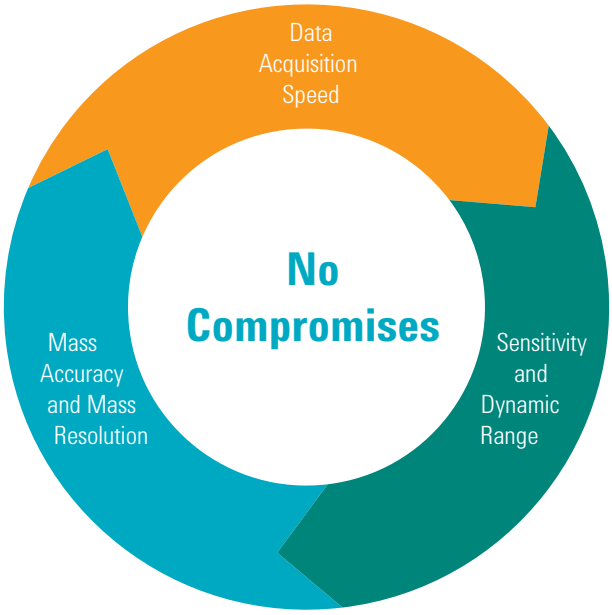
Achieving Unmatched Speed and Confidence for Complex Sample Analyses: Agilent True High-Definition TOF Technology

