Speeding Gas Chromatography – Mass Spectrometry to Analyze >150 Pesticide Residues in <10 min

Steven J. Lehotay, Urairat Koesukwiwat, and Lucía Geis-Asteggiante

USDA UNITED STATES DEPARTMENT OF AGRICULTURE

ars Agricultural Research Service

Department of Chemistry; Chulalongkorn University; Bangkok, Thailand

Urairat (Oil) Koesukwiwat

Lucía Geis-Asteggiante

Cátedra de Farmacognosia y Productos Naturales, DQO; UdelaR Montevideo, Uruguay

The Pesticide Analysis Challenge

- >500,000 possible pesticide/commodity pairs
- Low detection limits
  - EU and US "default" MRLs = 10 ng/g
- Minimum Cost
- Speed of Analysis
  - Perishable foods need results as soon as possible

Goal: Two chemists perform a batch of 32 samples for 300 pesticides from 9 am receipt of samples to determination and identification report by 5 pm on the same day.

But How?

Unified QuEChERS Method

extraction

1 g sample per 1 mL of MeCN w/ 1% HOAc
for fruits and vegetables

add internal standard

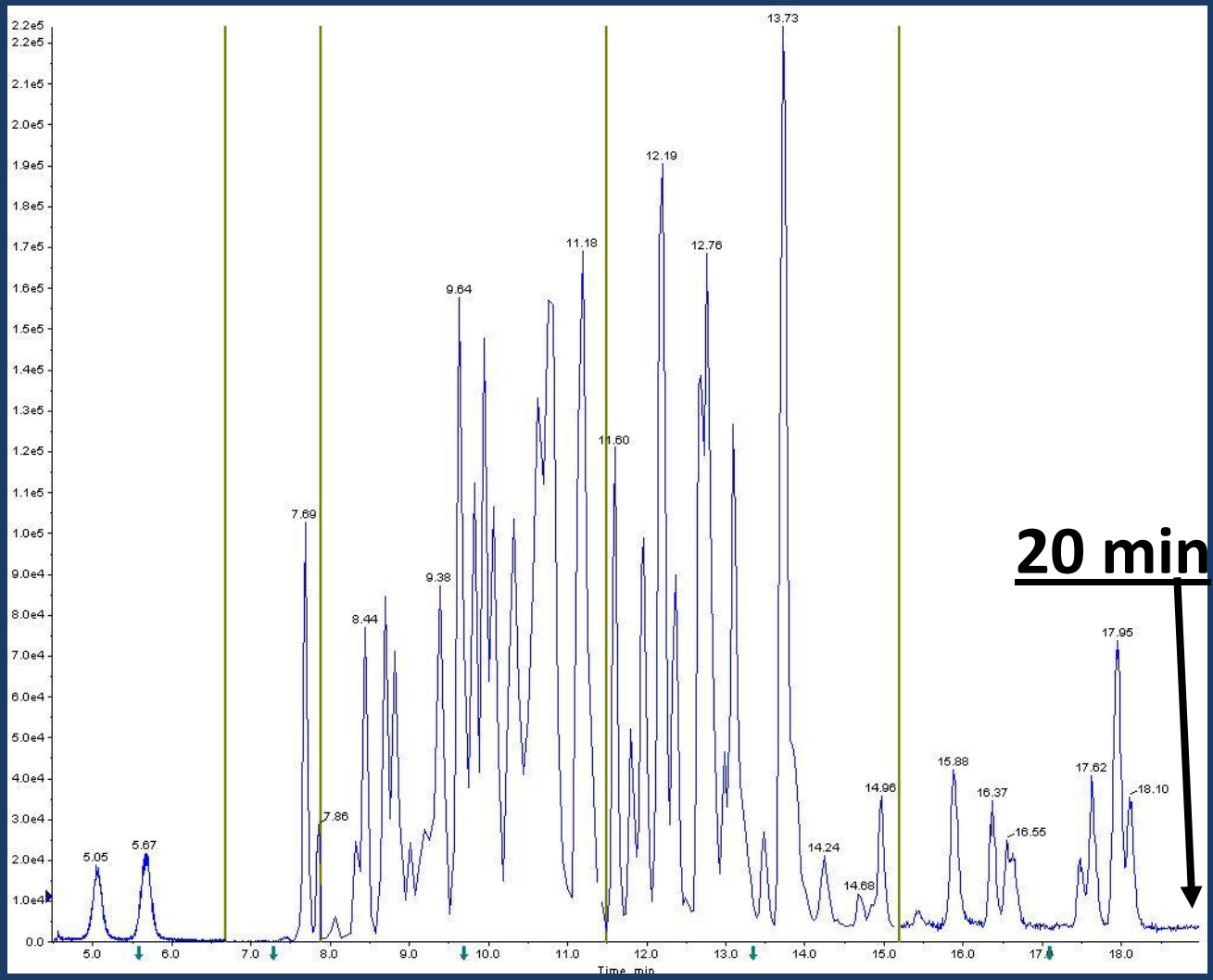
per g sample, add 0.4 g anh. $MgSO_4$
+ 0.1 g anh. NaOAc
shake or blend

centrifuge

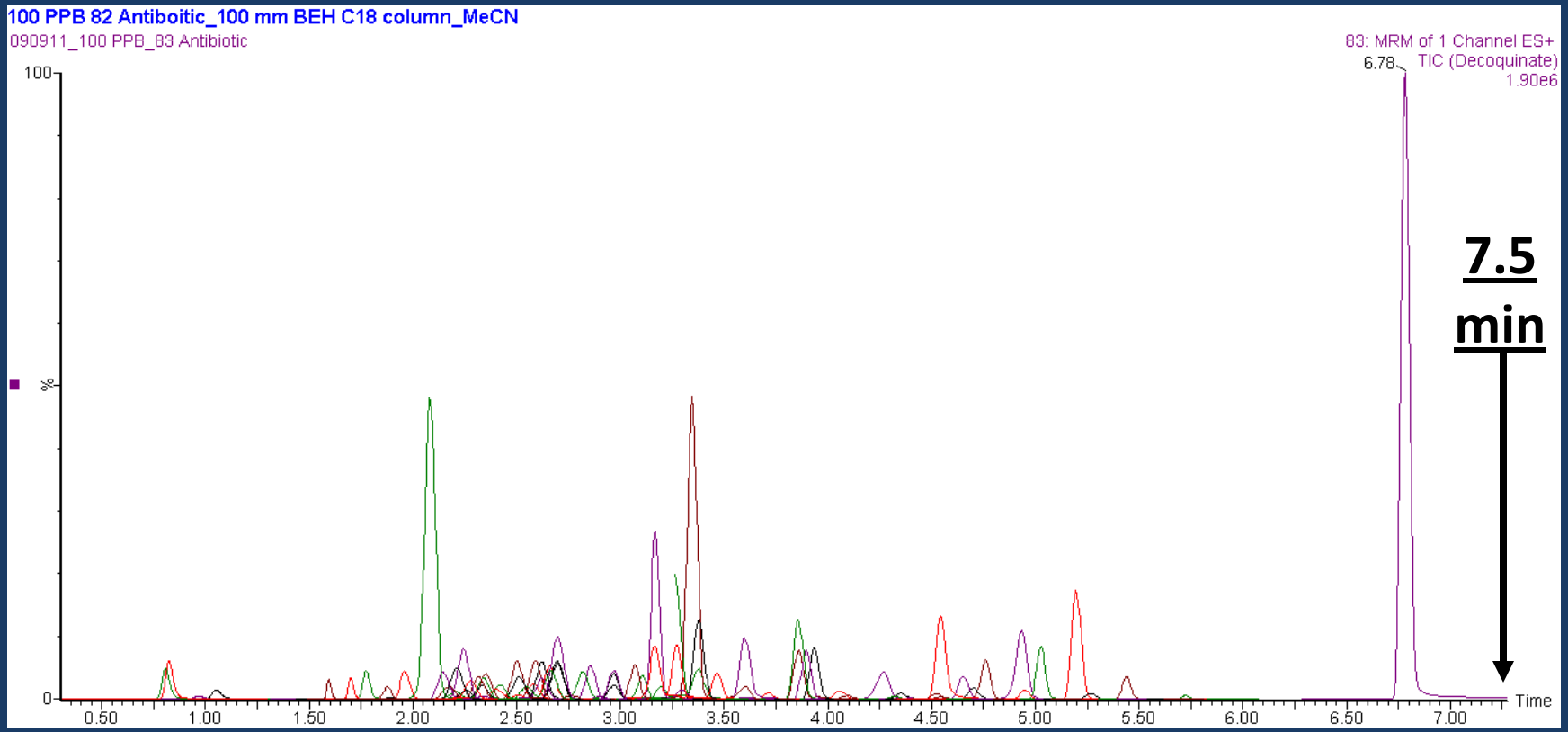
(dispersive) SPE clean-up

per mL of the upper layer:
150 mg $MgSO_4$ + 50 mg PSA
+ 50 mg C18 + 7.5 mg GCB
mix and centrifuge

HPLC-MS/MS Chromatographic Profile of 121 Veterinary Drugs

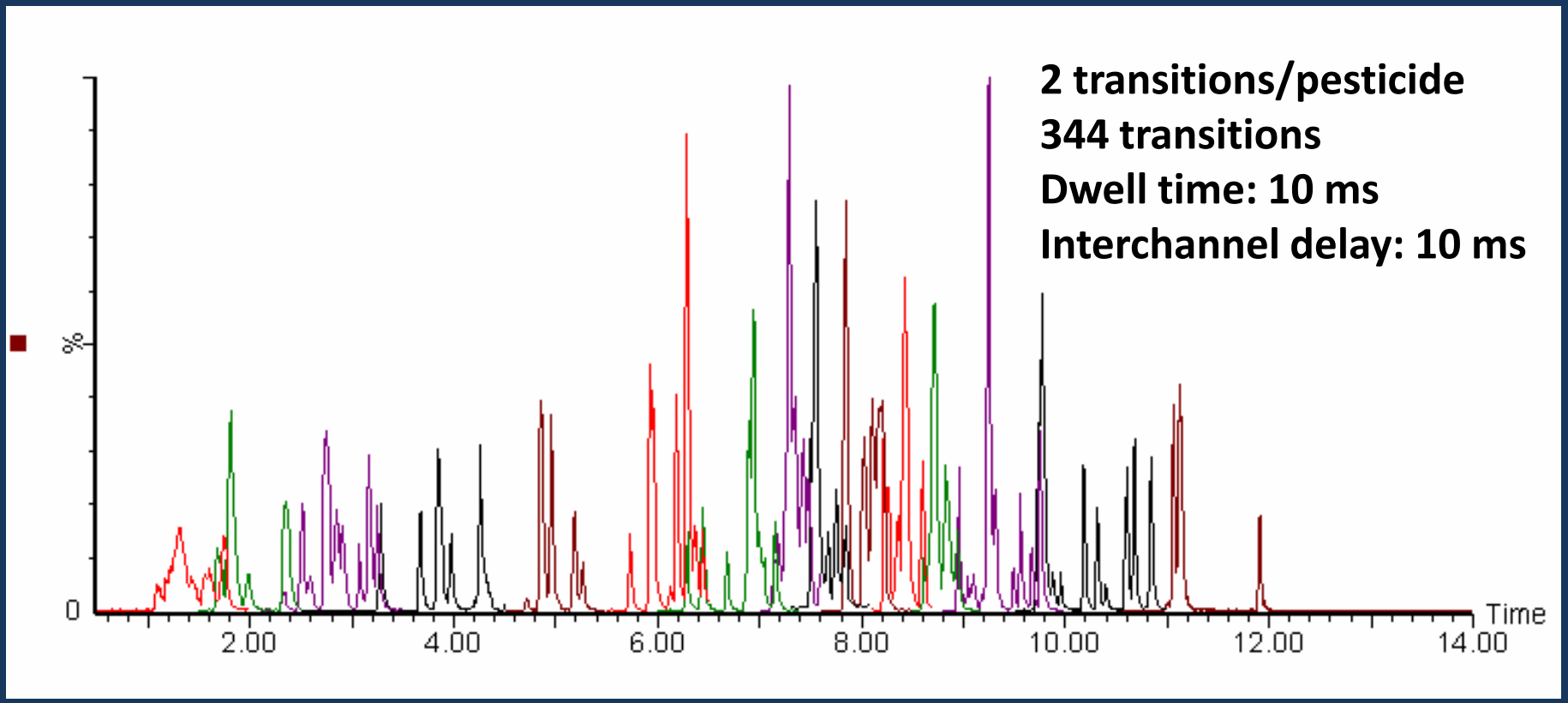


UHPLC-MS/MS Chromatographic Profile of 82 Antibiotics



Mobile Phase: A – 95% water / 5% MeCN / 0.1% formic acid
B – 100% MeCN / 0.1% formic acid

172 Pesticides in 14 min by UHPLC-MS/MS



Column: Acquity UPLCTM BEH C18, 2.1 x 150mm, 1.7µm
Flow rate: 0.45 ml/min
Temperature: 65°C

Slide adapted from André de Kok

How to speed GC analysis?

