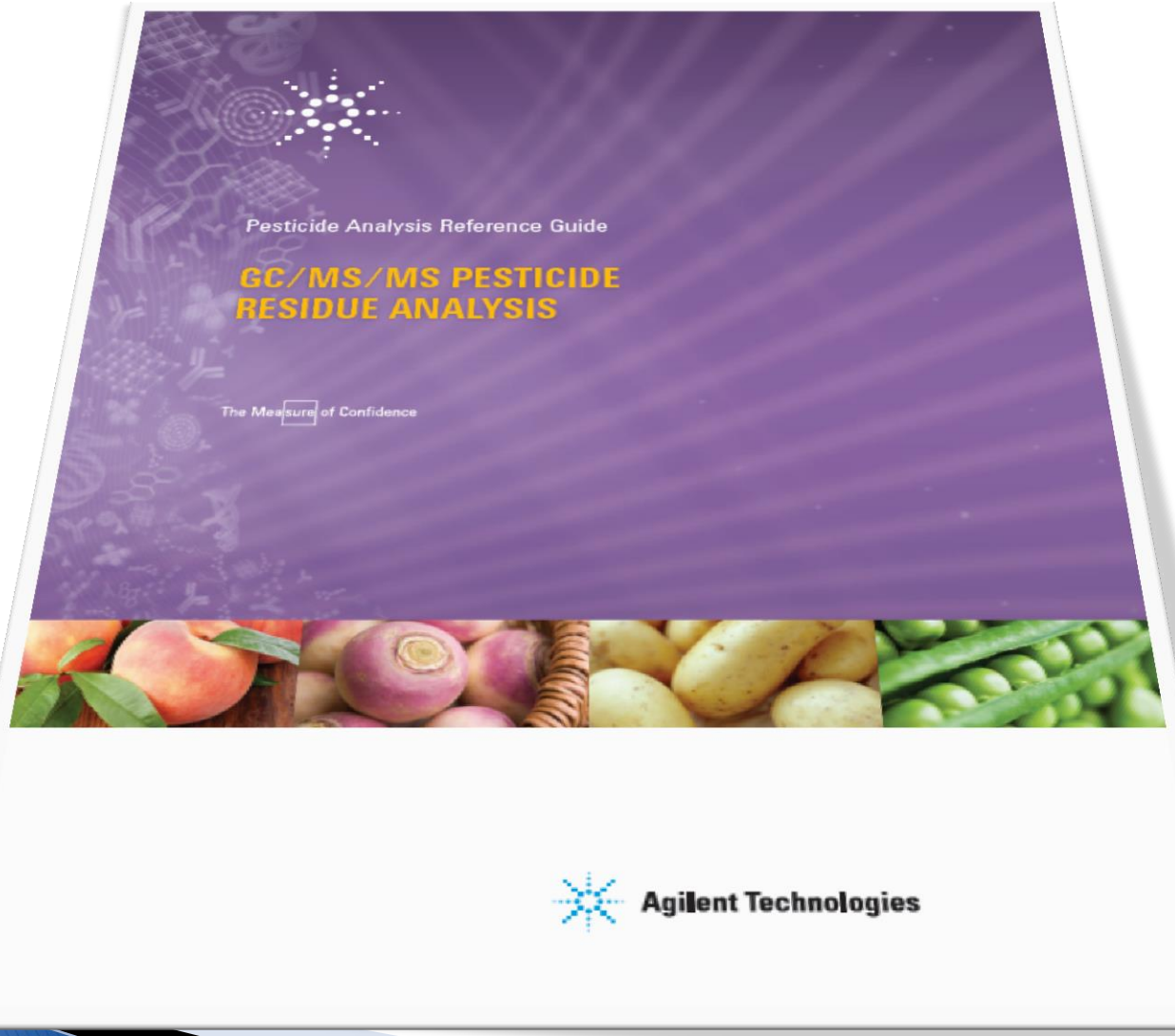
# Introducing a Rugged Core Method for GC/MS/MS Pesticide Residue Analysis

## Offering New Reference Guide for Pesticide GC/MS/MS Analysis

**Katerina Mastovska**

Excellcon International LLC


# New Reference Guide for GC/MS/MS Pesticide Residue Analysis



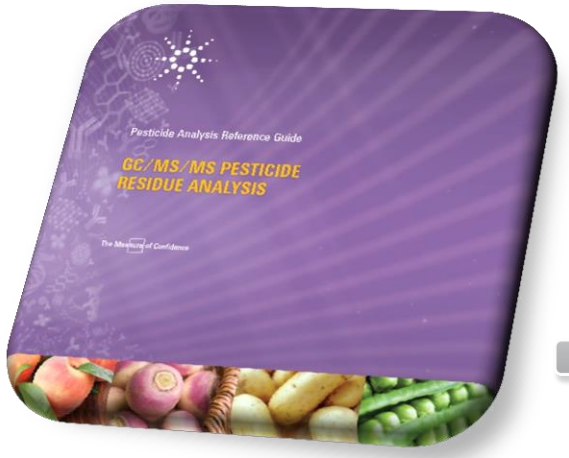
# New Reference Guide for GC/MS/MS Pesticide Residue Analysis

Developed by:

Katerina Mastovska, Ph.D. (Excellcon International LLC)

in collaboration with scientists from:

- California Department of Food and Agriculture
- Florida Department of Agriculture and Consumer Services
- Agilent Technologies



# Reference Guide

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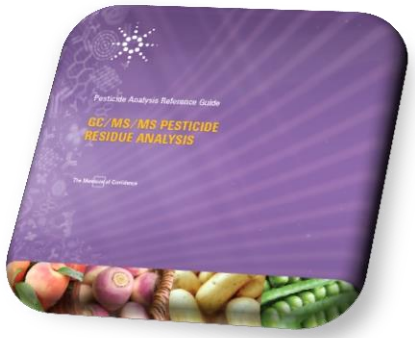




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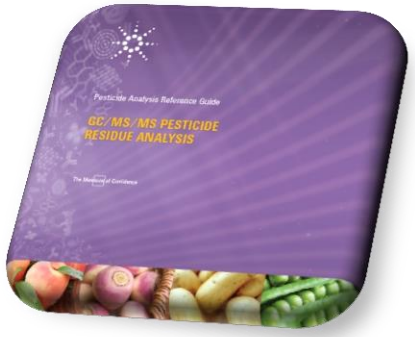




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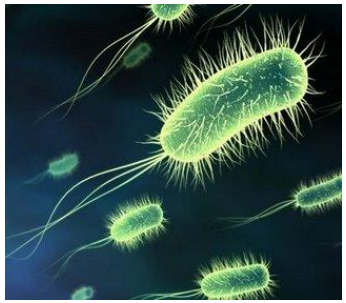
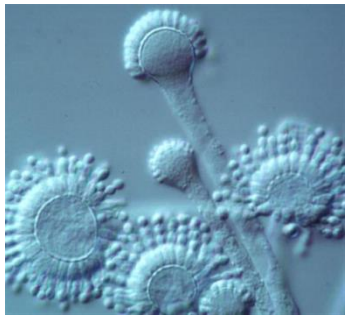
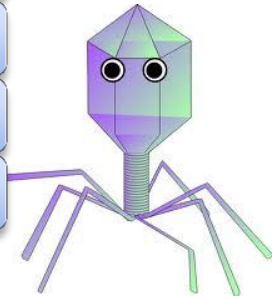
# Pesticides





- Algicides
- Avicides
- Bactericides
- Fungicides
- Herbicides
- Insecticides
- Miticides/acaricides
- Molluscicides
- Nematicides
- Rodenticides
- Virucides
- Plant growth regulators
- Defoliants
- Desiccants



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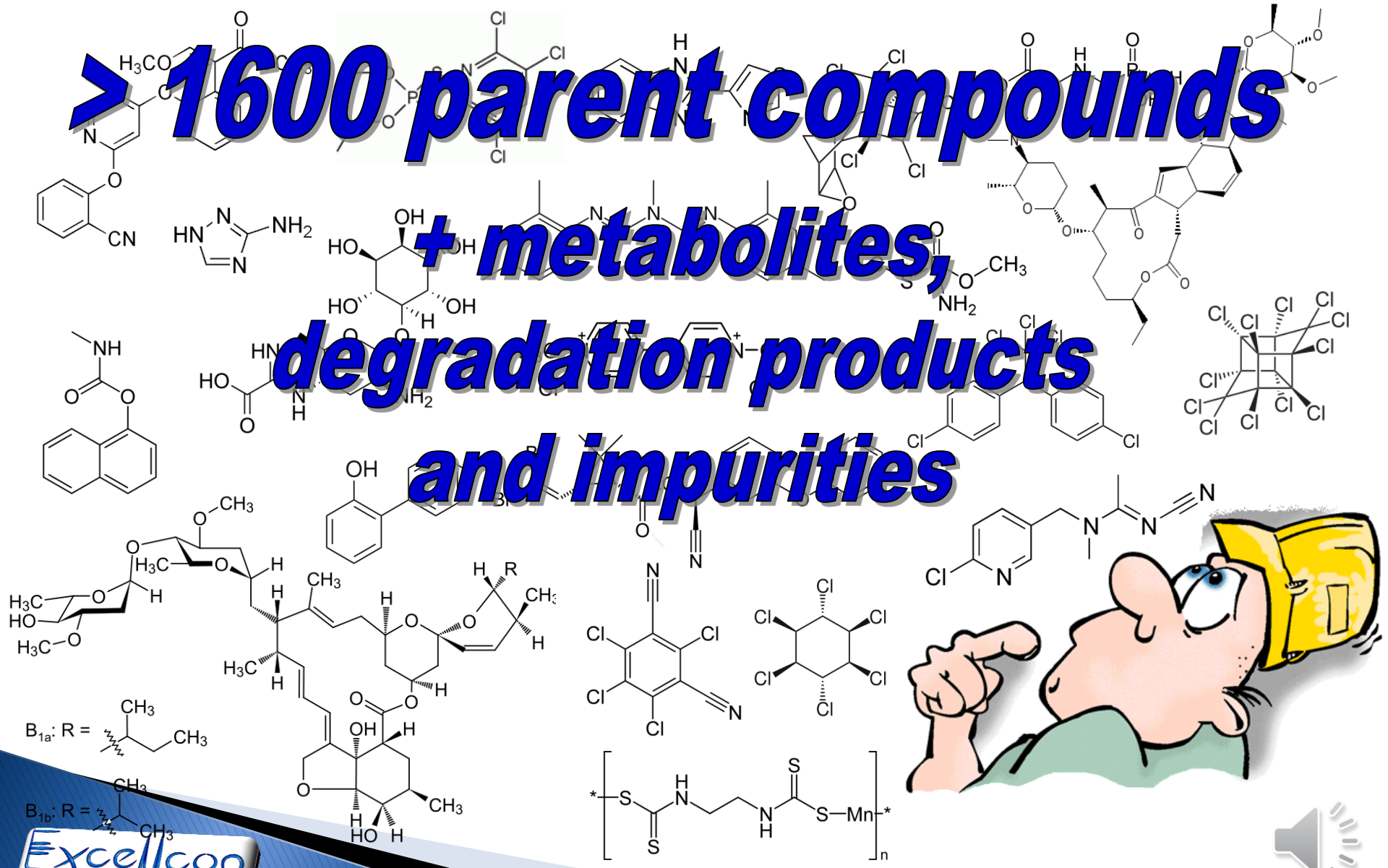
# Pesticides


LEAST HARMFUL
SORT OF HARMFUL
KIND O' DANGEROUS
DEFINITELY HARMFUL
QUITE DANGEROUS
OMIGOSH REALLY DANGEROUS

# How Many Pesticides Are There?

**>1600 parent compounds**

**+ metabolites, degradation products and impurities**



The Pesticide Manual, 16th Edition, 2012

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# Pesticide Multiresidue Analysis

## WHY?

Need for analysis of a large number of analytes

Unknown “history” of the analyzed sample (field treatment, post-harvest application, contamination)

Economic aspects (costs, time, and labor)

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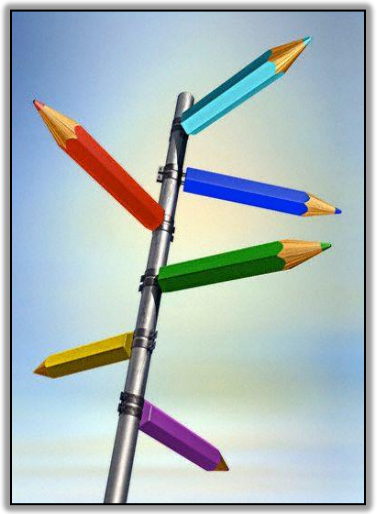
# Pesticide Multiresidue Analysis



## Classical approach:

Multiple methods and techniques to cover different analytes/classes of interest

e.g.: GC-FPD, NPD, ECD, ELCD
LC-UV or LC-FLD

## Modern approach:

Multi-analyte methods with simultaneous quantification and identification using

**GC-MS** and **LC-MS**, especially with **MS/MS** capabilities for increased selectivity

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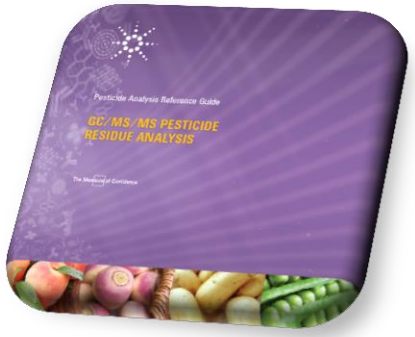
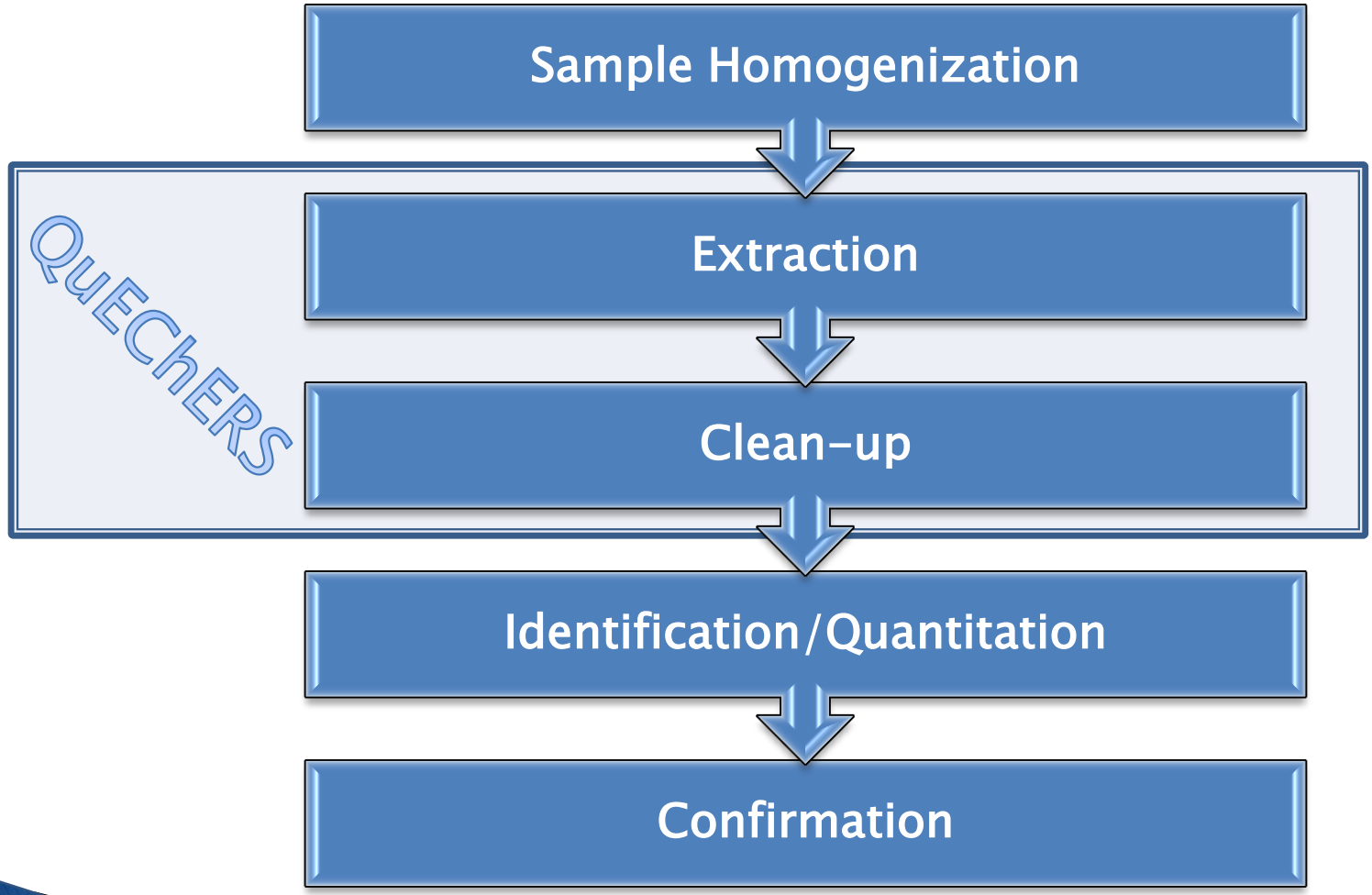


