

Comprehensive analysis of pesticides, herbicides, mycotoxins and other exogenous chemicals in foodstuffs using UHPLC / High-Resolution TOF MS

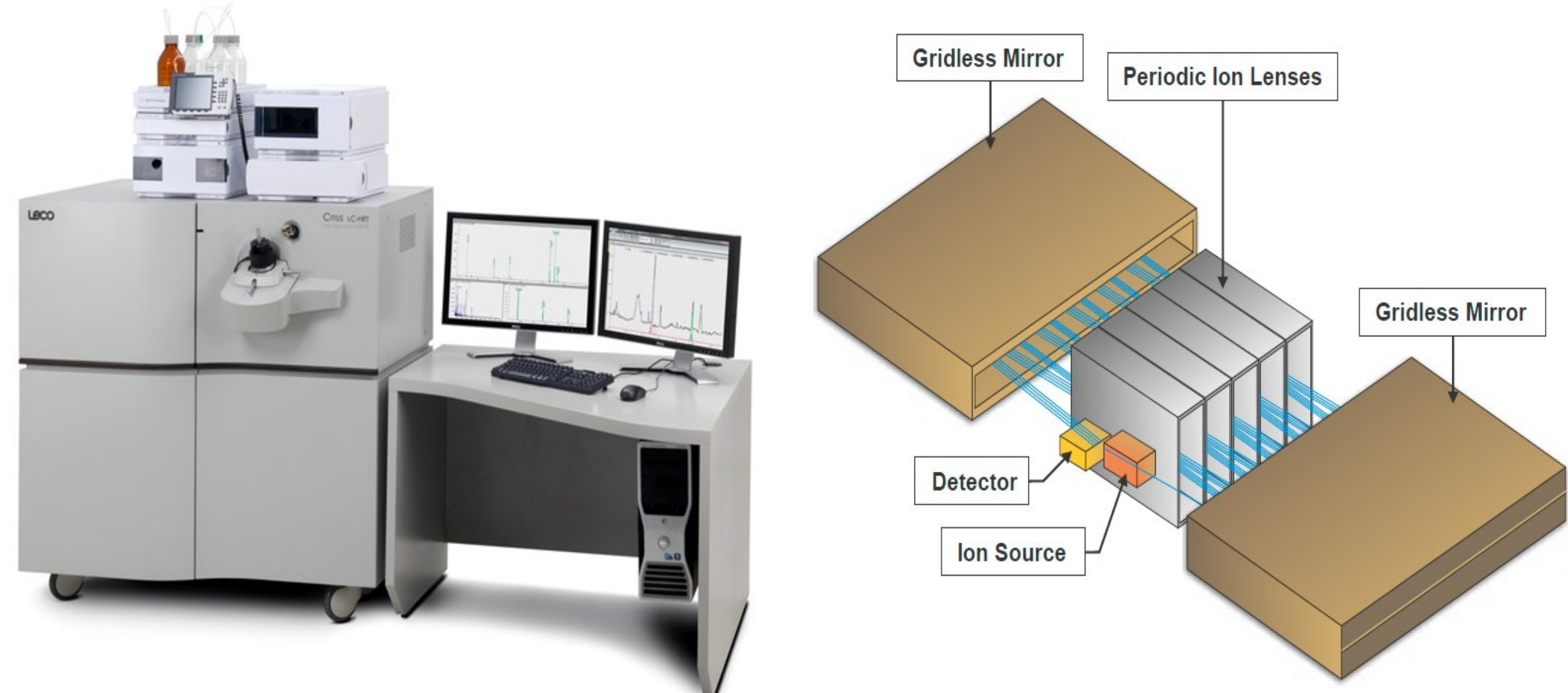
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Introduction

High resolution mass spectrometry has grown appreciably in use in the recent past with availability of new instrumentation being a significant contributor to that growth. Recognition of the value of accurate mass data, ultra high resolving power, and accurate isotope abundance in compound identification and formula determination has become ever more prominent. Simultaneously, the need for accurate global analysis of regulated compounds in complex matrices has created pressure on analytical chemists to apply better tools in the analysis of these compounds (i.e. environmental toxins). Accurate mass analysis and accurate relative isotope abundance, provided by a ultra high resolution TOF on a novel Folded Flight Path™ platform (Figure 1), serves as the framework for the development of a comprehensive method for the analysis of diverse compounds of environmental interest. The ability to simultaneously detect compounds of interest (targeted) and survey other possible contaminants offers substantial opportunity in environmental and food/safety efforts.

