

Tricks of the Trade, Keeping Your GC System Up Longer for the Analysis of Pesticides (and More!!)

Michelle Misselwitz, Jack Cochran, Julie Kowalski,
Christopher Rattray

The Main Problems with GC-MS

- GC inlet

- Liner
- Seal



- Dirty samples contaminate liner wool and bottom seal

- Poor compound transfer
- Compound degradation



- GC column

- Guard column
- Analytical column



- Dirty samples contaminate front of column system

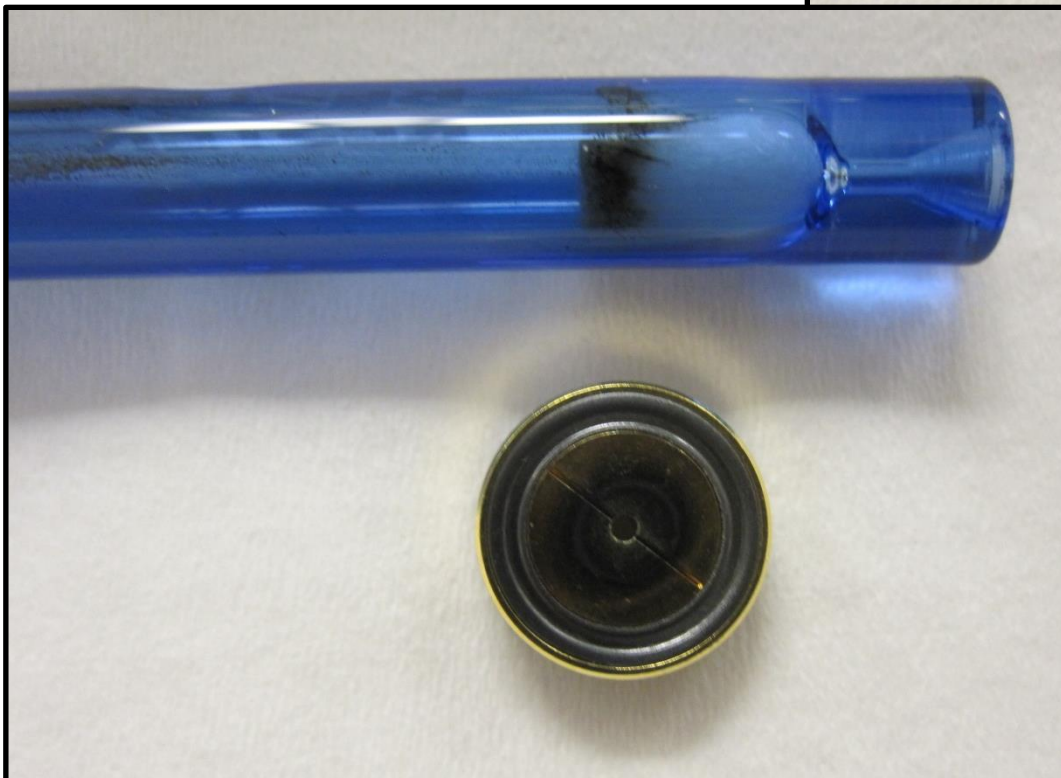
- Poor compound transfer
- Compound degradation



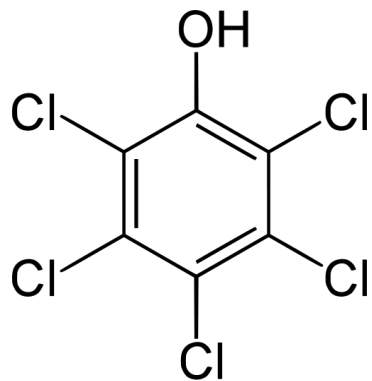
Both issues lead to poor data quality and downtime for maintenance...

Splitless injection

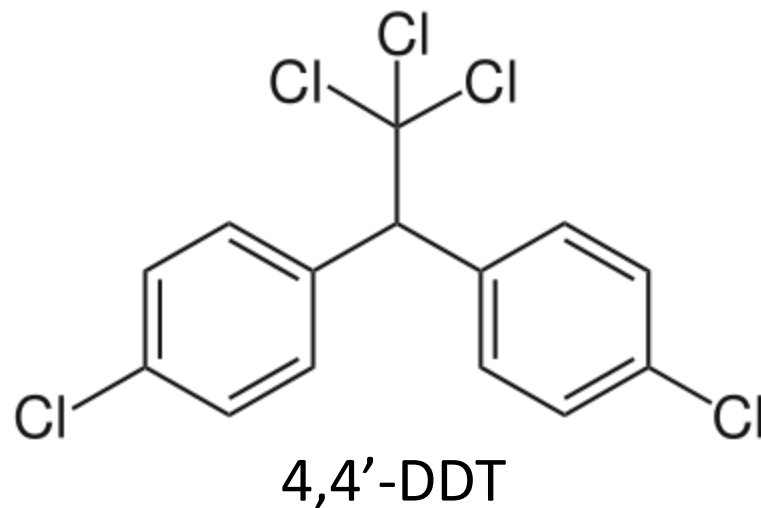
27 × Used Motor Oil
50,000 $\mu\text{g}/\text{mL}$



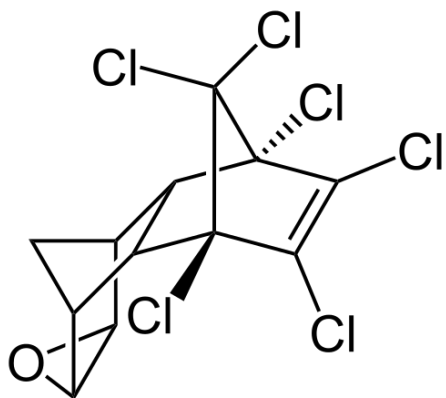
39 × Sediment Extract
(no dilution)



