

Targeted GC Pesticide Analysis at FDA: Transitioning to GC/MS/MS from GC/MS with Selected Ion Monitoring



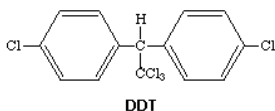
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Overview

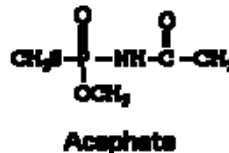
- Briefly discuss evolution and current use of SIM methods
- Impact of SIM methods on FDA program
- Discuss GCQQQ system & current method
- Expected improvements and goals
- Observations during method development and real-world sample analyses
- 20 minutes goes by FAST (for me anyway...)

Traditional FDA Pesticide Detection Procedures

1) GC with element selective detection



Need selective detector for Cl/Br
(ELCD/XSD or ECD)



Need selective detector for P
(FPD or NPD)

2) Confirmation using a different type of GC column

"Official" SIM Methods

NSO Method (J. AOAC Int. 87, (2004) 1224-1236)

- * Now targets 121 pesticides (mostly without X or P heteroatoms)
- * Average ~10-fold improvement in sensitivity vs. NPD

Halogen Method (J. AOAC Int. 88, (2005) 1452-1462)

- * Now targets 129 pesticides (mostly with F, Cl and/or Br)

Organophosphorous Method

- * Targets 75 OP pesticides – good for high sulfur matrices



Impact of SIM methods on FDA Pesticide Program

Year	# Samples	Violations (%)	MSD only (%)
1998	8570	198 (2.3)	3 (1.5%)
1999	9425	190 (2.0)	5 (2.6%)
2000	6529	170 (2.6)	6 (3.5%)
2001	6462	247 (3.8)	23 (9.3%)
2002	6749	210 (3.1)	47 (22.4%)
2003	7128	318 (4.5)	173 (54.4%)
2004	7850	327 (4.2)	182 (55.7%)

Total violations and MSD usage clearly jump from 2001 onward. Even more pronounced jump after the purchase of 25 instruments in late FY'02.



Increasing the Sample Load...

Electronics Upgrade for 5973 GC/MSD's (FY'10)

- * Cheap way to increase sample capacity for MSD's
- * Faster electronics allow more ions per SIM window (doubled from 30 to 60 ions plus you can set dwell times lower for higher scan rates)
- * NSOSIM and HALOSIM methods now 21 minute GC runtimes
- * Faster methods having more problems with co-elution



Use of GCQQQ at PRLNW

- * Received Agilent GCQQQ late in fiscal year 2009
- * Working on a method to target almost every pesticide FDA has detected on regulatory samples by GC - within last ~15 years or so.
- * Why spend the extra time with standard prep, calibration and data review for pesticides we never find? (qual screen for those by full scan)
- * Current method targets ~230 pesticides.
- * System also has multimode inlet with backflushing (more than just a change from GC/MS to GC/MS/MS – even more variables!)



GCQQQ Method Conditions

- GC Program: 60C (hold 1min) to 170C @ 40C/min then
170C to 310C @ 10C/min (hold 1.25min)
- Column 1: (HP5-MS UI 15m x 0.25mm x 0.25 μ m) 1.0mL/min
- Column 2: (HP5-MS UI 15m x 0.25mm x 0.25 μ m) 1.2mL/min
- Backflush: 4mL/min for 2min at end of GC program
- Inlet Program: 60C (hold 0.2min) to 270C @ 600C/min
Splitless 1 μ L
- Inlet Liner: “Dimpled” single taper 2mm id liner (5190-2296)
- Transfer Line: 300C EI Source: 300C Quads: 180C

GCQQQ Acquisition Program

MRM Start	Ret. Time	Compound	Std Mix #	Quant			Qual		
				Q1	Q3	CE	Q1	Q3	CE
3.4	3.736	Metaldehyde	1	89	45	10	89	43	30
4	4.373	Propoxur IP Frag	2	110	63	30	110	38	42
	4.473	D8 Naphthalene	IS	136	108	30	136	82	40
	4.469	Clofentzine frag	1	137	102	20	137	75	30
	4.620	Methamidophos	1	141	95	5	141	79	20
	4.700	Dichlorvos	2	184.9	93	15	184.9	109	15
	4.793	Novaluron frag 1	1	334.9	167.9	20	334.9	139.9	40
4.9	5.000	Carbofuran IP frag	2	164	103.1	30	164	77	40
	5.051	2,6-Difluorobenzamide	1	141	63	33	141	113	15
	5.062	Linuron IP frag	2	187	124	30	187	159	10
5.2	5.269	Nicotine	2	161	119	20	161	130	20

