Application Note 573

Qualitative and Quantitative Analysis of Pesticides in Horse Feed Matrix Using Orbitrap MS

Olaf Scheibner and Maciej Bromirski, Thermo Fisher Scientific, Bremen, Germany

Key Words

Exactive Plus, UHPLC, high resolution, accurate mass, high throughput, Orbitrap, Data-Dependent All-Ion Fragmentation, ExactFinder

Goal

To test the ability of a high-resolution, accurate-mass benchtop Orbitrap™ mass spectrometer to achieve high sensitivity and selectivity when analyzing modern, very-short-gradient UPHLC separations of complex samples.

Introduction

Productivity of a liquid chromatograph-mass spectrometer (LC-MS) system is measured in samples per day. To achieve higher productivity, modern ultra-high-performance LC-MS (UHPLC-MS) methods use very short gradients. Chromatographic peak widths are often below 5 seconds at the base. A high-resolution, accurate-mass (HR/AM) mass spectrometer operating in full-scan mode must be able to provide a sufficient number of scans (≥10) across the chromatographic peak without compromising sensitivity and selectivity. As reported earlier, a resolving power in excess of 50,000 (FWHM at *m/z* 200) combined with a mass extraction window of 5 ppm is necessary to ensure selectivity comparable to established MS/MS techniques.[1]

The Thermo Scientific™ Exactive™ Plus Orbitrap mass spectrometer (Figure 1) is the second generation of the Exactive product family. It features two major changes over the first generation instrument. First, in the ion optics the tube-lens / skimmer assembly has been replaced by an S-Lens (Figure 2) that provides significantly higher ion transmission, increasing the instrument's sensitivity. Second, the Orbitrap mass analyzer and related electronics have been improved,[2] resulting in higher scan speed and resolution, as well as improved polarity switching. As a result, the range of resolving power is from 17,500 to 140,000 at *m/z* 200, with a maximum scan rate of 12 Hz.

In this research, the Exactive Plus instrument was used to analyze extracts of horse feed spiked with common pesticides.

Figure 1. Exactive Plus mass spectrometer with Accela 1250 UHPLC

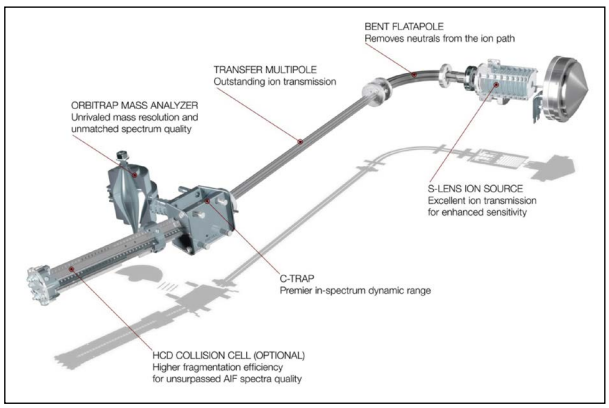

Figure 2. Exactive Plus ion optics and mass analyzer components

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Experimental

Sample Preparation

QuEChERS extracts of horse feed were spiked with 85 common pesticides (Table 1) at levels of 10 and 100 ppb, and diluted 1:1 with acetonitrile. Six calibration standards with the 85 pesticides in acetonitrile were mixed 1:1 with horse feed matrix that, through previous analysis, was proven to be free of pesticides. The final calibration levels were 5, 10, 25, 50, 100, and 150 ppb (5–150 µg/kg).

