High Speed Quantitative GC/MS/MS Data Acquisition

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Introduction

There is an ever increasing need for high speed quantitative GC/MS/MS acquisition particularly in the analysis of pesticides in foods. As pesticide target lists get ever longer the instrument requirements become greater. In particular there is a need to quantify hundreds of compounds in a single analysis. Due to the narrow chromatographic peak widths and peak density common to GC, very high speed MS/MS acquisition is required.

An EI GC/MS/MS "Triple Quad" instrument was designed specifically for high speed quantitative acquisition. This involves a highly optimized combination of software, acquisition firmware, circuit design, quad drive electronics, and collision cell design. Numerous "real world" tests were developed to prove the instrument performance.

Design Background and Goals

Rapidly switching between transitions on a quadrupole MS/MS instrument involves the rapid switching of numerous elements in several dimensions to accomplish the following:

- Changing m/z settings on two quadrupoles
- Changing DC offsets of the many ion optical elements with changes in collision energy
- Changing the DC offset of the front quadrupole analyzer with changes in collision energy
- Detecting, processing and transferring the ion data
- Smoothly transitioning between time segments
- Clearing the collision cell of ions from the previous MRM transition

For this poster, the focus shall be on the speed of switching the quadrupoles and clearing the collision cell. Results of experiments designed to demonstrate the "real world" capabilities of these designs will be shown.

The design goal was to show quantitative results with 500 transitions/second, while maintaining a duty cycle of at least 50%. An m/z 500 Thomson (Th, Dalton/unit charge) ion has a flight time through the instrument of about 0.6ms. This yielded a minimum dwell time for a single transition of 1ms and a settling time of about 1ms.

At these speeds, the goal was that ion cross-talk be less than 0.1%.

Quadrupole Drive Electronics Design

The quad drive electronics were designed and optimized to make large mass changes and still be able to settle in around 1msec. Figure 1 shows an example where a 40Th mass shift yields a total settling time of about 150µsec.

The required settling time was found to be dependent on the size mass shift taken by the electronics. To that end, the actual settling time was a dynamically calculated variable dependent on the actual mass change (see Figure 2) .

Figure 1: Measured Signal Stabilization Times for a 40Th Mass Shift

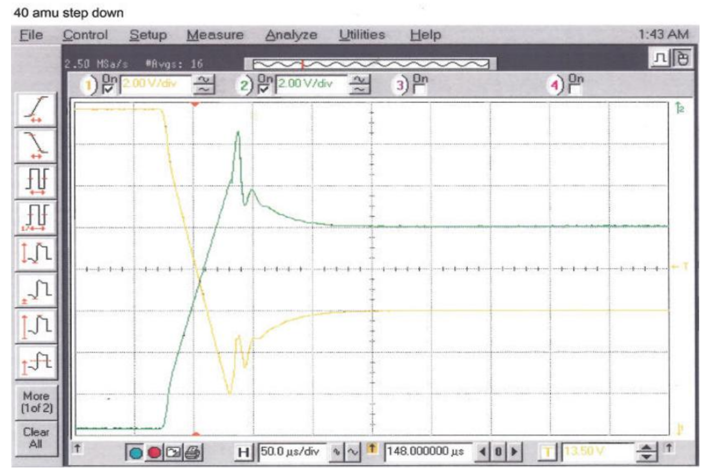
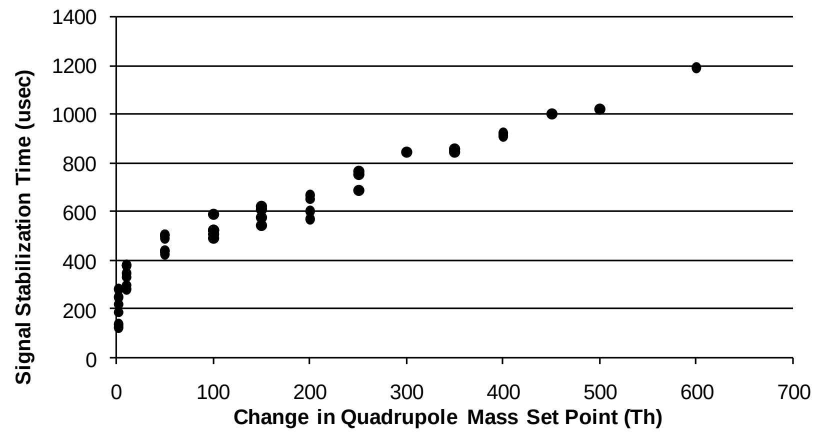


