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Orbitrap quantitation: lab of the future

Close your uncertainty gap with the selectivity and confidence of HRAM

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Quantitation: historical perspective

Thermo Scientific™ Orbitrap™ mass spectrometer technology is disrupting the use of mass spectrometry (MS) for qualitative, and more importantly for quantitative analyses, in applied markets. Why is this occurring?

For many years, liquid chromatography-triple quadrupole mass spectrometry (LC-QQQ MS) technology has been the standard for quantitation of many types of compounds, from pesticide residues in food and feed, to testosterone in plasma and urine for research applications. Due to their selectivity and sensitivity, quadrupole-based mass analyzers provide reliable, cost-efficient quantitation and identification of target analytes.

For targeted analyses, nominal-mass-resolution quadrupole-based instruments are operated in either the selected ion monitoring (SIM) or selected reaction monitoring (SRM) data acquisition modes. During SIM and SRM data acquisition, the scope of analysis is limited to the target ions selected and therefore suffers from non-detection of analytes that are present. Undetected compounds and unknown metabolites can be a source of uncertainty and concern.

Set-up of QQQ instruments require careful tuning and optimization of data acquisition parameters for each target analyte and ongoing monitoring of data acquisition time windows to ensure analyte detection. These tasks require time and effort, and limit the number of possible measurements that can be made during an analytical run.

Time-of-flight (TOF) technology has also been used for quantitation, but its dynamic range is limited due to detector saturation effects. Moreover as shown in Figure 1, TOF instruments do not have adequate resolution, especially at the lower masses measured in small molecule analyses, to provide adequate selectively. Poor selectivity often results in false identification (false negatives and false positives) of an analyte when it is not present and the inability to quantitate the analytes of interest.[1]

1. Rajski, Ł.; Gómez-Ramos Mdel, M.; Fernández-Alba, A. R. Large pesticide multiresidue screening method by liquid chromatography-Orbitrap mass spectrometry in full scan mode applied to fruit and vegetables. J. Chromatogr. A. 2014, Sep 19;1360:119-27.

High resolving power: quantitative capability for the lab of the future

Included in approximately 5000 peer-reviewed publications, Orbitrap mass spectrometer technology is well established as a powerful LC-MS platform. Its high-resolution, accurate-mass (HRAM) measurements, together with its high dynamic range, have enabled rigorous characterization of complex mixtures.[2]

Why is high resolution important? High resolving power is particularly important for experiments involving complex mixtures, such as biological, environmental, and food samples generated from a matrix, since these will contain a significant number of background ions in addition to the analytes of interest. In such cases, high resolving power will make the difference between detecting and not detecting analytes at low concentrations due to the masking effect of isobaric interferences. In other words, accurate quantitation relies on high selectivity, which is the ability to resolve compounds of interest from background interferences.

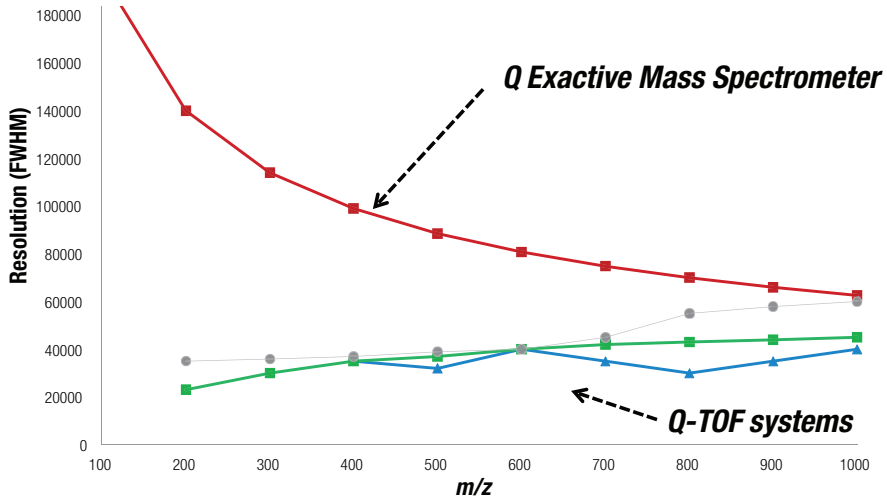

Figure 1. Comparison of Q Exactive mass spectrometer and Q-TOF resolution.

2. The Thermo Scientific Exactive Mass Spectrometer. The ultimate screening machine and overview of scientific literature. Thermo Scientific Literature Review: XX63548_E 06/12M.

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Orbitrap mass spectrometer technology has revolutionized the field of mass spectrometry. The Thermo Scientific™Q Exactive™ series of quadrupole-Orbitrap mass spectrometers are the next step in that revolution, combining high-performance quadrupole precursor selection with HRAM detection. The Thermo Scientific Q Exactive mass spectrometer and Thermo Scientific Q Exactive Focus mass spectrometer are benchtop LC-MS systems composed of six unique sections: RF-lens, bent-flatapole, hyperbolic quadrupole mass filter, C-Trap, HCD cell, and the Orbitrap mass analyzer (Figure 2).

Ions generated in the heated electrospray (H-ESI II) ion source enter the RF-lens through a heated capillary. The RF-lens acts as an ion funnel that focuses and transmits ions to the "bent-flatapole" where they are redirected by 90 degrees. This reduces instrument footprint and allows neutrals to be removed before the ions stream into the mass analyzer. Next, ions enter the hyperbolic quadrupole, which allows for the selection of ions in a broad or narrow mass window down to 0.4 amu. Ions then are captured in the C-Trap, an ion storage device where the user can choose to (1) send ions to the Orbitrap mass analyzer for full-scan detection (MS-level) or (2) fragment the ions inside the nitrogen-filled higher collisional dissociation (HCD) cell, send them back to the C-Trap, and then to the Orbitrap mass analyzer for fragment (MS/MS) detection. Mass spectra are acquired via image current detection. The vacuum inside the Orbitrap mass analyzer is maintained within the 10-10 mbar range.

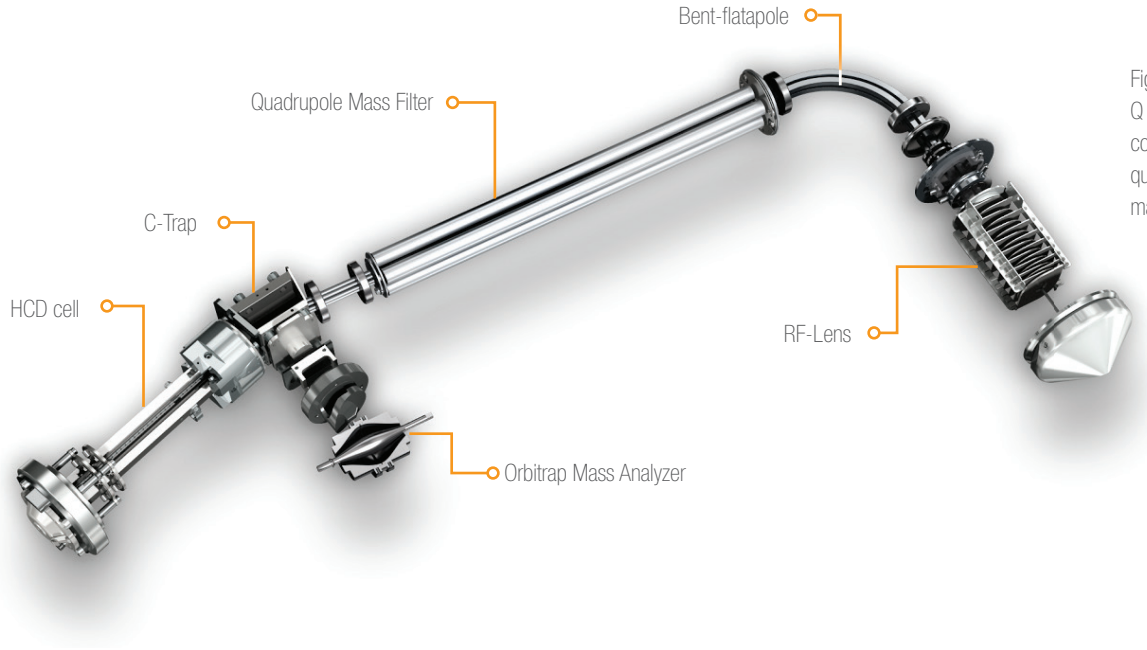

Figure 2. Building on its predecessors, the Q Exactive series of mass spectrometers consist of RF-lens, bent-flatapole, hyperbolic quadrupole, C-Trap, HCD cell and the Orbitrap mass analyzer.

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Orbitrap mass analyzer

Comprised of two outer electrodes and a central electrode, the Orbitrap mass analyzer acts both as an analyzer and a detector (Figure 3). When ions enter the Orbitrap analyzer they are captured via "electrodynamic squeezing." Once captured, the ions oscillate around the central electrode and in between the two outer electrodes. Different ions oscillate at different frequencies resulting in mass separation. Mass spectra are acquired via image current detection by measuring the oscillation frequencies induced by ions on the outer electrodes. In this way, the Orbitrap mass analyzer is a Fourier Transform (FT) mass analyzer analog of well-known FT- ion cyclotron resonance (ICR) technology, with the advantage of allowing smaller instrument size and easier instrument operation.

The Orbitrap mass analyzer's uniquely high resolution is due to three factors: 1) nanometer-range accuracy Orbitrap electrodes, 2) high voltage supplies, and 3) mass-to-charge measurements delivered as a function of oscillation frequency.

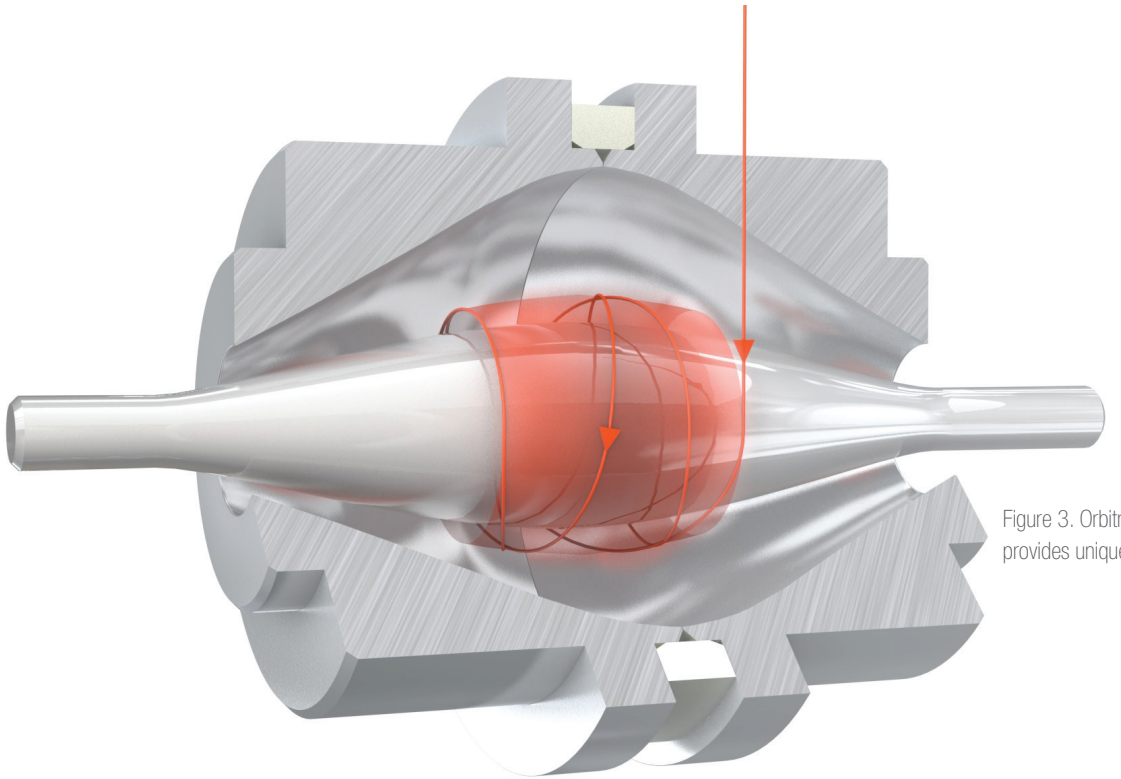

Figure 3. Orbitrap mass analyzer technology provides uniquely high resolution.

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Close your uncertainty gap: identify, quantitate, and confirm with selectivity and confidence

Orbitrap mass spectrometer technology is rapidly growing in popularity because it uniquely provides quantitative and qualitative (Quan/Qual) HRAM analytical capability in a single platform and often in a single analytical run (Table 1). Accurate mass assignment and high resolution in both MS and MS/MS modes increase selectivity and reduce uncertainty, benefiting most applications imaginable. Full-scan HRAM data acquisition captures all sample data, enabling identification of "unexpected" compounds and retrospective data analysis without need to re-run samples.

Application/Need	HRAM	QQQ
High Sensitivity	●	●
Confirm Analyte Structure	●	●
Analysis of "Unknowns"	●	●
Retrospective Search for New Compounds	●	●
Low Cost	●	●
Established Methods	●	●

Table 1. Comparison of HRAM and QQQ mass spectrometer capabilities.

Powerful quantitative capability

Q Exactive mass spectrometers can be operated in several scan modes, including: full-scan MS (full MS), all-ion fragmentation (AIF) in the HCD collision cell, SIM for single ion detection, and parallel reaction monitoring (PRM) for detecting all fragment ions in parallel. Fragments generated in-source or in the multipole collision cell by AIF provide additional information that complements full-scan data.

AIF fragments all ions in a single fragmentation event without precursor ion isolation and records all fragment ions in a single mixed spectrum. MS/MS (MS^2) data can be collected using data-dependent acquisition (DDA) where precursor ions selected using predetermined rules are subjected to MS/MS, or by using data-independent acquisition (DIA) where a full scan is followed by a number of wide isolation MS^2 scans, which together cover the same isolation range as the preceding full scan. The benefit of DIA is the ability to capture both all data and MS^2 HR data scans without need for additional sample injections, enabling the acquisition of full information for all compounds and their fragments.

The ability to perform fast polarity switching allows acquisition of both positive and negative scans within a single acquisition method, eliminating the need for extra sample injections, which is of particular importance when only limited amounts of sample are available. Positive and negative polarity switching can be performed on the chromatographic timescale and does not require instrument recalibration.

The Q Exactive series of mass spectrometers provide up to six orders of magnitude of linear quantitative dynamic range (Figure 4) and up to five orders of intra-scan dynamic range (Figure 5) for accurate quantitation in low-level components in complex matrices.* In addition, these mass spectrometers are easily calibrated in just a few minutes and maintain that calibration for days at a time.

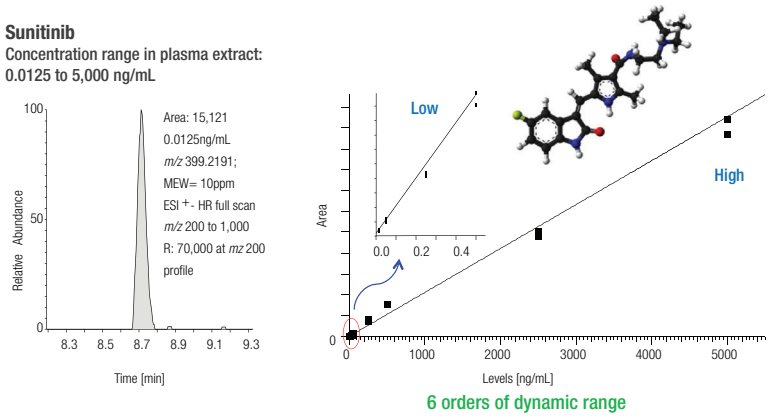

Figure 4. The Q Exactive mass spectrometer provides up to six orders of linear dynamic range in high-resolution full-scan acquisition mode for Sunitinib. The concentration range in plasma extract was 0.0125 to 5,000 ng/mL. MEW refers to mass extraction window.