$\downarrow L$ $R_S \propto \sqrt{L}$

- shorter capillary column

$t_R = \frac{L}{\bar{u}}(k+1)$

$\downarrow k$

- faster temperature programming

- $\uparrow d_c$ larger diameter capillary column (for fixed column length)

- altered stationary phase to adjust selectivity

- $\downarrow d_f$ thinner film of the stationary phase $Q_S \propto d_f$

$\uparrow \bar{u}$

$\bar{u} > \bar{u}_{opt} \ldots H > H_{min}$

- higher than optimum carrier gas velocity

$\uparrow \bar{u}_{opt} \ldots H = H_{min}$

- $\downarrow d_c$ smaller diameter capillary column (for fixed resolution) $Q_S \propto d_c^3$

- higher diffusivity of the solute in the gas phase:
  - H_2 as a carrier gas;
  - low-pressure GC (LP-GC)

Slide by K. Mastovska

Ways to Speed GC Analysis

- Shorter columns
- Wider columns
- Less viscous carrier gas
- Faster temp ramps
- Higher flow rates
- Thinner films

More Speed Means a Sacrifice:

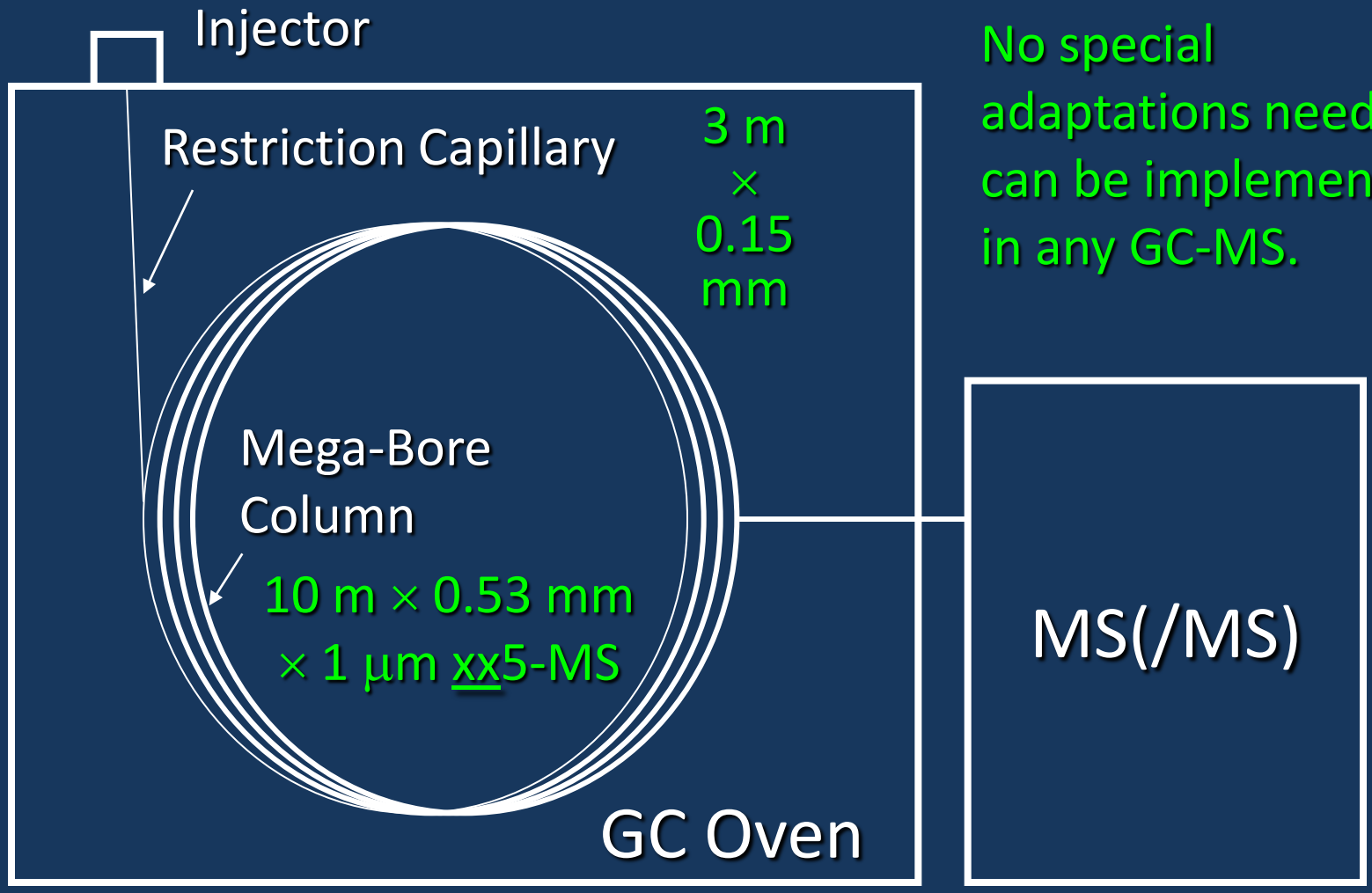
Reduced separation efficiency, Lower sample capacity, Complicated instrument design, Higher detection limits, and/or Less ruggedness

Mastovska and Lehotay, *J. Chromatogr. A* **1000** (2003) 153-180

Fast GC and GC-MS(/MS)

- Micro-Bore GC Columns
  - Gains separation efficiency but loses sample capacity, ruggedness, and ease of use
- Rapid Temperature Ramps (resistive heating)
  - Loses separation efficiency with gain in speed; but also loses of easy access to column
- Low-Pressure GC/MS (LP-GC/MS)
  - Loses separation efficiency but *gains* sample capacity and sensitivity with normal GC-MS
- Fast Flow Rates (Supersonic GC-MS)
- Pressure Tunable GC • Loses analytical scope

Low-Pressure GC-MS(/MS) Set-up

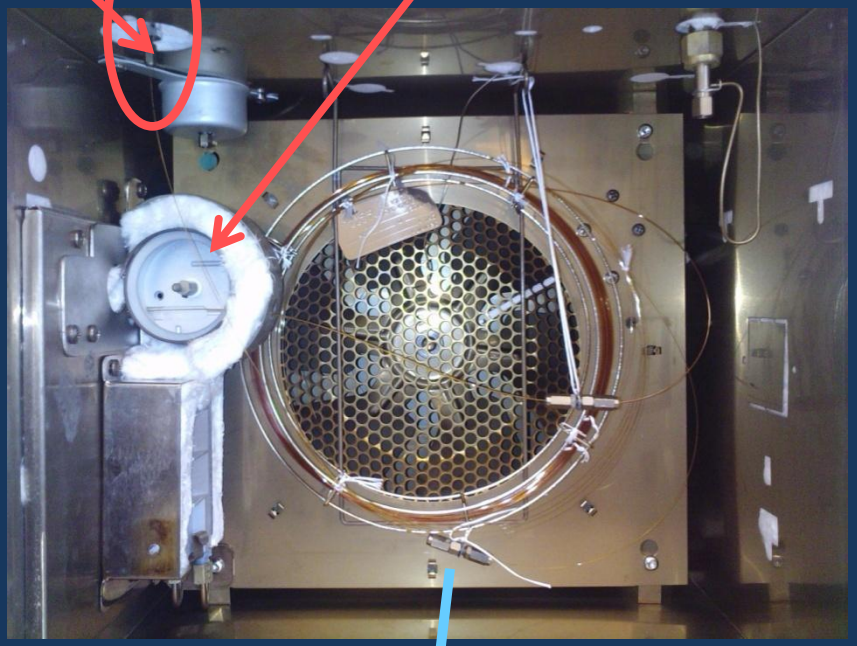


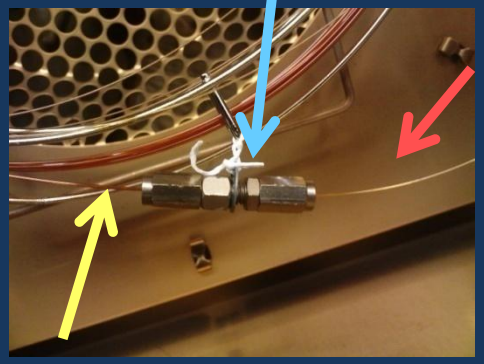
No special adaptations needed; can be implemented in any GC-MS.

Mastovska, Lehotay, Hajslova, *J. Chromatogr. A* **926** (2001) 299-316

Mastovska, Hajslova, Lehotay, *J. Chromatogr. A* **1054** (2004) 335-349

LP-GC/MS(-MS)

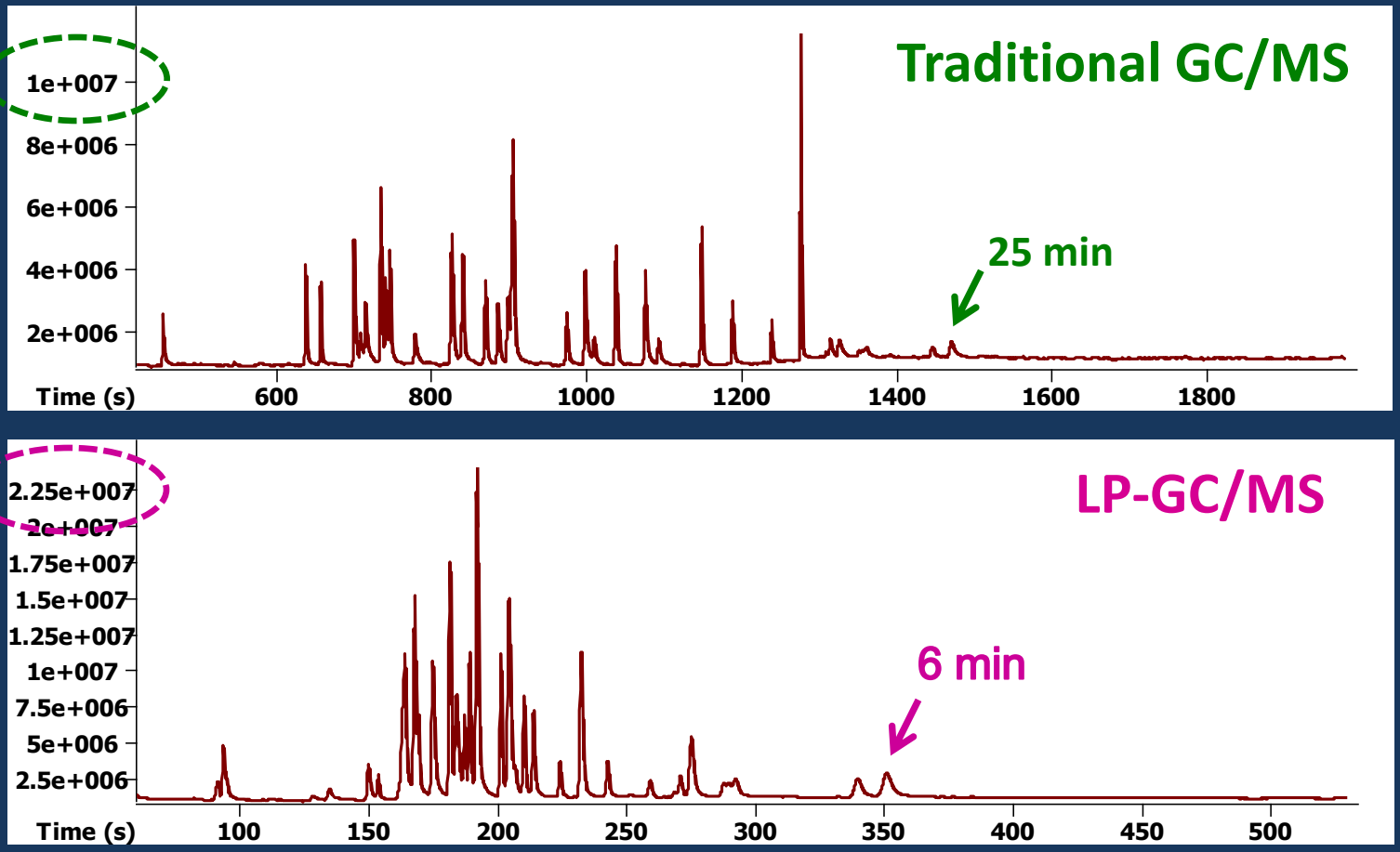




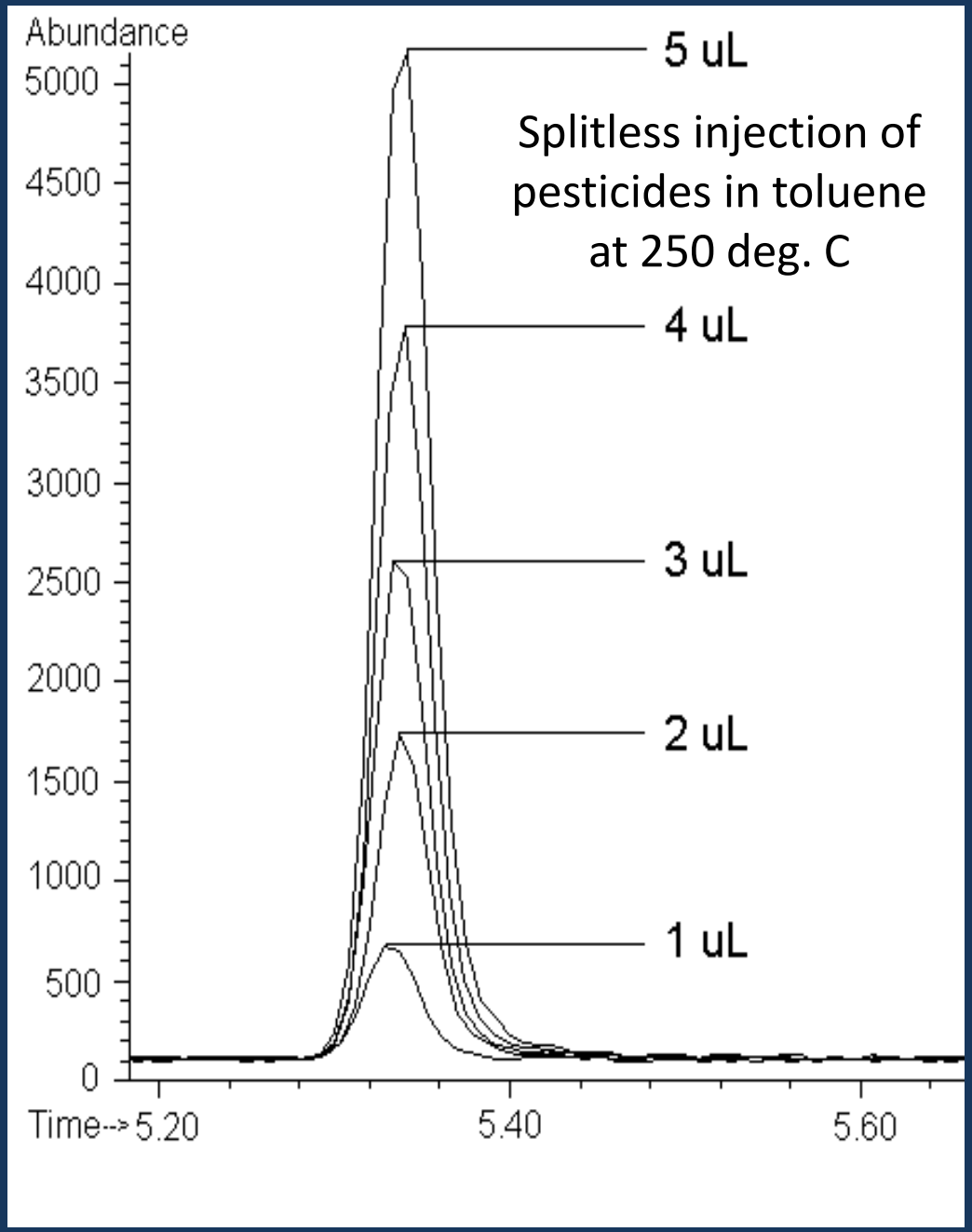
Features

- ↑ Speed
- ↑ Sample capacity
- ↓ Elution temperature
- ↑ S/N ratio
- ↓ Degradation of thermally-labile pesticides
- ↓ Peak tailing
- ↓ Separation efficiency, but compensation by MS(/MS)

