A Modified QuEChERS Procedure for the UPLC-MS/MS Analysis of Pesticides in Fresh Produce

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Outline

- One multiresidue method for GC, GC-MS and HPLC-MS/MS
- Development of a method based on a modified QuEChERS procedure
- Applications to sample analysis

Tandem GCB/PSA Cleanup

Advantages:

1) Removes coloring pigments (chlorophyll, carotenoids, polyphenols), sterols and acids in produce
2) Effective clean-up procedure for most produce commodities
3) Widely used procedure
4) One multiresidue procedure that can be both GC and LC compatible

Disadvantages:

1) Requires toluene (High boiling point)
2) ACN:toluene (3:1) not compatible for GC or LC analysis
3) Must evaporate to full dryness for LC-MS/MS
4) Lower recoveries for aromatic, planar pesticides

Multiresidue Residue Methods using Tandem GCB/PSA Cleanup

- **Fillion et al.** (PMRA, Canada)
  ACN extraction + C18 + GCB/aminopropyl SPE clean-up for GC-MS/SIM and HPLC-PCD/FLD analysis
  (*J. AOAC Int.* **1995**, *78*, 1252-1266, **2000**, *83*, 698-713)

- **Pang et al.** (Qinhuangdao Entry-Exit Inspection and Quarantine Bureau, PRC)
  ACN extraction: 1) C18 + GCB/aminopropyl SPE for GC-MS and 2) GCB/aminopropyl SPE for LC-MS/MS analysis
  (*J. AOAC Int.* **2006**, *89*, 740-71)

- **Mol et al.** (Rikilt Institute of Food Safety, Netherlands)
  Ethyl acetate extraction and GCB/PSA dispersive clean-up for GC-MS analysis
  (*Anal. Bioanal. Chem.* **2007**, *389*, 1715-1754)

- **Okihashi et al.** (Osaka Prefectural Institute of Public Health, Japan)
  Buffered ACN extraction and GCB/PSA SPE cleanup using GC-MS, GC-FPD and GC-MS/MS analysis
  (*J. Pest. Sci.* **2005**, *30*, 368-377; *J. AOAC Int.* **2007**, *90*, 1165-79)

- **Anastassiades** (CVUA, Stuttgart) 2.5 or 7.5 mg of GCB, 25 mg PSA and 150 mg $MgSO_4$ added to each mL of acetonitrile extract (quechers.com)

Multiresidue Procedure based on Canadian Method and QuEChERS

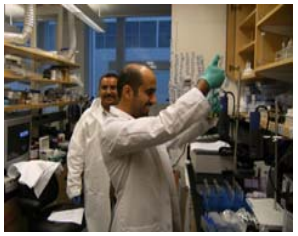
J AOAC Int. 2000, *83* (3): 698-713 (PMRA)	Proposed Method	*J AOAC Int.* 2003, 86(2):412-31 (QuEChERS)
50 g sample 100 mL ACN Blend and add 10 g NaCl	15 g sample + 15 mL ACN Blend	10 g sample + 10 ml ACN Vortex or shake
15 mL ACN extract C-18 SPE cartridge	6 g $MgSO_4$, 1.5 g NaCl Shake, add I.S., Centrifuge	4 g $MgSO_4$ + 1 g NaCl Vortex (or shake), add I.S., Centrifuge
Add Na_2SO_4, Centrifuge, and Evaporate 13 mL ACN extract to ~0.5 mL	Quant. Transfer 12 mL 0.4 g PSA + 0.2 g GCB + 1.2 g $MgSO_4$ Vortex 5 s	Transfer 1 mL ACN extract 0.025 g PSA + 0.15 g $MgSO_4$ Shake and centrifuge
Load extract for GCB/PSA Solid phase extraction	Add 4 mL toluene Shake and centrifuge	Transfer to ALS vials
Elute with 20 mL 3:1 ACN:toluene	Evaporate, add Q.C. std, and bring to volume Transfer to ALS vials	GC –MS and/or LC-MS Analysis
Evaporate, add I.S., and bring to volume Transfer to ALS vials	GC, GC-MS, HPLC and/or LC-MS Analysis	
GC, GC-MS and/or HPLC Analysis		

Modified Procedure

15 g cryo-grind sample

15 mL ACN + I.S.
+ $MgSO_4$ + NaCl

Shake +
centrifuge

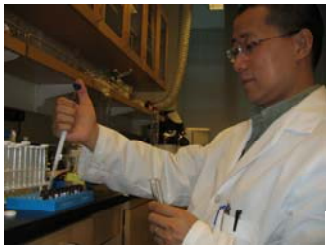


Transfer to tube containing GCB +
PSA + $MgSO_4$ and vortex

(Transfer)

Add toluene (3:1 ACN:toluene),
Shake + centrifuge

Transfer and
evaporate to
dryness



Add LC buffer + QC std,
filter, transfer to vials

UPLC-MS/MS

Modifications from QuEChERS

UPLC-MS/MS conditions

- UPLC Conditions

Column: 100 mm x 2.1 mm x 1.7 µ particle size Acquity BEH C18

UPLC: Acquity system, 3 µL injection volume

Time (min)	Flow (ml/min)	%A	%B
0.0	0.2	100	0
1.0	0.2	100	0
11.0	0.2	0	100
15.5	0.2	0	100
16.0	0.2	100	0
18.0	0.2	100	0

A: 10 mM NH_4OAC 90:10 H_2O:acetonitrile

B: 10 mM NH_4OAC 90:10 acetonitrile:H_2O

UPLC-MS/MS conditions (cont.)

- MS/MS Conditions

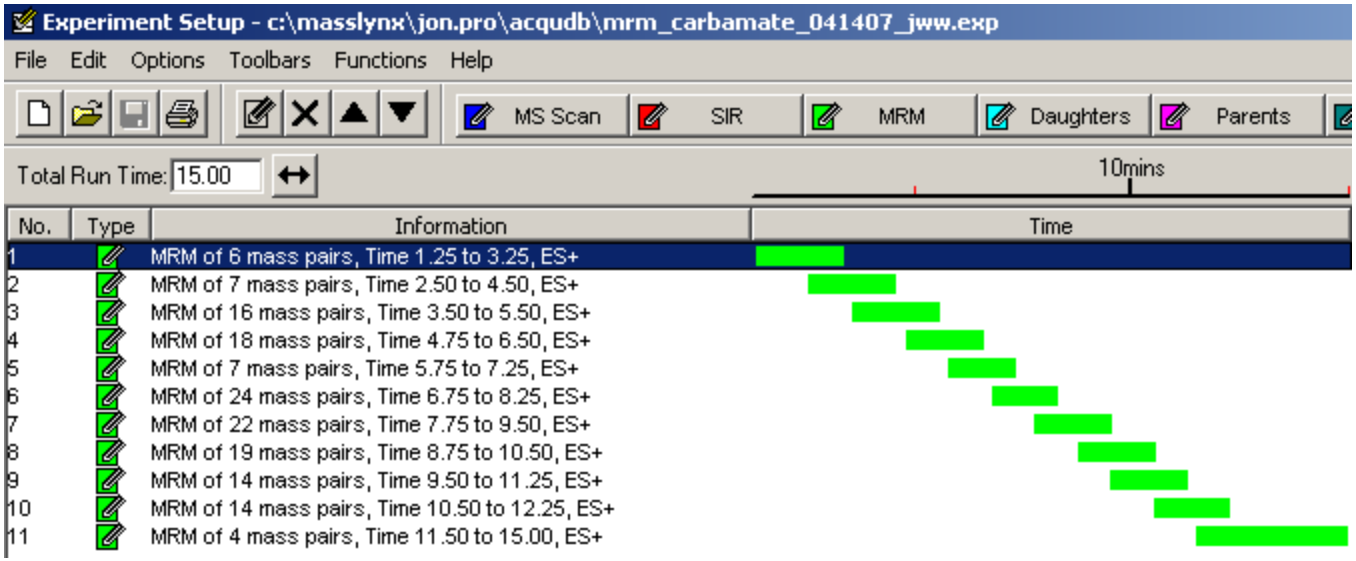
  Quattro Premier XE mass spectrometer (Waters Corp.)