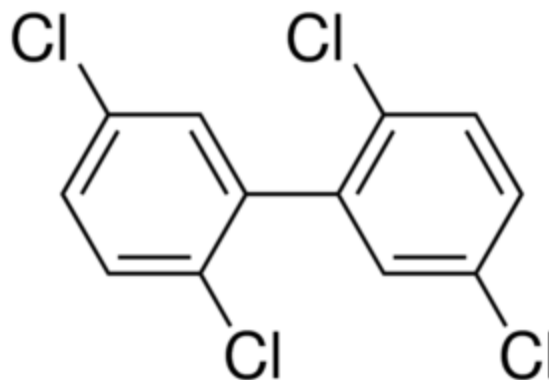
Pentachlorophenol



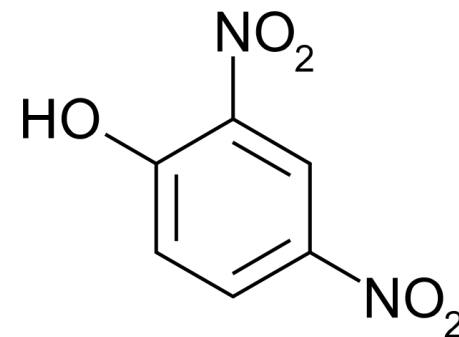
4,4'-DDT



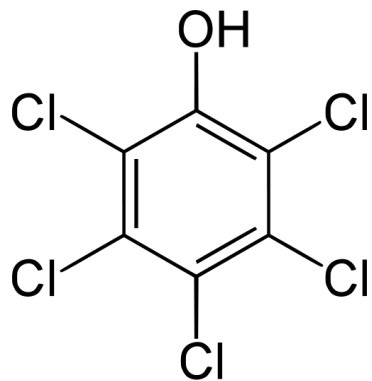
Endrin



PCB 52



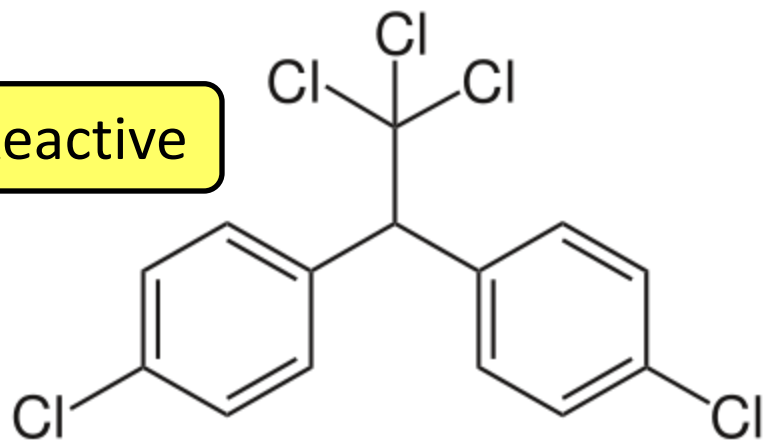
2,4-Dinitrophenol



Active

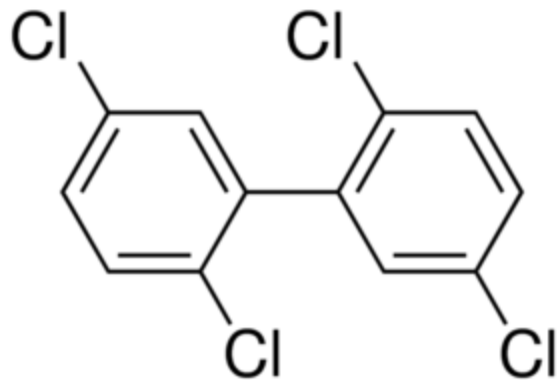
Pentachlorophenol

Reactive



4,4'-DDT

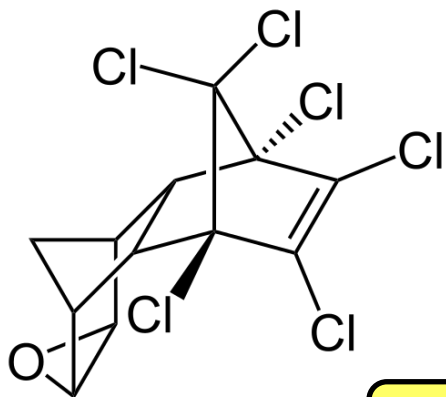
Internal standard



PCB 52



