

# LC-MS innovations: review of the technology and its advancements

## Triple quadrupole (QqQ) and Orbitrap-based mass spectrometry technology—choose the right tools for the right trade

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### Summary

Separation technologies, such as liquid chromatography (LC) coupled with mass spectrometry (MS), are the principal analysis techniques for identifying and quantifying compounds. The ever-increasing demands of modern analysts, and novel application workflows, have led to the evolution of these instruments. When working with complex matrices, [triple quadrupole \(QqQ\) mass spectrometers](#) have established popularity in quantifying analytes at very low detection limits, with specificity and selectivity. Similarly, [high-resolution accurate-mass \(HRAM\) spectrometers](#) are also known for their outstanding selectivity, specificity, and sensitivity when working with challenging matrices.

The ability to develop robust and reliable assays with both QqQ and HRAM systems has made these technologies a common choice when it comes to quantitation of analytes in complex matrices. Both technologies offer unique advantages, enabling supreme confidence in the quantitation of a variety of molecule types. Regardless of the chosen technology, we are still facing challenges in the development of robust and appropriate methods, the time taken to become operational, reducing cost per sample, etc. This paper outlines the latest developments with both of these MS techniques and highlights the suitability of each for varying application demands and challenges.

## What is triple quadrupole MS?

In mass spectrometry, quadrupoles are responsible for filtering ions by applying an oscillating electric field that results in the separation of ions from the sample based on their mass-to-charge ratios ( $m/z$ ). In this way, single quadrupole mass spectrometers are adept at selecting a specific mass from a sample. However, they might have some limitations when required to differentiate between molecules that have the same mass, or discerning between analytes in a complicated mixture, or in a complex matrix. When dealing with a complex sample matrix, isobaric masses that are produced in the source could also be selected along with the ion of interest and enter the analyzer. This will modify the data collected, alter the peak shape, and affect the validity of the results. This collection of unwanted isobars is referred to as similar mechanism interference. It broadens the chromatogram peaks to resolutions that would not be acceptable across almost every type of application.

Fortunately, quadrupoles lend themselves to being coupled to one another. Despite being known as 'triple quadrupole' instruments, a QqQ system is actually a tandem quadrupole mass analyzer. The two quadrupole mass analyzers are referred to as Q1 and Q3 with q2 acting not as an analyzer, but as a collision cell. When the triple quadrupole is operating, a precursor ion is selected in Q1 along with other isobaric masses. The molecular ions then enter the q2 collision cell, along with neutral gas molecules such as helium or nitrogen. The selected masses are then bombarded with the gas until they fragment to produce unique masses from each of the ions selected in Q1. This is known as collision-induced dissociation (CID). The mass or masses of interest are then selected in Q3, and the other interfering masses that were produced are discarded. This method yields greater structural information, along with enhanced selectivity and sensitivity, and ensures a sharp, clean signal. Figure 1 indicates this process when used in conjunction with an LC-MS.