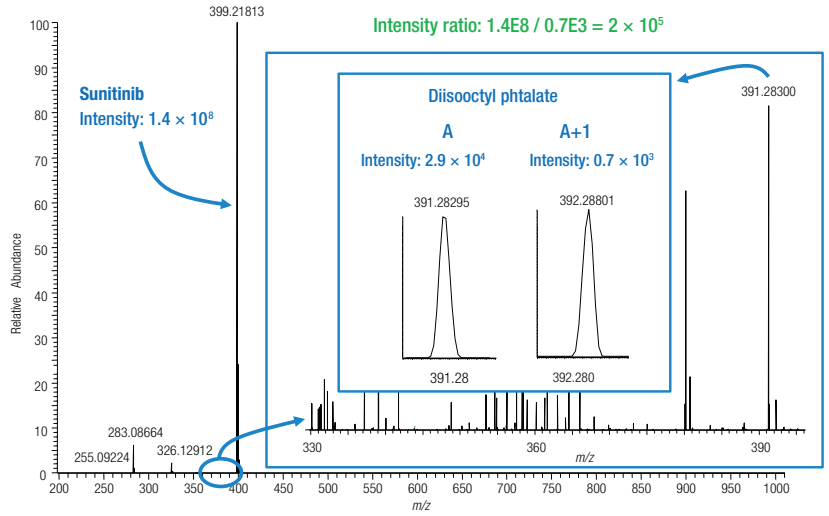

Figure 5. The Q Exactive series of mass spectrometers provide up to five orders of intra scan dynamic range for Sunitinib. MA refers to mass accuracy.

* Courtesy of Dr. Bertrand Rochat (ASMS 2015), CHUV, Lausanne, Switzerland.

Choice of quantitation experiments

Using the Q Exactive mass spectrometer's powerful modes of operation, there are generally three types of targeted quantitation experiments, and one type of non-targeted quantitation experiment, that are performed.

Targeted quantitation

1) Full-scan MS with data-dependent tandem mass spectrometry (full MS/ddMS^2 with inclusion list). In this workflow, the user defines a list of target parent ions and a retention time window for each. During the analysis, data acquisition is performed in full-scan mode. If any of the parent ions in the target list are detected within the defined retention time window, the instrument automatically performs an MS/MS scan, and then resumes full-scan operation. Data obtained in the full-scan mode are used for detection and quantitation, whereas data obtained from the MS/MS scans are used for identification with confirmation. Characteristics of the full MS/ddMS^2 quantitation workflow include:

- Peak areas of the extracted ion chromatograms of the parent ions of interest are used for quantitation
- Selectivity is based on high resolution
- Enables multi-residue methods in complex matrices
- Minimal method development required for food safety, clinical research, forensic toxicology, or environmental applications

2) Targeted SIM with data-dependent tandem mass spectrometry (t-SIM/ddMS^2). Characteristics include:

- Peak areas of the extracted ion chromatograms of the parent ions of interest are used for quantitation
- Scheduled target list (retention time, *m/z*)
- Most useful where ultimate sensitivity is needed
- Minimum MS method development needed (the user must define parent ions and mass isolation width)

3) Parallel reaction monitoring (PRM). Characteristics include:

- Peak areas of the extracted ion chromatograms of the precursor ion selected fragment ions are used for quantitation
- Post-acquisition processing of the fragment extracted ion chromatograms because all fragments are recorded
- Scheduled target list (retention time, *m/z*, collision energy)
- Provides the most sensitivity and selectivity for quantitation of compounds in very complex matrices

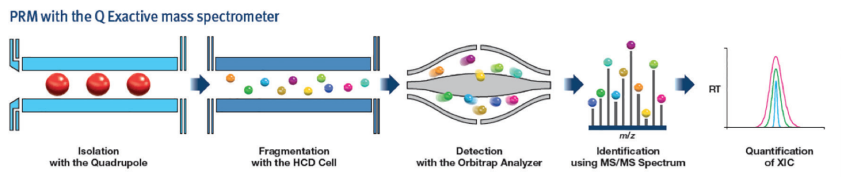

Figure 6. The Q Exactive analyzer provides parallel detection of all target product ions in one coordinated HRAM analysis.

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Choice of quantitation experiments

Non-targeted quantitation

Full-scan MS with DIA delivers reproducible quantitation with complete full-scan MS/MS qualitative information for unknown analysis. In the DIA approach, a number of fragmentation events with wide isolation windows are used to cover the entire range of precursor ions of interest. Compared with AIF, DIA can improve selectivity because the product ions are derived from a smaller range of precursor ions. Furthermore, it improves the sensitivity of detection of fragment ions because a higher number of analyte precursor ions are delivered to the C-Trap. The DIA approach allows for absolute quantitation through the use of reference standards, as well as relative quantitation when using Thermo Scientific™ TraceFinder™ software to compare different samples from one data set in one workflow. If needed, this workflow allows general unknown screening as an extension to analyses done previously with several options for suspect identification.

Q Exactive mass spectrometers are now used extensively for the quantitation of small molecule analytes in a broad range of clinical research, forensic toxicology, pharmaceutical discovery, environmental analysis, and food testing applications. The result is a steadily-increasing collection of peer-reviewed publications highlighting the benefits of Orbitrap mass spectrometer-based quantitation (Figure 6). In the overview of scientific peer-review publications that follows, three important themes emerge:

- Easier environmental method development: Compared to QQQ MS multi-residue methods that involve optimization of hundreds of SIM or SRM transitions in numerous time windows, Orbitrap mass spectrometer-based quantitation methods do not require special tuning and optimization nor comprehensive MS knowledge to set up.
- Greater confidence due to less possibility of false detection of the analytes of interest: The higher resolving power provided by the Orbitrap mass analyzer of 70,000 (FWHM) at *m/z* 200 or greater, provides better analytical selectivity for low molecular weight compounds in complex matrices.
- Unique ability to detect non-target compounds and to analyze data retrospectively, without re-running samples: Full-scan HRAM data can be acquired across the full mass range of interest.

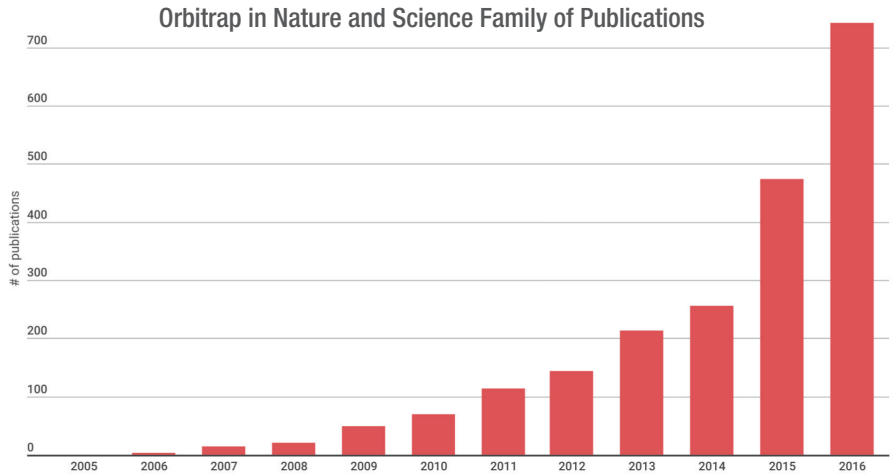

Figure 6. Growing collection of scientific peer-reviewed publications highlighting Orbitrap mass spectrometer-based quantitation.

Food safety testing

- Targeted screening and quantitation of food contaminants: workflow
- Pesticides in fruits and vegetables
- Synthetic hormones and other veterinary drugs in animal products
- Mycotoxins in dairy products
- Multiclass contaminants in food
- Marine biotoxins
- Dyes in wine
- Herbal medicines and dietary supplements

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Targeted screening and quantitation of food contaminants: workflow

Ensuring the safety of food supplies is of utmost importance for public health and safety. Screening and quantitating foodstuffs for known and unknown contaminants, such as pesticides, herbicides, antibiotics, and adulterants in complex matrices must be fast and simple. Orbitrap mass spectrometer-based HRAM analysis is ideally suited for screening and quantitating hundreds or thousands of contaminants in a single analysis. These analyses are performed using variations of the workflow shown in Figure 7.

Didyouknow?

Screening, identification, and quantiation of food contaminants is faster and more certain with Thermo Scientific™ High-Resolution Accurate-Mass MS/MS Spectral Libraries. To learn more: click here

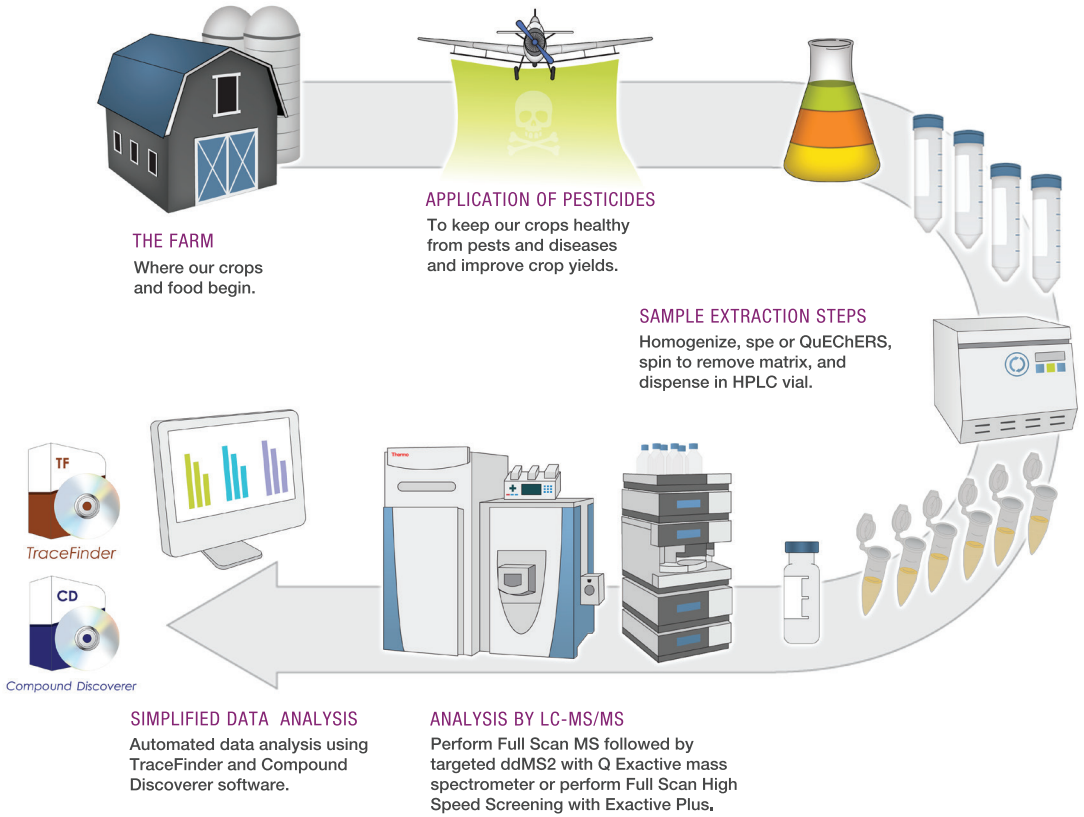

Figure 7. Q Exactive mass spectrometer workflow for targeted screening and quantitation of food contaminants. Samples are processed and contaminants are extracted using various methods such as QuEChERS and online and offline solid phase extraction (SPE), and then are analyzed on the Q Exactive mass spectrometer. Data is scrutinized and the amount of contaminate is determined using automated software.

Pesticides in fruits and vegetables

Del Mar Gómez-Ramos et al. applied the Q Exactive mass spectrometer to LC-MS analysis of 139 pesticide residues in QuEChERS extracts of tomato, pepper, orange and green tea.[3] An analysis of 100 real samples was performed to evaluate the mass spectrometer's identification and quantitation capability and the results were compared to a QQQ MS/MS analysis of the same samples. When the authors analyzed the samples using QQQ MS/MS, the results were consistent with those produced by the Q Exactive mass spectrometer–the same pesticides were found and false positives and false negatives were not reported. The authors noted that compared to the QQQ instrument, the Q Exactive mass spectrometer increased selectivity and, in full-scan mode, permitted retrospective analysis of data.

Wang et al. applied the Q Exactive mass spectrometer to LC-MS analysis of 166 pesticide residues in fruits and vegetables.[4] Pesticides were extracted using the QuEChERS method. Full-scan data (full MS) were used for quantitation. Data-dependent MS/MS ($ddMS^2$) product-ion spectra were used for confirmation. Matrix-matched standard calibration curves with isotopically labeled standards, or chemical analogues as internal standards, were used for quantitation. The authors evaluated overall recovery, intermediate precision, and measurement uncertainty. Approximately 90.3 to 91.5% of the pesticides had good recoveries between 81 and 110%, 92.1 to 97.6% had intermediate precision of ≤ 20%, and 89.7 to 95.2% had measurement uncertainty of ≤ 40%. Confirmation of targets was based on a mass accuracy ≤ 5 ppm and LC retention time tolerance within ±2.5%. The authors concluded that for quantitation and confirmation of pesticide residues in fresh fruits and vegetables, the Q Exactive mass spectrometer demonstrated good performance.

- *Attain selectivity surpassing QQQ instruments*
- *Analyze full-scan data retrospectively*

3. Del Mar Gómez-Ramos, M.; Rajski, Ł.; Heinzen, H.; Fernández-Alba, A. R. Liquid chromatography Orbitrap mass spectrometry with simultaneous full scan and tandem MS/MS for highly selective pesticide residue analysis. Anal. Bioanal. Chem. 2015 Aug; 407(21):6317-26.

4. Wang, J.; Chow, W.; Leung, D.; Chang, J. Application of ultrahigh-performance liquid chromatography and electrospray ionization quadrupole orbitrap high-resolution mass spectrometry for determination of 166 pesticides in fruits and vegetables. J. Agric. Food Chem. 2012, Dec 12;60(49):12088-104.

Pesticides in fruits and vegetables

Wang et al. went on to apply the Q Exactive mass spectrometer to the determination of 451 pesticide residues in ten fruit and vegetable matrices.[5] As before, the QuEChERS method was used to extract target pesticides. Full-scan data were used for quantitation and full MS/dd-MS^2 generated product ion spectra were used to identify compounds. To achieve optimal method accuracy, quantitation was performed using matrix-matched standard calibration curves along with isotopically labeled standards, or a chemical analogue, as internal standards. The method was validated based on overall recovery, intermediate precision, and measurement uncertainty. In the ten matrices studied, 94.5% of the pesticides in fruits and 90.7% in vegetables had recoveries between 81 and 110%; 99.3% of the pesticides in fruits and 99.1% of the pesticides in vegetables had an intermediate precision of ≤ 20%; and 97.8% of the pesticides in fruits and 96.4% of the pesticides in vegetables showed measurement uncertainty of ≤ 50%. The measurement uncertainty met the recommended default value of the European Commission SANCO/12495/2011 method validation and quality control procedures for pesticide residues analysis in food and feed. The authors concluded that the method demonstrated acceptable performance for the quantitation of pesticide residues in fruits and vegetables. Further, full MS/dd-MS^2 with library matching demonstrated the potential to improve pesticide identification in routine practice.

5. Wang, J.; Chow, W.; Chang, J.; Wong, J. W. Ultrahigh-performance liquid chromatography electrospray ionization Q-Orbitrap mass spectrometry for the analysis of 451 pesticide residues in fruits and vegetables: method development and validation. J. Agric. Food Chem. 2014, Oct 22;62(42):10375-91.