Table 1. Pesticides spiked into QuEChERS extracts

Pesticide	Chemical Formula
Acephate	$C_4H_{10}NO_3PS$
Acetamiprid	$C_{10}H_{11}ClN_4$
Aldicarb	$C_7H_{14}N_2O_2S$
Aldicarb-sulfone	$C_7H_{14}N_2O_4S$
Azinphos-ethyl	$C_{12}H_{16}N_3O_3PS_2$
Azinphos-methyl	$C_{10}H_{12}N_3O_3PS_2$
Azoxystrobin	$C_{22}H_{17}N_3O_5$
Bromacil	$C_9H_{13}BrN_2O_2$
Bromuconazole	$C_{13}H_{12}BrCl_2N_3O$
Carbaryl	$C_{12}H_{11}NO_2$
Carbendazim	$C_9H_9N_3O_2$
Carbofuran	$C_{12}H_{15}NO_3$
Carbofuran-3-hydroxy	$C_{12}H_{15}NO_4$
Chlorfluazuron	$C_{20}H_9Cl_3F_5N_3O_3$
Clofentezine	$C_{14}H_8Cl_2N_4$
Cymiazole	$C_{12}H_{14}N_2S$
Cymoxanil	$C_7H_{10}N_4O_3$
Cyproconazole	$C_{15}H_{18}ClN_3O$
Cyromazine	$C_6H_{10}N_6$
Demeton-S-methyl-sulfone	$C_6H_{15}O_5PS_2$
Dichlorvos	$C_4H_7Cl_2O_4P$
Diethofencarb	$C_{14}H_{21}NO_4$
Difenoconazole	$C_{19}H_{17}Cl_2N_3O_3$
Diflubenzuron	$C_{14}H_9ClF_2N_2O_2$
Dimethoate	$C_5H_{12}NO_3PS_2$
Disulfoton	$C_8H_{19}O_2PS_3$
Disulfoton-sulfone	$C_8H_{19}O_4PS_3$
Diuron	$C_9H_10Cl_2N_2O$
Ethiofencarb	$C_{11}H_{15}NO_2S$
Fenamiphos	$C_{13}H_{22}NO_3PS$
Fenazaquin	$C_{20}H_{22}N_2O$
Fenhexamid	$C_{14}H_{17}Cl_2NO_2$
Fenobucarb	$C_{12}H_{17}NO_2$
Fenoxycarb	$C_17H_{19}NO_4$
Fenthion	$C_{10}H_{15}O_3PS_2$
Flucycloxuron	$C_{25}H_{20}ClF_2N_3O_3$
Flufenoxuron	$C_{21}H_{11}ClF_6N_2O_3$
Formetanate	$C_{11}H_{15}N_3O_2$
Furathiocarb	$C_{18}H_{26}N_2O_5S$
Hexaflumuron	$C_{16}H_8Cl_2F_6N_2O_3$
Hexythiazox	$C_{17}H_{21}ClN_2O_2S$
Imazalil	$C_{14}H_{14}Cl_2N_2O$
Imidacloprid	$C_9H_{10}ClN_5O_2$