LP-GC/MS is Much Faster



and more sensitive

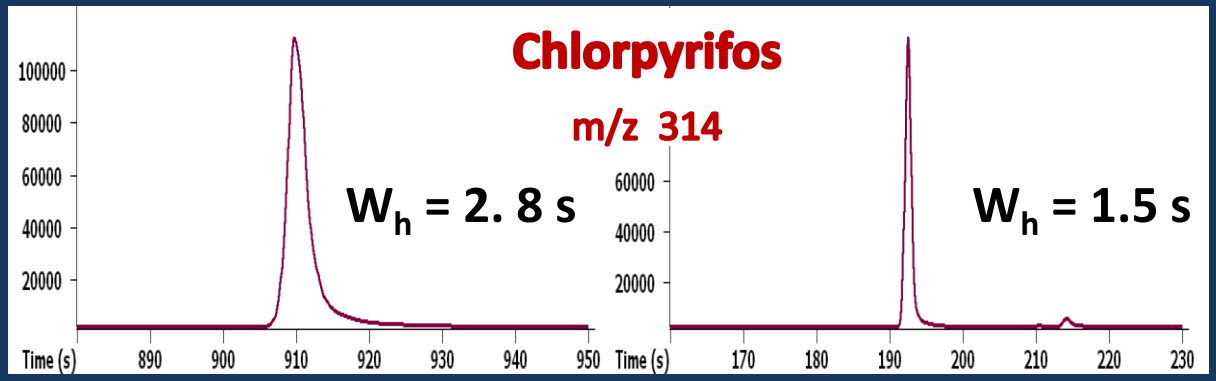


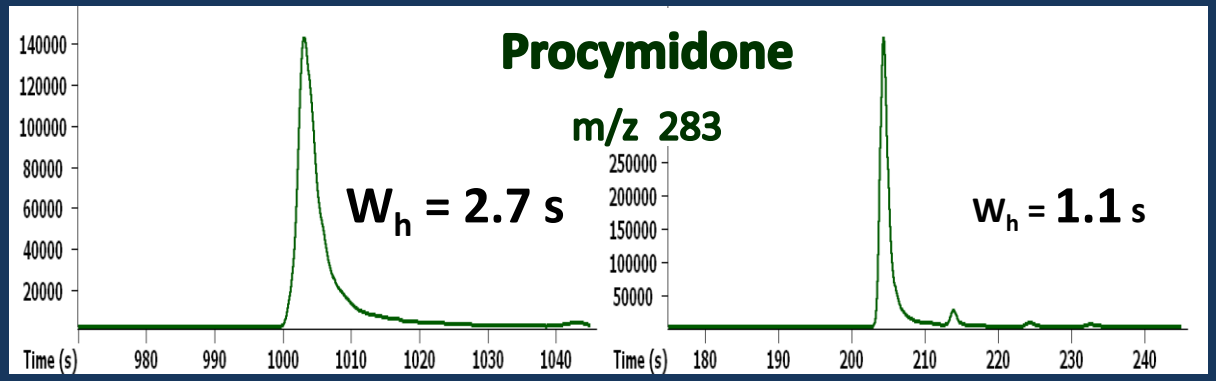
Sample Capacity is *Increased* in LP-GC/MS

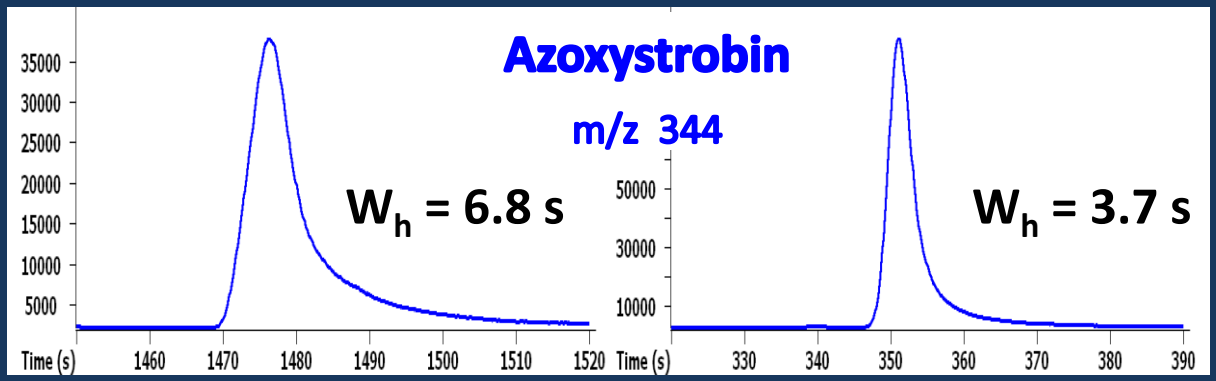
Well, in clean standards, anyway. Actually, LOQ is typically limited by the amount of matrix present.

Peak Widths

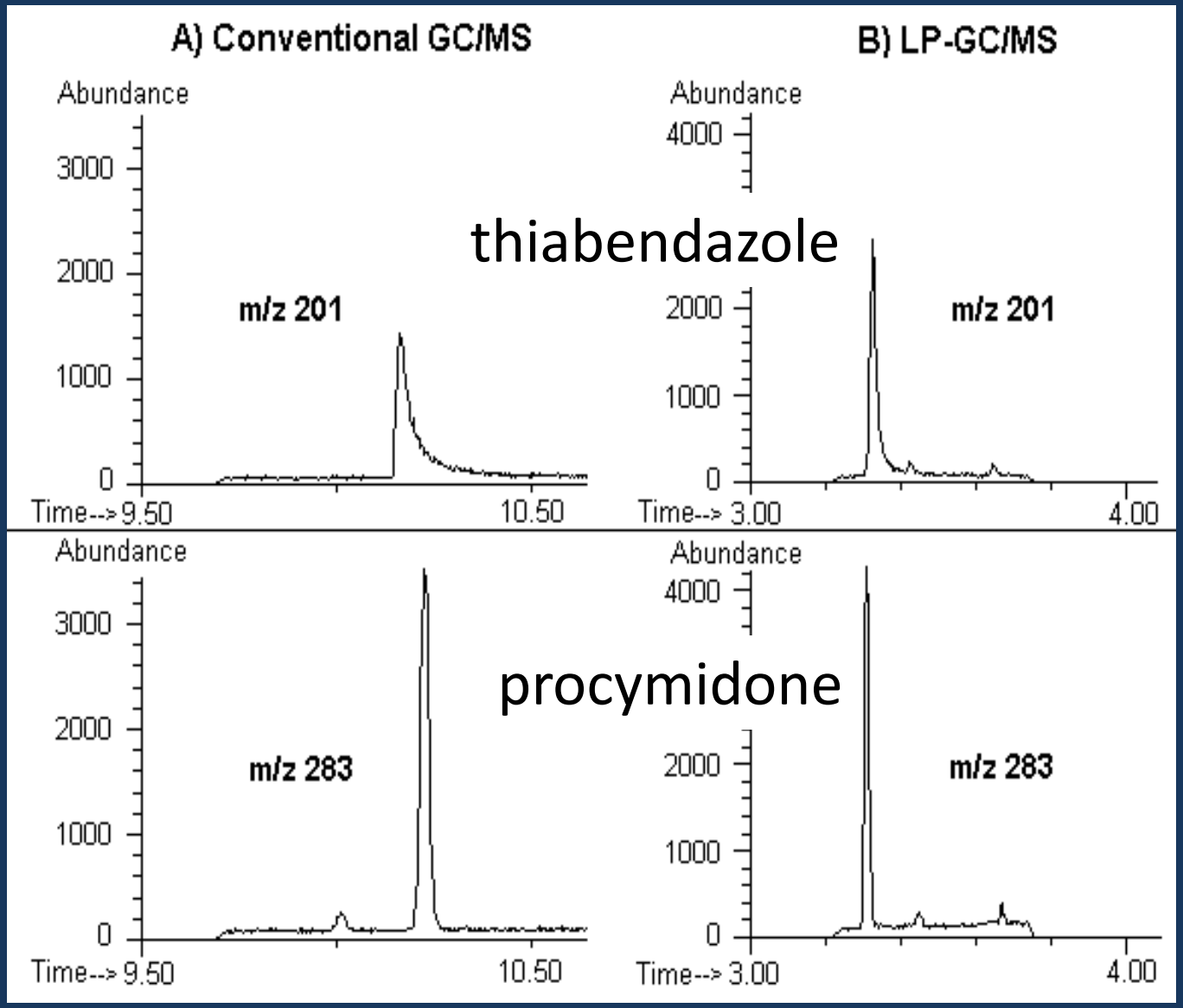
GC-MS LP-GC/MS







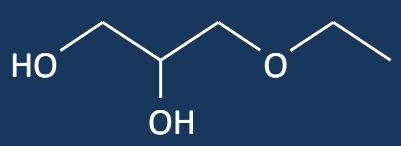
Thicker Film and Higher Flow Reduces Tailing



Analyte Protectants

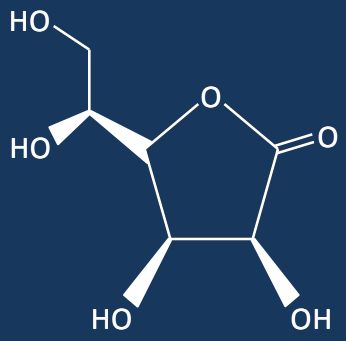
Strongly interact with active sites in GC system (inlet, column and ion source) to decrease degradation and adsorption of co-injected analytes.

Sharper peaks, less tailing, more ruggedness, lower LOD



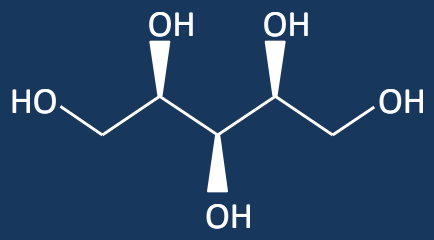
ethylglycerol

1 mg/ mL



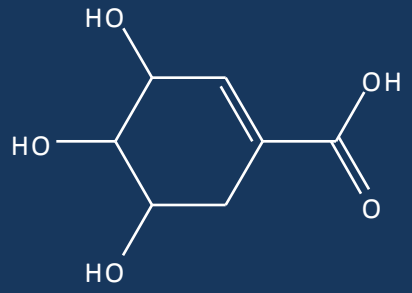
gulonolactone

0.1 mg/ mL



sorbitol

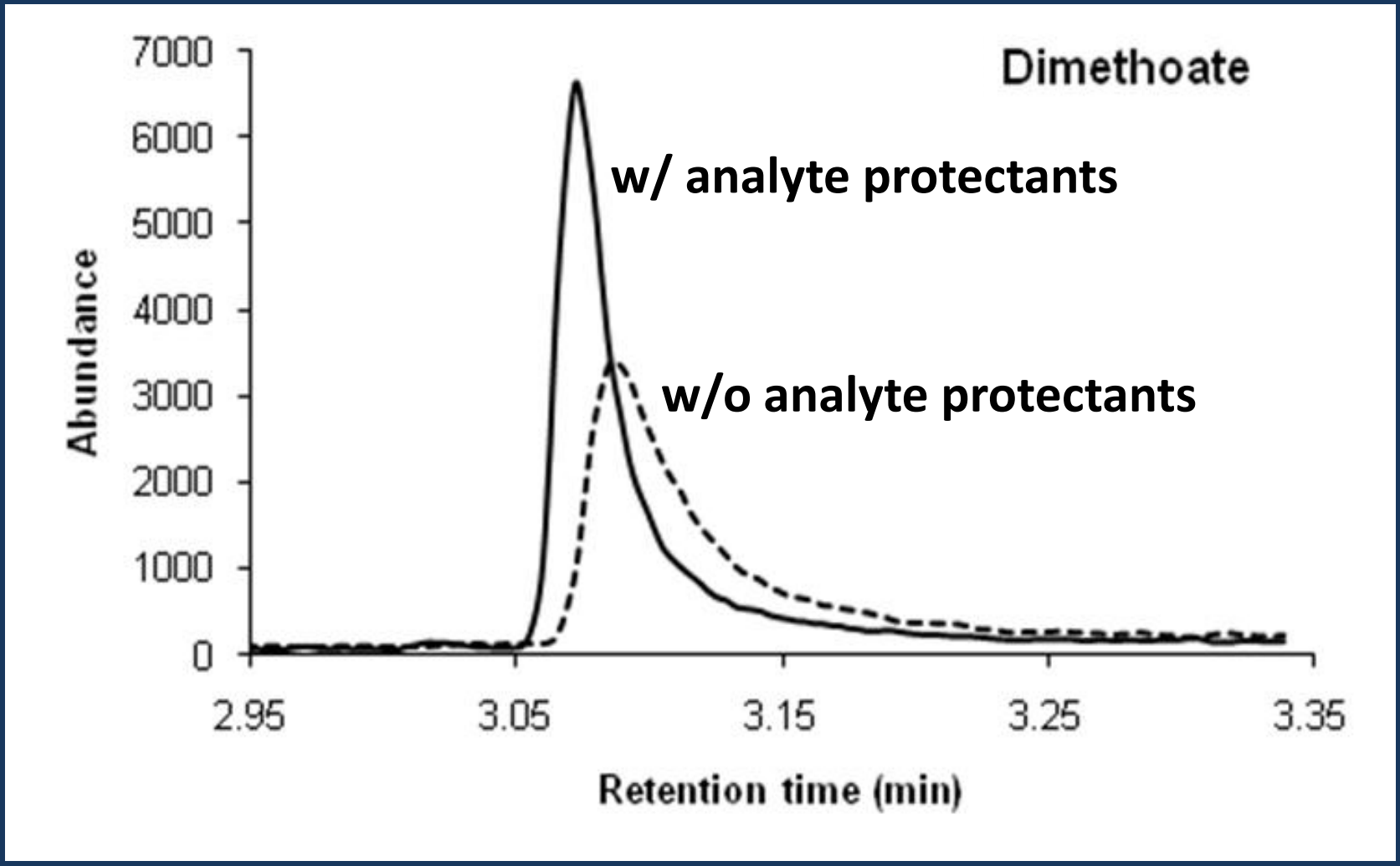
0.1 mg/ mL



shikimic acid

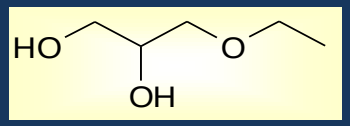
0.05 mg/ mL

Effect of Analyte Protectants



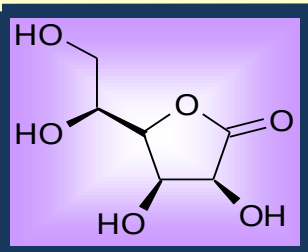
Anastassiades, Maštovská, Lehotay, *J. Chromatogr. A*, 1015, 163-184 (2003)

Combination of Analyte Protectants for GC Pesticide Residue Analysis

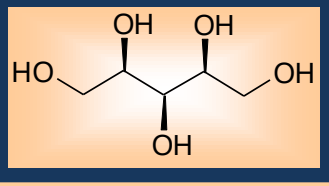


ethylglycerol (10 μg)

Signal enhancement:
- moderate
- strong



gulonolactone (1 μg)



