

# The Thermo Scientific Exactive Benchtop LC/MS Orbitrap Mass Spectrometer

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## Overview

Review of a new benchtop mass spectrometer based on a stand-alone Thermo Scientific Orbitrap™ mass analyzer. Key features of the instrument layout, analytical parameters and typical applications are described.

## Introduction

Over the past three years, the combination of Orbitrap technology with a linear ion trap has become an established platform for high resolution, accurate mass LC/MS<sup>n</sup> analysis. The high resolving power, mass accuracy and dynamic range of the Orbitrap analyzer allow rigorous characterization of complex mixtures even in the absence of precursor ion mass selection. We now describe the development of a non-hybrid mass spectrometer comprising of an atmospheric-pressure ion source (API) and a standalone Orbitrap mass analyzer.

## Methods

All experiments were performed on a prototype of the new Thermo Scientific Exactive™ mass spectrometer using an electrospray ionization (ESI) source.

## Instrument Layout Overview

Figure 1 shows the schematic layout of the instrument. Samples can be introduced into the API source by a variety of methods including direct infusion or an U-HPLC system (Thermo Scientific Accela™). The source is similar to the commercial source of the Thermo Scientific TSQ Quantum Ultra™.

Ions are transferred from the source through four stages of differential pumping using RF-only multipoles into a curved RF-only trapping quadrupole (the C-trap). In the C-trap, ions are accumulated and their energy dampened using a bath gas (nitrogen). Ions are then injected through three further stages of differential pumping using a curved lens system into the Orbitrap analyzer where mass spectra are acquired via image current detection. The vacuum inside the Orbitrap mass analyzer is maintained below 1E-09 mBar.

## Automatic Gain Control (AGC)

Automatic control of the number of ions in the Orbitrap is performed by measuring the total ion charge using a pre-scan and by calculating the ion injection time for the analytical scan from this. For very high scan rates, the previous analytical scan is used as a prescan to optimize the scan cycle time without compromising automatic gain control. Ion gating is performed using a fast split lens setup that ensures the precise determination of the ion injection time.

## Higher Energy Collision Induced Dissociation (HCD)

In a HCD experiment, ions are passed through the C-trap into a multipole collision cell where they are fragmented. After that, the HCD cell voltages are ramped, and ions are transferred back into the C-trap from where they are injected into the Orbitrap for detection.

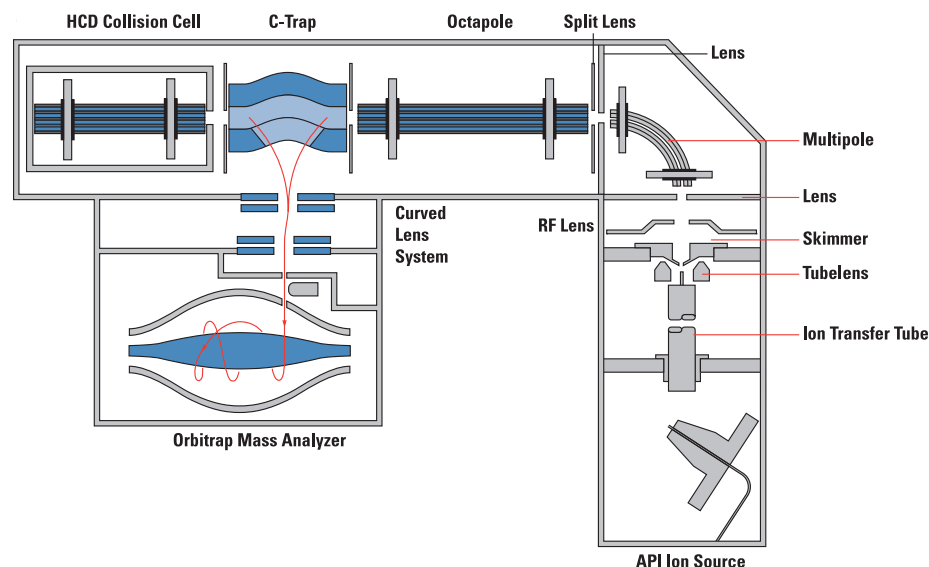


Figure 1: Schematic layout of the instrument.