# Pesticide Multiresidue Analysis




QuEChERS = Quick, Easy, Cheap, Effective, Rugged, and Safe

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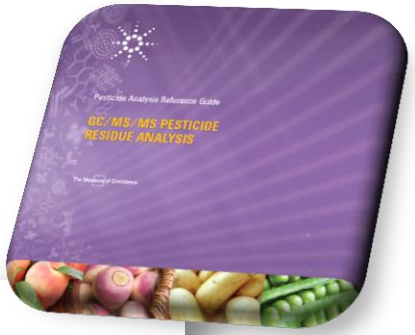




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# Pesticides with Special Consideration in QuEChERS

| Pesticide type | Examples of compounds | Problem | Solution |
|---|---|---|---|
| Base-sensitive | Captan, chlorothalonil, dichlofluanid, dicofol, folpet, tolylfluanid | Degradation in the presence of basic | Use buffering during the extraction/partition step and acidify the final extract (at about 0.1% acetic or formic acid) (prepare stock solutions in |
| Lipophilic | Aldrin, chlordan DDE, DDT, dicof endosulfan, end hexachlorobenzene, mirex, pentachlo pentachloroanis pentachlorothioa permethrin, prot quintozene | layer | content, decrease the educing sample size to ated from the acetonitrile extract), thus increase the partition of lipophilic pesticides Use a suitable QC standard losses of lipophilic gher fat content. Use accurate quantitation or overies, which are typically consistent for a given sample type. |
| Planar | Carbendazim, ch coumaphos, cyp hexachlorobenz pentachloroanili pentachlorothioanisole (MF-SPS), thiabendazole | | eanup, especially for gher amounts of chlorophyll ly pigmented matrices, use (*e.g.* 7.5 mg per mL extract), nts in the extract but gives acceptable recoveries for planar pesticides. Use a suitable QC standard (*e.g.* $d_{10}$-anthracene or $d_{10}$-phenanthrene) to monitor losses of planar compounds if GCB is used. |

| Pesticide type | Exar |
|---|---|
| Acidic | Aryl free feno fluaz dica herb imaz imaz phen 2,4-D MCP 2,4,5 (clop triclo acids |
| Basic | Amir pym |
| Acid-sensitive | Amit carb herb azim chlo etho halo meth rims meth trias |

Acidic

Basic

Acid-sensitive

Base-sensitive

Lipophilic

Planar

*cides (captan, folpet, dicofol are also known to degrade in the GC system, thus their analysis is or their degradation alimide (THPI), henylsulfonyldiamid ylsulfonyldiamide e, DMST), and 4,4'-dichlorobenzophenone, respectively. Unfortunately, tabolites are not part of xcept for THPI in captan modities in the US or due definition. Thus in erance, monitoring of these compounds can mostly serve only as an indication of the for compliance purposes.*

carbamate and phenolic metabolites.

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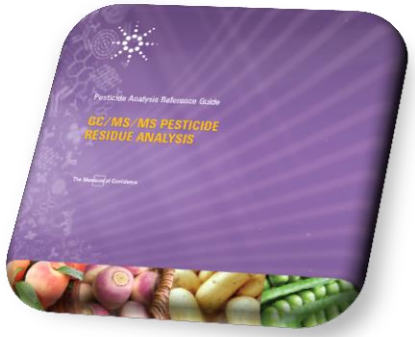




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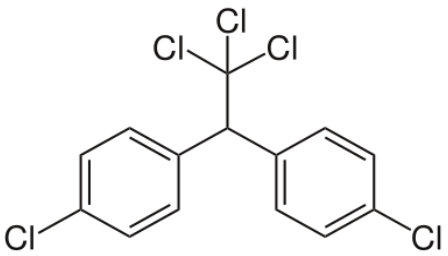
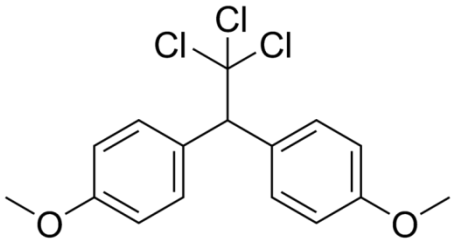
# Use of Internal Standards

Pre- or post-extraction addition to improve precision and trueness

| ISTD | *p,p'*-DDT | | *p,p'*-Methoxychlor | |
|---|---|---|---|---|
| | Mean Accuracy (%) | RSD (%) | Mean Accuracy (%) | RSD (%) |
| None | 95.5 | 14 | 94.3 | 13 |
| Triphenyl phosphate | 100 | 7.8 | 98.0 | 6.9 |
| $^{13}C_{12}$ – *p,p'*-DDT | 100 | 1.5 | 98.3 | 2.0 |

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K. Mastovska, Agilent Application Note 5991-1054EN

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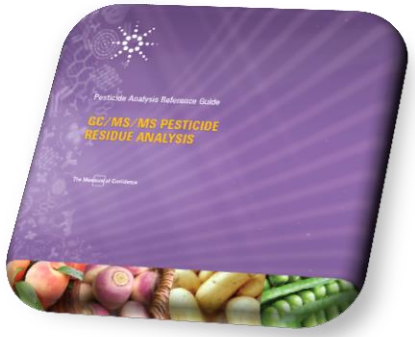




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# Matrix Effects in GC

**Adverse phenomena caused by unavoidable presence of co-extracted matrix components**

False negative results due to co-elution (masking of analytes)

False positive or overestimated results due to co-elution

Overestimation or underestimation of the results due to signal enhancement or diminishment

## INACCURATE QUANTITATION

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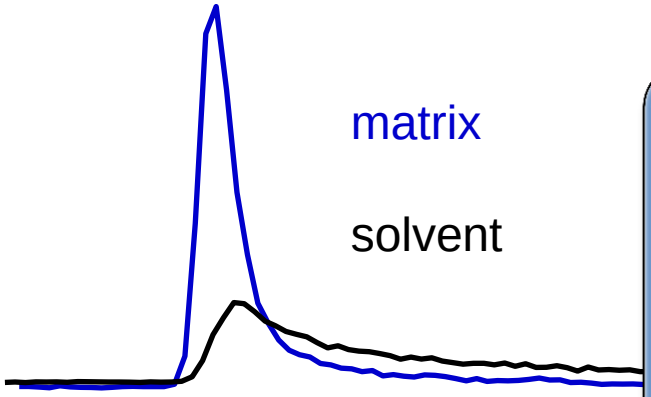
# Matrix-Induced Response Enhancement

**Reduced degradation and/or adsorption of susceptible analytes in the presence of matrix components that tend to block active sites in the inlet and column**




**Inaccurate quantitation when analyte solutions in matrix-free solvent are used for calibration**

**(overestimation of the results)**

Erney *et al.*, *J. Chromatogr. A* **638** (1993) 57-63

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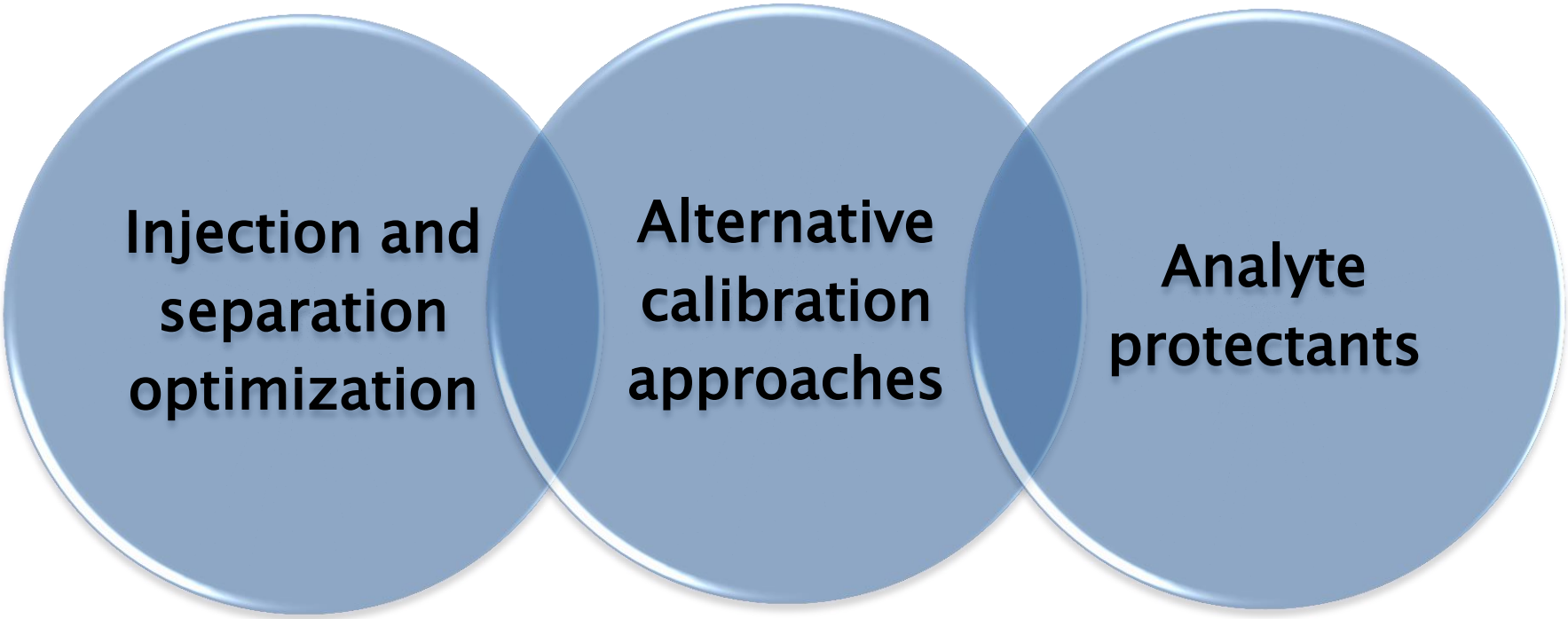
# Matrix-Induced Response Enhancement

## Practical Solutions



# GC Injection Techniques

## Multi-Mode Inlet (MMI) options:

- Hot split/splitless
- Cold split/splitless
- Pulsed split/splitless
- PTV solvent vent

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# Calibration Approaches

- Solvent-based standards
- Matrix-matched standards
- Isotopically labeled internal standards
- Standard addition

# Analyte Protectants (AP)

**Compounds that strongly interact with the active sites in the GC system, thus protecting susceptible analytes against adsorption and/or degradation**

M. Anastassiades, K. Mastovska, S.J. Lehotay, *J. Chromatogr. A* 1015 (2003) 163–184

**<u>Concept idea</u> = take advantage of the response enhancement effect rather than eliminate it**

matrix extract + AP
solvent standard + AP

**Same response enhancement**

**Equalization of the matrix-induced response enhancement effect**

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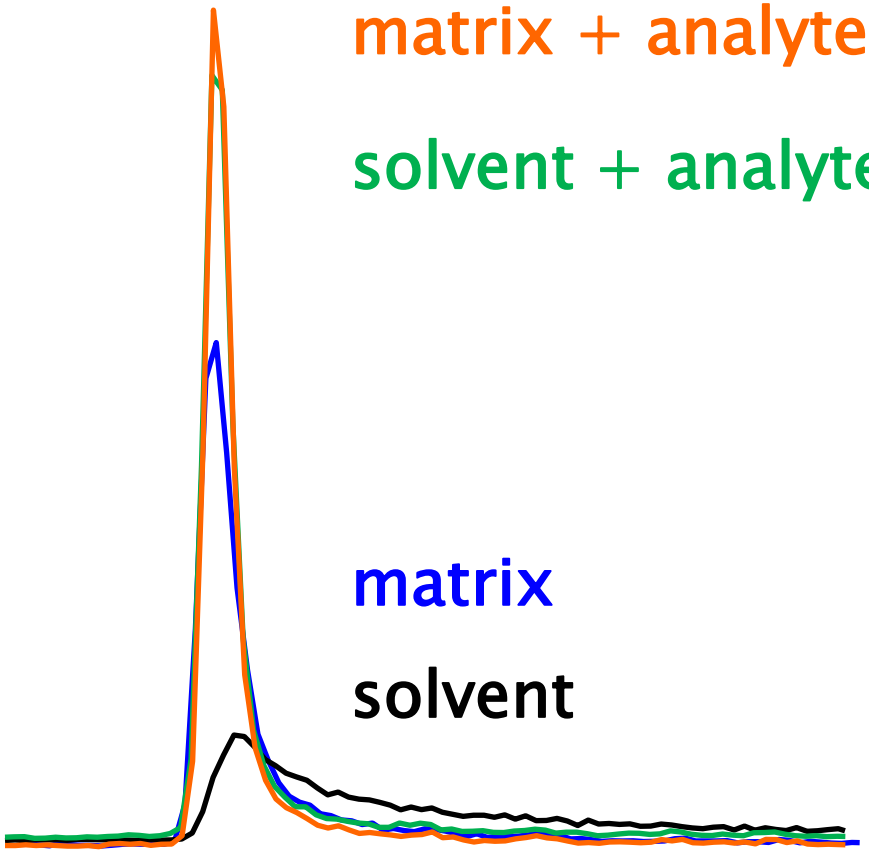
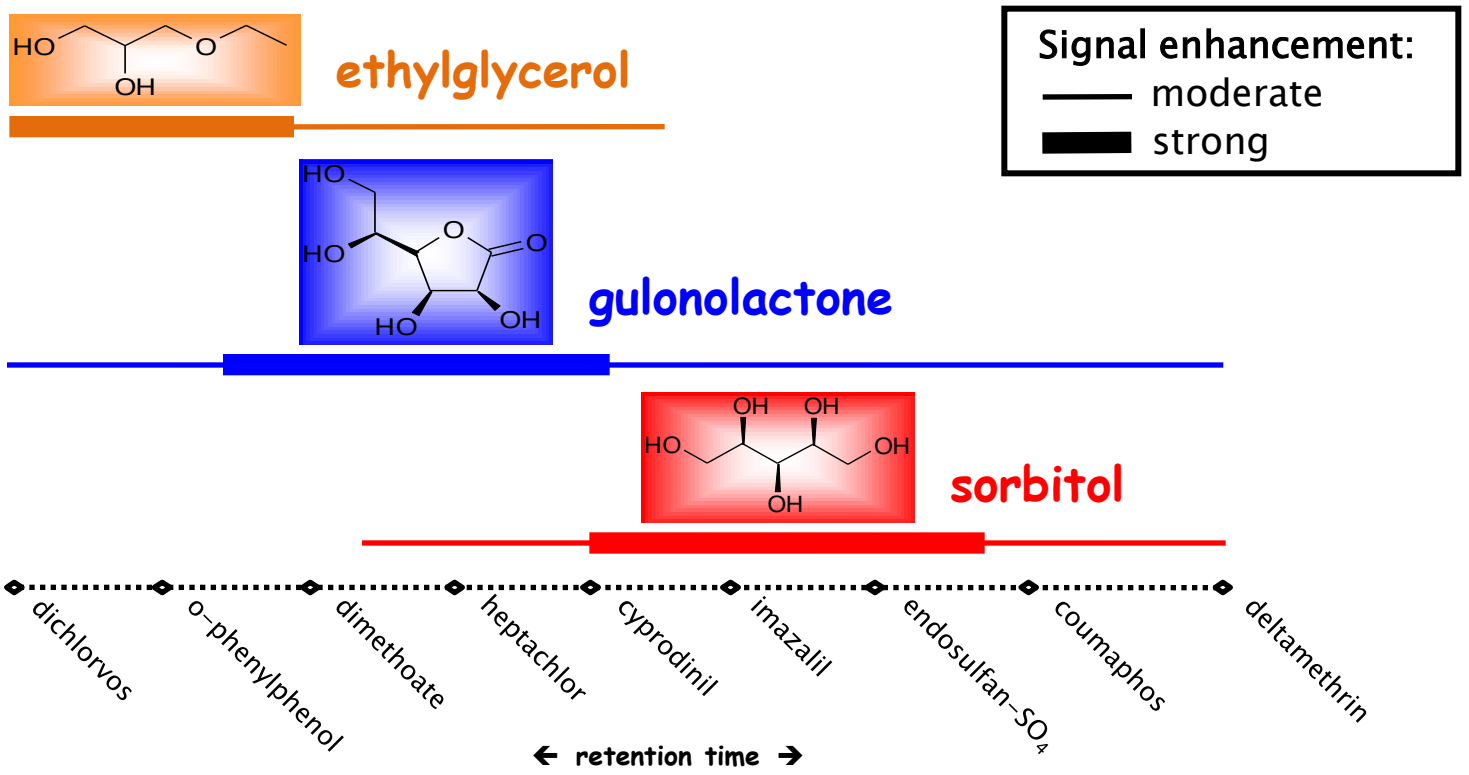
# Analyte Protectants (AP)



