

Thermo Scientific Q Exactive HF  
Orbitrap LC-MS/MS System



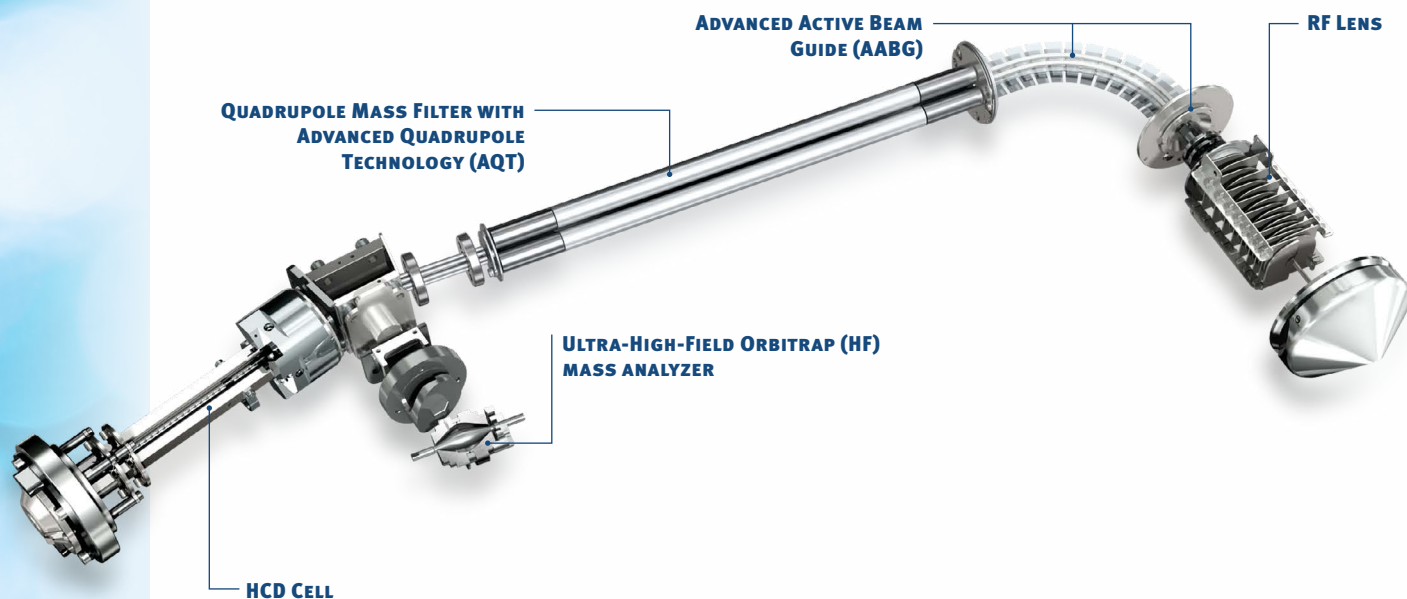
# Higher-Quality Data, Faster Than Ever

Speed • Productivity • Confidence

**Thermo**  
SCIENTIFIC

## Transforming discovery quantitation

The Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap mass spectrometer features an ultra-high-field Orbitrap analyzer which doubles its speed and resolution – more sample runs and amazing data quality. Built on the proven performance of the Q Exactive Plus platform, the Q Exactive HF mass spectrometer utilizes the same Active Beam Guide technology and Advanced Quadrupole design for very stable system operation and exceptional analytical performance. The Q Exactive HF mass spectrometer is designed for your toughest discovery and quantitation challenges where the combination of speed and highest performance are essential for the highest quality scientific results.



- Ultra-High-Field Orbitrap (HF) mass analyzer for faster scan speed and higher resolution
- Advanced Quadrupole Technology (AQT) improves precursor selection and transmission for more accurate quantitation of low abundant analytes in complex matrices
- Sophisticated Data-Independent Acquisition (DIA) and Parallel Reaction Monitoring (PRM) deliver reproducible quantitation with complete qualitative confidence
- The Advanced Active Beam Guide (AABG) reduces noise and improves instrument robustness
- Optional intact Protein Mode provides superior trapping of large molecules for improved analysis of intact proteins



## Analyze complex mixtures faster than ever!

The Q Exactive HF mass spectrometer, with an ultra-high-field Thermo Scientific™ Orbitrap™ detector, provides new levels of speed and resolution for every scan while maintaining high spectral quality for sensitive detection and confident quantitation of peptides, lipids, metabolites, contaminants and other important compound classes.

