

The Switch Is on: from triple quadrupoles and Q-TOF to Orbitrap High Resolution Mass Spectrometry

Success stories from Orbitrap users

Mass spectrometry (MS) users are continuing to make the switch from low resolution MS to high-resolution accurate mass (HRAM) MS systems. Specifically, scientists in routine labs are turning to Thermo Scientific™ Orbitrap™ mass analyzer technology. With its ease of use, robustness and performance, Orbitrap technology is now utilized more widely for applications in forensic toxicology, food safety, drug monitoring research, pharmacokinetics, environmental analysis, as well as various qualitative analyses in pharma and academia. What is it about Orbitrap technology that is so attractive to scientists?

The real power of the Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ series platforms, is that **they deliver qualitative and quantitative analysis in one instrument** (and even more in one method and one analytical run). Historically, users have relied on time-of-flight (TOF) instruments for screening and triple quadrupole systems for quantitation. A number of publications¹ have demonstrated that the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometers, are as sensitive as traditional triple quadrupole mass spectrometers. In addition, the Q Exactive Focus system provides industry leading capability for qualitative screening applications.

The Q Exactive and Q Exactive Focus mass spectrometers are used extensively for the quantitation of small molecule analytes in a broad range of applications because users are able to operate the instrument in three different targeted quantitation experiments:

- Full-scan MS
- Selected ion monitoring (SIM) - single ion detection
- Parallel reaction monitoring (PRM) - similar to MRM on a triple quadrupole MS

Both SIM and PRM are typically the most sensitive types of experiments enabling up to six orders of linear dynamic range, and up to five orders of intra-scan dynamic range. With SIM and PRM the LOD and LOQ typically go down to ppt levels and near perfect linearity.²

The Q Exactive Focus mass spectrometer provides the capability to operate in full scan acquisition to capture all of the ions in one run, overcoming the limitations of triple quadrupoles without compromising quantitative performance. Additionally, due to its high and ultra-high resolutions, from 70,000 up to 280,000, the Q Exactive series MS can resolve almost any matrix interference from analytes of interest, as well as determine fine isotopes (³⁴S, ¹⁸O, ¹⁵N etc.) for easier identification, see Figure 1. There is no time-of-flight instrument on the market that can match the performance of the Q Exactive series MS.³