  ~210 pesticides analyzed

  3 separate runs (each MS Program analyzes ~70 pesticides classified by group)

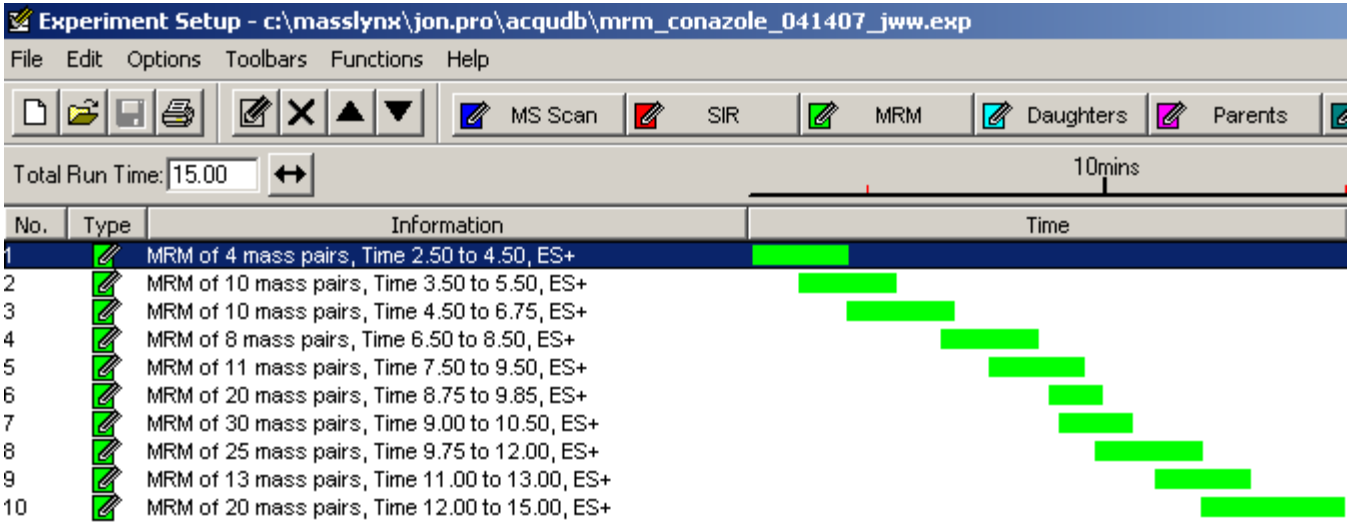
  2 transitions per each pesticide

Acquisition Methods



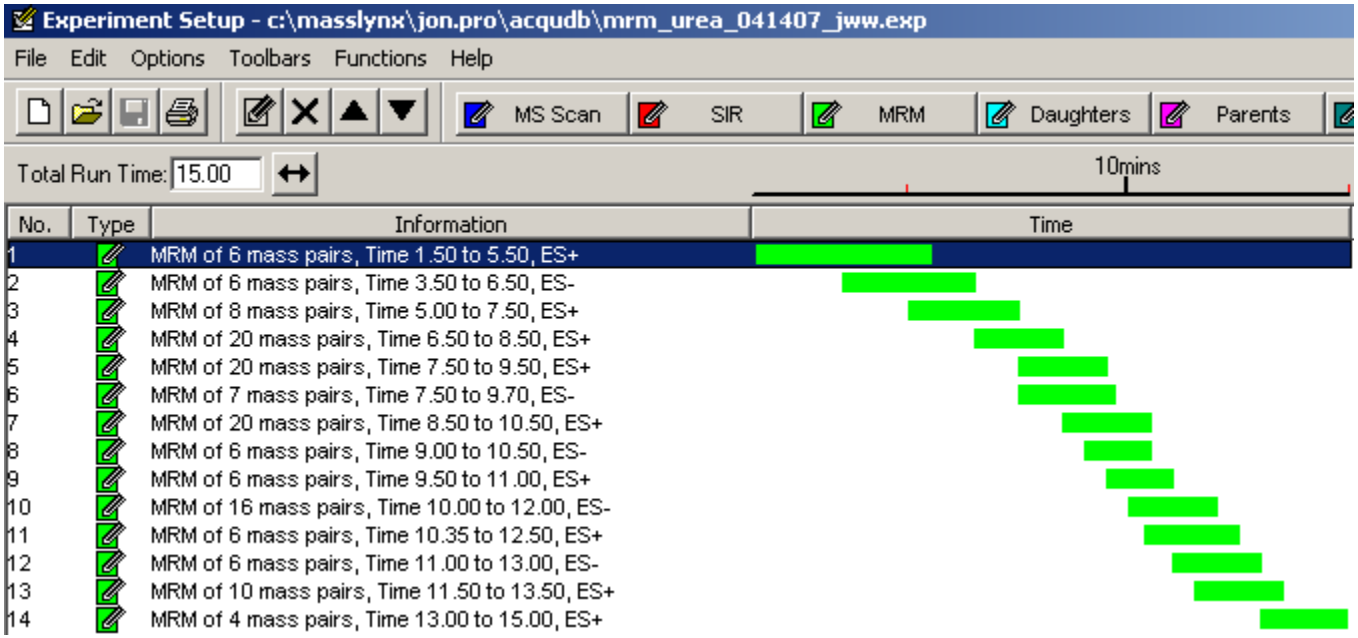
<u>Major groups</u>: amides, benzimidazoles, carbamates, polar organophosphates

Acquisition Methods (cont.)



<u>Major groups</u>: conazoles, neonicitinoids, macrocyclic lactones

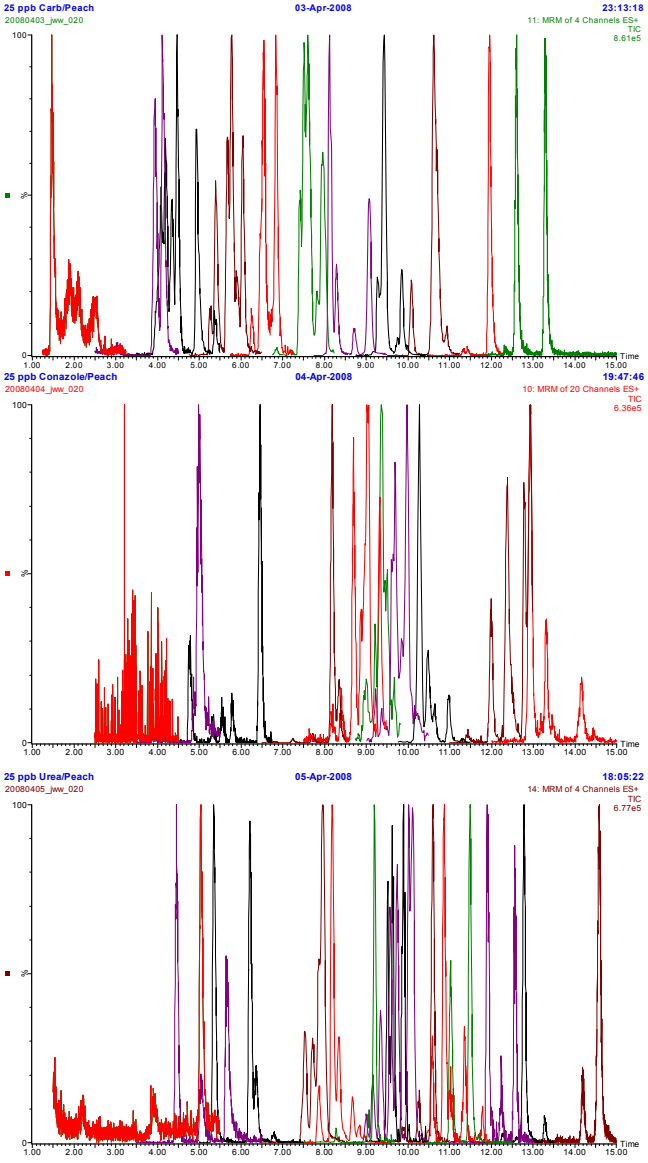
Acquisition Methods (cont.)



<u>Major groups</u>: phenylureas, strobilurins, triazines and ESI(-) pesticides

Chromatographic Analysis

Apple extract fortified at 25 ng/ml pesticide STD



Validation

- ~210 pesticides (25, 100, 500 µg/kg)

  ~70 amides, benzimidazoles, carbamates, organophosphates (polar)

  ~70 conazoles, neonicitinoids, macrocyclic lactones

  ~70 phenylureas, strobilurins, triazines

- Produce samples (5 fruits + 5 vegetables)

  apple, blueberry, orange, peach, strawberry

  broccoli, cabbage, carrot, pepper, spinach

Validation (cont.)

