

High Resolution and Precise Mass Accuracy: A Perfect Combination for Food and Feed Analysis in Complex Matrices

Markus Kellmann, Andreas Wieghaus, Helmut Muenster, Thermo Fisher Scientific, Bremen, Germany
Lester Taylor, Dipankar Ghosh, Thermo Fisher Scientific, San Jose, CA, USA

Overview

Purpose:

To demonstrate the analytical advantages of using high resolution (> 40,000) for the accurate screening of pesticides in complex matrices using a new benchtop Thermo Scientific Orbitrap detector.

Methods:

Use of a Ultra High Pressure Liquid Chromatography (U-HPLC) coupled with Orbitrap™ detector (Thermo Scientific Exactive) operating in high resolution mode.

Results:

The combination of high resolution (15,000 – 50,000) accurate mass is required for the detections of pesticides and mycotoxins.

Introduction

Screening of pesticides, mycotoxins and veterinary drugs is of great importance in regulated environments, such as food and animal feed analysis. Traditionally, these type of experiments have been carried out using triple quadrupole instruments. This approach has certain limitations:

- no post acquisition re-interrogation of data
- limited number of compounds per analysis
- cannot screen unidentified unknowns

Because of these limitations, there is currently a trend towards full scan MS experiments in residue analysis. Current screening approaches are performed using high performance ToF instruments, with mass accuracies of < 5ppm and resolutions of about 15,000, coupled to Ultra High Performance Liquid Chromatography (U-HPLC).

In complex sample matrices (e.g. food, feed, hair, honey) this limited resolution leads to inaccurate mass measurements caused by unresolved background matrix interferences. In this work, we show a full scan screening approach using a novel single stage Orbitrap mass spectrometer coupled to U-HPLC, capable of providing high mass accuracy at resolutions of up to 100,000.

Additionally, we will discuss two aspects of the analysis which also greatly benefit from very high resolution:

- resolving co-eluting, isobaric target compounds
- elemental composition determination

Methods

A new non-hybrid single stage Orbitrap mass spectrometer (Exactive™, Thermo Fisher Scientific, Bremen, Germany) coupled to a U-HPLC chromatograph (Thermo Scientific Accela™, Thermo Fisher Scientific, San Jose, USA) was used to evaluate a highly complex mixture of 116 pesticides, mycotoxins and plant toxins in different concentrations. A 12 min gradient was applied to a 50 x 2 mm RP C18 column (Thermo Scientific Hypersil GOLD™ 1.9 µm particles, Thermo Fisher Scientific, Madison, USA) with water/acetonitrile eluents. The method developed was evaluated with respect to sensitivity, selectivity and linearity in standard solutions and extracts from animal feed. Mass measurements were performed at different resolution settings ($R = 15,000$ and $R = 50,000$) to enable comparisons to data acquired by ToF instruments and to demonstrate the advantage of ultra high resolution. Orbitrap detection was carried out using automatic control of the number of ions entering the detector (AGC, target value = 10^6).

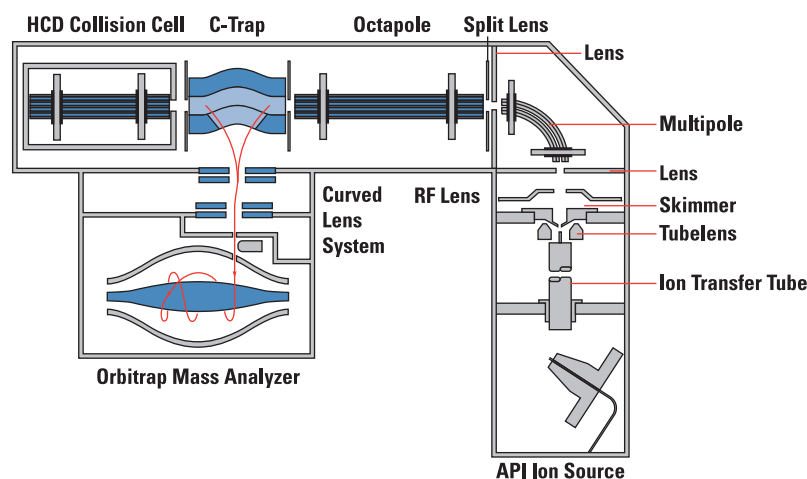


Figure 1: Schematic of the new Orbitrap benchtop mass spectrometer, including HCD collision cell for "All Ion Fragmentation".