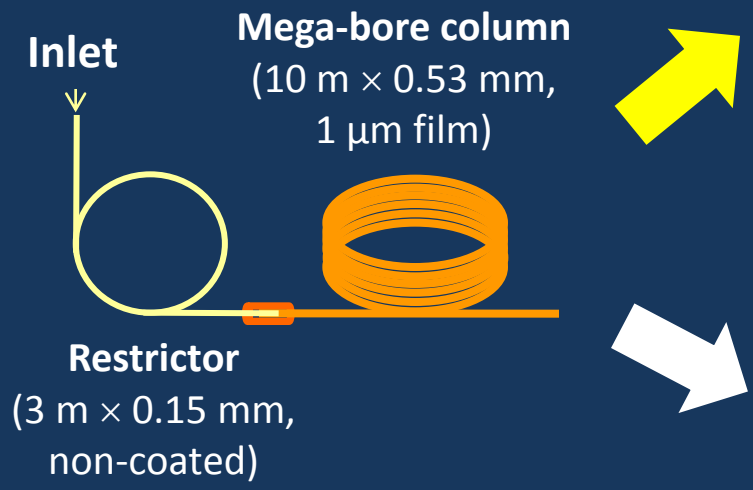
sorbitol (1 μg)

dichlorvos
o-phenylphenol
dimethoate
heptachlor
cyprodinil
imazalil
endosulfan-SO4
coumaphos
deltamethrin

← retention time →

K. Mastovska, S.J. Lehotay, M. Anastassiades, *Anal. Chem.*, 77, 8129-8137 (2005)

Comparison of LP-GC/ ToF with QqQ



LP-GC/TOF

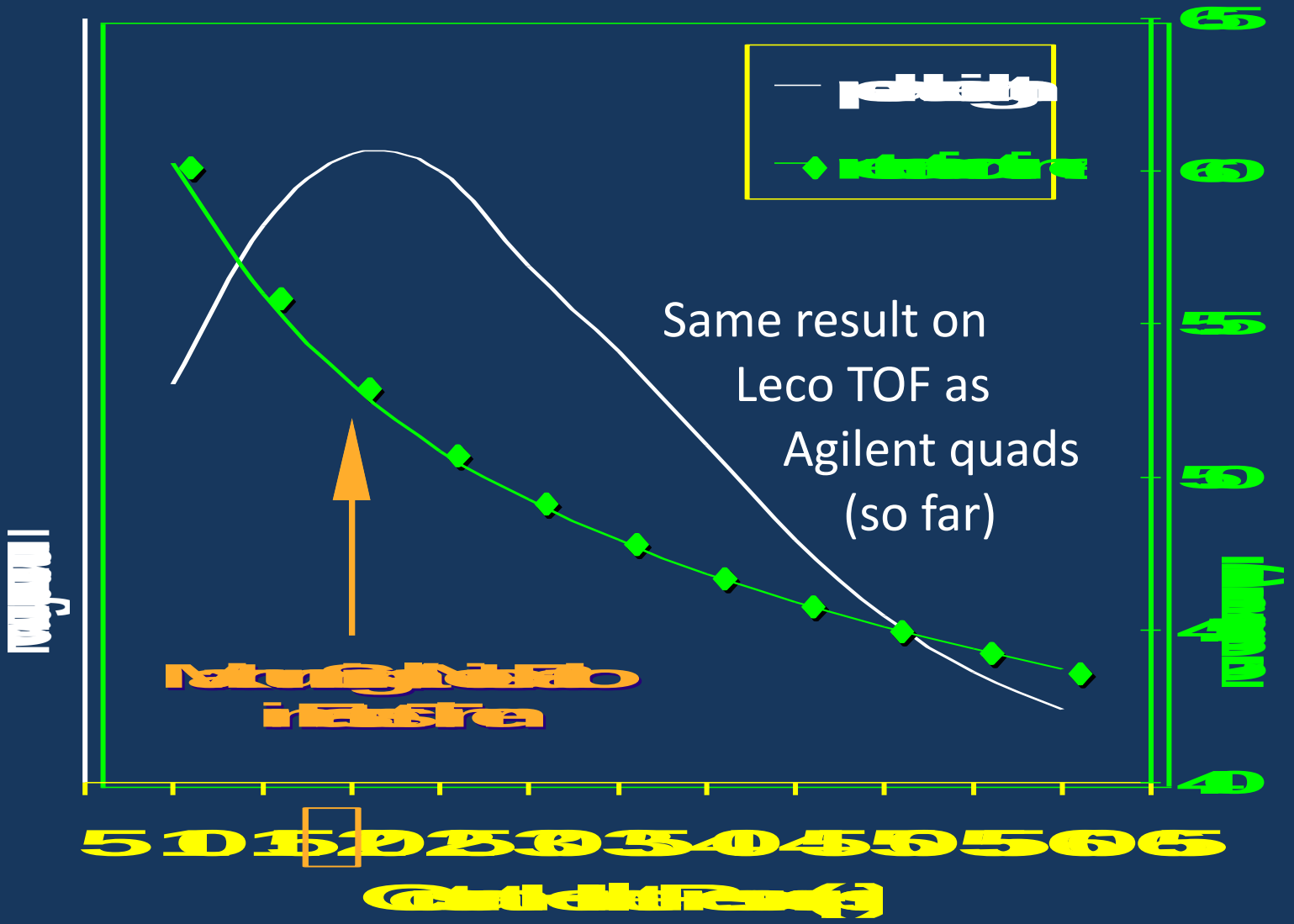
LP-GC/MS-MS

Comparison conducted for >100 QuEChERS extracts from 5 matrices spiked or not with 150 pesticides at 3 levels

List of 153 GC Analytes

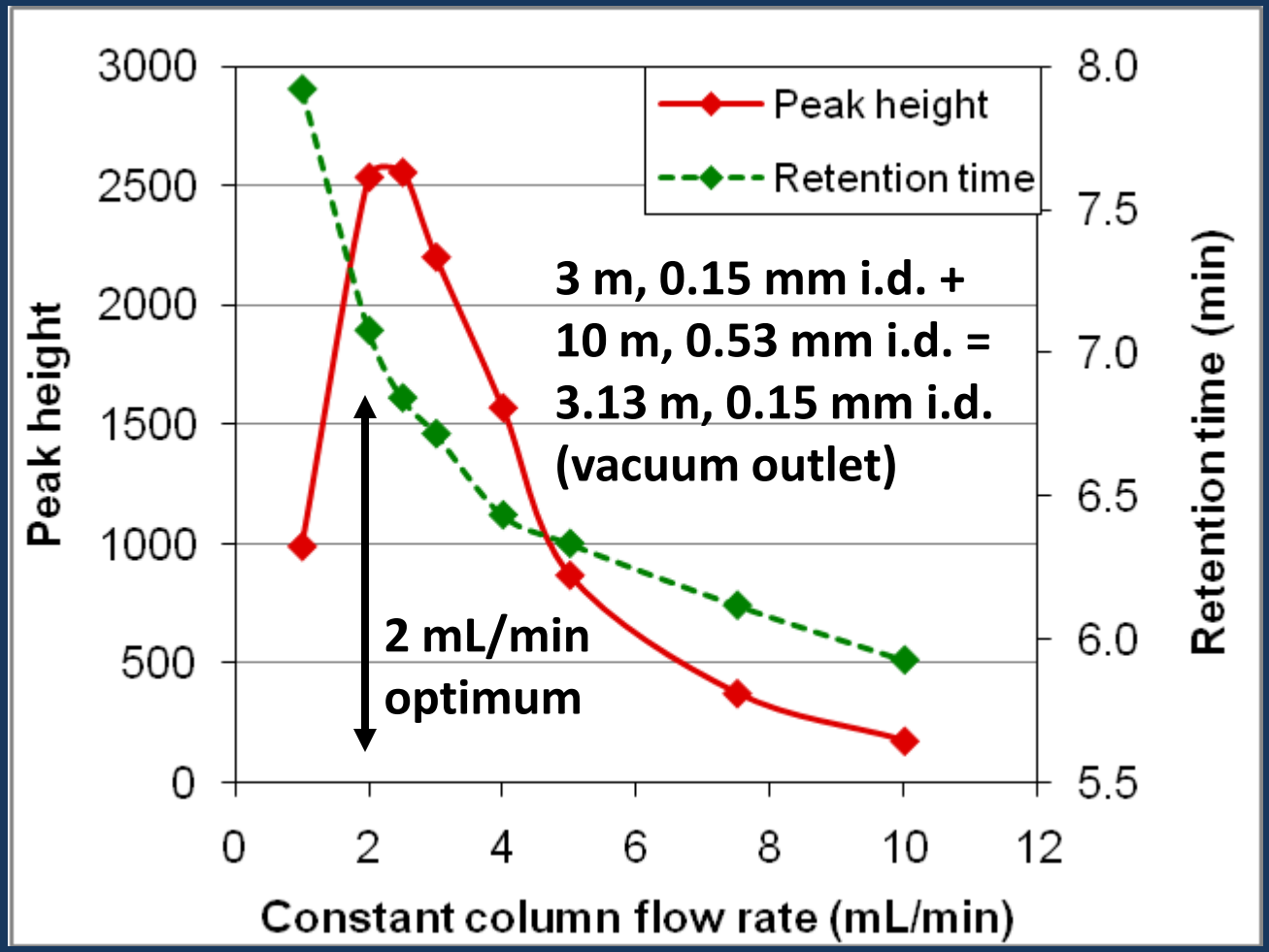
Alachlor
Aldrin
Atrazine
Atrazine-d_5 (I.S.)
Azinphos-ethyl
Azinphos-methyl
BHC, *alpha-*
BHC, *beta-* + Lindane
BHC, *delta-*
Bifenthrin
Bromophos
Bromophos-ethyl
Bromopropylate
Bupirimate
Buprofezin
Cadusafos
Captafol
Captan
Carbaryl
Carbofuran
Carbophenothion
Carfentrazone-ethyl
Chinomethionate
Chlordane, *cis-*
Chlordane, *trans-*
Chlorfenvinphos
Chlorothalonil
Chlorpropham
Chlorpyrifos
Chlorpyrifos-methyl
Coumaphos
Cyanophos
Cyfluthrin
Cyhalothrin, *lamda-*
Cypermethrin *(sum)*
Cyprodinil
DDD, *o,p'-*
DDD, *p,p'-* + DDT, *o,p'-*
DDE, *o,p'-*
DDE, *p,p'-*
DDT, *p,p'-*
Deltamethrin
Demeton-s-methyl
Demeton-s-methyl-sulfone
Diazinon
Dichlorfenthion
Dichlorobenzophenone, 4,4'-
Dicloran
Dicrotophos
Dieldrin
Dimethoate
Dioxathion
Diphenylamine
Disulfoton
Disulfoton sulfone
Endosulfan sulphate
Endosulfan, *alpha-*
Endosulfan, *beta-*
Endrin
Endrin ketone
EPN
Esfenvalerate
Ethafluralin
Ethion
Ethoprophos
Ethoxyquin
Famphur
Fenamiphos
Fenarimol
Fenchlorphos
Fenitrothion
Fenoxycarb
Fenpropathrin
Fensulfothion
Fenthion
Fenthion sulfone
Fenthion-d_6 (I.S.)
Fenvalerate
Fipronil
Flucythrinate *(sum)*
Fluvalinate
Folpet
Fonofos
Heptachlor
Heptachlor-epoxide
Heptenophos
Hexachlorobenzene
Iprodione
Isofenphos
Kepone
Kresoxim-methyl
Leptophos
Malathion
Metalaxyl
Methacrifos
Methidathion
Methiocarb
Methoxychlor
Metolachlor
Metribuzin
Mevinphos
Mirex
Myclobutanil
Nonachlor, *cis-*
Nonachlor, *trans-*
Oxadixyl
Oxyfluorfen
Parathion
Parathion-methyl
Penconazole
Pendimethalin
Pentachloroanisole
Pentachlorothioanisole
Permethrin, *cis-*
Permethrin, *trans-*
Phenylphenol, *o-*
Phorate
Phosalone
Phosmet
Phosphamidon
Phthalimide
Piperonyl butoxide
Pirimiphos-ethyl
Pirimiphos-methyl
Procymidone
Profenofos
Propachlor
Propargite
Propazine
Propetamphos
Propham
Propiconazole I-II
Propoxur
Propyzamide
Pyrimethanil
Quintozene
Resmethrin
Simazine
Sulprofos
Tebuconazole
Tecnazene
Terbufos
Terbuthylazine
Tetrachlorvinphos
Tetraconazole
Tetradifon
Tolclofos-methyl
Triadimifon
Triazophos
Trifluralin
Triphenylphosphate (QC)
Vinclozolin

Optimization of Speed and Sensitivity



He carrier gas at 20 psi constant pressure (2.57 mL/min at start and 1.2 mL/min at end of analysis = 103 - 70 cm/s)

LP-GC/MS-MS of Deltamethrin



Stronger pumping of MS/MS allowed higher flow rate and use of constant flow rather than constant pressure

LP-GC/MS Conditions

Leco Pegasus 4-D + Agilent 6890 GC + Atas Optic 3 PTV Injector

3 m, 0.15 mm i.d. restrictor + Rtx-5Sil-MS 10 m, 0.53 mm i.d., 1 µm film thickness

10 µL injection (MeCN extracts) 7°C initial for 18 s (15 s vent) to 280°C at 8°C/s (>2 min vent)

He carrier gas at 20 psi constant pressure

Oven program: 90°C for 1 min to 180°C at 80°C/min, to 250°C at 40°C/min to 290°C at 70°C/min, and hold for 5 min

Added oven insert pad to reduce volume and speed cool-down

280°C transfer line and 250°C ion source temperature

10 Hz data acquisition rate of *m/z* 70-600 (126 s delay), -70 eV

LP-GC/MS-MS Conditions

Agilent 7890 GC + 7000A MS/MS with Multi-Mode Injector

3 m, 0.15 mm i.d. HydroGuard capillary + Rti-5ms 10 m, 0.53 mm i.d., 1 µm film thickness

5 µL injection (MeCN extracts) 80°C initial w/ 50 mL/min vent for 19 s then to 320°C at 7°C/s (vent >4 min) – 9.5 min total

He carrier gas at 2 mL/min constant flow

Oven program: 70°C for 1.5 min to 180°C at 80°C/min, to 250°C at 40°C/min to 290°C at 70°C/min, and hold for 4.3 min

250°C transfer line; 320°C ion source; 150°C quad temperatures

2.5 ms dwell time with 1 ms interchannel delay

Wide setting (1.2 amu) for transitions (not “unit” or widest”)

Little Important Details

Needed 220 V oven heating upgrade to save 1 min in method and yield consistent t_R (critical in MS/MS)

Added oven insert pad to reduce volume and speed heat up by ≈0.8 min and cool-down by ≈0.15 min

Used MMI (or similar type) as PTV Injector (5-10 µL MeCN)

Liner can be dimpled, wall-coated sintered glass, or glass wool to keep matrix away from column restrictor inlet

Can backflush inlet, but can't backflush column in LP-GC

Used analyte protectants to improve results for pesticides affected by matrix-induced effect (wash syringe well!)