- **LOD/LOQ**

  LOD S/N = 3 for secondary transition

  LOQ S/N = 10 for primary transition and S/N = 3 for secondary transition

  Can easily detect 10 ppb or lower for most pesticides

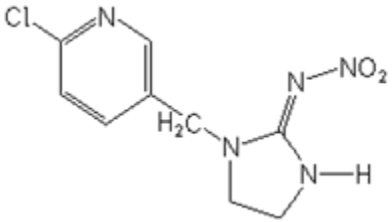
- **Linearity**

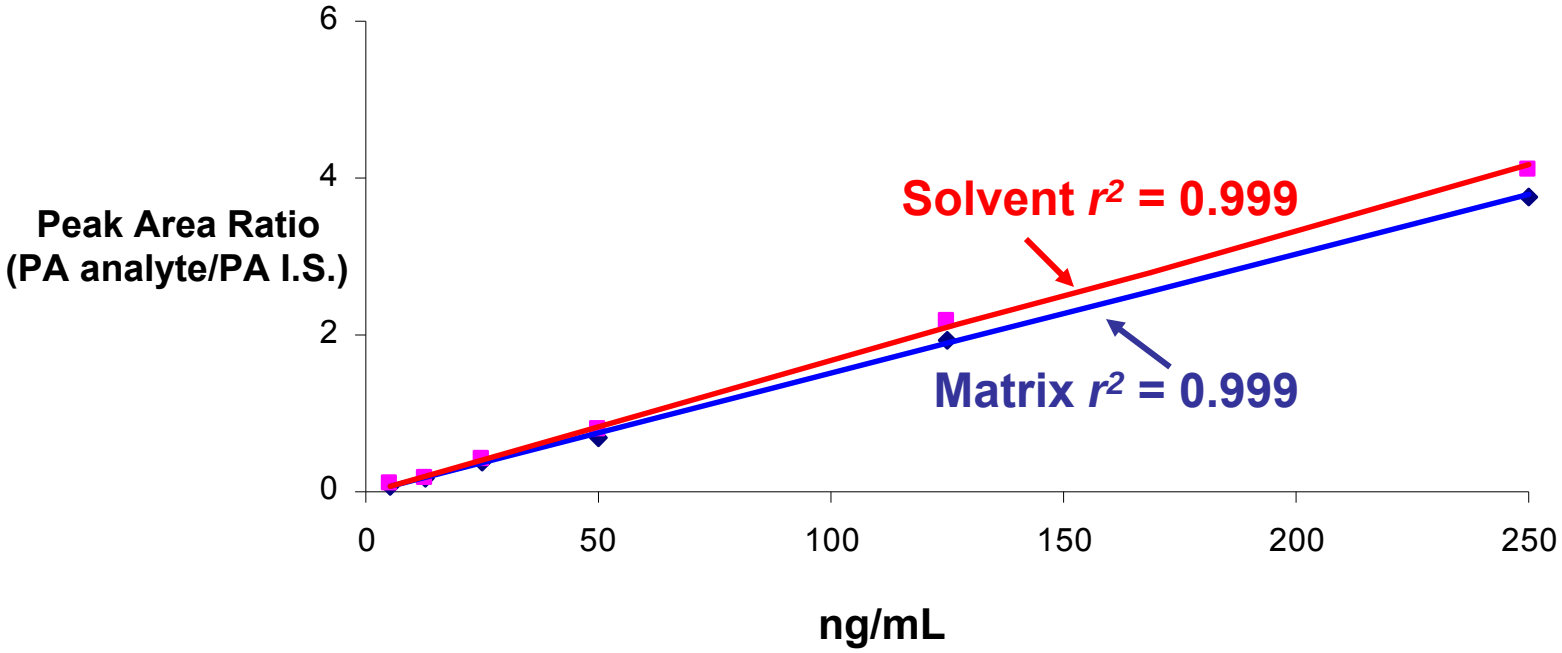
  r^2 = 0.99 or better for most pesticides

Validation (cont.)

Matrix effects – minor for some

Imidacloprid in Bell Pepper

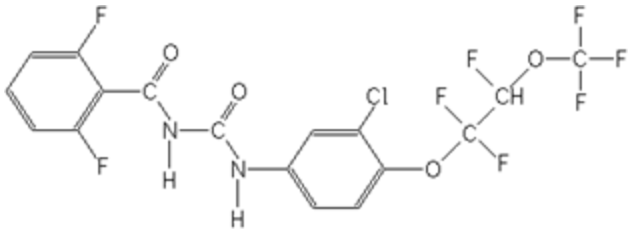


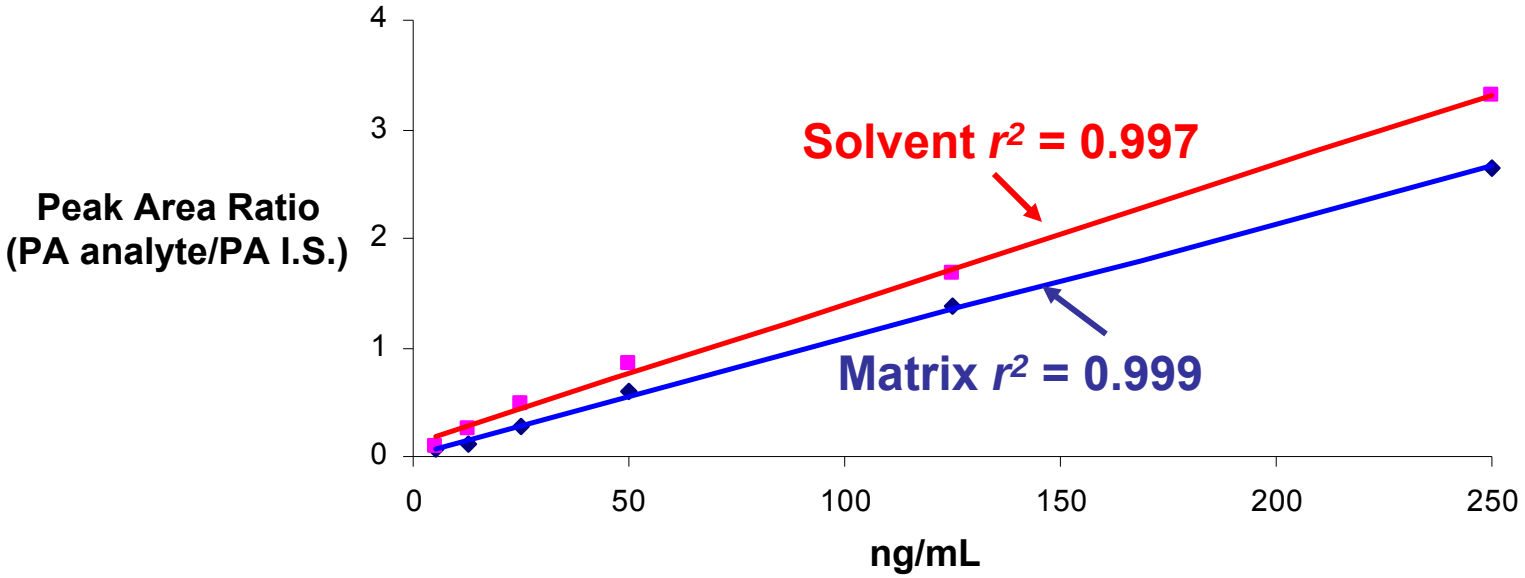


Validation (cont.)

Matrix effects – consistent for others

Novaluron in Peppers

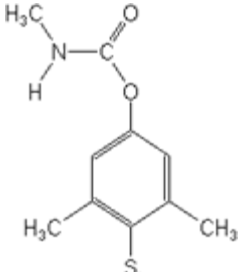


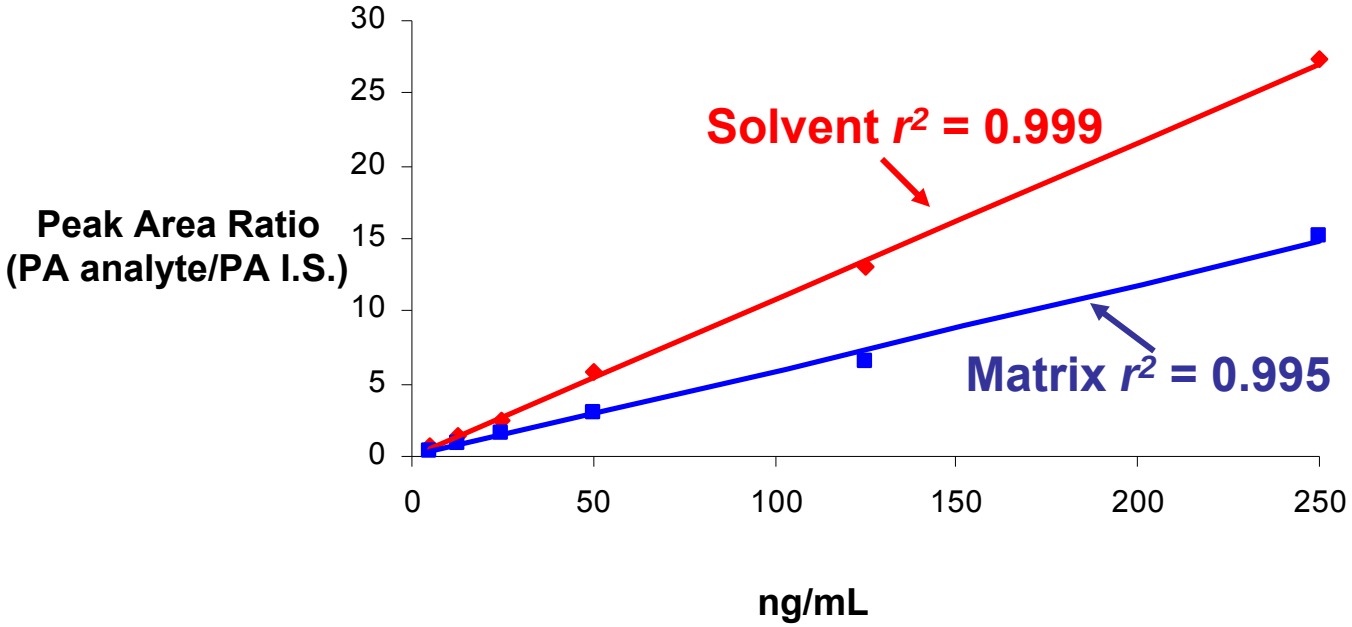


Validation (cont.)

Matrix effects – major for some

Methiocarb in Orange





<u>Future Studies</u>: Dilution of samples to minimize matrix effects

Validation (cont.)