Pesticides in fruits and vegetables

Zomer et al. developed and validated a Q Exactive mass spectrometer-based method using DIA for the simultaneous quantitation, identification, and qualitative screening of pesticides in fruits and vegetables.[6] Thermo Scientific TraceFinder software was used to process data. Both quantitative and qualitative performance was evaluated. The goal of the quantitative assessment was to determine whether non-targeted full-scan MS can replace QQQ MS. Quantitative performance was assessed by spiking 184 pesticide compounds in lettuce and orange matrices at 10 and 50 ng/g and quantitative validation was performed according SANCO/12571/2013. The goal of the qualitative assessment was to determine the ability of the method to detect the presence or absence of pesticides in an automated fashion. Qualitative performance was tested by analyzing nine additional matrices (apple, French bean, broccoli, carrot, celery, grape, leek, nectarine, and tomato) spiked with the same 184 compounds at 10, 50, and 200 ng/g. Reproducibility of qualitative performance was tested by repeating the analysis on new extracts, about 4 weeks later.

Data-independent acquisition provided a fully non-targeted approach for data acquisition. As described by the authors, a full-scan acquisition event without fragmentation at resolving power 70,000 was followed by five consecutive DIA fragmentation events at resolving power 35,000. According to the authors, the advantages of using a full-scan technique are that it is not necessary to decide or know beforehand which compounds should be targeted and the ease with which the number of compounds in a method can be increased beyond the practical maximum number of compounds typical of a QQQ MS method.

The quantitative validation demonstrated that the majority of the compounds met the criteria for trueness and precision set forth in SANCO/12571/2013. For the qualitative validation of the untargeted screening capabilities of the method, an overall detection rate of 92% was achieved at 10 ng/g, which increased to 98% at 200 ng/g. A screening detection limit (SDL) of 10 ng/g was achieved for 134 of the pesticides. For 39 of the compounds, the SDL was 50 ng/g. For two pesticides, the SDL was 200 ng/g. For the other nine compounds no SDL could be established. The recommended ion ratio identification criteria were met for 93% of the detected pesticide/matrix/concentration combinations. Based on these results, the authors concluded that the method can be used to combine, in one measurement, the quantification and identification of pesticides typically detected using QQQ MS/MS with qualitative screening to find a range of less frequently detected compounds. Qualitative screening uses the same data with another data-processing approach involving automatic detection by the TraceFinder software.

- *Detect both target and untargeted compounds in full scan mode*
- *Analyze more compounds per run than practical using QQQ instruments*
- *Quantify, identify, and screen in a single run*

6. Zomer, P. and Mol H. G. J. Simultaneous quantitative determination, identification and qualitative screening of pesticides in fruits and vegetables using LC-Q-Orbitrap™-MS. Food Addit. Contam: Part A. 2015 Sep 15:1-9. [Epub ahead of print].

Pesticides in fruits and vegetables

Because complex matrices present a challenge to fast and accurate screening and quantitation of pesticides in food, **Yang et al**. studied the relationship between matrix effects and LC separation and elution of pesticides and matrix components using an Orbitrap mass spectrometer.[7] The study used two LC columns containing different adsorbents. The 108 samples were prepared in solvent and five different sample matrices (avocado, spinach, orange, hazelnut, and honey) using calibration standards of 381 pesticides at three dilution levels of 1×, 1/10×, and 1/100×.

Principal component analysis and slope ratios of calibration curves demonstrated that the 1/100× sample dilution could minimize ion suppression (matrix effects) for most of the pesticides analyzed. If a pesticide coeluting with matrix components had a peak intensity of 25 times or higher, the suppression for the pesticide persisted to 1/100× dilution.

The effect and optimization of mass spectrometer parameters on the analysis of pesticide residues in complex food matrices is of great interest. **Rajski et al**. evaluated of the impact of Orbitrap mass spectrometer operating parameters such as resolution on analytical performance.[8] Analyses were performed on QuEChERS extracts of tomato, pepper, orange, and green tea. The extracts were spiked with 170 pesticides at concentrations of 10 µg/kg, 50 µg/kg, 100 µg/kg, and 500 µg/kg and were diluted 5-fold prior to UHPLC-MS analysis in the full-scan mode. Three resolution settings—17,500, 35,000, and 70,000—were tested at each concentration level.

Using a resolution setting of 17,500 with 5 ppm of mass tolerance, the percent pesticides detected at 10 µg/kg ranged from 91% in tomato to 83% in green tea. The percent pesticides detected increased when higher resolution settings were used. Analysis at 35,000 resolution produced better results—peak areas were more reproducible and more pesticides were detected—and was sufficient for analysis of the tomato, pepper and orange matrices. Green tea, the most problematic matrix, required analysis at 70,000 resolution. The rates of compounds detected at 70,000 resolution ranged from 98% in tomato to 88% in green tea, thus the authors determined the resolution of 70,000 to be best with the smallest percentage of false negatives at low concentrations. False negative detects were mainly due to a lack of sensitivity for a particular compound, combined with ion suppression effects of the matrix.

Reproducibility improved at resolution levels of 35,000 or higher. Linearity was evaluated from 2–100 ng/mL (10–500 µg/kg in the sample). Unlike other high-resolution MS technologies such as TOF, the Orbitrap mass spectrometer did not experience poor linearity due to detector saturation. The results obtained in this study were comparable those obtained using QQQ MS technology.

- *Detect more pesticides using higher-resolution settings (70,000)*
- *Avoid detector saturation and poor linearity of Q-TOF technology*

7. Yang, P.; Chang, J. S.; Wong, J. W.; Zhang, K.; Krynitsky, A. J.; Bromirski, M.; Wang, J. Effect of Sample Dilution on Matrix Effects in Pesticide Analysis of Several Matrices by Liquid Chromatography-High-Resolution Mass Spectrometry. J. J. Agric. Food Chem. 2015, Jun 3;63(21):5169-77.

8. Rajski, Ł.; Gómez-Ramos Mdel, M.; Fernández-Alba, A. R. Large pesticide multiresidue screening method by liquid chromatography-Orbitrap mass spectrometry in full scan mode applied to fruit and vegetables. J. Chromatogr. A. 2014, Sep 19;1360:119-27.

Synthetic hormones and other veterinary drugs in animal products

Because the synthetic steroid hormones used to treat animals have been found to affect cancer risk, consumers are concerned about exposure via the animal products they eat. However, the complexity of food matrices makes it difficult to confidently detect and quantify these contaminants at low concentrations. **Kumar et al**. studied the application of the Q Exactive mass spectrometer in four acquisition modes: full MS/AIF, full MS/tMS^2, full MS/ddMS^2, and t-SIM/ddMS^2, to the analysis of synthetic hormones.[9]

Finding it most suitable, the authors developed a t-SIM/ddMS^2 confirmation method for the analysis of eight synthetic hormones (trenbolone, 17α ethinylestradiol, zeranol, stanozolol, dienestrol, diethylstilbestrol, hexestrol, taleranol) and one naturally occurring hormone (zearalenone) in animal urine. Quadrupole precursor ion selection (SIM) acted as a powerful filter to reduce ion suppression. The method was validated according to the European Commission Decision 2002/657/EC for analysis of residues of veterinary medicinal products. The decision limit (CCα) and the detection capability (CCβ) ranged from 0.11 µg/L to 0.69 µg/L, and 0.29 µg/L and 0.90 µg/L, respectively. Overall, the results suggested that the Q Exactive mass spectrometer provides sensitivity similar to that of QQQ instruments, with enhanced selectivity.

Kaufmann et al. applied the Q Exactive mass spectrometer to the quantitation of over 100 compounds belonging to a variety of veterinary drug classes in milk.[10] Instead of traditional extraction and clean up approaches such as solid phase extraction (SPE), QuEChERS and ultra-filtration, the authors introduced a new technique—salting out supported liquid extraction (SOSLE)—to enhance extraction efficiency and sample clean-up of polar analytes. The method was validated based on European Commission Decision 2002/957/EC as it applies to quantitative veterinary drug analysis methods. The authors concluded that the Q Exactive mass spectrometer provided good quantitative data and superior compound confirmation. The SOSLE technique produced extracts of equal or superior cleanliness and with higher average recoveries than those obtained using QuEChERS or SPE. The largest improvement was obtained for polar analytes as such penicillines, quinolones, and tetracyclines.

- *Reduce ion suppression with quadruple precursor ion selection*
- *Attain sensitivity equal to QQQ instruments, with improved selectivity*

9. Kumar, P.; Rúbies, A.; Centrich, F.; Granados, M.; Cortés-Francisco, N.; Caixach, J.; Companyó, R. Targeted analysis with benchtop quadrupole-orbitrap hybrid mass spectrometer: application to determination of synthetic hormones in animal urine. Anal. Chim. Acta. 2013, May 30;780:65-73.

10. Kaufmann, A.; Butcher, P.; Maden, K.; Walker, S.; Widmer, M. Multi-residue quantification of veterinary drugs in milk with a novel extraction and clean up technique: salting out supported liquid extraction (SOSLE). Anal. Chim. Acta. 2014, Apr 11;820:56-68.

Mycotoxins in dairy products

Mycotoxin contamination can occur when molds grow on dairy products or when dairy cattle eat contaminated feeds. **Jia et al**. developed a Q Exactive mass spectrometer-based UHPLC-MS method for simultaneous analysis of 58 mycotoxins in dairy products.[11] The QuEChERS sample preparation method was used. The authors validated the method using the guidelines specified in European Commission Decision 2002/657/EC and 401/2006/EC.

Extraction recoveries ranged from 86.6 to 113.7%, with a coefficient of variation of < 6.2%. All target compounds were detected within the range of 0.001 to 100 µg/kg, with a correlation coefficient of > 0.99. The limits of detection (LOD) ranged from 0.001 to 0.92 µg/kg. The decision limit (CCα) values were comparable to those of previously reported QQQ methods. Repeatability was less than 6.4%. Three consecutive on-column injections of 1 µg/kg were made to detect any decrease in mass accuracy. No significant decrease was observed and the maximum mass deviation ranged from 0.2 to 2.5 ppm, demonstrating the wide dynamic range of Q-Exactive mass spectrometer at resolution 70,000 (FWHM). Compared with other methods, the method increased sensitivity and mass accuracy by more than five times. In summary, the authors found the method useful for fast screening of mycotoxins in dairy products.

- *Realize a wider dynamic range of detection*
- *Increase sensitivity and mass accuracy by a factor of five*

11. Jia, W.; Chu, X.; Ling, Y.; Huang, J.; Chang, J. Multi-mycotoxin analysis in dairy products by liquid chromatography coupled to quadrupole orbitrap mass spectrometry. J. Chromatogr. A. 2014, Jun 6;1345:107-14.

Multiclass contaminants in food

Analytical methods for screening and quantitating the many contaminants found in foods must be reliable and meet regulatory requirements for recoveries and limits of quantification (LOQ). **Dzuman et al**. presented a reliable and sensitive method that used HPLC separation in combination with HRAM Orbitrap mass spectrometer-based detection for the determination of 323 pesticide residues, 55 mycotoxins, and 11 plant toxins.[12] The efficiency of a special core-shell HPLC column with relatively high particle size (2.6 mm) with lower operational pressures was also examined. The authors validated the method in three sample matrices, leek, wheat, and tea, which differed in the type and amount of components causing matrix effects. QuEChERs was used for sample preparation and extraction of target analytes. A HRAM MS/MS spectral library containing the spectrum of fragment ions for each analyte was created to facilitate identification and confirmation of the target compounds.

The core-shell analytical column demonstrated good separation efficiency and robustness, yielding retention-time RSDs of less than 0.3% after 2000 injections. Method LOQs for the target analytes were less than 10 µg/kg for 82%, 81%, and 61% for the leek, wheat, and tea matrices, respectively. For the majority of the target analytes, recoveries were 70 to 120%, a range acceptable according to SANCO/12571/2013 analytical quality control and validation procedures for pesticide residues analysis in food and feed. The exception was the highly polar mycotoxin deoxynivalenol-3-glucoside with recoveries of 35%, 47%, and 42% for leek, wheat, and tea matrices, respectively. Calibration curve linearity expressed as coefficients of determination ranged from of 0.9661 to 1.000. Repeatability at the LOQ ranged from 0.25 to 13.51% RSD.

- *Screen and quantify multiclass contaminants, reliably and in accordance with regulatory requirements*
- *Identify and confirm target compounds*

12. Dzuman, Z.; Zachariasova, M.; Veprikova, Z.; Godula, M.; Hajslova J. Multi-analyte high performance liquid chromatography coupled to high resolution tandem mass spectrometry method for control of pesticide residues, mycotoxins, and pyrrolizidine alkaloids. Anal. Chim. Acta. 2015, Mar 10;863:29-40.

Multiclass contaminants in food

The method was validated according to the European Commission Decision. Ensuring the safety of baby food is of utmost importance, but screening and quantitating hundreds of known and unknown contaminants such as pesticides and antibiotics can be challenging. **Jia et al**. developed an UHPLC-ESI Q Exactive mass spectrometer method for simultaneous analysis of 333 pesticides and veterinary drug residues in baby food.[13] QuEChERs was used for sample preparation and extraction.

The method was validated according to the European Commission Decision 2002/657/EC and SANCO/12571/2013. QuEChERs extraction recoveries ranged of 79.8 to 110.7%, with coefficients of variation $< 8.3\%$. The 333 compounds ranged from 0.1 to 1000 µg/kg in concentration, with a correlation coefficient > 0.99. The LODs ranged from 0.01 to 5.35 µg/kg. The LOQs were in the range of 0.01 to 9.27 µg/kg. After successfully screening 93 commercial baby food samples for pesticide and veterinary drug residues, the authors concluded the method is appropriate for rapid screening of foods. In particular, tilmicosin, fenbendazole, tylosin tartrate, and thiabendazole were detected in some samples.

More than 300 compounds belonging to several classes of veterinary drugs and pesticides have been found in animal feed. Because medium and high-resolution mass spectrometers provide advantages in multi-residue analysis, **Gómez-Pérez et al**. compared the performance of medium-resolution (MRMS) TOF and high-resolution Orbitrap mass spectrometers for the analysis of toxic compounds in 18 different chicken, hen, rabbit, and horse feed samples.[14] Sample cleanup procedure was evaluated and several validation parameters were established including matrix effect, linearity, recovery and sensitivity.

The authors obtained better results using the Orbitrap mass spectrometer with sensitivity of 1 to 12.5 µg/kg (below MRL), recovery values of 60–125%, and fewer compounds experienced signal suppression or enhancement. The TOF LOQ values ranged from 5 to 100 µg/kg. Sulfadiazine, trimethoprim, robenidine and monensin sodium veterinary drugs, and the pesticide chlorpyrifos, were identified when the method was applied to the feed samples, demonstrating its applicability as a quantitative method regardless of the type of feed. Other advantages provided by the Orbitrap mass spectrometer important for routine analysis were short analysis time (14 minutes), ability to perform fast screening using Thermo Scientific™ ToxID™ automated screening software (which processed more than 450 compounds in less than 5 minutes), and lack of need for a lock mass.

- *Obtain better sensitivity and recovery for multi-residue analysis*
- *Reduce signal suppression and enhancement compared to Q-TOF technology*
- *Rapidly screen and save time using ToxID software*

13. Jia, W.; Chu, X.; Ling, Y.; Huang, J.; Chang, J. High-throughput screening of pesticide and veterinary drug residues in baby food by liquid chromatography coupled to quadrupole Orbitrap mass spectrometry. J. Chromatogr. A. 2014, Jun 20;1347:122-8.

14. Gómez-Pérez, M. L.; Romero-González, R.; Martínez Vidal, J. L.; Garrido Frenich, A. Analysis of veterinary drug and pesticide residues in animal feed by high-resolution mass spectrometry: comparison between time-of-flight and Orbitrap. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk. Assess. 2015, Mar 18:1-10.