Key Words

- Exactive
- Food Analysis
- Pesticide Screening
- High Resolution MS

Results

Resolution of Isobaric Pesticides

In cases where isobaric compounds co-elute, erroneous mass accuracy and elemental composition assignment will occur if the resolving power of the mass spectrometer is insufficient to separate these compounds. Figure 2 shows two pesticides Thiamethoxam ($C_8H_{10}ClN_5O_3S$) and Parathion ($C_{10}H_{14}NO_5PS$), which have protonated molecular ions (MH^+) at 292.02656 and 292.04031, respectively. A resolution higher than 40,000 is needed to resolve the protonated molecular ion of these two compounds completely. This is a pre-requisite for analysis of low concentration compounds in the presence of higher abundant ones. The example in Figure 2 shows an approximate 1:3 mixture of both pesticides measured and simulated.

Influence on Elemental Composition Determination

A limited resolution of 15,000 results in two major limitations. First, the detection of unresolved doublets may result in significant mass errors, which are outside the characteristic accuracy specification of the Exactive instrument. As a consequence, at lower resolution settings, the mass windows for elemental composition determination have to be increased, resulting in much larger number of elemental composition proposals for the unknown or targeted compounds. This can be seen for the example (Figure 3) of Pirimicarb at m/z 239.1503. Due to the presence of an isobaric interference, the peak at 239 shows a mass error of 6.5 ppm. At a resolution of 15,000, the underlying interference causes an apparent shift to higher mass, whereas at higher resolution (here 80,000), the doublet is clearly resolved, and the mass accuracy is well within instrument specifications.

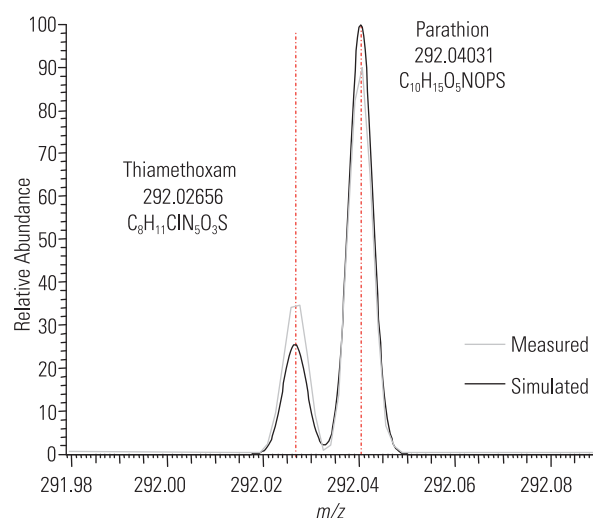
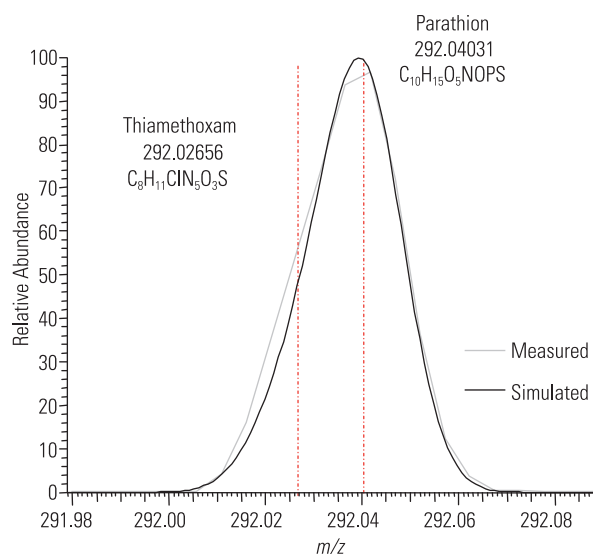


Figure 2: Mass chromatogram of two isobaric pesticides measured at a resolution of 15,000 (left) and 50,000 (right). Superimposed is the simulated mass trace at each resolution setting.

