Improved Screening for 250 Pesticides in Matrix using a LC-Triple Quadrupole Mass Spectrometer

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Overview

Purpose: Demonstration of the effect of a drag field in Q2 on signal intensity at short dwell times.

Methods: Pesticides were analyzed at various dwell times and in the presence of matrix on a Thermo Scientific™ TSQ Quantiva™ triple quadrupole mass spectrometer.

Results: We demonstrate effectiveness of a Q2 drag field in maintaining signal intensity at extremely low dwell times enabling data acquisition rates of 500 SRMs/sec which facilitate the monitoring of 250 pesticides simultaneously in an onion matrix.

Introduction

Both food safety and environmental regulatory requirements are demanding greater sensitivity on an increasing number of analytes. In addition, there are an increasing number of matrices to be evaluated. To meet these requirements, it is necessary to analyze a large number of analytes quickly at low levels. Triple quadrupole mass spectrometers are the industry standard for fast and reliable analysis achieved through the selected reaction monitoring experiment (SRM). This experiment is extremely efficient however, it too is limited by intrascan delays and dwell times required to get the maximum sensitivity and reproducibility. The experiments described here utilize a triple quadrupole MS equipped with a new Q2 collision cell enabling the rapid analysis of 250 pesticides in a screening application.

Methods

Liquid Chromatography

Separations were performed using a Thermo Scientific™ Dionex™ UltiMate™ 3000 XRS LC system with a Thermo Scientific™ Hypersil™ Gold aQ, 100x2.1mm ID, 1.9µm particle HPLC Column. The flow rate was 300 µL/min using the following chromatographic gradient:

Mobile Phase: A: 0.1% Formic Acid + 5 mM Ammonium Formate (aq)
B: Methanol + 0.1% Formic Acid + 5 mM Ammonium Formate

Gradient:

Time (min)	%B
0.00	2
0.25	30
35.00	100
40.00	100
40.01	2
45.00	2

Mass Spectrometer Method

Samples were analyzed using a TSQ Quantiva triple-quadrupole mass spectrometer in SRM mode. Ionization was performed using the new Ion Max NG ion source operated in heated electrospray ionization mode H(ESI), shown in Figure 1, with a vaporizer temperature of 425 °C and a capillary temperature of 350 °C. Sheath and auxiliary gas flows were 45 and 10 (arbitrary units) respectively with an ionization voltage of 3000 volts in both positive ion mode. Collision gas pressure was set at 1.5 mTorr throughout the experiments. Preliminary data was acquired for the mid-level standard (10 ppb) at dwell times of 1, 2, 5, 20, and 100 MS to assess the effect of dwell time on peak area; all subsequent data was acquired with a dwell time of 1 msecond with a total interscan delay of 1 msecond.

FIGURE 1. Ion Max NG source displaying drain extension for efficient removal of solvent and matrix reducing background, thereby improving sensitivity and overall system robustness in the presence of dirty matrices such as the onion matrix used in this study.

Results

Dwell Time Assessment

It is well known that in the absence of a drag field in Q2, decreasing dwell times result in a decrease in signal intensity, thus affecting both sensitivity and reproducibility, especially at low concentration levels of analytes. In the presence of the drag field, signal can be minimized even at dwell times as low as1 msec. The TSQ Quantiva MS creates a drag field by applying an axial DC potential to the 90° Q2 as shown in Figure 2. This is referred to as the "active collision cell". To assess the effect of the drag field, studies were performed 100, 20 ,5, 2, and 1 msec dwell times using the pesticide azoxystrobin. These studies assess the effect of the drag field and differing dwell times on signal intensity and reproducibility. It is clear from the results shown in Figure 3, that even at dwell times as low as 1 msec, there is no drop in signal and the reproducibility across all dwell times is only 3.14 % RSD.

The capability to reduce dwell times enables us to monitor more transitions per unit time thus, reducing HPLC run times, enabling the utilization of UPLC technology, or monitor numerous compounds at a single time.

