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Triumphs and Challenges of High-Resolution Mass Spectrometry in Comprehensive Pesticide Residue Screens

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Introduction

To continually expand analytical capabilities and allow for post-acquisition data mining food safety, laboratories can no longer rely solely on triple-quadrupole instruments for rapid, single-injection, easy-to-manage analyses. The Florida Department of Agriculture and Consumer Services pesticide regulatory program now has several single-stage high resolution mass spectrometers (ST-HRMS), which have a mass resolving power of ~100,000 and continually scan over a large mass-to-charge range. ST-HRMS allows for the mass resolution of commonly applied pesticides with similar masses and chromatographic retention times, a scenario that arises frequently in pesticide analysis. After a year of analyzing approximately 800 samples for 226 pesticides using high resolution mass spectrometry (HRMS) and systematically comparing the results to those acquired using a leading triple quadrupole instrument, the data indicates that ST-HRMS is valid analytical technique when analyzing a large number of compounds simultaneously in complex food matrices. This approach has the potential for transformational improvements in analytical capabilities by allowing a broad scope, single-injection analysis to be paired with a broad scope, analytical extraction for more efficient analyses.

Pesticide Residue Screen Challenges

The goal of the routine analysis of pesticides at residue levels is to identify and quantify as many pesticides as possible in a quick, reliable, and simple way. One way to accomplish this task is through the use of HRMS. However when incorporating HRMS technology into the laboratory, challenges can be encountered, from the complexity and variety of sample matrices, to the mass or structural similarities of the compounds involved.

One of the easiest and most cost-effective opportunities for improvement in analyses can be realized by simply switching from traditional HPLC to ultra-high performance liquid chromatography (UHPLC). UHPLC can provide chromatographic resolution of compounds with the same mass, or compounds that are structurally similar as illustrated in **Figure 1**. In this example, propazine and sebuthylazine both have the same exact mass, but can be chromatographically resolved with UHPLC (left pane of Figure 1), as can the structurally similar Dimethomorph E and Z isomers (right pane of Figure 1). Chromatographic resolution is also useful to distinguish secbumeton from terbumeton, as shown in **Figures 2 and 3**, respectively. In this example, secbumeton has a retention time of 8.23 minutes and has product ions corresponding to masses 170.1033 and 142.0723. Terbumeton also has product ions corresponding to masses 170.1033 and 142.0723 as a result of a higher energy collisional dissociation (HCD), however at a retention time of 8.32 minutes is easily

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Figure 1: UHPLC Provides Chromatographic Resolution for Compounds with Identical Exact Masses.

