ₆	80.18
Calc m/z	Diff (ppm)	Mass Match	Abund Match
487.280263	1.03	98.88	84.39
			Spacing Match
			37.71

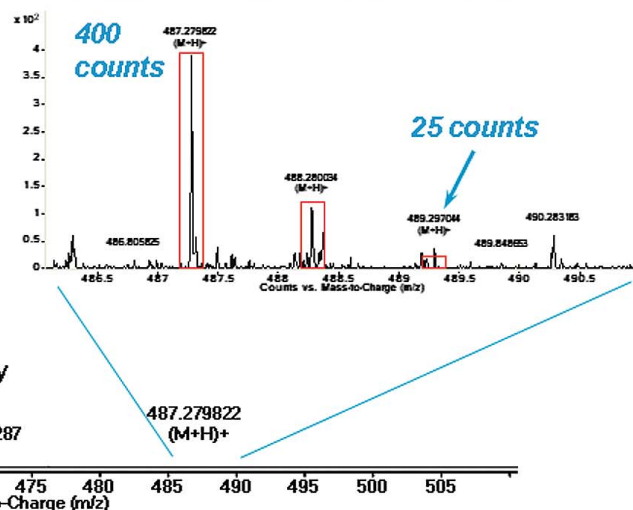


Figure 2. Agilent Ultra High Definition Q-TOF technology has the combined dynamic range, sensitivity, and mass resolution to detect and accurately assign mass in this MS-only analysis of trace metabolites that coelute with the parent drug. This figure shows a single spectrum, taken in 330 ms, of a sample from an S9 liver microsomal incubation of verapamil. The mass peak of verapamil [M+H]⁺ has an abundance of 2.68 million counts, while that of a minor peak in the spectrum is only 25 counts, demonstrating a dynamic range of more than 100,000. The Agilent molecular formula generation (MFG) software provides the correct result for the parent drug, with a mass error of 0.25 ppm and a superb match score. By zooming in on the spectrum, one can see that minor metabolites are present. Buried close to the baseline is the signal from a dihydroxy metabolite. Selection of m/z 487.2 and running MFG gives a formula for the dihydroxy metabolite with a mass error of 1.03 ppm. Significantly, the A+2 isotope is present and recognized by MFG with an abundance of around 25 counts. This example clearly shows the power of the Agilent system for detection and identification of trace components.