Figure 2: Measured Signal Stabilization Times for Fixed Mass Changes



Experimental Conditions:

For all experiments, unless noted:
MS: Agilent G7000A
    All settings from Autotune
    Quad 1 PW = 1.2 and Quad 2 PW = 1.2 FWHM amu
    Collision Gas Flows: 1.5 ml/min N2 and 2.25 ml/min He
GC: Agilent 7890 GC with a dual taper liner
    HP-5MS column 30m x 250µm x 0.25µm
Injector: Agilent 7683 ALS
    1µl injected in pulsed splitless mode
Data System: Agilent MassHunter Qualitative Analysis
    Peak areas determined by Autointegration using the MS/MS (parameterless) integrator

Collision Cell Design

A high pressure collision cell design was required in order to achieve thorough collisional cooling and focusing of the ion beam without resorting to expensive collision gases such as argon and xenon. However, this would slow down the ions to near thermal velocities, and lead to significant ion "cross-talk" from one transition to another. Experiments and calculations showed that the ion transit times under such conditions could be measured in seconds. A collision cell with a hexapole ion guide with axial acceleration using a resistive coating of the rods was developed. In operation, a potential was applied end to end, resulting in an accelerating field of the order of 0.5V/cm (see Figure 3). Calculations, modeling and experiments all yielded an ion transit (or clearing) time of the cell of about 0.5msec for a 500Th ion.

Figure 4 Shows experimental results measuring the time for the ion beam to "turn off". A square wave was applied to the entrance lens at the front of the collision cell and the time required for the ion abundance to decay was measured.

Figure 3: Hexapole Collision Cell Design

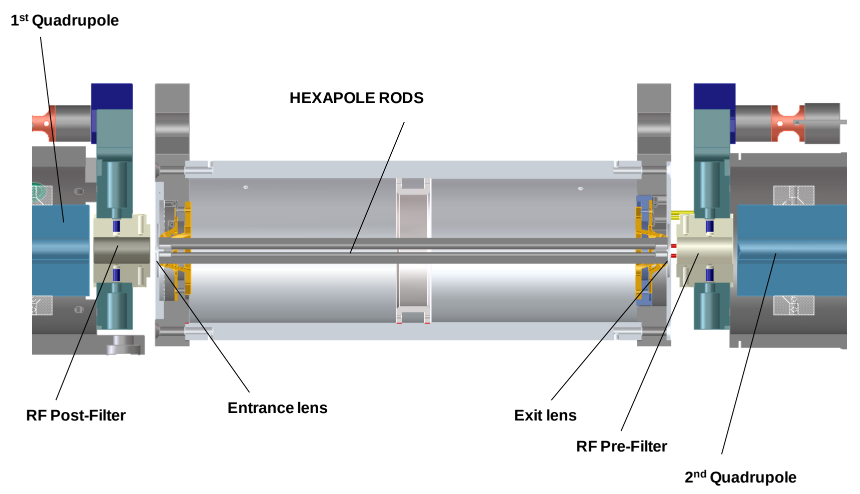
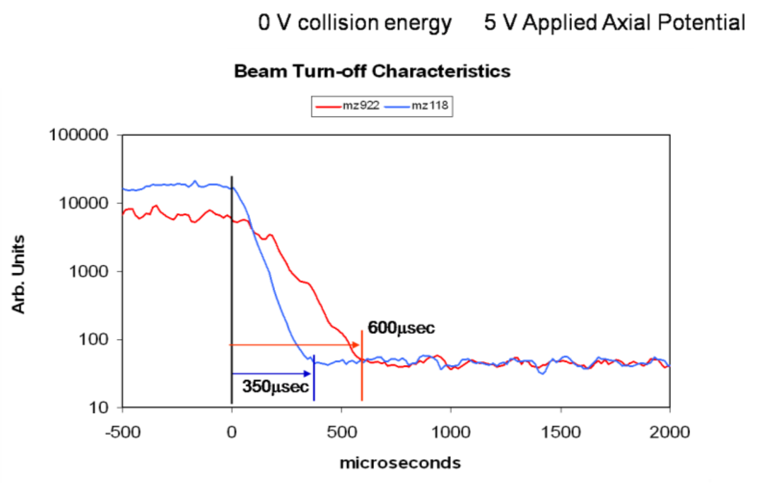