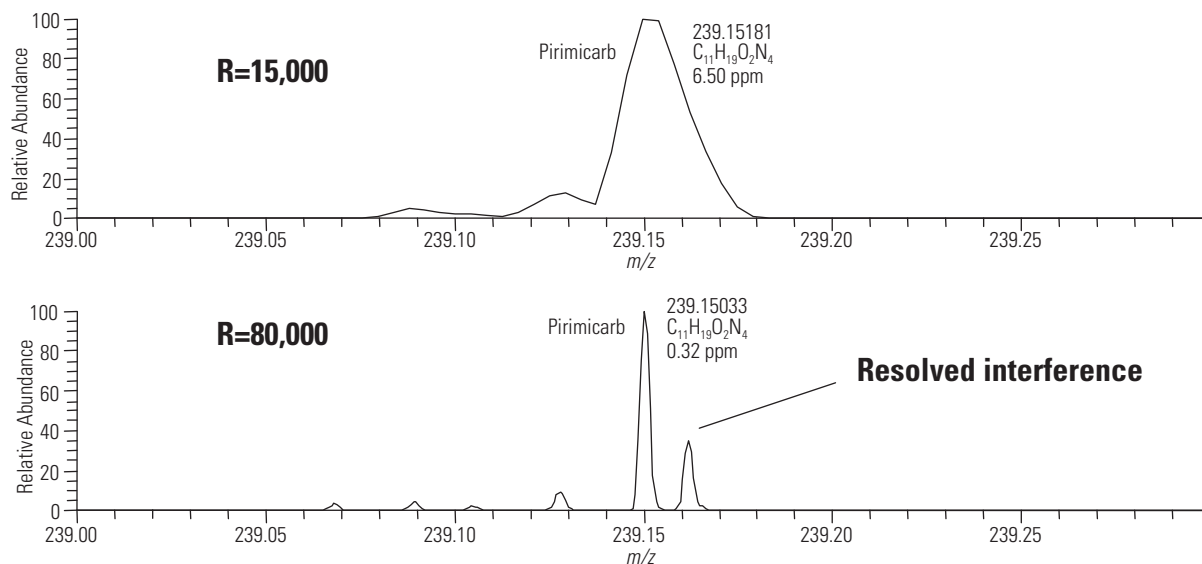


Figure 3: Improved mass accuracy simply by increasing the resolution in order to resolve the doublet. The peak at 15,000 resolution splits up into 2 by increasing the resolution to 80,000.

In order to limit the number of candidate elemental compositions to a single confident assignment, a sophisticated software algorithm is used. It takes into account the peak height and mass accuracy of the monoisotopic peak and its isotopes. However, in order to function correctly, all of the accurate mass values for the isotopic peaks must be within specified limits. The absence of interference peaks can only be assured by use of high resolution, (as can be seen in Figure 4). Here the fungicide, Azoxystrobin, is shown at resolutions of 15,000 and 80,000. The medium resolution spectrum shows very good mass accuracy for the monoisotopic peak, but gives unusually high mass errors for the A+1 and A+2 ions. This is due to an interference at m/z 405.1452, which cannot be resolved at medium resolution. Whereas, the high resolution spectrum shows excellent mass accuracy for all three measured isotopes. Determining elemental compositions using data acquired at $\sim 15,000$ resolution will result in misleading or incorrect data. Only sufficient high resolution allows the determination of the accurate mass of the complete molecular ion cluster, and therefore allows automated assignment of an elemental formula with a high degree of confidence.

Analyzing highly complex samples such as extracts from food or animal feed, and the screening of regulated substances including pesticides, mycotoxins and veterinary drugs is a major analytical challenge for mass spectrometry. On one hand, the methodology must have a high intra scan dynamic range in order to detect low concentrated compounds in presence of high abundant matrix ions, on the other hand high selectivity and high sensitivity is needed to avoid false positive, or even worse, false negative results. In our procedure, we analyzed an extract from horse feed as an example of extremely complex matrix, spiked with a mixture of 116 pesticides and mycotoxins. A dilution series ranging from 2 to 250 ppb (for each compound) was measured in duplicates at two different resolution settings. In addition, a 100 ppb sample of the same mixture was analyzed at a resolution of 50,000 in order to determine the maximum number of detectable substances for this method.