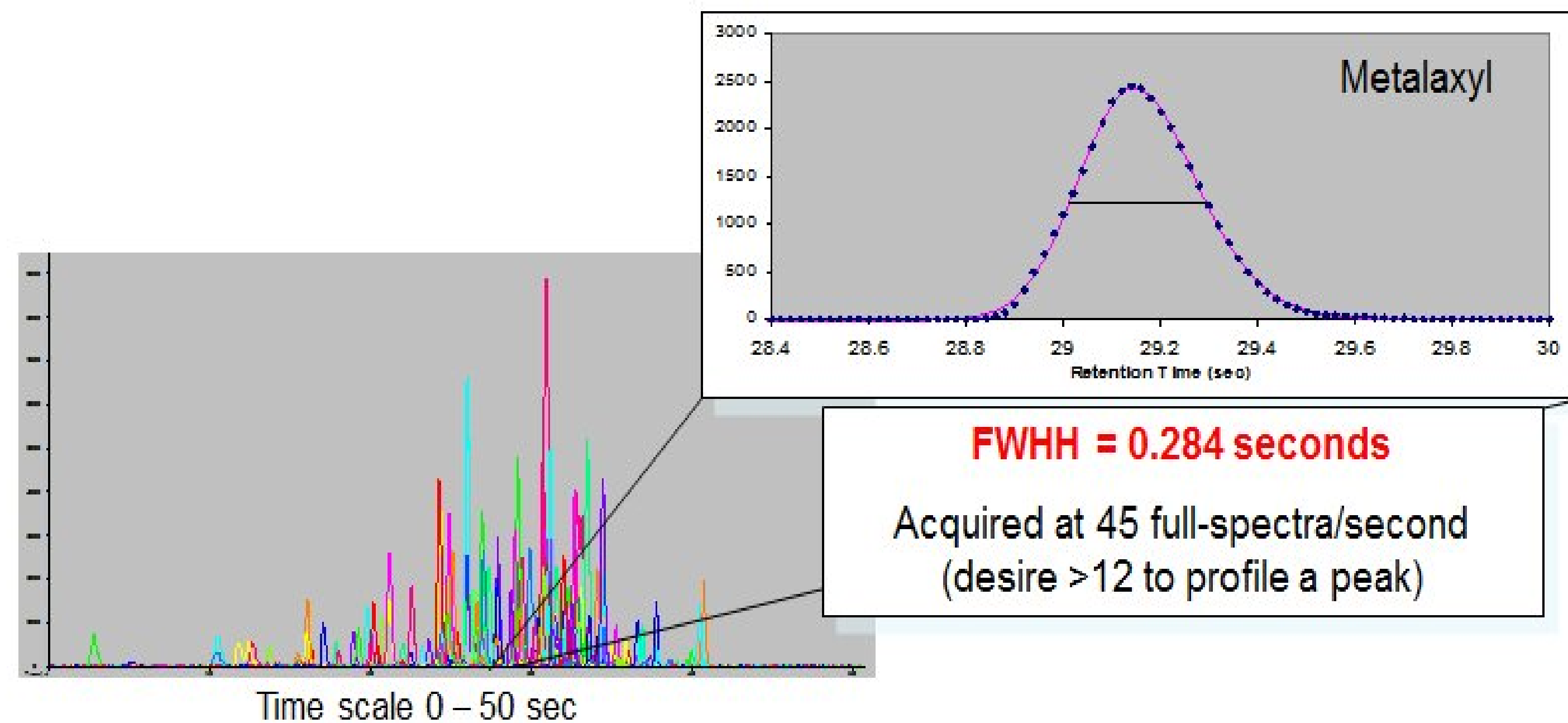
Figure 1: LECO CITIUS™ LC-HRT with Folded Flight Path™ Technology