Recovery Results, i.e., Carrot

Recoveries **between 70-120% for 195 of 213 pesticides**
Standard deviation < 20%

Not detected **at 25 µg/kg:**

Alanycarb, Amitraz, Benfuracarb, Bifenazate Clethodim, Dinotefuran, Flonicamid, 1-Napthol, Neburon, Nitenpyram, 2-Phenylphenol, Prothioconazole, Topramezone

Problematic **at all levels (not detected, low or high recoveries):**

Alanycarb, Aminocarb, Amitraz, Benfuracarb, Bifenazate, Clethodim, 1-Napthol, 2-Phenylphenol, Prothioconazole, Sulfentrazone, Thiophanate-methyl, Topramezone

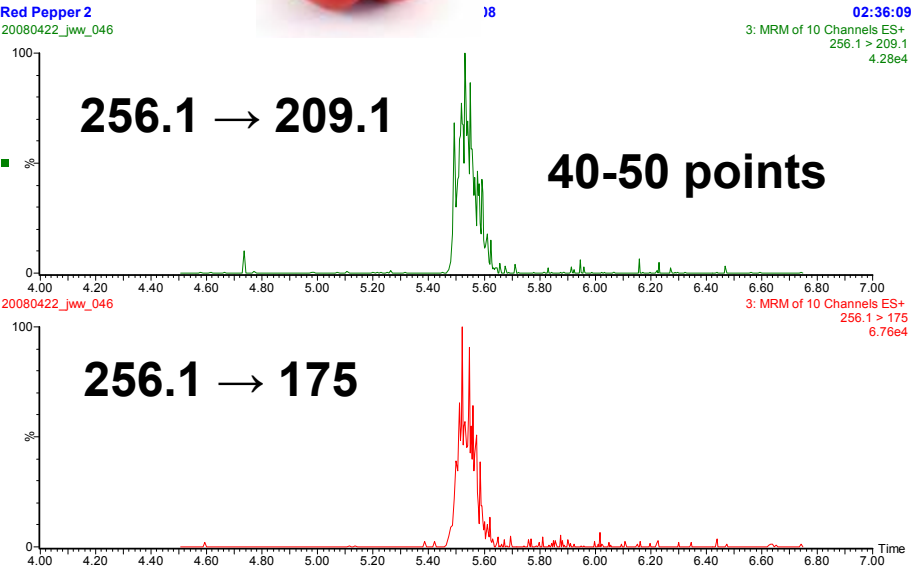
	.025 ppm	0.1 ppm	0.5 ppm		.025ppm	0.1 ppm	0.5 ppm		.025 ppm	0.1 ppm	0.5 ppm
2-phenylphenol	3±5	0±0	95±47	Bromuconazole 47	77±13	83±5	81±5	cyroxyzamin	*16±28*	49±4	52±3
Acephate	83±11	83±9	88±6	Bufencarb	126±49	133±9	137±13	Desmedipham	134±15	103±5	90±5
Acetamiprid	87±3	84±4	75±11	Bupirimate	80±9	81±6	91±11	Diclobutrazol	84±2	92±3	95±5
Acibenzolar-S-methyl	70±17	80±33	[illegible]	[illegible]	93±5	10±4	[illegible]	Dicrotophos	95±12	91±7	99±3
Alanycarb	19±2	18±2	19±0	Butafenacil	94±6	10±5	99±6	Diethofencarb	98±11	98±8	93±5
Aldicarb	94±14	94±8	102±5	Butocarboxin	102±1	99±9	105±8	Difenoconazole	90±6	98±4	96±4
Aldicarb sulfone	94±15	92±6	101±2	Butoxycarboxin	65±9	95±7	97±7	Diflubenzuron	107±5	107±3	120±3
Aldicarb sulfoxide	89±12	90±7	97±8	Cabaryl	92±12	87±7	87±5	Dimethoate	98±12	95±7	96±5
Ametryn	95±1	105±4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Dimethomorph	93±2	100±4	99±4
Aminocarb	440±258	477±150	550±49	Carbendazim	88±8	112±1	112±5	Dimoxystrobin	97±2	102±4	93±4
Amitraz	*17±1*	*12±5*	*18±1*	Carbofuran	100±13	103±1	103±7	Diniconazole	83±4	91±6	95±8
Avermectin B1b	128±98	106±3	90±5	Carbofuran, 3OH-	98±14	92±8	97±6	Dinotefuran	nd	109±3	91±11
Avermection B1a	97±16	100±3	90±1	Carboxin	85±8	78±8	79±4	Dioxacarb	93±12	94±8	99±6
Azoxystrobin	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	91±2
Benalaxyl	94±13	95±8	99±6	Chlorfluazuron	87±6	100±8	98±5	Doramectin	68±8	99.±8	91±4
Bendiocarb	89±12	90±8	87±5	Chloroxuron	95±2	98±3	87±1	Emamectin B1a	122±0	115±7	101±3
Benfuracarb	*12±0*	*9.±2*	*12±1*	Chlortoluron	98±18	96±1	81±3	Emamectin B1b	123±12	124±7	112±4
Bentazone	67±8	74±1	86±2	Clethodim	nd	nd	nd	Epoxiconazole	86±9	103±3	95±4
Benthiavalicarb	96±16	94±8	99±8	Clofentezine	200±48	101±2	88±7	Eprinomectin B1a	106±13	100±2	85±2
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	Etaconazole	[illegible]	[illegible]	soft
Bifenazate	*10±5*	*10±5*	*0.±0*	Cyazofamid	70±16	84±4	74±11	Ethiofencarb	89±6	81±3	84±4
Bitertanol	*114±8*	*108±6*	*10±4*	Cycluron	101±5.	100±3	94±4	Ethiprole	61±33	58±27	84±5
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	86±9
Bromoxynil	47±2	51±2	55±0	Cyproconazole	86±3	94±11	96±5	Etoxazole	93±2.	102±4	95±4
Bromuconazole 46	75±2	97±14	95±4	Cyprodinil	94±1	92±8	87±3	Famoxadone	78±2	110±8	105±1

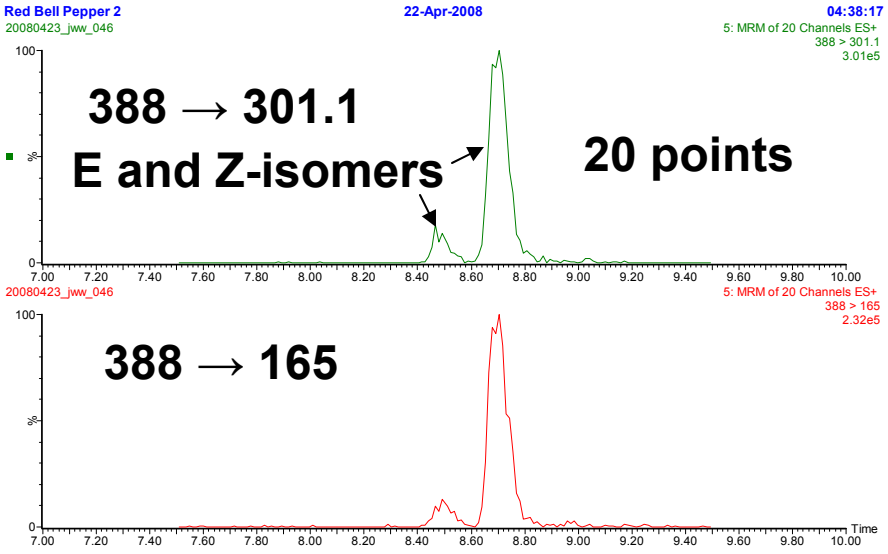
Incurred Residues in Produce

Bell Pepper

	UPLC-MS/MS Conc. (µg/kg), n=3	GC-MS/SIM Conc. (µg/kg), n=1
Bifenthrin	Not analyzed	16
Endosulfan	Not analyzed	12
Endosulfan sulfate	Not analyzed	6
Imidacloprid	53 ± 7	Not analyzed
Spinosyn A	5 ± 0.2	Not analyzed
Spinosyn D	0.6 ± 0.1	Not analyzed
Azoxystrobin	16 ± 2	8
Boscalid	50 ± 6	48
Cyproconazole	23 ± 3	23
Dimethomorph	44 ± 3	34
Famoxadone	25 ± 4	18
Myclobutanil	11 ± 2	10
Pyraclostrobin	23 ± 2	9

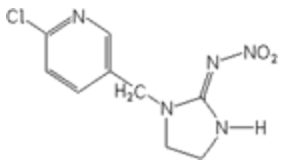
Incurred Residues in Bell Pepper (cont.)