FIGURE 2. Q2 from the TSQ Quantiva MS. Fragment ions are accelerated through the cell with the application of an axial DC potential, enabling data acquisition at dwell times as low as 1msec without signal loss. This facilitates the ability to acquire in excess of 500 SRMs/second.

FIGURE 3. Dwell time study on azoxystrobin. 10 ppb Azoxystrobin was analyzed at 100, 20, 5, 2, 1 msec dwell times. The %RSD for all dwell times combined was 3.14%.

	Sample Name	Area	ISTD Area	%RSD-RESP	RT	Exclude
1	Azoxy 1msec dwell	10459151	NA	3.14	2.51	
2	Azoxy 1msec dwell	10379082	NA	3.14	2.51	
3	Azoxy 1msec dwell	10494645	NA	3.14	2.50	
4	Azoxy 1msec dwell	10659557	NA	3.14	2.50	
5	Azoxy 1msec dwell	10648577	NA	3.14	2.51	
6	Azoxy 2msec dwell	11353046	NA	3.14	2.51	
7	Azoxy 2msec dwell	11636029	NA	3.14	2.50	
8	Azoxy 2msec dwell	11414801	NA	3.14	2.51	
9	Azoxy 2msec dwell	11415707	NA	3.14	2.51	
10	Azoxy 2msec dwell	11365536	NA	3.14	2.50	
11	Azoxy 5msec dwell	11204929	NA	3.14	2.51	
12	Azoxy 5msec dwell	11119550	NA	3.14	2.50	
13	Azoxy 5msec dwell	11124237	NA	3.14	2.50	
14	Azoxy 5msec dwell	11202701	NA	3.14	2.51	
15	Azoxy 20msec dwe	11032421	NA	3.14	2.50	
16	Azoxy 20msec dwe	10997641	NA	3.14	2.51	
17	Azoxy 20msec dwe	11506645	NA	3.14	2.50	
18	Azoxy 20msec dwe	11170524	NA	3.14	2.50	
19	Azoxy 20msec dwe	11294461	NA	3.14	2.51	
20	Azoxy 100msec dw	11141895	NA	3.14	2.51	
21	Azoxy 100msec dw	11127692	NA	3.14	2.51	
22	Azoxy 100msec dw	11138545	NA	3.14	2.51	
23	Azoxy 100msec dw	11423106	NA	3.14	2.51	

500 SRMs/second

After demonstrating no signal loss while monitoring a single transition at dwell times ranging from 1msecond to 100mseconds additional experiments were performed to assess the effect of monitoring 250 transitions per 0.5 seconds (rate equivalent to 500 SRMs/second. Two transitions (*m/z* 192→132,160) for pesticide carbendazim were acquired simultaneously with an additional 248 transitions for other common pesticide at a cycle time of 1.5 seconds (167 SRMs/second) and 0.5 seconds (500 SRM/sec.) . The results are shown in Figure 4 Panel A. The power of acquiring 500 SRM/sec. is clearly demonstrated in the increased number of data points across the peak shown in Panel A of Figure 4. Without the increase in data points it would not be possible to reproducibly integrate the peak and reproducibility would be impacted. However, as previously discussed, it is not typically possible to acquire data at these rates (corresponding to 1 msec. dwell times) without signal loss. Here we again demonstrate that even at 500 SRM/sec. (1 msec. dwell), in the presence of numerous additional transitions, we maintain the peak area, data shown in Figure 4, Panel B. Thus given the results we have demonstrated thus far we can monitor numerous compounds simultaneously with no signal loss and excellent reproducibility.

Our next experiment was to demonstrate the acquisition of 250 pesticides with two transitions each at a rate of 500 SRM/sec., this equates to a 0.5 second cycle time (1 msec. dwell), at 1-100 ppb.

FIGURE 4. Carbendazim acquired at the rate of 167 SRM/sec. and 500 SRM/sec. Panel A depicts the number data points across the peak at both acquisition rates. Panel B depicts the area response for the carbendazim transitions in the presence of 248 additional transitions at various dwell times.