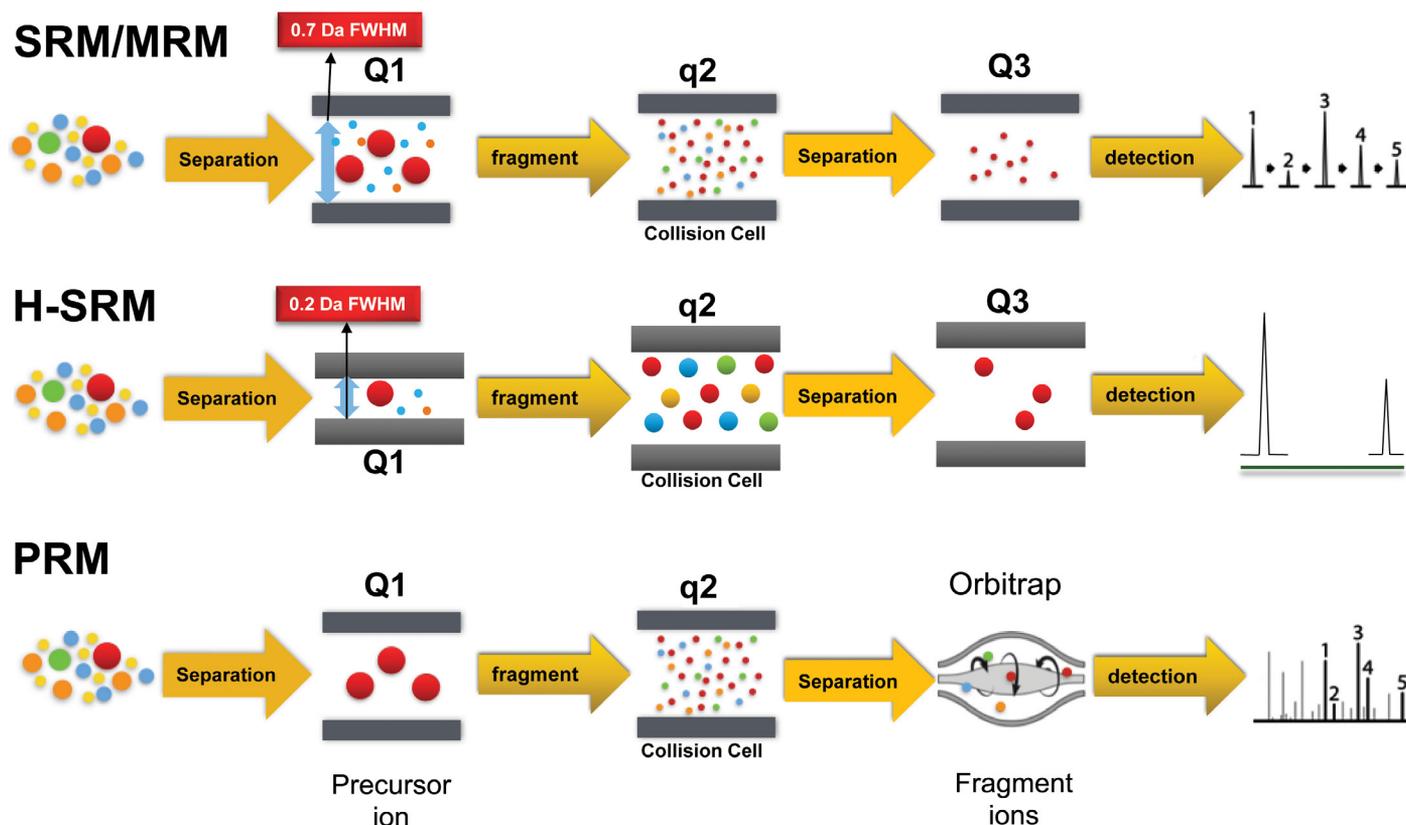


Figure 1. A schematic of a triple quadrupole.

## Advantages of triple quadrupole MS

Triple quadrupole setups can be used in scanning or filtering mode depending on the analysis requirements. A full scan mass spectrum is likely to only be used for qualitative analysis, because in full scan mode, quadrupoles rarely offer the same sensitivity as that in selected-ion monitoring (SIM) mode. If quantitative studies are to be undertaken, the quadrupoles will have to work in filtering mode. The most selective mode that a single quadrupole MS can operate is SIM, where the quadrupole functions at a fixed voltage, meaning that only an ion with a specific mass-to-charge ratio will be selected, and all ions with a different  $m/z$  will be filtered out.