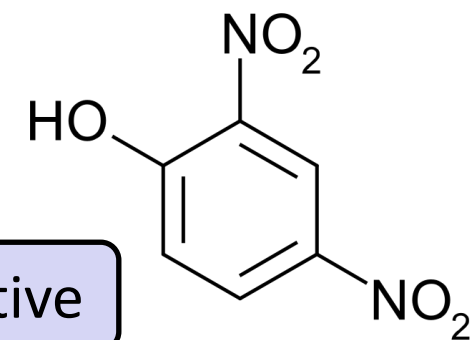
- Proof



Endrin

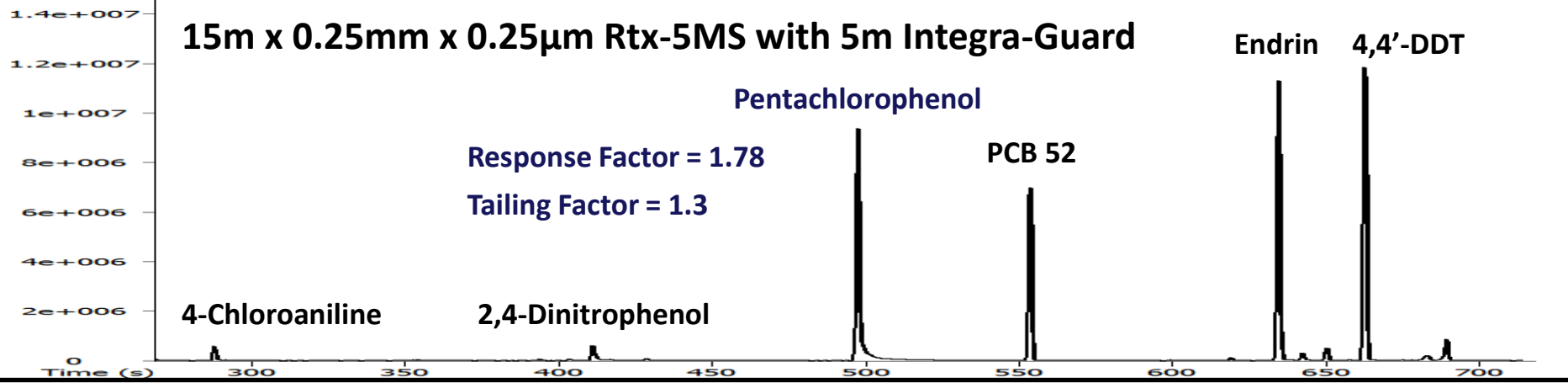
Reactive

Active

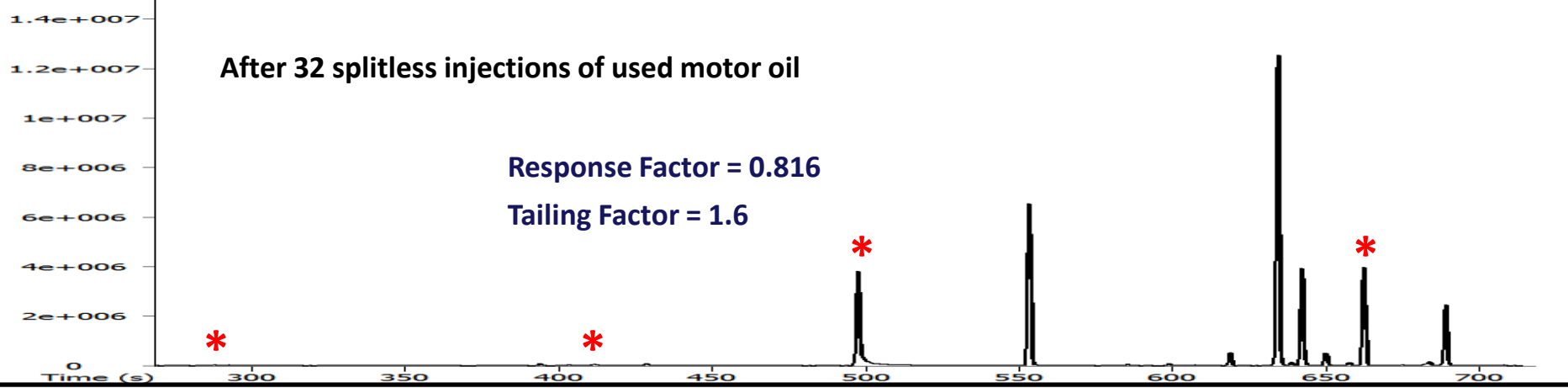


2,4-Dinitrophenol

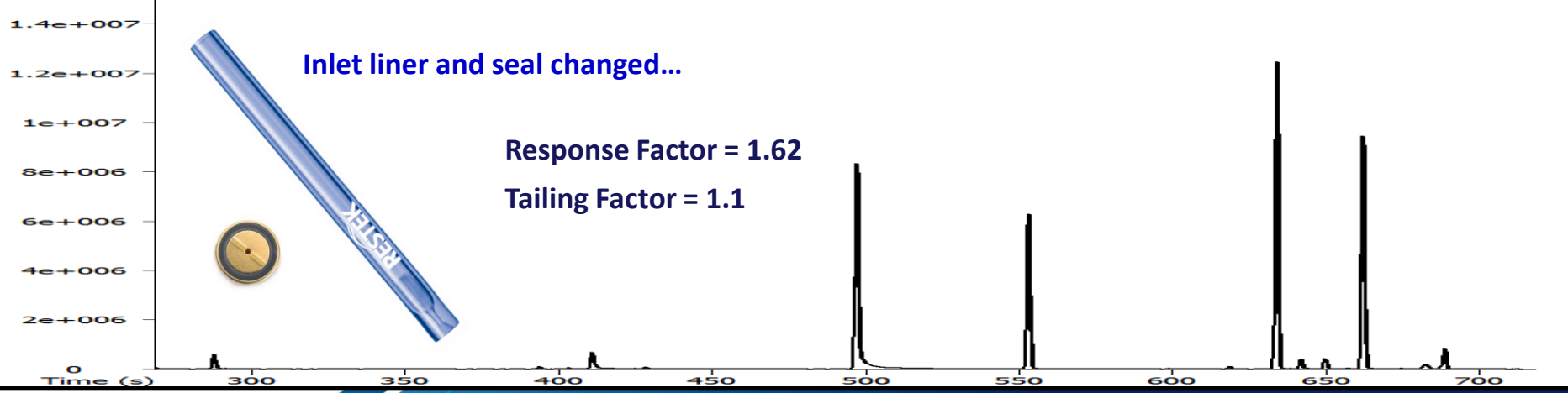
15m x 0.25mm x 0.25µm Rtx-5MS with 5m Integra-Guard



After 32 splitless injections of used motor oil



Inlet liner and seal changed...



What about wool-
packed versus non-
wool-packed liners?