# Analyte Protectants (AP)

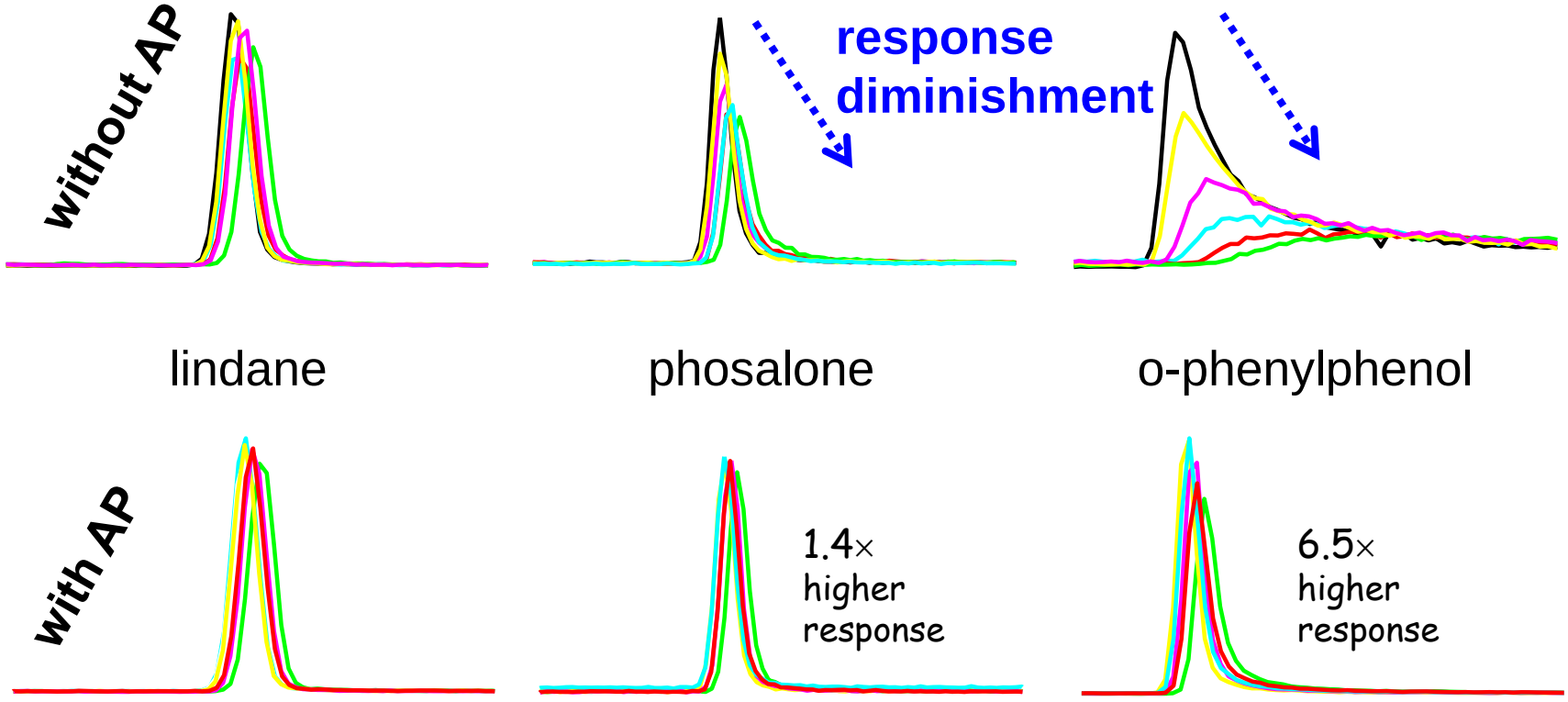
Combination of analyte protectants for GC pesticide analysis – inexpensive, nontoxic, and safe to use routinely in GC–MS




K. Mastovska, S.J. Lehotay, M. Anastassiades, *Anal. Chem.* 77 (2005) 8129.

# Analyte Protectants (AP)

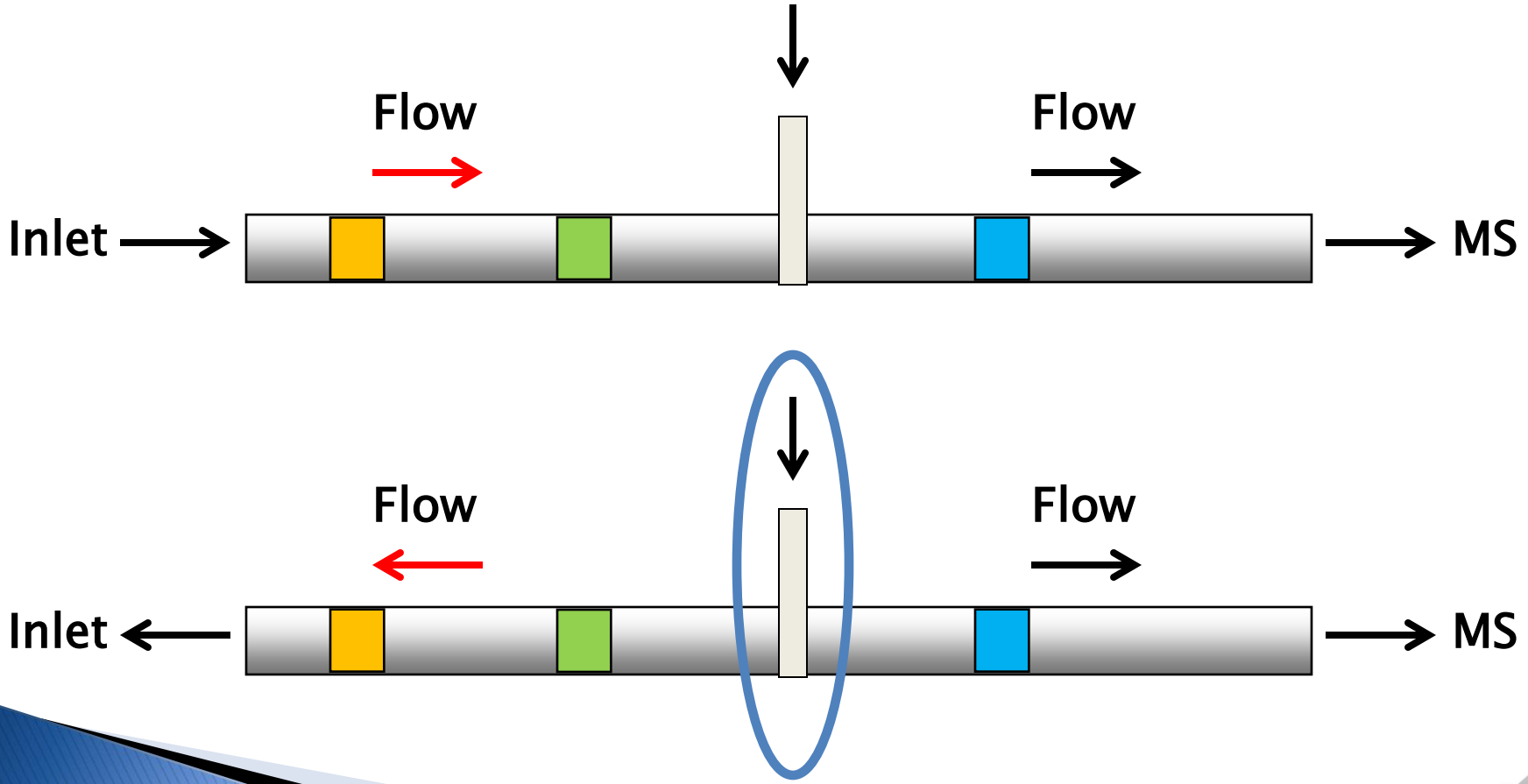
**Deactivation in Every Injection!**




K. Mastovska, S.J. Lehotay, M. Anastassiades
*Anal. Chem.* 77 (2005) 8129.

# Column Backflushing

Backflushing can eliminate less volatile matrix components from the GC column by reversing the column flow at a pressure junction point:



# Column Backflushing Configurations

- **Post-Run Backflushing**
  - begins after the last analyte has been detected
  - the entire column is backflushed
  - typically uses a short restriction capillary installed at the column outlet
- **Concurrent Backflushing**
  - begins after the last analyte has eluted from the first "column"
    - retention gap = a short uncoated capillary
    - mid-column set-up (e.g. two 15-m columns)
    - short column = a short coated capillary

Column 1: HP 5-MS UI, 5 m x 0.25 mm x 0.25 µm
Column 2: HP 5-MS UI, 15 m x 0.25 mm x 0.25 µm

K. Mastovska and P.L. Wylie,
*J. Chromatogr. A* **1265** (2012) 155-164

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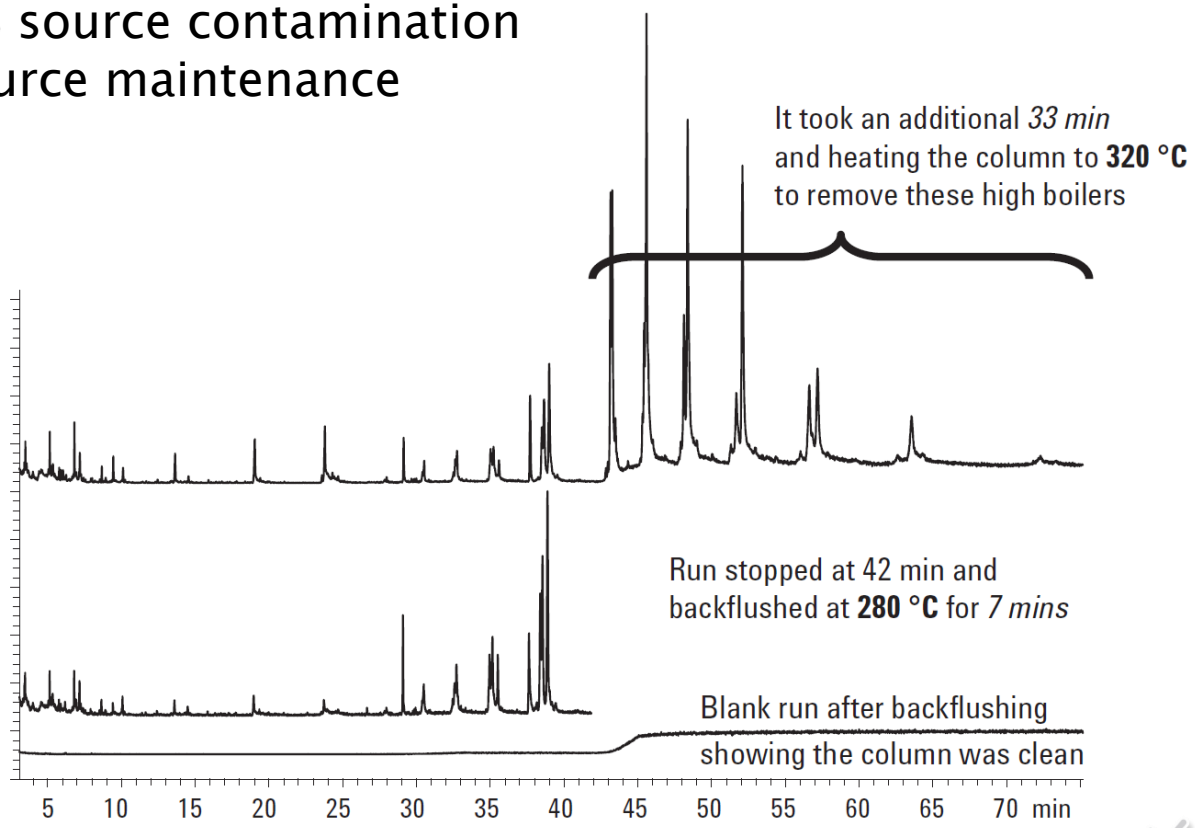
# Column Backflushing – Benefits

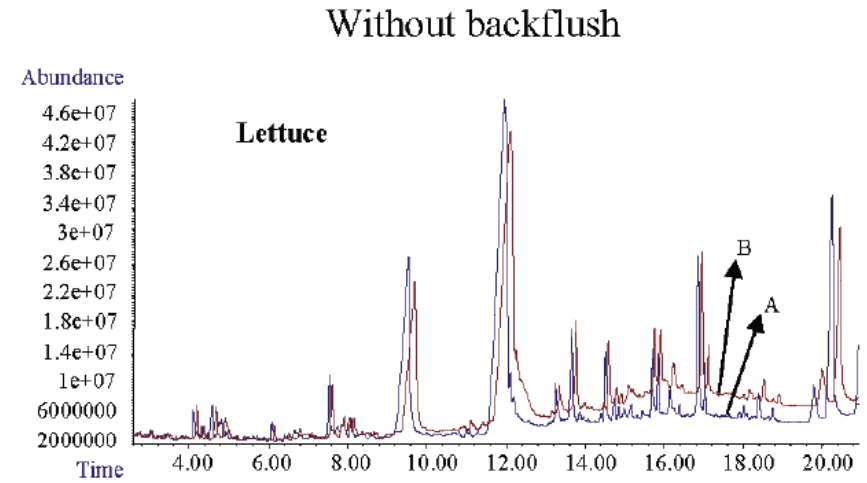
- Elimination of long “baked out” at a high temperature to remove less volatile, late eluting matrix components
- Reduced analysis time
- Increased column life time
- Prevention of the MS source contamination
- Less frequent MS source maintenance