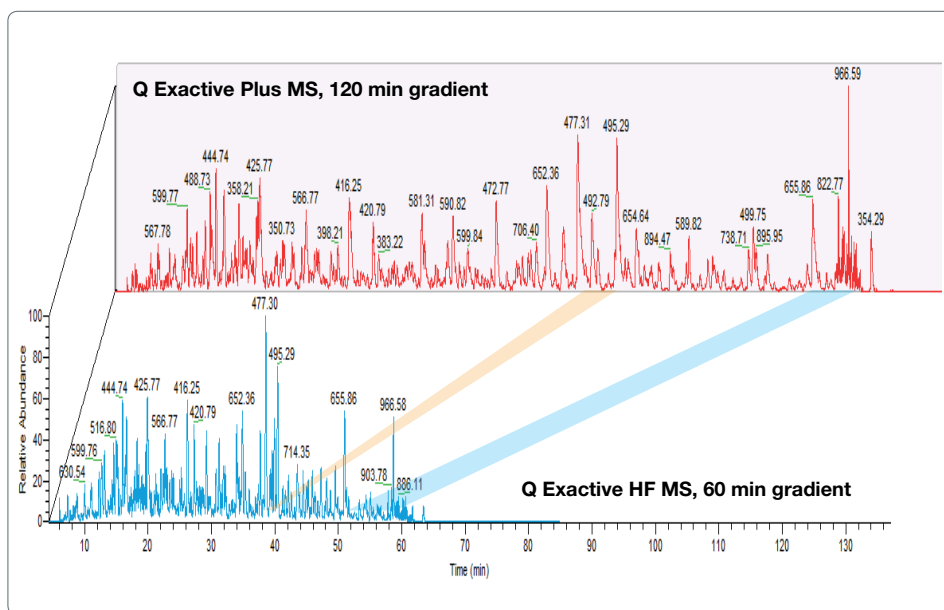
### Faster protein identification

The highest quality protein identification from complex biological samples over the widest dynamic range is an absolute requirement for all proteomics labs. The Q Exactive HF system delivers the mass accuracy and resolution you expect combined with far greater speed than ever before, enabling protein identification that is faster and deeper than ever, while maintaining the resolution and accuracy required for confidence in your results. Under the same LC gradient conditions, the Q Exactive HF MS identifies approximately 30% more unique peptides and 20% more protein groups than the Q Exactive Plus MS system. The speed of the system enables gradient runs that are twice as fast (steeper gradients) while maintaining the ability to identify roughly the same number of unique peptides and protein groups.

The faster scan speed of the Q Exactive HF MS results in higher number of precursor ions triggered for MS/MS—synonymous with improved productivity.

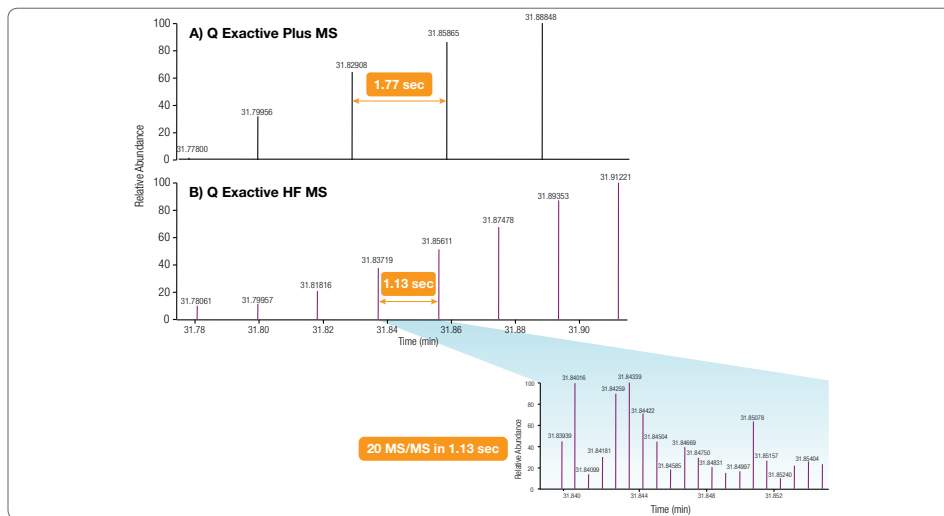
#### Improved productivity with Q Exactive HF MS

The Q Exactive HF MS system completes 1 full MS scan at 60,000 resolution and 20 HCD MS/MS scans at 15,000 resolution in 1.13 sec, 37% shorter compared to the Q Exactive Plus MS.



