



First choice in high resolution mass spectrometry with Orbitrap mass analyzer technology for screening, confirmative and quantitative analyses

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Summary

The first generation single-stage benchtop Thermo Scientific™ Exactive™ Orbitrap™¹ mass spectrometer was launched in 2008 and was first employed primarily for small compound and protein identification. This technology was also widely adopted for routine applications, including food and environmental analysis, forensic toxicology, anti-doping analysis and drug analysis. At the time of its launch, scientists desired high resolution and high mass accuracy data for untargeted analysis using full scan mass measurement. Screening, discovery and limited number of chemical composition hits with accurate mass and all ion fragmentation were conducted and a number of publications were published on these platforms².

In 2011, a new generation of benchtop high resolution, high mass accuracy mass spectrometer (HRMS) was introduced: the Thermo Scientific™ Q Exactive™ hybrid quadrupole-Orbitrap™ mass spectrometer³, which provided ion selection and fragmentation and thereby enabled more sensitive, selective and quantitative mass measurements. This breakthrough technology quickly developed into the most sought-after benchtop high resolution instrument for research analysis in omics applications including proteomics, metabolomics and lipidomics. The Q Exactive series mass spectrometers eventually evolved into the most sophisticated and sensitive HRMS on the market: the Thermo Scientific™ Q Exactive™ HF-X hybrid quadrupole-Orbitrap™ MS⁴, which was released in 2017 with the capability to identify up to 1,100 unique peptides

per minute⁵, perform dedicated TMT scans for maximum phosphopeptide quantitation and provide reproducible identification of tens of thousands of unique peptides in minutes using data independent acquisition. In addition, this new mass spectrometer employs smart algorithms to streamline top-down characterization as well as capture a wider picture of the proteins in a sample.

For routine applications, the breakthrough came in 2014, when the most affordable Thermo Scientific™ Q Exactive™ Focus hybrid quadrupole-Orbitrap™ MS (Figure 1) was launched⁶. This technology allows a much lower sample per analysis volume with the same sensitivity and mass accuracy obtained on the Q Exactive MS. In applied analysis labs, there are frequent needs for global approaches and identifications in order to reveal and identify compounds of interests; the works of Anton Kaufmann (KLZH)⁷ and Hans Maurer (UKS)⁸ are examples from the leaders in their field. The advantages of the benchtop Q Exactive series mass spectrometers

performing full scan or targeted acquisitions, were rapidly established based on sensitivity, robustness, ease of use and excellent mass accuracy and resolution. All these advantages are key parameters for high-resolution MS instruments: fewer false positive or negative detections and high versatility, for instance.

Today, according to peer-review publications⁹ with over a thousand citations and users' testimony¹⁰, it is firmly established that Orbitrap mass analyzer technology has enabled breakthroughs in qualitative and quantitative analyses. Indeed, Q Exactive series mass spectrometers can perform targeted and untargeted, quantitative and qualitative as well as routine and research analyses with equal or better performance than competing technologies⁹. In the following sections, we summarize advantage of this technology that underline why this MS technology has become the instrument of choice for both applied and research markets.