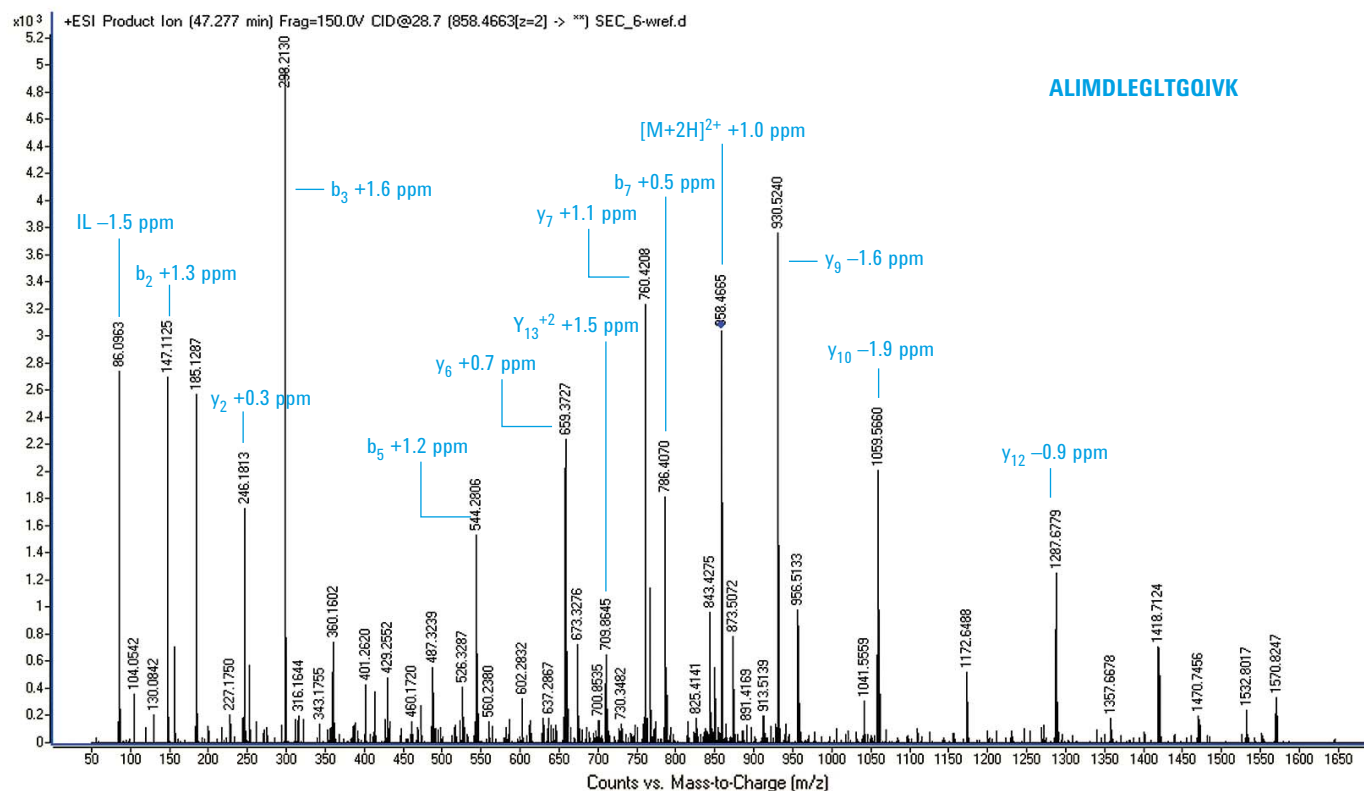


Figure 3. Accurate mass MS/MS data significantly reduce the chance of obtaining false positive results. Note the superb mass accuracy (< 2 ppm) in this MS/MS spectrum of a peptide, acquired on the Agilent 6538 Accurate-Mass Q-TOF LC/MS System. The peptide is from a size exclusion chromatography (SEC) fraction of a protein digest from *Schizosaccharomyces pombe*.

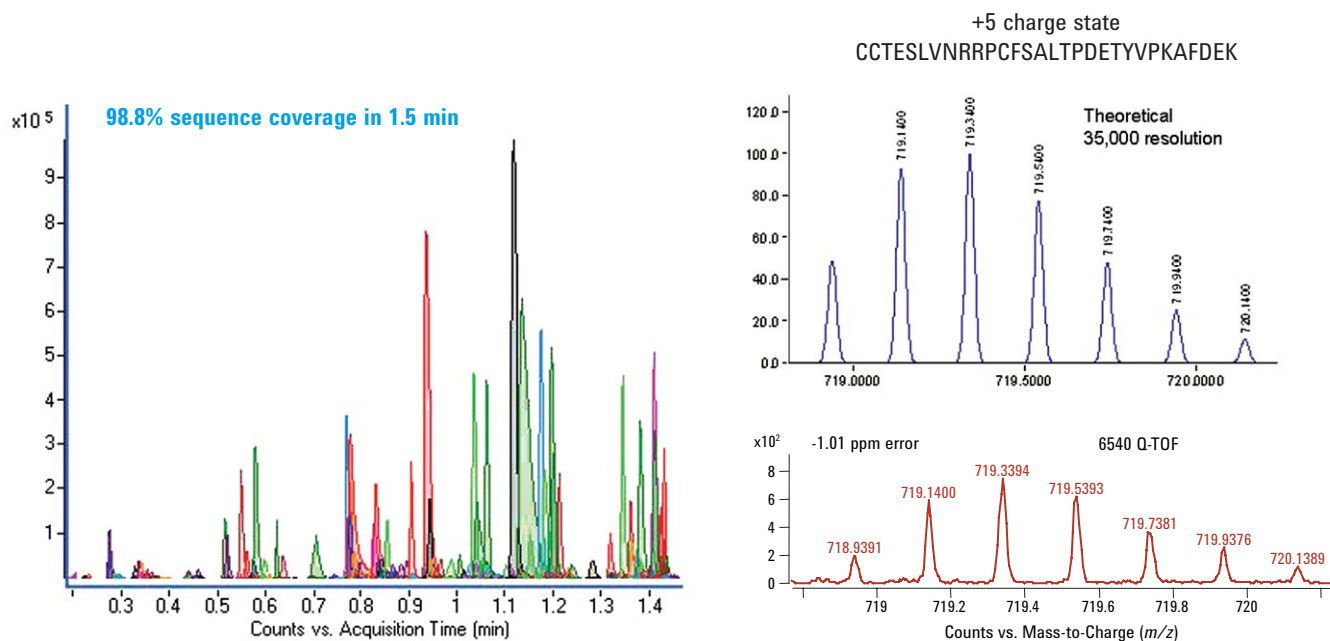


Figure 4. Rapid peptide mapping gives 98.8% sequence coverage in 1.5 min, using the Agilent 1290 Infinity LC System combined with the Agilent 6540 Q-TOF LC/MS System. In this ultrafast UHPLC analysis, the chromatographic peak widths (half-height) are only 0.3 to 0.8 seconds. To keep pace with rapid peak elution, the Agilent 6540 Q-TOF performs MS acquisition (m/z 300-3000) at 10 spectra/sec in high resolution mode at a resolution of 35,000 and mass accuracy of 1 ppm.