Results

To improve throughput, the HPLC method was transferred to a UHPLC system using a high efficiency C18 1.9µm particle column. High pressure (1100 Bar) and temperature (80°C) were used to generate high mobile phase velocity, which yielded a reduction in analysis time of approximately 20-fold while providing comparable chromatographic resolution (Figure 5). The full-width, half-height measurements of the chromatographic peaks were on the order of 0.28 seconds requiring data acquisition rates of greater than 40 spectra/ second for adequate characterization. Full spectrum data collection at acquisition rates greater than 40 spectra/second (written to disk) can only be achieved with state-of-the-art MRT technology.

Figure 5: HRT Technology for Ultra-High Speed Analysis



The implementation of accurate mass analysis and relative isotope abundance permit the clear identification and separation of closely related single analytes. The analytes spiked into the various extracts included 15 analyte pairs which differ by less than 10 ppm. One such example is the Methabenzthiazuron/Carbofuran pair which, under the UHPLC conditions used, co-eluted. Owing to the high mass accuracy and deconvoluting power of the supporting software, each of these compounds was still detected in extracts at less than 3 ng/ml concentrations. Other pairs also existed and showed similar results which are attributable to the high performance of the mass spectrometer.

Sample Preparation & LC-HRT Method Parameters

Plant, vegetable and food extractions were performed by the method of Mol, et al (Anal. Chem. 80 (2008), p 9450). These extracts were spiked at various levels with a mixture of 210 compounds, covering the range of 0.3 through 300 ng/mL. Of the 210 compounds (pesticides, mycotoxins and other exogenous chemicals) 181 were amenable to analysis by positive mode electrospray ionization. Samples were diluted and analyzed by UHPLC system interfaced to an high-resolution time-of-flight mass spectrometer.

LC: Agilent: 1290 Infinity

Injection Volume: 1-5 µL
Mobile phase A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

Mass Spectrometer: LECO CITIUS™ LC-HRT

Ion Source: LECO Electrospray Source
Polarity: Positive
m/z Calibration: 10 s co-infusion of Agilent tune mix

