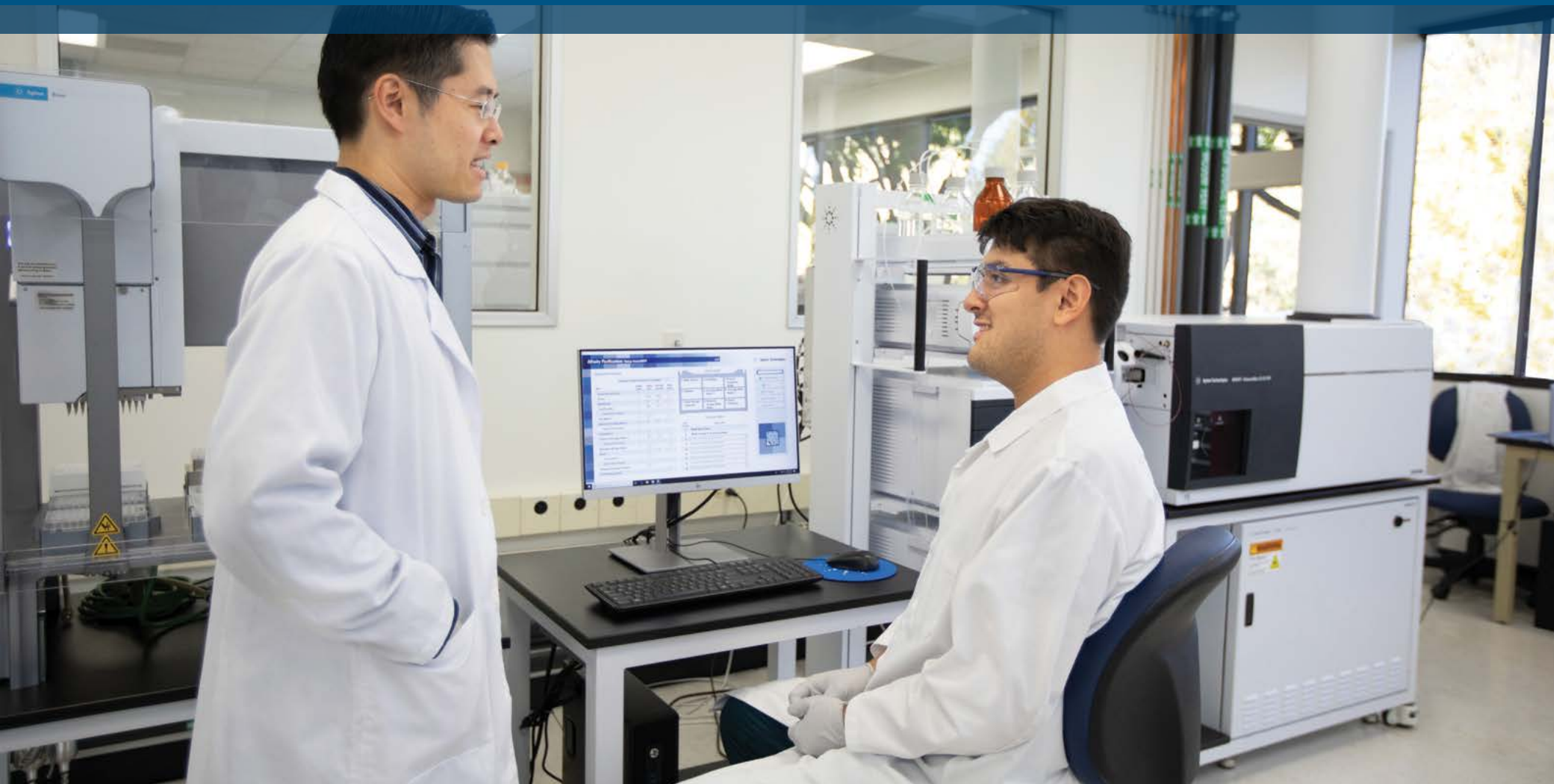
Agilent

Trusted Answers

Conquering Orbital Ion Trap Limitations

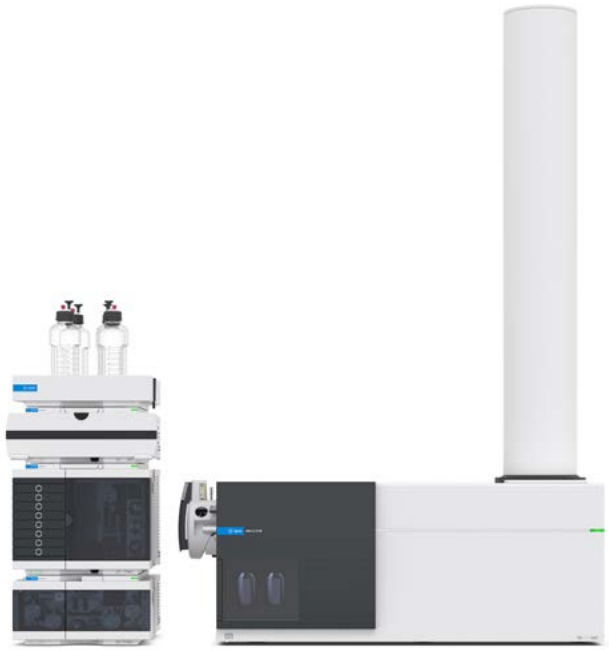
The proven data-quality demonstrating the exceptional capabilities of the Agilent 6546 LC/Q-TOF



Escape the Trap for Excellent Spectral Data

Unlike ion traps that only emphasize mass resolution and mass accuracy, the Agilent 6546 LC/Q-TOF delivers six coveted advantages in a single mass spectrometer. It helps you gain high confidence in your data, detect and resolve low-abundance compounds, attain higher mass accuracy, and improve isotope ratio fidelity. So, you can boost productivity and achieve better business outcomes.