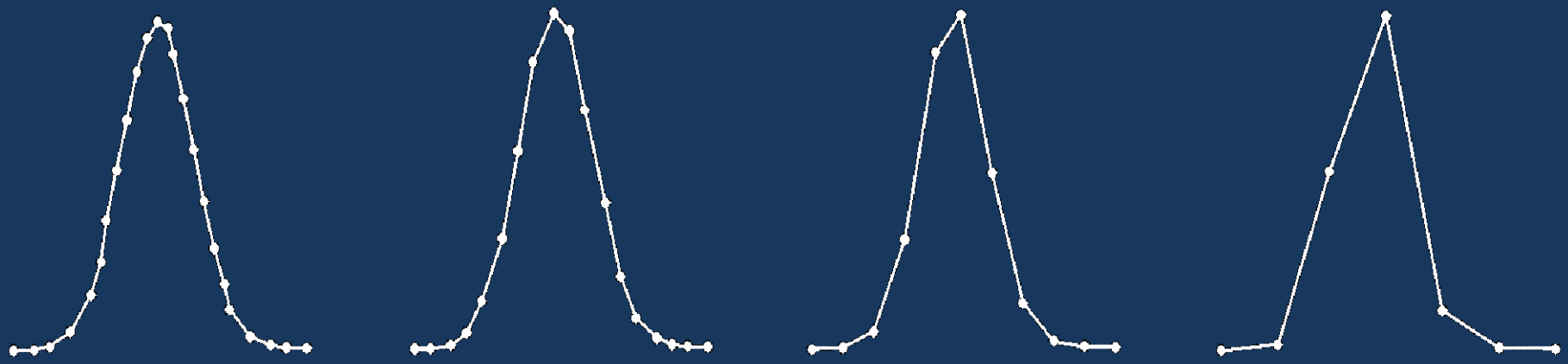
Can use 5 m, 0.18 mm i.d. restrictor + 10-15 m, 0.53 mm i.d., 1 µm film thickness→ COLUMN BLEED in full scan, but not seen in MS/MS Notes: Transfer Piece & Ultima Union

Peak Characteristics vs. Dwell Time

Data acquisition and peak width dictate the no. of data points across the peak

Cycle time = dwell time + interscan delay (1 ms) times the no. of ion transitions

Heptachlor



1 ms dwell	2.5 ms dwell	5 ms dwell	10 ms dwell
9.1 cycle/s	5 cycle/s	2.9 cycle/s	1.5 cycle/s
2.55 s pk width	2.97 s pk width	2.70 s pk width	2.85 s pk width
20 points/pk	14 points/pk	8 points/pk	4-5 points/pk

Longer dwell time results in worse chromatographic peak shapes

≥ 8 points across a peak for quantitative purposes are often overstated

Peak Characteristics vs. Dwell Time

If cycle time is not constant across peaks, then notches in peaks occur and quantitation is affected.

Thus, we included 30 analytes (60 transitions) in each of 26 segments with 2.5 ms dwell times (210 ms cycle times) for >10 points across each peak.

QuEChERS and LP-GC/TOF Exp't

Evaluate 4 different QuEChERS versions

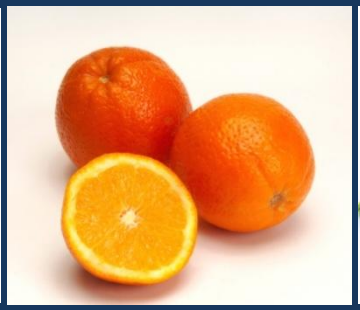
(original vs. AOAC 2007.01 each using d-SPE and DPX cleanup) for

150 + 3 QC pesticides spiked at 3 levels (25, 100, 400 ng/g) with

5 replicates at each level for each cleanup technique in

5 matrices (tomato, strawberry, potato, orange, and lettuces)




2 methods x 2 cleanups x 3 levels x 5 reps x 5 matrices = 300 spikes

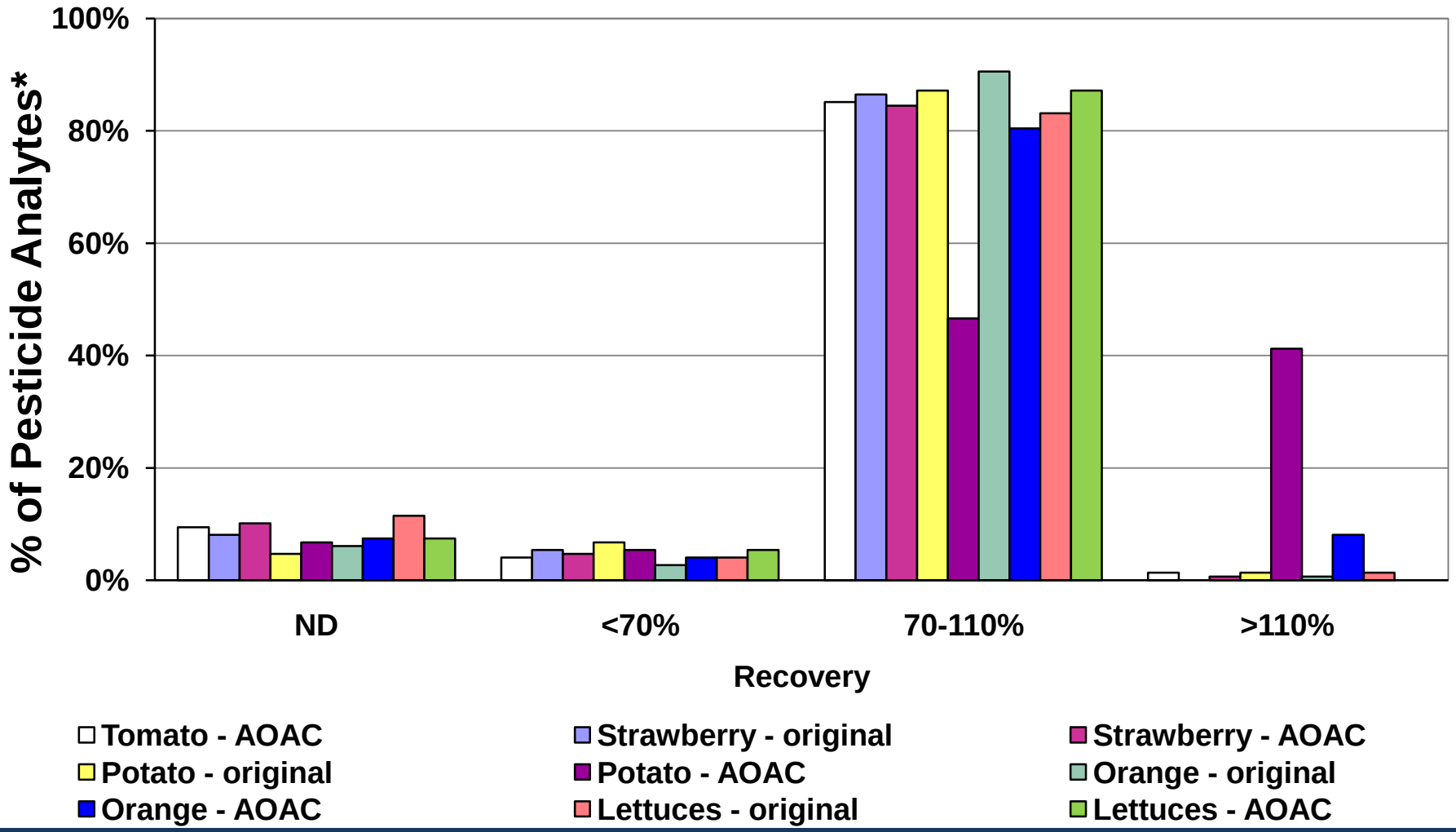
+ 15 cal stds + 3 blks per seq. x 153 analytes = 68,860 data points

2 chemists, 16 samples/day each, 10 days

(48 injections/day = 480 total)

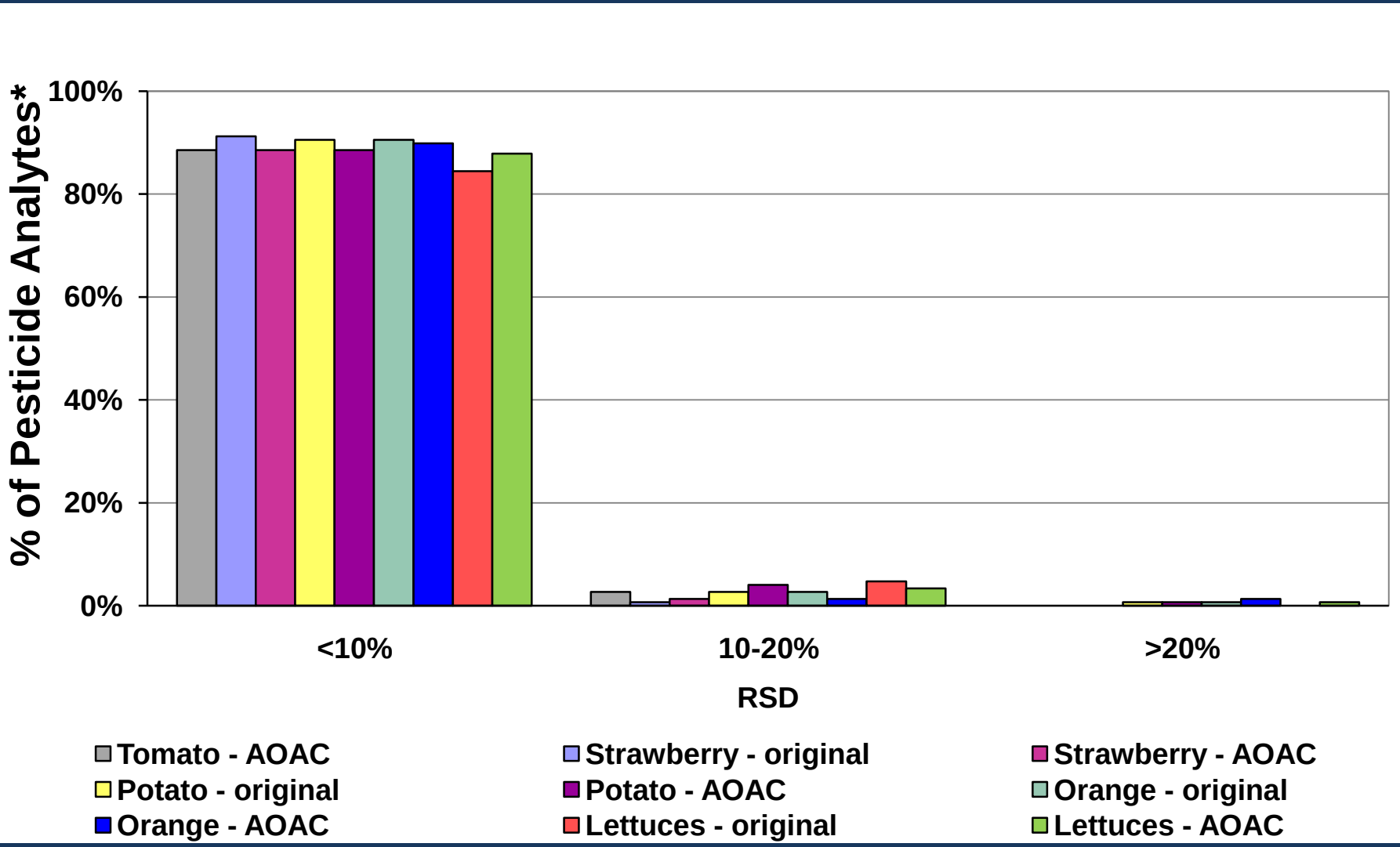
1 hr for sample prep and 8 hrs sequence per day

Recoveries in All Matrices



* 151 Total analyzed pesticides

RSD in All Matrices



* 151 Total analyzed pesticides

QuEChERS and LP-GC/MS-MS Exp't

Evaluate updated QuEChERS version for

150 pesticides (+ 3 I.S./QC compounds) spiked at

3 levels (10, 75, 400 ng/g) with

6 replicates at each level in

4 matrices (cantaloupe, sweet potato, lemon, and broccoli)

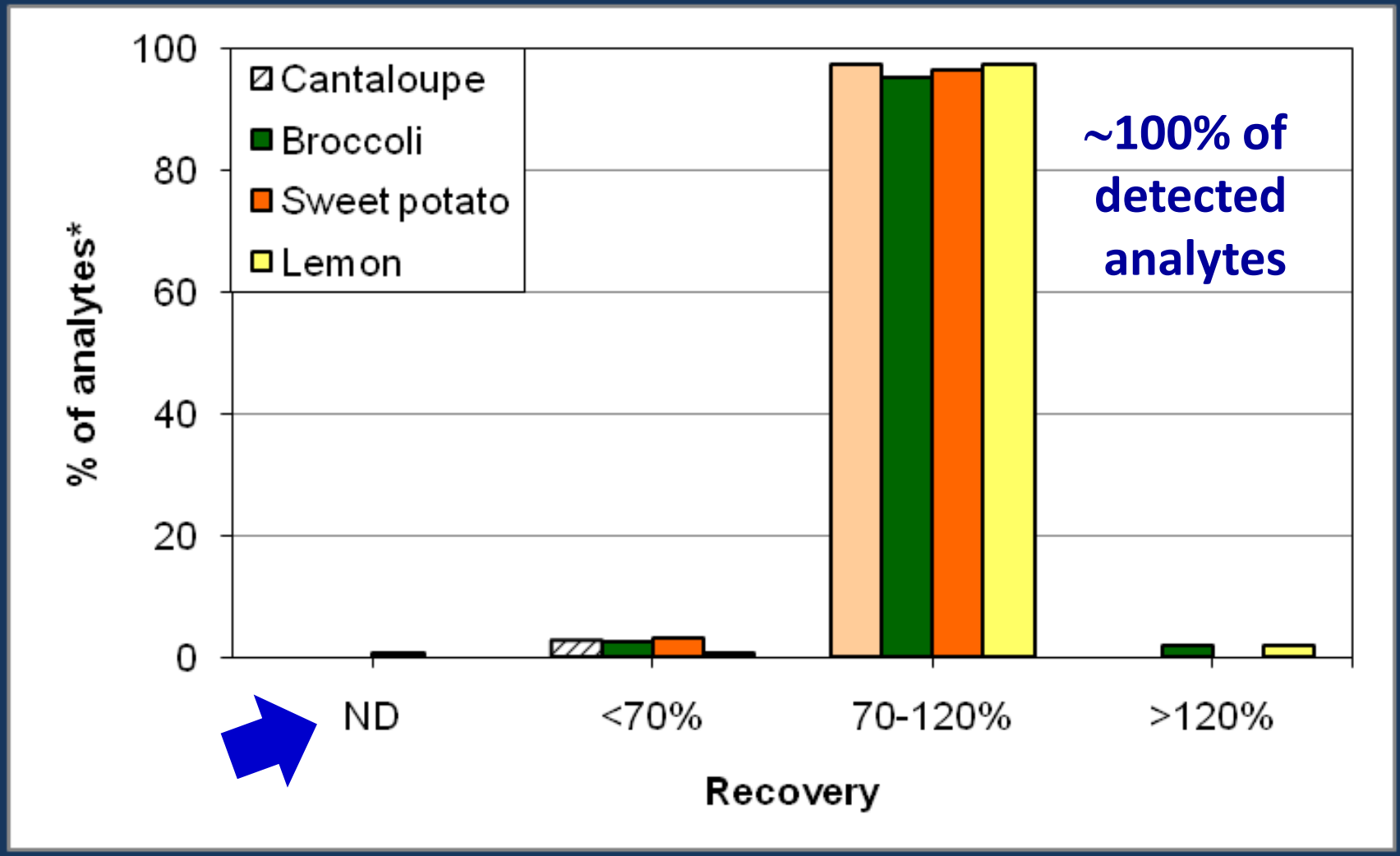



3 levels x 6 reps x 4 matrices = 72 spikes + 14 cal stds + 2 blks per seq. x 153 analytes = 13,464 data points