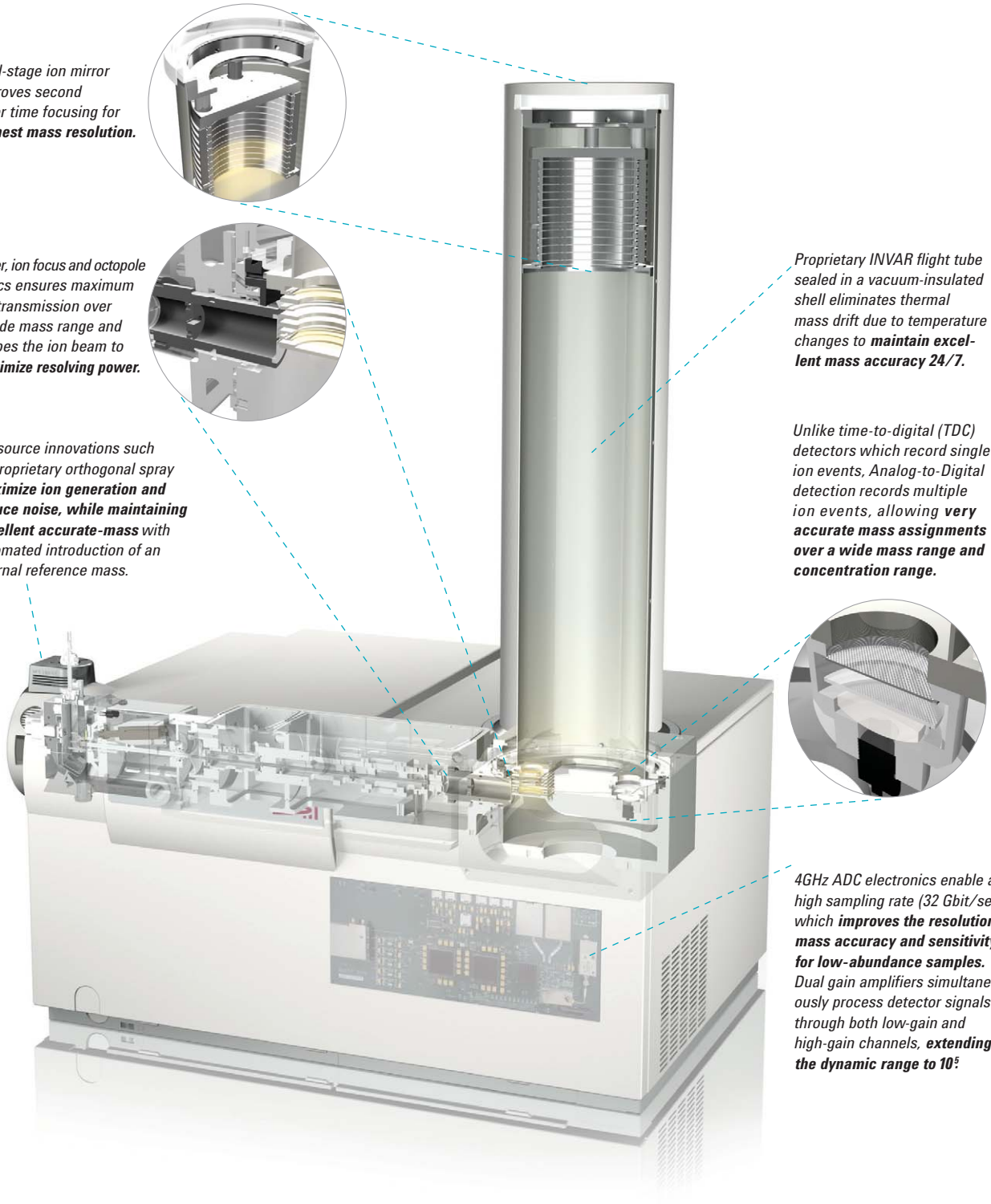
Evaluating Performance

Time-of-flight (TOF) and quadruple time-of-flight (Q-TOF) liquid chromatography/mass spectrometry (LC/MS) technologies are improving at a rapid pace and, as a result, are demonstrating impressive gains in their performance. When choosing a TOF or Q-TOF system you need to focus on what really matters: how the system performs when challenged with the complex samples encountered in real applications. It is essential to consider the performance that can be achieved in several dimensions. Mass accuracy and resolution are important, but so are dynamic range, sensitivity, and spectral acquisition speed. Unlike other TOF, Q-TOF and orbitrap systems, Agilent's TOF and Q-TOF instruments deliver excellent performance in all of these dimensions simultaneously, routinely and without compromise.



Agilent True Hi-Def TOF technology delivers exceptional, robust mass accuracy, high resolution, great sensitivity, wide in-spectrum dynamic range, and fast data acquisition rates without the compromises of other TOF systems or orbitrap mass analyzers.

Agilent Technologies



True Hi-Def TOF technology in the Agilent 6200 Series Accurate-Mass TOF systems brings together complementary technologies and proprietary engineering innovations to achieve TOF performance that measurably surpasses any other TOF system—and even rivals or exceeds other, more expensive MS technologies. The same technology is available on the Agilent 6500 Series Accurate-Mass Q-TOF systems.

True Hi-Def TOF technology

Agilent has relentlessly refined the performance and reliability of every component of Agilent's TOF and Q-TOF systems. The result is True High-Definition (Hi-Def) TOF technology. "True" refers to performance without compromise and to finding the correct answers to difficult analytical questions. "Hi-Def" refers to obtaining the maximum amount of useful information from real-world samples. This is accomplished by providing the combination of exceptional data quality, unmatched sensitivity and dynamic range—all at outstanding speed. Agilent's TOF and Q-TOF instruments deliver up to 40 mass-accurate, high-resolution spectra-per-second and are the only systems which capture sufficient high-quality data to keep pace with ultra-fast rapid-resolution liquid chromatography (RRLC) and ultra-high-pressure LC (UHPLC).

Agilent's 6200 Series Accurate-Mass TOF LC/MS and 6500 Series Accurate-Mass Q-TOF LC/MS are the only LC/MS systems available today which deliver the speed and certainty of True Hi-Def TOF performance:

- **Wide dynamic range** up to 5 orders of magnitude
- **Exceptional, high-femtogram level sensitivity** to find low abundance components in complex mixtures
- **High mass accuracy** of <1 ppm for MS and <3 ppm for MS/MS spectra
- **High mass resolving power** of 20,000 to enhance identification of nearly-isobaric compounds
- **Fast data acquisition** of up to 40 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** of 25 to 20,000 Da 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 True Hi-Def TOF technology allows you to come remarkably close. Coupled with fast, high-resolution chromatography, Agilent's TOF and Q-TOF systems can routinely screen samples for the presence of pesticides or contaminants. With more thorough fractionation, they can perform global differential analysis of proteins or metabolites.

- **Proteomics and metabolomics** applications require the analysis of exceptionally complex samples. For example, proteomics requires detection and quantitation of thousands of proteins. Metabolites exhibit even greater chemical diversity.
- **Product degradation studies and impurity analyses** require the detection and measurement of low abundance impurities in the overwhelming presence of native compounds.
- **Target compound analyses** require screening for hundreds of compounds, such as pesticide residues or drugs of abuse, in complex matrices.

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. True Hi-Def TOF technology delivers the unique combination of wide dynamic range and high-femtogram level sensitivity, making it possible to perform these applications quickly and easily **(Figure 1)**. Compound-centric data reduction strategies make post-acquisition data analysis effective and efficient.

High resolution and mass accuracy for high-confidence compound characterization

Agilent True Hi-Def TOF technology delivers data of exemplary quality, with high resolution and exceptional, stable mass accuracy. Routine sub-1 ppm mass accuracy allows you to quickly narrow search results to a few compounds and accurate MS/MS information enables precise structural assignment and increases confidence in results **(Figures 2 & 3)**.

- **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**—High MS and MS/MS mass-accuracy enables confident, simultaneous screening for hundreds of compounds using confirmatory MS/MS data.