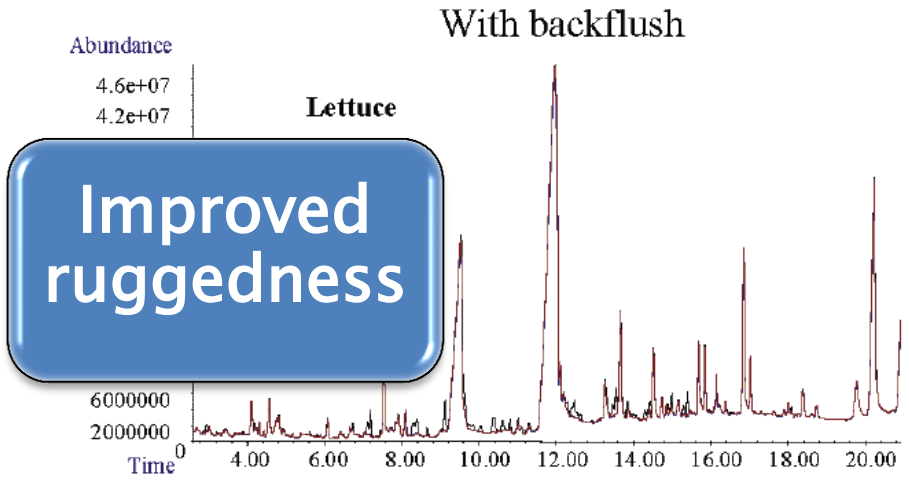
**Increased sample throughput**



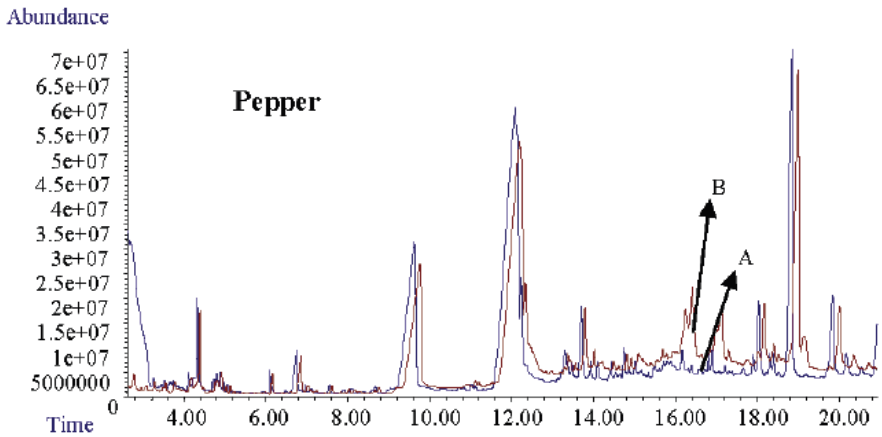

C.-K. Meng, Agilent Application Brief 5989-6018EN

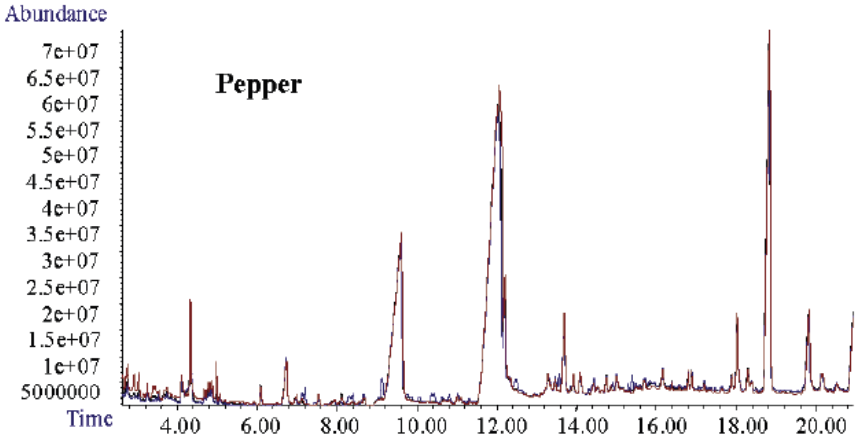
# Column Backflushing – Benefits




Improved ruggedness

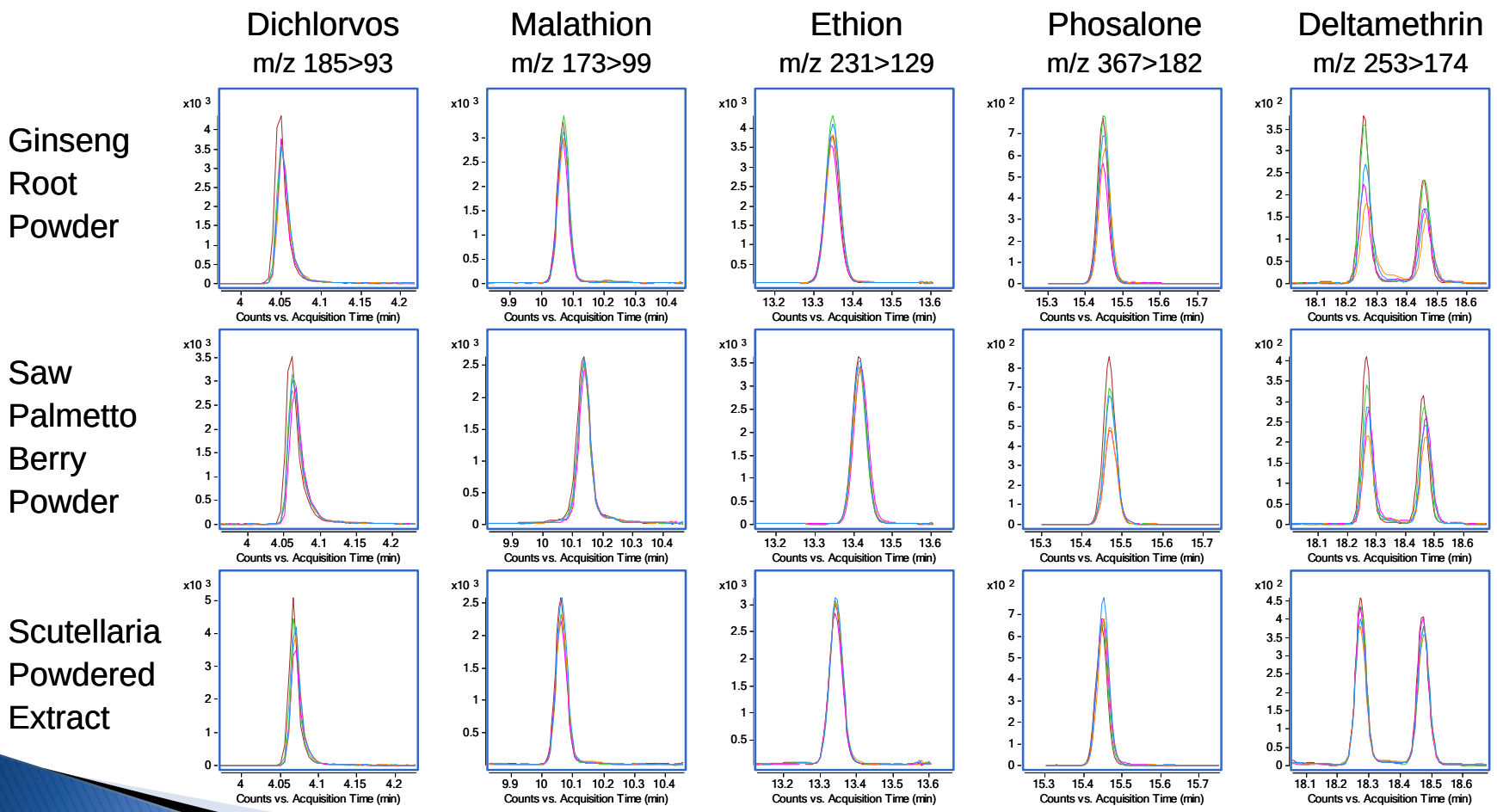



M. Mezcua, M.A. Martinez-Uroz, P.L. Wylie, A.R. Fernandez-Alba, *J. AOAC Int.* **92** (2009) 1790-1806.

# Column Backflushing – Benefits

**Overlays of GC-MS/MS chromatograms for selected analytes (at 50 ng/g ) obtained within a 2.5-day sequence of 125 dietary supplement sample injections :**




K. Mastovska and P.L. Wylie,
*J. Chromatogr. A* **1265** (2012) 155–164

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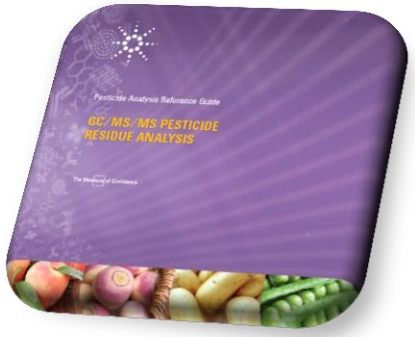




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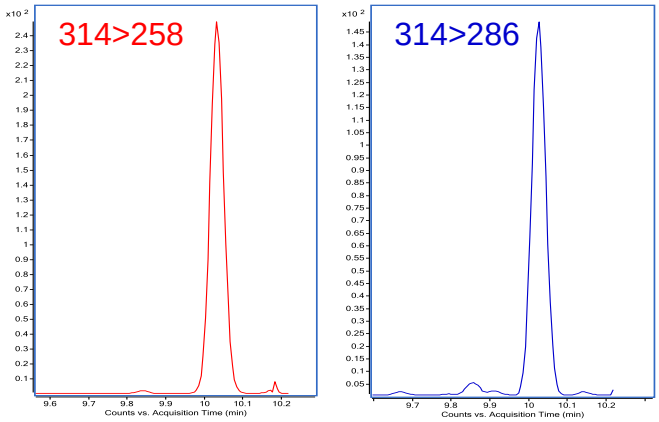
# Why Triple Quadrupole GC-MS/MS?

- Selectivity
- Sensitivity
- Ruggedness




**0.5 ng/mL chlorpyrifos in ginseng root extract (5 ng/g in the sample) using Agilent 7000B GC-QQQ**

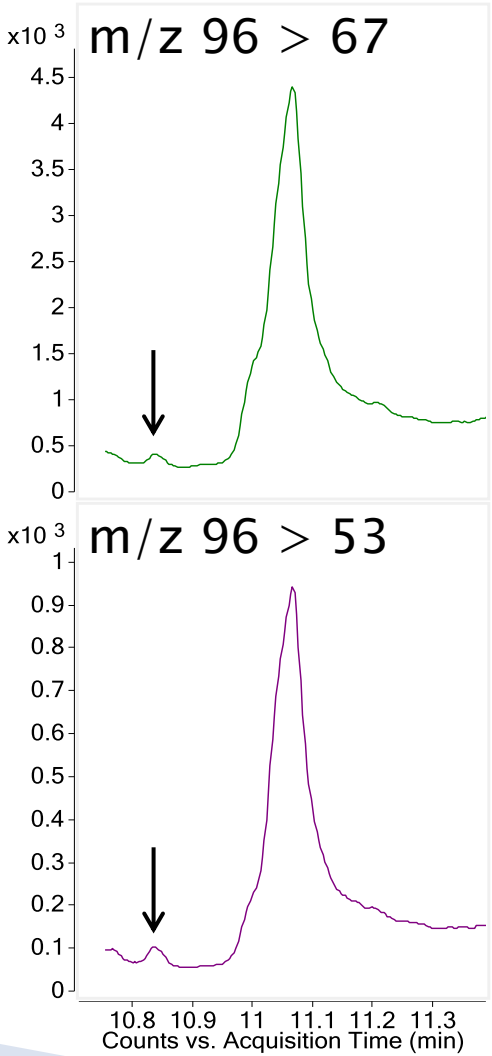
## MS/MS is TARGETED ANALYSIS

- Analyte-specific conditions required
- We don't see what we don't look for!

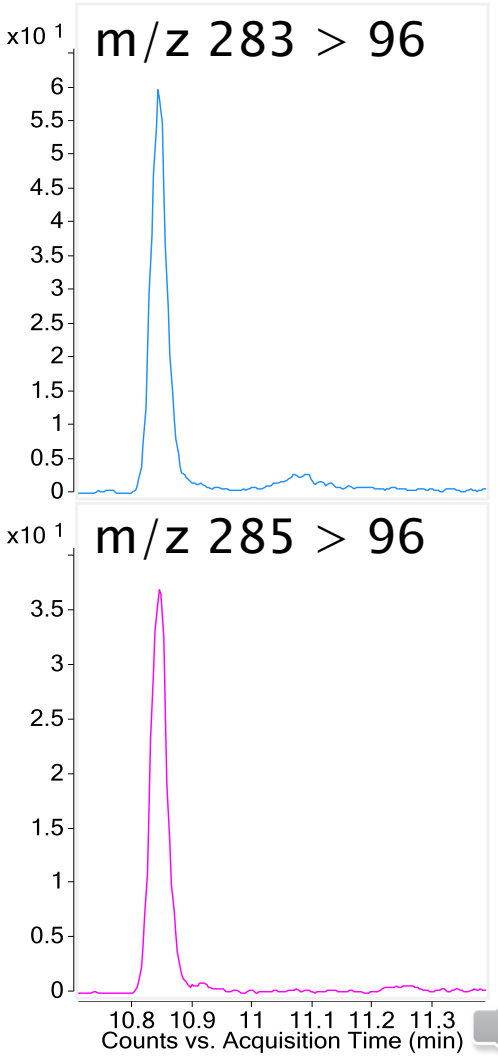
# Selectivity in GC–EI–MS/MS

Procymidone
in spinach
(at 5 ng/g)