Figure 4: Measured Collision Cell Clearing Time



Analytical Experiments

Collision Cell Ion "Cross-talk"

Purpose: To determine what portion of a large concentration of ions "spills over" into the adjacent acquisition period as ion "cross-talk"

Experiment: Injected1ng of hexachlorobenzene (HCB)

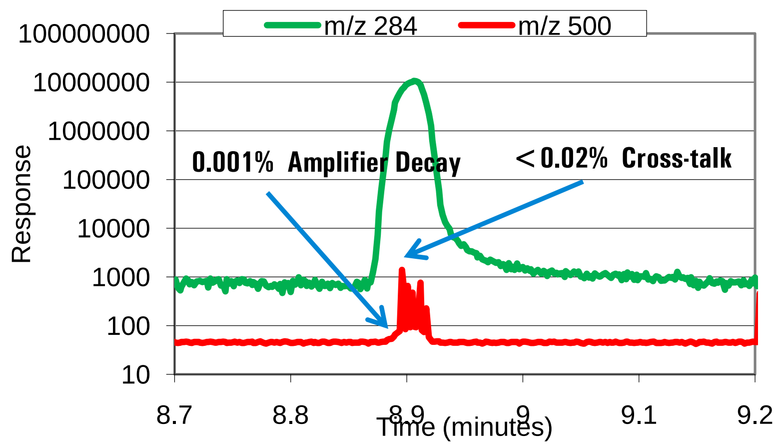
Analyzed the following two sequential transitions:

1.284→284 @ 0V, 1ms dwell & 1ms intra-MRM time
2.500→284 @ 0V, 1ms dwell & 1ms intra-MRM time

Notes: 0V collision energy was chosen to show the worst-case scenario of lowest-energy ions.

Results: See Figure 5. The jagged peaks on the lower (red) trace are single ions, and are cross-talk ion events. The smooth curve is the result of amplifier decay and does not represent actual ions. The cross-talk ions exist at less than 0.02% of the analyte ions. The amplifier decay is less than 0.001%.

Figure 5. Measured Ion "cross-talk" for 1ng of HCB



Measured MRM Dwell Time Equivalence

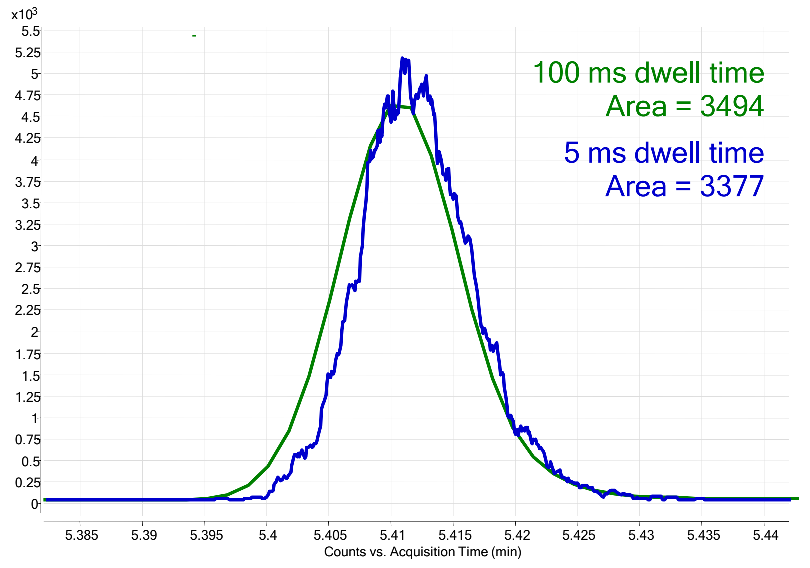
Purpose: Show that quantitation is independent of dwell time