RESTEK

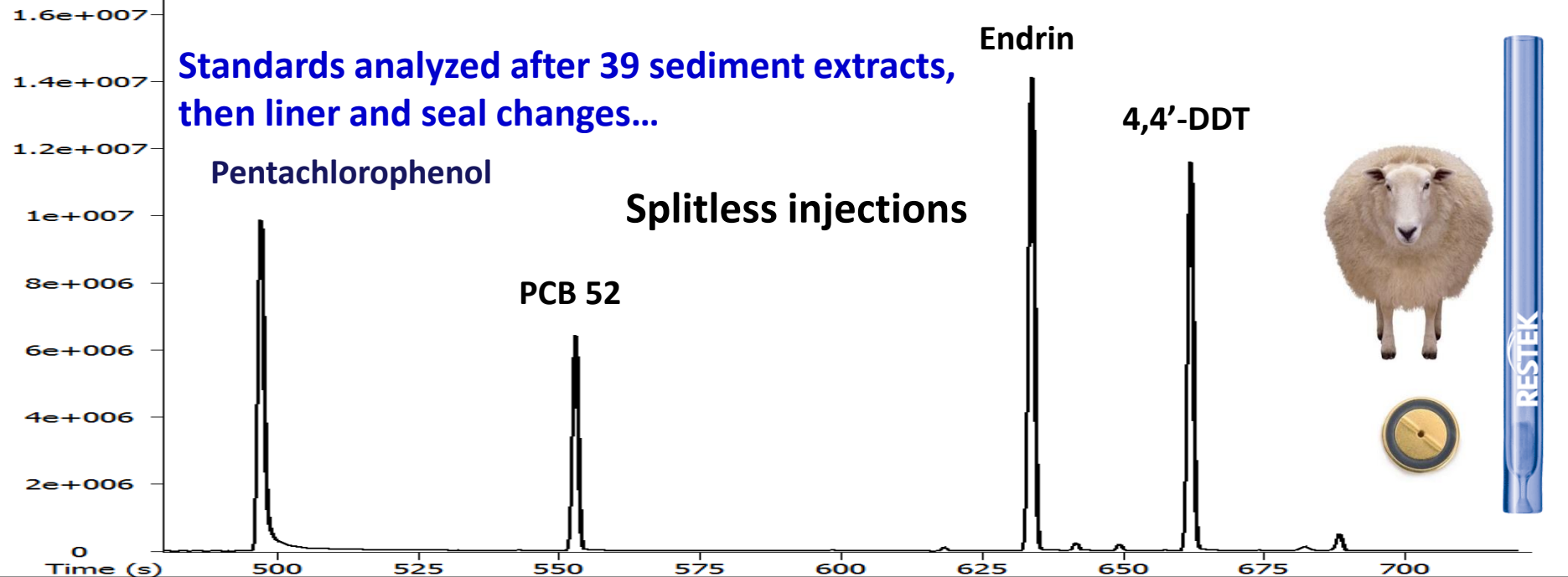
RESTEK

REST

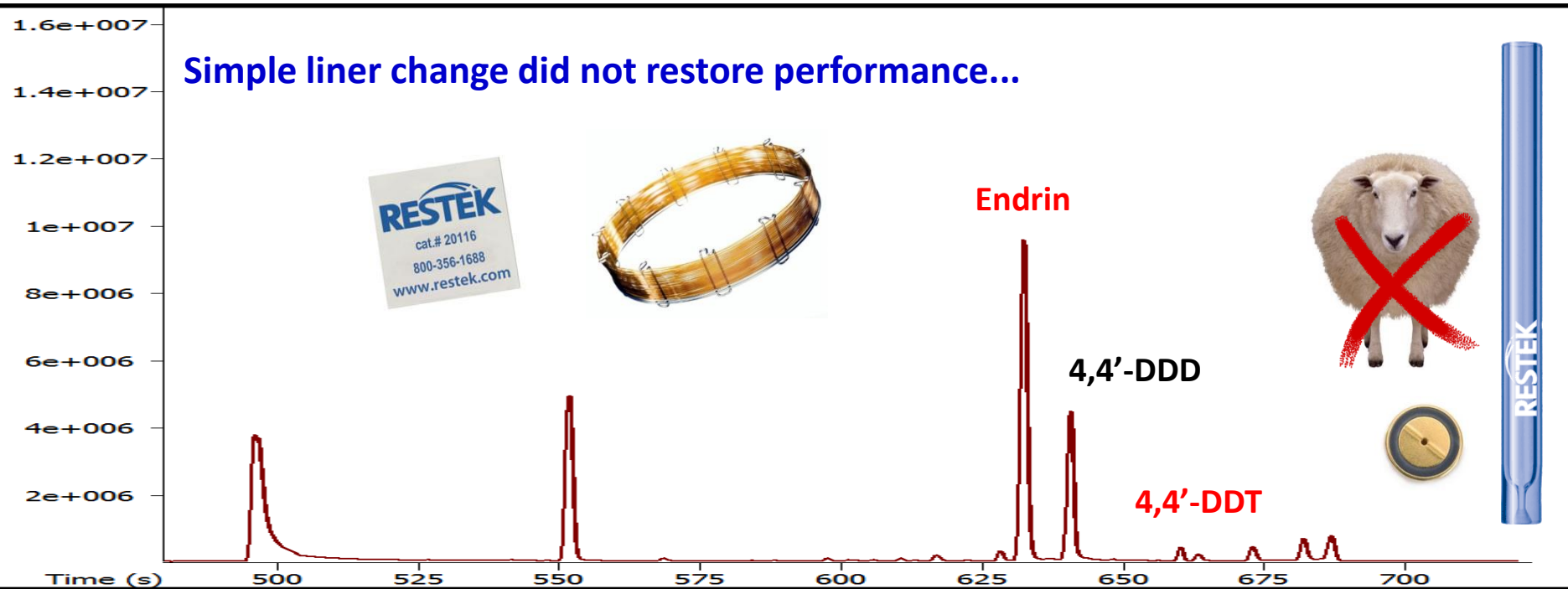
matography

estek.com

Standards analyzed after 39 sediment extracts,
then liner and seal changes...



Simple liner change did not restore performance...



A Better Way?



- **Analyte Protectants**
 - GC system active site masking compounds
 - Improve compound transfer from GC inlet
 - Allow better peak shape from GC column
- **Split injection (shoot-and-dilute)**
 - High GC inlet flow improves compound transfer
 - Less “dirt” on GC column improves peak shapes
 - System stays up longer



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CHROMATOGRAPHY A

Journal of Chromatography A, 1015 (2003) 163–184

www.elsevier.com/locate/chroma

Evaluation of analyte protectants to improve gas chromatographic analysis of pesticides[☆]

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*US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center,
600 East Mermaid Lane; Wyndmoor, PA 19038, USA*

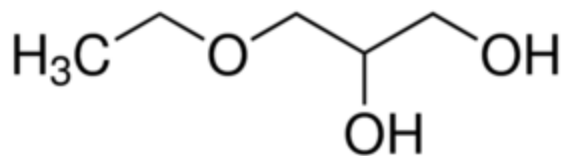
Received 9 August 2002; received in revised form 3 February 2003; accepted 4 February 2003

Significant peak quality improvements obtained when matrix components are present to fill active sites and reduce analyte interactions.