Powerful software takes full advantage of True Hi-Def TOF data

True Hi-Def TOF technology produces unprecedented amounts of high-quality raw

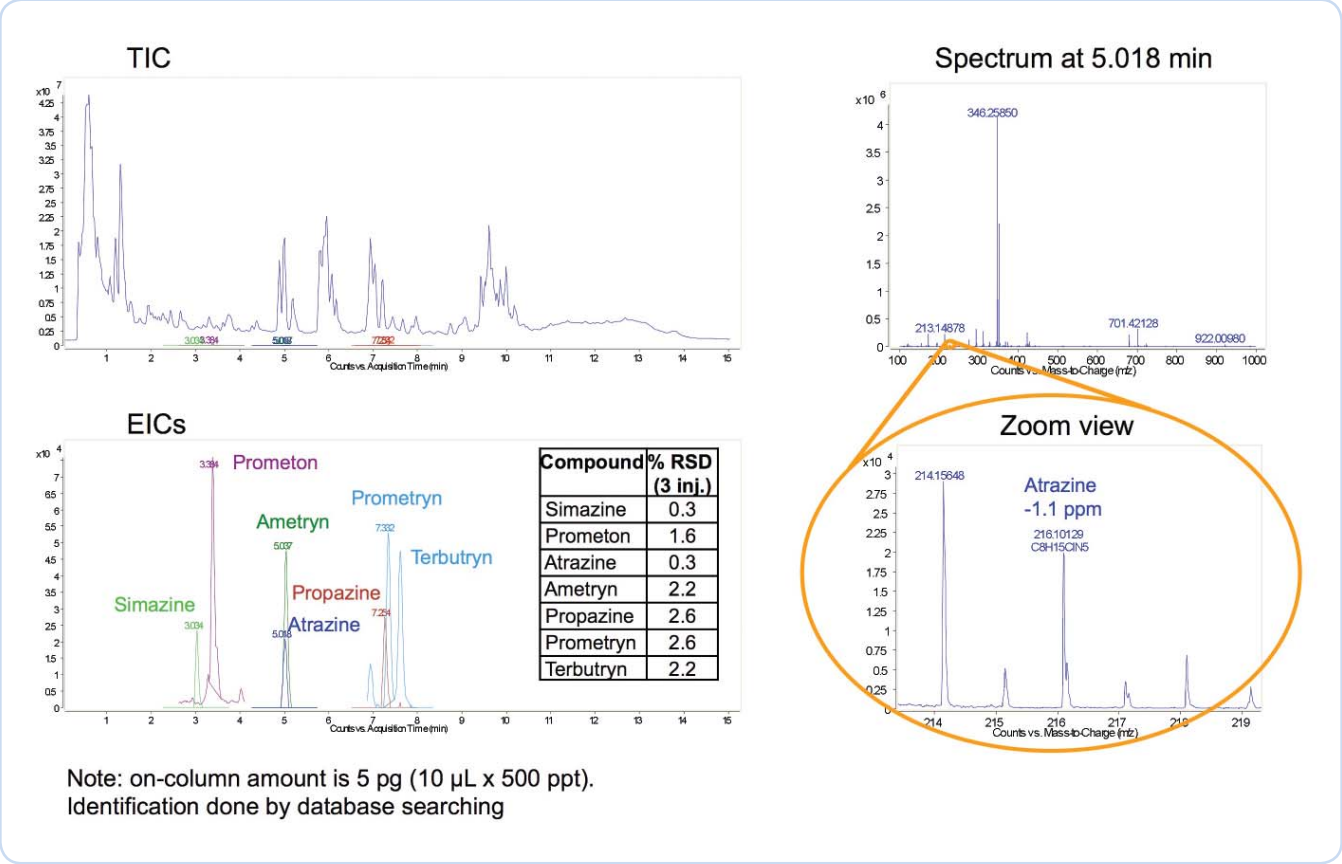


Figure 1. Agilent True Hi-Def TOF technology has the combined dynamic range, sensitivity and mass resolution to detect and accurately assign mass to within 1.1 ppm. In this MS-only analysis of a tomato extract, triazines were spiked in at 500 pg/µL of matrix. Ten mL of sample was injected, yielding 5 pg on column. The TIC shows many abundant components in the matrix. All seven pesticides were found by database searching against a user-created library, demonstrating exceptional mass accuracy, dynamic range and sensitivity. At 1,000 times lower abundances (lower left), the extracted ion chromatograms (EICs) for the triazine pesticides were found. On the right is the spectrum at the retention time of atrazine. Though the matrix (upper right) dominates the analyte (lower right) by 1,000 times, the combined dynamic range, sensitivity and mass resolution capabilities of True Hi-Def TOF technology detect and accurately assign mass to within 1.1 ppm.