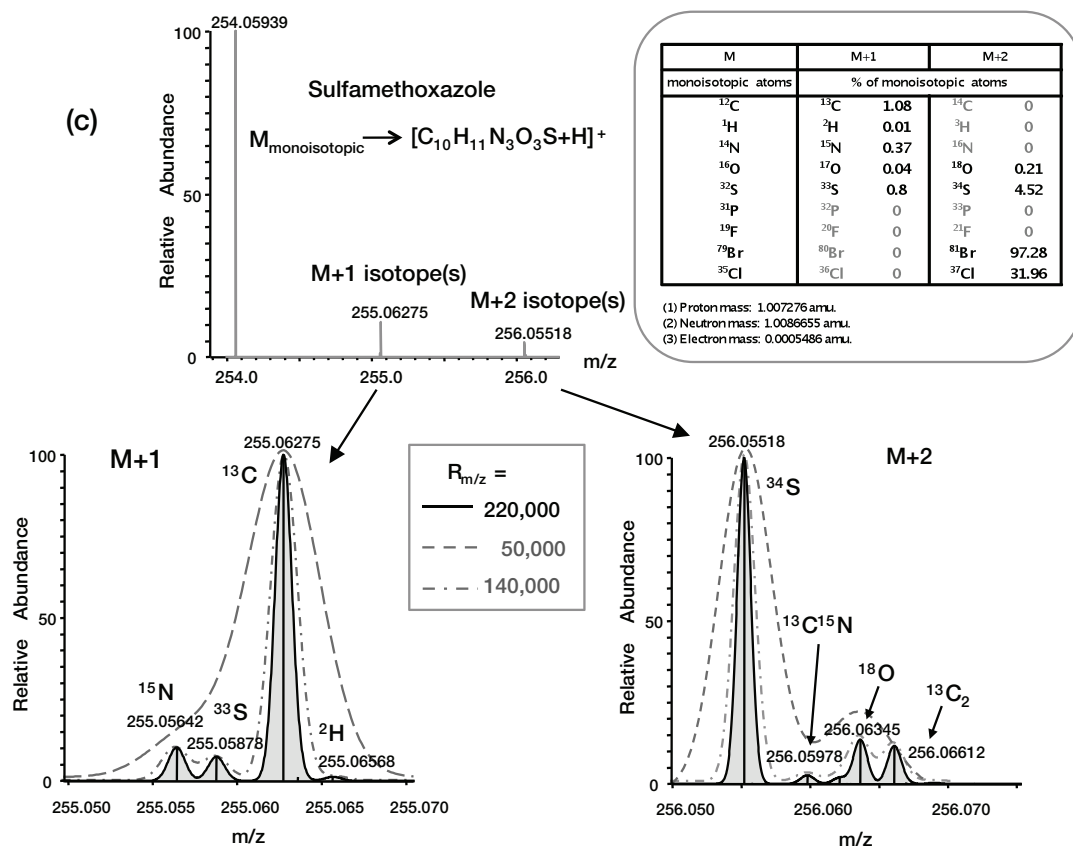


Figure 1. FID examples. Fine Isotopic Distribution (FID) of sulfamethoxazole ($[C_{10}H_{11}N_3O_3S+H]^+$) at M+1 and M+2 isotopes and for 3 different $R_{m/z}$. High, and especially ultra-high, resolution can resolve ³³S, ¹⁵N and ¹³C isotopes in M+1 as well as ³⁴S, ¹³C¹⁵N, ¹⁸O and ¹³C₂ isotopes. It also facilitates the determination of chemical compositions of unknown compounds. On the right, atom isotopes and their abundances are depicted.

The power of these systems is realized by scientists who expect and require more from their results. Thus, a steadily increasing collection¹ of peer-reviewed publications highlighting the benefits of Orbitrap mass spectrometer-based qualitative and quantitative applications continues to grow. In an overview of these publications, five important themes emerge:

- Easy method development for multi-residue analysis, especially in complex matrices
- Easy troubleshooting with the detection of all adducts, degradation products and contaminants
- Higher detection specificity of the analytes of interest
- The capability to analyze data retrospectively for new compounds of interest without re-running samples
- Simultaneous quantitative and qualitative analysis

	Orbitrap	QQQ	Q-TOF
Sensitivity	Green	Green	Yellow
Resolution	Green	Red	Yellow
Identification	Green	Yellow	Yellow
Unknowns	Green	Red	Yellow
Selectivity	Green	Yellow	Red
Quantitation	Green	Green	Yellow
Retrospective data mining	Green	Red	Yellow
Ease of troubleshooting	Green	Yellow	Yellow
Cost	Yellow	Green	Yellow

Table 1. A comparison of triple quadrupole, Q-TOF and Orbitrap technology as a function of instrument performance.

Customer success stories

The laboratory of Dr. Susanne Ekroth from Swedish National Food Agency (NFA) is benefiting from the combined benefits of quantitation and unknown screening using Orbitrap technology. Dr. Ekroth uses the Q Exactive Focus MS for its ability to quantitate target analytes, as well as to screen for unexpected pesticides, all in the same run. Dr. Ekroth points out that such technology provides a more efficient workflow by using one system instead of two: an LC-MS/MS for quantitation and a second HRAM instrument for unknown screening.



Dr. Susanne Ekroth

Dr. Ekroth states: "Orbitrap technology, as well as the software, is far more powerful and easy to handle than our LC-Q-TOF."

Her experience includes calibration curves with very good correlation factors for several compounds down to 0.001 mg/kg (0.0005 µg/ml). Thermo Scientific™ TraceFinder™ software with its compound database provides a good structural overview as well as ease of use.