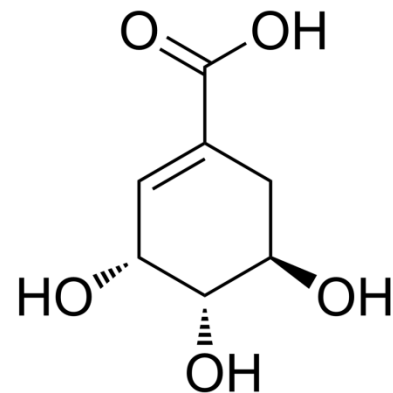
“Matrix-induced chromatographic response enhancement”.

Addition of “analyte protectants” (e.g. sugars, acids, etc.) to standards and samples.

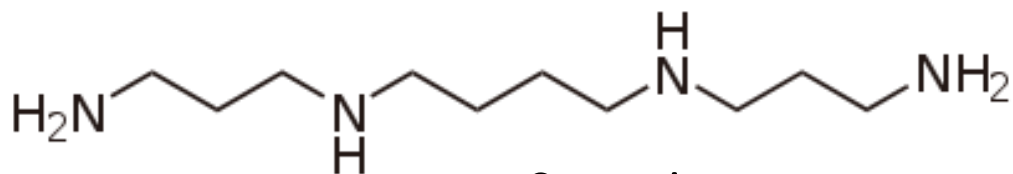
Provides chromatographic enhancement effect for analytes in a very dirty GC system.



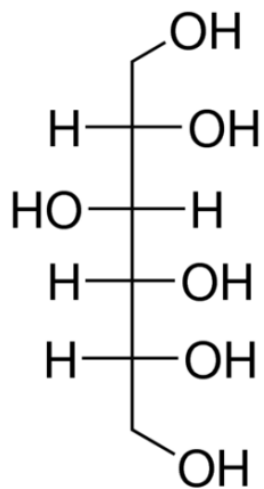
3-Ethoxy-1,2-propanediol



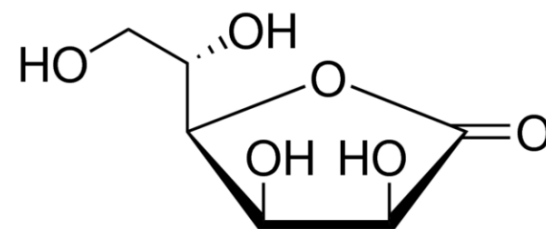
Shikimic acid



Spermine



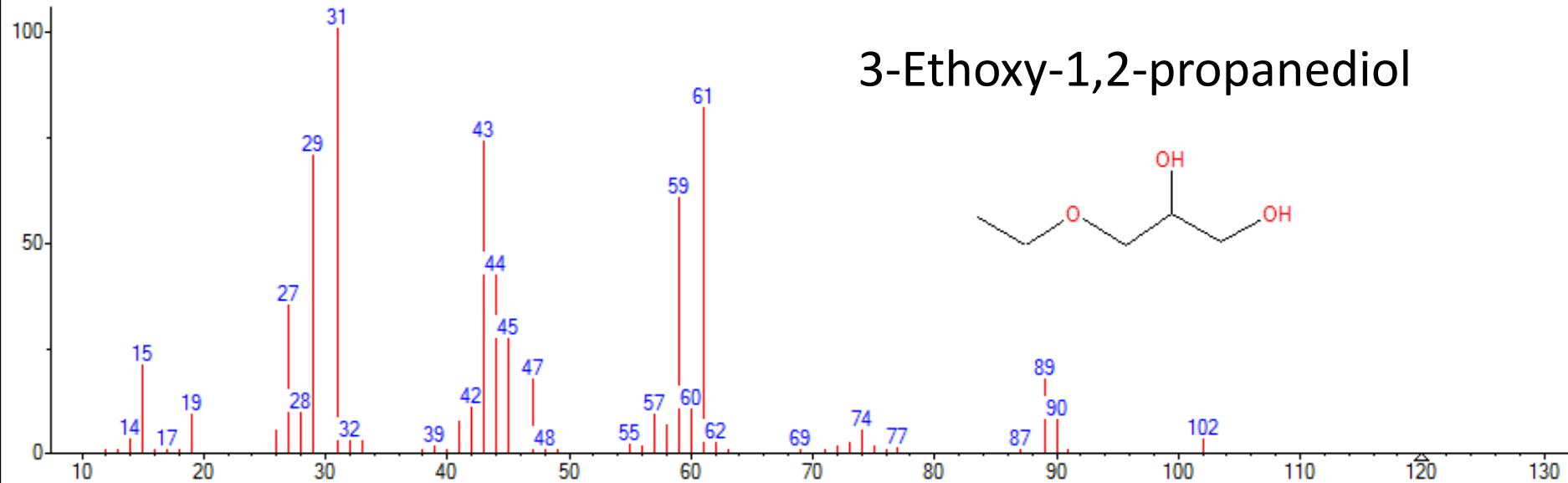
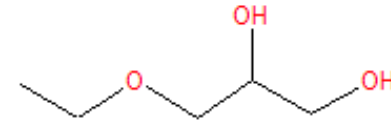
D-sorbitol