The Agilent 6546 LC/Q-TOF delivers remarkably accurate results for high-performance metabolomics, food, and environmental laboratories.



Wide dynamic range | Outstanding sensitivity | Constant resolution | Higher precision | Isotope ratio fidelity | Increased productivity

Wide Dynamic Range

Five orders of in-spectrum dynamic range

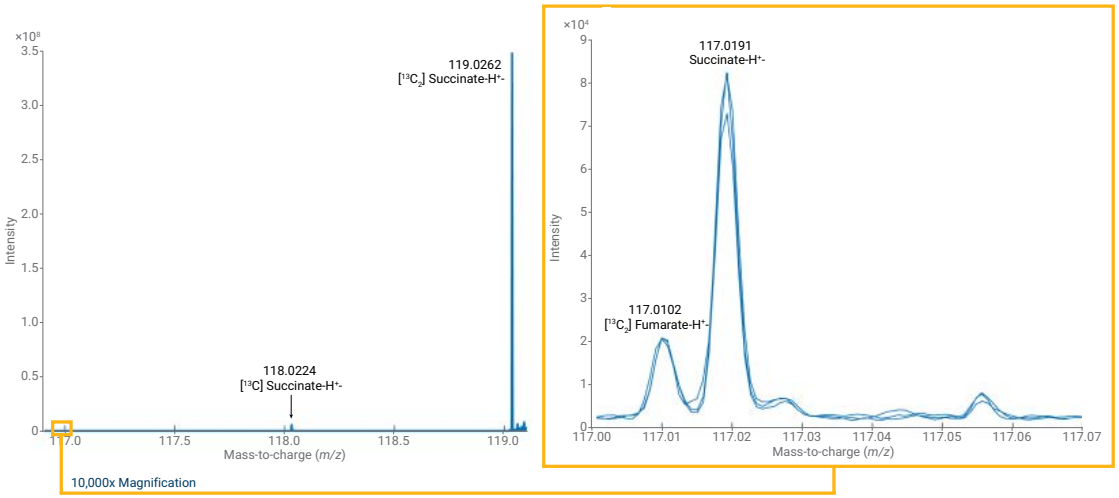
Quantify more compounds.
Improve resolution across the entire mass range at fast acquisition rates.

Complex biological systems require high-resolution mass spectrometers that can efficiently capture trace-level analytes among highly abundant compounds—and at a higher mass accuracy.

While ion traps can detect analytes up to 3.5 orders of magnitude for in-spectra dynamic range, the Agilent 6546 LC/Q-TOF provides up to five orders. That's more than 30 times more than high-resolution ion traps, enabling confident metabolite detection in complex matrices.

In one study,[1] *E. coli* samples were spiked with $^{13}C_2$-succinate. The 6546 LC/Q-TOF provided continuous access to a full intrascan dynamic range without compromising resolution, accuracy, sensitivity, or acquisition rate.

Despite the vast difference in intensities (104 to 108 counts), accuracy and resolution are preserved and allow precise determination of both abundant and rare peaks within the same spectrum.

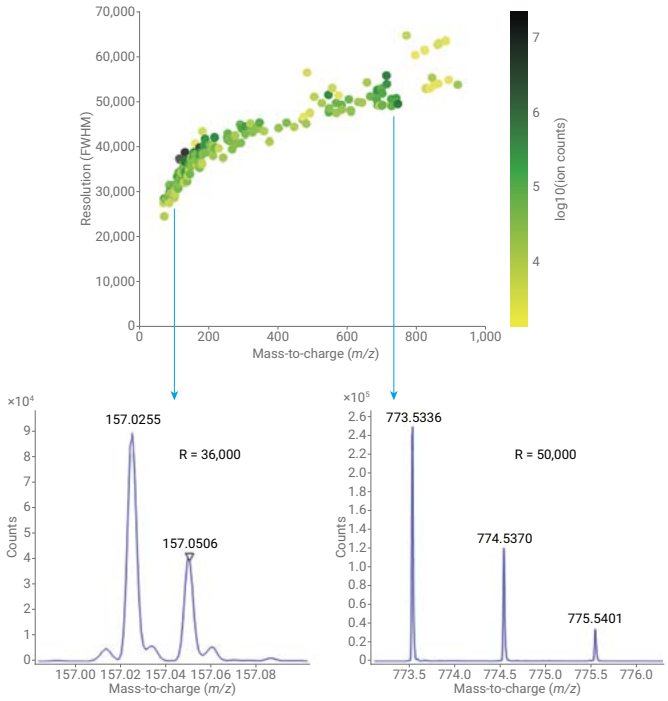


Intrascan dynamic range of the 6546 LC/Q-TOF. A 1X ^{13}C-enriched E. coli extract was spiked with $^{13}C_2$-succinate. Three technical replicates are superimposed.

Wide Dynamic Range

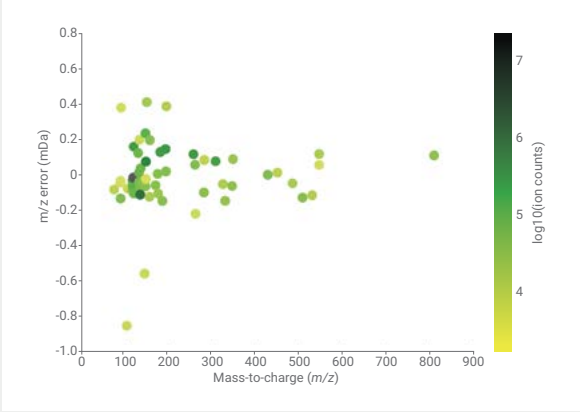
Five orders of in-spectrum dynamic range

The study also reported >30,000 resolution for analytes with *m/z* over 118 and >60,000 for *m/z* more than 1,521.