Marine biotoxins

During algal blooms, biotoxins produced by certain types of algae can become concentrated in filter feeders such as shellfish. Though the biotoxins don't harm the shellfish, but they can accumulate to levels that cause serious illness or even death in humans and other mammals when eaten. **Rúbies et al**. developed and validated a high-throughput method to analyze lipophilic marine biotoxins (okadaic acid, dinophysistoxins, azaspiracids, pectenotoxins, yessotoxins, spirolids) in fresh and canned bivalves.[15] The method coupled QuEChERS sample cleanup and extraction with LC-MS analysis using the Q Exactive mass spectrometer operating in tandem MS mode, with resolution set to 70,000 (FWHM) at *m/z* 200. Separation of analytes was performed in about ten minutes in gradient elution mode with a BEH C18 column and mobile phases based on 6.7 mM ammonia aqueous and acetonitrile mixtures. For each analyte, the molecular ion and one or two product ions were acquired, with a mass accuracy better than 5 ppm. Quantitation was performed using surrogate matrix matched standards, with eprinomectin as the internal standard.

The QuEChERS sample clean up and extraction procedure yielded high absolute recoveries. High-resolution MS/MS data acquisition was powerful in avoiding matrix interferences. Accurate mass data for both the molecular ion and the selected fragments could be obtained for each target analyte, enabling confirmation of compound identity and avoidance of false positives. The authors concluded that the method is straightforward, reliable, and suitable for routine confirmatory quantitative analysis of lipophilic marine biotoxins in fresh and canned bivalves at regulated levels. The method meets the requirements of the EU food safety regulations and is in routine use in a public health laboratory.

- *Avoid matrix interferences with high-resolution*
- *Confirm compound identity and avoid false positives using accurate mass*

15. Rúbies, A.; Muñoz, E.; Gibert, D.; Cortés-Francisco, N.; Granados, M.; Caixach, J.; Centrich, F. New method for the analysis of lipophilic marine biotoxins in fresh and canned bivalves by liquid chromatography coupled to high resolution mass spectrometry: a quick, easy, cheap, efficient, rugged, safe approach. J. Chromatogr. A. 2015, Mar 20;1386:62-73.

Marine biotoxins

Likewise, **Domènech et al**. developed and validated a Q Exactive mass spectrometer-based LC-MS method for the quantitation and confirmation of lipophilic marine biotoxins in mussels.[16] Compounds representative of each lipophilic toxin group were analyzed: Okadaic acid (OA), yessotoxin, azaspiracid-1, gymnodimine, 13-desmethyl spirolide C, pectenotoxin-2 and Brevetoxin B. Identification and confirmation criteria were established. Fragment and isotope ions and ion ratios were evaluated for use in confirmation. The authors found that both fragment ion and isotope ion ratios can be used to confirm a positive result, but for each compound one or the other can be more suitable.

Accuracy (trueness and precision), linearity, calibration curve check, LOQ and specificity were used as method validation parameters and the validation was performed at 0.5 times the European Union permitted levels. Overall, the method performed very well for the parameters investigated. Trueness (recovery) ranged from 80% to 94%, precision (intra-laboratory reproducibility), ranged from 5% to 22%, and LOQs were from 0.9 to 4.8 pg on column. Overall method uncertainty of 38% was estimated for OA, using certified reference material and a top-down approach considering contributions arising from the trueness and precision studies. The authors concluded that the Orbitrap mass spectrometer-based method enables full-scan acquisition with good sensitivity and better selectivity than other approaches, and can help avoid false positives when confirmation criteria are used.

- *Exceed EU-specified levels for accuracy, linearity, LOQ, and specificity*
- *Enjoy full-scan data acquisition with enhanced sensitivity and selectivity*
- *Avoid false positives using confirmation criteria*

16. Domènech, A.; Cortés-Francisco, N.; Palacios, O.; Franco, J. M.; Riobó, P.; Llerena, J. J., Vichi, S.; Caixach, J. Determination of lipophilic marine toxins in mussels. Quantification and confirmation criteria using high resolution mass spectrometry. J. Chromatogr. A. 2014, Feb 7;1328:16-25.

Dyes in wine

White wines can contain dyes added to produce a richer color and these dyes can cause reactions in susceptible people. **Jia et al**. developed a method combining QuEChERS sample preparation and extraction with Q Exactive mass spectrometer detection for accurate and sensitive screening of 69 dyes in wine.[17] After optimization of the QuEChERS procedure, the maximum predicted recovery was 99.48% for canacert indigo carmine. Recovery rates of the other 68 compounds ranged from 87.2 to 107.4%, with a coefficient of variation < 6.4%. The mass accuracy obtained was routinely better than 1.6 ppm with once-per-week calibration. The LODs ranged from 1 to 1000 µg/kg. The authors concluded that the method is very useful for fast screening of dyes in commercial wines.

Screen rapidly, accurately, and with high sensitivity

17. Jia, W.; Chu, X.; Ling, Y.; Huang, J.; Lin, Y.; Chang, J. Simultaneous determination of dyes in wines by HPLC coupled to quadrupole orbitrap mass spectrometry. J. Sep. Sci. 2014, Apr;37(7):782-91.

Herbal medicines and dietary supplements

The active ingredients in herbal medicines and dietary supplements can augment or antagonize the actions of other prescription and non-prescription drugs. In the US, antidiabetics are prescription-only medicines designed to be taken only with physician supervision. **Guo et al**. developed a Q Exactive mass spectrometer-based method for rapid screening, confirmation, and quantitation of 11 illegal antidiabetic adulterants in herbal medicines and dietary supplements.[18] Sixty-three batches of herbal medicine, and 34 batches of dietary supplement samples were tested. The mass spectrometer was operated in the full MS/ddMS^2 mode. Full-scan data was used for identification and provided superior accuracy, precision, and sensitivity for quantitation. The data-dependent MS/MS scans produced product ion spectra for unambiguous compound confirmation. Quantitation was performed using matrix-matched standard calibration curves with phenacetin as the internal standard. Method validation parameters were selectivity, sensitivity, calibration curve, accuracy and precision, recovery, matrix effects, and stability.

Response was linear over wide analyte concentration ranges (e.g., 0.0004 to 1 µg/g for metformin) with coefficients of correlation $r^2 > 0.9991$. The LODs ranged from 0.05 to 0.5 ng/g. Recoveries were higher than 74.3%. Accuracy ranged from -6.75 to 3.85% and the intra- and inter-day precision ranged from 0.048 to 11.5%. The adulterants metformin, phenformin, and glibenclamide were detected in seven of the dietary supplements tested, but in none of the herbal medicines. The authors concluded that the method demonstrated very good performance for the identification, confirmation, and quantitation of antidiabetics in herbal medicines and dietary supplements.

- *Use full-scan data for compound ID as well as accurate quantitation*
- *Confirm compounds confidently using data-dependent MS/MS*

18. Guo, C.; Shi, F.; Jiang, S.; Gong, L.; Zhao, Y.; Zhang, J.; Zeng, S. Simultaneous identification, confirmation and quantitation of illegal adulterated antidiabetics in herbal medicines and dietary supplements using high-resolution benchtop quadrupole-Orbitrap mass spectrometry. J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci. 2014, Sep 15;967:174-82.

Herbal medicines and dietary supplements

Vaclavik et al. developed and validated a Q Exactive mass spectrometer method for the simultaneous determination of 96 pharmaceuticals, plant toxins, and other plant metabolites in herbal dietary supplements.[19] Target analytes were extracted using the QuEChERS method. The mass spectrometer was operated in full MS/ddMS^2 acquisition mode, which provided high-resolution full-scan data for quantitation and high-resolution MS/MS data for confirmation in a single analytical run.

The method provided excellent selectivity in both full MS and ddMS^2 modes. Confirmation of analytes was with a high degree of confidence. Method LODs and LOQs differed significantly depending on the sample matrix tested. Across the five different matrices, the LODs ≤ 10 µg/kg and LOQs ≤ 50 µg/kg were obtained for 48 to 81% of the target compounds. With the exception of the highly polar analytes, the QuEChERS extraction provided acceptable recoveries in the range of 70% to 120%. The precision of the method, defined as the relative standard deviation (RSD, n = 5), was ≤ 25% and ≤ 18% at spiked concentrations of 50 µg/kg and 500 µg/kg, respectively. Because of sample-to-sample variation in matrix effects of the extracts, the method of standard additions and an approach based on dilution of matrix components followed by quantitation using solvent standards were applied to the quantitative analysis.

Quantitate and confirm in a single run

19. Vaclavik, L.; Krynitsky, A. J.; Radar, J. Targeted analysis of multiple pharmaceuticals, plant toxins and other secondary metabolites in herbal dietary supplements by ultra-high performance liquid chromatography-quadrupole-orbital ion trap mass spectrometry. Anal. Chim. Acta. 2014, Jan 31;810:45-60.

Environmental contaminants analysis

- Targeted screening and quantitation of environmental contaminants: workflow
- Contaminants in drinking and wastewater
- Contaminants in surface water

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Targeted screening and quantitation of environmental contaminants: workflow

Ultimately the goal of any environmental analysis is the quantitative determination of contaminants. Water, soil, and air have regulated levels of contaminants established to protect human health. These levels are based on the degree of toxicity along with prevalence and cost of cleanup. Monitoring for and removing toxic compounds from the environment, especially drinking water, also increases costs. Therefore, quantitation of exact concentration is crucial to both preserving health and minimizing costs. Also important are investigations of non-regulated, emerging contaminants to assess if and when a future regulatory determination is to be made.

Ensuring the safety of drinking water is one area of interest. Because lakes, rivers, streams, and oceans are impacted by human and natural activities, monitoring these resources continues to be of interest. HRAM analysis is ideally suited to rapid screening and quantitation of water samples for targeted contaminants such as pesticides, herbicides, and other pollutants. Orbitrap mass spectrometer-based LC-MS analyses of water are performed using variations of the workflow shown in Figure 8.

Didyouknow?

Screening, identification, and quantitation of environmental contaminants is faster and more certain with Thermo Scientific™ High-Resolution Accurate-Mass MS/MS Spectral Libraries. To learn more: click here

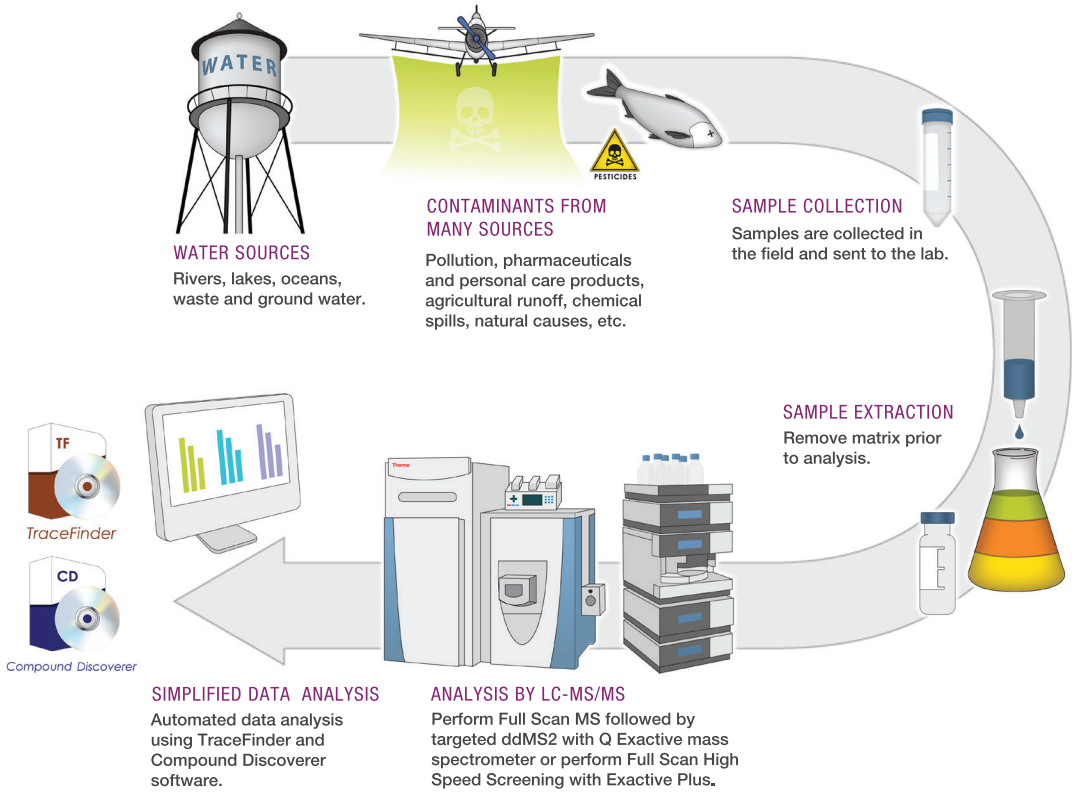

Figure 8. Q Exactive mass spectrometer workflow for targeted screening and quantitation of contaminants in water. Samples are processed and contaminants are extracted using various extraction techniques, from online solid-phase extraction to offline extraction, prior to analysis on the mass spectrometer.

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Contaminants in drinking and wastewater

1,2 N-nitrosamines (NAs) are receiving increased attention due to their high carcinogenic and mutagenic potential at low concentrations. Though a countrywide maximum level in drinking water is not yet established in North America, California and Ontario have set limits of 10 ng/L and 9 ng/L, respectively. N-Nitrosamine compounds are produced by industrial activity and resist removal during wastewater treatment. In addition, N-Nitrosamines such as NDMA are by-products of disinfection by chlorination and chloramination. Because of their hydrophobicity and polarity, extraction and detection of NAs at low levels is a challenge. **Ngongang et al**. developed a selective and robust Q Exactive mass spectrometer-based SPE-UHPLC-MS method for the analysis of nine N-nitrosamines in drinking and wastewater matrices.[20] SPE was employed as a cost-effective method that helps in achieving low detection limits and high sample throughput.

The authors validated the method in HPLC grade water, drinking water and wastewater matrices and determined that the method sensitivity is comparable with that of GC-MS and LC-MS/MS methods. HRAM data produced by the Q Exactive mass spectrometer helped the authors identify and quantify the target NAs unambiguously, and the selectivity of the HRAM method eliminated matrix interferences. The authors concluded that although GC-MS can provide better sensitivity for N-nitrosamines, LC-MS provides significant time savings because of the longer retention times of GC-MS. Furthermore, LC-MS enabled detection of both GC-detectable and GC-undetectable NAs such as NDPhA.

- *Identify and quantify with confidence using HRAM data*
- *Eliminate matrix interferences with high selectivity*

20. Ngongang, A.D.; Vo Duy, S.; Sauve´, S. Analysis of nine N-nitrosamines using liquid chromatography-accurate mass high resolution mass spectrometry on a Q-Exactive instrument. Anal. Methods. 2015.

Contaminants in drinking and wastewater

New ways to correlate the amounts of drug residues in wastewater with drug consumption are driving growing interest in their quantitation. Due to this interest, **Fedorova et al**. compared the quantitative performance of hybrid quadrupole-Orbitrap-based mass spectrometry with that of QQQ MS for LC-MS analysis of drug residues in wastewater.[21] The Q-Exactive mass spectrometer was operated at resolution of 70,000 (FWHM) at *m/z* 200 in full-scan (HRFS) mode and 17,500 (FWHM) at *m/z* 200 in product ion scan (HRPIS) mode. For the SRM analyses, the first and third quadrupoles of the QQQ mass spectrometer were set to 0.7 FWHM. A mass-extracted window of 5 ppm around the theoretical *m/z* of each target analyte was used to construct mass chromatograms.