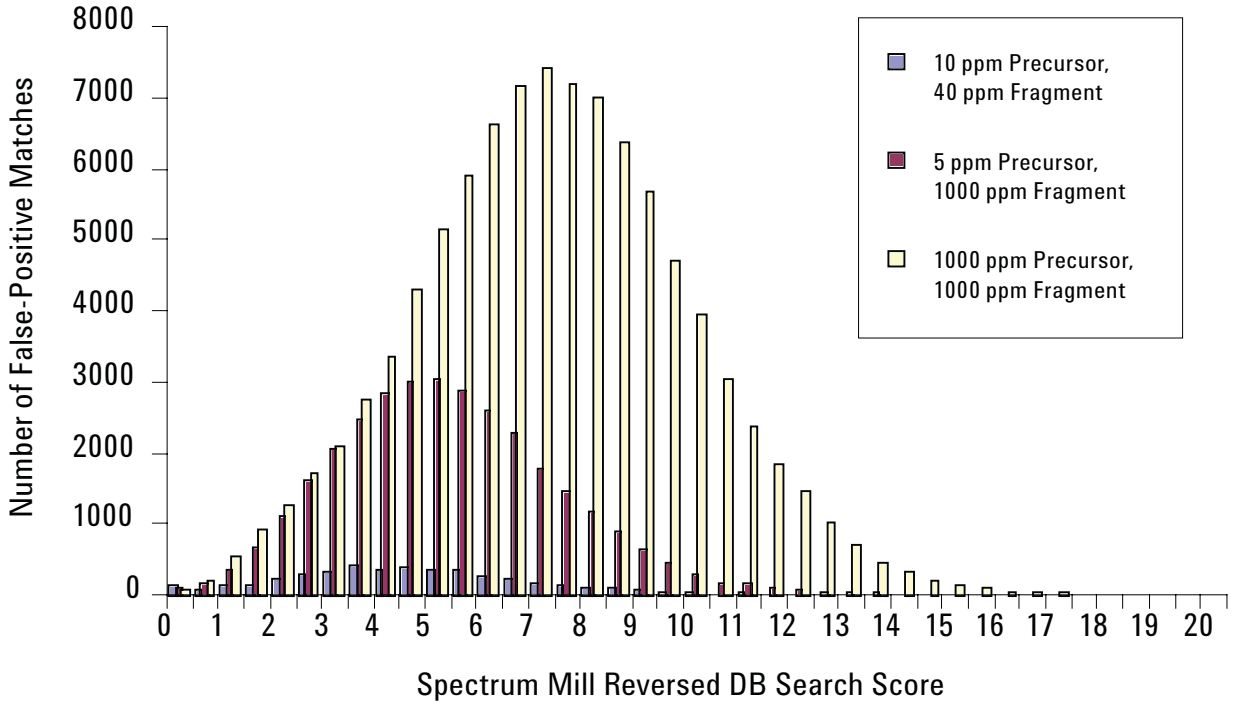


Figure 2. False-positive rate diminishes rapidly using accurate-mass MS/MS data. Database searches with 6500 Series Q-TOF data using different sets of precursor and fragment mass accuracy settings that represent three different MS instruments—an ion trap, a high-resolution hybrid linear ion trap, and the Q-TOF itself—demonstrate that the high precursor ion mass and fragment ion mass accuracy of the 6500 Series Q-TOF can significantly increase valid protein identification by reducing the number of false positives.

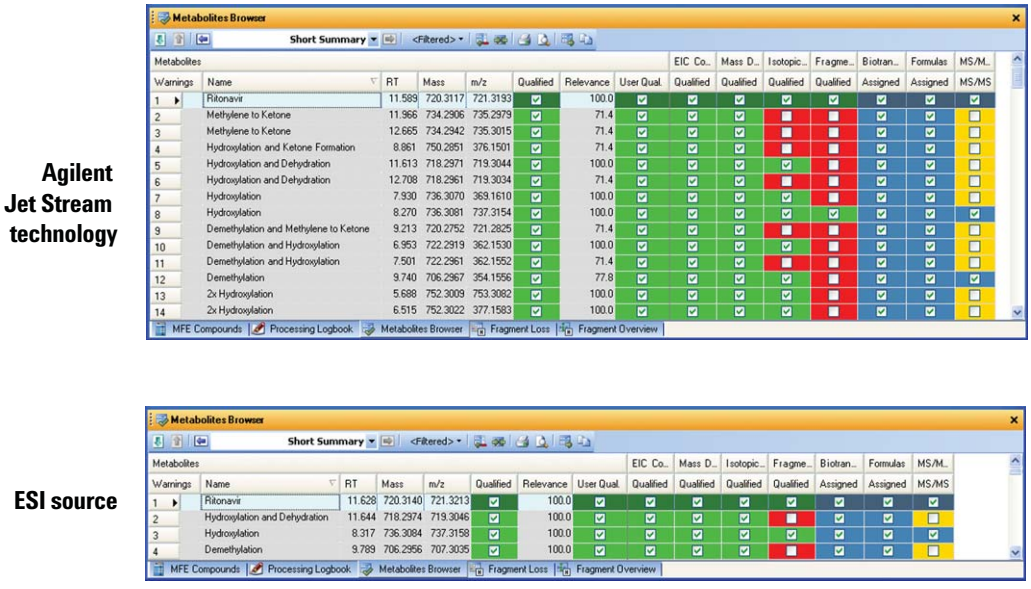


Figure 3. Improved sensitivity with Agilent Jet Stream technology enabled the identification of four times more low-level metabolites compared with the standard ESI source. Summary of the ritonavir metabolites in liver microsomes identified by LC/MS using a conventional Agilent ESI source with the Agilent 6520 Q-TOF instrument, and using Agilent Jet Stream technology integrated into the Agilent 6530 Q-TOF platform.

data. In this context, a high-definition answer is the final result of a process that starts with billions of raw data points acquired from complex samples and ends in a crisp image 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 advanced 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 4)**.
- Molecular formula generation (MFG) software provides high-confidence identification of unknowns, using multiple dimensions of information to generate and score lists of possible molecular formulae. The MFG reduces the number of plausible formulae by using the accurate-mass TOF and Q-TOF data.
- MassHunter profiling software finds variations between sample groups
- GeneSpring MS software performs sophisticated differential and statistical analysis of complex sample sets
- MassHunter Metabolite ID software identifies drug metabolites
- METLIN Personal Database of over 23,000 endogenous metabolites
- Spectrum Mill Workstation software for protein database searching
- MassHunter Personal Compound Database for pesticides