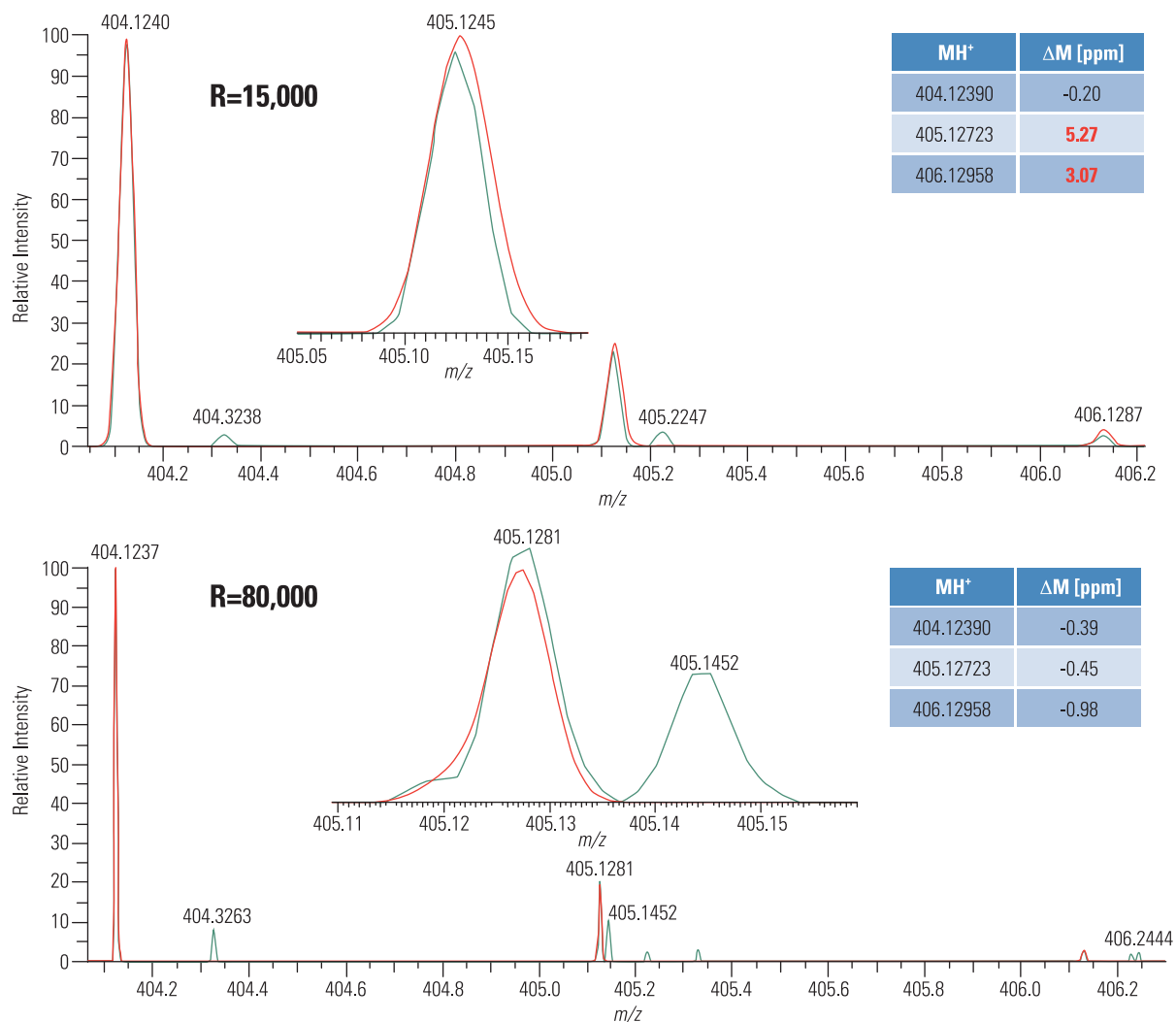


Figure 4: The importance of resolution for the molecular ion AND its isotopes. At high resolution the complete molecular ion cluster is correctly detected.