## Key Words

- Exactive™
- Accurate Mass
- High Resolution
- Polarity Switching
- Scan Speed

## Results

### Automatic Gain Control (AGC)

A requirement of any ion trap device is the ability to control the ion population within the trap. When the ion population is not accurately maintained, it can result in large variations in the quality of data. The correct AGC functionality of the Exactive instrument is exemplified in Figure 2 by two mass spectra acquired in the middle and at the end of an eluting LC peak of Buspirone.

### Scan Speed

The use of a single mass analyzer with very high transmission characteristics, in combination with the use of fast digital and analog electronics, allow high resolution mass spectra to be detected, processed and recorded at

In both, cases the mass resolution, mass accuracy and signal-to-noise ratio are excellent. The AGC feature, in combination with the precise determination of the ion injection time, allows the instrument to be used for accurate quantitative analyses.

high scan rates of up to 10 Hz. This is compatible with the narrow peak widths observed in fast chromatography analyses (Figure 2).

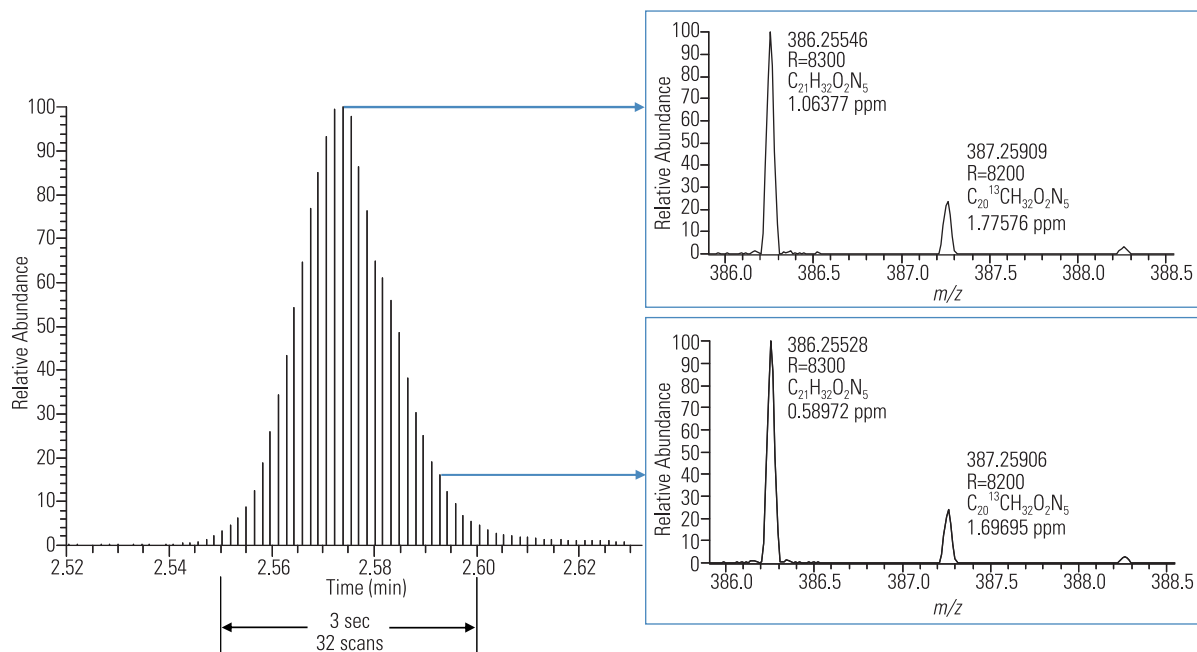


Figure 2: LC peak and mass spectra of Buspirone acquired at a scan rate of 10 scans per second.

### Mass Resolution

At a scan rate of 10 Hz, the resolving power of the instrument is  $> 10,000$  at  $m/z$  200. Increasing the transient detection time by a factor of 10 (corresponding to a scan rate of 1 Hz), the mass resolution can be increased beyond 100,000.

To demonstrate the resolving power of the instrument, a pesticide mixture was measured showing well resolved isobaric peaks of Dimethon ( $m/z$  231.0273) and Asulam ( $m/z$  231.0434) within a full scan spectrum (Figure 3).