E. coli sample analyzed by flow injection coupled with the 6546 LC/Q-TOF. Resolution is plotted over the mass range, and color denotes ion intensity. Insets show the peak shapes for low- and high-mass ions.

A mass error of approxinately less than 0.2 mDa was consistently observed, enabling higher confidence in results across the entire range of analytes.



E. coli sample analyzed by flow injection with the 6546 LC/Q-TOF. Mass error (mDa) is plotted over the mass range for extracted analytes, and color denotes ion intensity.

Outstanding Sensitivity

Detecting low-abundant analytes

Detect low molecular weight compounds at extremely low concentrations. Maximize accuracy and precision across the entire mass range.

Low-abundant ion detection is crucial to identifying new and emerging contaminants. Identifying the smallest concentrations of analytes can enhance your lab's ability to detect new and emerging contaminants in early stages.

Agilent Q-TOF technology separates ions in space, ensuring maximum coverage, accuracy, and precision across the entire mass range. In doing so, it effectively overcomes the limitations of ion trap technologies where low-abundant ions are hard to detect.

In the following study, the Agilent 6546 LC/Q-TOF showed exceptional results when detecting nitrosamine impurities in Angiotensin II receptor blocker (ARB) drugs at low detection limits[2].

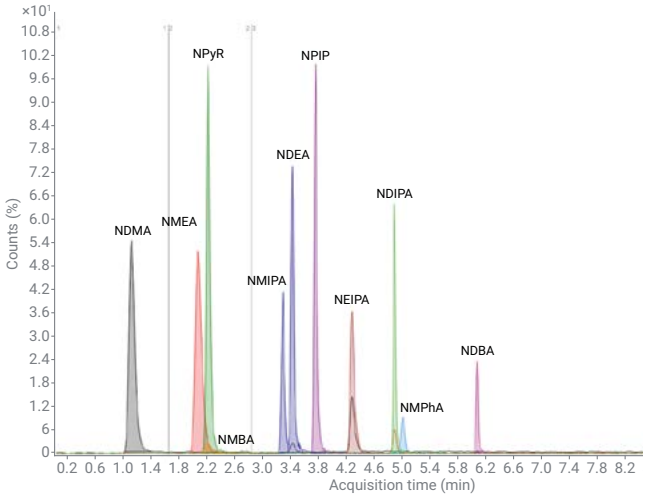
Compound	Detection Limit (ng/mL)	Detection Limit (S/N)	LOQ (ng/mL)	LOQ (S/N)	R^2	Cal. Curve	Linearity Range (ng/mL)
NDMA	0.1	16.54	0.25	35.72	0.999	Linear	0.1 to 100
NDEA	0.05	29.56	0.1	79.2	0.999	Linear	0.1 to 100
NMBA	0.25	12.16	0.5	27.88	0.996	Linear	0.5 to 100
NEIPA	0.1	11.22	0.25	80.6	0.998	Linear	0.1 to 100
NDIPA	0.075	16.65	0.15	21.99	0.999	Linear	0.075 to 100
NDBA	0.1	44.60	0.25	61.56	0.997	Linear	0.1 to 100
NMEA	0.05	18.42	0.1	23	0.998	Linear	0.05 to 100
NPyR	0.1	29.73	0.15	50.39	0.999	Linear	0.1 to 100
NPIP	0.075	12.18	0.1	25.30	0.998	Linear	0.075 to 100
NMPhA	0.25	24.22	0.5	32.30	0.997	Linear	0.5 to 100
NMIPA	0.075	29.79	0.1	47.46	0.997	Linear	0.075 to 100

Analysis of 11 nitrosamine impurities at low detection limits using the 6546 LC/Q TOF. These impurities have low molecular weights ranging from 74 to 158. Data include signal-to-noise (S/N), calculated LOQ, coefficient of regression, calibration curve fit, and linearity range. All standards used a linear function and a 1/x weighted calibration curve.

Outstanding Sensitivity

Detecting low-abundant analytes

Representative overlaid extracted ion chromatogram for all nitrosamine compounds tested at 10 ng/mL concentration.



The Agilent 6546 LC/Q-TOF high-resolution LC/MS/MS can analyze nitrosamine impurities at low concentration levels. High-resolution mass spectrometry reliably detects the presence of nitrosamine compounds in the drug products.

The Agilent 6546 LC/Q-TOF also showed excellent accuracy and reproducibility at various concentration levels for all compounds analyzed.

Concentration (ng/mL)	NDMA		NDEA		NMBA		NDIPA		NDBA		NMEA	
	Average	CV	Average	CV	Average	CV	Average	CV	Average	CV	Average	CV
0.1	86.67	5.57	83.53	1.59	NA	NA	91.30	0.55	101.03	6.19	93.93	1.67
0.25	87.00	3.96	88.33	2.58	NA	NA	91.33	4.67	95.13	5.69	89.40	4.55
0.5	97.23	4.51	101.17	3.62	92.40	1.96	94.6	2.27	102.30	3.38	102.53	3.28
1.0	104.93	2.44	110.80	2.47	98.37	5.80	109.83	2.13	101.80	1.15	103.23	0.78

Representative accuracy and reproducibility for different concentration levels determined using the Agilent 6546 LC/Q-TOF.