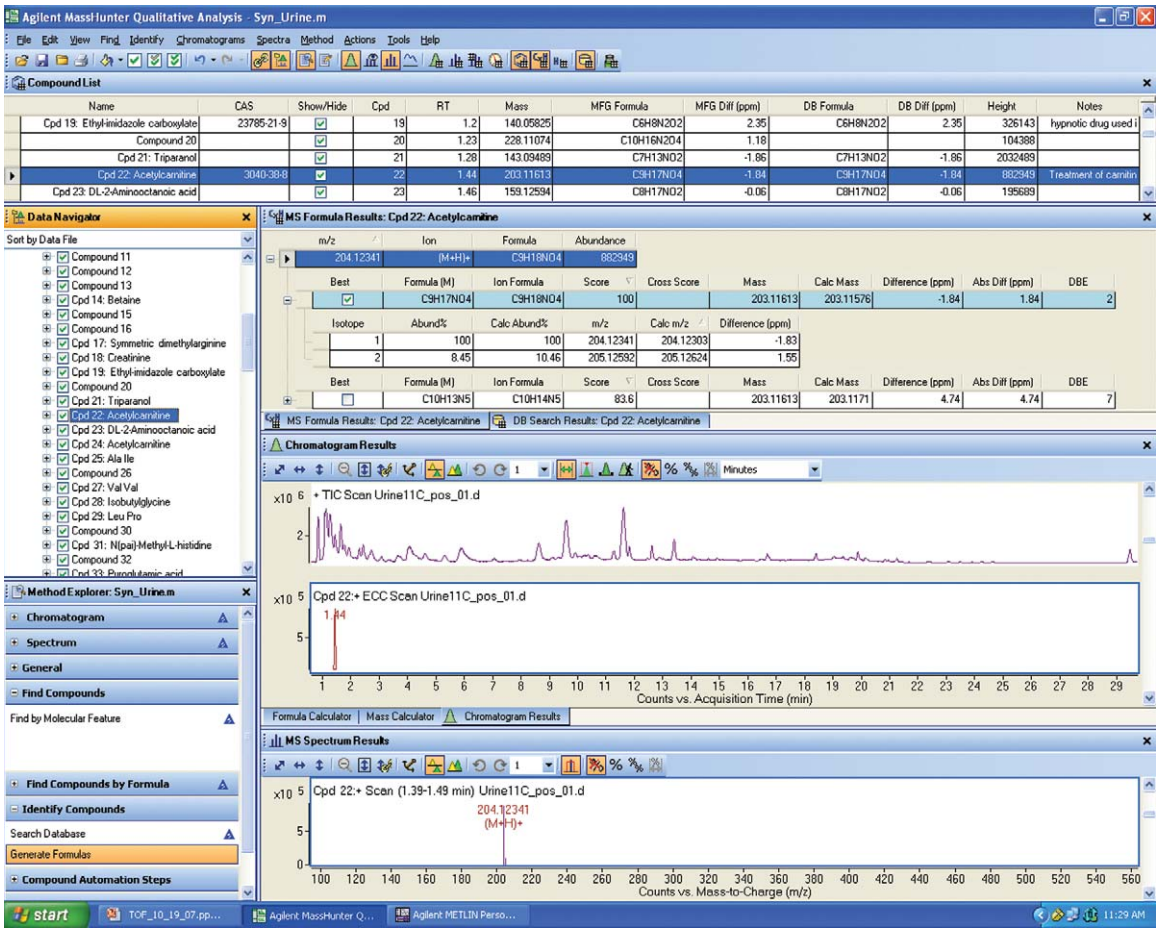


Figure 4. Molecular feature extraction and molecular formula generation capabilities of the MassHunter software save hours of analysis time by automatically locating sample components and extracting spectral and chromatographic information, and then using this information to suggest molecular formulae.

Fundamentals of True Hi-Def TOF technology

Agilent's history of innovation over the last 37 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 TOF and Q-TOF systems **(Table 1)**. The Agilent 6200 Series Accurate-Mass TOF and 6500 Series Accurate-Mass Q-TOF LC/MS systems achieve a completely new level of LC/MS performance—True Hi-Def 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

Table 1. Patented innovations enabling True Hi-Def TOF performance

Dimension of True Hi-Def 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
Five decades of in-spectrum dynamic range and high resolving power	Signal processing hardware and software, and 4GHz analog-to-digital (ADC) spectral acquisition electronics with dual-gain signal amplifiers	2
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 in **Figure 5** on page 9.

HPLC-Chip/MS, dramatically improve ionization efficiency to deliver remarkable sensitivity for both conventional and nanoflow-rate applications.

Agilent Jet Stream thermal-gradient focusing technology produces a dramatic, more than five-fold, increase in sensitivity, thereby 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 requiring 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 guides **(D and I)**, 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 and chemical background. 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 new analog-to-digital (ADC) detector electronics enable resolving power up to 20,000 so users can distinguish between compounds with identical nominal masses. Agilent's ADC detector technology makes it possible to achieve up to 5 decades of in-spectrum dynamic range, a necessity for applications where the concentrations of sample components vary by many orders of magnitude.