LC-MS analysis of the extracted spiked samples showed the presence of 95 out of 116 compounds at 100 ppb in matrix. Figure 5 shows the overlaid ion chromatograms for all 116 compounds (3 ppm window) at 50 ppb (in matrix). The number of recovered pesticides in different concentrations is shown in Figure 5. The data illustrates that a greater number of detected compounds (higher sensitivity) with an extraction window of 3 ppm at higher resolution setting. This is exemplified in Figure 6, where extracted ion chromatograms of Sulcotrion at 50 ppb for R = 15,000 and R = 50,000 are shown. The higher resolved spectrum displays two peaks, of which the smaller one is Sulcotrion.

The lower resolved peak masks the pesticide signal completely. The only indication for the presence of Sulcotrion is a slightly broader peak or shoulder and a mass shift of the interfering ion towards higher masses. This would lead to a false negative result, if the analysis was only performed at a resolution of 15,000, and is the major reason why the number of identified components in the case of R = 15,000 decreases disproportionately to the measurements at higher resolving power (diagram, Figure 5).

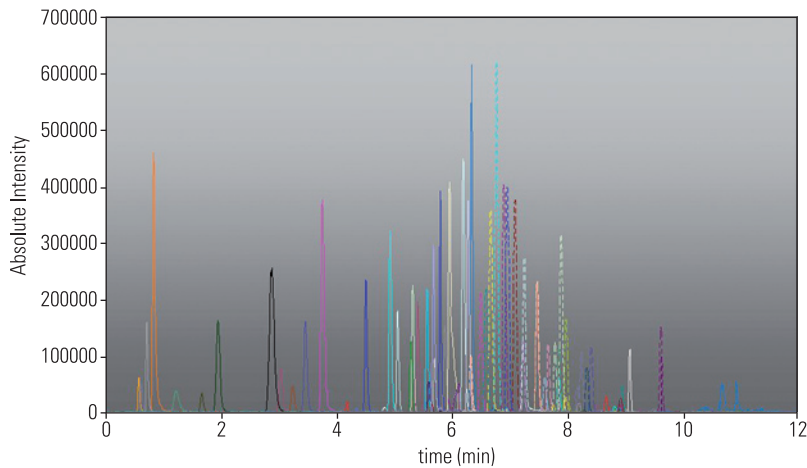


Figure 5: Overlaid extracted ion chromatograms from a mixture of 116 pesticides and mycotoxins at a 100 ppb level. Extraction was done with 3 ppm mass window. The inset chart shows the number of detected compounds at different concentrations (in matrix) at two different resolution settings.