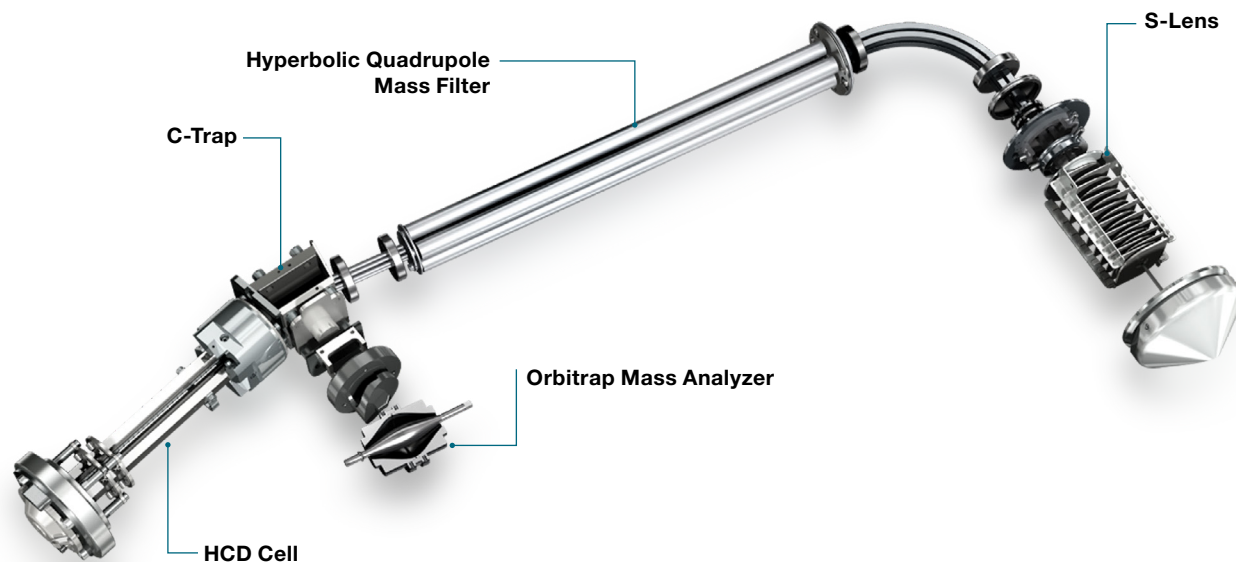


Figure 1. A three-dimensional schematic of the Q Exactive Focus MS ion path

The power of Orbitrap mass analyzer technology

Unquestionably, the biggest difference between the Q Exactive series mass spectrometer and any other high resolution instrument currently on the market, such as quadrupole-time-of-flight (Q-TOF) mass spectrometer is the high and ultra-high mass resolution (Figure 2) offered by the Orbitrap mass analyzer. It enables users to resolve analytes of interest from interferences. This key differentiator is particularly valuable for discriminating between ions of interest and interfering ions in the very low and low mass-to-charge (m/z) ranges (respectively: m/z 50–300 and m/z 300–1000), which is critical for the analysis of endogenous metabolites, xenobiotics, lipids, small molecules, and peptides, including their fragment ions.

The discrimination and resolution of ions of interest from interfering ions can be particularly advantageous for obtaining accurate mass assignment, resulting in increased confidence in the determination of elemental composition.

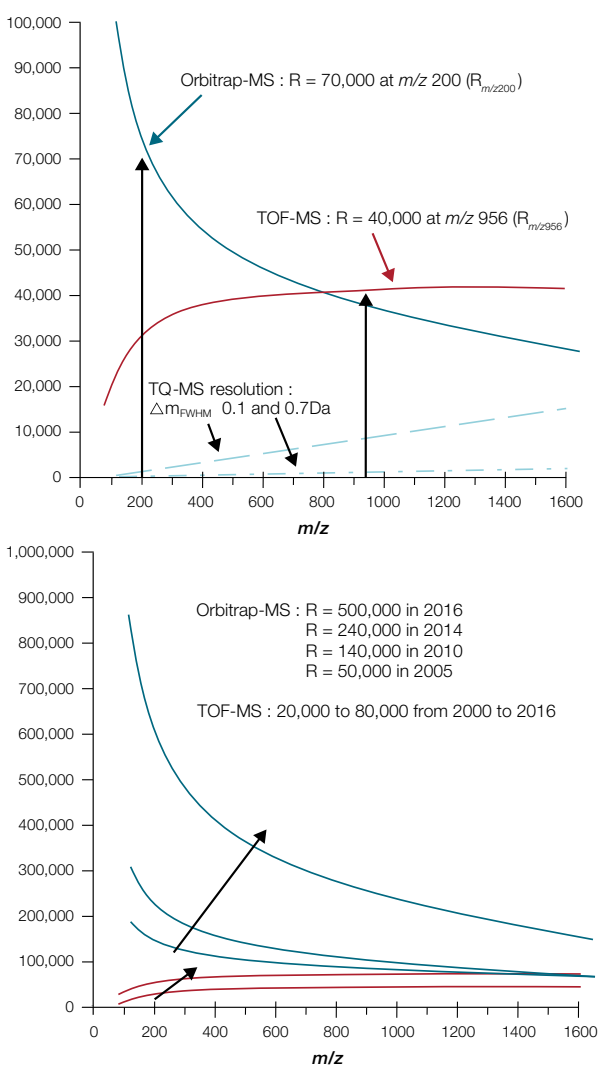


Figure 2. An illustration of resolution vs mass range for high resolution Orbitrap mass analyzers and typical Q-TOF MS.