Constant Resolution

Resolution independent of acquisition rate

Obtain deep-density data with multiple data points per peak.

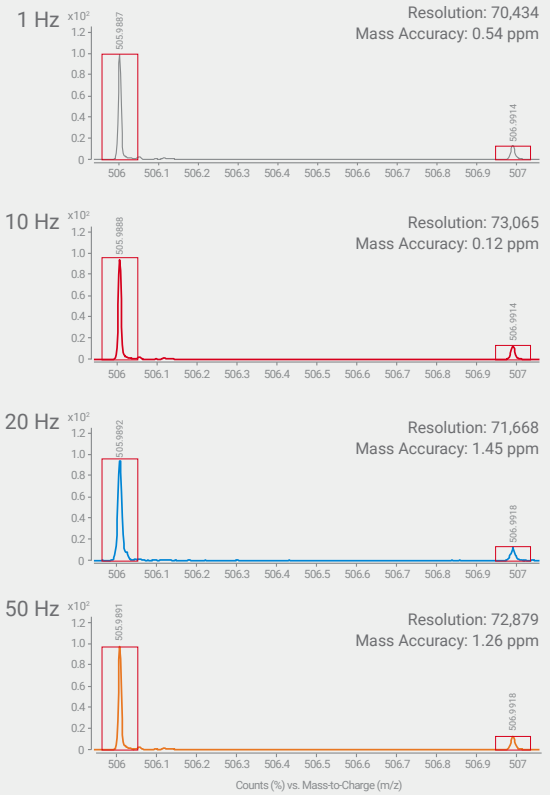
Agilent Q-TOF technology provides constant mass resolution independent of acquisition rate. This capability accommodates high-throughput, high-speed chromatography requirements where peak widths can be very narrow.

At high speeds, the resolution in ion traps drops substantially—leading to unresolved compounds and interferences—whereas Agilent Q-TOF technology offers uncompromised resolution at all speeds.

To demonstrate constant resolution, several metabolites were run at the acquisition rates of 1 to 50 Hz. The results showed consistent resolution at all rates.

See how Chris Elliott, Professor of Food Safety and founder of the Institute for Global Food Security at Queen's University, Belfast, employs mass spectrometry to help reliably analyze food fraud.

This graph shows high resolution and mass accuracy at all acquisition rates from 1 to 50 Hz for succinic acid. Resolution was independent of acquisition speed.

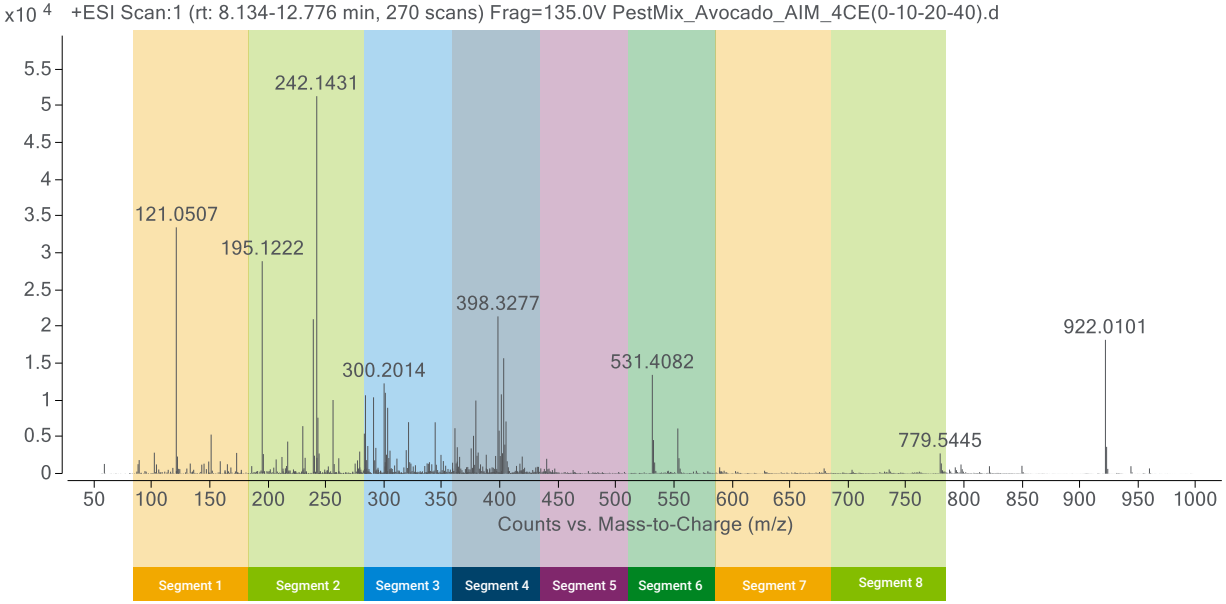


Constant Resolution

Resolution independent of acquisition rate

Perform data-independent acquisition with Q-RAI.