An advantage of triple quadrupole MS is that it can be operated in selected-reaction monitoring (SRM) mode for quantitative analysis. In SRM mode, the tandem quadrupoles act as a double mass filter, which in turn dramatically reduces background noise, and increases the selectivity of the instrument. In addition, some of the more advanced triple quadrupoles offer high resolution selected-reaction monitoring (H-SRM), in which the Q1 and Q3 width can be varied between unit resolution (0.7 Da FWHM) and a much lower width (0.2 Da FWHM). The ability to modify the Q1 and Q3 widths offers additional selectivity while also increasing the signal-to-noise ratio. [Thermo Scientific™ TSQ Altis™ triple quadrupole MS](#) and [Thermo Scientific™ TSQ Quantis™ triple quadrupole MS](#) systems are designed with this unique H-SRM feature, along with extremely fast scan speeds (600 SRMs/s). Both the TSQ Altis MS and TSQ Quantis MS systems are equipped with segmented quadrupoles for better ion transmission and management.

The features and benefits found in triple quadrupoles allow for faster, more efficient, and more robust method development, while ensuring increased throughput. With their higher sensitivity, triple quadrupoles allow for increased confidence in quantitation for multiple molecules in a variety of complex matrices. SRM based quantitation is extremely sensitive, as well as being reliable and suitable for analyzing large sample numbers. In addition, SRM with short dwell times allows for the detection of many different transitions.

The first step of any MS experiment is to produce the selected reaction ions. This involves setting up experiments to identify precursor masses and product masses, as well as optimizing the collision energies, which is a lengthy and laborious process. This time-consuming

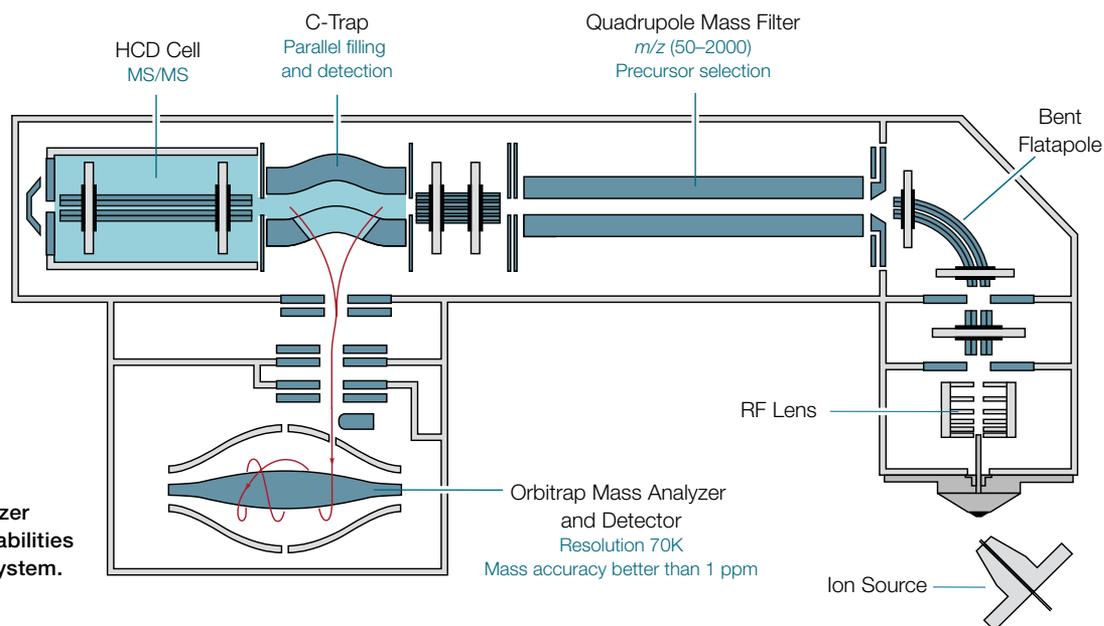
procedure can be avoided by using a triple quadrupole instrument which allows for automated optimization of selected-reaction monitoring methods. For example, the [Thermo Scientific™ XCalibur™ software](#) of the TSQ Altis LC-MS/MS and TSQ Quantis LC-MS/MS systems ensures faster method development and optimization of a host of molecule types in variable and complex matrices.