Importance of Peak Discrimination at low m/z range

Another example of the utility of Orbitrap mass analyzer technology is its ability to discriminate between compounds of interest from interfering species at low mass to charge ratio, seen in Figure 3¹¹, where most compounds in both the ChemSpider™ (intact compounds) and/or mzCloud™ (fragment ions)¹² databases have m/z below 600 and <200, respectively. mzCloud is a state-of-the-art mass spectral database that assists analysts in identifying compounds in areas such as life sciences, metabolomics, pharmaceutical research, toxicology, forensic investigations, environmental analysis, food control and various industrial applications.

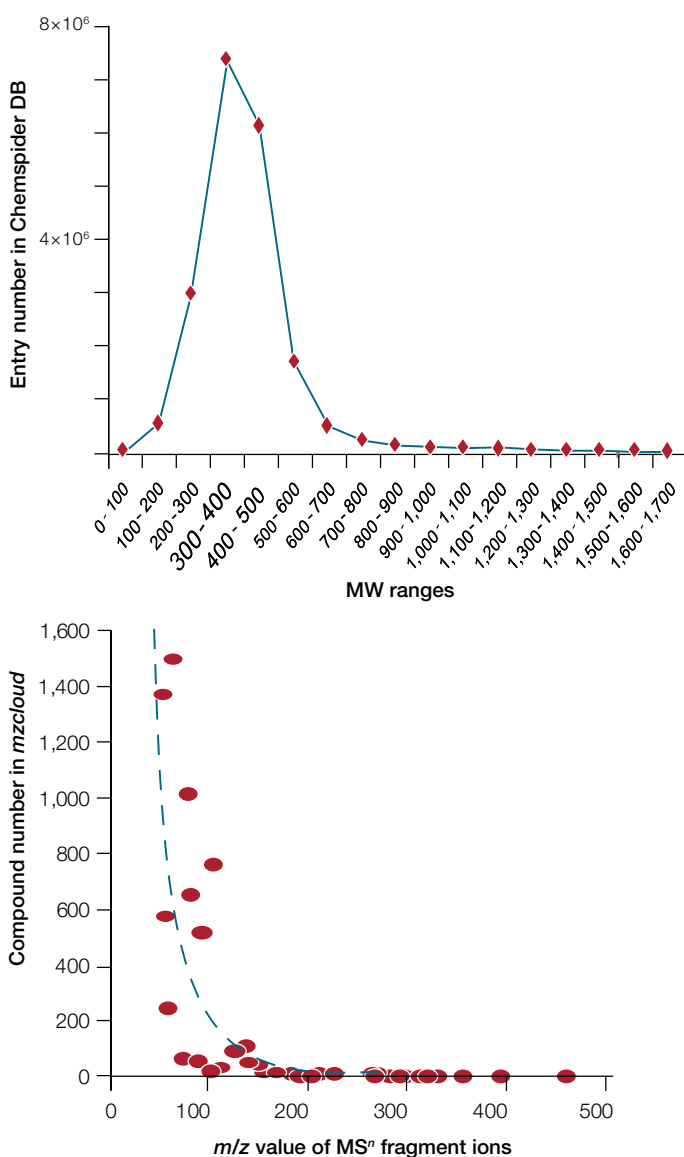


Figure 3. Distribution of compounds in ChemSpider and mzCloud databases as a function of molecular weight.

Fine isotopic distribution—why it matters!

Also, high and ultra-high resolution is crucially important for correct fine isotopic distribution assignment, as depicted in Figure 4¹¹. Correct assignment of isotopes, including fine isotopes, is used for analyte confirmation along with ion fragments, accurate mass and retention time (RT). This information gives additional confidence in unknown identification and could at least partially replace information derived from MS² fragments when they are not intense enough to be detected or when the precursor ions have poor fragmentation.

Data-mining: always an option with Orbitrap mass analyzer data

Retrospective data analysis, also known as data mining, is easily and reliably done with Orbitrap mass analyzer data from previously acquired full scan MS. This technique is often employed for drugs of abuse and doping applications, where new generation drugs are created and consumed before they appear on the list of targeted compounds, often years later. The same applies to food safety analysis for post acquisition detection of harmful and unwanted pesticides in food and feed matrices.