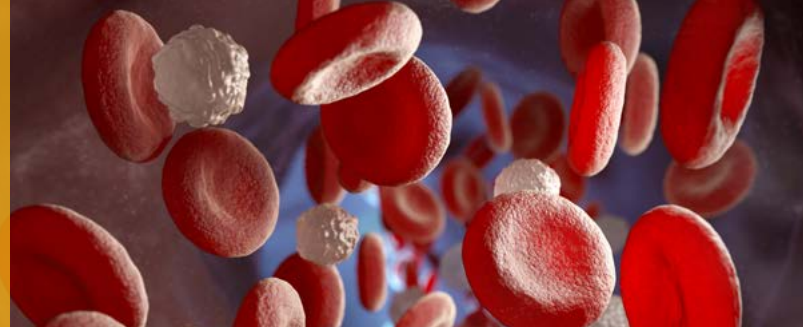
The Agilent 6546 LC/Q-TOF includes quadrupole-resolved all ions (Q-RAI), a data-independent acquisition mode that produces a less complex MS/MS spectrum than all ions experiments. In Q-RAI mode, the quadrupole filters a wide mass range before fragmentation, so the data are less noisy and more specific to the fragment origin. However, this technique requires high-speed data acquisition. Because the Agilent 6546 LC/Q-TOF acquires high-resolution data independent of speed, it is an ideal choice for Q-RAI[3].



Data obtained for avocado samples using Q-RAI mode. Quadrupole-wideband filtering reduces the complexity of fragment spectra. The user sets the number of quadrupole windows, and fragments are obtained only from those windows. With multiple windows, data need to be acquired at higher acquisition speeds while maintaining resolution.

Higher Precision

Creating low variance spectra

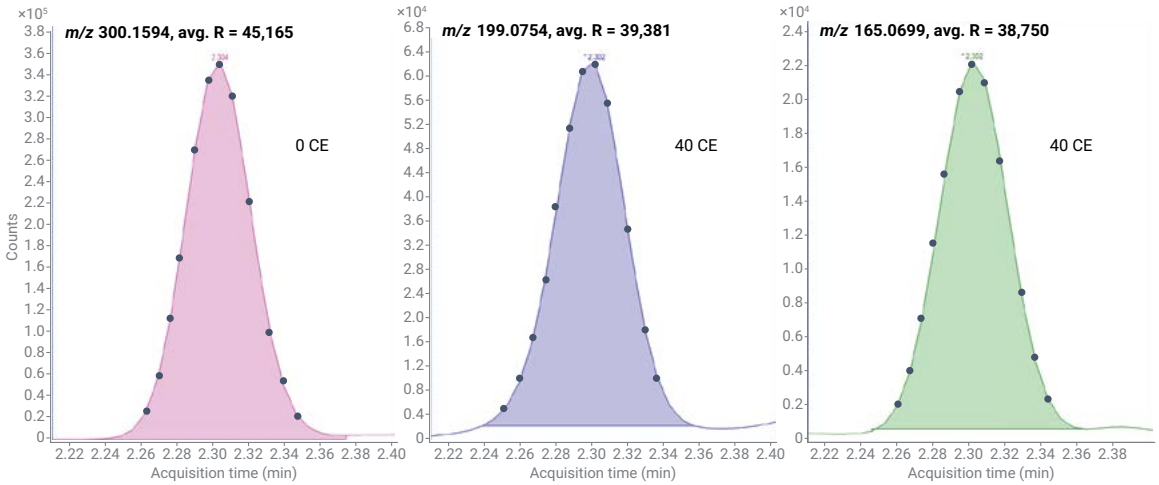


Ensure excellent chromatographic integration. Reduce spectra variability.

Multiple data points per peak are key to creating the low-variant, high-quality peaks that are essential to confident mass spectrometry results.

Unlike single-ion data collected by ion trap instruments, Agilent Q-TOF technology relies on thousands of ion transients to create a high-quality, low-variance mass spectrum. The result? Mass data with low relative standard deviations.

The following chromatographic peaks are for codeine in blood samples[4]. Multiple data points were collected across the peaks, leading to good quantitation of compounds.



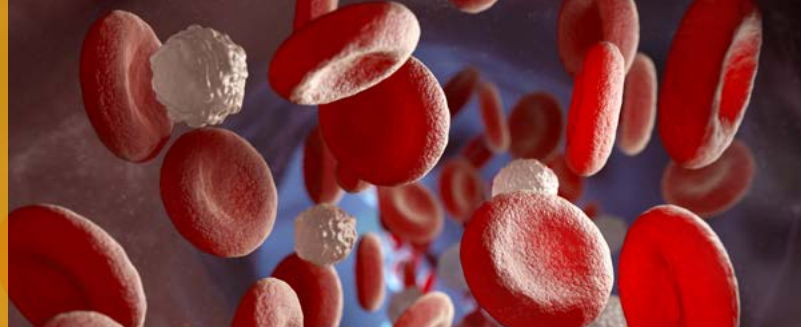
Blood sample spiked with drugs at 25 ng/mL. Chromatographic peaks of codeine precursor (m/z 300.1594, CE 0) and two fragments (m/z 199.0754 and 165.0699, CE 40). Twelve data points were collected across each peak using an acquisition rate of eight spectra/second, which provided the necessary number of spectra for quantitative integration. Even at this fast acquisition, the resolution of the precursor averaged 48,712 and the fragments had a resolution of 39,381 and 38,750.

"In our lab, the 6546 will be a great instrument for challenging applications, such as 13C metabolic flux analysis, high-throughput mass spectrometry, and metabolite identification."