Experiment: Injected 1pg of octafluoronaphthalene (OFN) in the following modes:

1.272→222 @ 25V, 5ms dwell & 1ms intra-MRM time
2.272→222 @ 25V, 100ms dwell & 1ms intra-MRM time

Results: See Figure 6. One run each at 5 and 100ms dwell are shown. Average of 5 peak areas agree to within 3.3%.

Figure 6: Measured MRM Dwell Time Equivalence for 1pg OFN



Results and Discussion

Collision Energy Change

Purpose: Demonstrate change in collision energy between transitions does not affect quantitation

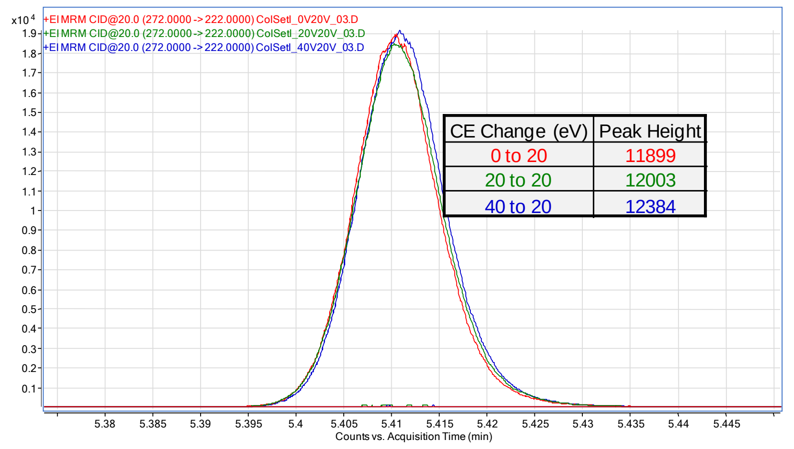
Experiment: 10pg OFN injected. Alternate runs were done with the following MRM tables (3 injections each):

1.273→221 @ 0V, 1ms & 272→222 @ 20V, 1 ms
2.273→221 @ 20V, 1ms & 272→222 @ 20V, 1 ms
3.273→221 @ 40V, 1ms & 272→222 @ 20V, 1 ms

Each quant transition was preceded by another, (not shown) only one Th away (to minimize the intra-MRM time) with either 20V higher or 20V lower collision energy.

Results: See Figure 7. The plot shows one set of three runs from the series; the numbers represent the average values from 3 runs.