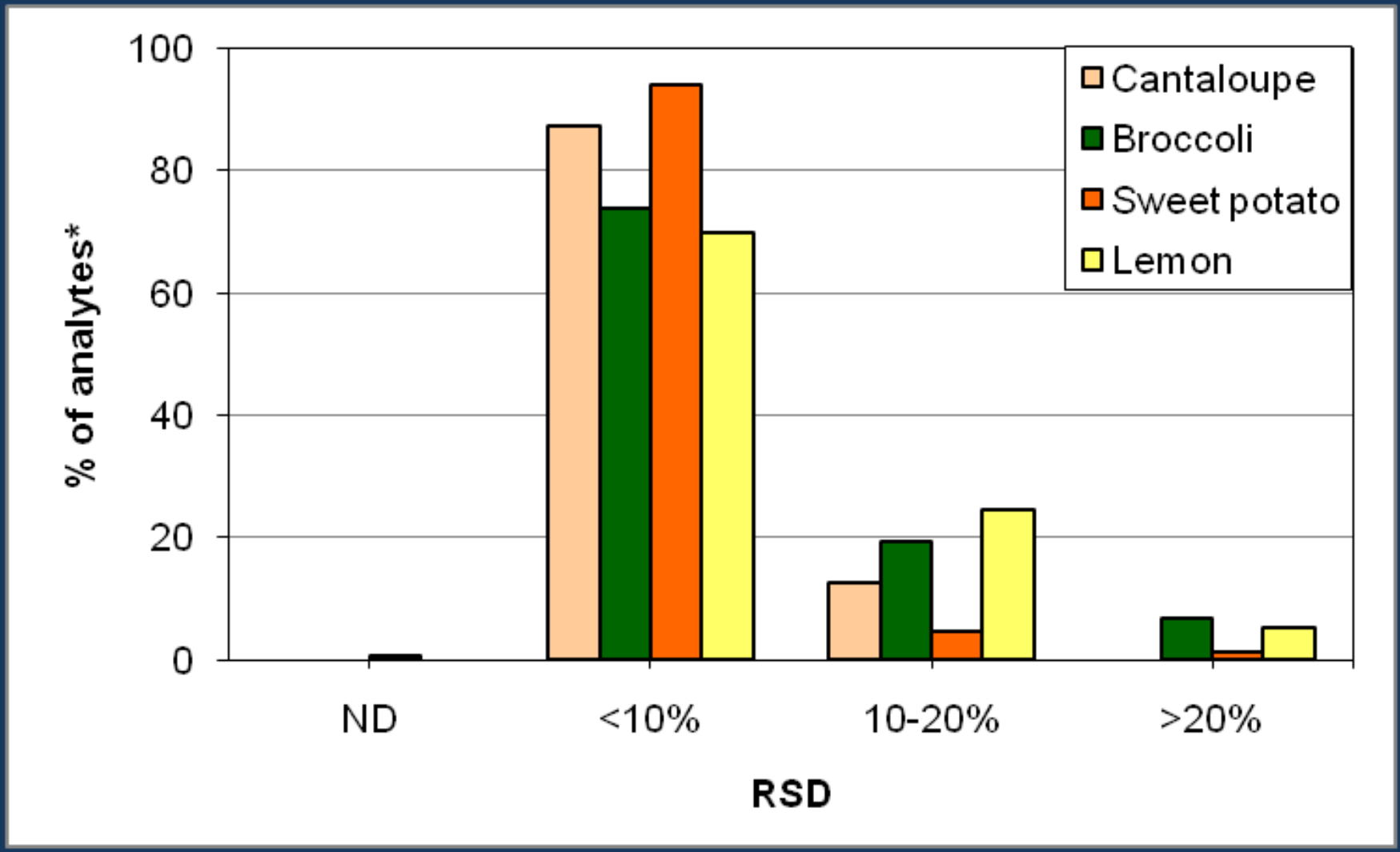
1 chemist, 18 samples/day, 4 days (34 inj'ns/day = 136 total)

1 hr for sample prep and 8 hrs sequence per day

QuEChERS + LP-GC/MS-MS Results



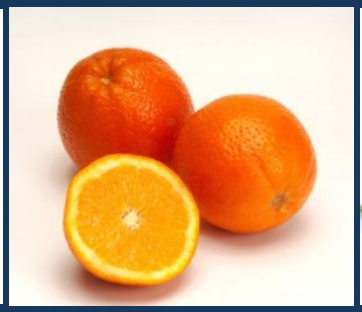
QuEChERS + LP-GC/MS-MS Results



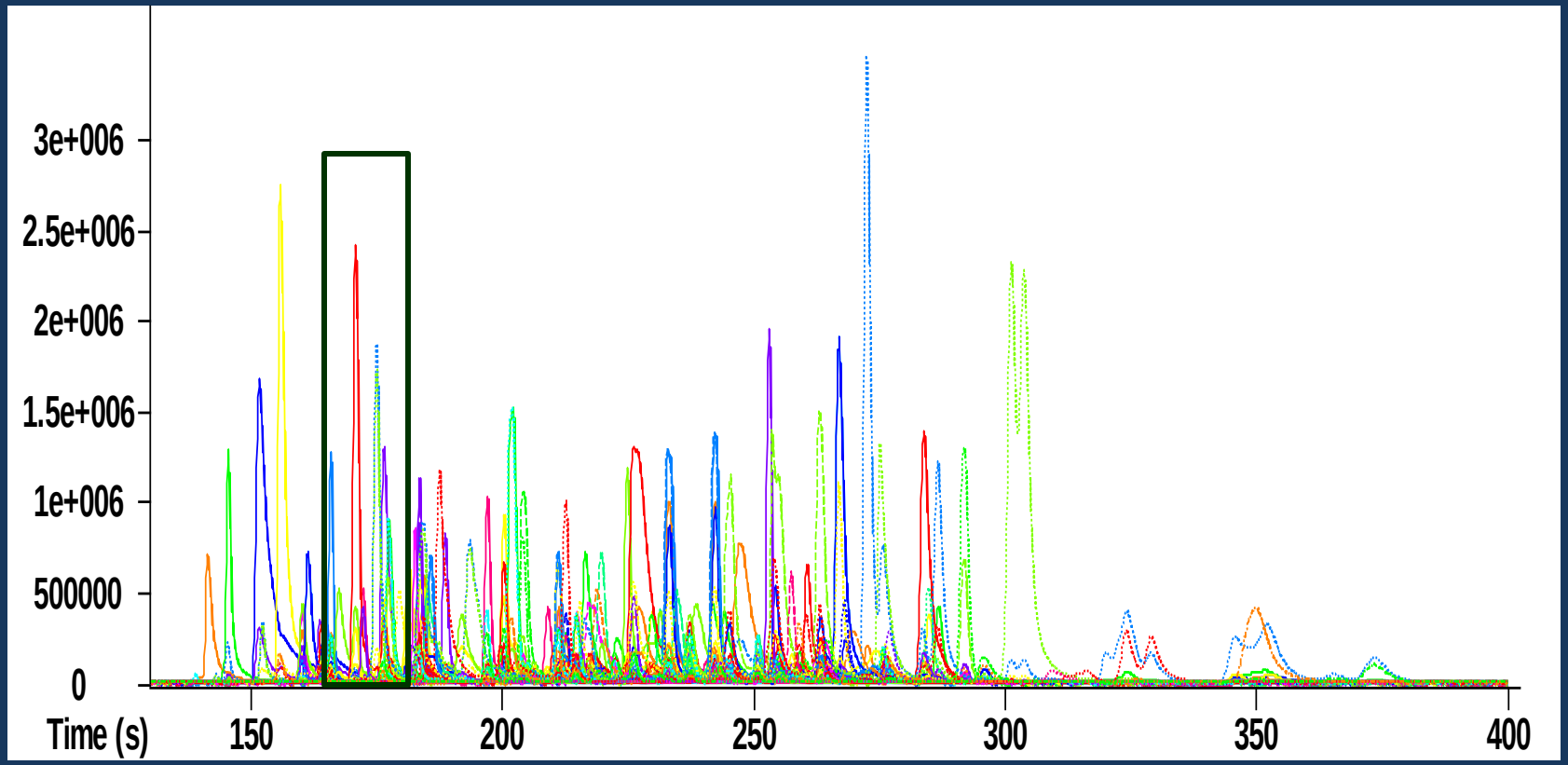
Qualitative Assessment LP-GC/ToF

100 Final Extracts Prepared (20 for each Matrix - Tomato, Strawberry, Potato, Orange, and Lettuces) and Random Spike Additions Made (or not) among the 150 Pesticides from 25-1,000 ng/g Analyzed in Blind Fashion to Determine Quantitative Accuracy and Rates of False Positives and Negatives by the LP-GC/ToF Method