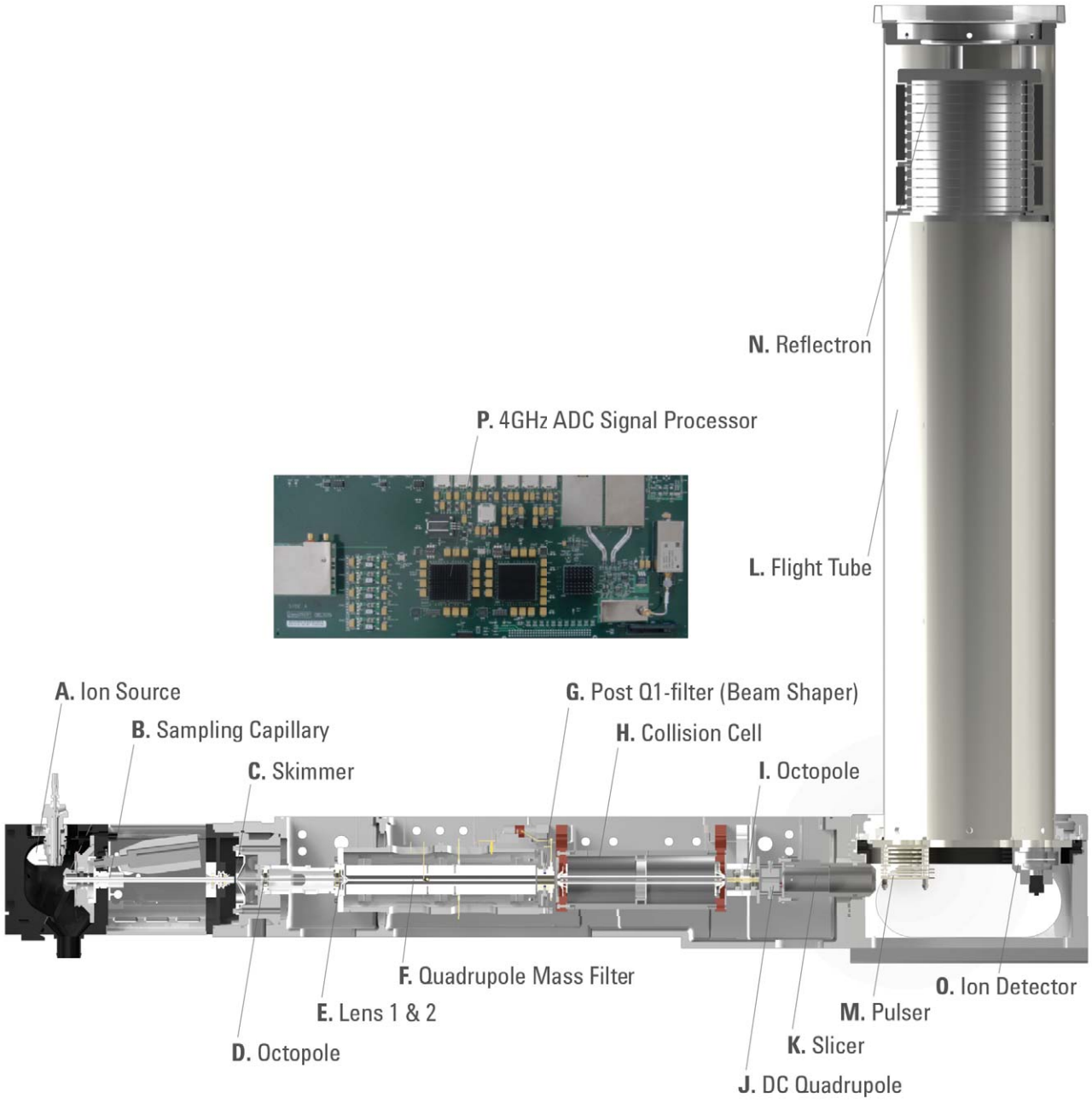
Precision beam shaping enables Agilent's TOF and Q-TOF systems to achieve extraordinary resolving power. Before ions are pulsed into the flight tube, the DC quadrupole and beam slicer **(J and K)** shape the ion beam into a tight ribbon. This equalizes the starting positions of the ions before they are pulsed into the flight tube and it reduces the random variation in the vertical momentum of the ions before they are pulsed. As a result, ions with nearly identical *m/z* values are distinguished more precisely.

The innovative two-stage reflectron (N) 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, thereby improving mass resolution.

Fast ADC signal processing, made possible by Agilent's patented dual-gain microprocessor 4GHz ADC **(P)**, provides up to 5 decades of in-spectrum dynamic range. All 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 time frame, 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.

*Letters in parentheses refer to corresponding labels in **Figure 5** on page 9.



Note: The Agilent Q-TOF system is represented here; the Agilent TOF system incorporates the same components as the Q-TOF except the quadrupole mass filter (F), the post Q1-filter (G), the collision cell (H), and the second octopole (I).