Figure 7: Collision Energy Change for 10pg OFN



CE Change (eV)	Peak Height
0 to 20	11899
20 to 20	12003
40 to 20	12384

Time Segment Switching

Purpose: Demonstrate data integrity when switching time segments

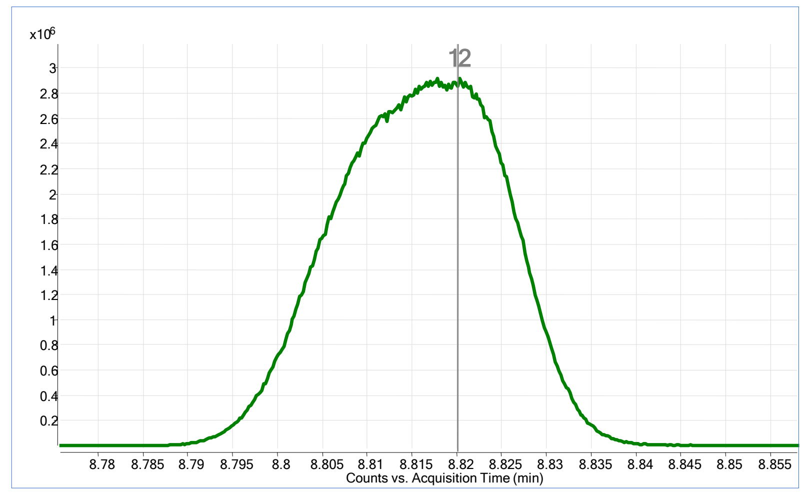
Experiment: 1ng of HCB injected and the time segment was changed in the middle of an eluting peak from (1) to (2):

1. 500→501 @ 35V, 5ms and 283.8→213.9 @ 35V, 5 ms
2. 501→502 @ 35V, 5ms and 283.8→213.9 @ 35V, 5 ms

Analyzed 283.8→213.9 @ 35V transition

Results: See Figure 8: Vertical line indicates the time segment change. No perturbation in the signal was observed.

Figure 8: Time Segment Switching for 1ng HCB



500 MRM/sec

Purpose: Show the high quality results obtainable at the highest speed

Experiment: Injected 2µl of 10ppb propyzamide at varying dwell times with fixed cycle time (See Table 1)

1.173→145 @ 15V

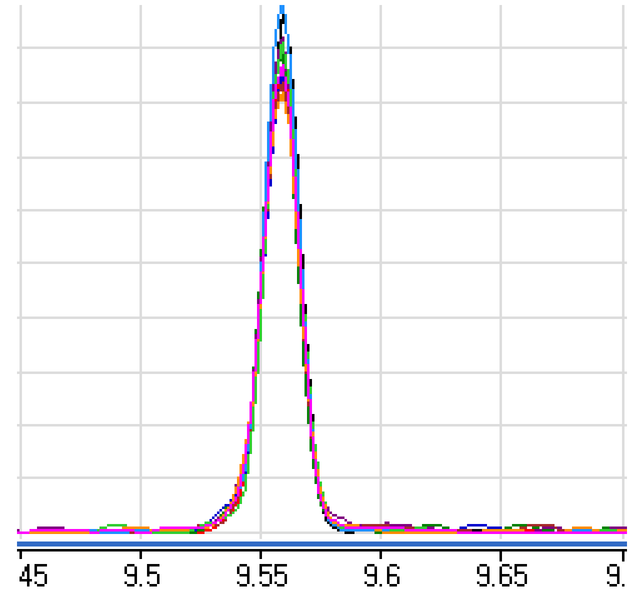
Notes: Peak Areas shown in Table 1.

Results: See Table 1 and Figure 9. Virtually no measureable speed affect was observed.

Table 1: MRM Speed for 10ppb propyzamide

Dwell Time (msec)	10	5	3	2	1
# Transitions	18	33	50	66	99
Cycle Time	198	198	200	198	198
MRM/sec	91	167	250	333	500
n=1	37453	39629	38589	37722	36246
2	37854	38444	35487	34584	34524
3	36464	39402	37690	35916	35007
4	38547	37270	35756	36243	33895
5	38372	38376	37277	37373	36955
6	36349	37881	38076	38625	33315
7	39439	32900	36179	38984	34861
8	37654	37787	37455	38018	37986
9	37588	38620	40348	34537	34307
10	37140	36425	37795	36869	34700
Average Area	37686	37673	37465	36887	35180
SD Area	940	1925	1439	1557	1447
% RSD	2.5%	5.1%	3.8%	4.2%	4.1%

Figure 9: Propyzamide, 1 ms Dwell, 500 MRM/sec, 4.1% RSD



Conclusions

Quantitative high speed GC/MS/MS data acquisition has been demonstrated via several simple real world tests. Signal integrity has been shown to be maintained over a broad range of speeds.