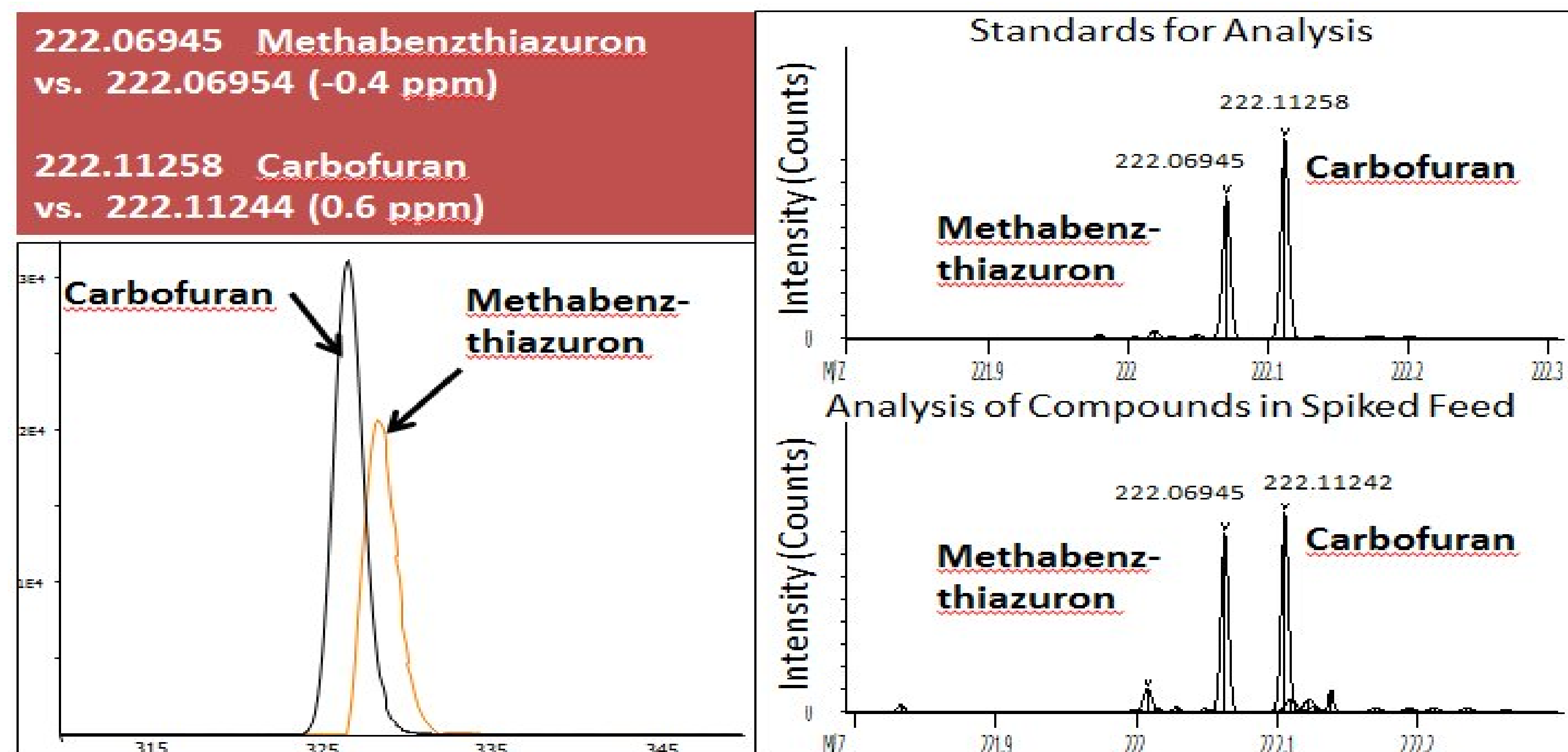
10 min Analysis

Column: 1.8µm HSS T3 100 mm x 1.0 mm ID
Gradient: 2.5 – 97.5% B
Flow: 0.180 mL/min
Column Temperature: 40°C
Acquisition Mode: High Res (50,000 FWHM)
Spectral Acquisition: 2.5 spectra / sec
m/z Range: 50 - 2250