With LC-QQQ-MS/MS technology there are limitations with the number of analytes that can be included with the acquisition method. This is due to the duty cycle of hundreds of ions during an LC-MS run. Therefore, the method is divided into two separate LC-QQQ-MS/MS runs, in order to cover the full range of target analytes. However, with the Q Exactive Focus, both methods can be combined into one LC-MS/MS analysis. "This allows higher laboratory throughput, less data manipulation, fewer chances for interpretation errors, and a better global view of what is in the samples and faster result delivery, making the laboratory more efficient," says Dr. Ekroth.

According to Dr. Ekroth, one interesting aspect has been the variable data-independent acquisition (vDIA)* capability which provides fragmentation data of everything in the sample throughout the LC-MS run within a limited m/z range. This aids in the analysis of complex matrices and enables compliance with the SANTE guidelines for pesticide identification. An additional benefit is that it enables retrospective analysis of unexpected residues present in your samples.

* vDIA mode is not available in the United States of America



Dr. Bertrand Rochat

Dr. Bertrand Rochat (based in CHUV, Lausanne, Switzerland) is on the front line of absolute quantification using HRMS Orbitrap MS. His last review published in Trends in Analytical Chemistry⁵, advocates that high-resolution mass spectrometry can be used for both targeted quantification of

metabolites as well as for untargeted metabolomics. Similar to colleagues in various fields, Dr. Rochat was interested in knowing if the Q Exactive Focus could perform targeted quantitation as good as a triple quadrupole MS for his clinical research applications. Dr Rochat's lab performed the following on one Q Exactive Focus:

- Absolute quantification of hepcidin
- Absolute quantification of 25-hydroxyvitamin D3
- Quantitative method validation
- Simultaneous quantitative and qualitative analysis for drug quantification
- Simultaneous quantitative determination and biotransformation study of anticancer agents
- Drug metabolite identification and profiling
- Global screening (HRAM-full scan) of pollutants and drugs in toxicological samples
- Discovery of cancer biomarkers (untargeted metabolomics)

Dr. Rochat was impressed with the ability of the Q Exactive Focus to look for unknown compounds and to quantify known molecules in a wide variety of matrices. Using full scan acquisition modes, he has tested many compounds in many matrices for detection sensitivity, selectivity and robustness.^{5,6} In his own experience, Dr. Rochat has demonstrated that high-resolution mass spectrometry is comparable to triple quadrupole mass spectrometry for quantification, but outperforms triple quadrupole mass spectrometry for qualitative analysis.

Today, Dr. Rochat truly believes that, "Once you work with the Q Exactive Focus mass spectrometer, it is *really hard* to go back to a low resolution instrument."

"One of the major benefits of accurate mass analysis over triple quadrupole analysis is the ease with which new compounds can be added to an analysis," says Dr. Rochat. To add a new compound to a triple quadrupole analysis generally requires optimization using a reference material. This is not necessary for Orbitrap high-resolution accurate mass MS: compound optimization is not needed as a generic method with sufficient mass range, resolution setting and collisional energy can be used, saving time and effort.



Ali W. Haghani

"The diverse application capabilities as well as the ease of creating a variety of workflows makes the Q Exactive the gold standard technology in our laboratory. The system is used to analyze the polar compounds required for EPA compliance samples that need MS² data. However, its power and versatility is in the ability to quantify and unequivocally confirm emerging contaminants in a variety of water matrices from drinking water to waste water when concentrations are extremely low as well as the capability to identify transformational products and non-targeted site-specific emerging contaminants" says Ali W. Haghani, R&D and Technical Manager for the LC-MS Department at Eurofins Eaton Analytical, Inc.

Additional scientific endeavors by Dr. Haghani are published in the following article: Publication on Quantitative Comparison of Hormones in Drinking Water Between Low and High Resolution Mass Spectrometry.⁷



Dr. Simon Hudson

Full scan data acquired is fully retrospective enabling old data to be reviewed to search for new analytes or analytes not previously included in the initial experiment. "This is not possible using triple quadrupole instruments," says the technical director Dr. Simon Hudson at LGC Group, based in Cambridgeshire, U.K. In addition,

he adds that the Q Exactive Focus has the mass stability common to all Orbitrap systems and as a result, will operate very successfully with a single daily, or weekly calibration.⁸ Internal lock masses can be used to further improve mass measurement and stability.