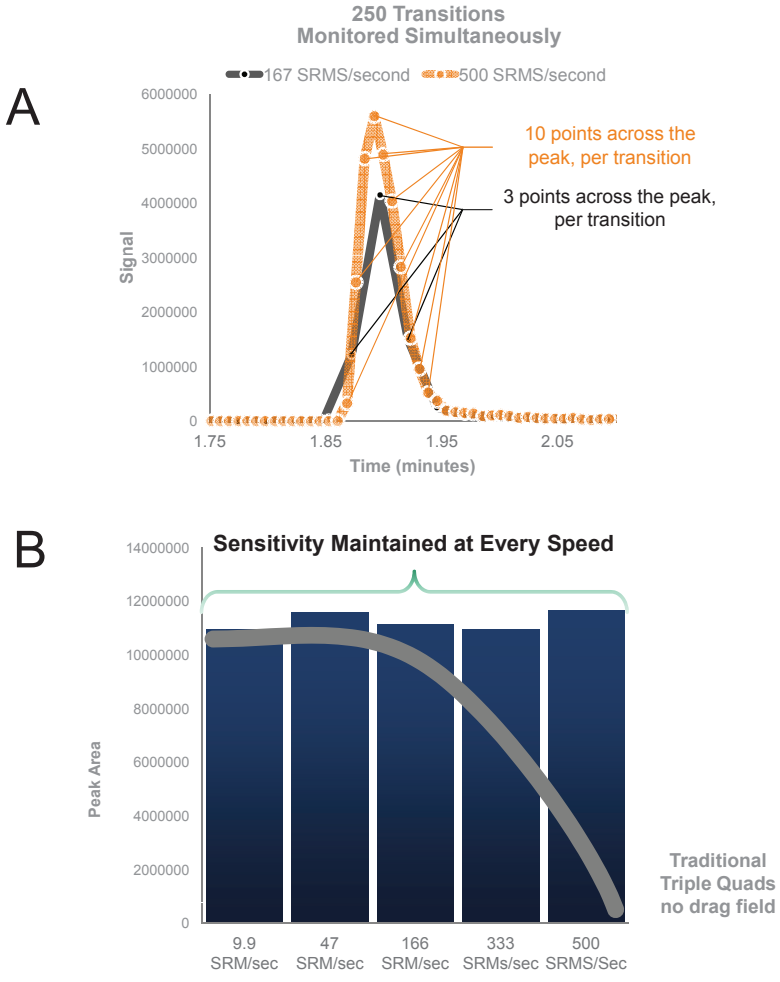


FIGURE 5. 1 ppb level of 250 pesticides (500 transitions) in onion matrix, acquired at a rate of 500SRM/sec. Panel A depicts the chromatograms for all 250 pesticides; Panel B depicts the peaks for 5 randomly chosen pesticides- displaying the number of data points across the peak and %RSD.