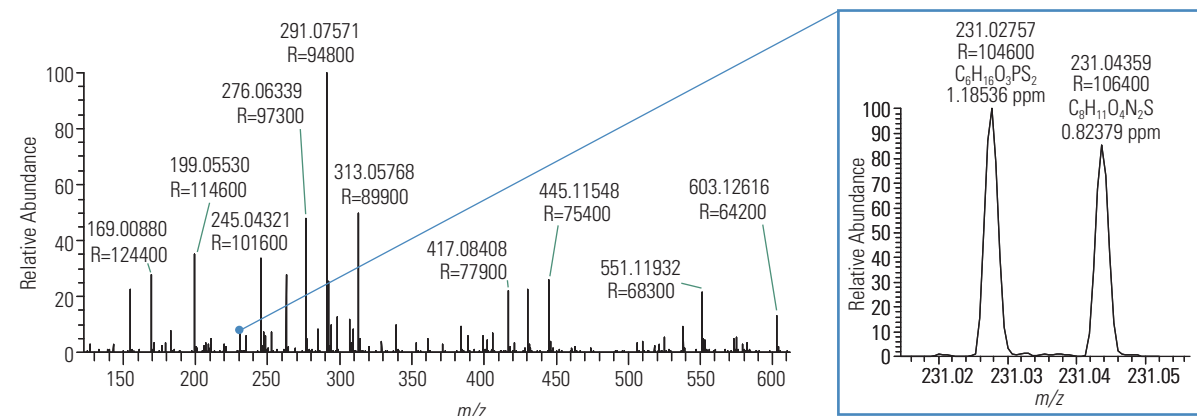


Figure 3: Full scan spectrum of a pesticide mixture demonstrating a resolving power of up to 100,000.

### Mass Accuracy and Stability

Using fully automated AGC and mass calibration procedures, mass spectra with high mass accuracy are recorded. The mass accuracy, precision and stability is equally as good as that obtained in ion trap based hybrid instruments, i.e. Thermo Scientific LTQ Orbitrap™ or LTQ FT Ultra™.

Figure 4 shows the mass accuracy and its stability over time for different molecular ions of an ESI calibration mixture. The full scan spectra were acquired at a resolution setting of 100,000 in an infusion experiment applying an external calibration, i.e. no lock masses were used.

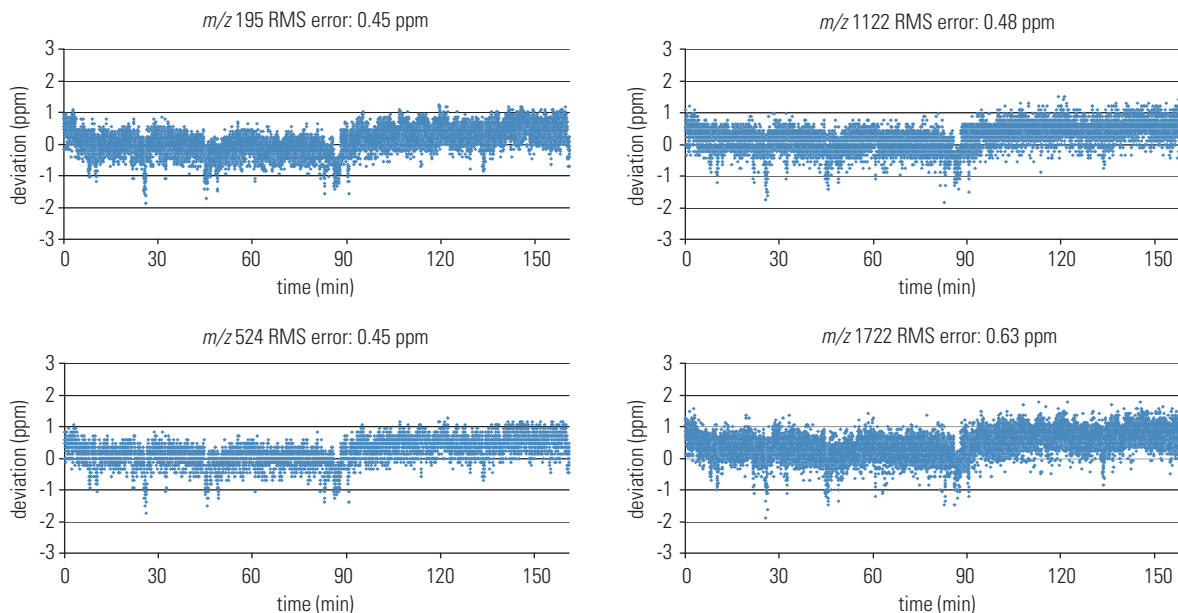


Figure 4: Mass accuracy and stability of ions at different  $m/z$  values acquired in an infusion experiment without using lock masses.