(A) Low selectivity




(B) High selectivity

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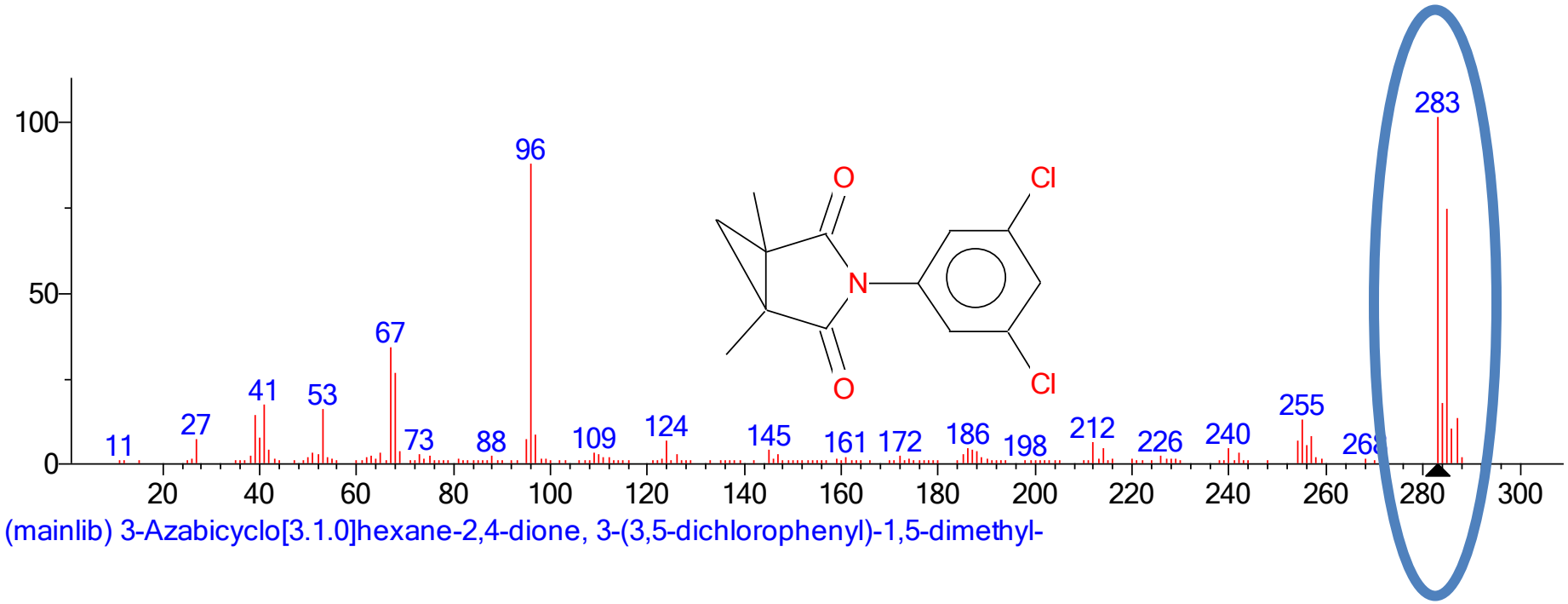
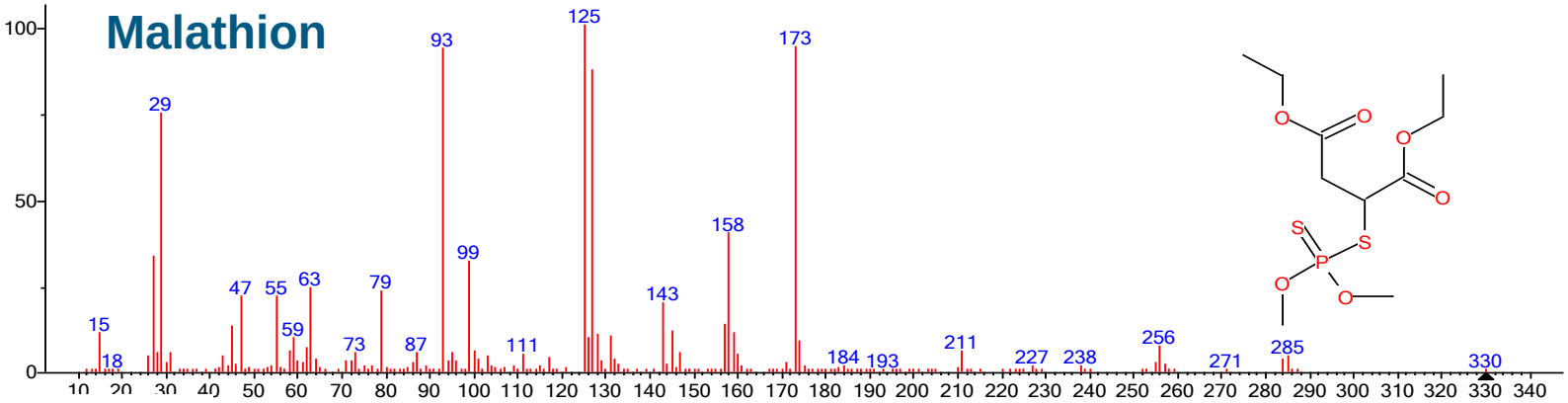
# Selectivity in GC-EI-MS/MS

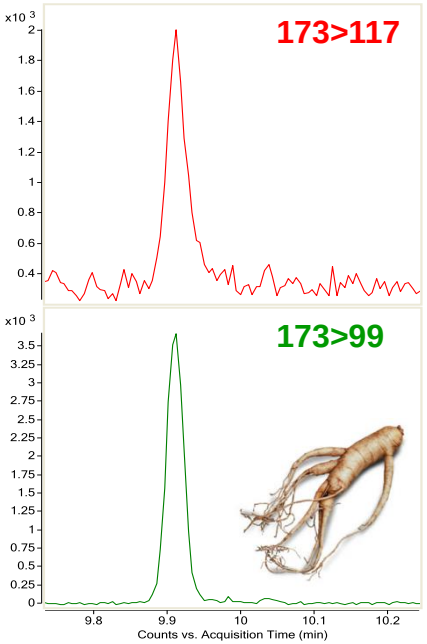
Procymidone EI-MS spectrum (NIST)

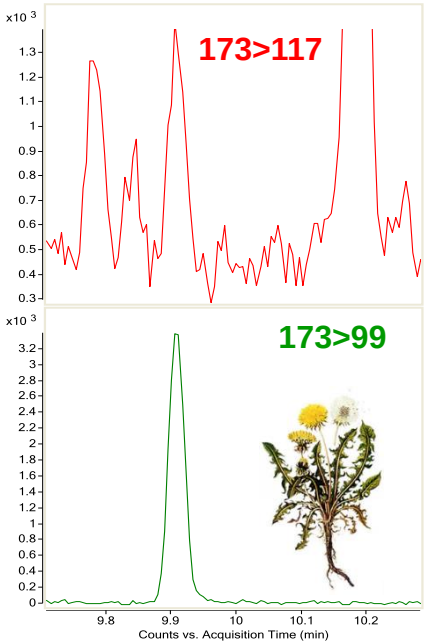


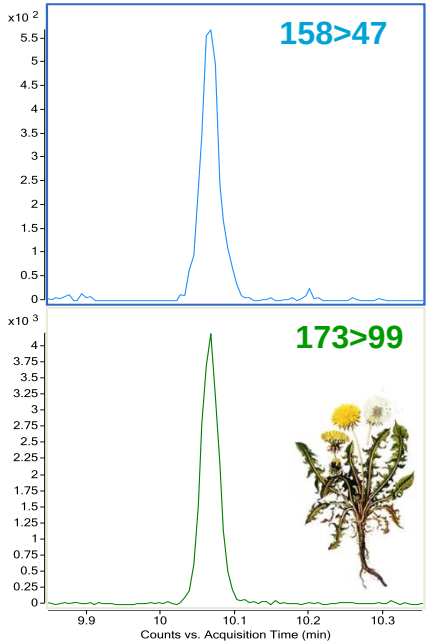

(mainlib) 3-Azabicyclo[3.1.0]hexane-2,4-dione, 3-(3,5-dichlorophenyl)-1,5-dimethyl-

# Selectivity in GC–EI–MS/MS




K. Mastovska and P.L. Wylie,
*J. Chromatogr. A* **1265** (2012) 155–164

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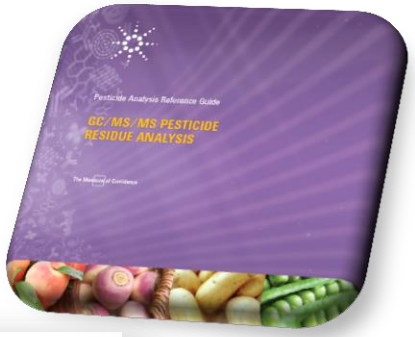




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# MS/MS Optimization

(1) Full scan

→ Selection of precursor ions

(2) Product ion scans at various CEs

→ Selection of MS/MS transitions

(3) Optimization of CEs for each MRM

(4) Evaluation of MRMs in various matrices

→ Selection of 2-3 MRMs for routine analysis

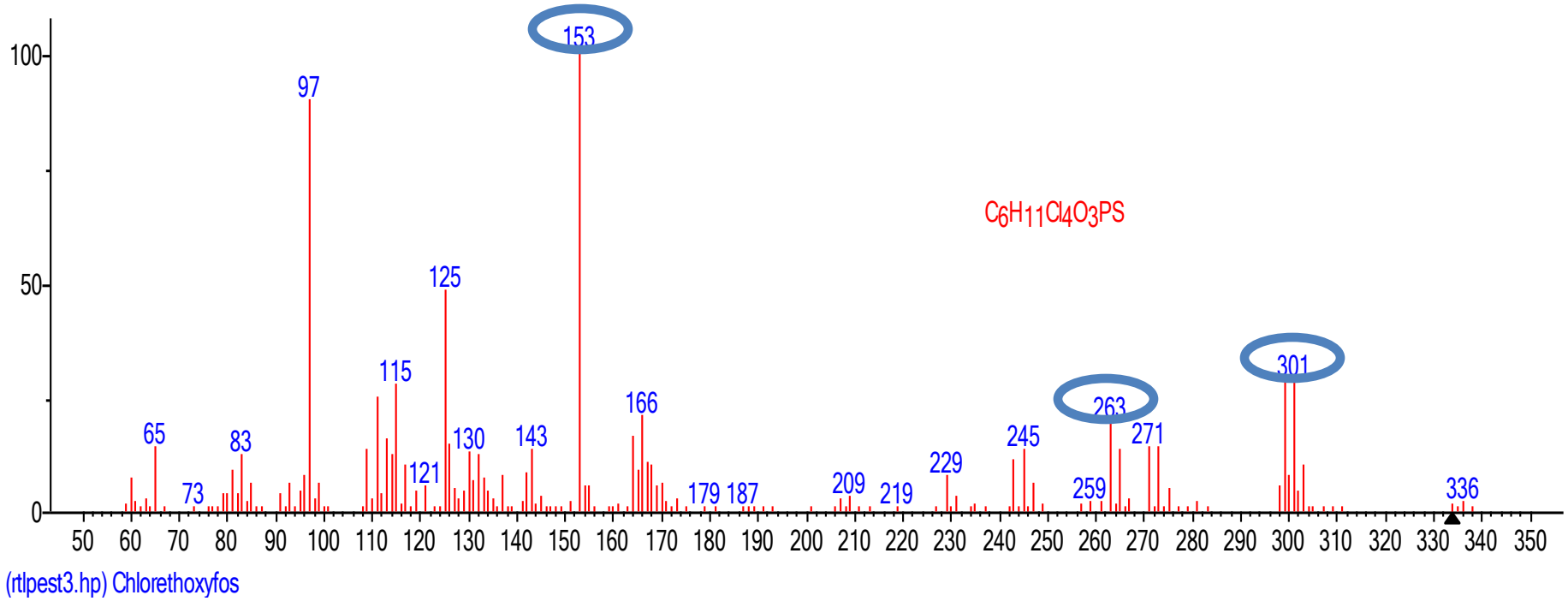
Note: Steps (1)-(3) can be eliminated by using Pesticides and Environmental Pollutants MRM Database (G9250AA)