## What is high resolution Orbitrap MS?

Another option in state-of-the-art MS is to link a quadrupole analyzer to a [Thermo Scientific™ Orbitrap™ mass analyzer](#), to achieve high resolution spectra. Orbitrap mass analyzer technology is capable of screening, confirming, and quantifying all in the same run, and allows for improved resolution and selectivity of the compounds. In this way, Orbitrap mass analyzer instruments have advantages over alternative high resolution technologies (such as Quadrupole Time-of-Flight (QTOF technologies)) in that they can identify all compounds in a complete matrix, achieve accurate quantitation with excellent dynamic range, without detector saturation and experimental flexibility<sup>1</sup>.

Orbitrap mass analyzers are comprised of two outer electrodes wrapped around a central electrode and in this configuration, it acts as both a mass analyzer and detector. When ions enter the Orbitrap mass analyzer they are captured by a process called 'electrodynamic squeezing'. These captured ions then revolve around the Orbitrap mass analyzer's central electrode and between the two outer electrodes. The charge differences of the ions generated mean that they will oscillate at different rates which results in mass separation.

The unique high resolution that is achievable by the Orbitrap mass analyzer is due to the nanometer-range accuracy of the electrodes, the high voltage that is supplied, and the mass-to-charge measurements that can be measured as a function of the frequency of the ion's oscillation. Most analyte compounds will contain certain amounts of isotopic elements, for example C<sup>12</sup>, C<sup>13</sup>, C<sup>14</sup>. The Orbitrap mass analyzer system can resolve all these different isotopes and differentiate between them, giving distinct peaks or spectra related to that compound of interest. Figure 2 indicates an Orbitrap mass analyzer setup, with the [Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ MS](#) system as an example.



**Figure 2. Orbitrap mass analyzer setup—performance and capabilities of the Q Exactive Focus MS system.**

### Advantages of high resolution Orbitrap MS

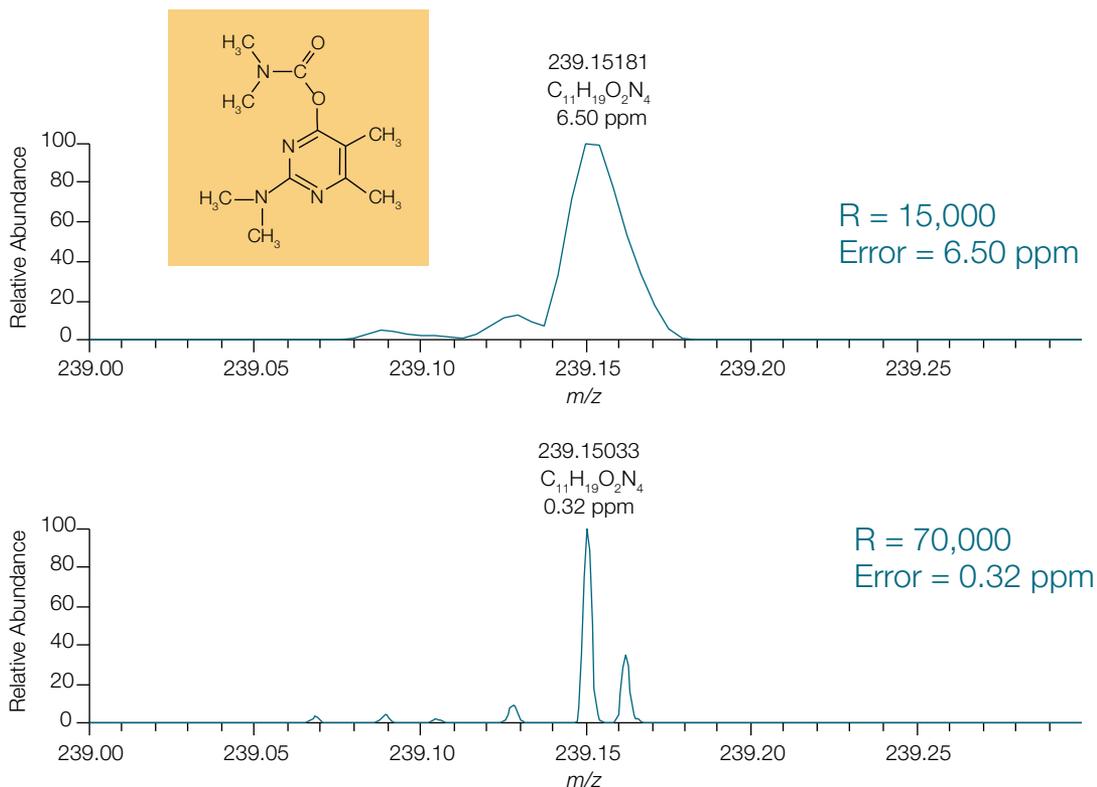
High resolution setups based on Orbitrap mass analyzer technology can collect data in full scan, MS/MS, SIM, or parallel reaction monitoring (PRM) modes. PRM operates by performing a full scan of each transition by a precursor ion, in other words, parallel monitoring of all fragments from the precursor ion, unlike selected-reaction monitoring which only performs one transition at a time. The Orbitrap mass analyzer replaces the Q3 from the triple quadrupole set-up, and scans all of the product ions with high resolution, accuracy, and efficiency of operation. This means that the parallel reaction monitoring technology has the same selected-reaction monitoring quantitative analysis capabilities, but can also perform qualitative analysis.