#### Comparable number of protein IDs in half the time

Base peak chromatogram (BPC) of a 120 min gradient on the Q Exactive Plus MS (red) and a 60 min gradient on the Q Exactive HF MS (blue) showing one half the analysis time.



## Quantify with the highest confidence

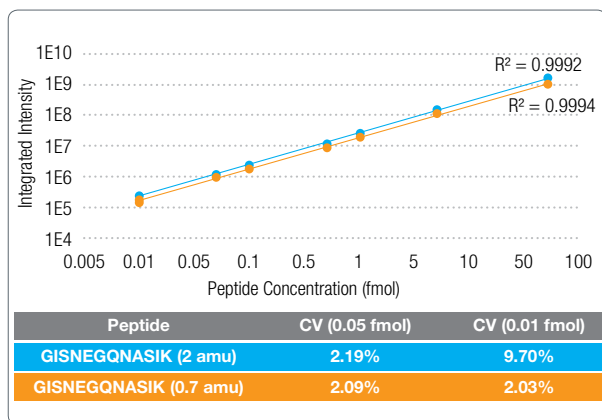
With its unique spectrum-multiplexing capabilities and faster scan speed, the Q Exactive HF mass spectrometer provides the speed necessary for precise quantitation even for narrow ultra-high-performance liquid chromatography (UHPLC). Industry leading high resolution accurate mass (HR/AM) MS provides you with a more complete quantitative picture.

### Parallel Reaction Monitoring – the new paradigm for high-throughput targeted quantitation

The high resolution accurate mass (HR/AM) capability of Orbitrap MS enables a new mode of quantitation – Parallel Reaction Monitoring (PRM). PRM relies on HR/AM to provide exquisite selectivity, high sensitivity, accurate and high throughput quantitation of analyte targets, and excellent MS/MS data to confirm target identification.

### Enabling quantitative accuracy

Quantitative accuracy is achieved by removing interferences from co-eluting analytes. The unique Advanced Active Beam Guide technology offers the ability to narrow the quadrupole isolation width to as little as 0.4 amu, significantly reducing the possibility of signal interference that can decrease selectivity and accuracy. For low level peptide quantitation by PRM, the extreme sensitivity of the Q Exactive HF system enables routine quantitation down to 0.01 fmole and provides extremely high precision using 0.7 amu isolation —as demonstrated by the very low coefficients of variation (CV) at the lowest levels of quantitation.

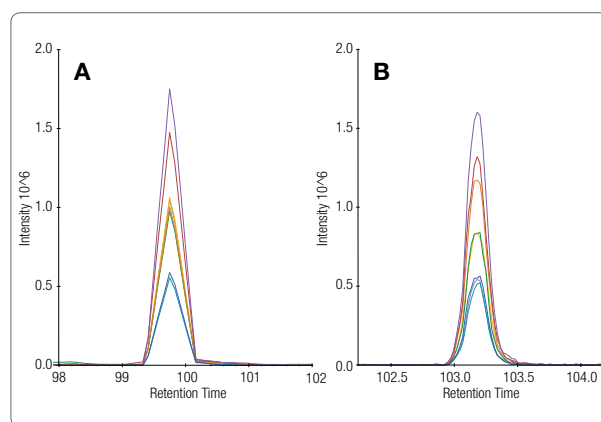


### Improved quantitation linearity and reproducibility with PRM

As a result of the improved selectivity, better quantitation over a large linear dynamic range is obtained. This demonstrates great reproducibility for very low amounts of target peptide in a complex background, in this example, 200 ng HeLa digest. The 2 amu isolation window clearly shows higher variability at the 0.01 fmol level vs. the narrower isolation width of 0.7 amu.