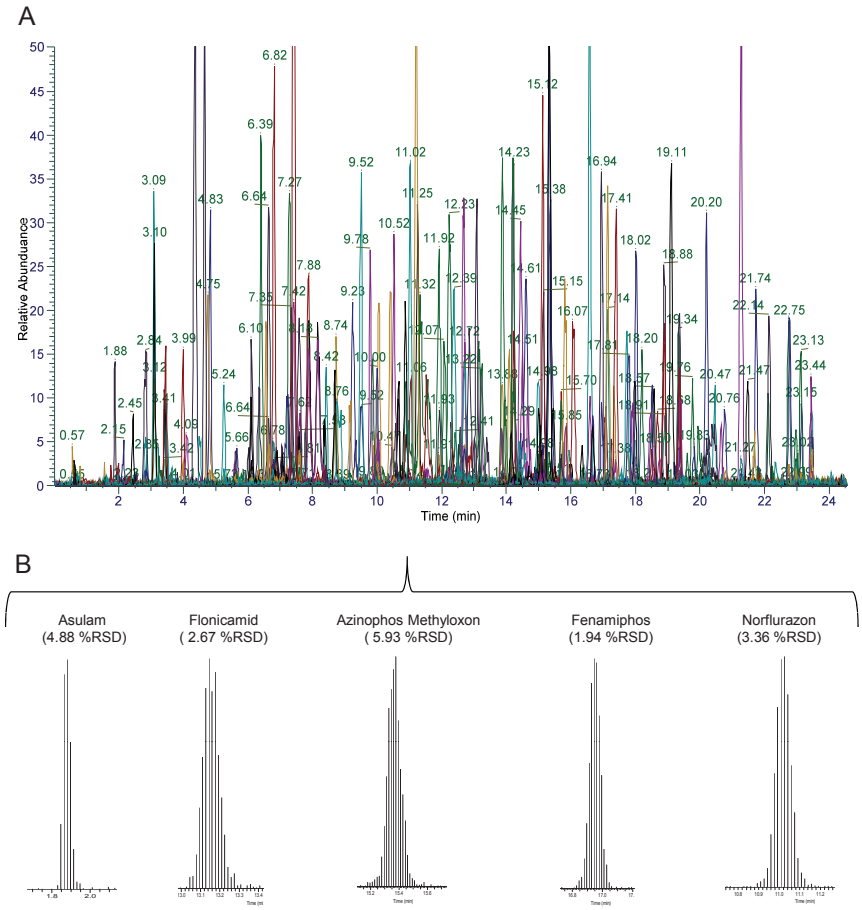
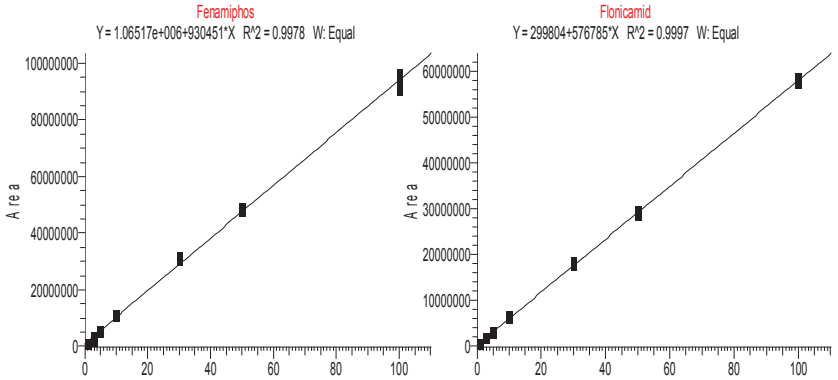


FIGURE 6. Calibration curves for fenamiphos and norflurazon, two of the compounds shown in Figure 5 above. Triplicate injections were made at each level; Note the excellent precision and linearity.



500 SRM/sec. in Onion Matrix

Having demonstrated the ability to, and the benefit of acquiring data at rates of 500 SRM/ sec., we now demonstrated the application of this method to 250 pesticides spiked into an onion matrix. Pesticides were spiked into an onion matrix at levels of 1 ppb through 100 ppb.

A representative chromatogram for a 1 ppb sample is shown in the Figure 5A. Figure 5B depicts the peaks for 5 randomly chosen pesticides, the number of data points acquired across the peak and the %RSD for triplicate injections.

Figure 6 contains the calibration curves for 2 of the 5 previously mentioned compounds. The data show that even in the presence of matrix, we are able to easily detect the compounds with excellent %RSDs, even at the 1 ppb level, while maintaining superb linearity.

The ability to acquire data for numerous compounds without specifying specific retention time is particularly helpful when running samples in matrix. This ability eliminates the need to adjust retention times that can change due to matrix effects on chromatographic conditions, thus simplifying the analysis of pesticides in food matrices.

Conclusion

- Q2 drag field enables 1 msec. SRM acquisitions without signal loss
- 1 msec. dwell times, with no signal loss, facilitates data acquisition rates of 500 SRM/sec.
- 500 SRM/sec. data acquisition rates allow us to
    - Reduce HPLC run times, enable the utilization of UPLC technology, or monitor numerous compounds in a single run.
    - Greatly simplify method setup by removing the need to set up specific time windows for compounds – run all the compounds, all the time.
- The analysis of pesticides in an onion matrix was simple, robust, sensitive, precise and linear.
- The new Ion Max NG source efficiently evacuates the source region therefore reducing the background and improving LODs and long term system robustness.

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