The mass accuracy that is achievable with an Orbitrap mass analyzer is at or below ppm levels—eliminating the background interference and false positive data to a greater level than SRM methods. It also improves the detection limit and sensitivity in all kinds of samples types and matrices. Full scan of the product ions is possible without the need to select the ion pair and optimize the fragmentation energy, meaning that it is easier to establish the assay. There is also a wider linear dynamic range that is available—increased by 5 orders of magnitude.

When the Orbitrap HRAM-MS system is operating in a screening mode, it is examining four criteria: exact mass, retention time, isotopes and fragment ions. Once the scan is complete, the mass spectra with isotopic pattern can be imported into a library search to identify the compound. Whereas a triple quadrupole or a single quadrupole system would only see one peak in

detection, a high resolution system can look at multiple peaks at the same time, and give multiple spectra related to different compounds mixed in the sample. High resolution is therefore very important, since it enables similar analytes that have only very subtle changes in their structure to be differentiated, as demonstrated in Figure 3. The main advantage of this is the ability to determine the elemental composition of individual molecular or fragment ions, creating a powerful tool for structural elucidation or confirmation. Furthermore, it is possible to repetitively analyze extremely low concentrations and quantitate (as well as identify) compounds using this technique.

In addition to this, an unknown peak can also undergo retrospective analysis. Once the data has been acquired and stored, various libraries and databases can be used to find the compound based on the experimental fragmentation of the compound of interest. This is a powerful tool for discovering unknown compounds or metabolites present in a sample. Software such as [Thermo Scientific™ Compound Discoverer™ software](#) and [Thermo Scientific™ Sample Profiler™ software](#) can match fragmentation spectra against the [mzCloud™](#) spectral library, which is regularly updated with a wide variety of analytes of interest. Databases such as ChemSpider™ can also narrow down compound searches by molecular weight or formula. There is also the possibility to build a compound database specifically tailored to the needs of a particular analysis with Thermo Scientific™ mzVault™ spectral library, or the opportunity to download a premade database and import it into the high resolution setup.



**Figure 3. Standard pesticide mixture in horse feed matrix.** Unusually high error after 6.5 ppm: acquired at low resolution (ca. 20,000 (FWHM) at  $m/z$  200, typically obtained on Q-TOF instruments) indicates the need for higher resolution re-analysis at 70,000 (FWHM) at  $m/z$  200 resolving power.