Similar to SIM windows (46 total) – currently using unit-unit resolution

Potential Improvements

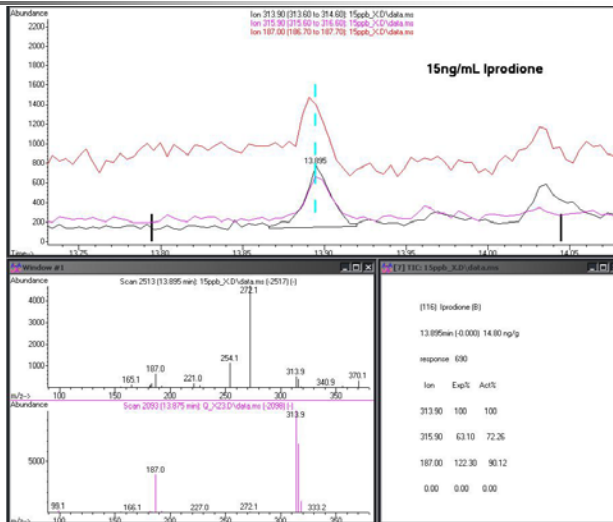
- Large drop in background responses and increase in S/N using MS/MS with multi-reaction monitoring (MRM)
- Co-elution much less of a problem (better for faster runs)
- Calibrate all target compounds with one injection (≥ 4 for SIM)
- More dilute extracts may be analyzed for required sensitivity
- More dilute extracts + backflushing = less maintenance
- Might skip concentration steps during extractions
- Cool initial temp of MMI better for thermally labile analytes?

15ng/mL Each Target Compound by HALOSIM (5975 System)

Probably a little under the LOQ for Ipridione

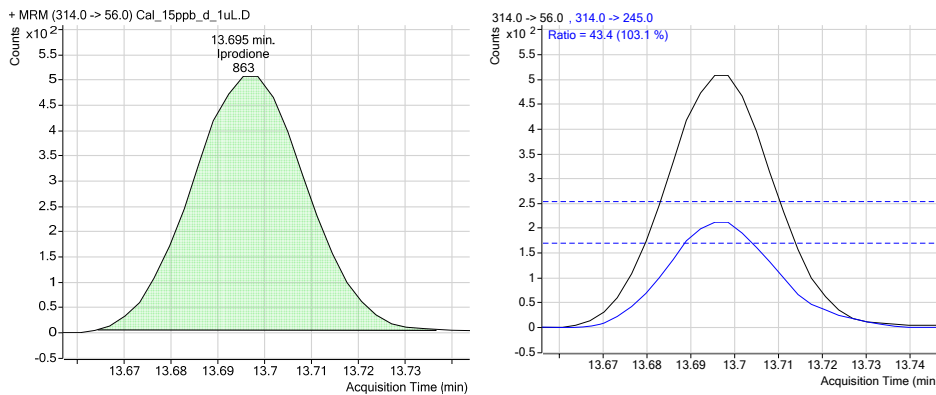
Coelutes with spiromesifen -->

(Using same liner and very similar GC program)



Same 15ng/mL Std on GCQQQ

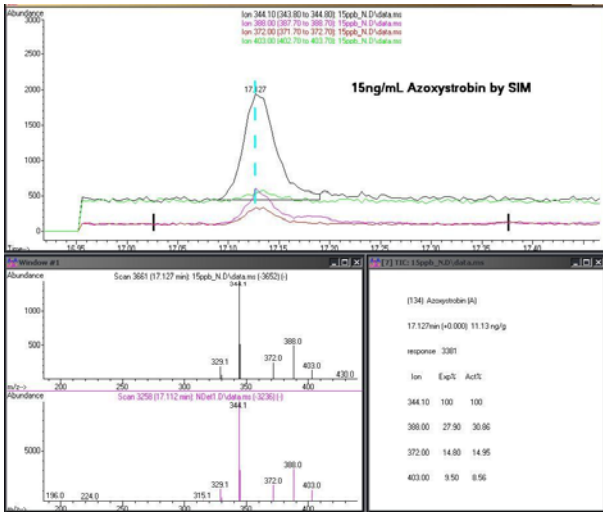
Ipridione results using current GCQQQ method



S/N is excellent and coelution is not an issue – pretty small peak area though (863)