Figure 5. True Hi-Def TOF performance is achieved by the combination of many innovations. Complementary technologies and proprietary engineering innovations come together to achieve True Hi-Def TOF performance that measurably surpasses any other system.

Four GHz ADC-based signal processing also enables extraordinary mass resolution. By virtue of its speed—it samples ion signals every 250 picoseconds—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 to 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 True Hi-Def TOF technology leads in providing stable, accurate and repeatable mass measurements. It rivals more costly and complicated magnetic sector, Fourier Transform Ion Cyclotron Resonance (FT-ICR) TOF and orbitrap systems that have been used to obtain exact 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 ±1°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 greater than 1-ppm MS and 3-ppm 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

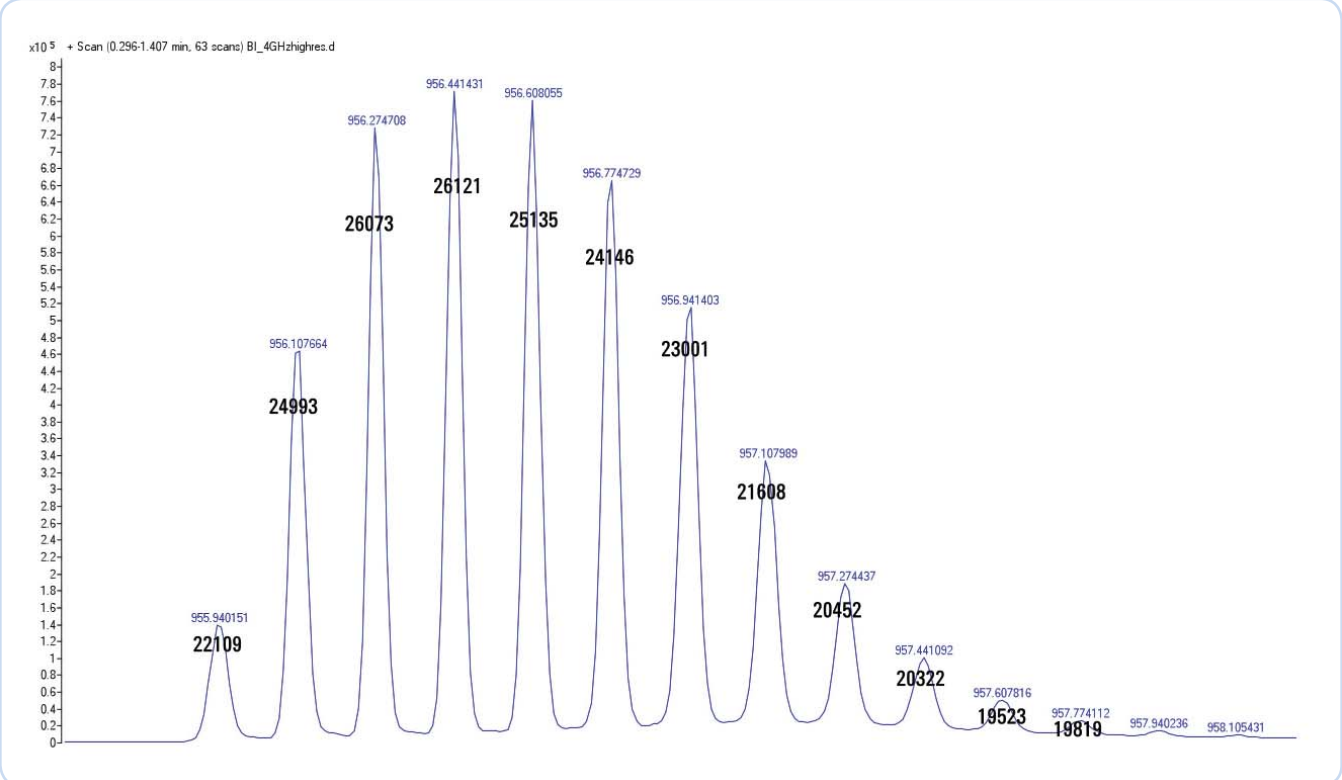


Figure 6. True Hi-Def TOF technology results in high resolution of small proteins thus enabling easy confirmation of charge state and monoisotopic peak as shown here for the +6 charge state of insulin. Resolution values are listed in black.

*Letters in parentheses refer to corresponding labels in **Figure 5** on page 9.

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 3-ppm MS/MS mass accuracy.

Fast MS and MS/MS ensure compatibility with RRLC and UHPLC

The use of fast chromatography has grown due its ability to speed method development, increase lab throughput, and extract more information from complex mixtures. 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 the mass spectrometer system. To preserve the resolution provided by the LC, the mass spectrometer must sample the chromatographic peak at a very fast rate.

True Hi-Def TOF technology provides fast data acquisition rates unmatched by other Q-TOF or orbitrap system, up to 10 MS/MS spectra per second, to ensure compatibility with fast chromatography.

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 picoseconds. There is no dead-time in which ion signal, and therefore valuable information, is lost.