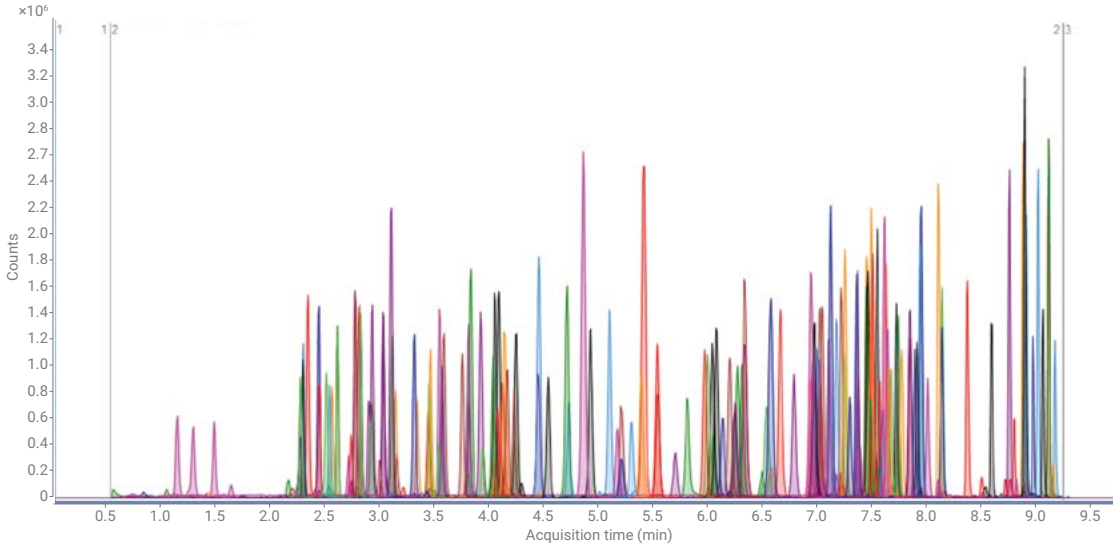
— Nicola Zamboni, head of the Swiss Federal Institute of Technology in Zurich

Higher Precision

Creating low variance spectra



This chromatograph shows clearly separated and well-integrated peaks for 153 analytes spiked in blood samples. Good separation and peak shape were achieved for all analytes.



Chromatographic separation of 153 analytes at 25 ng/ml in whole blood over a 10-minute gradient.

"The Agilent 6546 LC/Q-TOF is a major advancement compared to previous time-of-flight systems, because it combines dynamic range with excellent resolution in all conditions (speed, mass range, intensity)."

— Nicola Zamboni, head of the Swiss Federal Institute of Technology in Zurich

Isotope Ratio Fidelity

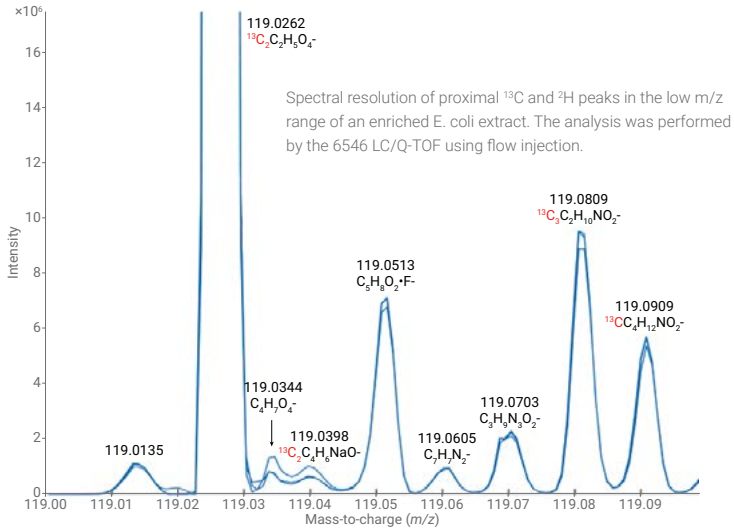
Gaining spectral accuracy

Ensure accurate isotope ratios. Perform stable isotope tracing.

Stable isotope labeling experiments can probe metabolic changes in complex biological systems. Isotope ratio fidelity, along with high resolution and mass accuracy, is essential to compound identification.

Agilent Q-TOF analog-to-digital (ADC) detection systems provide accurate isotope ratios. ADC detection records multiple ion events, allowing accurate mass assignments over a wide mass range and a dynamic concentration range. This capability helps overcome isotope ratio errors observed in ion trap instruments.

Here, the high resolution of a 6546 LC/Q-TOF enabled clear separation of most ^{13}C and ^{2}H isotopologues in crowded regions of analytes in an *E. coli* extract[1].



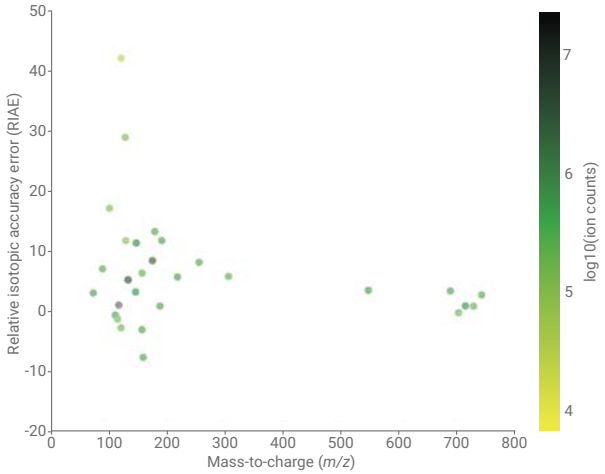
Spectral resolution of proximal ^{13}C and ^{2}H peaks in the low m/z range of an enriched E. coli extract. The analysis was performed by the 6546 LC/Q-TOF using flow injection.