Pesticide	Chemical Formula
Indoxacarb	$C_{22}H_{17}ClF_3N_3O_7$
Iprovalicarb	$C_{18}H_{28}N_2O_3$
Isofenphos-methyl	$C_{14}H_{22}NO_4PS$
Isofenphos-oxon	$C_{15}H_{24}NO_5P$
Isoprothiolane	$C_{12}H_{18}O_4S_2$
Isoproturon	$C_{12}H_{18}N_2O$
Linuron	$C_9H_{10}Cl_2N_2O_2$
Mepanipyrim	$C_{14}H_{13}N_3$
Metconazole	$C_{17}H_{22}ClN_3O$
Methiocarb	$C_{11}H_{15}NO_2S$
Methiocarb-sulfone	$C_{11}H_{15}NO_4S$
Methoxyfenozide	$C_{22}H_{28}N_2O_3$
Metobromuron	$C_9H_{11}BrN_2O_2$
Monocrotophos	$C_7H_{14}NO_5P$
Napropamide	$C_{17}H_{21}NO_2$
Nitenpyram	$C_{11}H_{15}ClN_4O_2$
Omethoate	$C_5H_{12}NO_4PS$
Oxamyl	$C_7H_{13}N_3O_3S$
Pencycuron	$C_{19}H_{21}ClN_2O$
Phenmedipham	$C_{16}H_{16}N_2O_4$
Pirimicarb	$C_{11}H_{18}N_4O_2$
Prochloraz	$C_{15}H_{16}Cl_3N_3O_2$
Propamocarb	$C_9H_{20}N_2O_2$
Propoxur	$C_{11}H_{15}NO_3$
Prosulfocarb	$C_{14}H_{21}NOS$
Prosulfuron	$C_{15}H_{16}F_3N_5O_4S$
Pymetrozine	$C_{10}H_{11}N_5O$
Pyraclostrobin	$C_{19}H_{18}ClN_3O_4$
Pyridaphenthion	$C_{14}H_{17}N_2O_4PS$
Spinosyn-A	$C_{41}H_{65}NO_{10}$
Spinosyn-D	$C_{42}H_{67}NO_{10}$
Spiroxamine	$C_{18}H_{35}NO_2$
Tebufenozide	$C_{22}H_{28}N_2O_2$
Tebufenpyrad	$C_{18}H_{24}ClN_3O$
Teflubenzuron	$C_{14}H_6Cl_2F_4N_2O_2$
Tetraconazole	$C_{13}H_{11}Cl_2F_4N_3O$
Thiabendazole	$C_{10}H_7N_3S$
Thiacloprid	$C_{10}H_9ClN_4S$
Thiodicarb	$C_{10}H_{18}N_4O_4S_3$
Trichlorfon	$C_4H_8Cl_3O_4P$
Trifloxystrobin	$C_{20}H_{19}F_3N_2O_4$
Triflumuron	$C_{15}H_{10}ClF_3N_2O_3$

Liquid Chromatography

A Thermo Scientific Accela™ UHPLC system consisting of an Accela open autosampler in combination with an Accela 1250 UHPLC pump was used. A 2 minute chromatographic gradient of water and methanol, both spiked with 0.1% formic acid, was applied resulting in a total chromatographic cycle time of 5 minutes (Figure 3). Ten microliters of each sample were injected onto a Thermo Scientific Hypersil™ GOLD PFP column (50 x 2.1 mm, 1.9 µm particle size) with a flow rate of 800 µL/min. This resulted in peak widths of 3–6 seconds for the analytes of interest.

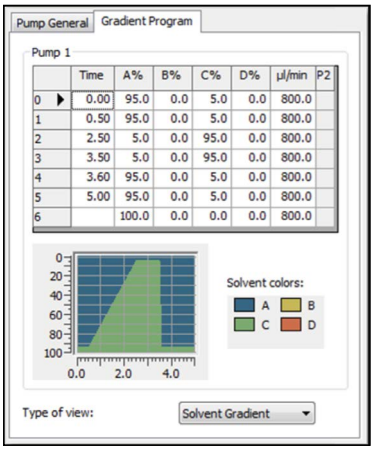

Figure 3. Chromatographic gradient

Mass Spectrometry

Given that resolution in excess of 50,000 was needed for this application, the Exactive Plus system was set to a resolving power of 70,000 at *m/z* 200, resulting in a scan rate of 3.7 Hz. As shown in Figure 4, this provided 13 scans across a 3.2 second peak.