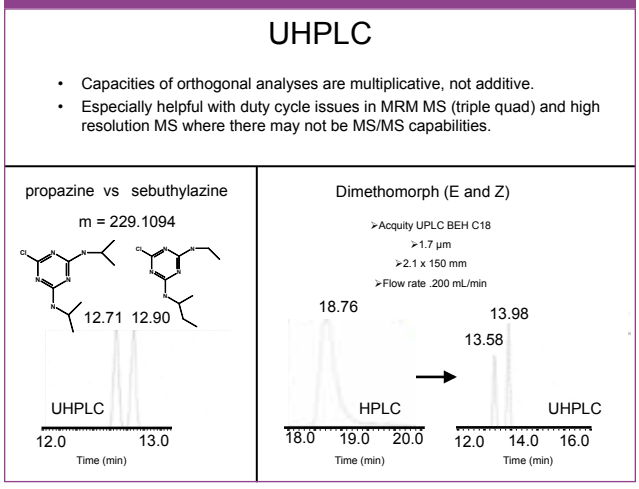
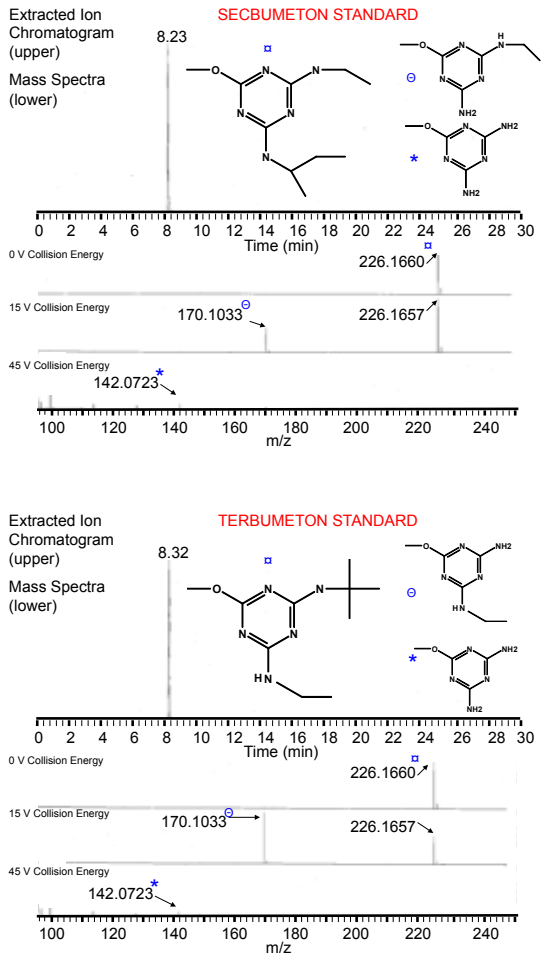


Figure 2-3: Orthogonal Chromatographic Analysis Aids in Identification of Compounds with the same Exact Mass.



distinguishable from secbumeton. Examples such as these demonstrate how an orthogonal chromatographic analysis can be a valuable tool when used in combination with using single stage high-resolution mass spectrometry.

ST-HRMS versus Triple Quadrupole MS

In a triple quadrupole mass spectrometer, ion isolation occurs in the first quadrupole; fragmentation occurs in a slightly elevated pressure region in the second quadrupole, and the product ions are scanned or measured in the third quadrupole. This type of MS only looks for what it's instructed to look for; consequently only product ions of known masses will be observed. In comparison, ST-HRMS instruments scan across a wide mass to charge ratio constantly, and the product ions that are generated in the HCD cell correspond very closely to the product ions that are generated in the quadrupole instrument because the same type of collisional activation occurs. So when using ST-HRMS, often the traditional product ions commonly observed using triple quadrupole MS can also be found.

Another fundamental difference between these two types of mass spectrometers is the duty cycle. With a triple quadrupole instrument, analysis occurs sequentially, but ST-HRMS can scan across a large mass to charge ratio, with a reliable scan speed. While some ST-HRMS instruments might scan slower than a triple quadrupole, because the analysis is done for all the ions in the cell, a slower scan rate that is dependable can be considered more than adequate.

One advantage that ST-HRMS instruments offer is the ability to mine data post-acquisition. If a contaminant is found in any sample that has been analyzed by ST-HRMS, data can be retroactively interrogated to determine if that contaminant was present in the past, and when it first appeared in a food matrix.

Approaches to Mitigate Potential Challenges to ST-HRMS Analyses

There are several options that can be used to mitigate potential challenges when using ST-HRMS technology in the laboratory. One challenge is related to the complexity of the method, and how easily it can be maintained and new staff trained due to turnover. In one application, The Florida Department of Agriculture Laboratory has a 226 pesticide residue screen, requiring about 450 entries into the triple quadrupole method. Taking into account additional fields for each method entry, over 3,000 pieces of data have to be entered into the method. While triple quadrupole MS has been the gold standard in this type of work for a long time, providing excellent confirmation and quantitation, the amount of upkeep that is required for a triple quadrupole method to screen for 200–500 compounds or more can be daunting. It can be done, but the scientist maintaining a triple quad instrument with 500 pesticides is usually somebody with considerable experience who understands how duty cycle must be managed, and also understands how to check for chromatographic drift and manage retention time windows