### Fast Polarity Switching

Due to the use of a novel power supply design, it is possible to perform fast polarity switching without sacrificing mass accuracy in any scans. Figure 5 demonstrates this feature by means of two experiments. In the first experiment, the polarity was changed from scan to scan to check mass accuracy at fast alternating polarity

switching corresponding to a full cycle of 1 positive and 1 negative scan within 1 second. In the second experiment, the polarity was switched every 5 minutes to check for potential drift effects. In both cases, full scan spectra were acquired at a resolution setting of 30,000 in an infusion experiment using an ESI calibration solution applying an external calibration, i.e. no lock masses were used.

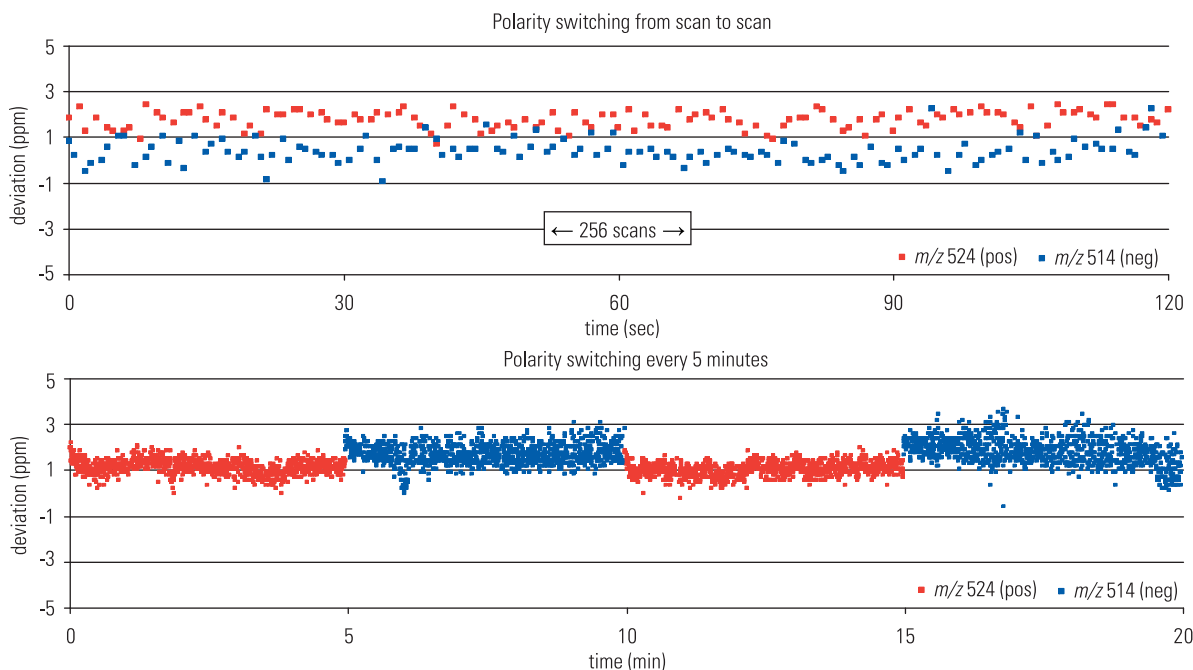


Figure 5: Mass deviations of  $m/z$  524 (positive ions) and  $m/z$  514 (negative ions) observed in polarity switching experiments.

## Dynamic Range

The dynamic range of the instrument varies by sample and with the instrument settings, but it is typically about 3 to 4 orders of magnitude. Figure 6 shows that it is possible to acquire full scan spectra with an in-scan dynamic range of more than 13,000. The spectrum was acquired in an infusion experiment using a mix of Buspirone ( $m/z$  386) and Caffeine ( $m/z$  195).

The ratio of the Buspirone signal to the Caffeine signal is greater than 13,000. Both peaks show mass accuracies of less than 1 ppm. Thus, this spectrum demonstrates not only the high in-scan dynamic range in terms of signal, but also the high dynamic range in terms of mass accuracy of this instrument – analogous to the performance of a hybrid LTQ Orbitrap mass spectrometer.