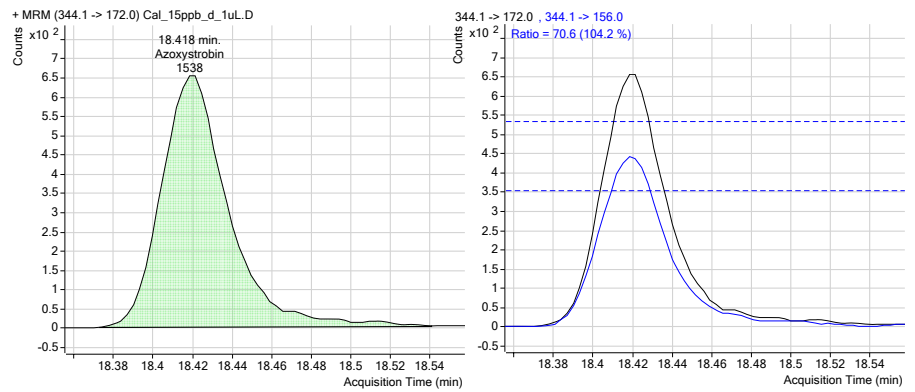
15ng/mL Each Target Compound by NSOSIM (5975 System)

Above the LOQ for Azoxystrobin



Same 15ng/mL Std on GCQQQ

Azoxystrobin results using current GCQQQ method



S/N is excellent and MRM ratio good – pretty small peak area though (1538)

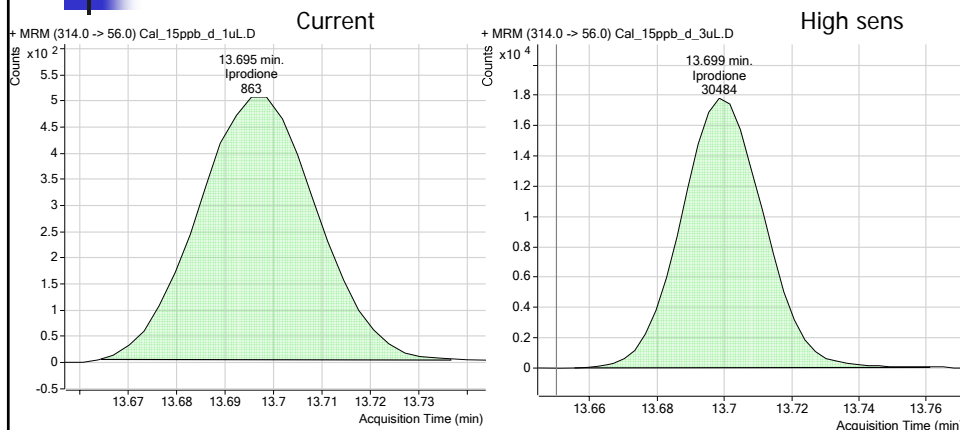


Time to Give it a Little Gas...

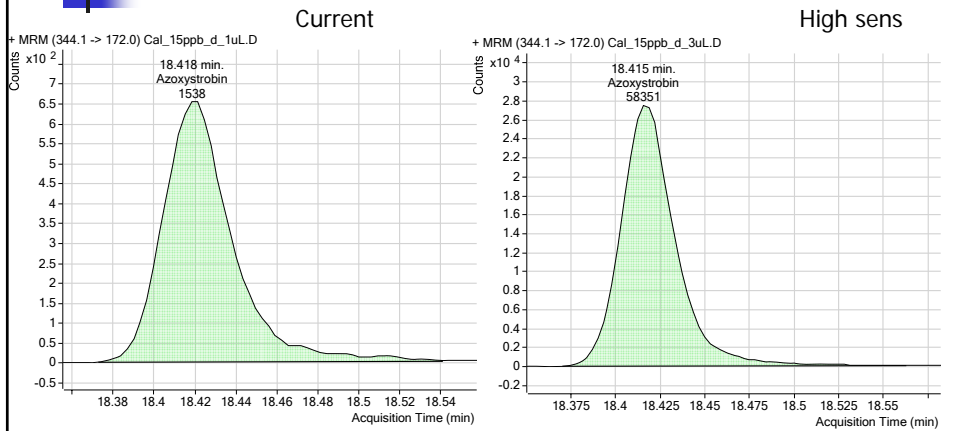
- I've been driving this thing like granny (but granny's car usual lasts longer!)
- Several ways to increase sensitivity
 - Inject more sample (upped to 3uL)
 - Increase the Gain (up from 20 to 100)
 - Use wider resolution setting (switched from Unit to Wide for Q1 – Q3 still unit)