While all three methods showed good linearity and repeatability, the HRPIS mode delivered better sensitivity and selectivity for certain compounds. The selectivity and sensitivity advantages of the Q Exactive mass spectrometer result from quadrupole precursor ion selection combined with HRAM Orbitrap mass-analyzer detection. The LOQs ranged from 0.46 to 20 ng/L. Both the QQQ and the HRPIS Orbitrap mass spectrometer-based MS/MS methods showed good selectivity. The HRFS-based method suffered from more interferences and showed some false positive results when confronted with co-extracted matrix interferences. The authors concluded that the Q Exactive mass spectrometer is suited for trace-level detection and quantitation of most of the drugs tested in complex wastewater matrices and offers potentially better selectivity than QQQ MS instruments for certain compounds.

When exposed to chemical disinfectants used in wastewater treatment, many pharmaceuticals degrade into by-products that can be more toxic than the parent compound itself. To address this problem, **Negregaria et al**. investigated the stability of a widely used cytostatic etoposide in chlorinated water using the HRAM capability of the Q Exactive mass spectrometer.[22] The authors identified two new etoposide oxidation by-products and were able to measure the time course of etoposide degradation into its by-products at different pH values and free chlorine concentrations, and in different water matrices.

- *Detect and quantify trace-level compounds in wastewater matrices*
- *Experience selectivity and sensitivity surpassing QQQ instruments*

21. Fedorova, G.; Randak, T.; Lindberg, R.H.; Grabic, R. Comparison of the quantitative performance of a Q-Exactive high-resolution mass spectrometer with that of a triple quadrupole tandem mass spectrometer for the analysis of illicit drugs in wastewater. Rapid. Commun. Mass Spectrom. 2013, Aug 15;27(15):1751-62.

22. Negreira, N.; López de Alda, M.; Barceló, D. Degradation of the cytostatic etoposide in chlorinated water by liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry: identification and quantification of by-products in real water samples. Sci. Total Environ. 2015, Feb 15;506-507:36-45.

Contaminants in drinking and wastewater

Organic contaminants in drinking water have diverse chemistry and are found in varying amounts ranging from mg/L to ng/L. Until now, analyses focused on pesticides and industrial contaminants instead of those emerging such as pharmaceuticals, personal care products, and flame-retardants. Most methods are based on LC-QQQ/MS and thus limit the number of target contaminants that can be screened in one analysis. Because high-resolution mass spectrometers that provide HRAM full scan data can address a virtually unlimited number of organic compounds, **Cotton et al**. developed and evaluated a fast automated method using on-line SPE, UHPLC, and the Q Exactive mass spectrometer to screen and identify several hundred pesticides and drug residues in drinking water. [23]

The method allowed simultaneous semi-quantitative analysis of 539 pesticides and drug residues in 5 mL samples, in only 36 minutes. Repeatability, selectivity, linearity, and matrix effects were used to validate the method. When applied to 20 tap water samples, the method detected 34 different compounds at concentrations below 0.1 mg/L, the European Union limit for drinking water. Drug residues not commonly monitored, such as valsartan and carbamazepine, were found. The authors concluded the multi-analyze method is very promising as HRAM full scan data enables both post-target and non-target analysis.

23. Cotton, J.; Leroux, F.; Broudin, S.; Poirel, M.; Corman, B.; Junot, C.; Ducruix, C. Development and validation of a multiresidue method for the analysis of more than 500 pesticides and drugs in water based on on-line and liquid chromatography coupled to high resolution mass spectrometry. Water Res. 2016, Nov 1;104:20-27.

Contaminants in surface water

Moschet et al. developed a Q Exactive mass spectrometer-based LC-MS method that relies on exact mass rather than reference standards to assess rarely-investigated pesticides and their transformation products (TPs) in 76 surface water samples.[24] One hundred eighty five water-soluble insecticides, fungicides and their major TPs were analyzed. A SPE LC-MS method was developed using 45 known, persistent, and high-sales-volume pesticides. Seventy percent of these targets had LOQs < 5 ng/L. This compound set was then used to develop and optimize a screening method using only exact mass as a priori information. This method resulted in a 70% success rate. False negatives were mainly due to low intensity peaks. The authors then applied the method to the remaining 140 compounds. Nineteen additional substances were detected including two TPs that had never been found in the environment before. The authors concluded that the screening approach was fast, successful, and easily expanded to other micropollutant classes for which reference standards are not accessible.

Due to the large number of potential contaminants, comprehensive assessment of pesticides in surface waters is challenging. Most scientific studies and routine monitoring programs include only 15 to 40 pesticides. Routine, comprehensive screening has not been feasible due to labor-intensive analysis with QQQ mass spectrometers. For this reason, **Moschet et al**. developed a comprehensive LC-MS screening method relying on the HRAM capability of the Q Exactive mass spectrometer.[25] The method covered 86% of all polar organic pesticides sold in Switzerland that are applied to agricultural or urban land (249 compounds) and 134 TPs. Between March and July 2012, the authors regularly drew samples from five medium-sized rivers containing large areas of diverse crops and urban settlements within their catchments. The authors detected over 100 parent compounds and 40 TPs and the sum of the pesticide concentrations was above 1000 ng/L in 78% of samples. The chronic environmental quality standard was exceeded for 19 single substances and the “exceedances” occurred over the entire measurement period in all rivers.

- *Screen for surface water contaminants routinely and comprehensively*
- *Avoid the labor-intensive method set-up of QQQ instruments*

24. Moschet, C.; Piazzoli, A.; Singer, H.; Hollender, J. Alleviating the reference standard dilemma using a systematic exact mass suspect screening approach with liquid chromatography-high resolution mass spectrometry. Anal. Chem. 2013, Nov 5;85(21):10312-20.

25. Moschet, C.; Wittmer, I.; Simovic, J.; Junghans, M.; Piazzoli, A.; Singer, H.; Stamm, C.; Leu, C.; Hollender, J. How a complete pesticide screening changes the assessment of surface water quality. Environ. Sci. Technol. 2014, May 20;48(10):5423-32.

Contaminants in surface water

Advances HRAM Orbitrap mass spectrometers have allowed development of non-targeted screening methods, where data can be archived and in the future re-interrogated when new contaminants are identified. Renaud et al. developed a spectral counting approach to calculate the product and precursor ion selectivities of Q Exactive mass spectrometer acquisition modes--high resolution MS alone or in combination with AIF, DIA or DDA—for retrospective screening of 95 pharmaceuticals in surface water samples. Samples were reanalyzed using targeted LC-MS/MS to confirm the accuracy of each acquisition mode and to quantify the putatively detected drugs. Of the modes evaluated, DIA selectivities were better than traditional LC-MS/MS, showed no bias towards the most intense signals, achieved low LODs, and confidently detected the greatest number of pharmaceuticals (22) with only one false positive. Overall, the authors concluded that DIA offered the best compromise between selectivity and detection.

Moschet et al. also applied the Q Exactive mass spectrometer to a large field study designed to evaluate the in-situ calibration of a passive sampler (styrene divinylbenzene (SDB) covered by a polyether sulfone (PES) membrane for 322 polar organic micropollutants.[26] As before, 5 rivers with different agricultural and urban influences were sampled between March and July 2012 using two methods: i) two-week time-proportional composite water samples, and ii) two-week passive sampler deployment. All compounds of different compound classes (logKow -3 to 5, and neutral, anionic, cationic, and zwitterionic species) were analyzed using the Q Exactive mass spectrometer. Because the number of detected substances was similar, LOQs were comparable (median: 1.3 ng/L vs. 1.6 ng/L), and handling was fast and easy. The authors concluded the SDB passive samplers are well-suited for qualitative screening of polar micropollutants.

Of concern because it is poorly biodegradable, iodinated contrast media (ICM) is used to aid in visualization of human tissue, organs, and the cardiovascular system during radiographic medical procedures. **Zonja et al**. studied the applicability of Orbitrap mass spectrometer-based LC-MS analysis for screening and quantitation of six ICMs and their photo TPs in surface waters.[27] The authors began by performing a photodegradation study of ICMs using a sunlight lab-scale simulator. Differential analysis was used to determine the exact masses of molecular ions and retention times of the TPs. Hits were manually filtered to produce a list of 108 suspected TPs.

Next, solid-phase extraction of real surface water samples followed by LC-MS analysis was used to screen for the compounds detected in the photodegradation study. The eleven TPs detected in more than 50% of the samples were selected for structural elucidation using LC-MS and NMR. The median concentrations of parent ICMs ranged from 110 ng/L to 6 µg/L. TPs were detected at concentrations of 8 ng/L to 0.4 µg/L. The authors concluded that the LC-MS method facilitates characterization of ICM degradation products and detection of TPs without standards.

Detect, characterize, and quantitate without standards

26. Moschet, C.; Vermeirssen, E. L.; Singer, H.; Stamm, C.; Hollender, J. Evaluation of in-situ calibration of Chemcatcher passive samplers for 322 micropollutants in agricultural and urban affected rivers. Water Res. 2015, Mar 15;71:306-17.

27. Zonja, B.; Delgado, A.; Pérez, S.; Barceló, D. LC-HRMS suspect screening for detection-based prioritization of iodinated contrast media photodegradates in surface waters. Environ. Sci Technol. 2015, Mar 17;49(6):3464-72.

Contaminants in surface water

Anatoxin-a (ANA-a) is highly neurotoxic cyanotoxin produced by cyanobacteria that is a threat to humans and livestock when ingested. **Roy-Lachapelle et al**. developed a rapid method that relies on HRAM Orbitrap mass spectrometer-based detection to distinguish ANA-a from the natural amino acid phenylalanine (PHE).[28] The Q Exactive mass spectrometer was equipped with laser diode thermal desorption-atmospheric pressure chemical ionization (LDTD-APCI). Full-scan and targeted ion fragmentation modes were compared to determine which achieve highest selectivity and sensitivity. The method was then applied to eight lake water samples that showed signs of cyanobacterial blooms.

Even though the resolving power of the Q Exactive mass spectrometer was sufficient to distinguish the ANA-a and PHE, the targeted ion fragmentation mode greatly increased selectivity. Internal calibration with standard addition was validated using isotopically labeled phenylalanine (PHE-D5) as the internal standard. The method was validated and determined to be linear with correlation coefficients (r^2) above 0.999. The method yielded better signal-to-noise and thus the lower detection and quantification limits of 0.2 and 0.6 µg/L, respectively, for real samples than in a previous study of a QQQ-based method. Accuracy, inter-day, and intra-day relative standard deviations were below 15%, and signal recovery in the targeted ion fragmentation mode showed no significant matrix effects with values ranging from 96 to 108%. Using the lock mass feature, mass accuracy was below 1 ppm with low variation. In sum, the LDTD-APCI Q Exactive mass spectrometer system enabled ultra fast screening—in less than 15 seconds per sample–of anatoxin-a at concentrations below established guidelines (3.7 µg/L) in water matrices, with simplified sample preparation and high selectivity.

Advances HRAM Orbitrap mass spectrometers have allowed development of non-targeted screening methods, where data can be archived and in the future re-interrogated when new contaminants are identified. **Renaud et al.** developed a spectral counting approach to calculate the product and precursor ion selectivities of Q Exactive mass spectrometer acquisition modes--high resolution MS alone or in combination with AIF, DIA or DDA—for retrospective screening of 95 pharmaceuticals in surface water samples.[29] Samples were reanalyzed using targeted LC-MS/MS to confirm the accuracy of each acquisition mode and to quantify the putatively detected drugs. Of the modes evaluated, DIA selectivities were better than traditional LC-MS/MS, showed no bias towards the most intense signals, achieved low LODs, and confidently detected the greatest number of pharmaceuticals (22) with only one false positive. Overall, the authors concluded that DIA offered the best compromise between selectivity and detection.

- *Screen at concentrations below established guidelines, rapidly, with simplified sample preparation and high selectivity.*
- *Achieve lower detection and quantification limits than QQQ instruments*

28. Roy-Lachapelle, A.; Solliec, M.; Sinotte, M.; Deblois, C.; Sauvé, S. High resolution/accurate mass (HRMS) detection of anatoxin-a in lake water using LDTD-APCI coupled to a Q-Exactive mass spectrometer. Talanta. 2015, Jan;132:836-44.

29. Renaud, J.; Sabourin, L.; Topp, E.; Mark, W. Sumarah, M. A spectral counting approach to measure selectivity of high resolution LC-MS methods for environmental analysis. Anal. Chem., Just Accepted Manuscript. February 7, 2017.

Clinical research

- Clinical Research Overview
- Metabolism and Drug Monitoring Research

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Clinical research overview

Because it offers the required analytical sensitivity and selectivity, QQQ LC-MS/MS analysis in the SRM mode has been routinely employed for targeted analyte quantitation in a range of forensic toxicology and clinical research applications. However, the SRM duty cycle makes monitoring and quantitating of large numbers of analytes very difficult, if not impossible. For clinical research and forensic toxicology applications where quantitation of multiple targeted and untargeted analytes are required, Orbitrap mass spectrometers offer an outstanding orthogonal solution with QQQ-like analytical selectivity, sensitivity, and dynamic range, which are critical for performing successful quantitative analysis of both endogenous and exogenous compounds in plasma, serum, whole blood, urine, cerebrospinal fluid, and oral fluid. Accurate mass measurements help eliminate the risk of over- and underestimations, reduce the rate of false results, and improve sensitivity by minimizing background noise. Moreover, the ability to analyze and quantitate in the same analytical run, together with the potential for retrospective data analysis, makes Orbitrap mass spectrometers a must-have for clinical research, including drug monitoring research, forensic toxicology, and sports anti-doping (particularly for emerging drugs of abuse) applications.

Clinical research overview

Clinical Research

Being able to accurately quantify both endogenous and exogenous compounds in biological samples is of critical importance to clinical researchers. High-resolution MS technologies offer high identification power and easy development of quantitative research methods. Specifically, the advantages of high-resolution MS compared to QQQ MS for clinical research are:

- Comparable sensitivity can be achieved with easier research method development because MS optimization for each analyte is not needed.
- Identification of analytes using isotopic patterns and high-resolution product ion spectra is possible.
- Particularly with Orbitrap mass spectrometer technology, much higher analytical selectivity can be obtained with the ability to determine inaccurate detection of an analyte that is not present and is caused by interferences.
- High-resolution full-scan quantitation methods can monitor a much larger number of compounds and data can be re-evaluated later to address analytical questions that arise.
- Often a smaller amount of sample and a shorter sample preparation time can be used.

Though both Orbitrap and TOF mass analyzers provide high to medium resolving power, higher mass spectral noise can be a major limiting factor in the detection threshold and dynamic range of TOFs instruments.

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Metabolism and drug monitoring research

Though drug metabolism is thought to impact drug toxicity, determination of drug metabolites is not fully explored due to the challenges faced in metabolite profiling. Streamlining analysis of *in vivo* drug metabolism and pharmacovigilance studies could offer a more comprehensive understanding of drug biotransformation. To understand if the analytical challenges can be addressed, **Dahmane et al.** compared the quantification of the pharmaceutical drug tamoxifen, and three of its metabolites in plasma using high-resolution Orbitrap and QQQ mass spectrometer technologies.[30] Of particular interest was the ability of the Orbitrap mass spectrometer to collect full-scan mass spectral data with comparable analytical selectivity and sensitivity to the SRM data collected using a QQQ mass spectrometer.

Using simultaneous Quan/Qual capability, the Orbitrap mass spectrometer-based research method enabled identification and relative quantitation of 37 more tamoxifen metabolites than the QQQ method. Using multivariate analysis, the researchers associated metabolite patterns and ratios with the administration of tamoxifen and the CYP2D6 genotype, an enzyme important in drug metabolism. Two hydroxylated metabolites were identified as putative CYP2D6 substrates. Relative quantitation was demonstrated to be reasonably precise at $< 20\%$ and suggested that the metabolites found were in consequential amounts.