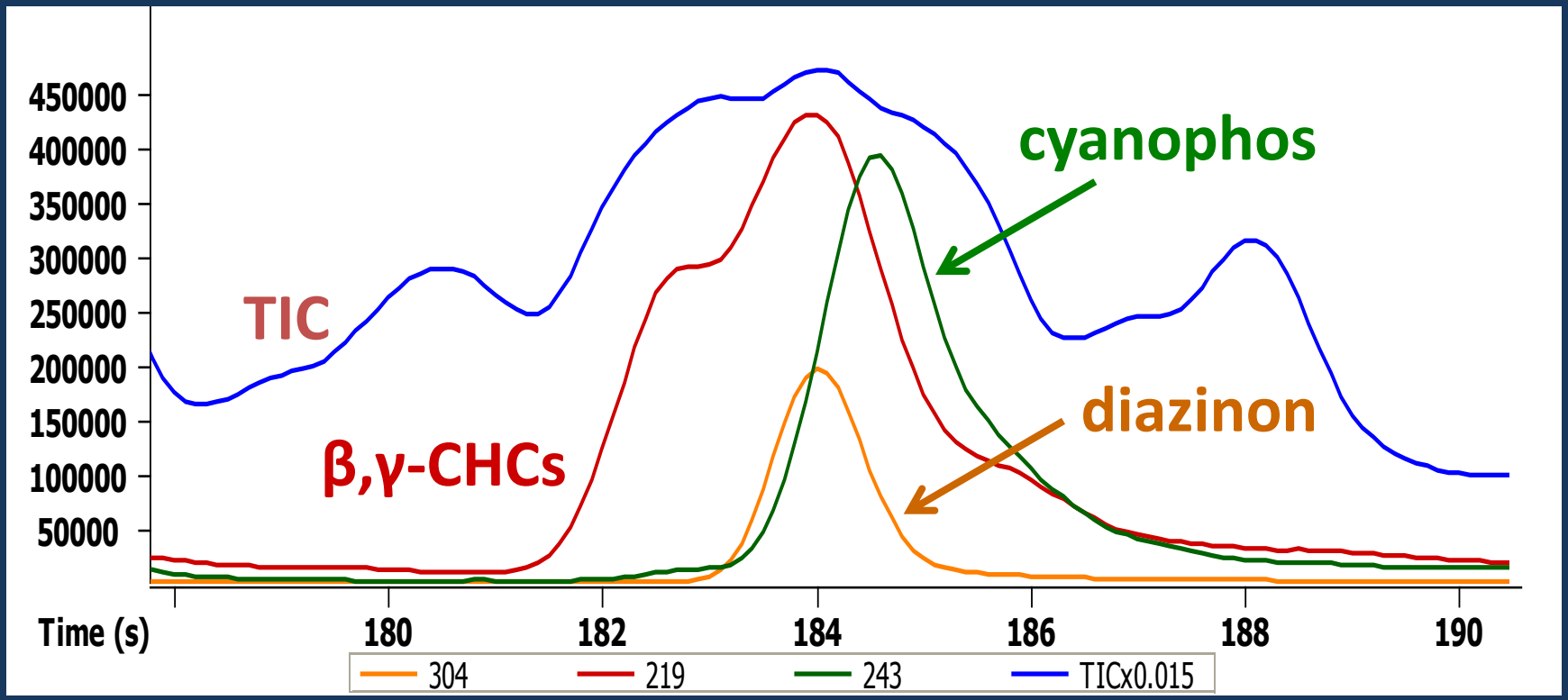



LP-GC/MS (ToF) of 153 Pesticides

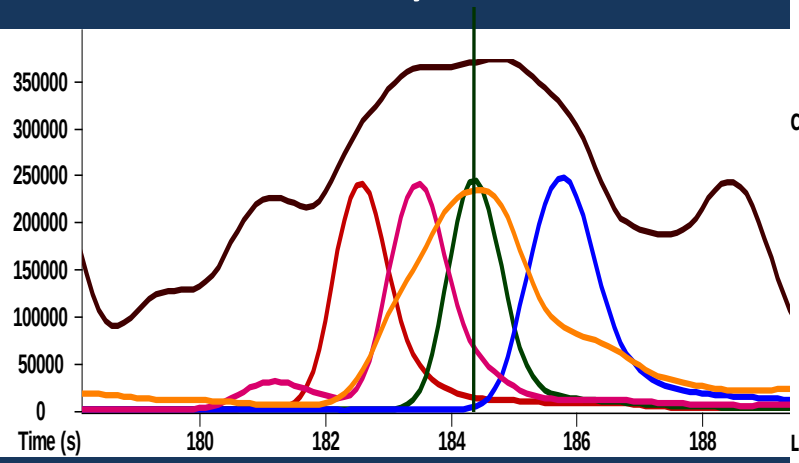


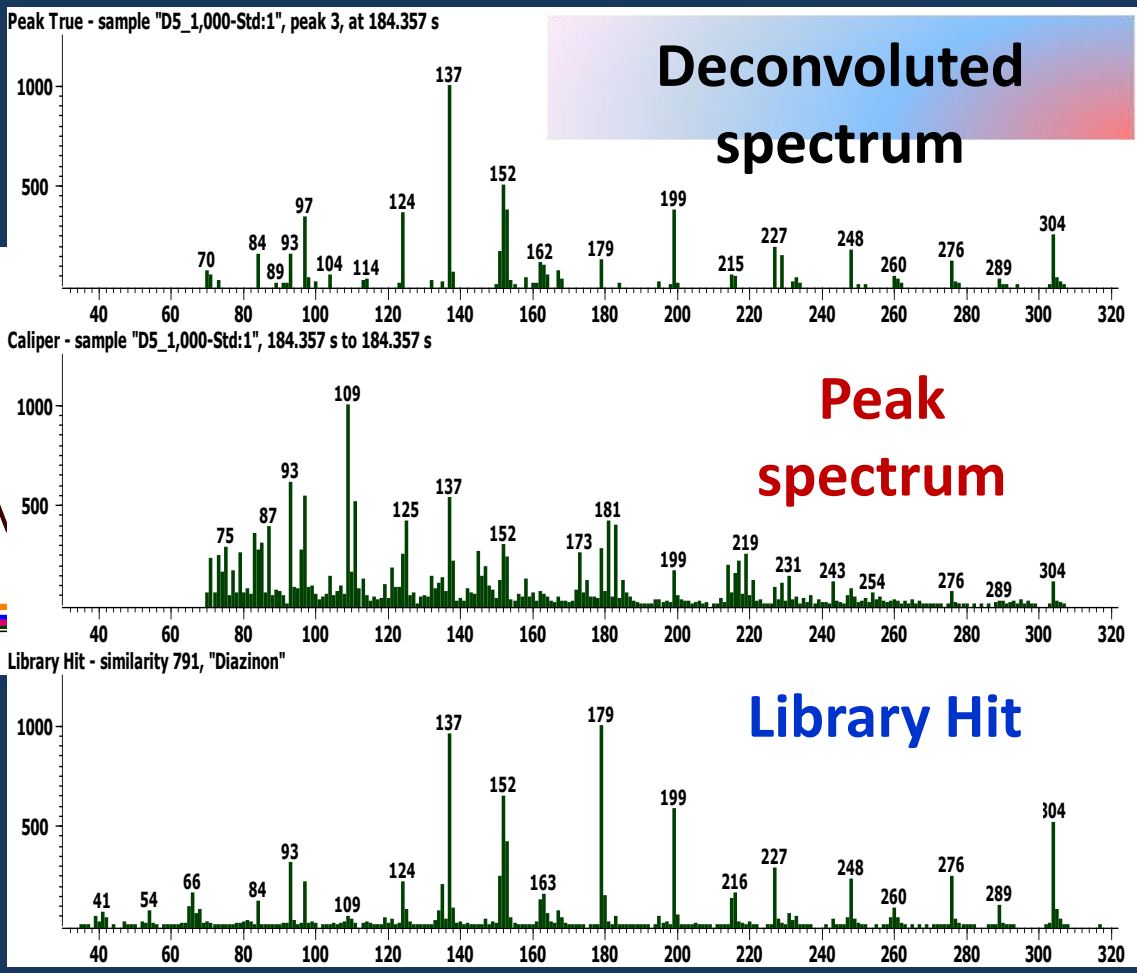
Deconvolution Full-Scan MS



Analyte Identification ToF

Diazinon
m/z 304





Rates of True and False Identifications

Factor	Human Judgment	Automated Software	
		Fit ≥ 600	Fit ≥ 700
	≥10 ng/g	S/N ≥ 100	S/N ≥ 100
False positives	0.8%	1.0%	0.5%
False negatives*	17.1%	23.1%	29.4%
True (added)	82.9%	76.9%	70.6%
True (overall)	98.4%	97.8%	98.0%

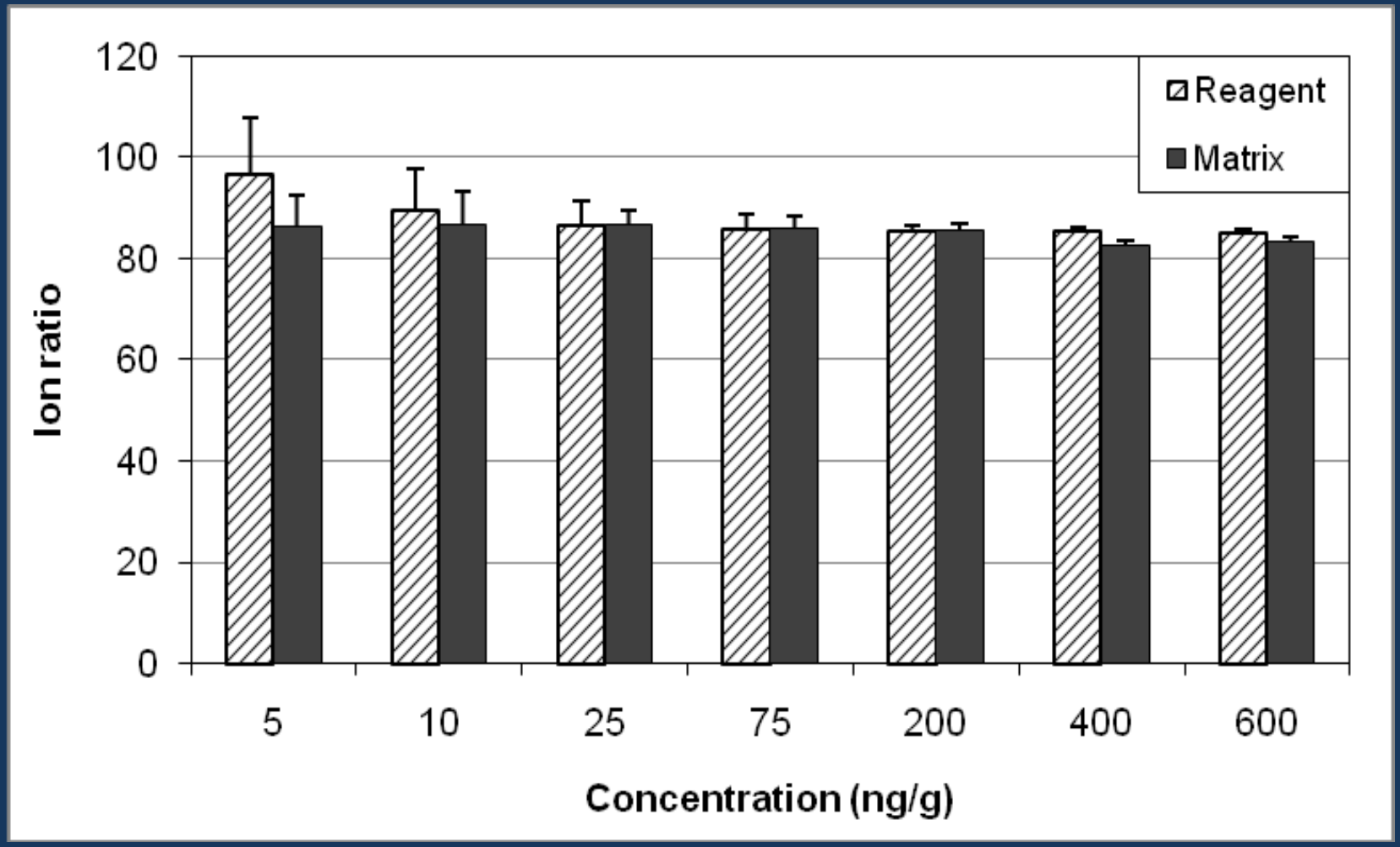
*** The false negatives were mainly caused by LC-amenable pesticides carbaryl, fenthion sulfone, and simazine, and degradable captafol, folpet, and phthalimide. False negative rates were 5-10% in the approaches otherwise.**

Qualitative Assessment of LP-GC/QqQ

100 Final Extracts Prepared (20 for each Matrix - cantaloupe, sweet potato, lemon, and broccoli) and Random Spike Additions Made (or not) among the 150 Pesticides from 10-600 ng/g Analyzed in Blind Fashion to Determine Quantitative Accuracy and Rates of False Positives and Negatives by the LP-GC/MS-MS Method




Identification Criteria



Results for Diazinon, n=35 at each level in 4-5 matrices in sequences on multiple days

Identification Criteria

1. Retention time (t_R) is within ±3 SD of average t_R and peak shape matches that of reference standard

2. t_R and peak shape of qual. ion(s) matches those of the quantification ion

3. Ion ratio is within ±3 SD (measured at 10 ng/g level) of average ion ratio

4. S/N ≥ 3 (or 10) for quant. and qual. ions

5. Absence of positive findings in blanks

Qualitative Validation Results

NO FALSE POSITIVES

OR FALSE NEGATIVES!

(for the targeted analytes)

Conclusions

The LP-GC/MS-MS method outperformed the LP-GC/ToF method for the same pesticides in similar matrices in several respects:

1) greater selectivity led to much easier peak integration and identification

2) at least 50% lower LODs were obtained w/ 50% lower inj'n vol.

3) data review time was reduced from several weeks to a few days

4) a wider scope of pesticides, including chlorothalonil, folpet, and captan gave excellent recoveries and precision in the results

The drawback of MS/MS vs. ToF is that only targeted analytes could be monitored whereas full scan allows looking for wider scope.

The LP-GC/ToF studies are published and the LP-GC/MS-MS papers are submitted.

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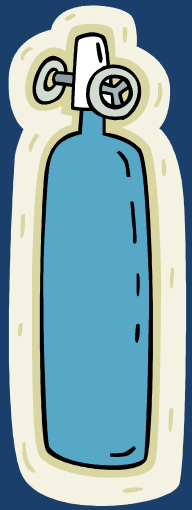

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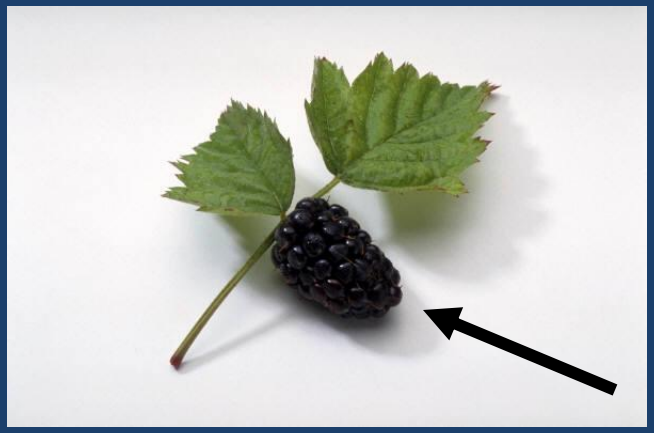

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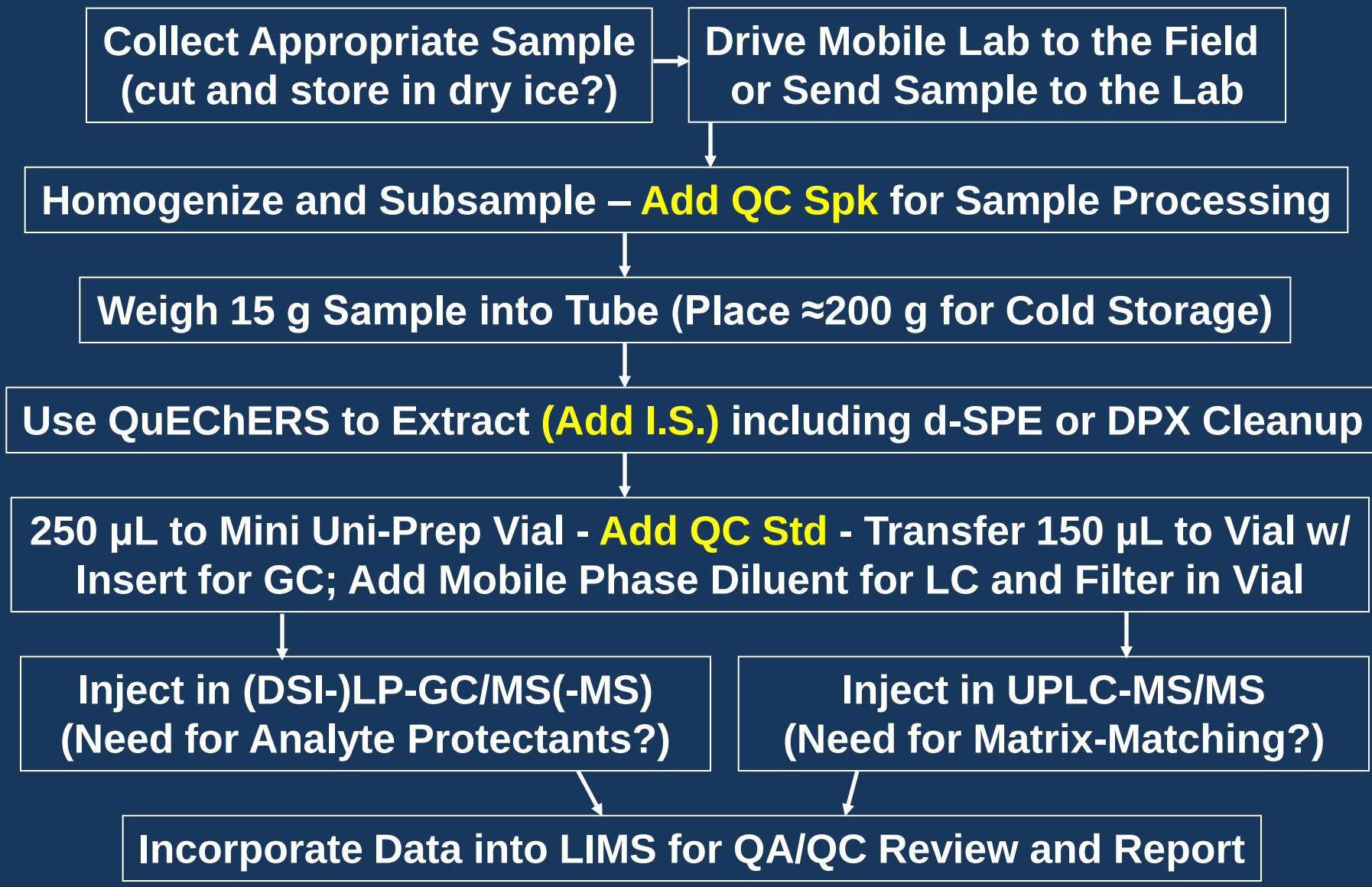
Tanks Berry Mulch!

Efficient Pesticide Residue Analysis



Conclusions

- QuEChERS is a well-proven, fast sample preparation method for hundreds of pesticide residues in different types of food matrices.
- UPLC-MS/MS can provide 10 min analysis of hundreds of LC-amenable pesticides.
- LP-GC/MS can also provide 10 min analysis of hundreds of GC-amenable pesticides.
- Currently, the **HUGE** sample throughput limitation is data processing and review!