Wide dynamic range | Outstanding sensitivity | Constant resolution | Higher precision | Isotope ratio fidelity | Increased productivity

Isotope Ratio Fidelity

Gaining spectral accuracy

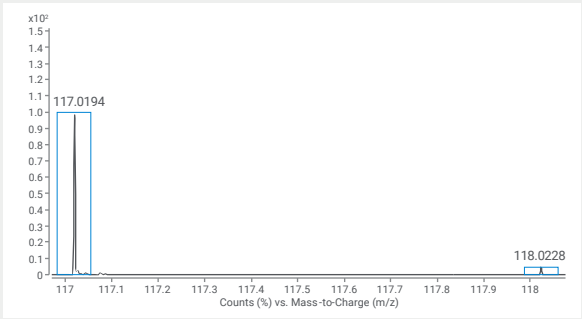
In the same *E. coli* extract study, the relative isotopic accuracy error (RIAE) was calculated for several representative metabolites. The RIAE was below 20% for most analytes.



Accuracy of isotopic measurements. The RIAE reports the percent error for the ratio between theoretical and measured M+1/M+0 RIAE values (<20 is considered excellent). Color denotes the log of the sum of ion intensity over 10 scans.

Higher RIAE values were observed only for peak ions of low intensity. M+1 ion counts accounted for about 5 to 10% of the monoisotopic peaks and fell within baseline range.

Similarly, good isotope ratio fidelity was observed for succinic acid in a yeast extract.

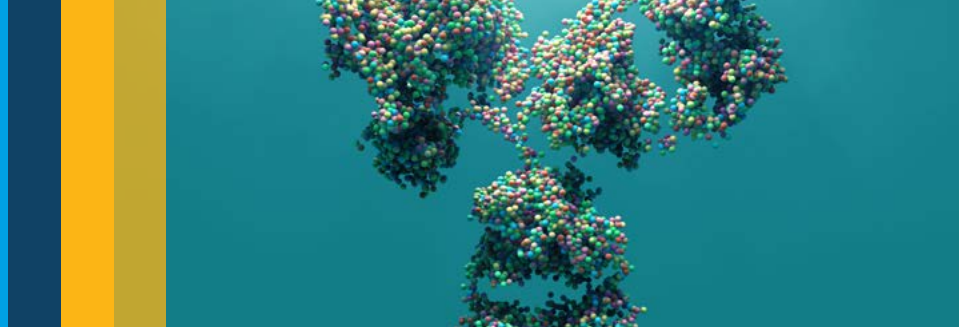


m/z	Height %	Height % (Calc)
117.0194	100	100
118.0228	4.67	4.54
119.0237	0.91	0.90
120.0269	0.06	0.04

Isotope ratio for succinic acid. Even the low-intensity isotopologues are accurately measured.

Increased Productivity

Efficient resource utilization

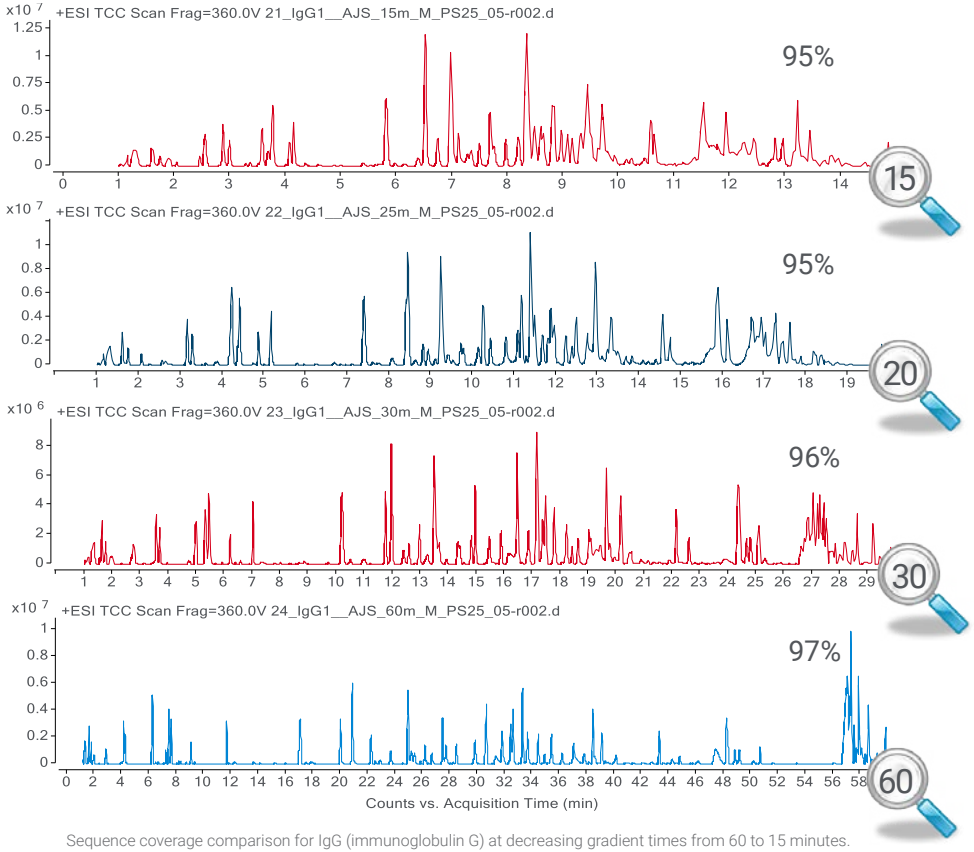


Match UHPLC resolution and speed.
Reduce peptide mapping time without losing resolution.

Data acquisition speed can be a limiting factor in maximizing UHPLC capabilities. A lab cannot fully utilize UHPLC systems if mass spectrometers do not have the capacity to acquire spectra with uncompromised data quality.