### Achieving quantitative precision

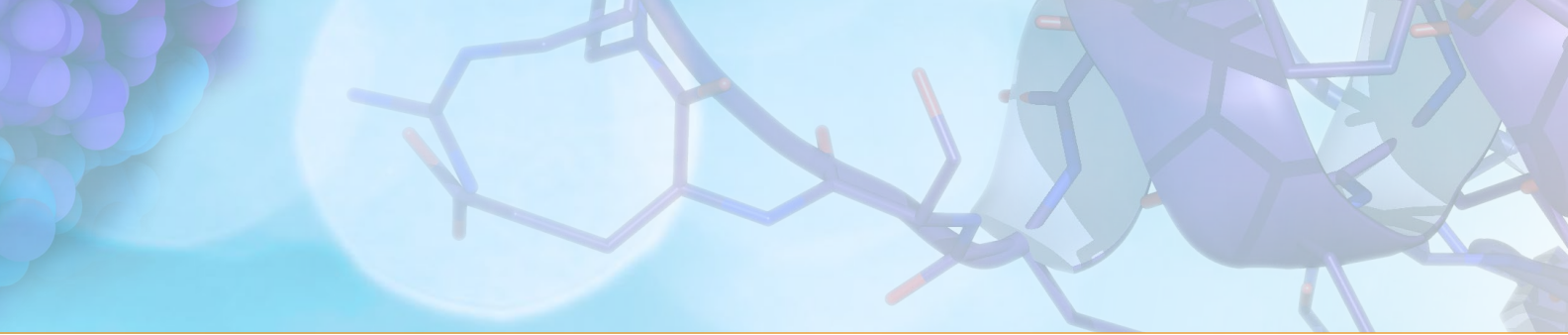
The best precision in quantitation is achieved with the highest possible ion transmission and rapid data collection to correctly define analytical peak shape. The ultra-high-field Orbitrap analyzer of the Q Exactive HF system scans fast enough to accurately define narrow UHPLC peaks for optimal quantitative results.



### Improved chromatographic sampling

Extracted ion chromatograms (XIC) of the same peptide showing (A) only 4 points across the chromatographic peaks, and (B) 8 points across the peak. The short cycle time on the Q Exactive HF system samples the peak more frequently, ensuring the best quality quantitation.





## A digital data archive – New levels of DIA capability

Targeted quantitative methods deliver the highest sensitivity, best precision, and lowest detection limits, but require a *priori* knowledge of the precursor ions (target molecules) and significant time and effort for SRM or PRM method development and optimization.

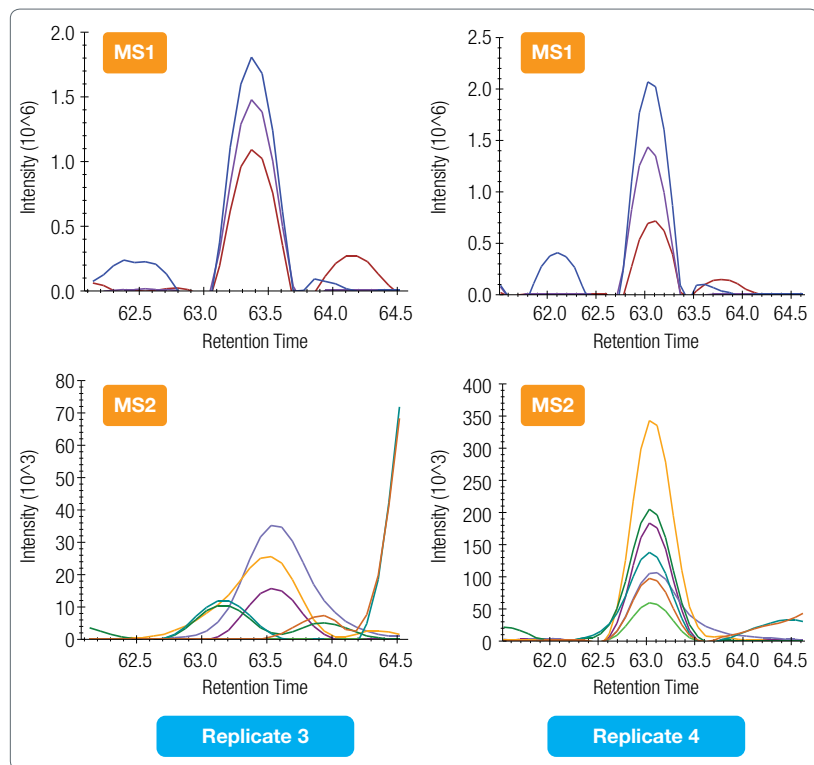
In a true discovery quantitation environment, the targets may not be known and relative quantitation may be sufficient. In such cases, data independent acquisition, is a viable quantitative approach. The Q Exactive HF MS enables several methods for DIA data acquisition, including BASIC-DIA, pSMART-DIA, and MSx-DIA. These DIA methods fragment all ions present in the sample to create a high-quality, permanent digital data archive of a sample that can be reanalyzed to look for new targets.