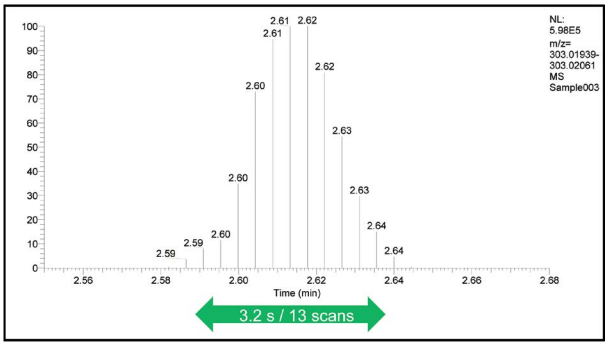

Figure 4. Scans achieved across a narrow chromatographic peak

For improved component identification, it would have been useful to have fragmentation scans on the analytes of interest. However, continual switching between full-scan and all-ion fragmentation scan modes (FS/AIF) would have required resolution to be reduced to maintain the number of scans. As an optimal solution, data-dependent AIF scans (dd-AIF) were introduced into the full scans (FS/dd-AIF) by means of a mass inclusion list containing the masses of the spiked components. One AIF scan was triggered for each target compound as soon as the abundance of the target compound crossed a given intensity threshold in a full scan. This significantly reduced the number of fragmentation scans and kept the overall data rate close to what could have been achieved in full-scan-only mode. Method details are shown in Figure 5.

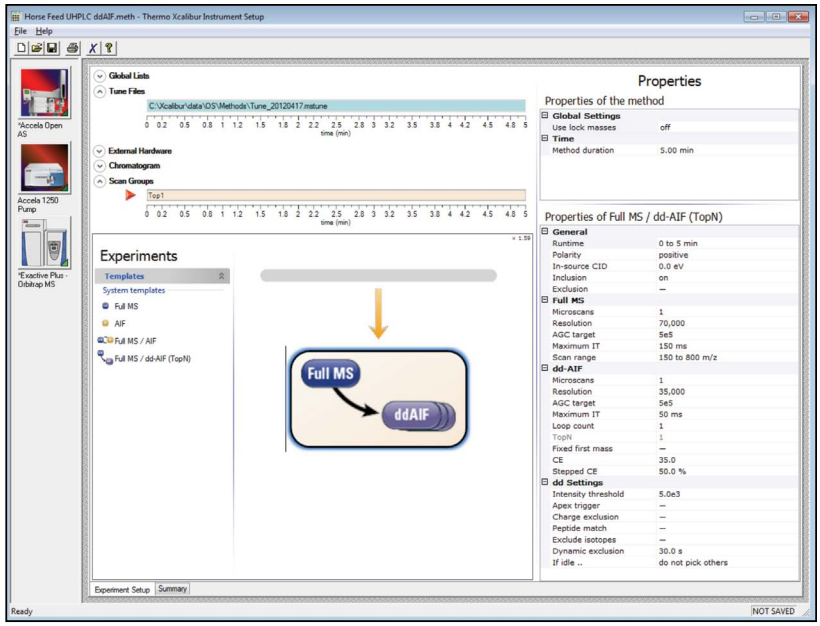

Figure 5. Exactive Plus instrument method setup

Data Analysis

The same data set was used for quantitative and qualitative data processing. Thermo Scientific ExactFinder™ software version 2.0 was used to process the data. Qualitative processing included targeted screening in combination with general unknown screening. The 85 common pesticides were selected using built-in databases from ExactFinder software. These selection could be exported directly into the mass inclusion list used by the Exactive Plus instrument method to trigger the dd-AIF scans. No further optimization of the LC-MS system was needed.

Results and Discussion

Quantitative Analysis

The six calibration standards, with spike levels ranging from 5 to 150 µg/kg, were analyzed to establish calibration curves for each of the target pesticides. The majority of pesticides eluted at between 1.3 and 3.0 minutes, so a number of target components and matrix components coeluted (Figure 6). However, the extracted ion chromatograms of most target components were free from additional peaks, demonstrating that the 5 ppm extraction window combined with the resolving power of the mass spectrometer provided very high selectivity. Linear calibration curves were achieved for nearly all target pesticides (example shown in Figure 7), confirming that the compounds could be clearly distinguished from the matrix.