for accuracy. Using ST-HRMS however, as the methods analyte list becomes increasingly long, instrument setup, and consequently the method, can be much simpler, as long as scan rate and duty cycle are taken into consideration. For example, using the newest triple quadrupole instruments, 60 transitions require a 25-millisecond dwell time. Assuming the instrument takes about eight milliseconds to reset, it will take about two seconds to get a scan, and the longer the scan rate, resulting in a fewer number of data points collected across the peak in a chromatogram. Since instrument software will automatically recalculate dwell times and cycle times based upon how many analytes are being analyzed at any given time, scan times can increase to the detriment of peak identification and quantification, because fewer data points will be collected across the peak. The consequences of fewer data points being collected across a peak are shown in **Figure 4**. As the number of cycles or the scan rate decreases, the peak is not adequately sampled leading to peak distortion when the red dots representing sampling points in Figure 4 are connected. The distortion can lead to a miss calculation of peak area, and provide a source of data ambiguity. When these kinds of failures occur, they're difficult to solve and require a lot of expertise to manage. Sometimes a rugged and adequate experimental method that avoids high maintenance acquisition methods is more desirable. Using the continuous acquisition of ST-HRMS, full-scan data allows retrospective data analysis, vastly simplifying MS method development. Fast scanning, fully compatible with narrow UHPLC peaks, and fast polarity switching can further streamline method development and increase sample throughput. All-ions fragmentation (AIF) with multiple dissociation techniques—in source collision-induced dissociation (CID) and higher energy collisional dissociation (HCD)—adds an additional dimension of information in ST-HRMS instruments.

Another challenge is to elucidate relevant fragments and to use those fragments, which have been used historically, or fragments who's structure may be unknown. This challenge is illustrated by the analysis of prometon and turbumeton; at retention times of 8.21 minutes and 8.32 minutes respectively, they have the same exact mass of 226.1660, but have product ions that have been historically observed. As shown in **Figure 5**, the two isopropyl moieties from the nitrogen's contained within prometon give this analyte the ability to lose a three-carbon moiety and form a product ion at mass 184.1190. Whereas as seen in **Figure 6**, terbumeton does not have a pathway to get to the same product ion mass as prometon because it has an ethyl group and an isobutyl group, and fragmentation of the isobutyl group leads to a product ion mass of 170.1033. So even though the two compounds have the same exact masses, the characteristic product ions for these analytes can be used to identify the precursor ions based upon their structural relevancy. This identification is possible since the product ions are formed in the same way in single-

Figure 4: Effect of Scan Rate on the Number of Data Points Collected Across a Chromatographic Peak.

transitions	dwell time (ms)	reset time (ms)	sec/scan
60	25	8	2
45	25	8	1.5
30	25	8	1
15	25	8	0.5