3 min Analysis

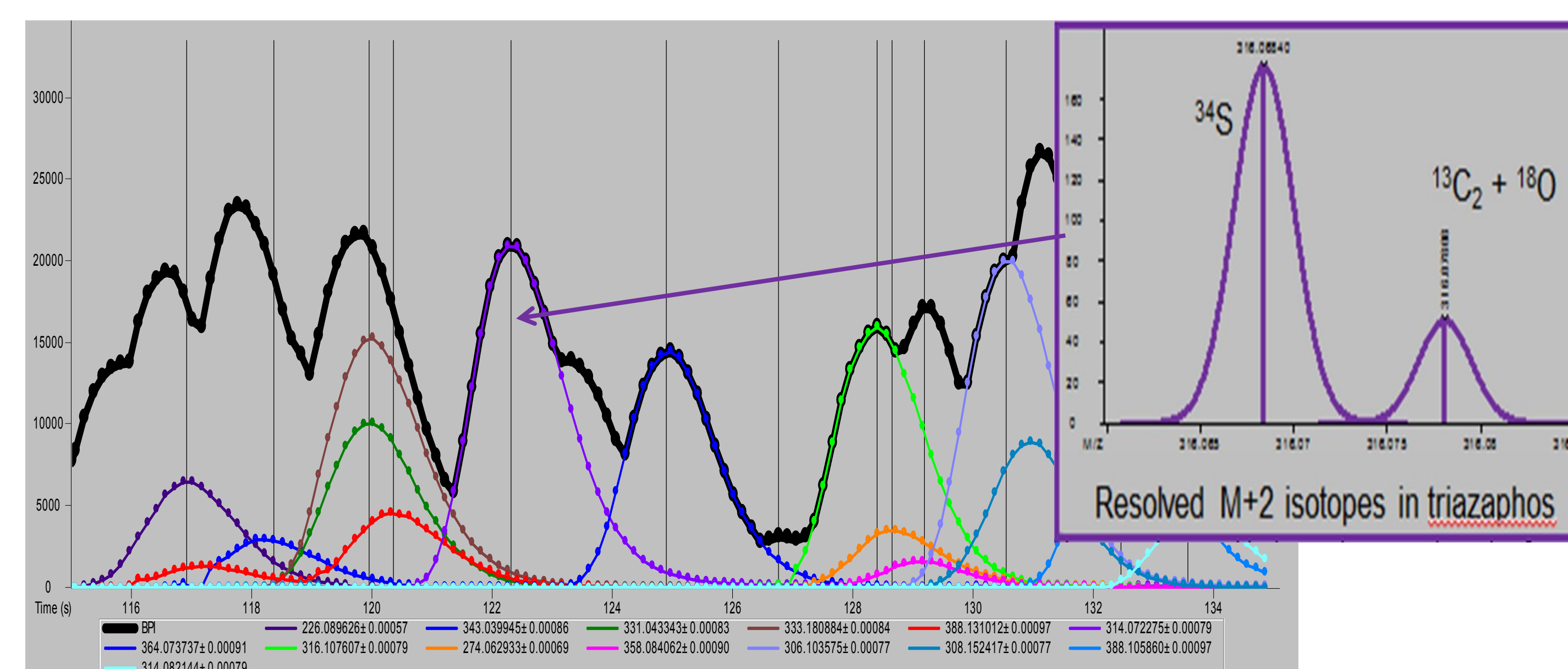
Column: 1.9µm Hypersil Phenyl 50 mm x 1.0 mm ID
Gradient: 2.5 – 97.5% B
Flow: 0.250 mL/min
Column Temperature: 80°C
Acquisition Mode: Ultra-High Res (1000,000 FWHM)
Spectral Acquisition: 5 - 150 spectra / sec
m/z Range: 150 - 600

Figure 6: Isobaric Chemicals in Animal Feed



Ultra-high resolution allows the deconvolution software to reject peaks without characteristic isotopic signatures, as shown for triazaphos (Figure 7). Xenobiotic substances containing S, Si, or Cl are shown in color below with XIC ± 2.5 ppm.

Figure 7: HRT Technology for Element –Selective Detection



Results

Accurate mass analysis has provided a powerful tool for the trace analysis of compounds in complex matrices. High resolution MS provided the means to selectively detect and quantify a broad range of analytes in a sensitive and selective fashion. At 10 pg/µL, all pesticides amenable to ionization were detected in a green tea sample. At 1 pg/µL, greater than 88% of the compounds were at sufficient signal-to-noise for detection. Figure 2 shows the extracted ion chromatogram of a pesticide standard mixture (1 pg/µL), spiked into a green tea extract. Retention time variability was less than 1 second for each sample set. These retention time measurements were used to aid in the identification of residual pesticides in complex matrix samples.

Figure 2: Extracted Ion Chromatograms of a Pesticide Standard (1pg/ul) in green Tea