L-gulonic acid γ -lactone

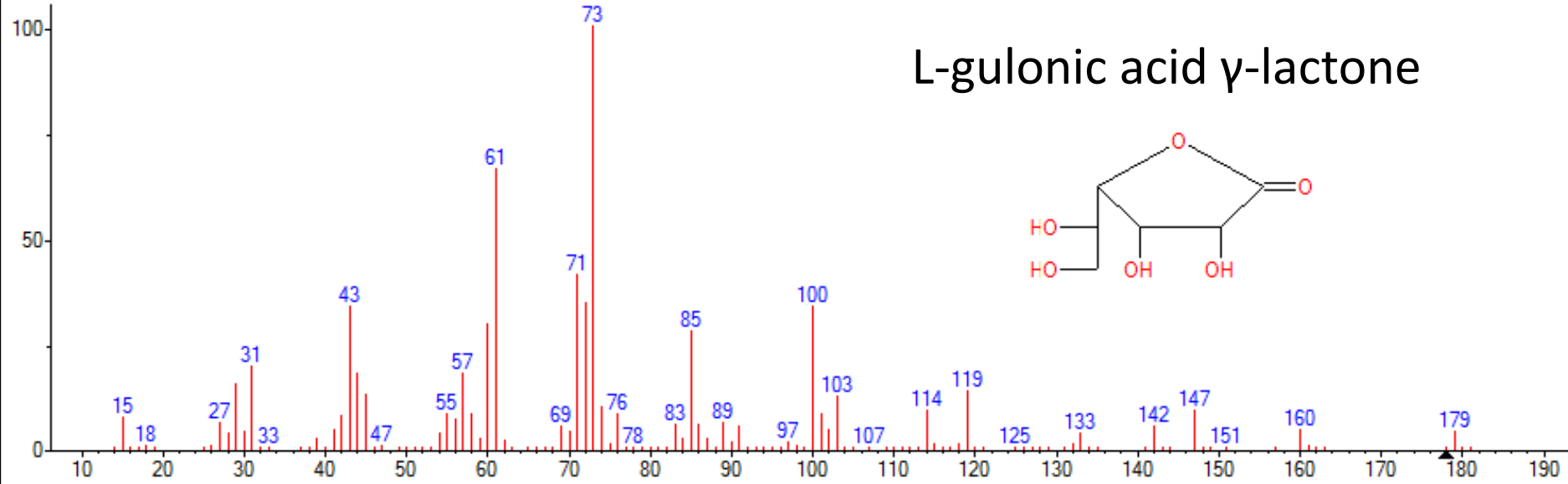
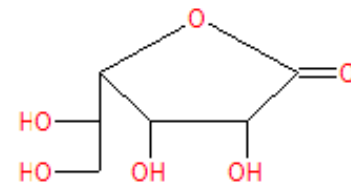
Analyte protectants

3-Ethoxy-1,2-propanediol



(mainlib) 3-Ethoxy-1,2-propanediol

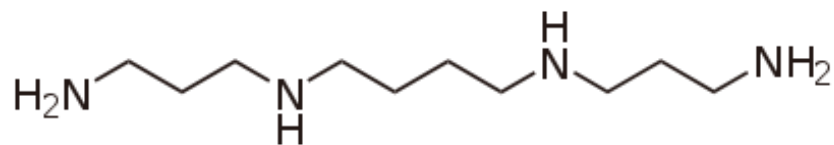
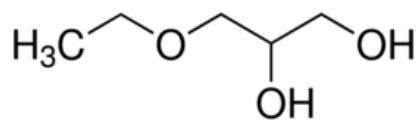
L-gulonic acid γ -lactone



(mainlib) L-Gulonic acid, γ -lactone

Analyte Protectant Criteria

- Rich in hydroxys/aminos to deactivate systems
- Volatile so they gas chromatograph
- Several may perform better than one
- Volatility range similar to compounds analyzed
- Added in high concentration
- Low m/z ions to avoid quantification bias
- Inexpensive
- Injected with each standard and sample



15m x 0.25mm x 0.25µm Rtx-5MS with 5m Integra-Guard

Pentachlorophenol

Standard 10 µg/µL AP

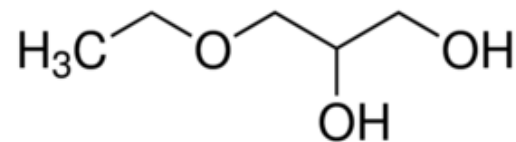
Standard 2.5 µg/µL AP

Standard 0.5 µg/µL AP

Standard No AP

Tailing Factor = 1.1

Tailing Factor = 2.1



3-Ethoxy-1,2-propanediol

PCB 52

Analyzed after one injection of used motor oil...

15m x 0.25mm x 0.25µm Rtx-5MS with 5m Integra-Guard

Response Factor = 1.60

Tailing Factor = 2.1

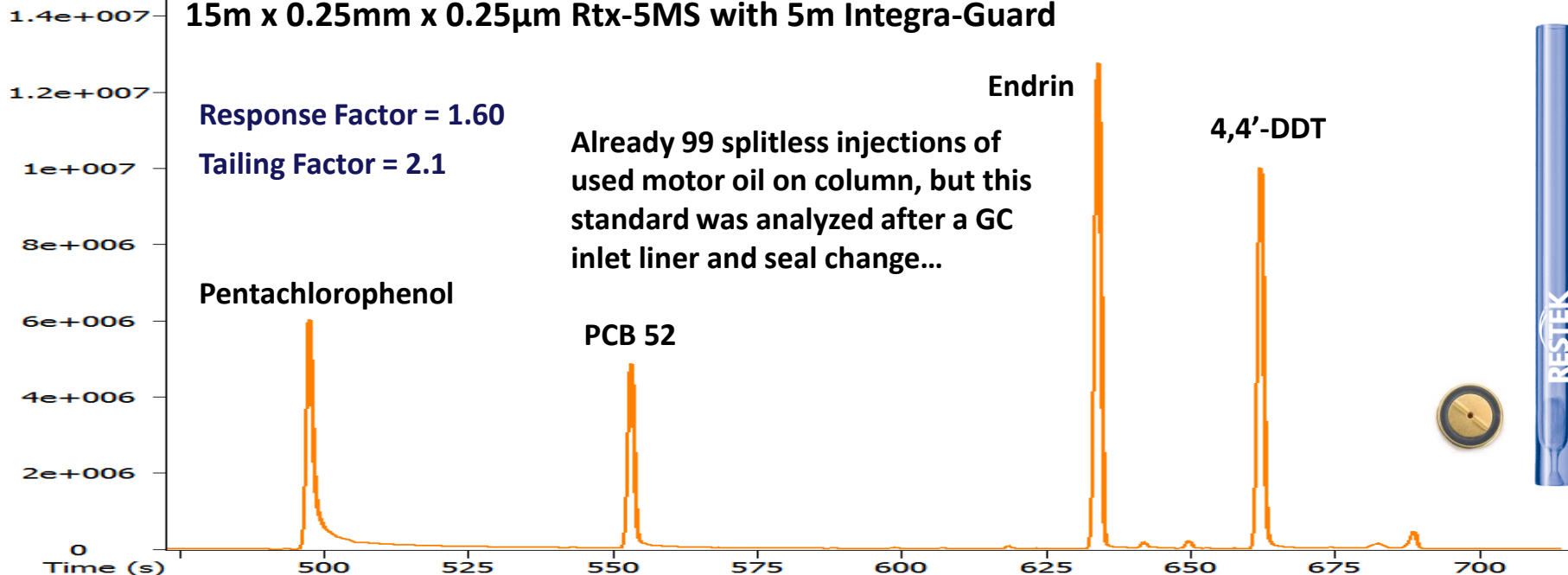
Already 99 splitless injections of used motor oil on column, but this standard was analyzed after a GC inlet liner and seal change...