Fine Isotopic Distribution and Resolution

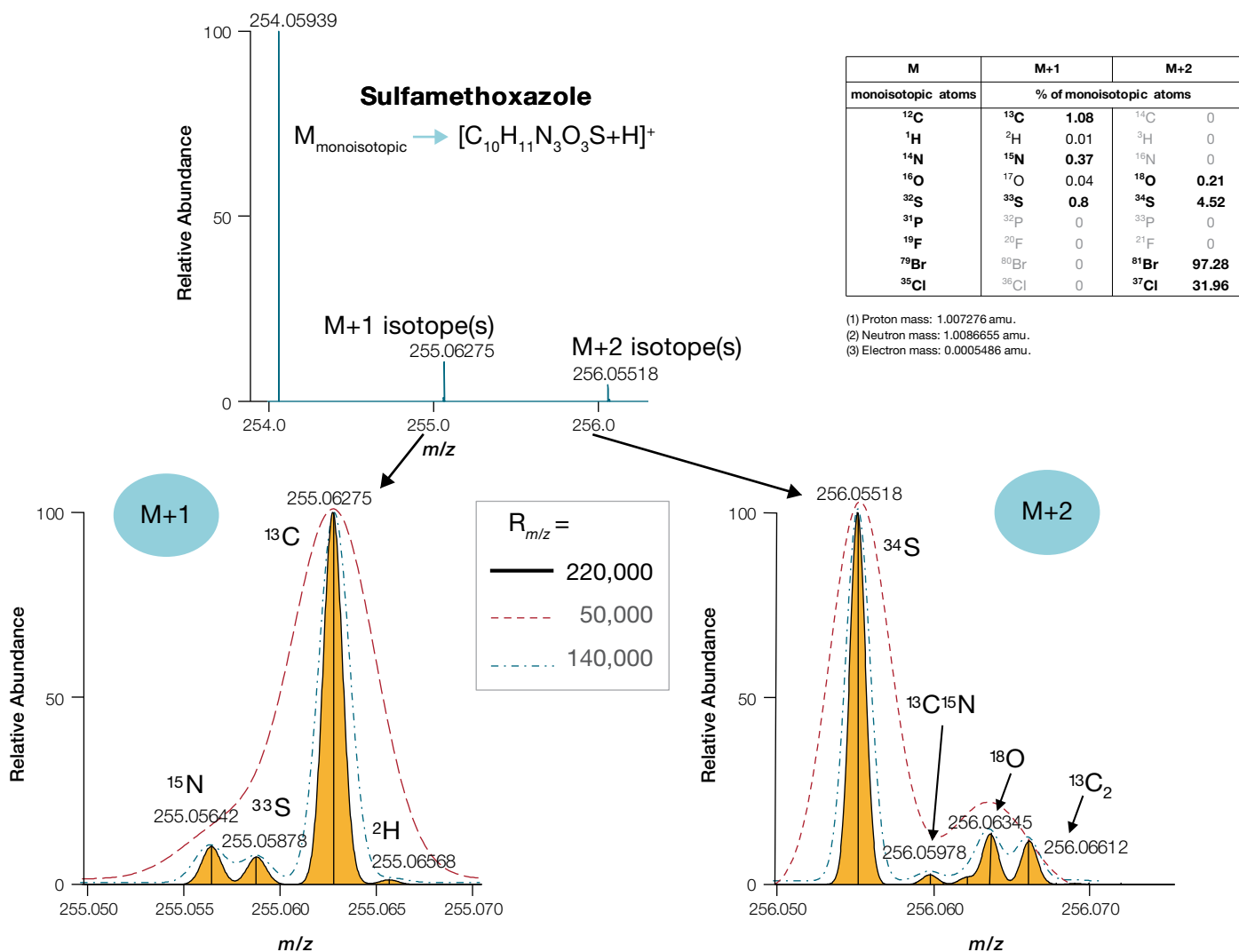


Figure 4. Isotopic fine structure obtained from high resolution Orbitrap mass analyzer at different resolution settings compared to theoretical values.

Robustness with flexibility at your fingertips

Orbitrap mass resolution can be easily adjusted, from the lowest figure often used for MS² scans to ultra-high (240,000 (FWHM) at *m/z* 200) in full-scan MS. With this flexibility, the user can increase or decrease the number of data points across the LC peak without losing sensitivity, which is often not the case for Q-TOF instruments.