Powerful software takes full advantage of Ultra High Definition Q-TOF data

Q-TOF and TOF instruments produce large amounts of raw data. In this context, an ultra high definition answer is the result of a process that starts with billions of raw data points acquired from complex samples and ends in a clear answer about the composition of those samples—a list of compound identities.

Agilent's advanced software tools automate qualitative and quantitative analysis of the most complex TOF and Q-TOF LC/MS data:

- The Molecular Feature Extraction (MFE) algorithm saves hours of analysis time by automatically locating all sample components down to the lowest-level abundance and extracting all relevant spectral and chromatographic information (**Figure 5**).
- Molecular formula generation (MFG) provides high-confidence identification of unknowns, using multiple dimensions of information to generate and score lists of the most likely molecular formulae.
- Additional tools include accurate mass/retention time (AMRT) database search, MS/MS library search, and deconvolution or charge state determination.
- MassHunter Mass Profiler software finds variations between sample groups, while MassHunter Mass Profiler Professional software performs sophisticated differential analysis and profiling of complex sample sets.
- MassHunter Metabolite ID software identifies drug metabolites.
- MassHunter Personal Databases include more than 23,000 endogenous metabolites, lipids and related compounds; 1,600 pesticides; and 6,700 compounds of interest in forensics/toxicology.
- Spectrum Mill software enables protein database search and rapid results review.

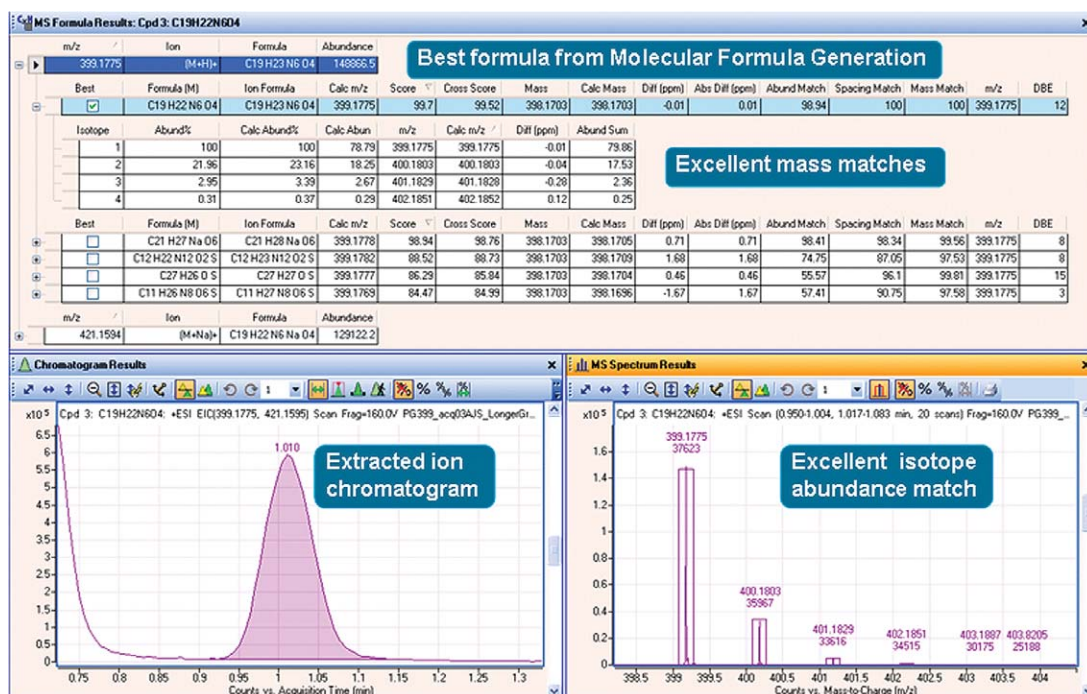


Figure 5. Molecular feature extraction and molecular formula generation capabilities of the MassHunter software save hours of analysis time by automatically locating sample components and extracting spectral and chromatographic information, and then using this information to suggest molecular formula.

Fundamentals of Ultra High Definition Q-TOF technology

Agilent's history of innovation over the last 40 years includes the development and introduction of many truly fundamental advances in mass spectrometry. These advances have led to many patented inventions that have been engineered into Agilent's Q-TOF systems (**Table 1**). The Agilent 6500 Series Accurate-Mass Q-TOF LC/MS Systems achieve a completely new level of LC/MS performance—Ultra High Definition Q-TOF performance—that can answer the most difficult and complex analytical questions.