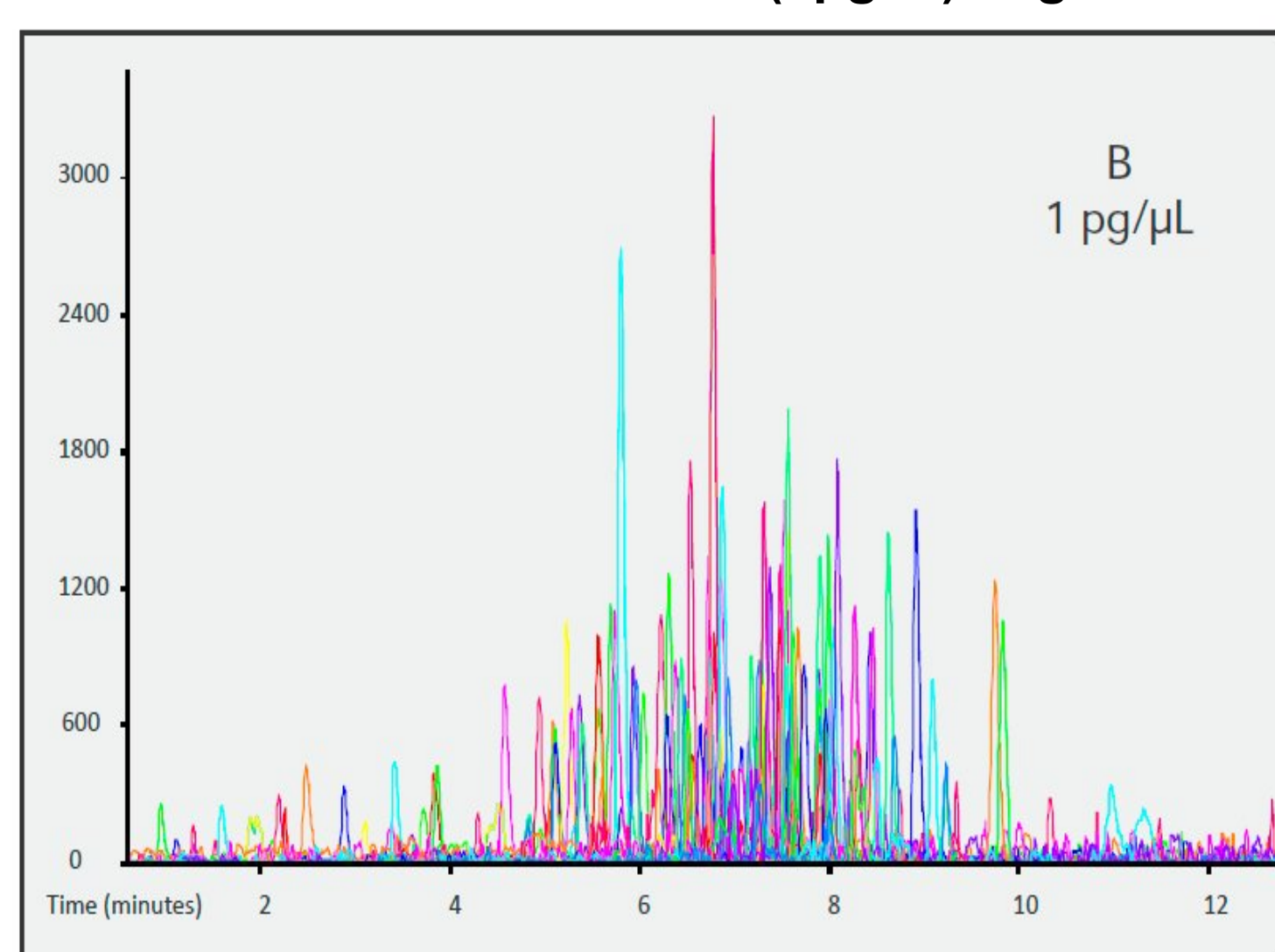


Figure 3: Mass Accuracy Distribution of 362 accurate mass measurements



The mass accuracy distribution as a function of chromatographic peak height is presented in figure 3. As expected, variability in accurate mass measurements increases with decreasing ion intensity. However, even at these very low concentrations, 80% of the 362 accurate mass measurements fall within +/- 1 ppm and 90% of the measurements fall within +/- 1.5 ppm. Also, it should be noted that the distribution is perfectly symmetric about the origin, indicating the absence of any systematic error dependence on intensity. This distribution was used to set accurate mass limits on formula generation.

Conclusions

The analysis of trace level analytes in complex matrices has been investigated using high performance time-of-flight mass spectrometry. Speed of acquisition and high performance capabilities have led to the detection and confident identification of over 200 analytes in complex food-based matrices including tomato, feed grain and egg. Analysis times below 3 minutes were achieved with no compromise to the quality of the analytical data. The analytical utility is demonstrated by selectively detecting compounds based on their isotopic signature. Resolution and mass accuracy also lend to a more robust deconvolution.

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