# MS/MS Optimization

(1) Full scan

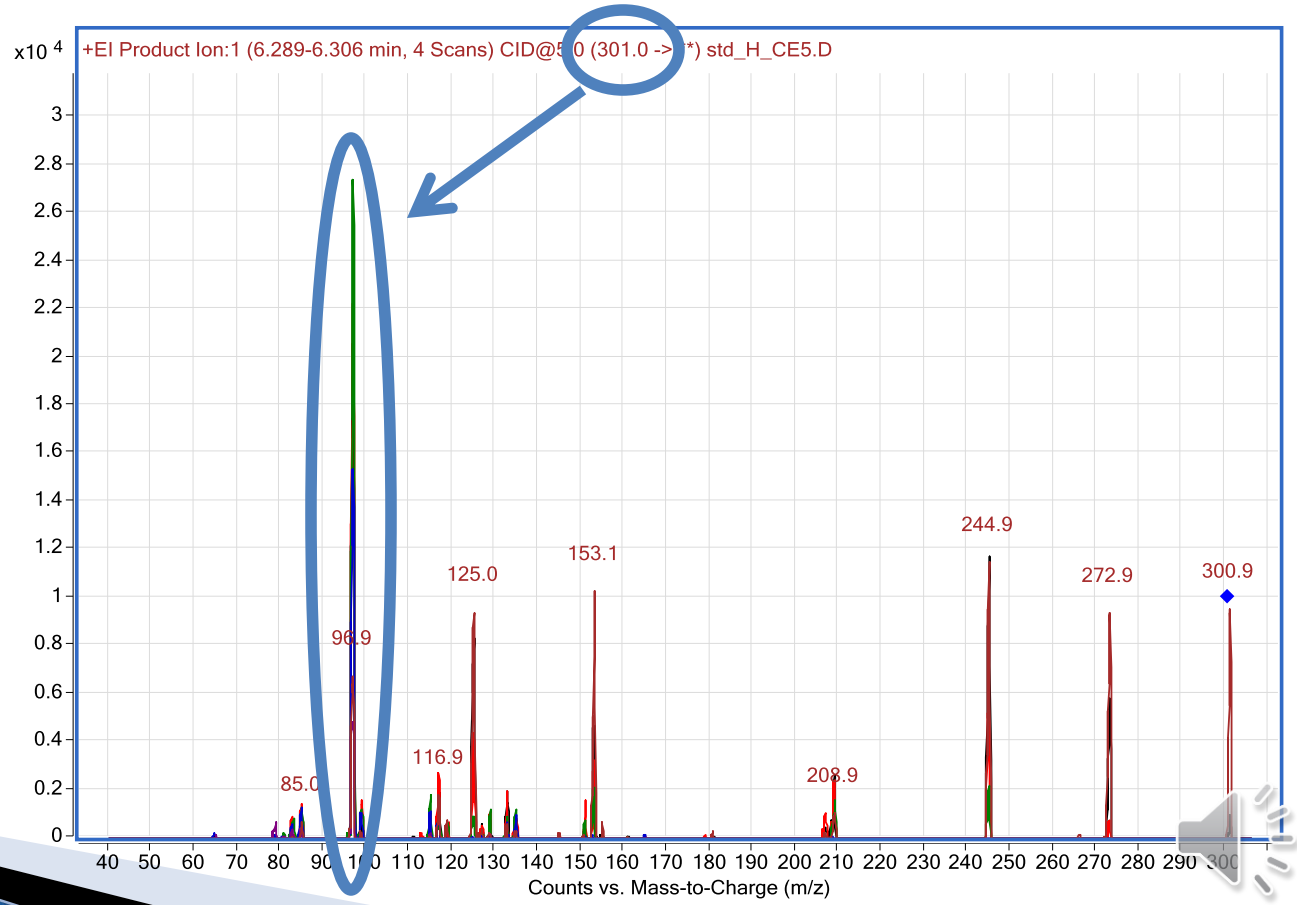
→ Selection of precursor ions



# MS/MS Optimization

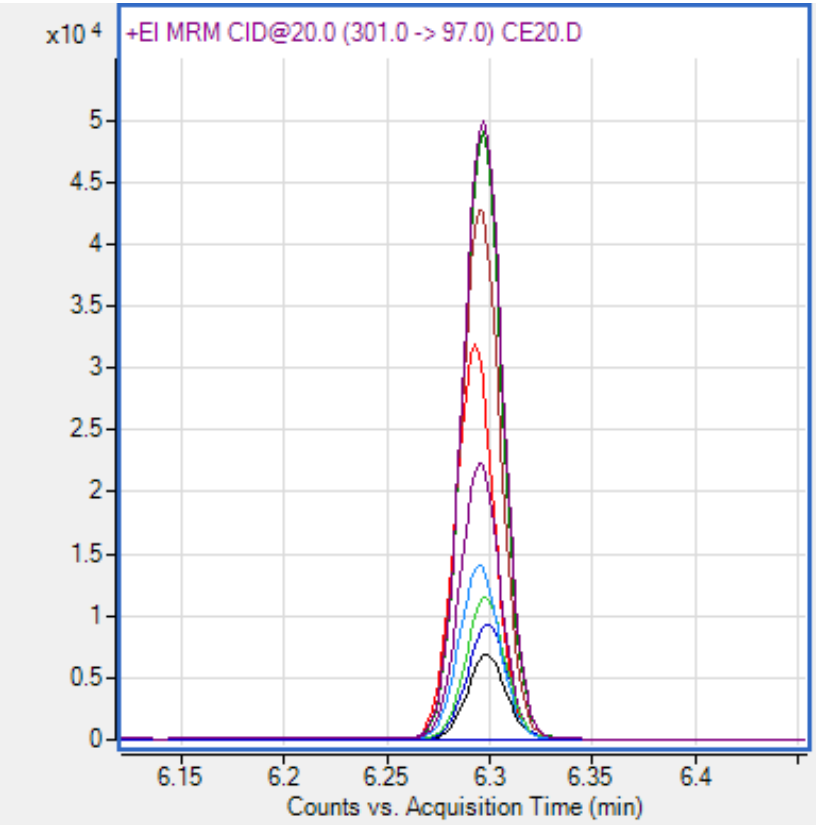
(2) Product ion scans at various CEs

→ Selection of MS/MS transitions



# MS/MS Optimization

(3) Optimization of CEs for each MRM



# MS/MS Optimization

(1) Full scan

→ Selection of precursor ions

(2) Product ion scans at various CEs

→ Selection of MS/MS transitions

(3) Optimization of CEs for each MRM

(4) Evaluation of MRMs in various matrices

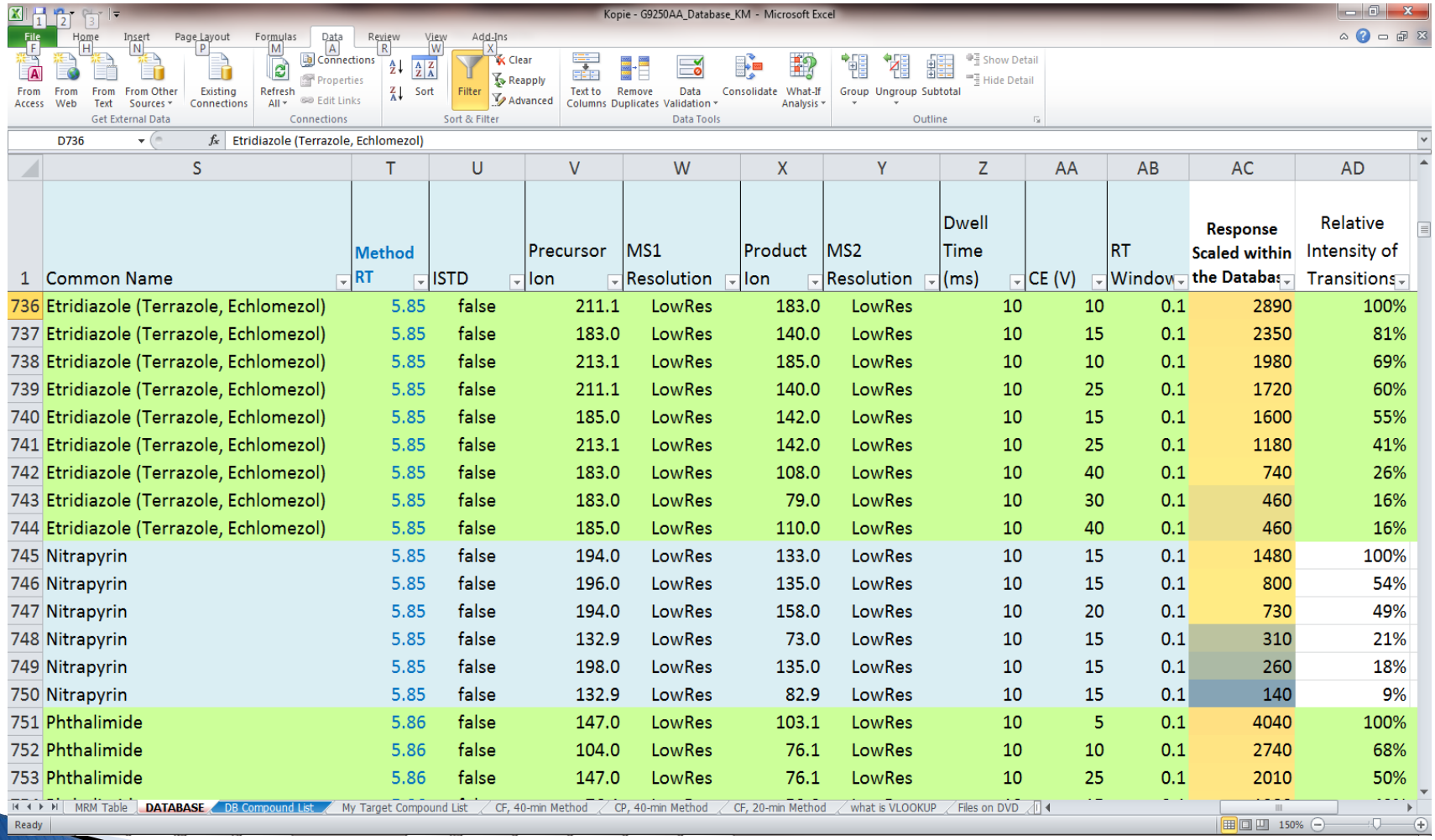
→ Selection of 2-3 MRMs for routine analysis

Note: Steps (1)-(3) can be eliminated by using Pesticides and Environmental Pollutants MRM Database (G9250AA)



# MS/MS Optimization

Etridiazole: Pesticides and Environmental Pollutants MRM Database (G9250AA)



Kopie - G9250AA_Database_KM - Microsoft Excel

D736 | Etridiazole (Terrazole, Echlomezol)

| 1 | Common Name | Method RT | ISTD | Precursor Ion | MS1 Resolution | Product Ion | MS2 Resolution | Dwell Time (ms) | CE (V) | RT Window | Response Scaled within the Databas | Relative Intensity of Transitions |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 736 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 211.1 | LowRes | 183.0 | LowRes | 10 | 10 | 0.1 | 2890 | 100% |
| 737 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 183.0 | LowRes | 140.0 | LowRes | 10 | 15 | 0.1 | 2350 | 81% |
| 738 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 213.1 | LowRes | 185.0 | LowRes | 10 | 10 | 0.1 | 1980 | 69% |
| 739 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 211.1 | LowRes | 140.0 | LowRes | 10 | 25 | 0.1 | 1720 | 60% |
| 740 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 185.0 | LowRes | 142.0 | LowRes | 10 | 15 | 0.1 | 1600 | 55% |
| 741 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 213.1 | LowRes | 142.0 | LowRes | 10 | 25 | 0.1 | 1180 | 41% |
| 742 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 183.0 | LowRes | 108.0 | LowRes | 10 | 40 | 0.1 | 740 | 26% |
| 743 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 183.0 | LowRes | 79.0 | LowRes | 10 | 30 | 0.1 | 460 | 16% |
| 744 | Etridiazole (Terrazole, Echlomezol) | 5.85 | false | 185.0 | LowRes | 110.0 | LowRes | 10 | 40 | 0.1 | 460 | 16% |
| 745 | Nitrapyrin | 5.85 | false | 194.0 | LowRes | 133.0 | LowRes | 10 | 15 | 0.1 | 1480 | 100% |
| 746 | Nitrapyrin | 5.85 | false | 196.0 | LowRes | 135.0 | LowRes | 10 | 15 | 0.1 | 800 | 54% |
| 747 | Nitrapyrin | 5.85 | false | 194.0 | LowRes | 158.0 | LowRes | 10 | 20 | 0.1 | 730 | 49% |
| 748 | Nitrapyrin | 5.85 | false | 132.9 | LowRes | 73.0 | LowRes | 10 | 15 | 0.1 | 310 | 21% |
| 749 | Nitrapyrin | 5.85 | false | 198.0 | LowRes | 135.0 | LowRes | 10 | 15 | 0.1 | 260 | 18% |
| 750 | Nitrapyrin | 5.85 | false | 132.9 | LowRes | 82.9 | LowRes | 10 | 15 | 0.1 | 140 | 9% |
| 751 | Phthalimide | 5.86 | false | 147.0 | LowRes | 103.1 | LowRes | 10 | 5 | 0.1 | 4040 | 100% |
| 752 | Phthalimide | 5.86 | false | 104.0 | LowRes | 76.1 | LowRes | 10 | 10 | 0.1 | 2740 | 68% |
| 753 | Phthalimide | 5.86 | false | 147.0 | LowRes | 76.1 | LowRes | 10 | 25 | 0.1 | 2010 | 50% |

MRM Table | DATABASE | DB Compound List | My Target Compound List | CF, 40-min Method | CP, 40-min Method | CF, 20-min Method | what is VLOOKUP | Files on DVD

Ready

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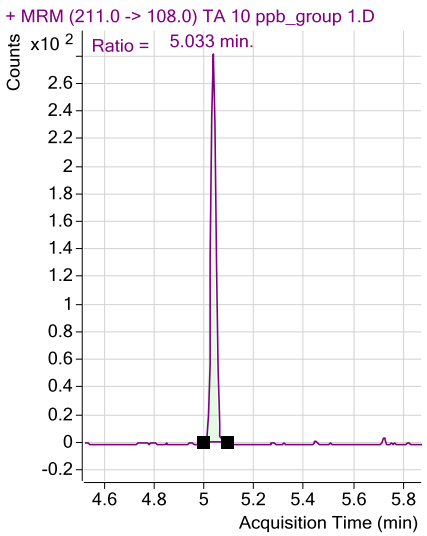
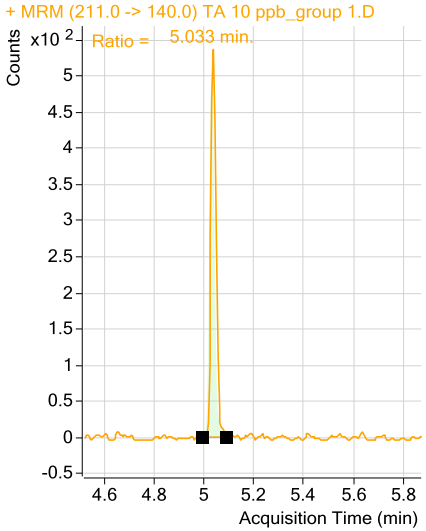
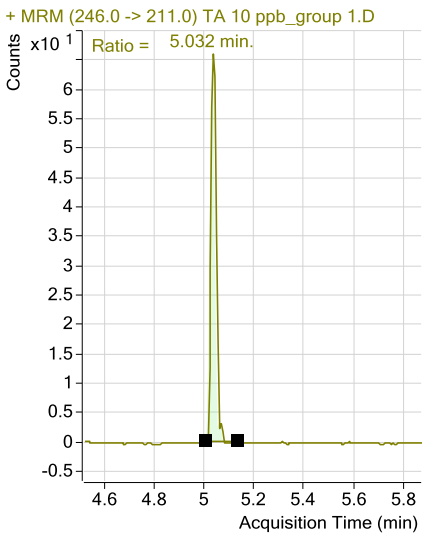
# MS/MS Optimization

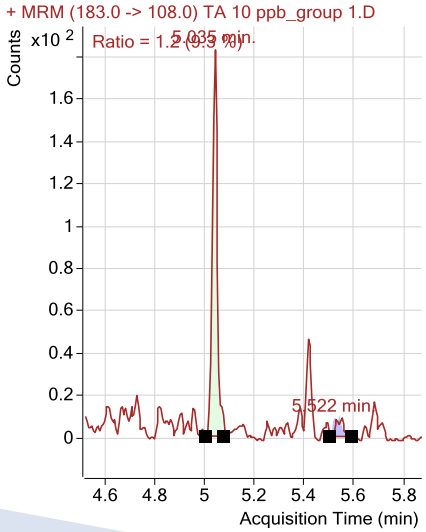
## Etridiazole: Evaluation of selected MRMs in representative/target matrices

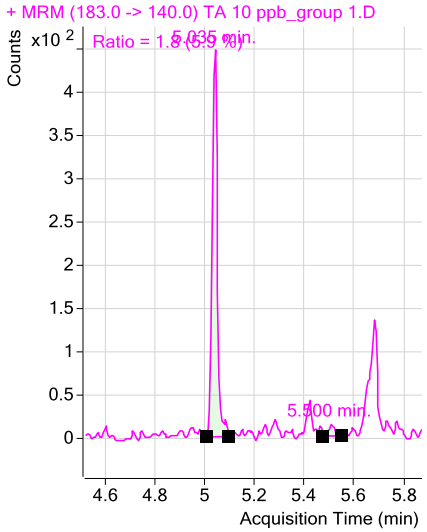


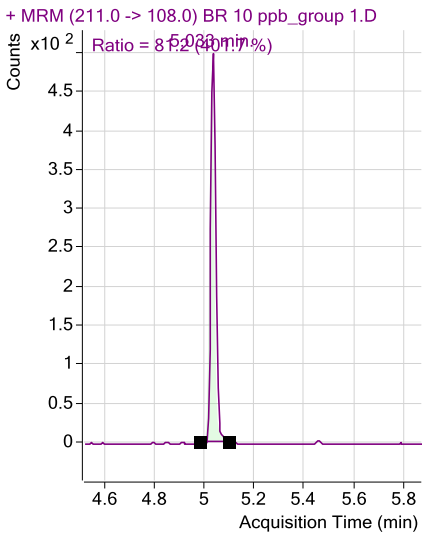

10 ng/g in tangerines

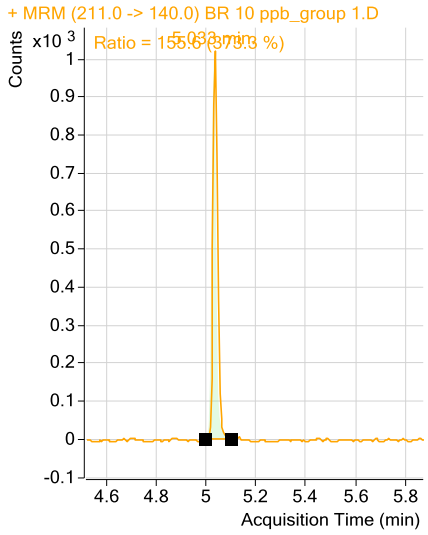


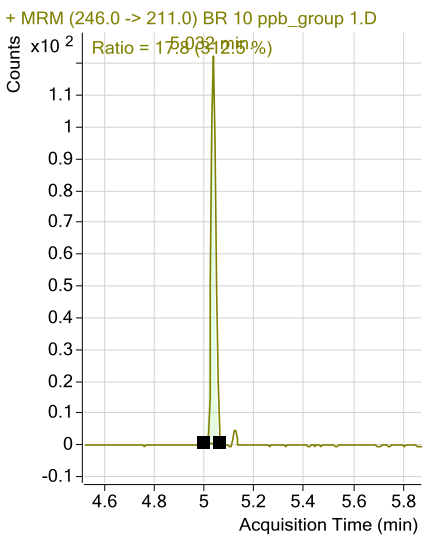



# MS/MS Optimization

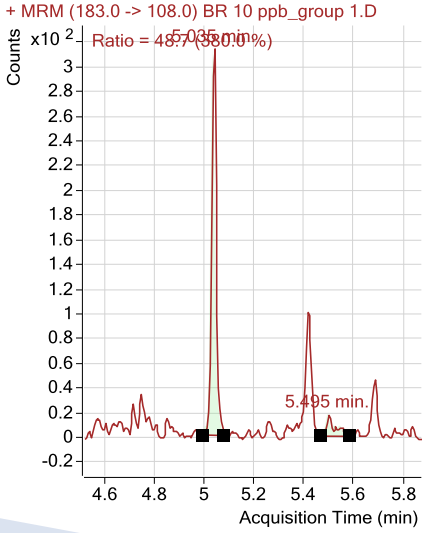
## Etridiazole: Evaluation of selected MRMs in representative/target matrices

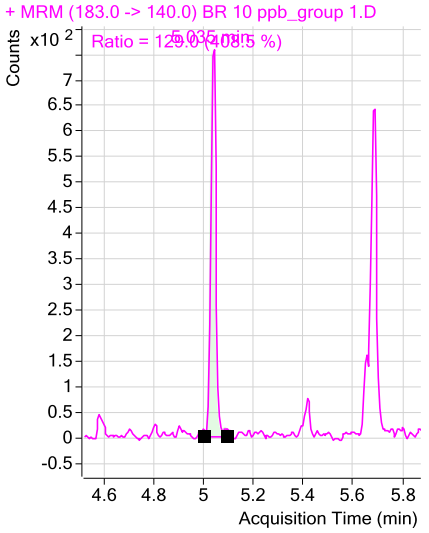



10 ng/g in broccoli



# PTV Solvent Vent Optimization

## Multi-Mode Inlet (MMI) options:

- Hot split/splitless
- Cold split/splitless
- Pulsed split/splitless
- PTV solvent vent