Pentachlorophenol

PCB 52

Endrin

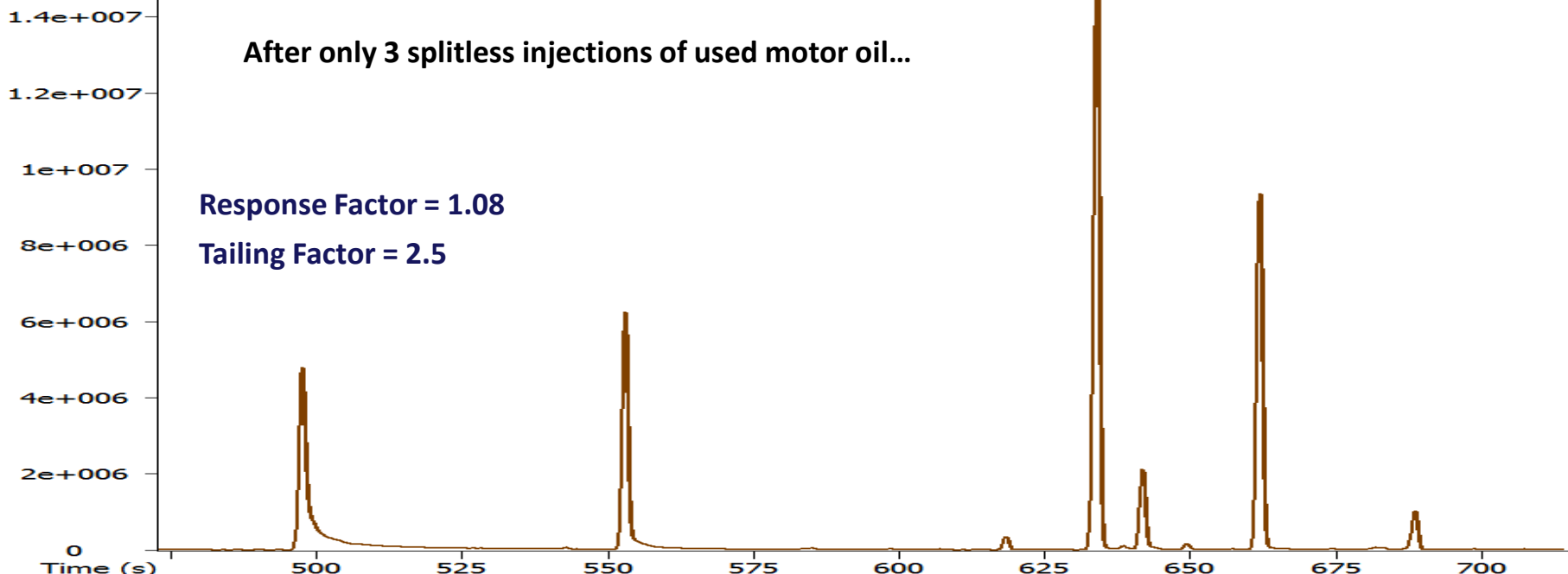
4,4'-DDT