More ions generated and transmitted, for remarkable sensitivity

The number of ions generated and introduced into the mass spectrometer is determined in the ion source (**A**)*. The ion source is also the greatest source of solvent droplets and mobile-phase adducts that cause chemical background. As a result, the ion source has an enormous impact on sensitivity. Agilent pioneered and patented several innovations that maximize ion source efficiency, including orthogonal spray, heated nebulizer gas, and a multimode source that can perform ESI and APCI simultaneously.

More recent innovations, Agilent Jet Stream thermal-gradient focusing inlet and HPLC-Chip/MS, dramatically improve ionization efficiency to deliver remarkable sensitivity.

Table 1. Patented innovations that enable Ultra High Definition Q-TOF performance

Dimension of Ultra High Definition Q-TOF performance	Innovation	Patents
Femtogram-level sensitivity	Agilent Jet Stream thermal-gradient focusing technology	6
	Nebulizers for superior ion formation and thus greater sensitivity	3
	Dynamic ion optics for increased ion transmission	1
	Agilent Ion Beam Compression	1
Five decades of in-spectrum dynamic range and high resolving power	Signal processing hardware and software, and 4 GHz analog-to-digital (ADC) spectral acquisition electronics with dual-gain signal amplifiers	2
High resolving power	Agilent Ion Beam Compression	1
	Agilent Enhanced Mirror Technology	1
Thermal stability	INVAR flight tube and thermally stable electronics	1
Robust, easy operation	Atmospheric pressure ionization (API) ion source technology	5
Diverse applications	Multimode source for simultaneous atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI)	3

* Letters in parentheses refer to corresponding labels indicated in **Figure 6** on page 9.

Agilent Jet Stream thermal-gradient focusing technology produces a dramatic, more than five-fold, increase in sensitivity, improving detection limits for both qualitative and quantitative analyses. Agilent Jet Stream technology uses a precisely collimated curtain of ultra-high-speed, super-heated nitrogen gas to enhance desolvation and ionization, and to better confine the ion beam. More ions and fewer solvent droplets enter the sampling capillary (B)*, resulting in greater sensitivity.

Agilent's HPLC-Chip/MS provides plug-and-play nanospray LC/MS operation for applications that require high sensitivity and low sample consumption. The chip integrates the sample enrichment and separation columns of a nanoflow LC system with the intricate connections and spray tip used in ESI-MS. It eliminates the traditional fittings, valves, and connections typically required in a nanospray LC/MS system, dramatically reducing the possibility of leaks and eliminating post-column dead volumes. Peak dispersion is virtually eliminated, resulting in narrower, better-defined peaks, greatly improved separations, and dramatically enhanced sensitivity.

Innovative ion optics provide unsurpassed ion transmission efficiencies. Agilent's dielectric sampling capillary (B)*, skimmer (C)*, high-frequency octopole ion guide (D)*, radio frequency (RF) lenses (E)*, hyperbolic quadrupole mass filter and post-Q1 filter (F and G)* are highly optimized to efficiently transmit ions and exclude

solvent clusters. The hexapole collision cell (H; Q-TOF only)* eliminates noise by reducing the number of excited neutrals and clusters transmitted through the cell. An accelerating voltage is used to avoid ion loss and noise due to ion latency.

High resolution and wide dynamic range

Better resolving power minimizes the chance that a mass peak of interest will be hidden by an interfering ion in the sample or background. Agilent's innovations in precision beam shaping, reflectron design, and analog-to-digital (ADC) detector electronics enable resolving powers up to 40,000 so users can distinguish between compounds with identical nominal masses. Agilent's ADC detector technology makes it possible to achieve up to five decades of in-spectrum dynamic range, a necessity for applications where the concentrations of sample components vary by many orders of magnitude.

Ion Beam Compressor (I)* provides a narrowed, cooled, and condensed beam of ions to the slicer (J)* and the pulser (K)*. This new technology equalizes the starting positions and velocities of the ions before they are pulsed into the flight tube. As a result, ions with nearly identical m/z values are distinguished more precisely. Some competitive designs simply use narrower slits, which discard ions needed for both sensitivity and mass accuracy. The proprietary IBC technology boosts mass resolution to 40,000 *while maintaining excellent sensitivity* and retaining the numbers of ions that you need to get *superb mass accuracies*.

Enhanced Mirror Technology (M)* normalizes variations in flight time caused by ion dispersion. Because ions closest to the pulser are accelerated more forcefully into the flight tube, ions of the same mass will have slightly different speeds simply because they had different starting positions relative to the pulser. Though beam shaping helps to tightly align ions before they are pulsed, no beam is perfectly flat and the pulse itself will inevitably introduce a small amount of ion dispersion.