A fungal infection of the central nervous system, cryptococcal meningitis (CM) is a common opportunistic infection with a mortality rate of 25–30% among those infected with AIDS. CM can be controlled with early antifungal treatment with amphotericin B (AMB), fluconazole (FZ), and fluorocytosine (FC). However, serious side effects require careful monitoring of plasma and cerebrospinal fluid (CSF) concentrations to produce the optimal clinical outcomes.

Because few fully automated sample pretreatment and simultaneous quantification methods for AMB, FZ and FC in plasma and CSF have been reported, **Qu et al.** developed a fast, automated online dual-solid phase extraction (SPE)-LC Q Exactive-based mass spectrometer method.[31]

Simultaneously analysis of AMB, FZ and FC in plasma and CSF was achieved in seven minutes and the method was fully validated according to FDA guidelines. The LLOQs were 0.04, 0.04, and 0.40 µg/mL for AMB, FZ, and FC, respectively. Levels were determined to be linear from 0.04–2.00 µg/mL for AMB and FZ, and from 0.40–20.00 µg/mL for FC. The method yielded good linearity, intra-day and inter-day precision, and recovery. The authors concluded the method rapid and reliable for monitoring AMB, FZ, and FC levels in plasma and CSF.

Detect, characterize and quantitate without standards

30. Dahmane, E.; Boccard, J.; Csajka, C.; Rudaz, S.; Décosterd, L.; Genin, E.; Duretz, B.; Bromirski, M.; Zaman, K.; Testa, B.; Rochat, B. Quantitative monitoring of tamoxifen in human plasma extended to 40 metabolites using liquid-chromatography high-resolution mass spectrometry: new investigation capabilities for clinical pharmacology. Anal. Bioanal. Chem. 2014, Apr;406(11):2627-40

31. Qu, L.; Qian, J.; Ma, P.; Yin, Z. Utilizing online-dual-SPE-LC with HRMS for the simultaneous quantification of amphotericin B, fluconazole, and fluorocytosine in human plasma and cerebrospinal fluid. Talanta. 2017, Apr 1;165:449-457

Metabolism and drug monitoring research

Mueller et al. presented a method to quantify the five main components of the therapeutic drug teicoplanin.[32] The method coupled turbulent-flow-based online extraction with LC-MS analysis performed using a Q Exactive mass spectrometer.[4] The results of the LC-MS method were compared to the commercially available immunoassay QMS® teicoplanin. Results obtained using the Q Exactive mass spectrometer-based LC-MS method correlated well with the results obtained using immunoassay, demonstrating within- and between-day precision and accuracy suitable for quantitative analysis of therapeutic drugs for clinical research.

Henry et al. compared the full-scan HRAM performance of the Q Exactive mass spectrometer with that of the Thermo Scientific™ TSQ Quantum Discovery™ and Thermo Scientific™ Quantum Ultra™ triple quadrupole mass spectrometers operating in the SRM mode for LC-MS quantitation of drugs in plasma for clinical research.[33] The Q Exactive mass spectrometer was set to mass resolution of 50,000 (FWHM) at *m/z* 200 and a mass extracted window of 5 ppm around the theoretical *m/z* of each analyte was used to construct chromatograms for quantitation. Seventeen drugs, including eight antifungal agents (anidulafungin, caspofungin, fluconazole, itraconazole, hydroxyitraconazole posaconazole, voriconazole, and voriconazole-N-oxide), four immunosuppressants (ciclosporine, everolimus, sirolimus, and tacrolimus) and five protein kinase inhibitors (dasatinib, imatinib, nilotinib, sorafenib, and sunitinib), were analyzed.

The quantitative results obtained from the Q Exactive mass spectrometer demonstrated detection selectivity, assay precision, accuracy, linearity, and sensitivity comparable and with good correlation to that obtained using the QQQ MS instruments in the SRM mode. The Q Exactive mass spectrometer method was compatible with sample preparation approaches used for QQQ-based SRM analyses. The authors commented that the Q Exactive mass spectrometer method offered several benefits: ease of research method development with no optimization of SRM parameters, ability to rapidly transfer the analysis from one mass spectrometer to another, more complete full-scan qualitative information, and easier troubleshooting. Considering the advantages, the authors suggested that there should be a shift in how routine quantitative analyses of small molecules are performed for clinical research.

- *Obtain high-resolution SIM results surpassing QQQ-based SRM*
- *Reduce the amount of sample needed for high-sensitivity analysis*

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32. Mueller, D. M; von Eckardstein, A.; Saleh, L. Quantification of teicoplanin in plasma by LC-MS with online sample clean-up and comparison with QMS® assay. Clin. Chem. Lab. Med. 2014, Jun;52(6):879-87.

33. Henry, H.; Sobhi, H. R.; Scheibner, O.; Bromirski, M.; Nimkar, S. B.; Rochat, B. Comparison between a high-resolution single-stage Orbitrap and a triple quadrupole mass spectrometer for quantitative analyses of drugs. Rapid Commun. Mass Spectrom. 2012, Mar 15;26(5):499-509.

Metabolism and drug monitoring research

Dafachronic acid (DA) is a steroid hormone required for the reproductive development of *Caenorhabditis elegans* into fertile adults. **Li et al.** developed a simple, sensitive research method for the absolute quantification of DA that employs derivatization with 2-picolylamine and the high-resolution SIM capability of the Q Exactive mass spectrometer.[34] Due to the research method sensitivity, only relatively small amounts of worms were needed for the analysis. Orbitrap mass spectrometer-based high-resolution SIM analyses outperformed targeted MS/MS analysis (on the same instrument) and QQQ-based SRM. The LOQ was determined to be as low as 1 pg of DA, enabling absolute quantification of endogenous DA during the worms' reproductive development. The DA levels at different developmental stages and in different Daf-c mutants showed that during the L2 larval stage, DA is highly elevated to ensure complete reproductive development of *C. elegans*.

Understanding how cellular metabolism is coordinated with biological processes is an important goal for biologists. Of particular interest to **Miyazawa et al**., is how energy metabolism adapts to changing bioenergetics demands during mammalian embryonic development.[35] As part of their multi-technique study, the authors quantified trace-level pentose phosphate pathway metabolites using a Q Exactive Focus mass spectrometer connected to a Dionex ICS-5000+ ion chromatography system. The author's model of glucose metabolism suggested that for normal development during chorioallantoic branching, embryos rewire glucose metabolism by redirecting glucose carbon flow into the pentose phosphate pathway via suppression of the key glycolytic enzymes PFK-1 and aldolase.

34. Li, T. M.; Chen, J.; Li, X.; Ding, X.J.; Wu, Y.; Zhao, L.F.; Chen, S.; Lei, X.; Dong, M.Q. Absolute quantification of a steroid hormone that regulates development in Caenorhabditis elegans. Anal. Chem. 2013, Oct 1;85(19):9281-7.

35. Miyazawa, M.; Yamaguchi, Y.; Sugiura, Y.; Honda, K.; Kondo, K.; Matsuda, F.; Yamamoto, T.; Suematsu, M.; Miura, M. Rewiring of embryonic glucose metabolism via suppression of PFK-1 and aldolase during mouse chorioallantoic branching. Development. 2017, Jan 1;144(1):63-73.

Metabolism and drug monitoring research

Oxyntomodulin (OXM) has been the focus of many clinical and preclinical research studies because of its effects on weight loss, food intake, and energy balance. However, complete understanding of the role of OXM has been limited by the lack of sensitive and specific tools for its reliable in vivo characterization. For this reason, **Cox et al.** developed and validated an immunoaffinity (IA) capture-Q Exactive mass spectrometer based method to quantify OXM 1-37 and its primary catabolites 3–37, pE 3–37 and 4–37, in human and rat plasma, pre- and post-prandially. Method data provided evidence of key differences between human and rat OXM secretion in response to nutrient ingestion, which could potentially further understanding of OXM biology and facilitate clinical translation. Full scan HRAM analysis using mass resolution (R = 70,000 FWHM) was shown to offer another approach to SRM-based methods for low-level peptide biomarker quantification.

In the past, high-resolution MS has been associated with qualitative research applications, and QQQ-MS with routine quantitative methods. Because there are now many quantitative high-resolution MS methods validated for many different pharmaceutical analytes and matrices, **Grund et al.** challenged this view.[37] Their study compared the performance of a Q Exactive mass spectrometer with a QQQ instrument for quantitative analyses of protease inhibitors, tyrosine kinase inhibitors, metanephrines, and steroids spiked in plasma extracts. The concentrations analyzed covered over 5 or 6 orders of magnitude from 0.0125–5,000 or 10,000 ng/mL. External calibrations were determined to establish dynamic range, and LODs.

The authors concluded the Q Exactive mass spectrometer reliable and sensitive, with quantitative performance comparable to that of QQQ mass spectrometers. Most targeted analyses can be performed in high-resolution full-scan to record virtually all ions. In addition to absolute quantification, full scan analyses allow relative quantification of hundreds of metabolites in plasma, thus revealing metabolomes or exposomes. Analyses requiring increased sensitivity and detection selectivity can be performed in SIM mode. Taking into account the versatility, user-friendliness, and robustness of the Q Exactive system, the authors believe it will become more commonly used for quantitative LC–MS bioanalyses.

36. Cox, J.; Berna, M.; Jin, Z.; Cox, A.;, Sloop, K.; Gutierrez, J.; Ackermann, B. Characterization and quantification of oxyntomodulin in human and rat plasma using high-resolution accurate mass LC-MS. Bioanalysis. 2016, Aug;8(15):1579-1595.

37. Grund, B.; Marvin, L.; Rochat, B. Quantitative performance of a quadrupole-orbitrap-MS in targeted LC–MS determinations of small molecules. Journal of Pharmaceutical and Biomedical Analysis. 2016, May, Vol. 124, 48–56.

Metabolism and drug monitoring research

For targeted metabolite quantification, multiple reaction monitoring (MRM) using QQQ instruments has been considered the "gold standard." However MRM assays can produce nonspecific chromatographic peaks caused by compounds with similar molecular weights and fragments. Without a full mass spectrum, metabolite identification is difficult. Improvements to high-resolution, full scanning MS instruments have led to new approaches such as DDA, a powerful technique for non-targeted metabolite profiling. Because it has shown reliable performance for targeted peptide quantification, **Zhou et al.** sought to evaluate a quantitative metabolomics strategy using PRM.[38]

Using a Q Exactive system with scheduled PRM, the authors developed an efficient large-scale quantitative method targeting 237 metabolites involved in various metabolic pathways. Evaluation of different kinds of biological samples, including cells, serum, and cell media, showed excellent accuracy, sensitivity, and system suitability of the method for targeted metabolite quantification. Quantitative performance of the Q Exactive PRM and MS1-based assays, and the QQQ MRM assay, were compared. The PRM assay showed greater reproducibility and quantitative accuracy than MS1-based quantification. Compared to the QQQ MRM assay, PRM showed greater flexibility in post-acquisition assay refinement and in metabolite identification without standards. The authors concluded that Q Exactive mass spectrometer-based PRM provides a useful approach for large-scale metabolite quantification in complex biological samples.

Domon et al. also reviewed the benefits of hybrid quadrupole-Orbitrap mass spectrometer-based PRM when used to overcome the limitations of QQQ MS for performing clinical research studies.[39] The authors note in particular that hybrid quadrupole-Orbitrap systems: 1) more efficiently discriminate analyte from complex matrix, resulting in better data quality while mitigating the need for elaborate sample preparation approaches, 2) require little upfront effort and information to run experiments because few parameters need to be defined in advance, 3) have high compatibility with fast chromatographic separation, expediting research studies, 4) enable large scale screening of large sets of peptides, and 5) provide flexible data analysis because all fragment ions are recorded. Overall, the main benefit is a dramatic increase in selectivity that yields excellent analytical precision and reliable quantification, which in turn, provide more consistent biological results.

38. Zhou, J.; Liu, H.; Liu, Y.; Liu, J.; Zhao, X.; Yin, Y. Development and Evaluation of a Parallel Reaction Monitoring Strategy for Large-Scale Targeted Metabolomics Quantification. Anal. Chem. 2016, Apr 19;88(8):4478-86. 2016, May, Vol. 124, 48–56.

39. Domon, B.; Gallien, S. Recent advances in targeted proteomics for clinical applications. Proteomics Clin. Appl. 2015, Apr;9(3-4):423-31.

Metabolism and drug monitoring research

Unlike proteomics, untargeted metabolomics profiling based on LC-MS/MS, is hampered by a lack of metabolite databases. Though there are free web-based databases, they are not practical for routine high-throughput analyses, especially for MS/MS confirmation of large datasets. Commercial databases have limited compatibility with data produced from all manufacturers' systems. Databases created using chemical standards can be expensive and time consuming to build.

For this reason, **Tang et al.** investigated in-house creation of accurate MS/MS databases without using standards.[40] The authors constructed a lipid database of regularly observed species where accurate masses were calculated based on structures and chemical formulas. A polar metabolite database was also built, in this case by using the MS/MS information in public DDA data in Metlin, mzCloud, and others. Entries were stored in a TraceFinder software database for subsequent high-throughput analyses. To validate lipid and polar metabolite workflows, mouse brain and liver extracts were analyzed using a Q Exactive instrument. The lipid database enabled identification and simultaneous quantitation of about 500 lipids. DDA data provided compound confirmations. The polar metabolite database enabled assignment of 370 compounds in mouse liver with186 confirmed by MS/MS fragments. In mouse brain, 529 polar metabolites were identified with 156 confirmed by MS. All compounds were quantified with adequate information for PCA.

Because high-resolution MS is becoming important in many labs, **Rochat** reviewed why this trend is extending to clinical research.[41] Most high-resolution mass spectrometers perform sensitive and reliable quantification of a variety of analytes, in either full scan or targeted SIM or MS2 modes. In terms of sensitivity, selectivity, mass accuracy, detection reliability, calibration-curve linearity, level accuracy, ease-of-use, and costs, the quantitative performance of high-resolution MS is similar to QQQ-MS. Because all ionized compounds are detected, high-resolution full-scan data has the advantage of providing global sample information. The use of one platform for targeted quantification to untargeted 'omics, and targeted quantification streamlines workflows, enabling research to move forward more rapidly. In support of these assertions, the author cites a large body of research that describes the quantitative, qualitative, and Quan/Qual capabilities of high-resolution MS.

40. Tang, H.; Wang, X.; Xu, L.; Ran, X.; Li, X.; Chen, L.; Zhao, X.; Deng, H.; Liu, X. Establishment of local searching methods for orbitrap-based high throughput metabolomics analysis. Talanta. 2016, Aug 15;156-157:163-71.

41. Rochat, B. From targeted quantification to untargeted metabolomics: Why LC-high-resolution-MS will become a key instrument in clinical labs..

ic toxicology and sports doping

- Quantitation of drugs and metabolites in urine: workflow
- Equine doping
- Cannabis detection
- New psychotic substances (NPS)

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Quantitation of drugs and metabolites in urine: workflow

In the fields of forensic toxicology and doping, quantitation of drugs in body fluids is challenging due to varying concentrations and substantially different chemical and physical properties of drugs, interfering matrices, occasionally small volumes of sample to test, and the presence of many similar compounds. Further, the constant evolution of drugs and their analogs makes it harder to identify and quantitate them. Orbitrap mass spectrometer-based LC-MS analyses of body fluids are performed using variations of the workflow shown in Figure 9.