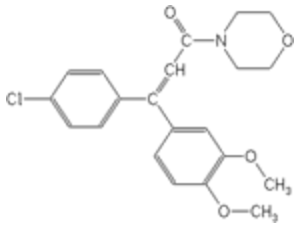
Imidacloprid

53 ± 7 µg/kg

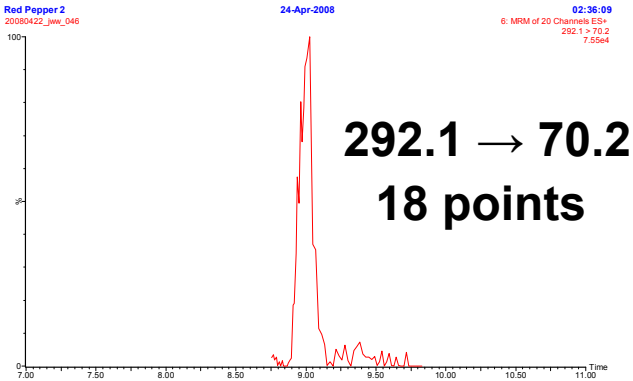


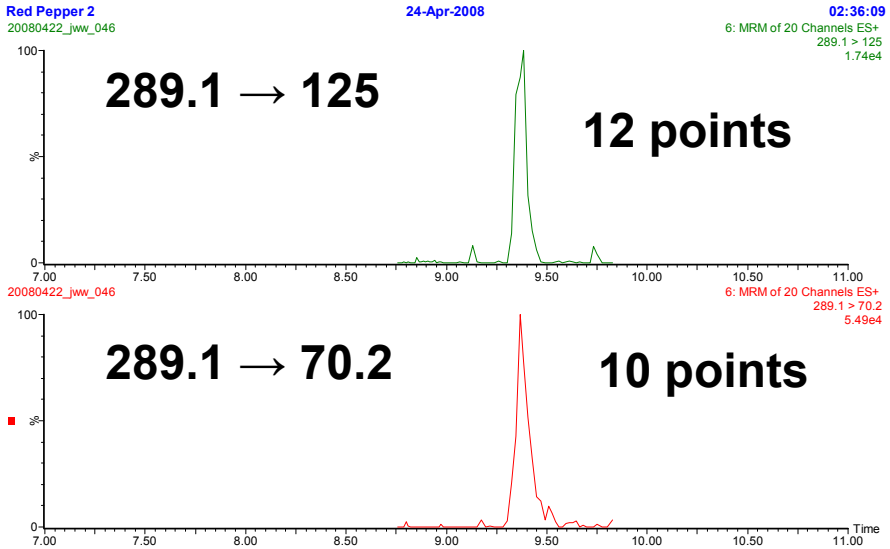
Dimethomorph

44 ± 3 µg/kg



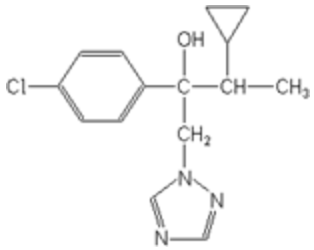
Incurred Residues in Bell Pepper (cont.)





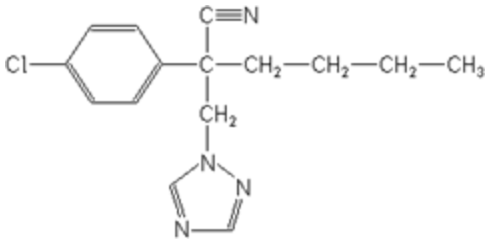
Cyproconazole

23 ± 3 µg/kg



Myclobutanil

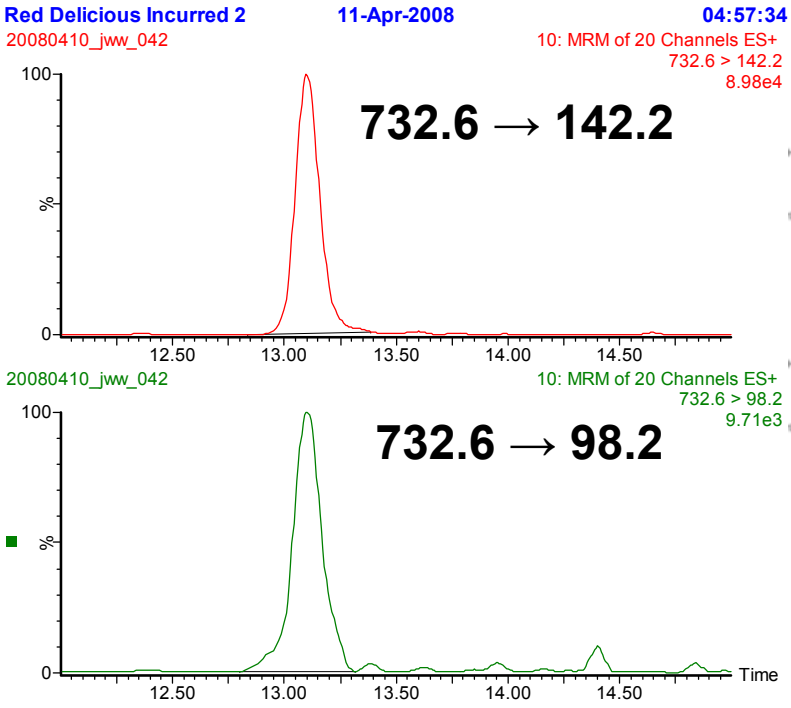
11 ± 2 µg/kg

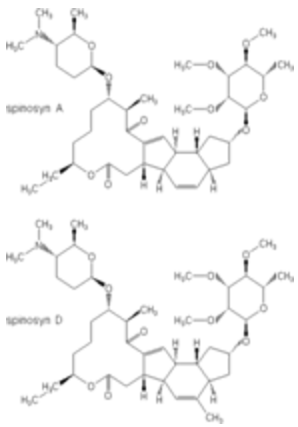


Incurred Residues in Produce (cont.)

Spinosad in Apple

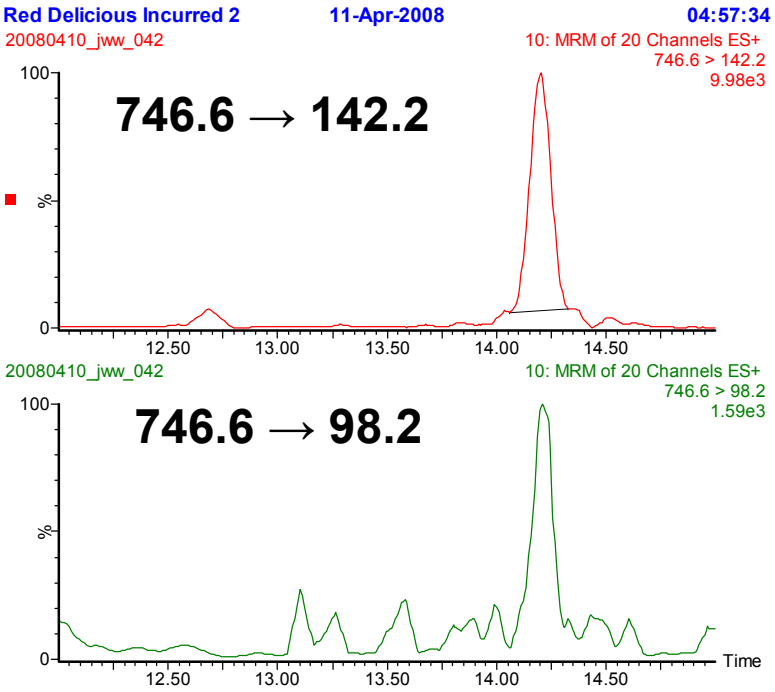
Spinosyn A





2.4 ± 0.3 µg/kg

Spinosyn D

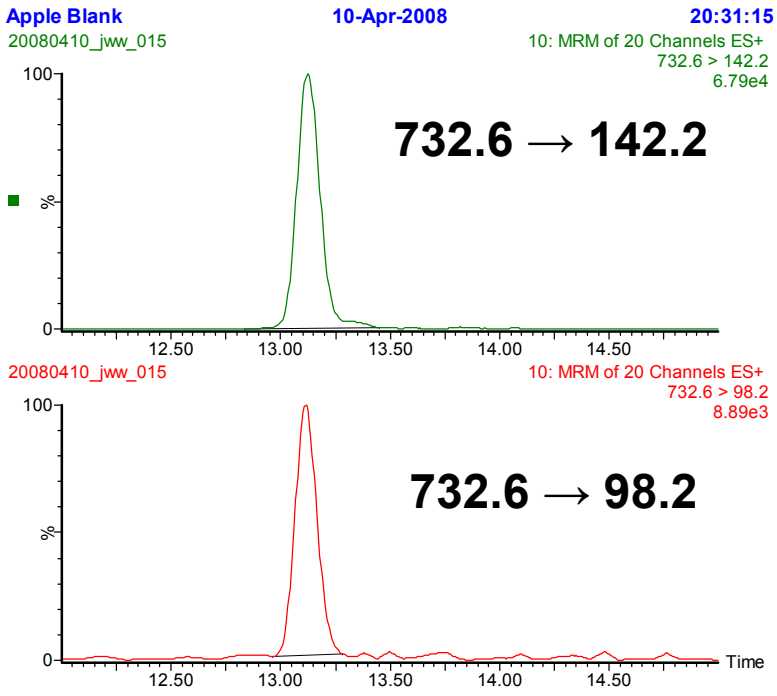


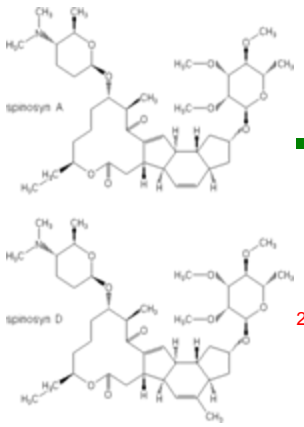
0.2 ± 0.04 µg/kg

Incurred Residues in Produce (cont.)