Ion traps are limited in their ability to acquire ions at a given point in time. They also suffer from resolution loss at faster speeds, such as speeds in shorter/steeper gradients. The Agilent 6546 GC/Q-TOF eliminates the requirement for trapping ions—countering the limitation posed by the number of ions that can be trapped in an ion trap. This feature enables much faster data acquisition workflows as well as complete UHPLC utilization. In addition, Agilent LC/Q-TOF systems show exceptional results at different gradient times.

The following experiment was performed to study[5] immunoglobulin G (IgG) at various gradients using AdvanceBio Peptide Mapping columns. An Agilent LC/Q-TOF showed sequence coverage between 97 and 95%, even with run times shortened from one hour to 15 minutes.

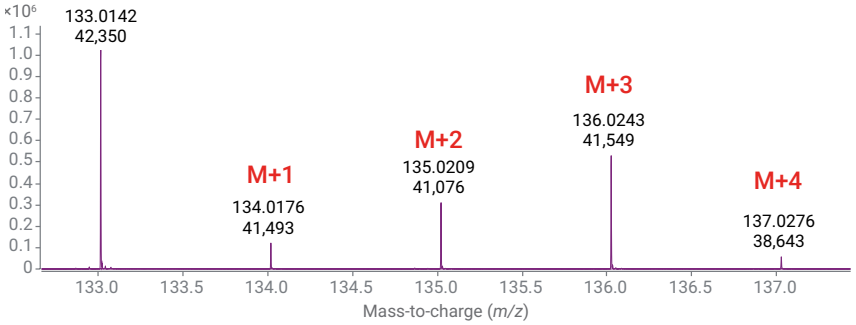


Sequence coverage comparison for IgG (immunoglobulin G) at decreasing gradient times from 60 to 15 minutes. The sequence coverage at each gradient was maintained at a high level between 97 to 95%.

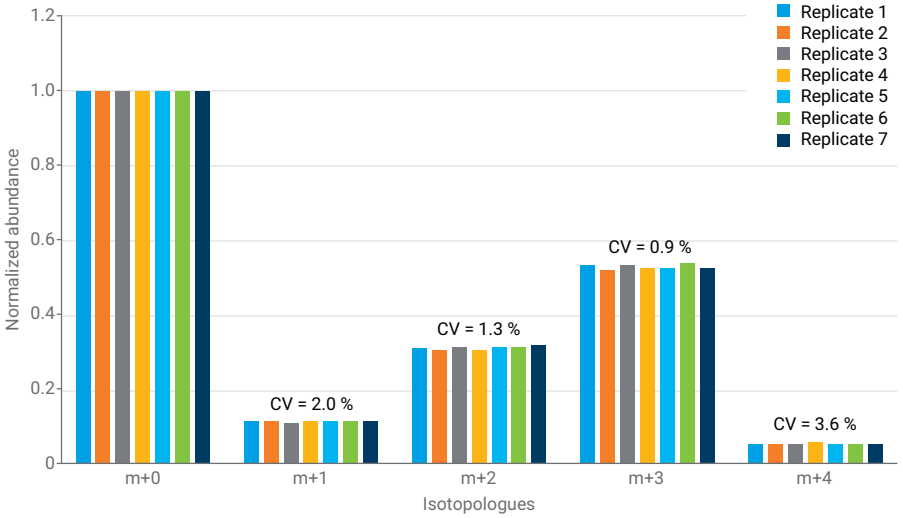
Find Answers
Beyond the Peak Shape

Agilent Accurate Mass Q-TOF instruments provide high-quality results by acquiring data with high resolution, mass accuracy, sensitivity, dynamic range, and isotopic fidelity. More precise data can enable your laboratory to obtain statistically significant results with fewer samples.

What's more, the outstanding isotope ratio performance of Agilent Q-TOF technology makes it a stable choice for isotope tracing experiments.[6]



Mass spectra of malate isotopologues with m/z and resolution labeled on each ion peak.



The coefficient of variation (CV%) of all malate isotopologues for seven technical replicates.

Wide dynamic range | Outstanding sensitivity | Constant resolution | Higher precision | Isotope ratio fidelity | Increased productivity

References

1. Agilent 6546 LC/Q-TOF: Gaining Higher Confidence and Throughput in Metabolite Analysis. *Agilent Technologies technical overview*, publication number 5994-0724EN, 2019.
2. Determination of Nitrosamine Impurities Using the High-Resolution Agilent 6546 LC/Q-TOF. *Agilent Technologies application note*, publication number 5994-1372EN, 2021.
3. Improving Food Safety Analysis with LC/Q-TOF. *Agilent Technologies brochure*, publication number DE.5521759259, 2020.
4. Drug Screening in Whole Blood Using the Agilent 6546 LC/Q-TOF and the LC Screener Tool with Automated Sample Preparation. *Agilent Technologies application note*, publication number 5994-1744EN, 2020.
5. Fast and Efficient Peptide Mapping of a Monoclonal Antibody (mAb): UHPLC Performance with Superficially Porous Particles, *Agilent Technologies application note*, publication number 5991-3585EN, 2013.
6. ^{13}C Glucose Qualitative Flux Analysis in HepG2 cells. *Agilent Technologies application note*, publication number 5994-0713EN, 2020.

Look deeper into samples than ever before

Confidently analyze target and nontarget analytes in complex matrices with Agilent Q-TOF instruments. Their simultaneous accuracy, speed, and isotopic fidelity let you minimize false positives, improve database search scores, and generate molecular formulas for unknowns.

What's more, you can move beyond targeted screening restrictions with accurate mass spectral libraries for pesticides, veterinary drugs, mycotoxins, extractables and leachables, forensics, and water contaminants.

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Agilent 6545XT
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LC/Q-TOF

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