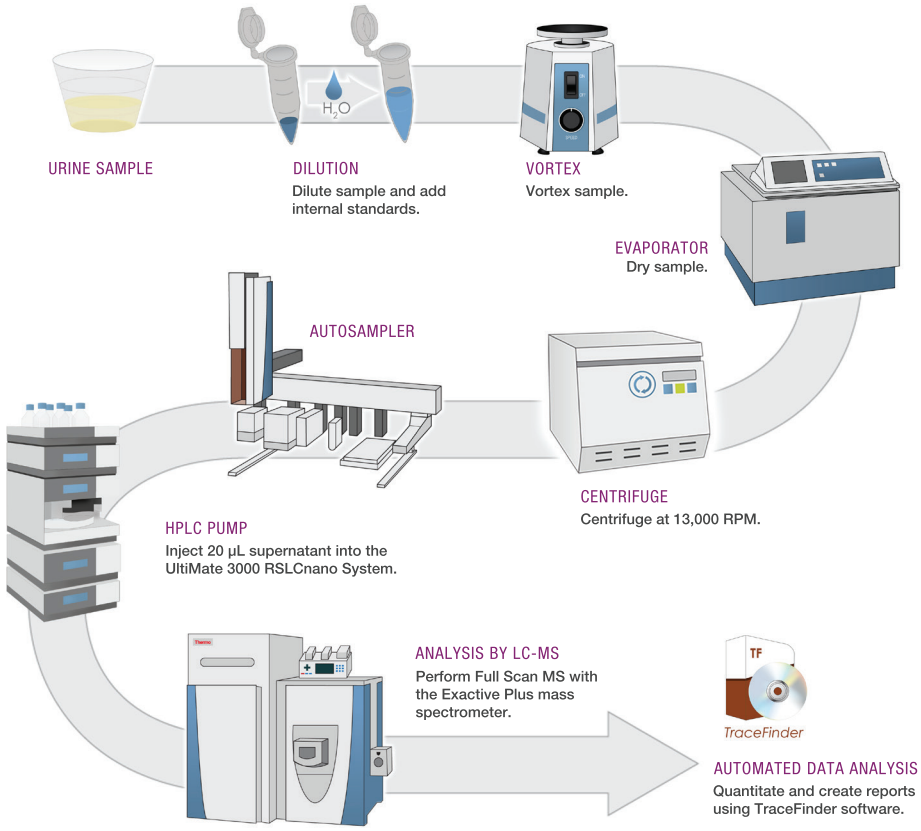

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Figure 9. Q Exactive mass spectrometer-based workflow for quantitation of drugs and metabolites in urine. Currently, urine is a matrix of choice for quantitative analysis in equine drug testing. Many drugs and their metabolites are present in conjugated form in urine and include isomers that need to be identified after separation using chromatography. The analytical workflow must handle a wide range of polarities, many similar molecules, matrix interferences, wide dynamic ranges and sample-to-sample differences caused by the varying nature of the matrix.

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Equine doping

The cobra venom toxin α-cobratoxin (α-Cbtx) has analgesic potency greater than morphine. After being found in the facilities of a thoroughbred trainer, the lack of a detection method for the protein became a serious problem in horseracing. To address the problem, **Bailly-Chouriberry et al.** developed the first method, a method that relies on the Q Exactive mass spectrometer, to detect and quantitate α-Cbtx in equine plasma.[42] Prior to LC-MS/MS analysis, 3 mL of equine plasma sample was treated with ammonium sulphate precipitation, methanol precipitation, SPE extraction, concentration via filtration, and then digestion with trypsin. LC-MS/MS analysis of the product ions of the doubly-charged precursor of the target peptide was performed at 70,000 resolution.

The method was validated and proved to be sufficiently specific, robust and sensitive to enable confirmation of the presence of α-Cbtx in 18 different equine plasma samples spiked at 5 g/L (640 pmol/L), thus meeting the Association of Official Racing Chemists (AORC) requirements. The LOD was determined to be 1 µg/L (130 pmol/L). The method makes it possible to confirm the presence of α-Cbtx in horse plasma from 30 minutes up to 24 hours after administration with an upper limit of 48 hours.

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Confirm compounds at regulated levels with sensitivity, specificity, and robust operation

42. Bailly-Chouriberry, L.; Cormant, F.; Garcia, P.; Kind, A.; Popot, M. A.; Bonnaire, Y. Identification of α-cobratoxin in equine plasma by LC-MS/MS for doping control. Anal. Chem. 2013, May 21;85(10):5219-25.

Cannabis detection

Due to advancements in analytical technology and because it is possible to obtain a specimen quickly and easily, oral fluid is gaining acceptance as an alternative matrix for forensic toxicology applications. Though Δ9-tetrahydrocannabinol (THC) is the primary target for detecting cannabis use in oral fluid, THC carboxylic acid (THCA) has been demonstrated more reliable because its presence in oral fluid does not occur from passive exposure. However, THCA quantitation is more difficult because it is found in very low concentrations in oral fluid. LC–MS/MS methods provide the requisite sensitivity, but can involve complicated sample preparation procedures. For this reason **He et al.** developed a sensitive LC–MS/MS forensic method for the simultaneous quantitation of THC and THCA in oral fluid using online sample extraction low-flow LC coupled to a Q Exactive mass spectrometer.[43]

The method proved simple, robust, and efficient with a total runtime of 12.5 minutes. The HRAM capability of the Q Exactive mass spectrometer enabled the method to achieve high specificity and low-pg/mL sensitivity. Quantitative results were linear from 7.5 to 300 pg/mL for THCA, with a lower limit of 7.5 pg/mL. Intra- and inter-batch precision of ranged from 3.3% to 9.3% for both THC and THCA.

Though Δ(9)-Tetrahydrocannabinol (THC) is the primary target in oral fluid for detecting cannabis use, additional biomarkers such as 11-nor-9-carboxy-THC (THCCOOH), cannabidiol (CBD), and cannabinol (CBN) are needed to address the possibility of passive inhalation. **Concheiro et al.** developed and validated a Q Exactive mass spectrometer-based microflow LC-MS method for the simultaneous quantitation of THC, THCCOOH, CBD, and CBN in oral fluid.[44] Authentic oral fluid specimens were collected using the Oral-Eze(®) and Quantisal™ collection systems. Proteins were precipitated and the supernatant extracted using CEREX™ Polycrom™ THC SPE. Target compounds were identified and quantified in targeted MS/MS experiments.

The forensic method was linear from 0.5 to 50 ng/mL for THC, CBD and CBN, and from 15 to 500 pg/mL for THCCOOH. Intra- and inter-day and imprecision were < 10.8% CV, and bias was from 86.5 to 104.9%. Extraction efficiency ranged from 52.4 to 109.2%, process efficiency from 12.2 to 88.9%, and matrix effect from -86 to -6.9%. The authors concluded that the method provides rapid simultaneous quantitation of THCCOOH, THC, CBD, and CBN with good selectivity and sensitivity, and thus presents an opportunity to improve interpretation of the results obtained from cannabinoid analyses of oral fluids.

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Quantify challenging low-level compounds, with high selectivity and sensitivity

43. He, X.; Marta Kozak M.; Nimkar, S. Ultra-Sensitive Measurements of 11-Nor-Δ9-Tetrahydrocannabinol-9-Carboxylic Acid in Oral Fluid by Microflow Liquid Chromatography–Tandem Mass Spectrometry Using a Benchtop Quadrupole/Orbitrap Mass Spectrometer. Anal. Chem. 2012, 84 (18), pp 7643–7647.

44. Concheiro, M.; Lee D.; Lendoiro, E.; Huestis, M. A. Simultaneous quantification of Δ(9)-tetrahydrocannabinol, 11-nor-9-carboxy-tetrahydrocannabinol, cannabidiol and cannabinol in oral fluid by microflow-liquid chromatography-high resolution mass spectrometry. J. Chromatogr. A. 2013, Jul 5;1297:123-30.

New psychotic substances (NPS)

New designer drugs and the various drug analogues developed to circumvent legislation have varying effects and potencies, which can complicate interpretation of forensic toxicology cases. Synthetic cathinones are a group of designer stimulants with amphetamine or cocaine-like effects. Comprehensive multi-analyte confirmation methods are needed due to the wide spectrum of synthetic cathinones available. **Concheiro et al.** developed an Orbitrap mass spectrometer-based method for simultaneous quantitation of 28 synthetic cathinones and four metabolites in urine.[45] The target cathinones included cathinone, methcathinone, and synthetic cathinones position-3'-substituted, N-alkyl-substituted, ring-substituted, methylenedioxy-substituted, and pyrrolidinyl-substituted. Extraction was via solid phase cation exchange extraction (SOLA SCX) followed by reverse-phase LC. Target compounds were identified and quantified using targeted MS/MS experiments. The method was then applied to the urine specimens containing synthetic cathinones.

The authors determined that the method was linear from 0.5-1 to 100 µg/L, with LODs of 0.25-1 µg/L. Imprecision (n = 20) was < 15.9% and accuracy was (n = 20) 85.2 to 118.1%. Extraction efficiency was 78.9 to 116.7% (CV 1.4 to 16.7%, n = 5), process efficiency was 57.7 to 104.9%, and matrix effects were from -29.5% to 1.5% (CV 1.9 to 13.1%, n = 10). The confirmation method proved comprehensive for the 28 synthetic cathinones, with good analytical specificity. The authors concluded that the research would help in the interpretation of test results in forensic toxicology cases, and in the evaluation of the toxicity of designer cathinone drugs.

NPS are continuously introduced to the illicit-drug market, rendering traditional immunological detection unusable. LC–MS is thus more frequently applied to these analyses, but with standards not always available, full-scan detection and identification strategies must be used. **Montesano et al.** present a sensitive, quantitative Q Exactive mass spectrometer method for analyzing NPS (16 cathinones and 9 synthetic cannabinoids) in plasma.[46] Detection was performed in targeted MS/MS mode, with identification based on retention time and exact mass of the precursor ion and two diagnostic fragments. A full-scan analysis was performed in parallel with MS/MS acquisition, allowing post-run processing and re-interrogation of data for unexpected compounds. Mass spectra were compared with an expandable, in-house developed TraceFinder software library containing over 300 NPS and their metabolites. The method allowed screening of substances not included in the 25 specifically tested. LOQs ranged from 0.03 to 0.4 ng/mL; levels appropriate for the analysis of synthetic cannabinoids, which generally are found in plasma at less than 1 ng/mL.

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Confirm and quantitate of a wide range of compounds, simultaneously and with high specificity

45. Concheiro, M.; Anizan, S.; Ellefsen, K.; Huestis, M. A. Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry. Anal. Bioanal. Chem. 2013, Nov;405(29):9437-48.

46.Camilla Montesano, C., Vannutelli, G., Adolfo Gregori, A., Ripani, L., Dario Compagnone, D., Curini, R., Sergi, M. Broad Screening and Identification of Novel Psychoactive Substances in Plasma by High-Performance Liquid Chromatography–High-Resolution Mass Spectrometry and Post-run Library Matching. Journal of Analytical Toxicology. 2016;40:519–528.

New psychotic substances (NPS)

The availability of robust, easy to use high-resolution mass spectrometers has allowed toxicology laboratories to realize the advantages of full scan over QQQ MRM and immunoassay methods. **Jagerdeo et al.** described an LC-Q Exactive mass spectrometer-based method to simultaneously screen for 38 drugs of abuse, including 19 benzodiazepines, 12 opiates, cocaine and three metabolites, and three "Z-drug" hypnotic sedatives, in blood and urine, in a ten minute run.[47] Urine samples were subjected to fast, high-temperature hydrolysis with abalone glucuronidase to convert glucuronic acid conjugates to the free drug or metabolite. All samples were processed using rapid, Supported-Liquid Extraction (SLE) prior to MS analysis. With the exception of the benzodiazepines clobazam and temazepam, all analytes were distinguished from each other and from other drugs commonly found in forensic toxicology samples. The method was validated at 1, 3, 5, or l 10 ng/mL in whole blood and urine in triplicate over three days. The high number of scans across chromatographic peaks at low concentrations suggested that the method can also be used for quantification. The authors concluded that the method is a suitable replacement for immunoassay screening of the drug classes targeted, with much better specificity and limits of LOD.

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47. Jagerdeo, E.; Schaff, J. Rapid screening for drugs of abuse in biological fluids by ultra high performance liquid chromatography/Orbitrap mass spectrometry. Journal of Chromatography B. 2016, Aug., Volume 1027, 1, 11–18.

Pharmaceutical discovery assays

- Drug discovery process: workflow
- Bioanalysis
- Discovery-stage screening
- ADME screening and DMPK research

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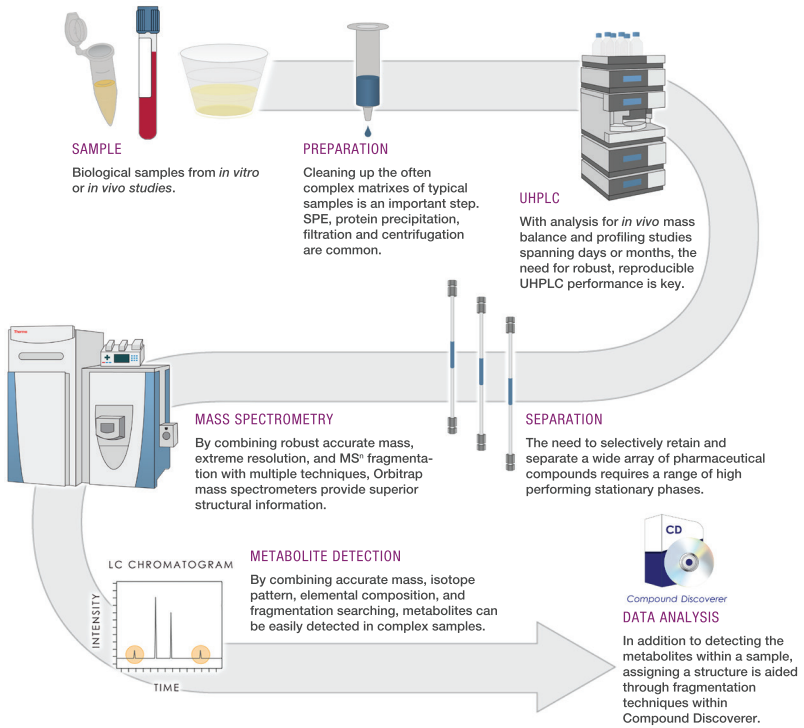
Drug discovery process: workflow

As potential leads for successful drug candidates move through the drug discovery process, there is a need for quantitative *in vitro* and *in vivo* analysis at each step. Though the requirement for quantitative LC-MS assays that provide sensitivity, ruggedness, and linear response has remained relatively constant over time, other considerations are emerging as MS technology improves. Ease of use, simplified method development, and troubleshooting tools are growing in importance when choosing the most appropriate MS technology for a particular assay and its corresponding method development. Orbitrap mass spectrometer-based LC-MS analyses of plasma are performed using variations of the workflow shown in Figure 10.



Quantitation using HRAM Orbitrap mass analyzer technology produces reliable analytical results at extremely low concentration levels and in complex matrices. The performance, in particular sensitivity and linear response, and the HRAM results produced by Orbitrap mass spectrometers are comparable to and in some cases exceed, that of QQQ mass spectrometers. Additionally, HRAM capability reduces the time and effort needed to develop quantitative methods. Full-scan and SIM modes of operation enable direct analysis and monitoring of target analytes without the need to remove interferences through fragmentation or MRM. Data collection in both full-scan and SIM modes also provides additional information about possible matrix interferences near the target analyte *m/z* that can facilitate quantitative method development and troubleshooting.

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Figure 10. Orbitrap mass spectrometer-based workflow for plasma-protein binding (PPB), an early ADME *in vitro* study that indicates the likelihood of a test compound binding to proteins in blood plasma. The sample matrices analyzed require solvent addition and centrifugation to remove the proteins prior to injection to the LC-MS system. Use of on-line sample preparation such as Thermo Scientific™ Turboflow™ Technology allows direct injection of the plasma matrix, thereby increasing sample throughput and reducing cost.