The two-stage reflectron uses an electrostatic field to reflect ions back toward the detector after they have been pulsed into the flight tube. More energetic ions penetrate deeper into the reflectron, and therefore take a slightly longer path to the detector. Less energetic ions of the same mass penetrate a shorter distance into the reflectron and take a shorter path to the detector. The net effect is that ions of the same mass arrive at the detector at more nearly the same time, improving mass resolution.

The new Enhanced Mirror Technology uses a deeper electrostatic ion mirror, which is optimized for the slightly longer flight tube in the Accurate Mass Q-TOF.

Fast Bipolar Detector (N)* was developed specially for the Agilent Q-TOF systems. It uses a new ultrafast, high-efficiency scintillator that reduces the pulse width for single ions to a mere 800 ps. The narrow pulse width increases resolution, especially at low mass and in the 2 GHz extended dynamic range mode.

* Letters in parentheses refer to corresponding labels indicated in **Figure 6** on page 9.

Fast ADC signal processing, made possible by Agilent's patented dual-gain 4 GHz ADC microprocessor (**O**)*, provides up to five decades of in-spectrum dynamic range. Many other TOF and Q-TOF systems use TDC detectors. A TDC only registers an ion arrival above a certain intensity level, and gives the same response regardless of whether the signal is the result of one or many ions. With its 32 Gbit/sec sampling rate, Agilent's ADC based detector generates a continuous digital representation of the signal. Dual-gain amplifiers simultaneously process signals through

both low-gain and high-gain channels. When multiple ions of a given mass arrive at the detector within a very short timeframe, the ADC translates this rising and falling signal into an accurate digital profile of the mass peak. Therefore, the detector output is accurately represented regardless of whether it is from a small or large ion current.