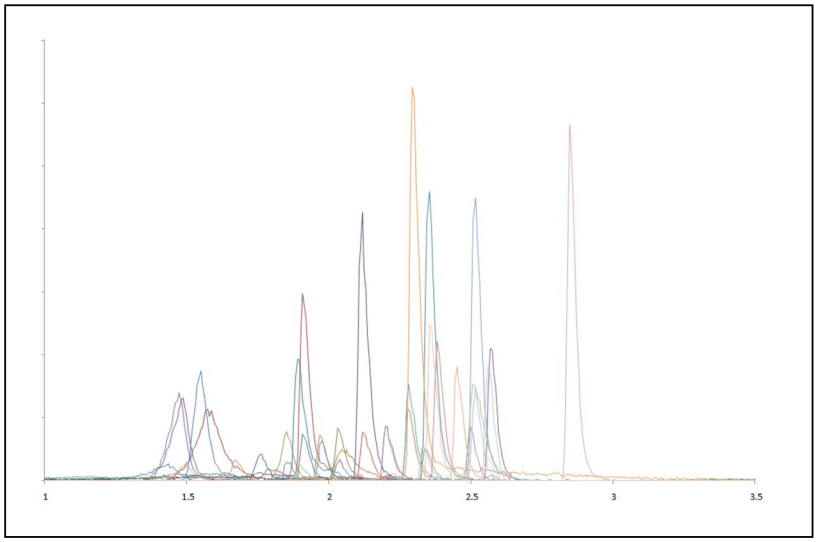

Figure 6. Extracted chromatograms demonstrate coelution of target and matrix compounds (only 20 traces compound shown)

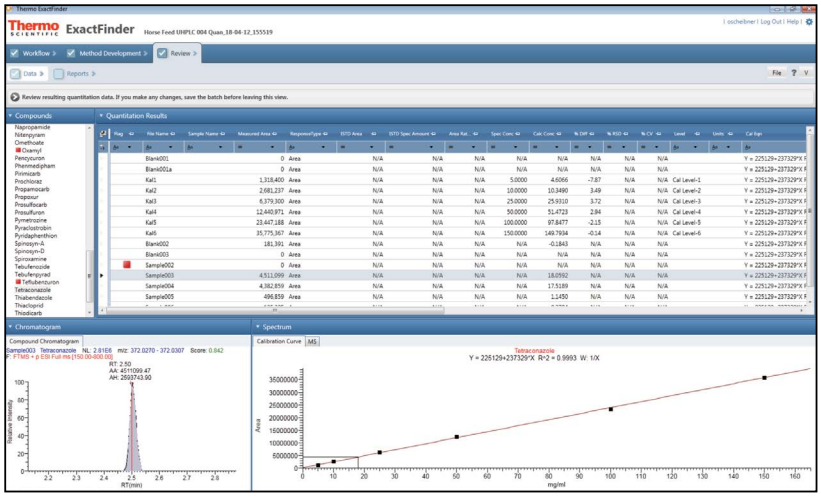

Figure 7. Example of quantitative results from one target compound (tetraconazole)

Qualitative Analysis

Qualitative analysis was carried out as a combination of targeted analysis and general unknown screening. In a first step, targeted analysis was carried out. In a second step, all peaks not identified in the targeted search were automatically forwarded for general unknown screening.

The same list of analytes used for quantitative analysis (Table 1) was applied for the targeted search. Retention time, isotopic pattern match, fragment search, and library search were used as confirmation criteria for targeted search. The fragment information for the analytes of interest and the fragmentation spectra for the library search were taken from databases included with the ExactFinder software. Even at the lower end of the concentration range, most components quantified could be easily confirmed on all four stages of confirmation (see Figure 8). With its built-in reporting capabilities, the ExactFinder software version 2.0 provided a quick, easy overview of the screening results.