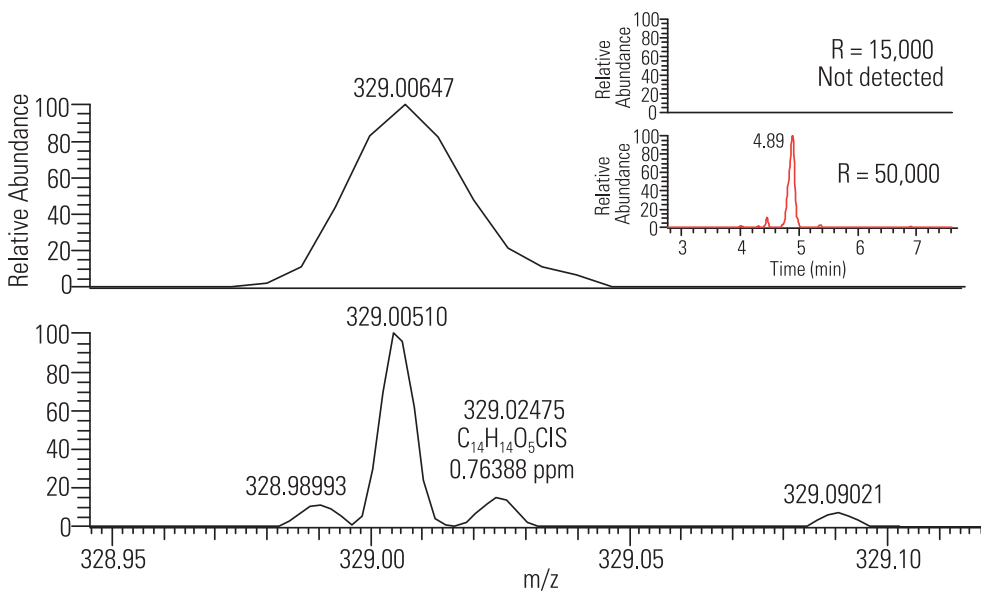


Figure 6: Expanded view of the pesticide mixture at different resolution settings (top: 15,000 and bottom: 50,000). Pesticide Sulcotrion (m/z 328.02475) is masked under background ions at a resolution of 15,000 but is easily detected at 50,000 resolution (see also mass chromatogram inset).

For this reason, a dilution series was measured at higher resolution settings. One example (Metabenthiazuron) is shown in Figure 7. It demonstrates excellent linearity and sensitivity down to 2 ppb level (2 ng/mL).

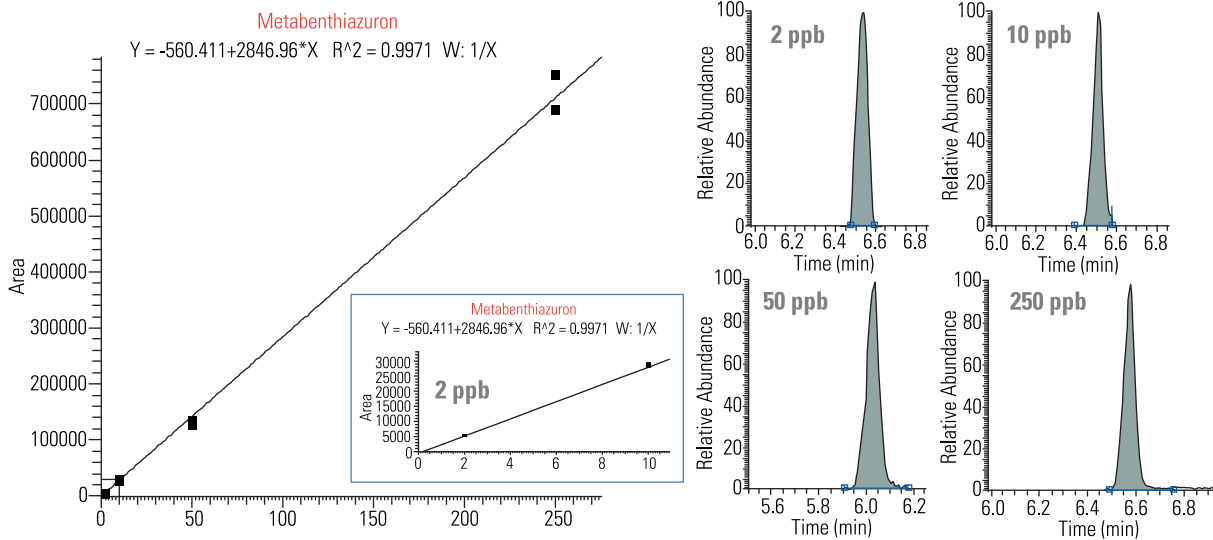


Figure 7: Quantitation curve for Metabenthiazuron ranging from 2 to 250 ppb. The quantified peak for each concentration level demonstrates the high quality data even at the lowest level.

Conclusions

- New benchtop Orbitrap mass spectrometer demonstrates superior mass resolving power compared to that obtained using TOF instruments.
- High resolving power (up to 100,000) provides precise mass accuracy for complex sample analysis.
- High resolving power provides excellent sensitivity, linearity and selectivity in multi-residue screening of complex matrices.
- Fast scan speeds (10 Hz) are fully compatible with the use of U-HPLC fast chromatography methods.

For the analysis of very complex samples, it is advantageous to select the appropriate scan speed and resolution in order to avoid unresolved isobaric compounds (matrix ions from analyte ions) and still allow unambiguous detection of low abundant species.

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