### Applied MS technology considerations

Some important factors to consider when implementing MS technologies in analytical applications are:

- **Sensitivity**—the ability of the technique to detect low-level signals
- **Selectivity**—the ability of the technique to detect the analyte(s) in a complex matrix without interference from other compounds in that matrix
- **Flexibility**—the ability of the technique to detect all kinds of sample types and matrices
- **Speed**—the ability to address throughput requirements where needed
- **Reliability**—the ability to be robust enough for everyday use while ensuring high reproducibility of results
- **Cost per sample**—the ability to constantly address organizational profitability goals by reducing the cost of analysis per sample
- **Upcoming analytical demands**—the ability to address sample complexity and regulatory requirements regardless of the user expertise

As well as these primary drivers, there are several additional factors to consider. For example, if the analyst is less experienced, they may prefer a more user-friendly system with established support from the respective IT team; it may be important that adding and expanding target analytes of interest is easy to do; there may have to be a certain amount of industry acceptance of the technology for the particular application. The most suitable MS technology is down to a combination of many factors.

As a further comparison of modern MS techniques, Table 1 highlights the critical benefits of each technology.

**Table 1. A summary of the current abilities of HRAM and QqQ techniques.**

Needs	HRAM	QqQ
Untargeted screening	●	●
Targeted screening	●	●
Untargeted and targeted quantitation	●	●
Targeted quantitation only	●	●

Least Fit ● ● ● ● ● Best Fit

## Conclusion

For sensitive, fast, targeted quantitation of analytes, triple quadrupole systems can be ideal for analysis, owing to their high sensitivity offerings, unmatched speed, robustness, and ease-of-use. Whereas for retrospective searching of compounds, as well as screening and quantitation of both targeted analytes and unknowns, HRAM-Orbitrap mass analyzer systems can prove to be aptly suited and critical for unknown analysis.

The ability to perform high resolution analysis is important for almost all applications. Any sample that is generated from complex matrices will also contain a number of background ions as well as the analytes of interest. So, in order to perform quantitation with confidence, high selectivity is necessary. In this way, Orbitrap mass analyzers allow for high resolution capabilities that can separate the compounds of interest and the sample background interferences.

The ability to proactively screen for both targeted analytes and unknowns is also incredibly useful for a host of applications, such as the detection of pesticides within food, contaminants in water, drugs of abuse in forensic toxicology, enhancement drugs in sports anti-doping and drugs/vitamins and their metabolites in clinical research and drug development. Features such as H-SRM in triple quadrupole systems are being leveraged more and more for quantitation of complicated mixtures and large molecules in complex matrices. For targeted quantitation of known analytes, sensitivity is an absolute requirement. Also, with the growing complexity of molecules, mixtures, and matrices, there is an ever-increasing demand for robustness, reproducibility, and reliability for all assays,

regardless of the expertise of the analyst. When an analyst is trying to determine the concentration of a known analyte, triple quadrupole MS instruments set the standard for quantitation, because they are known for their robustness and ability to deliver ultimate sensitivity. Due to its high sensitivity and selectivity, it allows analysts to confidently identify and quantify trace-levels of contaminants, while its reliability ensures that it is able to perform routine, high-throughput analysis cost-effectively because it enables hundreds to thousands of analytes to be analyzed and distinguished easily in a single run unlike other technologies available today.

Analyses by mass spectrometry, despite being a popular choice, has become increasingly intricate and complicated. The need to address the scientific challenges, while achieving business goals, is common for every analytical laboratory. To keep with the growing demands, one type of MS might not be sufficient for all assays undertaken. Both triple quadrupole and high resolution Orbitrap mass analyzer systems offer several unique benefits, and selecting the right technique should be done based on the requirements of the assay.

## References

1. Rajski Ł, Gómez-Ramos Mdel M, Fernández-Alba AR (2014) [Large pesticide multiresidue screening method by liquid chromatography-Orbitrap mass spectrometry in full scan mode applied to fruit and vegetables](#) *J Chromatog A* 1360: 119-127.

## Resources

1. [Orbitrap LC-MS Comparison chart](#).
2. [Can your QTOF keep pace with your needs?](#)
3. [Which QQQ is right for you?](#)

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