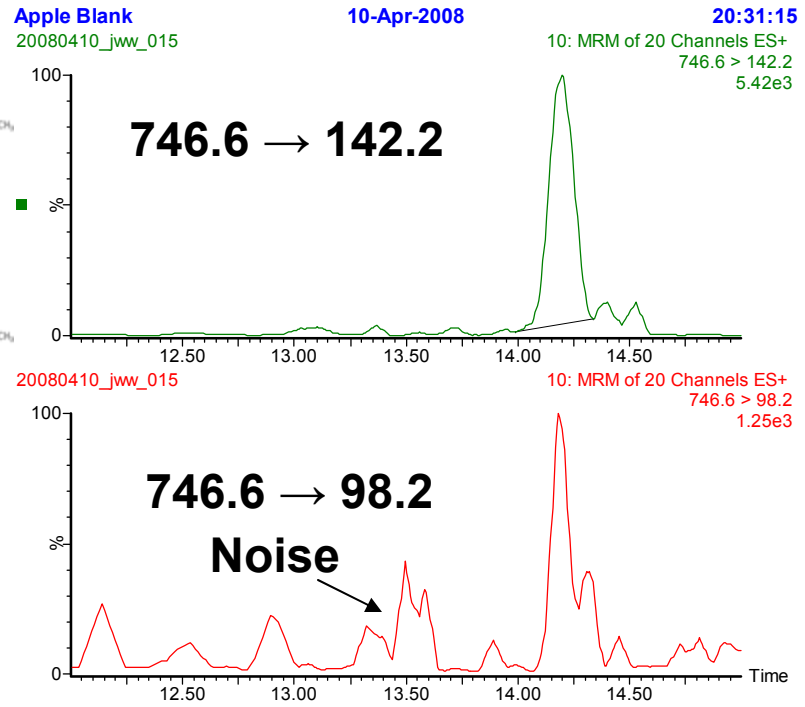
Spinosyn A also found in Organic Apple Blank

Spinosyn A





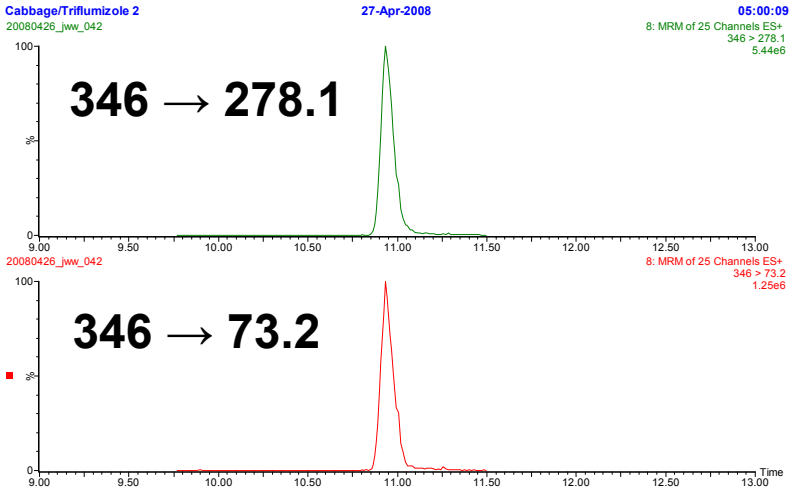
Spinosyn D

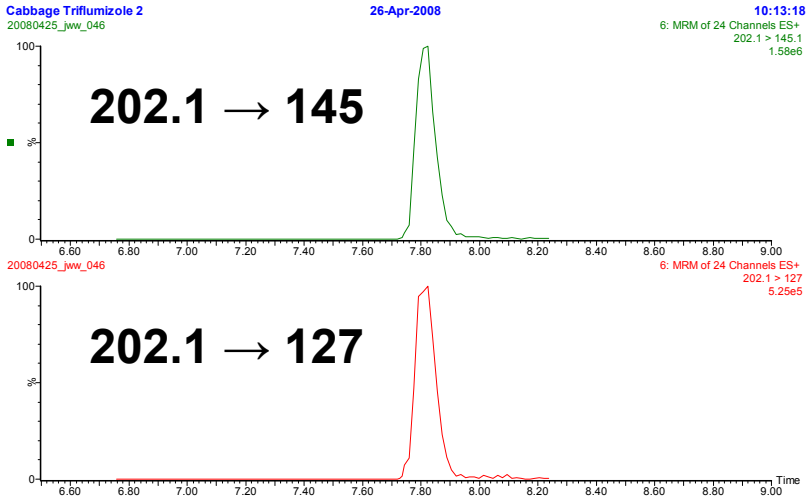


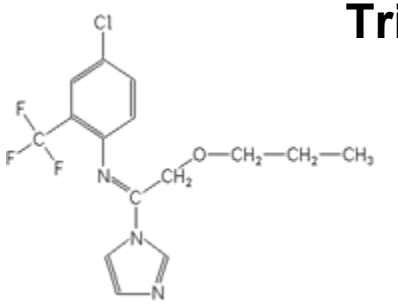
~ 1 µg/kg (est.)

Incurred Residues in Produce (cont.)

Triflumizole and Carbaryl in Cabbage

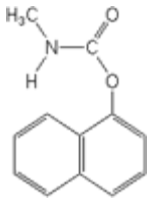






Triflumizole: 0.56 mg/kg

0.81 mg/kg including metabolites found in study for parent and metabolites

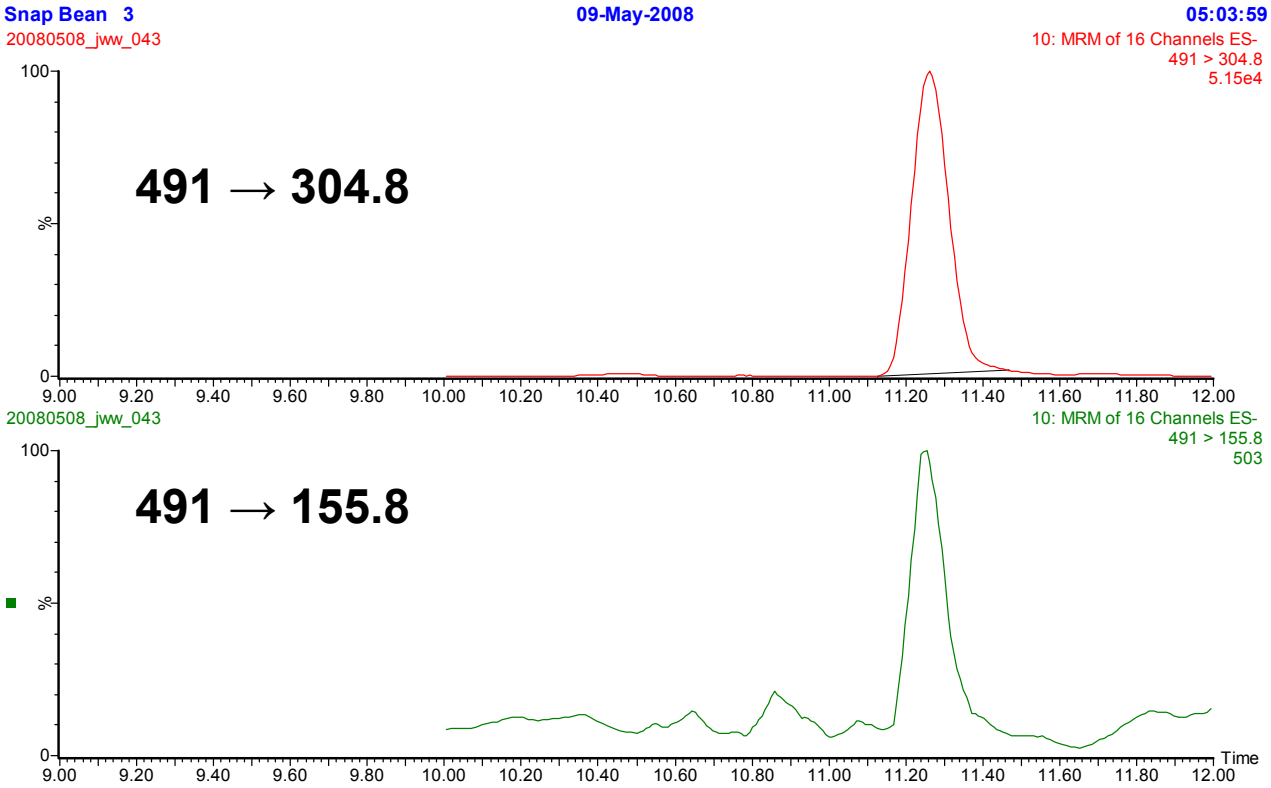


Carbaryl: 0.22 ± .005 mg/kg

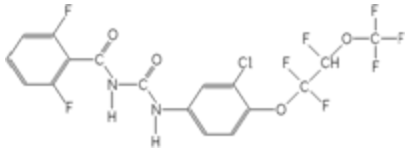
Not targeted for study in cabbage

Incurred Residues in Produce (cont.)