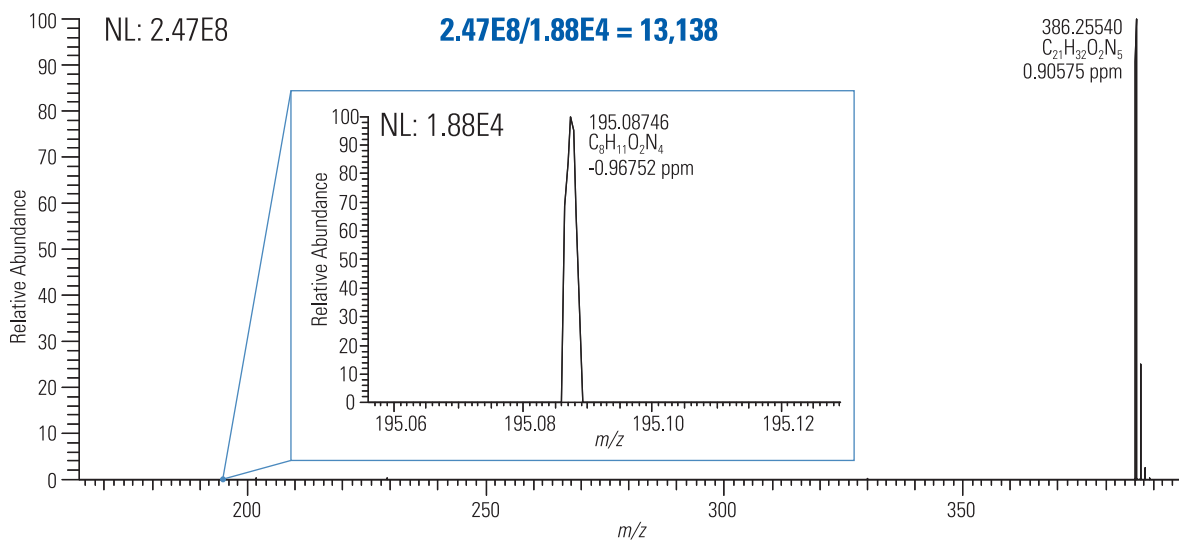


Figure 6: Spectrum of a mixture of Buspirone ( $m/z$  386) and Caffeine ( $m/z$  195) showing an in-scan dynamic range of > 13,000 and sub-ppm mass accuracies.

## All Ion Fragmentation (HCD)

The instrument design allows high efficiency “All Ion Fragmentation” experiments by means of Higher Energy Collision Induced Dissociation (HCD).

As an example, Figure 7 shows full scan spectra of Verapamil with and without HCD fragmentation and demonstrates the high fragmentation efficiency and the excellent mass accuracy of the HCD fragments.