Comparison of Current Method to Higher Sensitivity GCQQQ Method

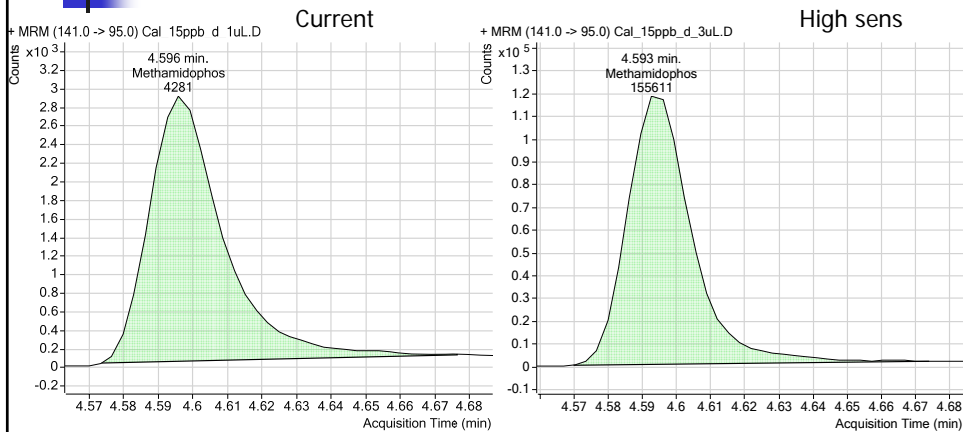


Comparison of Current Method to Higher Sensitivity GCQQQ Method



38-fold increase in peak area for azoxystrobin

Comparison of Current Method to Higher Sensitivity GCQQQ Method



36-fold increase in peak area for methamidophos
(I don't even get a peak for it by SIM)

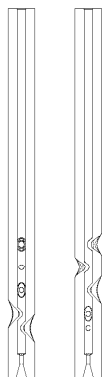
Now Less Sample Prep an Option...

- Less clean up
- Run more dilute
- Solvent mixtures OK? (might inject eluant as is from SPE)
- May depend on the liner

Inlet Liners



Dimpled 2mm id Liner – I have seen an improvement in reproducibility and linearity compared to helical liners



4mm id Helical Liner



Quechers at PRLNW

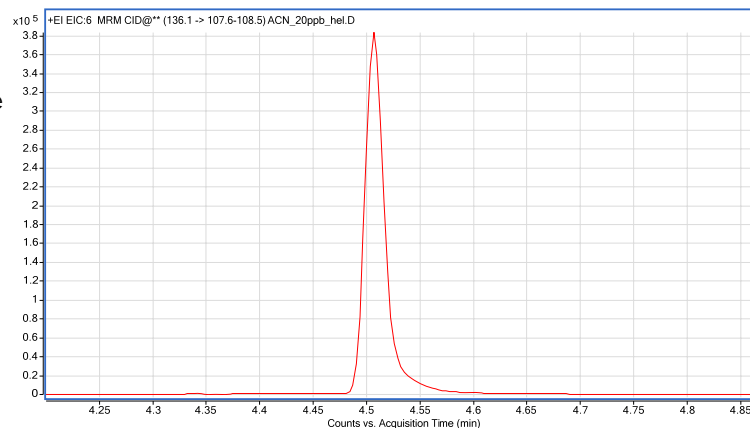
- Wanted to use same GC/MS internal standards with acetone extracts (FEDSO) or with quechers extracts
- Quechers extracts wind up with 10% acetone and 90% acetonitrile
- 2mm id liners + our SIM GC programs have problems with solvent mixtures