Novaluron in Snap Beans



Novaluron



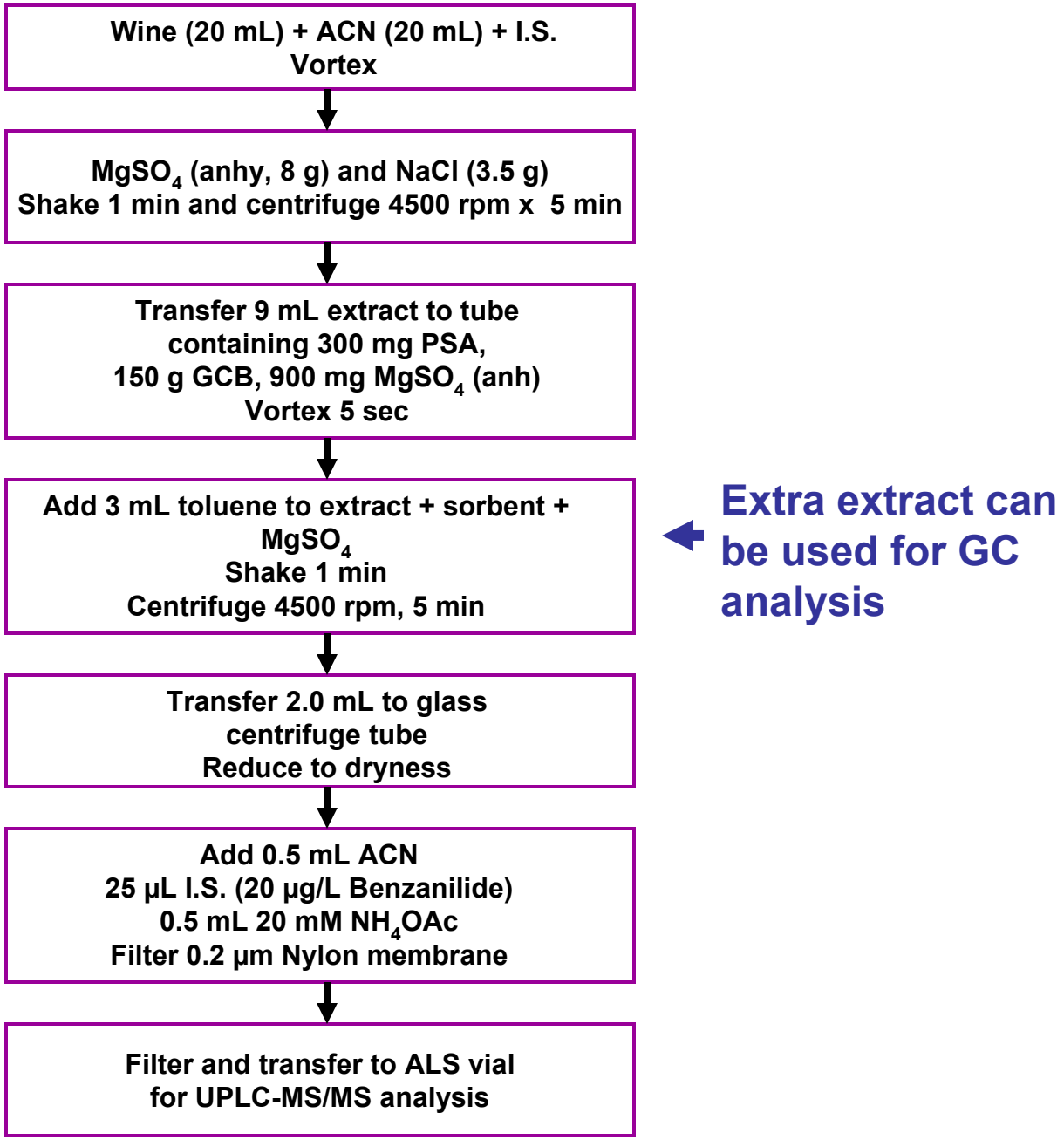
This study: 0.12 ± 0.01 mg/kg
Other study: 0.09 mg/kg

Analysis of Pesticides in Beverages

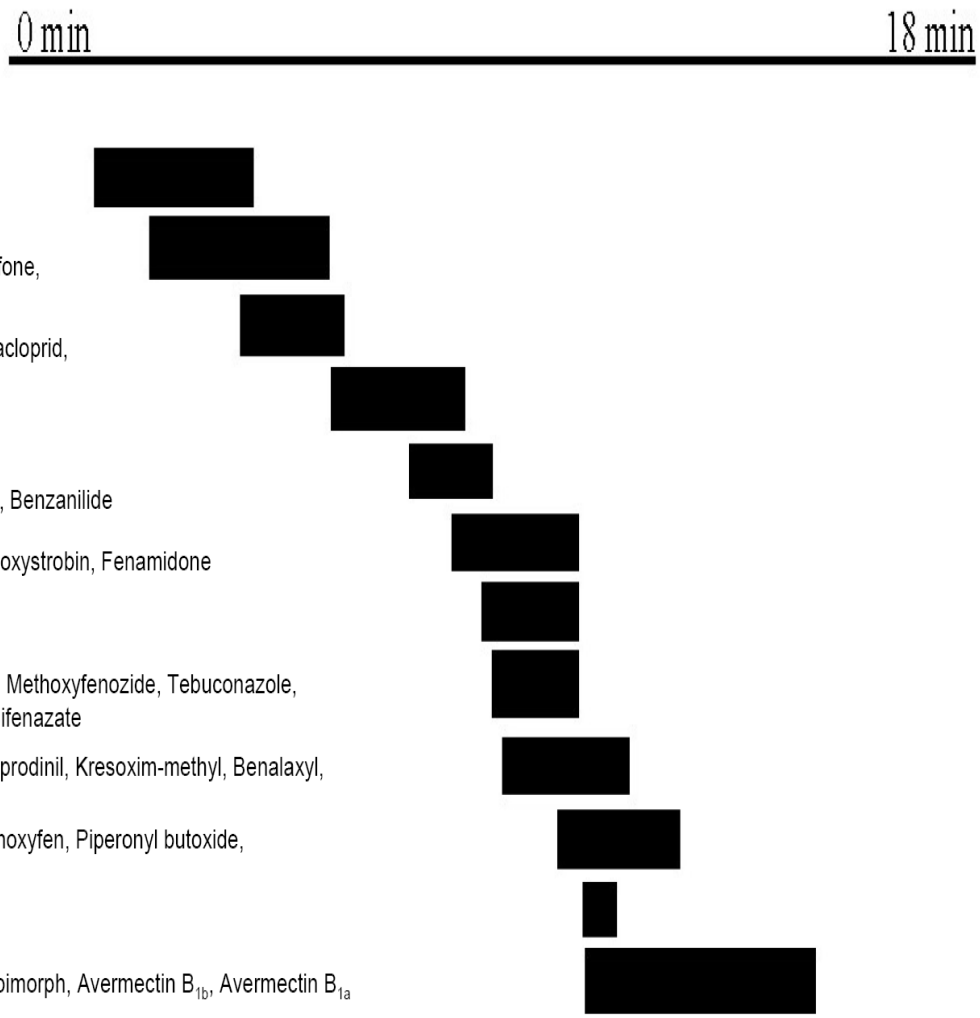
- Applications of QuEChERS to beverages

- Salt-out acetonitrile extraction and GCB/PSA applied to wines and juices (orange, apple, grape)