# PTV Solvent Vent Optimization

- PTV = Programmable Temperature Vaporizer
- PTV solvent vent – 2 steps:

  (1) Solvent elimination

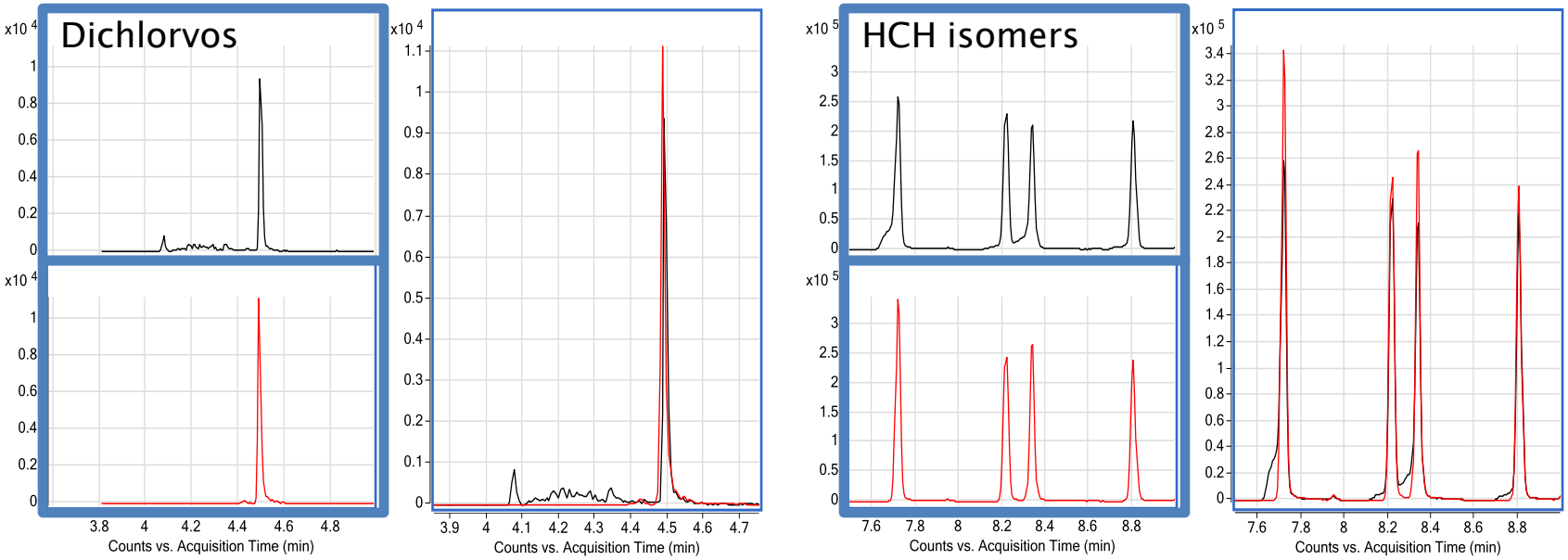
  = low temperature, split vent open

  (2) Analyte transfer to the column

  = increased temperature, split vent closed
- Benefits of PTV solvent vent injection:
  - Low injection temperature → less discrimination, better results for thermally-labile analytes, reduced matrix effects
  - Large volume injection (LVI) possibility

# PTV Solvent Vent Optimization

- Solvent elimination → LVI, better peak shapes of early eluting peaks in acetonitrile (QuEChERS extracts):




- Optimization of inlet conditions depends on the injection solvent, volume, inlet and liner design, target analytes (most and least volatile compounds) etc.

K. Mastovska and P.L. Wylie,
*J. Chromatogr. A* **1265** (2012) 155–164

# PTV Solvent Vent Optimization

**Important inlet parameters:**

- **Solvent vent period:**
  - vent temperature
  - vent time
  - vent flow
  - vent pressure
- **Analyte transfer period:**
  - inlet temperature program
  - splitless time
- **Post-transfer period:**
  - purge flow
  - gas saver flow and time

Dimpled liner
2 mm i.d.

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# Backflushing Optimization

## Post-Run Backflushing

- begins after the last analyte has been detected
- backflushing start easily determined
- effectiveness should be verified by subsequent solvent blank analysis in full-scan MS mode

## Concurrent Backflushing

- begins after the last analyte has eluted from the first column
- backflushing start determined experimentally
- effectiveness should be verified by subsequent solvent blank analysis in full-scan MS mode

Column 1: HP 5-MS UI, 5 m x 0.25 mm x 0.25 µm
Column 2: HP 5-MS UI, 15 m x 0.25 mm x 0.25 µm

K. Mastovska and P.L. Wylie,
*J. Chromatogr. A* **1265** (2012) 155-164

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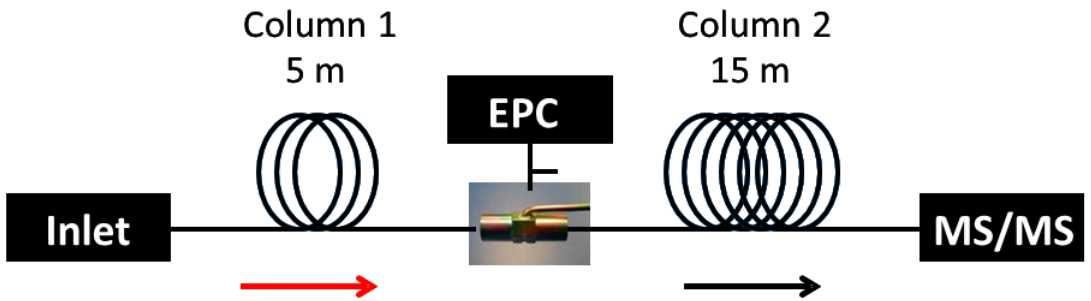
# Backflushing Optimization

**(A) Elution of the analytes from the first column**

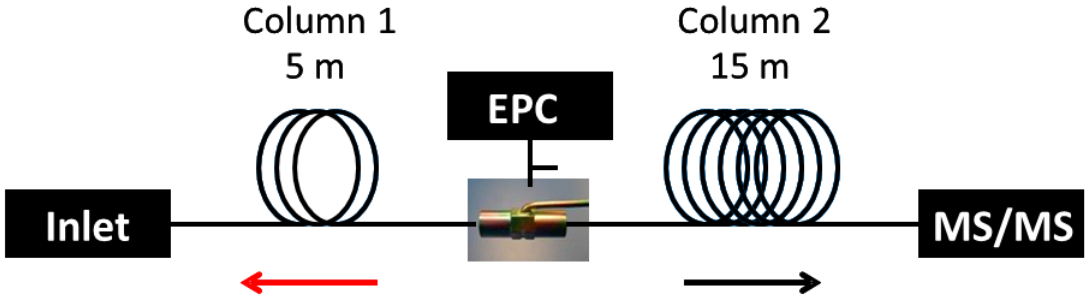



Time:
0 – 15.2 min

1.1 mL/min

1.2 mL/min

**(B) Backflushing of the first column to remove less volatile matrix components**

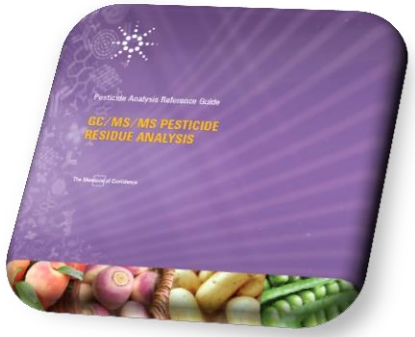



Time:
15.2 – 18.0 min
Post-run (0.5 min)

2.283 mL/min
10.683 mL/min

1.2 mL/min
4.0 mL/min

# Reference Guide
## Chapters



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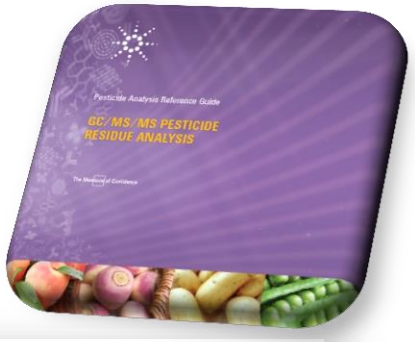
# Agilent Analyzer
## for the 5+15m column configuration

Coming soon!

Including MRM pesticide database with retention times for the Reference Guide core method conditions!

# Reference Guide
## Chapters



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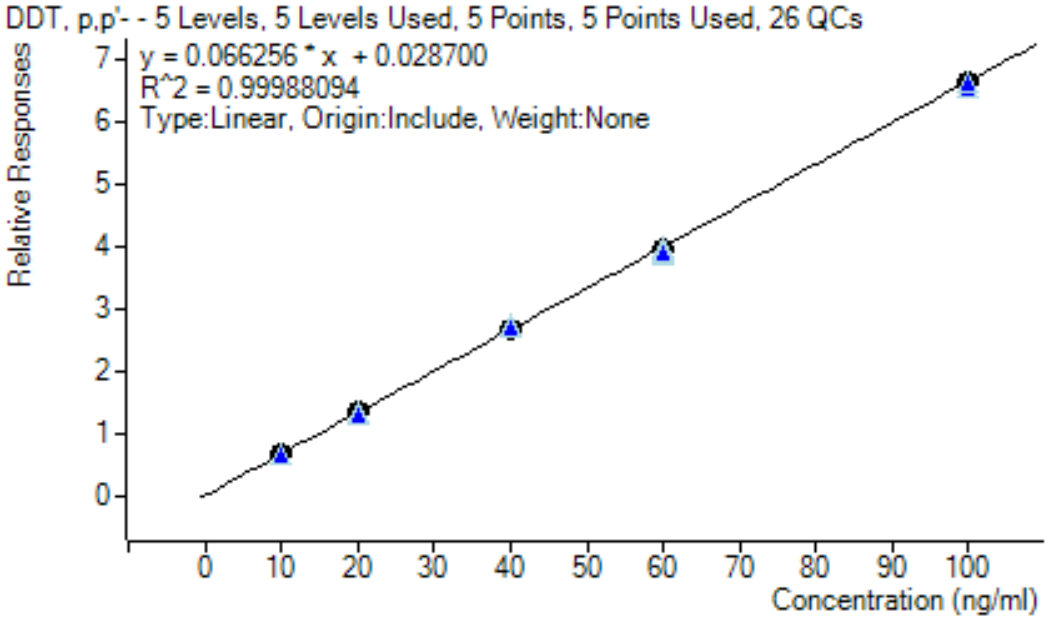
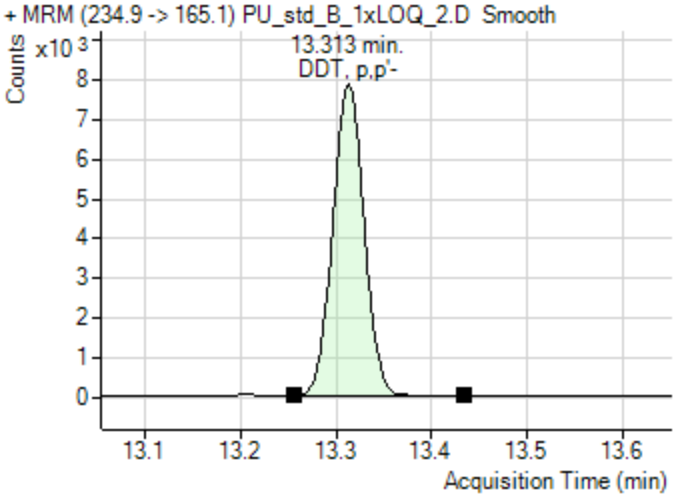
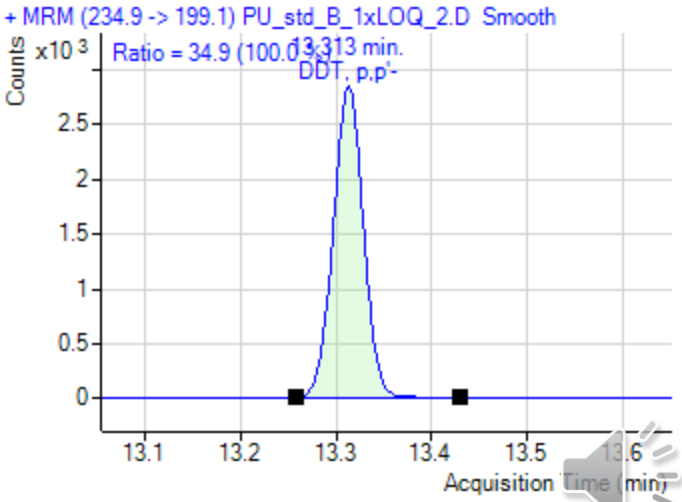
# Calibration Curve Examples

3 calibration curves and 16 QCs (at 20 and 60 ng/g) for *p,p'*-DDT in plum matrix obtained during a sequence mimicking a typical PDP sample batch




*p,p'*-DDT at 10 ng/g in plum

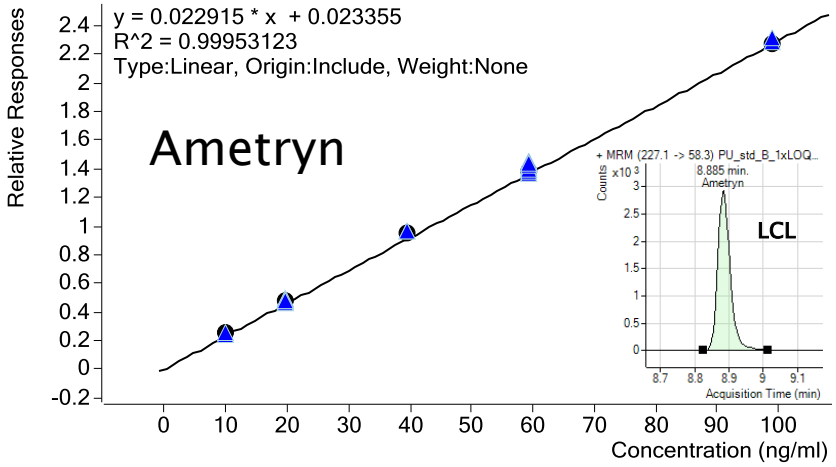


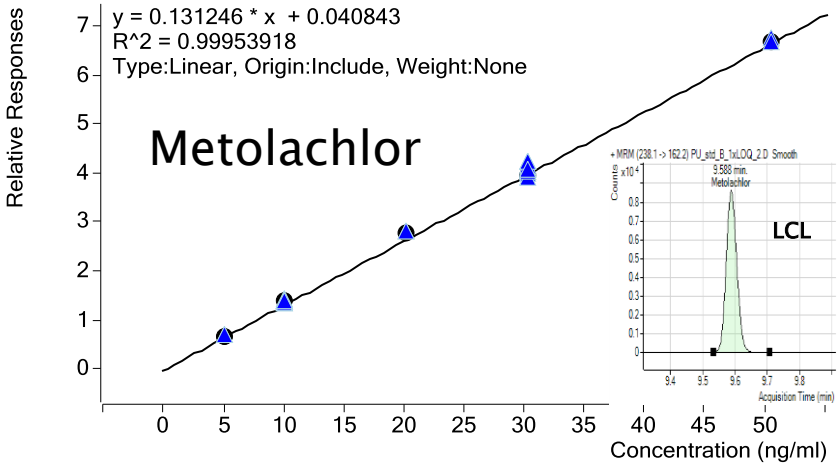

K. Mastovska, Agilent Application Note 5991-1054EN

# Calibration Curve Examples

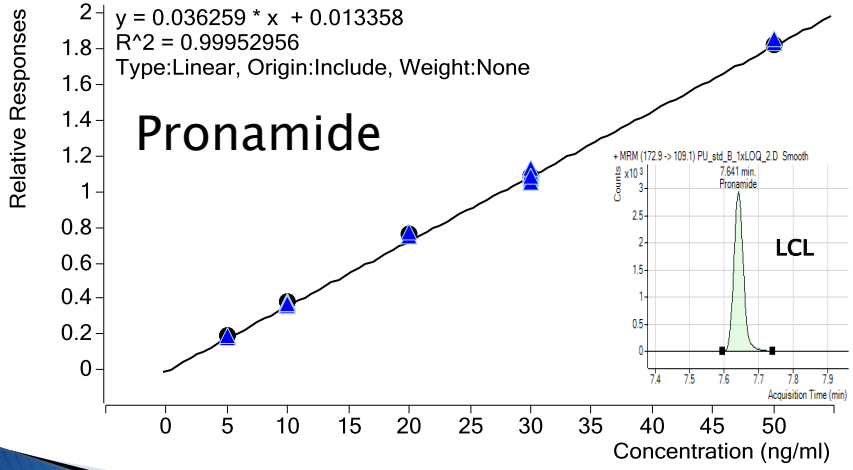
Ametryn - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 26 QCs