Conclusions

- **QuEChERS:** fast and easy sample preparation method
- **LP-GC/MS:** 10 min analysis of hundreds of GC-amenable pesticides
- Single method for wide scope of analysis!
- Limit of detection: ≥10 ng/g for ToF MS
  5-10 ng/g for MS/MS
- High sample throughput
- Low cost per sample (~40-50% reduction)
- Less hazardous waste

Objective

Develop a high throughput screening method (20 min extraction and determination) for determination of pesticide residues in fruits and vegetables using LP-GC/TOF

Experimental





Method validation

- recovery: 25, 100, 400 ng/g
- precision: 5 replicates/each cleanup met'd

3 Spks × 5 replicates × 2 cleanups × 2 ext'ns × 5 matrices

- linearity: 5 points (10, 25, 100, 400, 1000 ng/μL) for both of Stds in solvent and matrix-matched Stds
- matrix effects
- ruggedness: 200 ng/g I.S.

*48 injection per day, 8 hrs

LP-GC/TOF Conditions

Atas Optic 3 PTV Injector: 10 µL injection (MeCN extracts), 7ºC initial for 18 s (15 s vent) to 280ºC at 8ºC/s (>2 min vent)

Agilent 6890 GC:

- 3 m×0.15 mm i.d. restrictor + Rtx-5Sil-MS 10 m×0.53 mm i.d., 1 µm film thickness
- He carrier gas at 20 psi constant pressure entire the run
- oven temp program: 90ºC for 1 min, 80ºC/min to 180ºC, 40ºC/min to 250ºC, 70ºC/min to 290ºC (hold for 5 min)
- oven insert pad used to reduce the oven volume and speed cool-down

Leco Pegasus 4D TOFMS: 280ºC transfer line, 250ºC ion source temperature, solvent delay 126 s, m/z 70-600, 10 Hz data acquisition rate, -70 eV

Problematic Analytes

Degradation in MeCN/GC injection steps:

captan, captafol, chlorothalonil, folpet

LC-based analysis:

atrazine, captan, captafol, deltamethrin, dimethoate, phosmet, methiocarb

Missing in detection/integration:

azinphos-methyl, demeton-s-methyl, demeton-s-methylsulfone, fenthion sulfone, metribuzin, oxadixyl, and methyl parathion

Retention of planar pesticides on GCB sorbent:

chinomethionat, chlorothalonil etc.

Conclusions

The method provides identification and quantification for most of pesticides in a single method

TOF MS: automated peak searching and comparing with library, mass spectral deconvolution

QuEChERS and LP-GC/TOF MS = High throughput analysis (~10 min for 48 extractions and <10 min for determination)

80-91% of analytes give 70-110% recovery with <10%RSD for 84-91% of analytes

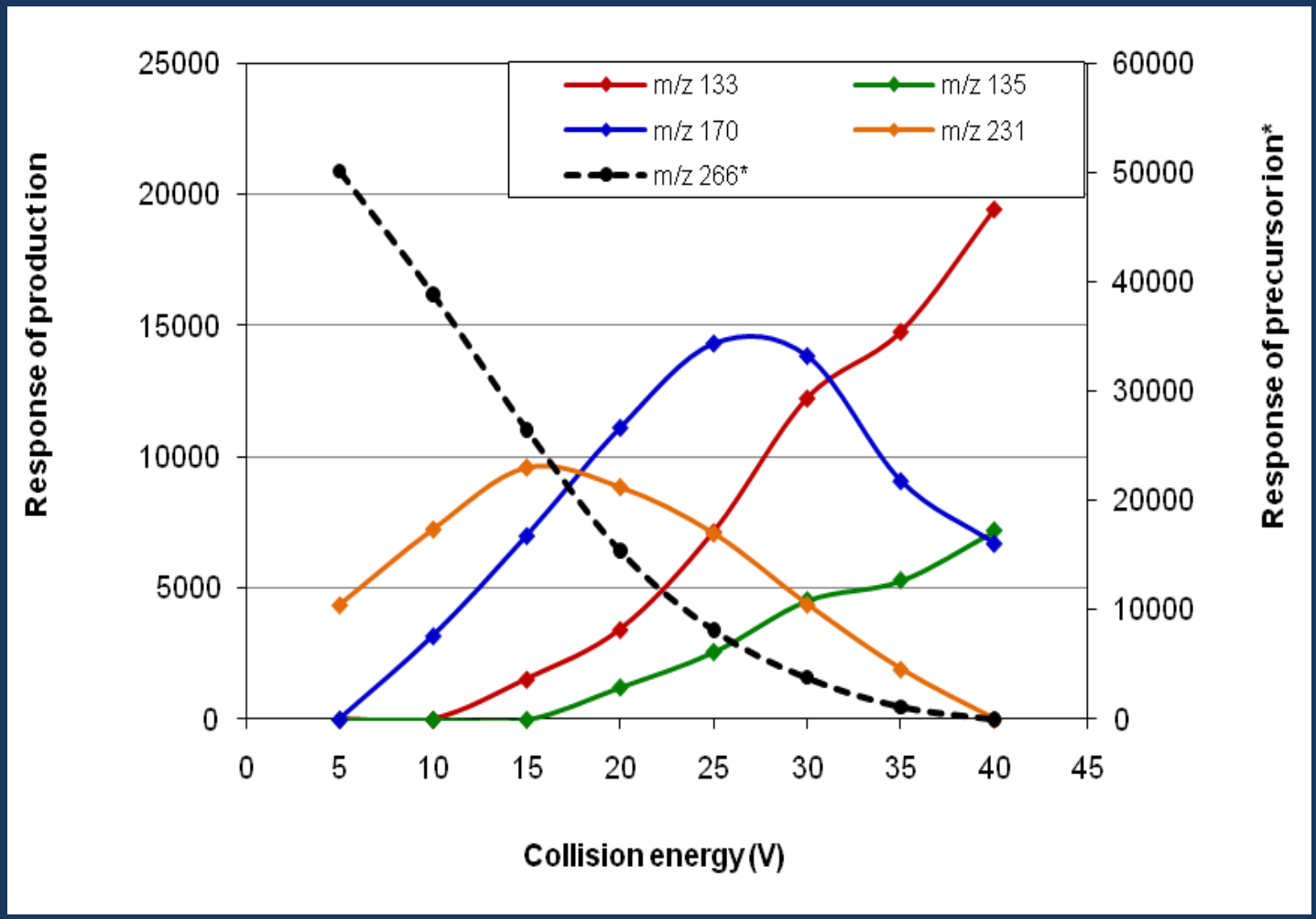
No significant difference of results between d-SPE and DPX tip, except cost and time

QuEChERS Sample Prep

(1) weigh 15 g homogenized sample into a 50 mL tube
(2) add spiking and I.S. solutions, and vortex for 1 min;
(3) add 15 mL of MeCN with 1% HOAc; shake for 30 s;
(4) add 6 g of anh. $MgSO_4$ and 1.5 g of anh. NaOAc;
(5) shake the tube immediately for 1 min;
(6) centrifuge the tube at 3,250 rcf for 2 min;
(7) transfer 1 mL extract to d-SPE tube containing 150 mg anh. $MgSO_4$ + 50 mg PSA + 50 mg C-18 + 7.5 mg GCB;
(8) mix for 30 s and centrifuge at 3,250 rcf for 2 min;
(9) transfer 0.5 mL into an autosampler vial;
(10) add 50 μL of the QC and analyte protectants mixture and 50 μL MeCN (to make sample volumes equal those of the calibration standards), and
(11) conduct LP-GC/MS-MS analysis.

Choice of Ion Transitions

Chlorothalonil: quant. *m/z* 266→133 at 40 V CE;
qual. *m/z* 266→168 at 25 V CE

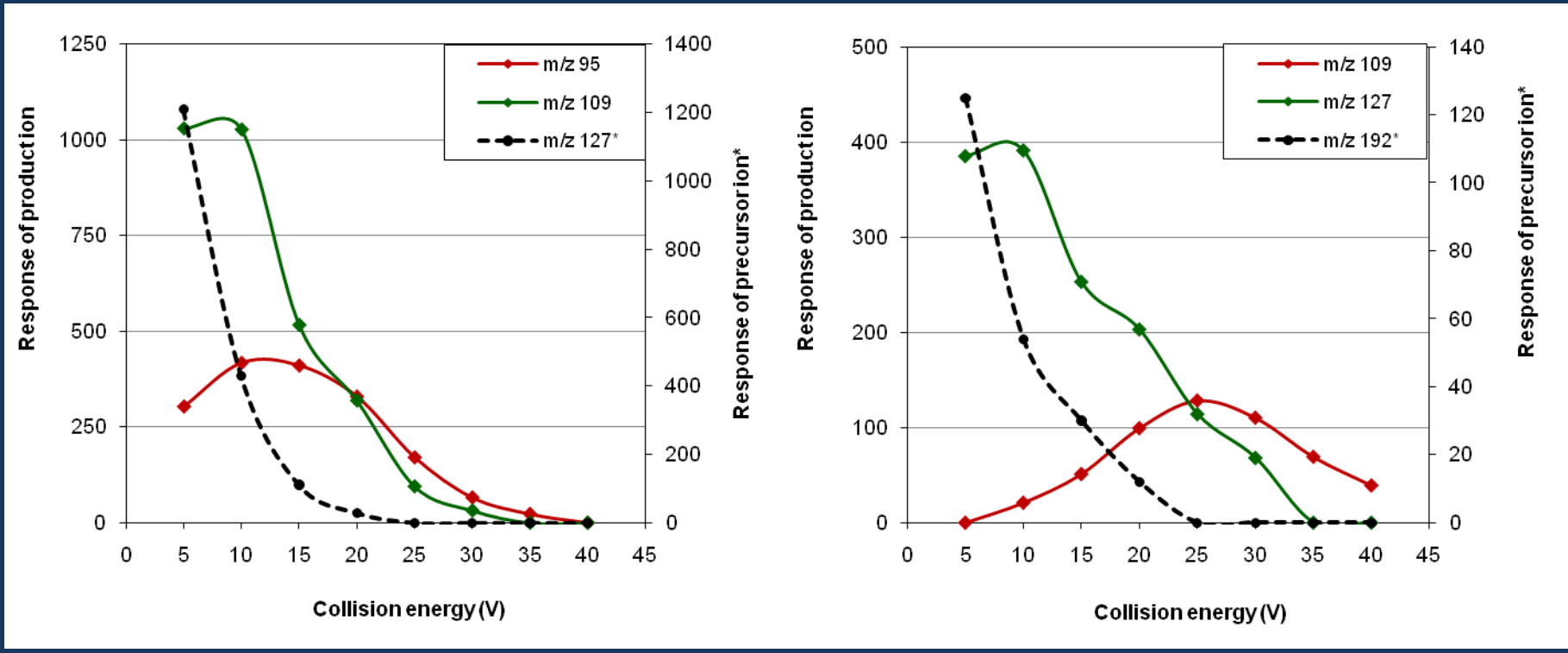


Choice of Ion Transitions

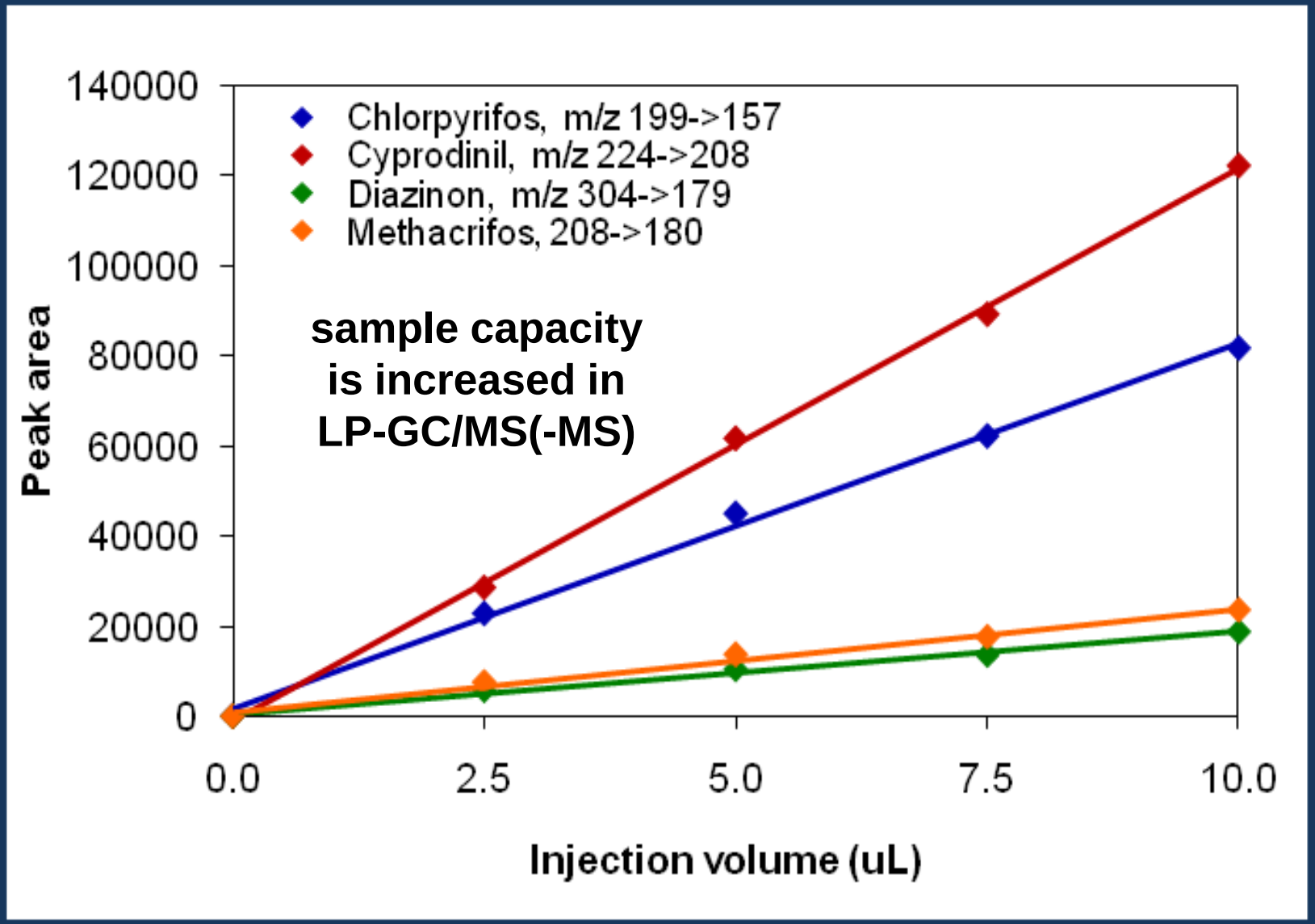
- high sensitivity and selectivity for each compound
- consideration of interfering ions from matrix and other analytes
- optimum collision energy → highest S/N for each transition

Mevinphos

quant. *m/z* 127→109 at 10 V CE; qual. *m/z* 192→127 at 10 V CE



Injection volume



Injection volume

Increase inj'n vol. → Increase peak ht. with const. peak width

Diazinon FWHM = 0.9 s

Chlorpyrifos FWHM = 0.96 s