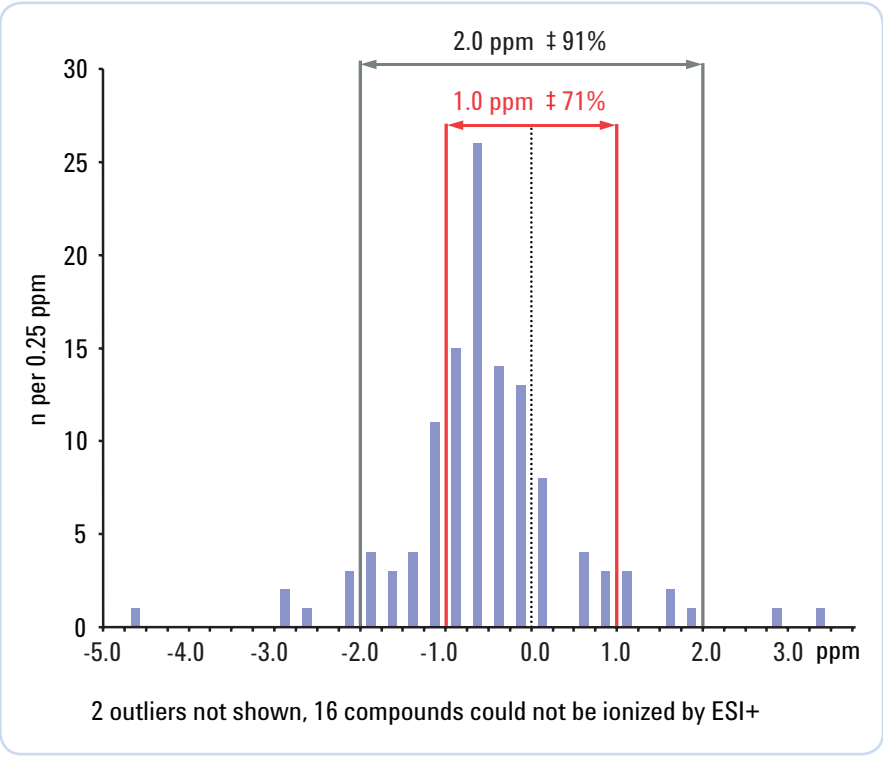


Figure 7. With Agilent's ADC-based detector electronics, mass accuracy is not compromised even at the fastest data acquisition rates. In this screening of a 140-compound library where one sample was injected every 90 seconds, over 70% of the compounds were measured to within 1 ppm of their actual mass; over 90% were within 2 ppm. Acquisition speed was 40 spectra per second.

*Letters in parentheses refer to corresponding labels in **Figure 5** on page 9.

Why choose True Hi-Def TOF technology?

Obtaining the correct analytical results is critical. Unlike any other TOF or Q-TOF system, Agilent True Hi-Def TOF technology delivers the performance needed to meet the demands of the most challenging analyses:

- Sensitivity to find compounds that other systems miss
- Highest data quality over the broadest range of concentrations and masses
- Fastest data collection—up to 40 spectra/second—to reduce the risk of missing an important analyte or contaminant
- Unique compound-centric data-processing to take full advantage of True Hi-Def TOF data to accurately identify and quantify analytes
- And most importantly—the entire process from system setup to final result is robust and automated

Further reading

1. Agilent LC/MS Ion Sources, Maximizing LC/MS Performance. April 2007. Agilent Literature P/N: 5989-4197EN.
2. Agilent 1100 Series HPLC-Chip/MS system. September 2005. Agilent Literature P/N: 5989-3627EN.
3. Ion Optics Innovations for Increased Sensitivity in Hybrid MS Systems. October 2007. Agilent Literature P/N: 5989-7408EN.
4. Superior Molecular Formula Generation from Accurate-Mass Data. January 2008. Agilent Literature P/N: 5989-7409EN.
5. Time-of-Flight Mass Spectrometry. December 2003. Agilent Literature P/N: 5989-0373EN.
6. Stroh, J.G. *et al.* (Published Online: October 16, 2008) Sub-2 mum HPLC coupled with sub-ppm mass accuracy for analysis of pharmaceutical compound libraries. *J Sep Sci*. DOI: 10.1002/jssc.200800399
7. Bereman, M.S. *et al.* (2008) Mass measurement accuracy comparisons between a double-focusing magnetic sector and a time-of-flight mass analyzer. *Rapid Commun Mass Spectrom*. **22**(10):1563-6.

About Agilent Technologies

Agilent Technologies is a leading supplier of life science research systems that enable scientists to understand complex biological processes, determine disease mechanisms, and speed drug discovery. Engineered for sensitivity, reproducibility, and workflow productivity, Agilent's life science solutions include instrumentation, microfluidics, software, microarrays, consumables, and services for genomics, proteomics, and metabolomics applications.

Buy online:
www.agilent.com/chem/store

Find an Agilent customer center in your country:
www.agilent.com/chem/contactus

U.S. and Canada
1-800-227-9770
agilent_inquiries@agilent.com

Asia Pacific
adinquiry_aplsca@agilent.com

Europe
info_agilent@agilent.com

Printed in the U.S.A. December 15, 2008

5990-3168EN