Scan-to-scan long-term mass accuracy and instrument robustness (Figure 5) are essential for consistent and accurate data collection, especially for long LC gradients, which are often used in multiresidue screening in food safety, forensic toxicology and other applications. Orbitrap mass analyzers have acquired an excellent reputation for mass stability. Once the instrument is calibrated, it holds its calibration for a number of days within low ppm range specifications without the need for run-to-run recalibration. Mass stability performance and ease of use to the user are attributable to Orbitrap mass analyzer technology and stable electronics. Mass stability is even maintained when on the fly polarity switching is utilized for maximum compound coverage. This is different from a Q-TOF instrument, where the mass resolution needs seconds to recover after polarity switching, making it impractical for LC experiments.

Performing quantification with Orbitrap

Non-targeted analysis, screening in full scan and confirmation with Q Exactive series mass spectrometers would have limited reach in applied and research markets if it wasn't for their ability to perform quantitation measurements⁹. Scientists are often surprised that Q Exactive series mass spectrometers can obtain similar LODs and LOQs as those obtained on triple quadrupole (QQQ) instruments. In addition, method transfer from QQQ MS to HRMS is easy and reliable. Q Exactive series mass spectrometers can also perform parallel reaction monitoring (PRM), which is more selective than the SRM acquisition on a triple quadrupole MS, because with PRM, narrower mass extraction window(s) can be applied on the detection of fragment ion(s).

Many authors from different fields have compared QQQ MS and HRMS for quantitative determinations on different biological matrices¹⁴. Comparative studies show that the robustness, ease of use, selectivity and sensitivity of HRMS data are comparable or better than the QQQ MS data in some cases.