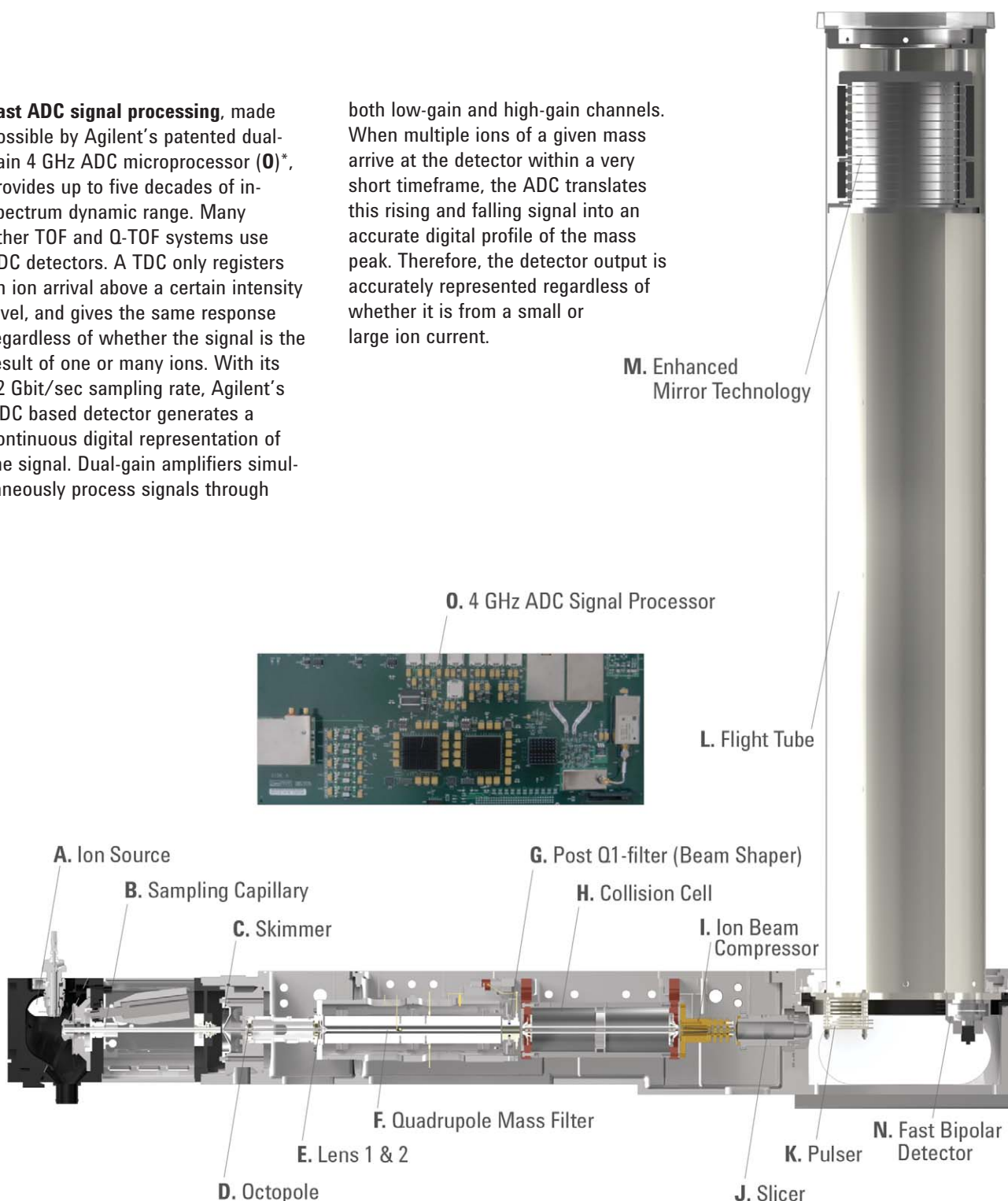


Figure 6. Ultra High Definition Q-TOF performance is achieved by the combination of many innovations. Complementary technologies and proprietary engineering innovations come together to achieve Ultra High Definition Q-TOF performance that measurably surpasses any other system.

* Letters in parentheses refer to corresponding labels indicated in **Figure 6**

Four GHz ADC-based signal processing also enables extraordinary mass resolution. By virtue of its speed—it samples ion signal every 250 ps—it can detect very rapid changes in signal intensity, such as the nearly instantaneous changes in signal that occur between ions with very small mass differences. Compared with other systems, Agilent’s proprietary peak signal processing algorithms process the detector’s signal in a much more precise manner, further enhancing mass resolution.

Excellent mass accuracy, rock-solid stability

Through a combination of innovative designs, Agilent Ultra High Definition Q-TOF technology leads in providing stable, accurate, and repeatable mass measurements. See **Figure 7** for sample data. It rivals more costly and complicated magnetic sector, Fourier Transform Ion Cyclotron Resonance (FT-ICR) TOF and Orbitrap systems that are used to obtain accurate-mass measurements.

Thermally-stable flight tube. Accurate-mass measurement requires a thermally stable flight tube. Unlike other systems, Agilent’s TOF and Q-TOF systems do not require tight control of lab temperature to within $\pm 1^\circ\text{C}$. Agilent’s systems use an Invar flight tube (L)* with an extremely low coefficient of thermal expansion. The tube resides in a vacuum-insulated shell to further protect it from temperature changes.

Effortless, accurate-mass calibration and correction are essential to achieving 250 ppb MS and 700 ppb MS/MS mass accuracy. To achieve this exceptional mass accuracy, Agilent’s system automatically corrects the most miniscule instrument variations that can occur during a sample run, by introducing two internal mass standards continuously via a reference mass nebulizer (A)*. The control software automatically corrects the measured masses of the samples using the known reference masses.

Therefore, the mass calibration is dynamically adjusted each time ions are pulsed into the flight tube. The system’s wide dynamic range allows the reference mass to be introduced at a very low concentration, preventing interferences from the reference compounds.

Superior MS/MS mass accuracy, higher quality MS/MS spectra, and better response for higher masses are made possible by Agilent’s hexapole collision cell (H)*. To avoid ion stall-out, a voltage potential is applied along the length of the rods. This creates linear axial acceleration that maintains ion momentum through the cell. Ions exit the collision cell with a negligible but uniform energy. Using this approach, the same correction factors can be applied to both MS and MS/MS mass assignments, enabling Agilent’s system to achieve better than 1 ppm MS/MS mass accuracy.