Acetone:ACN Mixture with Helical Liner

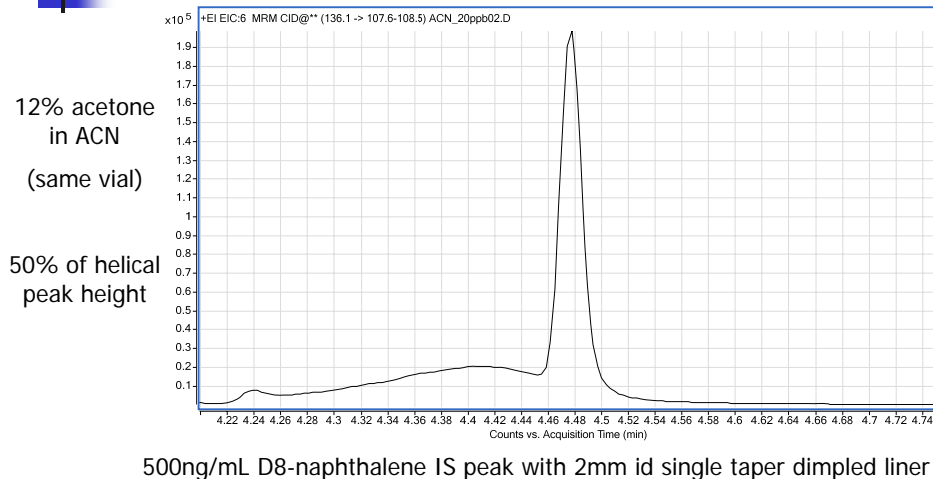
12% acetone
in ACN

Worked fine
here



500ng/mL D8-naphthalene IS peak with 4mm id single taper helical liner

Acetone:ACN Mixture with 2mm id Liner

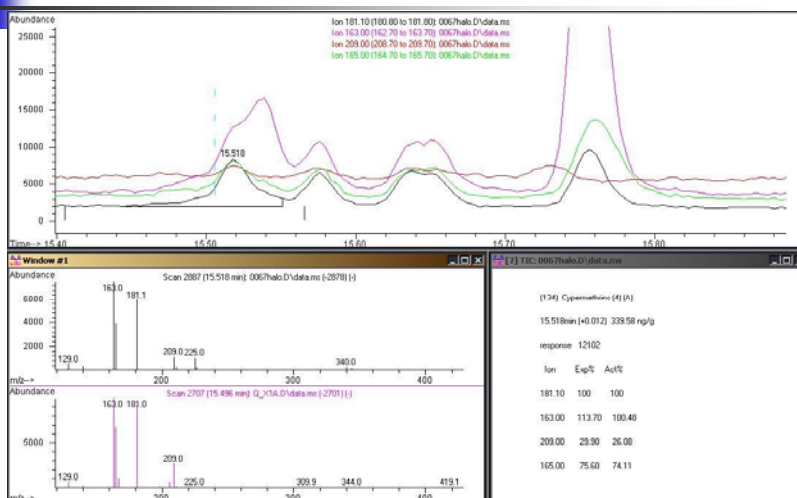


Pesticide EI MRM Selection



- Review of MRM data from a wide variety of samples is a nice way to determine which MRM will provide the best quantitative results
- There are several suspect MRMs floating around in the literature and on the internet
- Usually high background (loss of 15, 18, 28), daughter is a very common ion (77 or 91) or the MRM is not structurally relevant (loss of 1???)
- Sometimes you have no other options (my method is still full of them but most have ~low background responses – good parenting!)

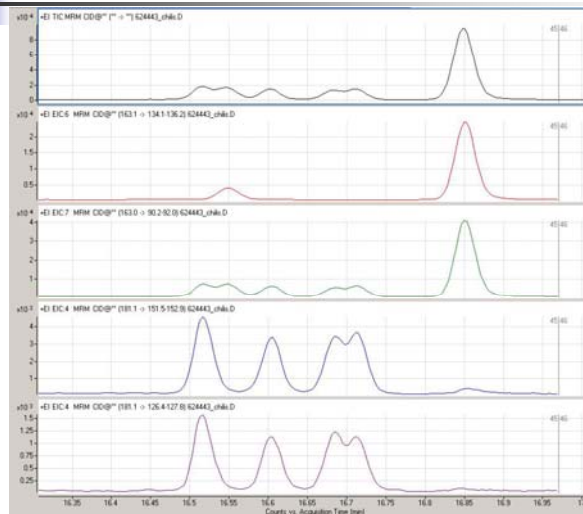
Incurring Cypermethrin by SIM



Dried Chili Pepper Sample

163 ion has co-elution problems

Incurring Cypermethrin by QQQ



All acquired MRMs

163 >>> 135 MRM
(loss of 28)

163 >>> 91 MRM

181 >>> 152 MRM

181 >>> 127 MRM



Preliminary Observations with GCQQQ Method Development

- Not a bad idea to have a third SRM for a few compounds and consider different parent ions for better confirmation (160->132 and 132->77)
- MRM ion ratios seem to be a little more variable than single quad GC/MS
- System needs to be HOT for late eluting pesticides – like azoxystrobin or dimethomorph (see method conditions)
- Multimode inlet seems a little picky about liners. Wool plugs seem to eat pesticides.



Conclusions

- The NSO and halogen SIM methods provided a large improvement over the traditional element selective GC detection systems.
- Similar improvements may result by replacing the 3 SIM methods with one targeted GCQQQ method that will be faster, more sensitive and selective.
- Need to be diligent about incorporating new findings into targeted methods



Acknowledgements

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