According to Dr. Hudson, a feature of the Q Exactive Focus MS, as with all instruments in the Q Exactive range, is the ability to rapidly switch polarity. Though possible for MS/MS systems, this is not possible with Q-TOF systems. "Hence, the Q Exactive Focus can do twice the amount of work that a Q-TOF system can do when both positive and negative modes are required," according to Dr. Hudson. Whether you need quantitation or screening for unknown analytes, switching polarity is important.

Scientists at Admescope Ltd. based in Oulu, Finland, have stated that as a response to increased demand for drug metabolism services, they installed a second Thermo Scientific™ Q Exactive mass spectrometer. "The Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer is a perfect tool for metabolite profiling and identification workflows due to its excellent dynamic range and spectacular sensitivity and specificity, also well suited for quantitative bioanalysis".⁹



Professor Mario Thevis

Professor Mario Thevis, head of the Center for Preventative Doping Research at the German Sport University Cologne, articulates that "over the past decade, a trend from employing nearly exclusively low resolution (tandem) mass spectrometry towards high resolution/high accuracy (tandem) mass spectrometry has been

recognized in doping controls. The underlying reasons are certainly manifold, among which the increasing use of alternative test matrices (e.g. dried blood spots) and the concomitant need to comprehensively and sensitively test for target analytes certainly fueled the growing utilization of systems such as the Q Exactive mass spectrometer."

Dr. Barbara Bojko, from Nicolaus Copernicus University in Poland, Department of Pharmacodynamics and Molecular Pharmacology, utilizes a workflow to search for unknown drug metabolites for their metabolomics and lipidomics research. These clinical and pharmaceutical applications use microextraction followed by unknown screening using the Q Exactive Focus MS.

According to Dr. Bojko, "While utilization of triple quadrupole technology is also used for targeted analysis, a key aspect of drug metabolism is the determination of unknown metabolites, which require an alternative research tool." Triple quadrupole MS/MS technology is poorly suited for unknown screening.



Dr. Barbara Bojko

“This aspect is what we appreciate in the Q Exactive Focus instrument - a chance to perform retrospective analyses on top of planned experiments. For instance, currently this lab is involved in a few projects on brain-related studies.” In such analyses, Dr. Bojko’s team looks at neurotransmitter changes. Due to the complexity of brain function, metabolite screening is vital to the

overall understanding and can shed new light on brain metabolism and brain function. The Q Exactive Focus is very sensitive reaching low ng/mL detection levels.¹⁰

Conclusion

Orbitrap mass analyzer technology has been successfully used for the quantitation of both small and large molecule analytes in a wide range of clinical research, forensic toxicology, pharmaceutical discovery, environmental analysis, and food testing applications. As observed in numerous publications and customer testimonials, the popularity of performing quantitative, qualitative and screening analyses using Orbitrap mass analyzer technology is growing rapidly.

This is due to the Orbitrap mass analyzer’s unique ability to provide high resolution accurate mass and both quantitation and qualification (accurate screening) data in a single analytical run. The quality of data produced provides ultimate confidence in the results obtained in a wide variety of qualitative and quantitative applications. High resolving power increases analytical selectivity for compounds in complex matrices and thus reduces the uncertainty associated with the detection of false positives. The unique ability to capture all relevant data using full scan allows retrospective data analysis, reducing the need for additional sample injections..

In addition, Orbitrap mass analyzers are enabling scientists to raise their productivity to new levels. Compared to triple quadrupole MS-based quantitation methods that involve time consuming optimization of hundreds of SIM or SRM transitions in numerous time windows, Orbitrap mass analyzer methods for the quantitation of virtually unlimited numbers of compounds are fast and easy to set up. Calibrations are easy to perform and remain stable for days. Fast scanning and spectral multiplexing capabilities make the instrument fully compatible with UHPLC and fast chromatography techniques. With several operating modes the Q Exactive mass spectrometers provide unmatched analytical versatility to meet the needs of routine laboratories today and into the future.

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