### Orbitrap-based HR/AM reduces false positives

The Q Exactive HF MS provides high resolution and practical, experimental mass accuracies of less than 3 ppm for both precursor and fragment ions enabling very narrow mass windows to be used for data extraction and comparison to library spectra, greatly reducing the risk of false positive identifications using the BASIC-DIA method, relative to standard SWATH™ methods.

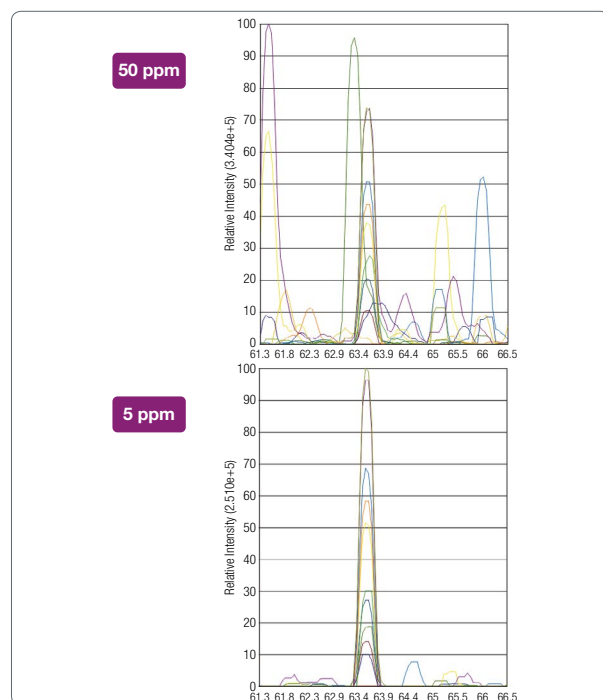
### High quality data to address complex samples

The high resolution capability of the Q Exactive HF MS is a key attribute in separating legitimate signal from potential interferences. With resolutions up to 240,000 FWHM @ 200 m/z and low-ppm MS and MS/MS mass accuracy, the Q Exactive HF MS system provides superior selectivity to resolve isobaric fragments—significantly better than Q-ToF systems with their lower resolution, and limited accuracies.



#### Comparison of replicate DIA quantitation results

While replicate 4 exhibits a good correlation to the spectral library (0.97), replicate 3 is only moderately correlated (0.73). However, data can be confirmed at the MS1 level with 0.4 ppm mass error and with isotopic dot products greater than 0.9. If one replicate is questionable the excellent mass accuracy of the MS1 peak can be used to match the peptide and “save or recover” a poor replicate, so quantitation is performed on the MS1 precursor.