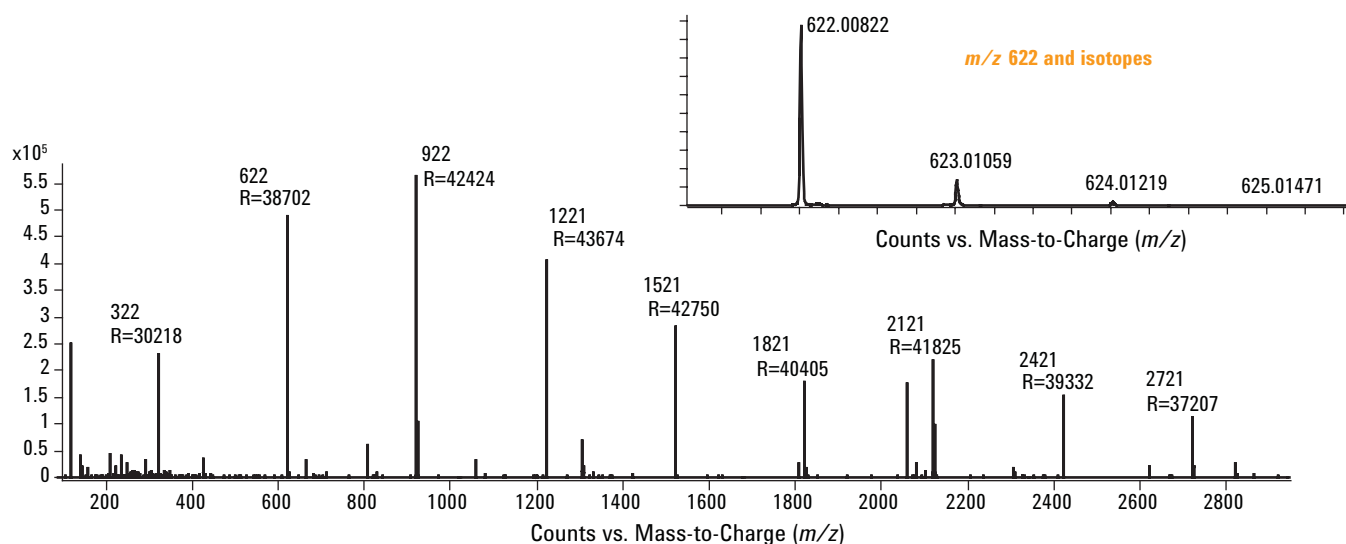


Figure 7. Ultra High Definition Q-TOF technology maintains exceptional resolving power across the mass range. Unlike Orbitrap instruments, UHD Q-TOF technology maintains this resolution regardless of how fast spectra are acquired. This characteristic makes the Agilent Accurate-Mass Q-TOF systems ideal for MS detection of subsecond wide peaks from UHPLC.

* Letters in parentheses refer to corresponding labels indicated in **Figure 6** on page 9.

Fast MS/MS ensures compatibility with RRLC and UHPLC

The use of fast chromatography has grown due its ability to accelerate method development, increase lab throughput, and extract more information from complex mixtures. See **Figure 8** for sample data. Separations using the latest sub-two micron technology with RRLC and UHPLC systems often result in analyte peak widths of one second or less. This reduction in the chromatographic time scale places new demands on MS/MS analyses. To preserve the resolution provided by the LC, the mass spectrometer must sample the chromatographic peak at a very fast rate.

Ultra High Definition Q-TOF technology provides fast data acquisition rates—up to 10 MS/MS spectra per second—to ensure compatibility with fast chromatography. Because UHD Q-TOF technology transfers more ions to the flight tube and detector, it produces best-in-class MS/MS mass accuracy at high speeds.

The hexapole collision cell (H)* uses a voltage potential to produce axial acceleration that sweeps ions out of the collision cell after fragmentation. By accelerating ions in this manner, Agilent's Q-TOF can perform more

fragmentation reactions per unit time, without cross-reaction memory effects due to ion latency.

Ultra-fast 4 GHz electronics and ADC-based signal processing (P)*, easily record ion signal every 250 ps. There is no dead-time in which ion signal, and therefore valuable information, is lost.