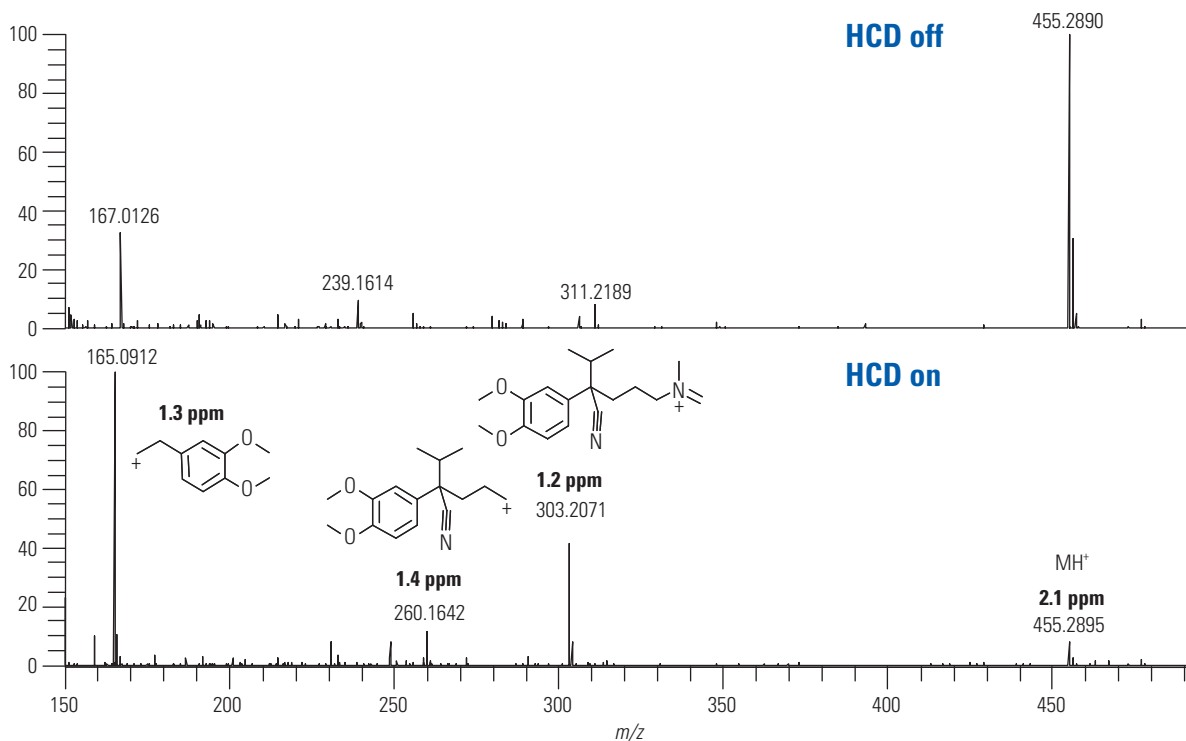


Figure 7: Full scan spectra of Verapamil with and without HCD fragmentation.

## Applications

As a result of the described performance characteristics of this new benchtop Orbitrap mass spectrometer, several key applications are ideally suited to the use of the Exactive mass spectrometer. Some of these are:

1. Exact mass measurements of organic compounds
2. Early drug discovery metabolism and pharmacokinetics (DMPK)
3. General unknown screening
4. Multiple residue analysis (Pesticides, Mycotoxins, veterinary drugs)
5. Metabolomics

For all of these applications, high resolution, accurate mass measurements, together with high dynamic range, is required for unequivocal results in full MS mode. Where it is needed, additional information can be provided by use of high resolution/high mass accuracy MS/MS experiments in an “All Ion Fragmentation” mode. Figure 8 shows an extracted ion chromatogram of 116 pesticides and mycotoxins at a level of 50 ppb in a very complex matrix of horse feed extract at a mass resolution of 50,000. This exemplifies the high selectivity and sensitivity of the instrument working in full scan mode, which is a prerequisite for a successful screening approach, since resolving matrix interferences from the target analytes is essential.

## Conclusions

A new benchtop mass spectrometer has been developed based on an API ion source combined with a stand-alone Orbitrap mass analyzer. The key performance features are as follows:

- Mass resolutions of up to 100,000
- Scan speeds of up to 10 Hz
- High in-scan dynamic range (4 orders of magnitude)
- Mass accuracies of better than 2 ppm in full scan and “All Ion Fragmentation” mode
- Fast polarity switching (full cycle of 1 positive and 1 negative scan within 1 second)
- High efficiency “All Ion Fragmentation” Higher Energy Collision Induced Dissociation (HCD)

The instrument is very easy to operate and its performance characteristics are ideally suited for discovery work, screening applications, quantitative analyses and elemental composition determinations.

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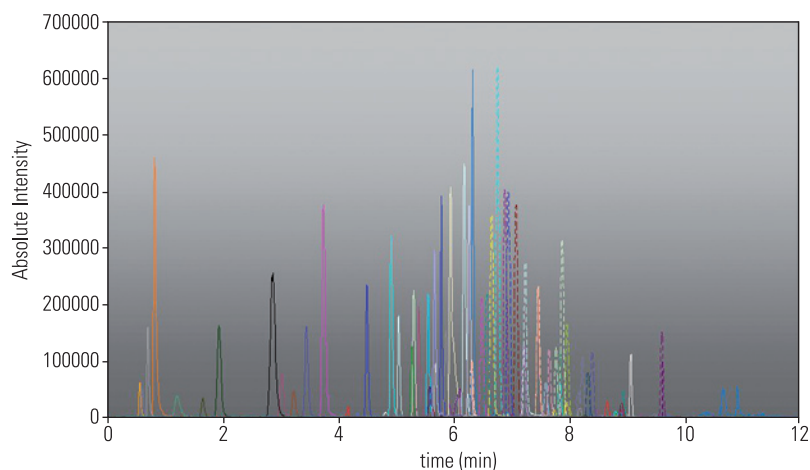


Figure 8: Extracted ion chromatogram of 116 pesticides and mycotoxins at a level of 50 ppb in a complex matrix of horse feed extract.

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