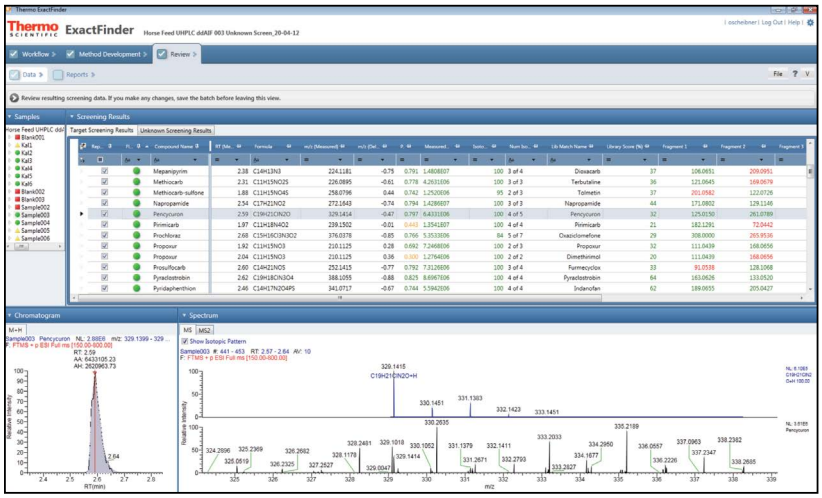

Figure 8. Qualitative results as displayed by the ExactFinder software

It quickly became clear that sufficient resolution was the key to successful full-scan quantitation and screening of complex samples like the ones analyzed in this work. As shown in Figure 9, most analyte signals were surrounded by numerous matrix signals. Only sufficient resolving power ensured proper separation of analyte and matrix signals. This applies to the monoisotopic signals used for analysis as well as for the isotopic signals used for confirmation. The peaks of interest showed a resolution of close to 60,000. It was apparent that significantly lower resolving power at these masses would have led to interference and merged signals, causing significant mass shifts. The mass shifts would have led to false negatives or would have required to widening of the extraction window. Widening the extraction window would have lowered the selectivity of the analysis and resulted in false positives.

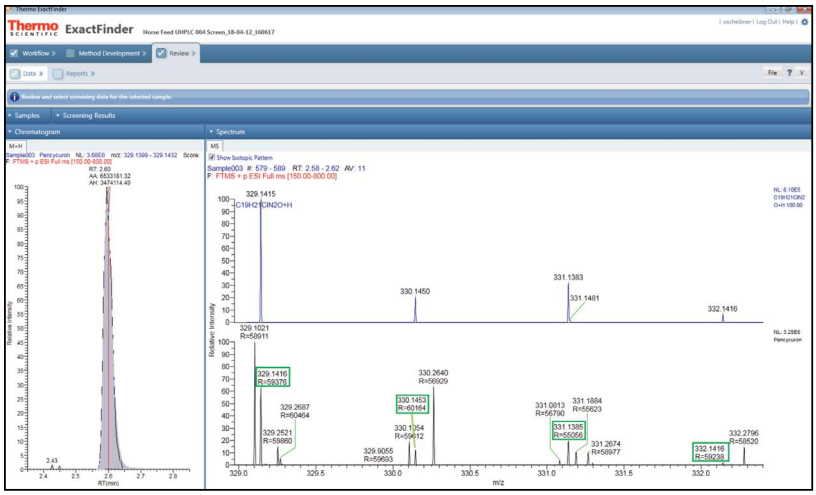

Figure 9. Isotopic pattern match of pencycuron. Green boxes mark the isotope signals surrounded by matrix signals

The general unknown screening carried out on the remaining peaks offers several options for automatic identification of the found peaks: database search, elemental composition determination based on isotopic pattern matching, spectral library search, and internet search. For the samples, roughly 15,000 components were detected; all of them went through the identification process. Database and spectral library searches were carried out using built-in resources. Internet search was carried out using a selection of databases listed in the ChemSpider® online search portal. Numerous additional contaminants could be identified, especially pesticides and a selection of aflatoxins (results not shown).

Conclusion

HR/AM analysis is a versatile method for residue analysis. It offers full quantitation capabilities in combination with unrestricted target and unknown screening options. Ultra-high resolution delivered by the Orbitrap mass analyzer in the Exactive Plus mass spectrometer provides reliability and selectivity comparable to established MS/MS techniques. The Exactive Plus mass spectrometer is compatible with UHPLC without compromising resolution or mass accuracy.

References

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Africa-Other +27 11 570 1840
Australia +61 3 9757 4300
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