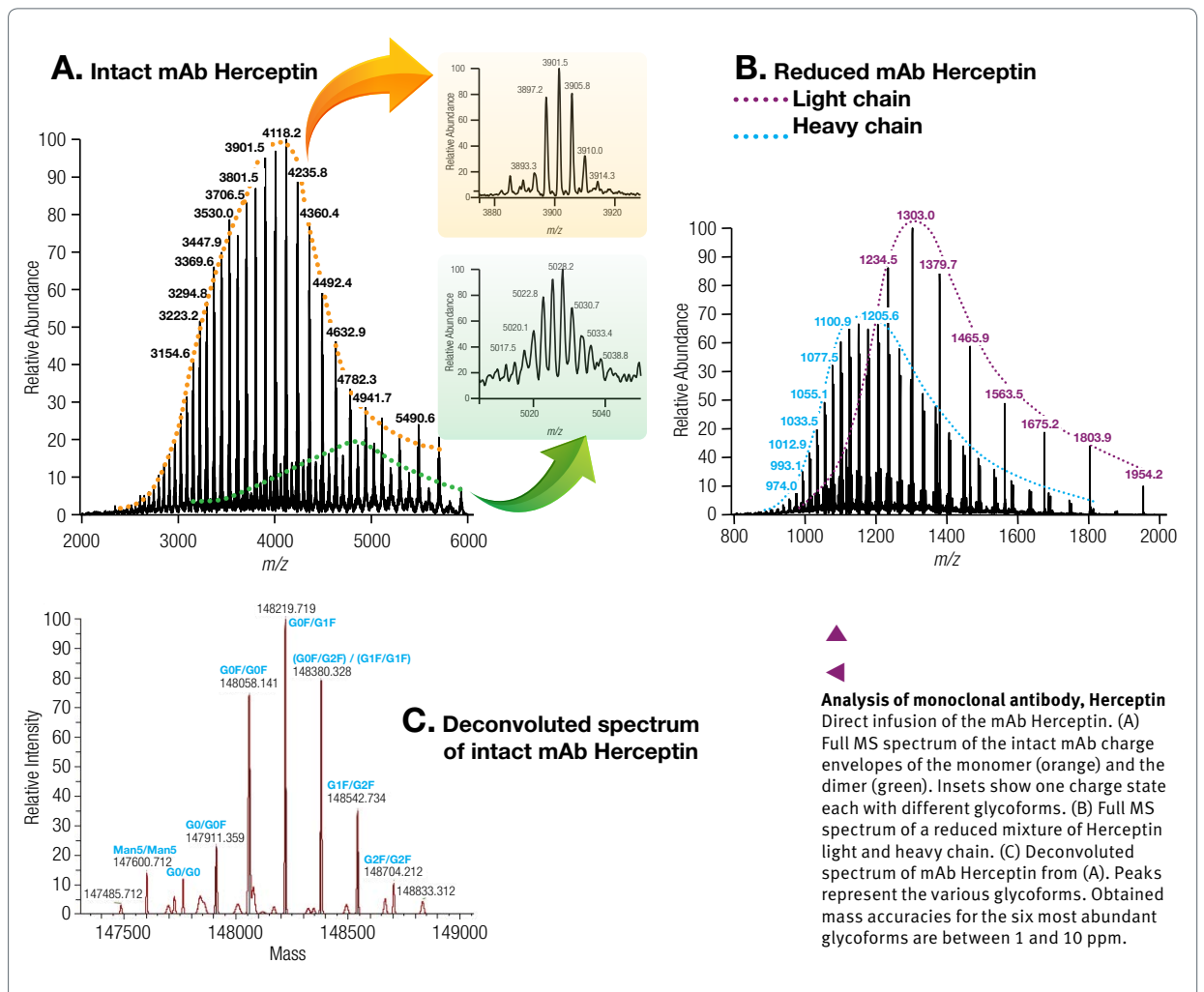
#### Selectivity through mass accuracy

Comparison of selectivity obtained with 50 ppm mass accuracy (typical of ToF systems) vs. 5 ppm mass accuracy in MS/MS analyses, and the selectivity it brings to DIA analyses—only obtainable on Orbitrap-based systems.

## Superior intact protein characterization

The analysis of intact proteins presents a different set of challenges in which maximum resolution of the spectral features can help to distinguish major protein isoforms from lower abundant species. Particularly in biopharmaceutical applications, the analysis of heterogeneous protein mixtures requires the highest resolving capabilities combined with the high mass accuracy provided only with Orbitrap technology.

The Q Exactive HF MS, with the optional Intact Protein Mode, enables high resolution analysis of intact proteins, allowing detailed characterization of larger, more highly charged protein species such as intact antibodies, as well as the individual heavy and light chains. With the additional resolving power of the Q Exactive HF MS, proteins as large as 50 kDa can be isotopically resolved. This improved resolution allows routine, detailed characterization of proteins and biopharmaceuticals.