Isotope ratios

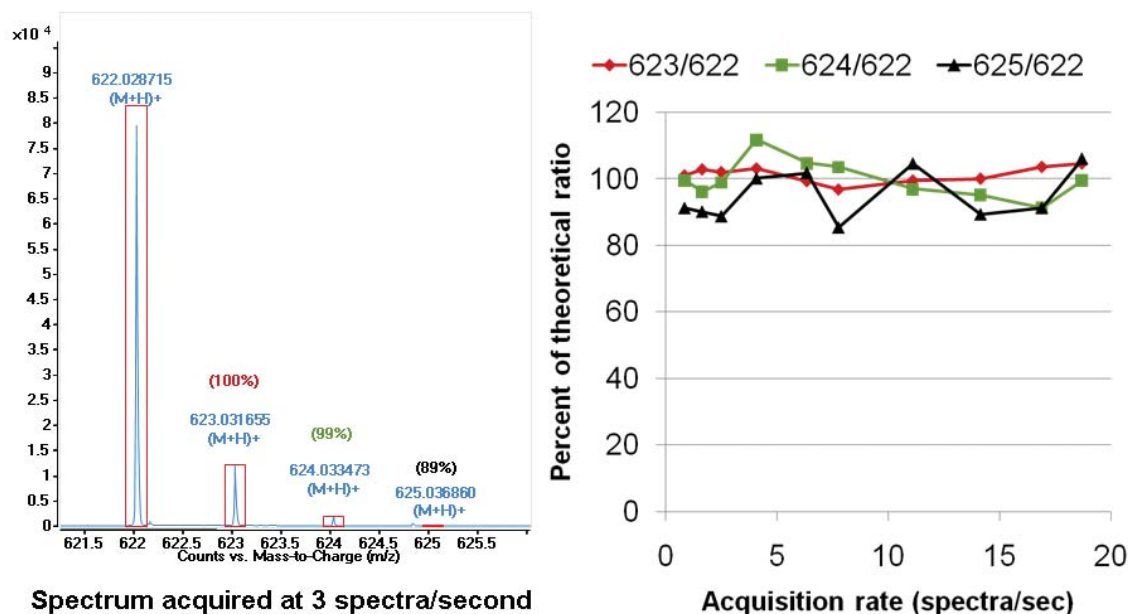


Figure 8. For maximum compatibility with UHPLC, UHD Q-TOF technology produces accurate and invariant isotope ratios over spectral acquisition rates of up to 20/sec.

* Letters in parentheses refer to corresponding labels indicated in **Figure 6** on page 9.

Why choose Ultra High Definition Q-TOF technology?

Obtaining the correct analytical results is critical. Unlike any other Q-TOF system, Agilent Ultra High Definition Q-TOF technology delivers the performance needed to meet the demands of the most challenging analyses. See **Figure 9** for sample data. This technology provides:

- Highest-quality data at the rapid spectral acquisition speeds that keep pace with UHPLC—unsurpassed

resolution, mass accuracy, dynamic range, and isotopic fidelity—all at 20 spectra/sec

- Sensitivity to find compounds that other systems miss
- Mass accuracy of up to 0.25 ppm and mass resolution up to 40,000, to improve confidence and reduce false positives
- High definition isotopic fidelity that confirms molecular formulas with greater confidence

- Unique compound-centric data-processing to take full advantage of Ultra High Definition Q-TOF data to accurately identify and quantify analytes
- And most importantly—the entire process from system setup to final result is robust and automated.

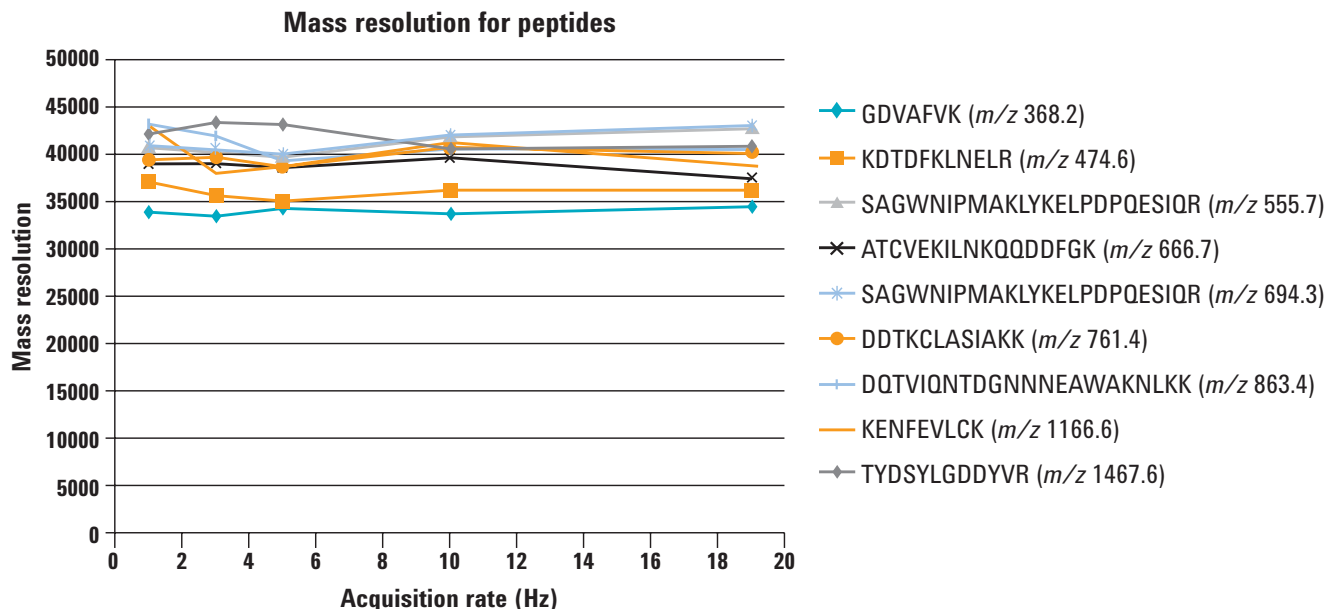


Figure 9. UHD Q-TOF technology produces excellent mass resolution at up to 20 spectra/sec, giving ultimate performance for UHPLC applications.

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