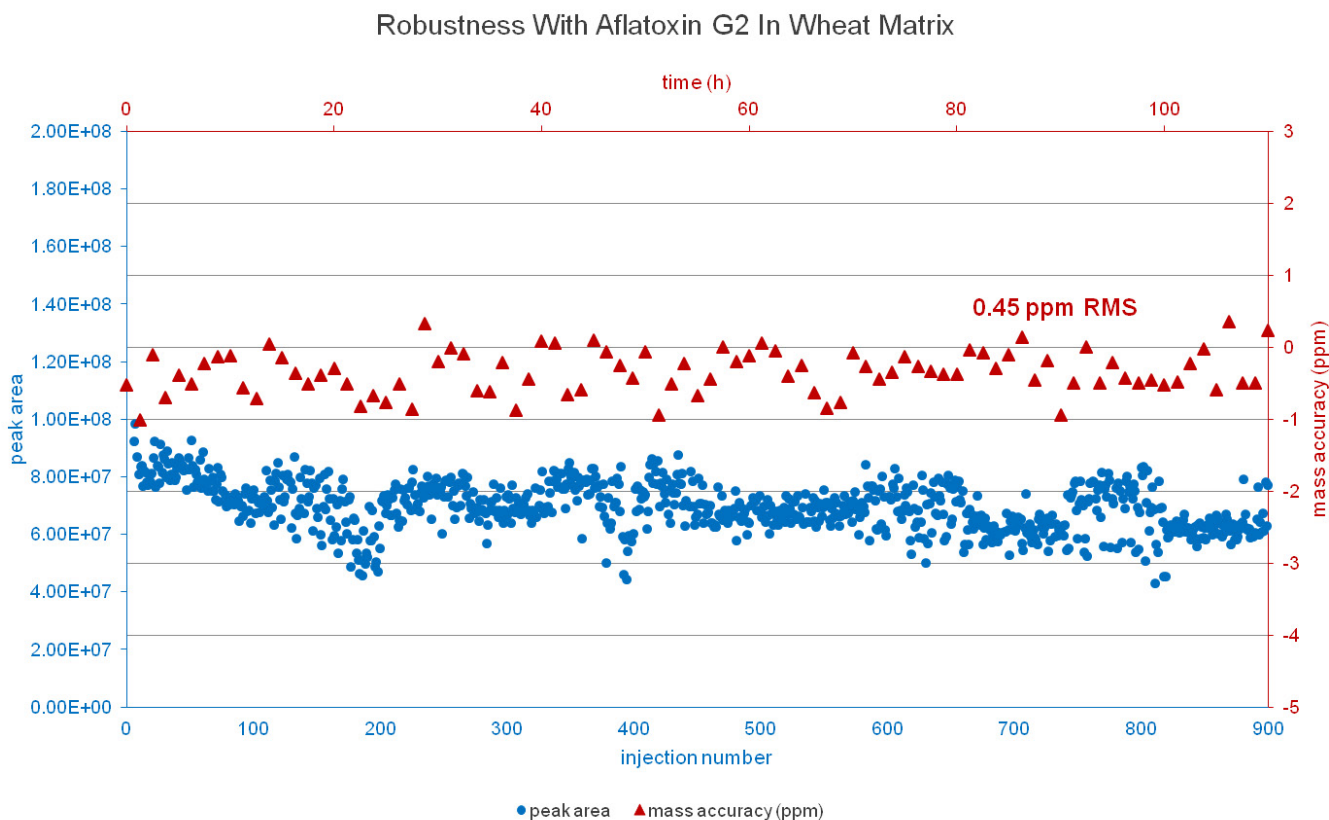


Figure 5. Mass accuracy and peak area of Aflatoxin G2 in wheat matrix for 900 continuous injections (120 hrs run) without instrument recalibration.

It is worth mentioning that Q Exactive series mass spectrometers guarantee a high linearity range independent of resolution and with no detector saturation effects (Figure 6). Inter-dynamic ranges and intra-dynamic ranges of up to 5 orders of magnitude have been demonstrated¹⁴. Other high resolution instruments, namely Q-TOF mass spectrometers, typically have serious problems with the detector's linearity of response caused by the rapid saturation of the detector¹⁵.

The Q Exactive MS can be used for routine, quantitative and/or qualitative analyses in any laboratory¹⁶⁻²⁰. This means that with the same Orbitrap mass analyzer it is possible to perform a very wide range of applications: from targeted quantitation to untargeted metabolomics²¹.

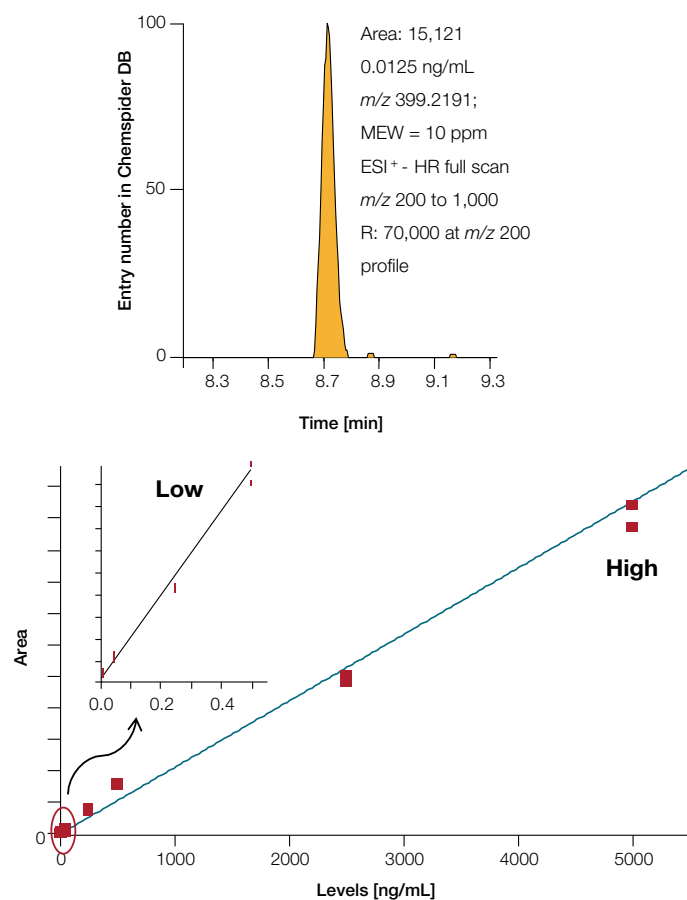


Figure 6. Five order linear dynamic range in full scan obtained on Q Exactive Focus MS for Sunitinib in plasma extract.

Conclusion

The outstanding performance of Orbitrap mass analyzer instruments is based on their high and ultra-high mass resolution capabilities, high sensitivity and selectivity in full scan (and MS/MS) acquisition, mass accuracy and precision (robustness), and ease of use. These instruments ensure not only high resolution but also very good linear dynamic range and analysis reproducibility.

Q Exactive series mass spectrometers have been extensively used in applied markets and research applications due to their flexibility in both targeted analysis as well as screening and unknown determination.

There are various Q Exactive mass spectrometer models and prices allowing laboratories to select HRMS according to budget and analytical needs. But all models have the same small (83 × 91 cm) footprint making them ideal for even the smallest laboratories.

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