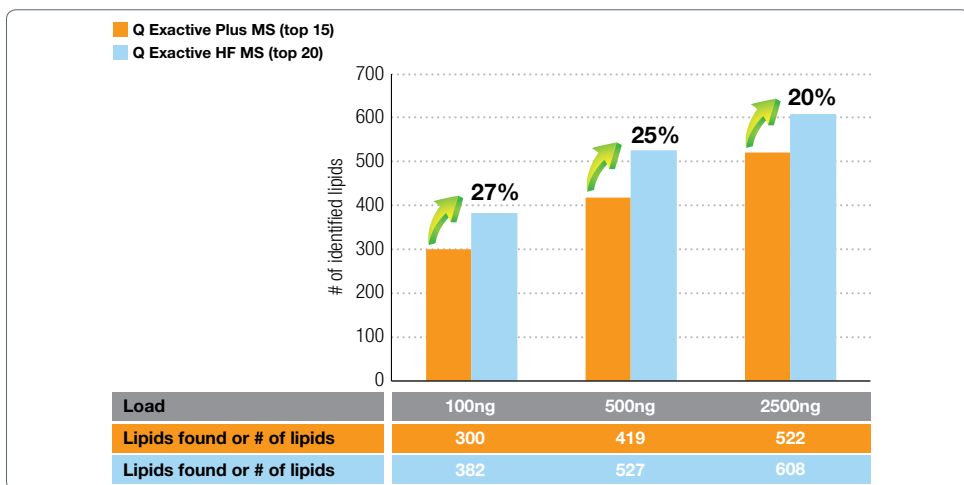


# Increased productivity and confidence for lipidomics

The analysis of lipids poses a unique analytical challenge in research, as the closely related molecular structure of many lipid species produce multiple isobaric species within a relatively narrow mass range. This creates the risk of ambiguous lipid structure assignments. The high resolving power of the Q Exactive HF system greatly reduces this

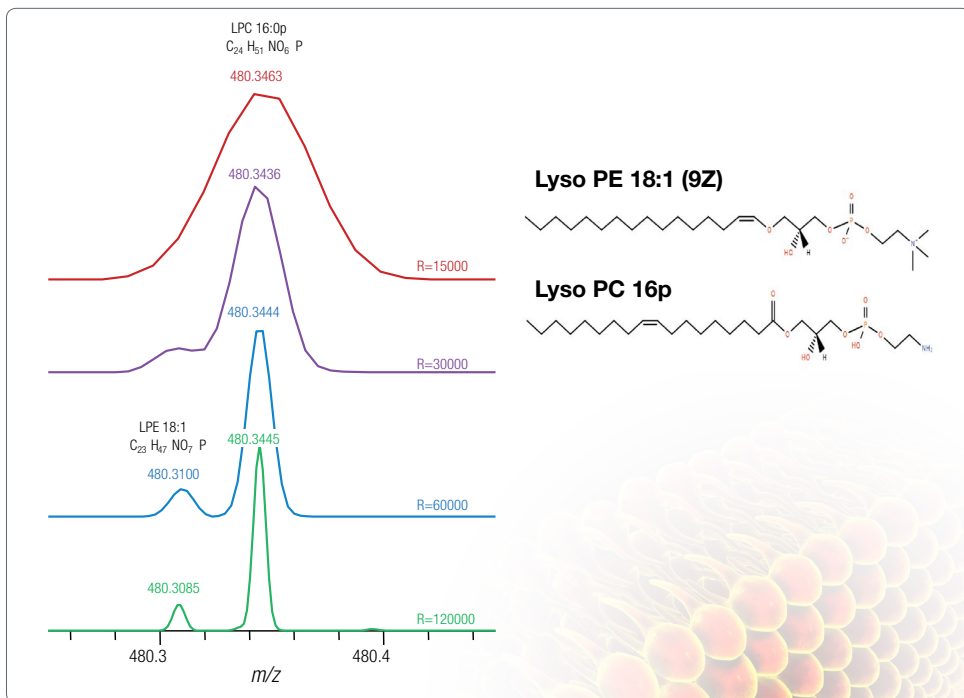
risk compared to instrumentation with lower resolving power.