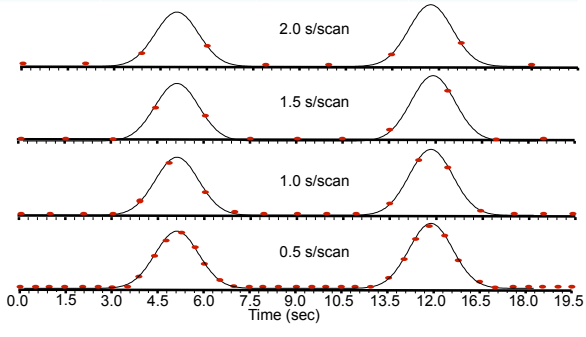
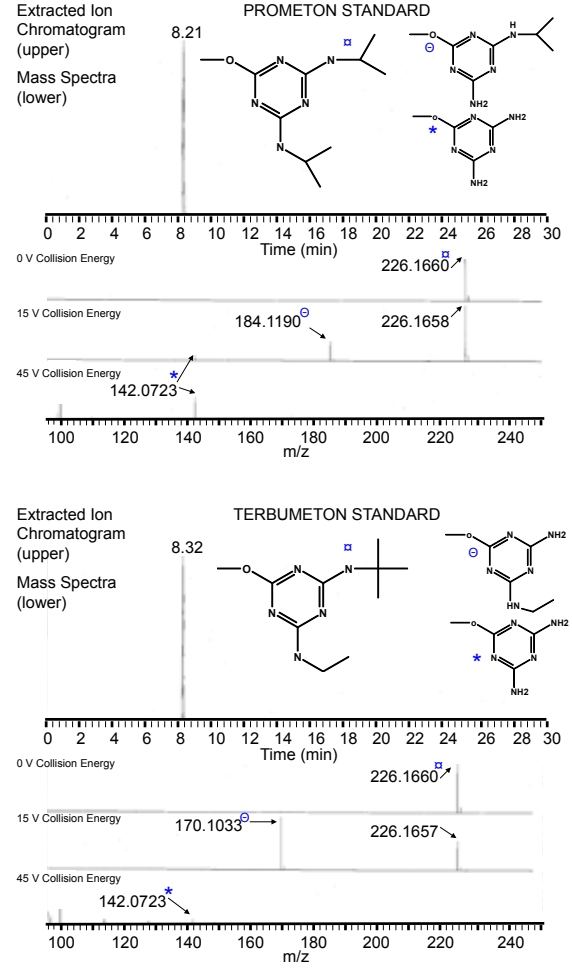


Figure 5-6: Identification of Compounds with Same Exact Mass, but Different Product Ions.



The Future: Incorporating ST-HRMS Into Everyday Work

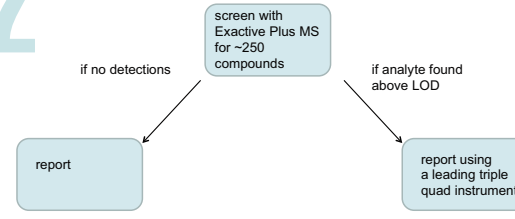
1 our *initial strategy and flow

Quantify and confirm (~ 120 compounds) using a leading triple quad instrument

identification and quantitation using ms/ms, ion ratios and a 6 point calibration curve

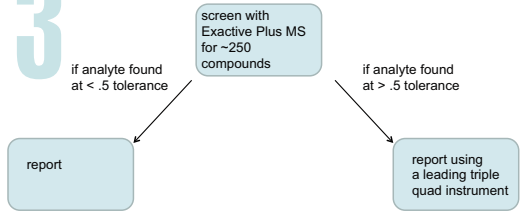
* This inital strategy in play at the FDACS. There was a base Exactive upon which the expanded pesticide screen was validated.
Now FDACS has two Exactive Plus instruments in the pesticide program.

2 our initial strategy and flow with Orbitrap MS



identification and quantitation using ms/ms, ion ratios and a 6 point calibration curve

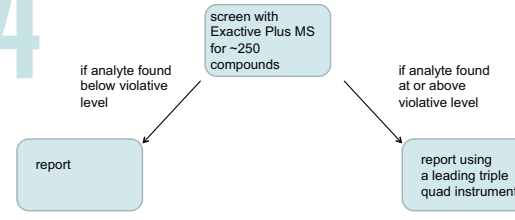
3 our next strategy and flow



estimate and characterization- 1 point calibration standard, and characteristic fragments for confirmation

identification and quantitation using ms/ms, ion ratios and a 6 point calibration curve

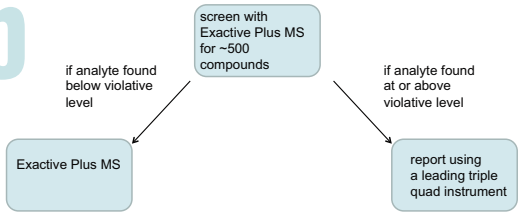
4 our current strategy and flow



estimate and characterization- 1 point calibration standard, and characteristic fragments for confirmation

identification and quantitation using ms/ms, ion ratios and a 6 point calibration curve