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Bioanalysis

Bioanalytical scientists strive to achieve lower limits of quantitation. The reasons range from smaller sample volumes available for analysis, to more potent analytes and the growth of biologics in drug development. As a result, scientists are exploring new LC-MS techniques, involving both high- and low-flow rate LC devices and HRAM. Because most biological samples are matrices comprised of plasma, serum, whole blood, urine, and various tissues, desirable new analytical technology should provide greater sensitivity and robustness. **Wang et al.** assessed application of a Q Exactive mass spectrometer combined with microspray ionization and microflow separation.[48] A standard flow rate sprayer needle was compared to a microspray needle and Q Exactive mass spectrometer performance was compared to QQQ MS. Specifically, the sensitivity and noise level of four ionization modes were evaluated: QQQ MS SRM, HRAM full scan, HRAM SIM, and HRAM MS/MS.

The authors determined that microflow LC-MS showed less matrix effects than UHPLC-MS, but the extent of suppression for both was compound-dependent and the amount of reduction was not proportional for each analyte. Because the precision was improved by using microflow LC-MS, relative quantitation might be more reliable. Microflow LC-MS did provide a substantial increase in sensitivity for some compounds without additional sample preparation or chromatographic method development. Carryover of high-concentration samples was a disadvantage of microflow LC-MS. When using SIM, the Q Exactive mass spectrometer was found to be as or more sensitive than the Thermo Scientific TSQ Vantage Triple Stage Quadrupole Mass Spectrometer, but was also compound-dependent. The authors also concluded that the HRAM full-scan capability of the Q Exactive mass spectrometer allowed observation of co-eluting compounds during method development, which in turn enabled reduction or elimination of undesirable matrix effects.

- *Obtain sensitivity equal to or exceeding QQQ instruments*
- *Observe co-eluting compounds, reduce matrix effects, using full-scan HRAM data*

48. Wang, H.; Bennett, P. Performance assessment of microflow LC combined with high-resolution MS in bioanalysis. Bioanalysis. 2013, May;5(10):1249-67.

Bioanalysis

While there is growing interest in studying native intact proteins and protein complexes, quantitative LC-MS analysis of intact proteins greater than 5 kDa is still new. Though ligand-binding assays (LBA) are commonly used for quantification, compared to LC-MS, LBA assay development is time consuming, dynamic range limited, and selectivity can be poor.

Because information about the quantitative performance of LC-MS for large intact proteins in biological matrices is scarce, **Buscher et al.** compared a QQQ and a Q-Exactive mass spectrometer for quantification of Cytochrome C (12.4 kDa), Human Serum Albumin (66.5 kDa), apo-Transferrin (79.5 kDa) and Infliximab (Remicade®, 148.5 kDa). QQQ LODs ranged from 10 to 100 ng/mL in the MRM mode and 200-500 ng/mL in the SIM mode.[49] Q Exactive instrument LODs ranged from 50-500 ng/mL. The mass spectrum of intact Infliximab was within the mass range of the Q-Exactive instrument (*m/z* up to 6000), but beyond that of the QQQ system (*m/z* up to 2000). Adding 0.5% (V/V) m-nitrobenzyl alcohol to the mobile phase was needed to increase the charge state of Infliximab to allow QQQ analysis. The authors concluded that the Q Exactive mass spectrometer provided best sensitivity for the largest protein (Infliximab), and is preferred when structural information is needed such as for the analysis of isoforms.

49. Buscher, B., Toersche, J., Holthoon, F., Kleinnijenhui, A. Comparison of Triple Quadrupole and Orbitrap Mass Spectrometry for Quantitative Bioanalysis of Intact Proteins. Journal of Research Analytica. 2015, Dec. 27.

Bioanalysis

MS/MS with CID enables QQQ and ion trap mass spectrometers to quantify biological analytes in complex matrices with high sensitivity and selectivity. However, the molecular ions produced from cyclic and large disulfide-containing peptides, sterols, and fatty acids are not amenable to CID, making their analysis using these technologies difficult. In particular, cyclic peptides are of growing interest in drug discovery because of their stability in blood and potential for oral dosing. As a solution, **Ciccimaro et al.** demonstrated a Q Exactive mass spectrometer method where the target ion is selectively isolated for quantitation while interfering matrix components undergo MS/MS fragmentation by CID (HCD)—an approach the authors termed HRAM survivor-SIM.[50] Fundamentally different than the traditional MS/MS with CID where a target analyte's unique fragments are monitored following CID (HCD) fragmentation, the new approach significantly enhanced selectivity by removing isobaric interferences. Comparative QQQ MS experiments were performed using a Triple Quad 6500 LC/MS/MS System (AB Sciex). Because molecular ion detection is more sensitive than fragment ion detection, the authors observed a five- to ten-fold improvement in LLOQs compared to traditional approaches for CID-resistant peptides in plasma extracts. The results demonstrated that the Q Exactive mass spectrometer is an ideal orthogonal platform for quantitation in situations where compounds are not amenable to MS/MS with HCD/CID fragmentation.

- *Quantitate using HRAM survivor-SIM when compounds are not amenable to HCD/CID fragmentation*
- *Eliminate isobaric interferences and enhance selectivity*

50. Ciccimaro, E;. Ranasinghe, A.; D'Arienzo, C.; Xu, C.; Onorato, J.; Drexler, D. M.; Josephs, J. L.; Poss, M.; Olah, T. Strategy to improve the quantitative LC-MS analysis of molecular ions resistant to gas-phase collision induced dissociation: application to disulfide-rich cyclic peptides. Anal. Chem. 2014, Dec 2;86(23):11523-7.

Discovery-stage screening

Though high-throughput screening using LC-MS is routinely implemented throughout the drug-discovery process, using it earlier in the lead discovery stage can provide significant benefits. For example, avoiding labeling agents can reduce costly sample preparation, and LC separation of analytes of interest from interferences and can increase selectivity and sensitivity. For this reason **Murphy et al.** applied high-resolution MS-LC multiplexing to a screening assay of phosphorylated peptides.[51] Phosphorylated peptide standards were prepared using common enzyme buffers and these were plated into a 96-well plate format prior to LC-MS analysis using a Q Exactive mass spectrometer.

Laboratories supporting the lead discovery stage must analyze several thousand samples per day, generally with complex matrixes that vary from project to project. Therefore, assay methods must be fast and robust. The authors concluded that Q Exactive mass spectrometer coupled with LC multiplexing provides robust, high-quality results at rapid sampling rates (up to 18 seconds per sample). Samples analyzed in both simple and complex sample matrices demonstrated an LOQ of 5 nM with linear response across the working range of the assay.

With the paradigm shift in drug discovery from SRM-based QQQ to high-resolution MS analysis, **Strum et al.** describe how, when, and why Q Exactive mass spectrometers should be considered for regulated quantitative bioassays.[52] "How" topics covered include Q Exactive instrument-based quantitative bioanalysis in a regulated environment, software validation, system suitability, and considerations and decision tree for developing and optimizing methods. "When and why" topics include the advantages of the Q Exactive instrument for assays in which sensitivity is limited by the selectivity of the SRM transition compared with the background, for analytes that do not fragment well such as disulfide-rich cyclic peptides, and for large whole-molecule bioanalysis rather than bottom-up, surrogate peptide quantification of proteolytic or chemical fragments. For these reasons, the authors expect that high-resolution MS will have a significant impact on large molecule biotherapeutic and biomarker applications.

Achieve robust, high-throughput screening of complex sample matrices

51. Murphy, K.; Bennett, P. K.; Duczak, N. Jr. High-throughput quantitation of large molecules using multiplexed chromatography and high-resolution/accurate mass LC-MS. Bioanalysis. 2012, May;4(9):1013-24.

52. Sturm, R., Jones, B., Mulvana, D., Lowes, S. HRMS using a Q-Exactive series mass spectrometer for regulated quantitative bioanalysis: how, when, and why to implement. Bioanalysis. 2016, Aug;8(16):1709-21.

ADME screening and DMPK research

Application of high-resolution MS to absorption, distribution, metabolism and excretion (ADME), and drug metabolism, and pharmacokinetics (DMPK) studies has generated considerable interest within pharmacokinetics and pharmacology laboratories. **Zhang et al**. explored the benefits of high-resolution MS for quantitative bioanalysis using full-scan data acquisition.[53] Of particular interest is the lack of compound-specific MS method development, simultaneous data collection for both targeted and non-targeted components, and suitability for simultaneous quantitation of an unlimited number of compounds, which is not possible using QQQ SRM methods. In addition, the information obtained from HRAM at the ADME phase can be transferred to reduce method development time during downstream routine quantitation analysis using QQQ MS.

The authors developed an *in vitro* ADME workflow involving cassette incubation of as many as 32 compounds, followed by quantitative analysis using an Orbitrap mass spectrometer in full-scan mode. The workflow was evaluated for serum protein-binding and parallel artificial membrane permeability (PAMPA) assays. The workflow was found to have acceptable sensitivity, selectivity, and linearity for all compounds, and the biological results obtained were similar to those obtained from discrete incubation and analysis, demonstrating the feasibility of the workflow.

- *Achieve a dynamic range exceeding Q-TOF instruments*
- *Attain scan speed equal to that of Q-TOF systems*
- *Conserve time, space, and budget with Quan/Qual capability*
- *Quantify with the instrument for the lab of the future*

53. Zhang, J.; Maloney, J.; Drexler, D. M.; Cai, X.; Stewart, J.; Mayer, C.; Herbst, J.; Weller, H.; Shou, W. Z. Cassette incubation followed by bioanalysis using high-resolution MS for in vitro ADME screening assays. Bioanalysis. 2012, Mar;4(5):581-93.

ADME screening and DMPK research

The ability to acquire quantitative data, along with qualitative data for parallel or retrospective data analysis—the quan/qual approach—is powerful in conserving time, laboratory space, and budgets. For this reason, **King et al**. described their evaluation and implementation of Q Exactive mass spectrometer-based Quan/Qual analyses at UCB's research DMPK department.[54] The authors compared the quantitative performance of Q-TOF and Q Exactive instruments. Both types of instruments performed equally in terms of mass accuracy, sensitivity, robustness, and scan speed. The key difference between the Q-TOF and Orbitrap instruments was the linear quantitative dynamic range achieved, four and greater than four orders of magnitude, respectively, for the compounds tested, a feature considered important in measuring all time points at all doses.

The authors concluded by summarizing the benefits of adopting the Q Exactive mass spectrometer-based Quan/Qual workflow: the additional information obtained produced a more integrated understanding of bioanalytical processes and the DMPK properties of compounds; a large amount of time is saved by not having to develop targeted MS/MS methods for individual compounds; and the ability to obtain both Quan/Qual data from a single analysis has reduced the need for *in vivo* and *ex vivo* studies involving animals, sample preparation and processing, consumables, and laboratory space. The authors expect that over time the development of less expensive high-resolution MS systems will lead to the demise of the use of QQQ mass spectrometers in routine bioanalytical applications.

54. King, L.; Kotian, A.; Jairaj, M. Introduction of a routine quan/qual approach into research DMPK: experiences and evolving strategies. Bioanalysis. 2014, 6(24):3337-48.

Omics

- Metabolomics
- Lipidomics

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Metabolomics

While targeted metabolomics focuses on quantification of a limited number of well-characterized preselected molecules, non-targeted methods are much more exhaustive and thus often lack certain characterization of metabolites of interest. For this reason, **Nzoughet et al.** presented a non-targeted metabolomics approach that provides unequivocal metabolite identification using the Q Exactive mass spectrometer and an in-house database, created from the Mass Spectrometry Metabolite Library of Standards (MSMLS), containing accurate *m/z* values, retention times, isotopic patterns, full MS, and MS/MS spectra.[55] The method was validated for extraction recovery, selectivity, repeatability, method precision, linearity, and instrumental precision. Multivariate and univariate statistical analyses were used to analyze data obtained from the analysis of plasma from remote ischemic preconditioning (RIPC) rats. Three biomarkers identified in previous targeted metabolomics studies were found, as well as three novel metabolites. The authors concluded the workflow allows identification and confirmation of new metabolite biomarkers, in a single experimental sequence.

Because high-resolution MS is becoming important in many labs, **Rochat** reviewed why this trend is extending to clinical research.[56] Most high-resolution mass spectrometers perform sensitive and reliable quantification of a variety of analytes, in either full scan or targeted SIM or MS2 modes. In terms of sensitivity, selectivity, mass accuracy, detection reliability, calibration-curve linearity, level accuracy, ease-of-use, and costs, the quantitative performance of high-resolution MS is similar to QQQ-MS. Because all ionized compounds are detected, high-resolution full-scan data has the advantage of providing global sample information. The use of one platform for targeted quantification to untargeted 'omics, and targeted quantification streamlines workflows, enabling research to move forward more rapidly. In support of these assertions, the author cites a large body of research that describes the quantitative, qualitative, and Quan/Qual capabilities of high-resolution MS.

55. Nzoughet, J.; Bocca, C.; Simard, G.; Prunier-Mirebeau, D,; Chao de la Barca, J.; Bonneau, D.; Procaccio, V.; Prunier, F. A Nontargeted UHPLC-HRMS Metabolomics Pipeline for Metabolite Identification: Application to Cardiac Remote Ischemic Preconditioning. Anal. Chem. 2017, 89 (3), 2138–2146.

56. Rochat, B. From targeted quantification to untargeted metabolomics: Why LC-high-resolution-MS will become a key instrument in clinical labs. Trends in Analytical Chemistry. 2016, Nov. " Volume 84, Part B, 151–164.

Lipidomics

Unlike proteomics, untargeted metabolomics profiling based on LC-MS/MS, is hampered by a lack of metabolite databases. Though there are free web-based databases, they are not practical for routine high-throughput analyses, especially for MS/MS confirmation of large datasets. Commercial databases have limited compatibility with data produced from all manufacturers' systems. Databases created using chemical standards can be expensive and time consuming to build.

For this reason, **Tang et al.** investigated in-house creation of accurate MS/MS databases without using standards.[57] The authors constructed a lipid database of regularly observed species where accurate masses were calculated based on structures and chemical formulas. A polar metabolite database was also built, in this case by using the MS/MS information in public DDA data in Metlin, mzCloud, and others. Entries were stored in a TraceFinder software database for subsequent high-throughput analyses. To validate lipid and polar metabolite workflows, mouse brain and liver extracts were analyzed using a Q Exactive instrument. The lipid database enabled identification and simultaneous quantitation of about 500 lipids. DDA data provided compound confirmations. The polar metabolite database enabled assignment of 370 compounds in mouse liver with186 confirmed by MS/MS fragments. In mouse brain, 529 polar metabolites were identified with 156 confirmed by MS. All compounds were quantified with adequate information for PCA.

57. Tang, H.; Wang, X.; Xu, L.; Ran, X.; Li, X.; Chen, L.; Zhao, X.; Deng, H.; Liu, X. Establishment of local searching methods for orbitrap-based high throughput metabolomics analysis. Talanta. 2016, Aug 15;156-157:163-71..

Quantitate for the future, close your uncertainty gap

Quadrupole-Orbitrap mass spectrometer technology has been successfully used for the quantitation of both small and large molecule analytes in a range of clinical research, forensic toxicology, pharmaceutical discovery, environmental analysis, and food testing applications. As observed from numerous peer-reviewed publications, the popularity of performing quantitation using Orbitrap mass spectrometer technology is growing rapidly due to its unique ability to provide HRAM Quan/Qual MS and MS/MS 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 chance of false detection of the analyte of interest. The unique ability to capture all relevant data in using full scan allows retrospective data analysis, reducing the need for additional sample injections.

In addition, quadrupole-Orbitrap mass spectrometers are enabling clinical research, forensic toxicology, food safety, and environmental laboratories to raise their productivity to new levels. Compared to QQQ MS-based quantitation methods that involve time consuming optimization of hundreds of SIM or SRM transitions in numerous time windows, quadrupole-Orbitrap mass spectrometer 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 such as full-scan MS, AIF, MS/MS, SIM, HCD, PRM, and rapid polarity switching, the Q Exactive and Q Exactive Focus mass spectrometers provide unmatched analytical versatility to meet the needs of laboratories today and into the future.

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