The Q Exactive HF MS is ideal for both infusion and LC-based workflows. Fast acquisition speed allows the system to keep pace with demanding lipid separations from complex samples.



## Identification of more lipid species at varying concentrations

While the LC gradient for the Q Exactive HF system was 33% shorter (steeper LC gradient), the speed and resolution of the system still allowed identification of 20% more lipid species from a bovine heart lipid extract. LC method, column and gradients for the Q Exactive Plus MS and for the Q Exactive HF MS were identical.



## Increased resolution improves identification/quantitation

The effect of resolving power for the identification of lipid species is illustrated for two Lyso phospholipid species, 18:1 LPE (lysophosphoethanolamine) and 16:0p LPC (lysophosphocholine). Identification of minor lipid species is challenging without sufficient mass resolution, leading to fewer identifications. In order to baseline-separate these two lipid species, a minimum of 60,000 resolving power (or higher) is required, easily achievable using high resolution Orbitrap technology.



# Accelerating productivity with software solutions



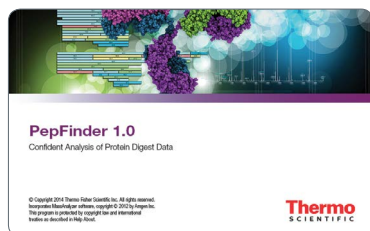
## Proteome Discoverer Software

Thermo Scientific™ Proteome Discoverer™ 1.4 software is a comprehensive and flexible data analysis platform for qualitative and quantitative proteomics research, offering a wide array of bio-software tools and customizable workflows for every proteomics experiment.



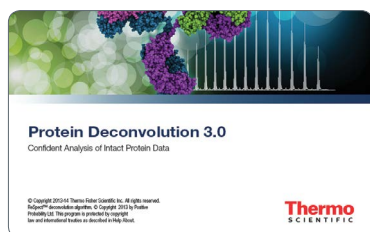
## Pinpoint Software

Thermo Scientific™ Pinpoint™ 1.4 software is a targeted proteomics quantitation software supporting the analysis of proteins, peptides, and now glycoproteins. Automated workflows facilitate all experimental aspects, from method development and acquisition to data verification and reporting.



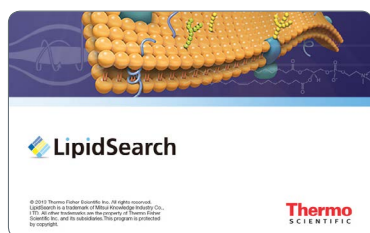
## PepFinder Software

Thermo Scientific™ PepFinder™ 1.0 software is a new peptide mapping tool for the characterization of biotherapeutic proteins, including relative quantitation of PTM's and amino acid substitutions, and location of disulfide bonds. A novel fragmentation prediction and scoring provides fast, comprehensive and confident assignments.



## Protein Deconvolution Software

Thermo Scientific Protein Deconvolution 3.0 software provides accurate mass measurement and isoform quantification for intact proteins such as biotherapeutics. Now includes enhanced deconvolution algorithms, and mirror plots for comparison of samples.



## LipidSearch Software

Thermo Scientific LipidSearch software processes high-resolution accurate-mass data, to provide accurate lipid identification. It automatically integrates complex data into reports and dramatically reduces data analysis time.

**For Research Use Only. Not for use in diagnostic procedures.**

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**Australia** +61 3 9757 4300  
**Austria** +43 810 282 206  
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**Canada** +1 800 530 8447  
**China** 800 810 5118 (free call domestic)  
400 650 5118

**Denmark** +45 70 23 62 60  
**Europe-Other** +43 1 333 50 34 0  
**Finland** +358 9 3291 0200  
**France** +33 1 60 92 48 00  
**Germany** +49 6103 408 1014  
**India** +91 22 6742 9494  
**Italy** +39 02 950 591

**Japan** +81 45 453 9100  
**Latin America** +1 561 688 8700  
**Middle East** +43 1 333 50 34 0  
**Netherlands** +31 76 579 55 55  
**New Zealand** +64 9 980 6700  
**Norway** +46 8 556 468 00  
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**Singapore** +65 6289 1190  
**Spain** +34 914 845 965  
**Sweden** +46 8 556 468 00  
**Switzerland** +41 61 716 77 00  
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