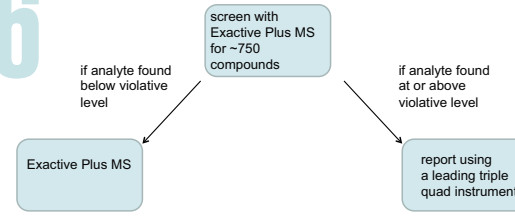
5 our (very near) future strategy and flow



quantitation and characterization- 6 point calibration curve, and characteristic fragments for confirmation

identification and quantitation using ms/ms, ion ratios and a 6 point calibration curve

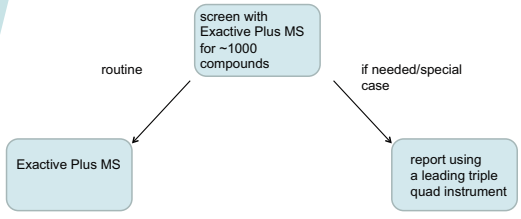
6 our future strategy and flow



quantitation and characterization- 6 point calibration curve, and characteristic fragments for confirmation

identification and quantitation using ms/ms, ion ratios and a 6 point calibration curve

7 our ideal strategy and flow



quantitation and characterization- 6 point calibration curve, and characteristic fragments for confirmation

identification and quantitation using ms/ms, ion ratios and a 6 point calibration curve

stage ST-HRMS instruments as they are in traditional triple quadrupole instruments.

Sometimes the structure of the product ion may be unknown. For the analysis of azoxystrobin, product ion masses at 372 and 344 are typically measured. The structures of these product ions are currently unknown, but can be found in fragmentation scans in a ST-HRMS instrument. Since the product ions are generated in the same way with the same mechanism compared to a triple quadrupole (collision into a neutral gas), historical data can be used to confirm The ST-HRMS instrument results. By extracting the ions and verifying the timeline, a high degree of confidence in the assignment can be obtained, even without true MS/MS capability.

Eventually, the objective is to continue to increase our capability to a >1,000 pesticide residue-level screen and report everything found from those analyses via ST-HRMS.

The Future: Incorporating ST-HRMS Into Everyday Work

Historically at the Department of Agriculture, all the residue work has been performed on triple quadrupole instruments, and ST-HRMS was recently introduced very methodically as part of a vetting process of the new technology. Initially, the approach was to use ST-HRMS to screen samples, and if nothing was observed, that's how it was reported, and if a response for any analyte or pesticide above the method's LOD was observed, it was re-measured on a triple quadrupole instrument for verification. During this process, however, it was noticed that there was good agreement between the ST-HRMS and triple quadrupole instruments, and the threshold for reporting with the ST-HRMS instrument became half of the tolerance. With this approach, if a response was observed above the LOD by ST-HRMS, it wasn't necessary to rerun it on the triple quadrupole. In other words, if the response is above the LOD, but it's below half of the tolerance for that analyte in that matrix, or below violative levels, the ST-HRMS result will be reported. If an analyte is found at or above a violative level by ST-HRMS, the matrix is re-extracted and the analysis is repeated on the triple quadrupole. If the results for both instruments are violative, then the sample is confirmed and reported as violative. This systematic evaluation of the two platforms has allowed a careful implementation strategy, knowing that there was good agreement in quantitation and in identification between the two platforms provided additional confidence in the results. To date, of about 800 samples screened via ST-HRMS for about 226 pesticides per sample, no false positives were reported, and the quantitated levels were in high agreement lending confidence that the approach satisfies regulatory requirements.

Ultimately, the goal for the future will be to expand to over 500 analytes, validate and implement the methods, and continue to vet new technology similar to our current process. Eventually, the objective is to continue to increase our capability to a > 1,000-pesticide residue-level screen and report everything found from those analyses via ST-HRMS. In this future approach, triple quadrupole mass spectrometers will be used only in special cases, as needed, for a second opinion, or to verify any controversial assignments.