QuEChERS Modifications Applied to Pesticide Analysis in Wines

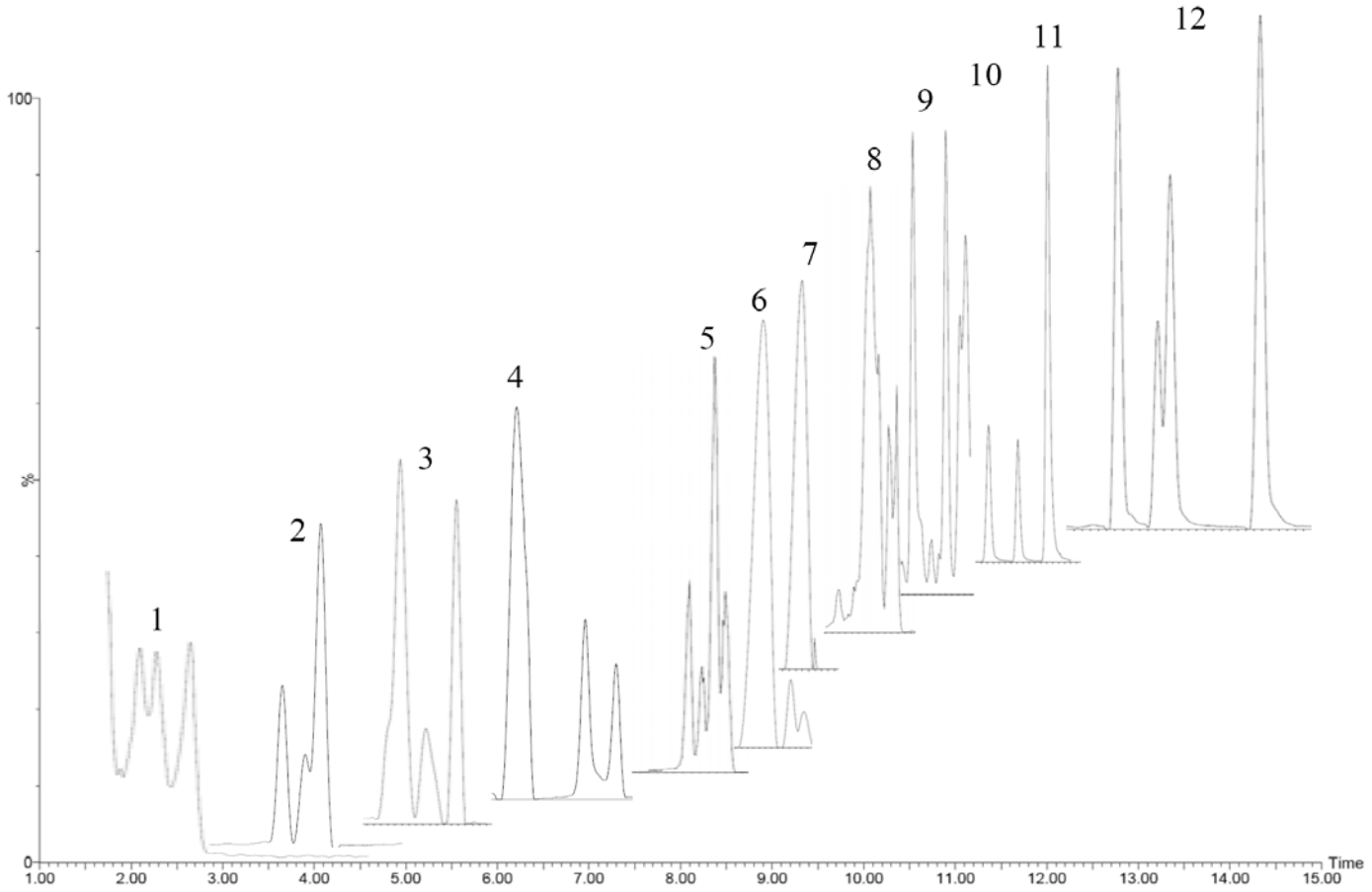


Screening of 72 pesticides

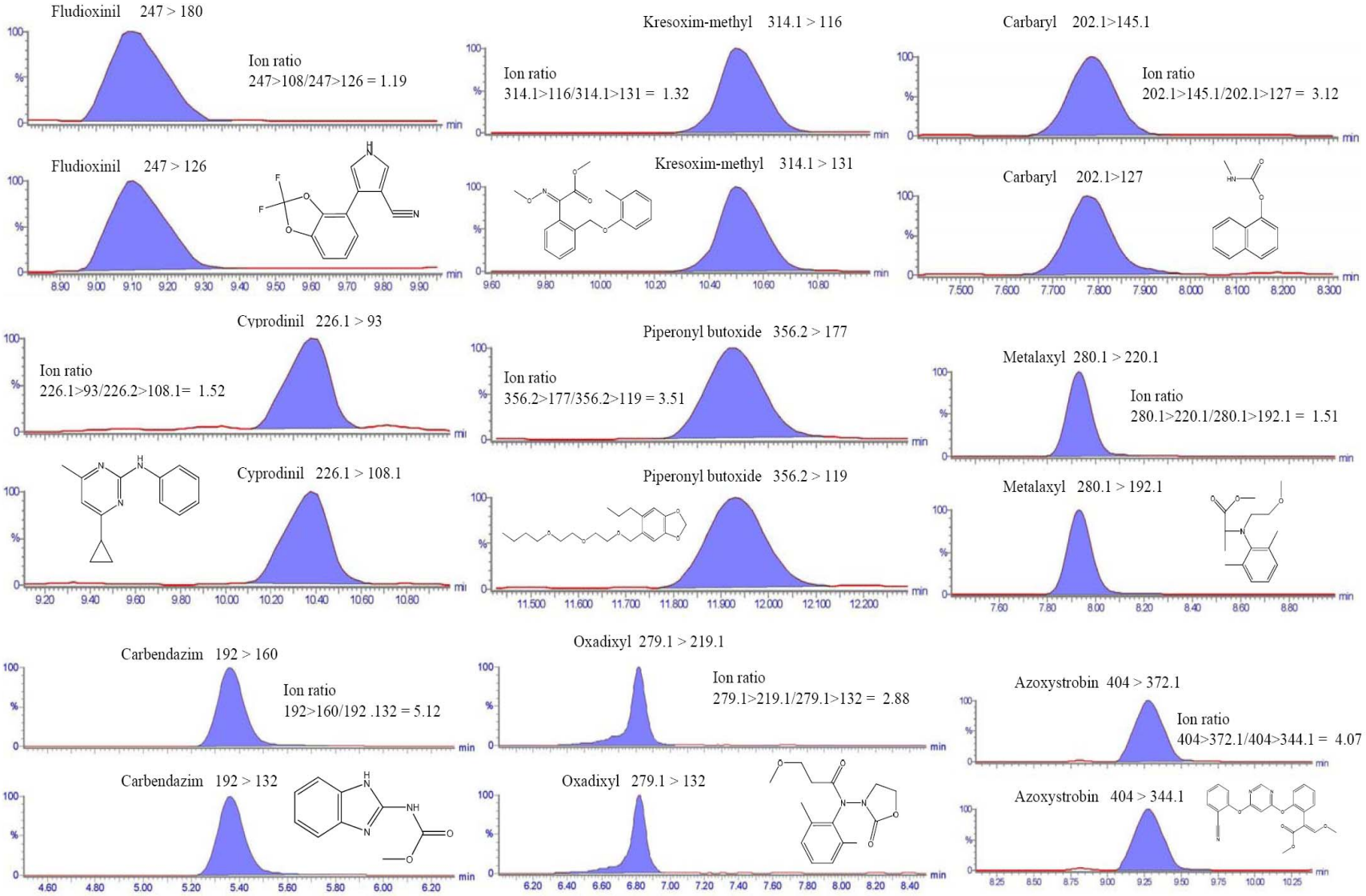


Group No.	
1 (1.5-4.5min)	Methamidophos, Acephate, Cyromazine, Omethoate
2 (2.5-6.0min)	Aldicarb sulfoxide, Dinotefuran, Propamocarb, Aldicarb sulfone, Methomyl
3 (4.5-6.3min))	Vamidothion, Carbendazim, Acetamiprid, Dimethoate,Imidacloprid, Thiabendazole, Mevinphos, Fluconazole
4 (6.0-8.5min)	Aldicarb, Oxadixyl, Simazine, Propoxur, Carbofuran
5 (7.4-9.0min)	Atrazin, Carbaryl, Metalaxyl, Atrazine, Diuron, Fenhexamid, Benzanilide
6 (8.1-10.0min))	Dimethomorph, Pyrimethanil, Iprovalicarb, Chloroxuron, Azoxystrobin, Fenamidone
7 (8.8-10.4min))	Fludioxnil
8 (9.0-11.0min)	Acibenzolar S-methyl, Myclobutanil, Triadmimifon, Imazalil, Methoxyfenozide, Tebuconazole, Fenbuconzaole, Mepanipyrim, Bitertanol, Eithofumesate, Bifenazate
9 (9.1-11.5min)	Spiroxamine, Hexaconazole, Rotenone, Propiconazole, Cyprodinil, Kresoxim-methyl, Benalaxyl, Diclobutrazol, Ipconazole, Dimoxystrobin
10 (10.2-12.3min)	Triflumizole, Pyraclostrobin, Zoxamide, Trifloxystrobin, Quinoxyfen, Piperonyl butoxide, Benfuracarb, Furathiocarb
11(10.5-11.1min)	Famoxadone
12(10.5-15.0min)	Propargite, Buprofezin, Pyridaben, Spinosyn A&D, Fenpropimorph, Avermectin B_{1b}, Avermectin B_{1a}

UPLC-MS/MS of 72 pesticides in wine



9 Pesticides in Red Wine



Pesticides found in Wine Sample

Azoxystrobin	33 ± 7 µg/L
Carbaryl	2.3 ± 0.7
Carbendazim	1015 ± 235
Cyprodinil	3 ± 1
Fludioxinil	1.3 ± 0.4
Kresoxim-methyl	239 ± 62
Metalaxyl	39 ± 9
Oxadixyl	9 ± 2
Piperonyl butoxide	3 ± 0.5

Conclusions

- QuEChERS modification with PSA/GCB cleanup applied to >200 pesticides and 10 commodities for UPLC-MS/MS analysis
- Effective application of the procedure to the analysis of incurred residues in fresh produce and beverages
- More work needed for faster analysis and to evaluate matrix effects

Acknowledgements

U.S. Environmental Protection Agency

National Pesticide Standard Repository, Fort Meade, MD for pesticide standards, produce samples and expertise (Terry Cole, Dr. Alaa Kamel, Betty Kolbe, Lynda Podhorniak, Dr. Yaorong Qian, Chuck Stafford)

U.S. Food and Drug Administration

Pacific Northwest Regional Laboratory, Bothell, WA for providing produce samples (Dr. Greg Mercer)

U.S. Alcohol and Tobacco Tax and Trade Bureau

for providing wine samples (Michael Webster and Leticia Drakeford)

Thank you for your attention

Please Join Us!

Pesticides Subgroup in Food Community

Currently, the **Chemical Contaminants and Residues in Food Community** is in the process of searching for and identifying potential stakeholders in several areas, such as pesticides.

From the government agency to the private sector or industry, stakeholders help AOAC to understand their analytical needs. This, in turn, helps the Association to better focus its efforts on the areas of greatest interest (priority methods).

Pesticides continue to be an important topic and we seek members including global representatives from **governments**, **academia**, **producers**, **processors**, **distributors**, **importers**, **exporters**, **private laboratories**, **equipment/instrument vendors**, and **consumer groups** working together to develop analytical standards of excellence in the area of pesticides.

The **Pesticide subgroup** will address these needs.

Please visit our website at:

http://www.flworkshop.com/community-1st.html

and join!