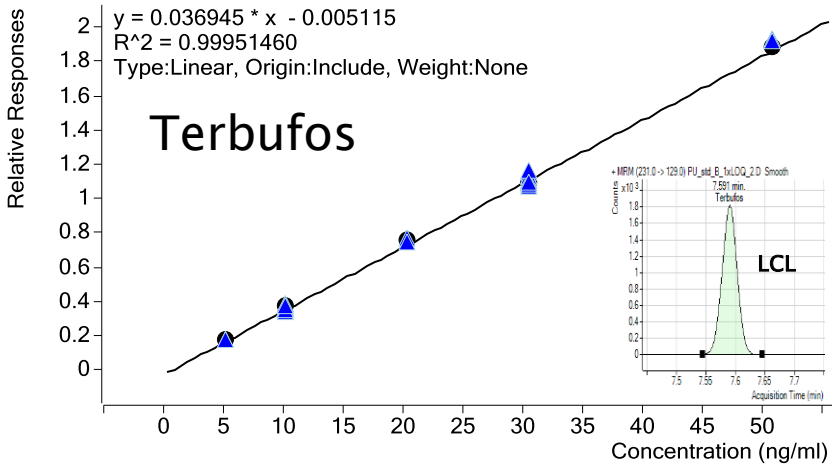

Metolachlor - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 26 QCs




Pronamide - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 26 QCs




Terbufos - 5 Levels, 5 Levels Used, 5 Points, 5 Points Used, 26 QCs

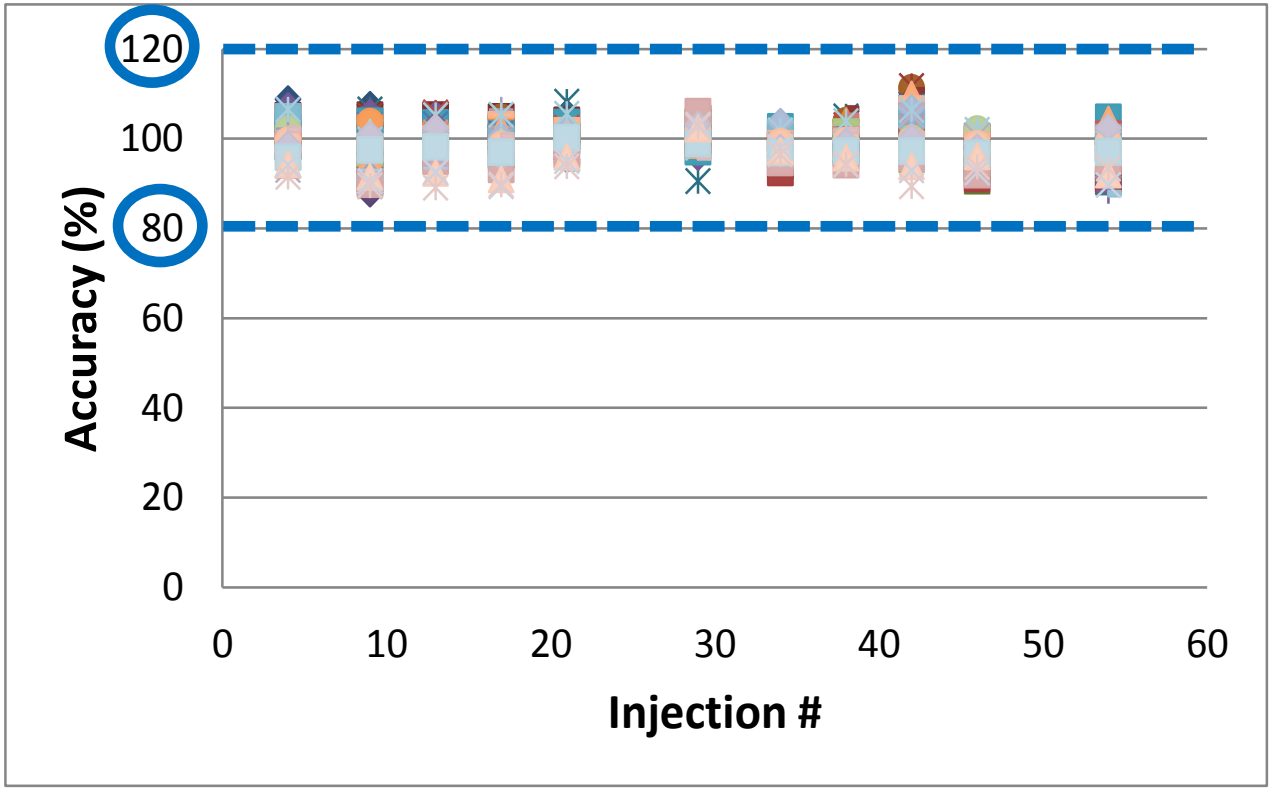



**LCL = Lowest Calibration Level**

K. Mastovska, Agilent Application Note 5991-1054EN

# Calibration Integrity

Accuracy (%) for > 70 analytes at 2xLOQ in calibration standards and QC samples injected throughout a sequence of plum samples mimicking a typical PDP sample batch

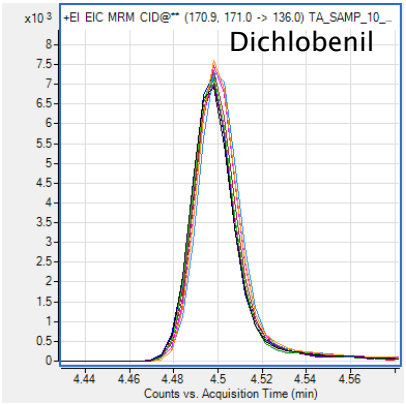


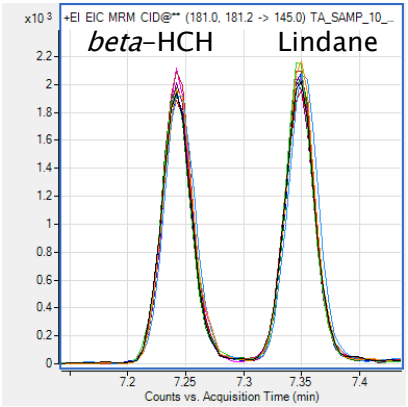


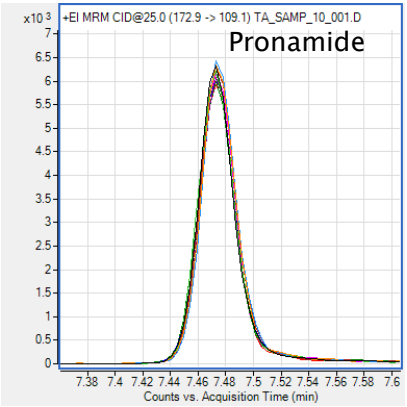

K. Mastovska, Agilent Application Note 5991-1054EN

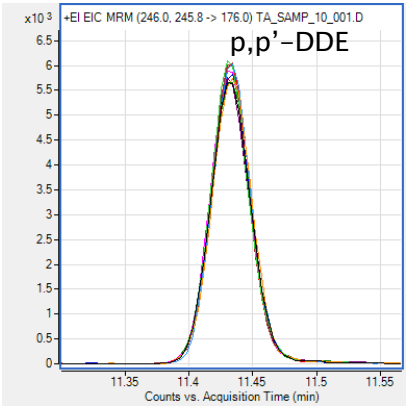
# Repeatability Results

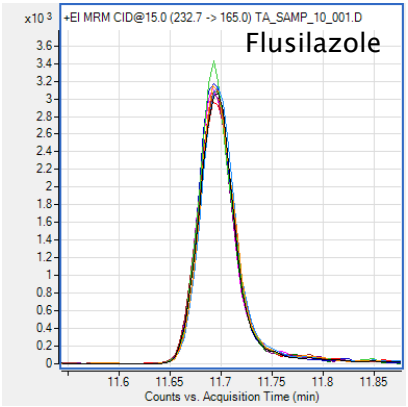
Overlay of 10 repeated injections (2 µL) of **tangerine** samples at **10 ng/g** obtained throughout a typical sequence in a PDP laboratory

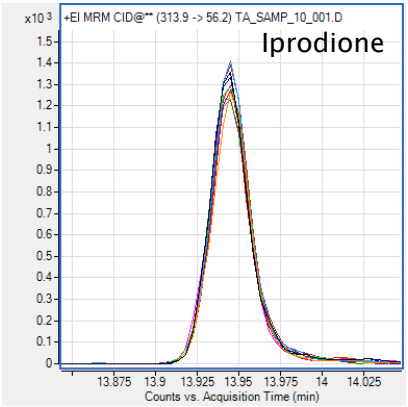


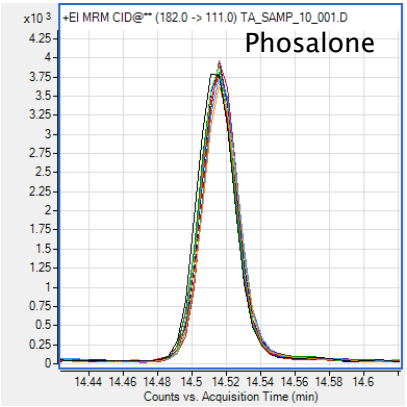



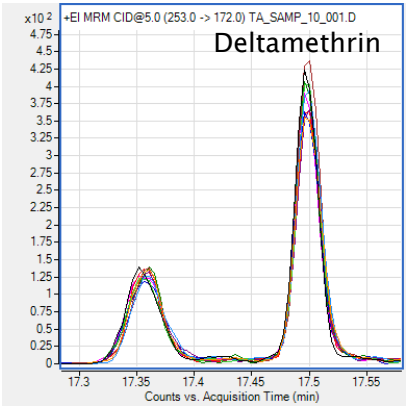



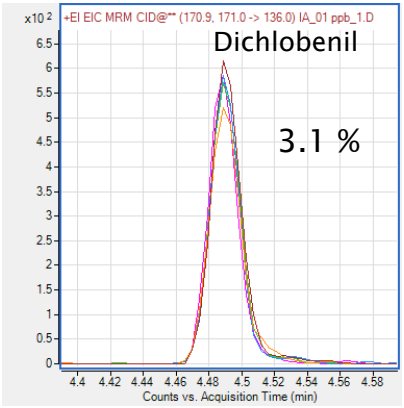



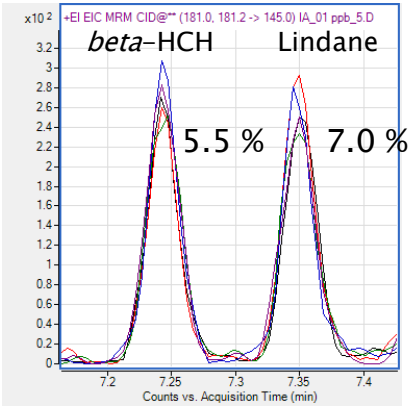



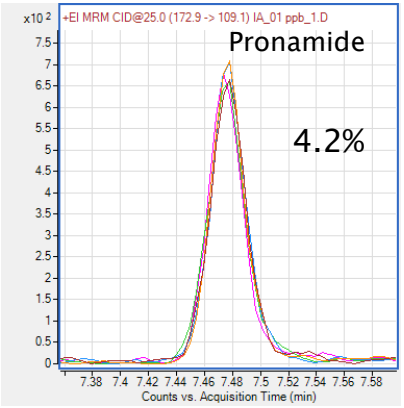



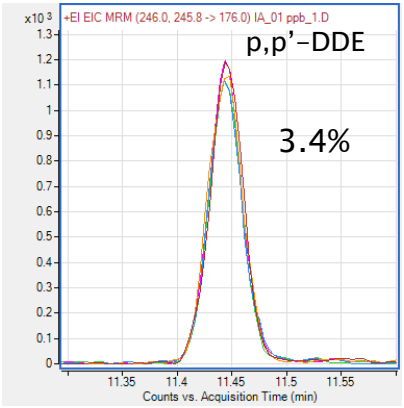
# Repeatability Results

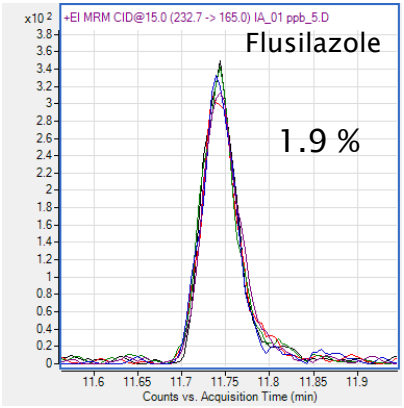
Overlay of chromatograms for 5 repeated injections (2 µL) of **apple sauce** at **1 ng/g** to estimate LOQ based on % RSD (without ISTD normalization)

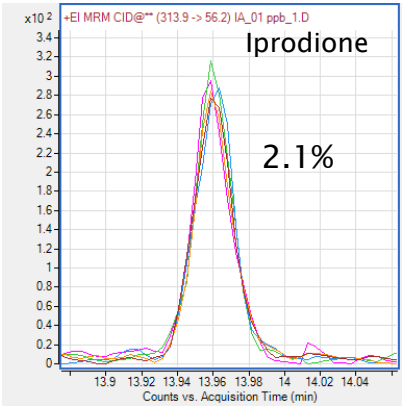


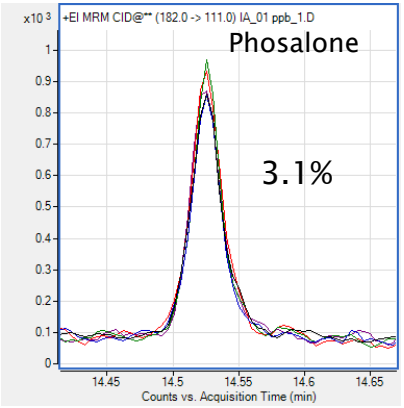



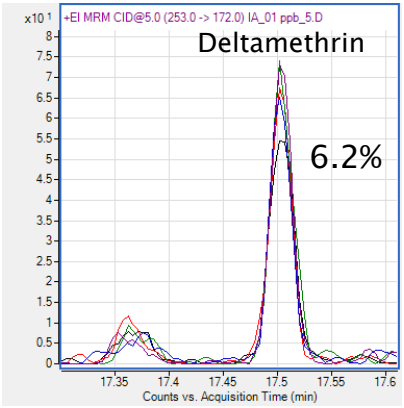

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# Recommendations

- Rugged and well-optimized GC-MS/MS method
- Internal and QC standards
- Column backflushing
- Analyte protectants

# Contact an Agilent Sales Representative or Product Specialist to obtain your copy of the Reference Guide.

# Acknowledgments



- **California Department of Food and Agriculture**, Center for Analytical Chemistry, Sacramento, CA, USA
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# Thank you for your attention!

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