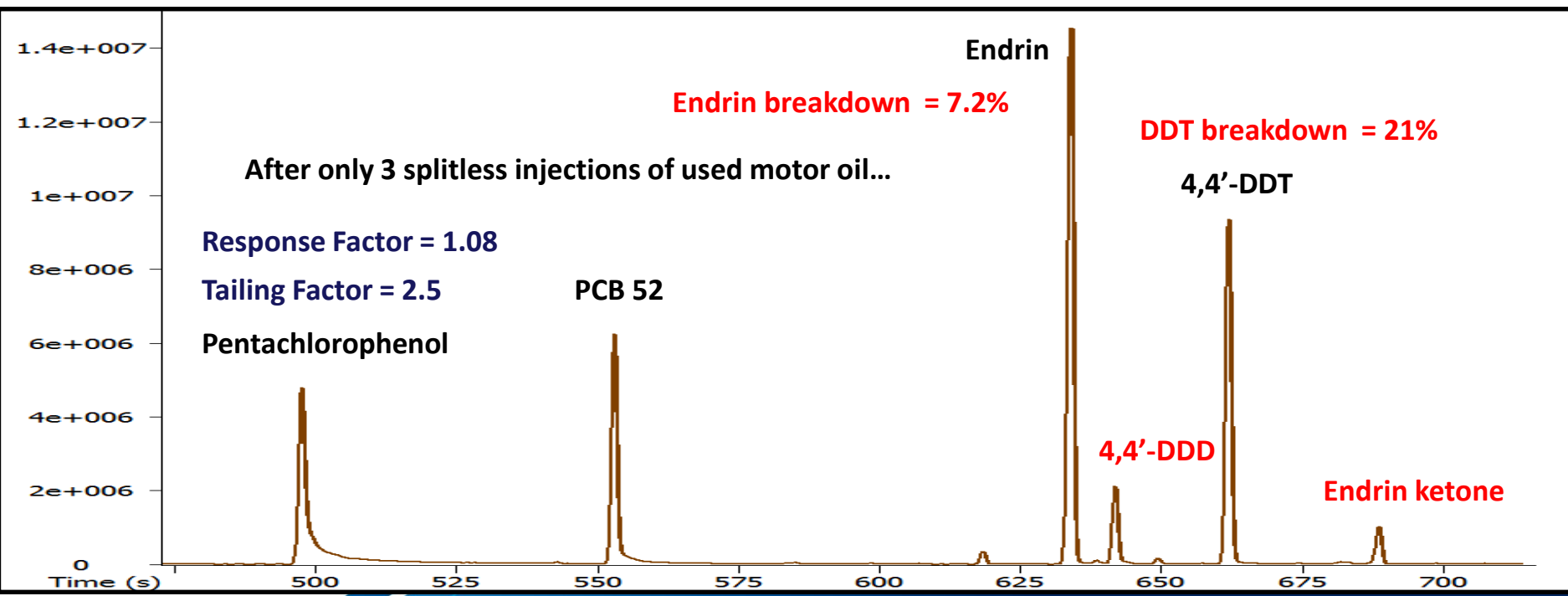
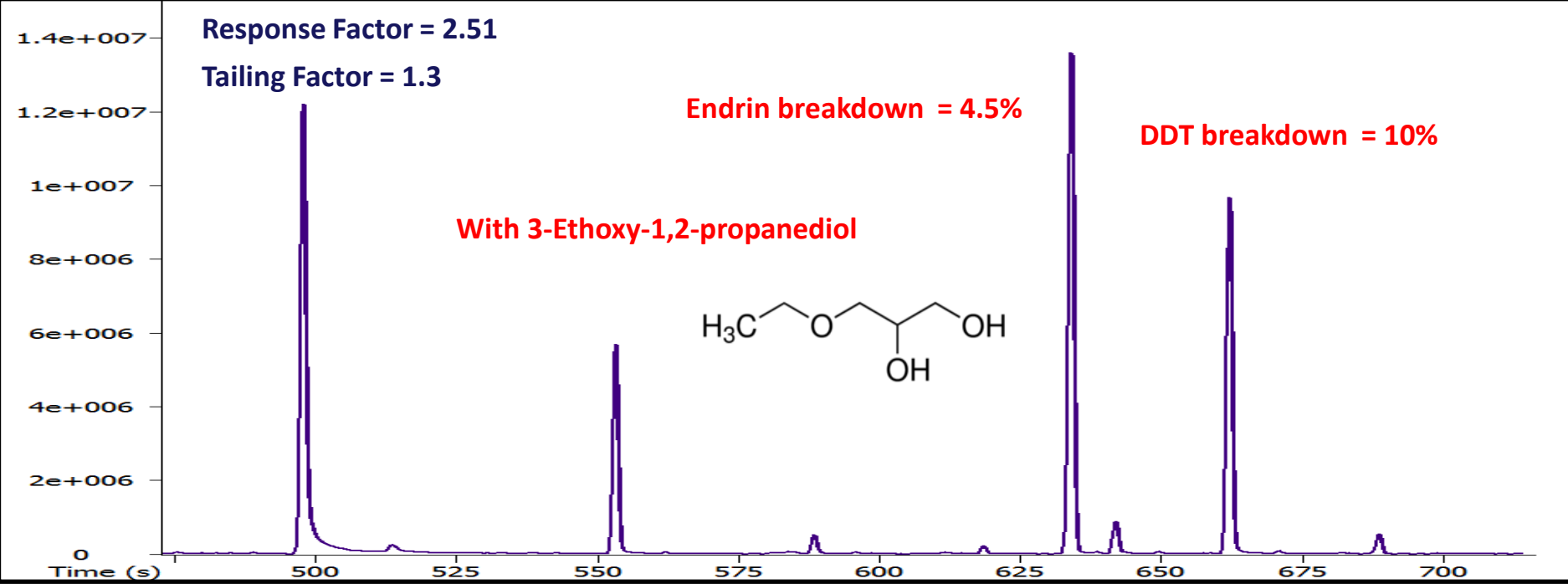


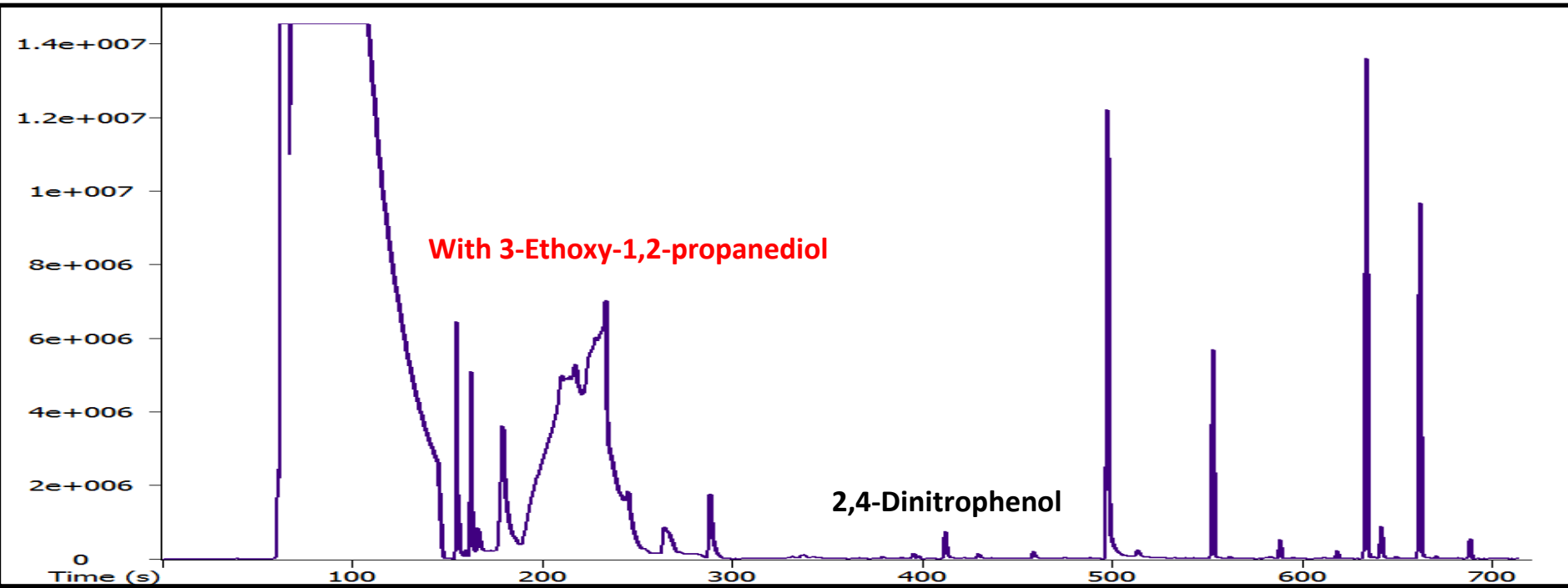
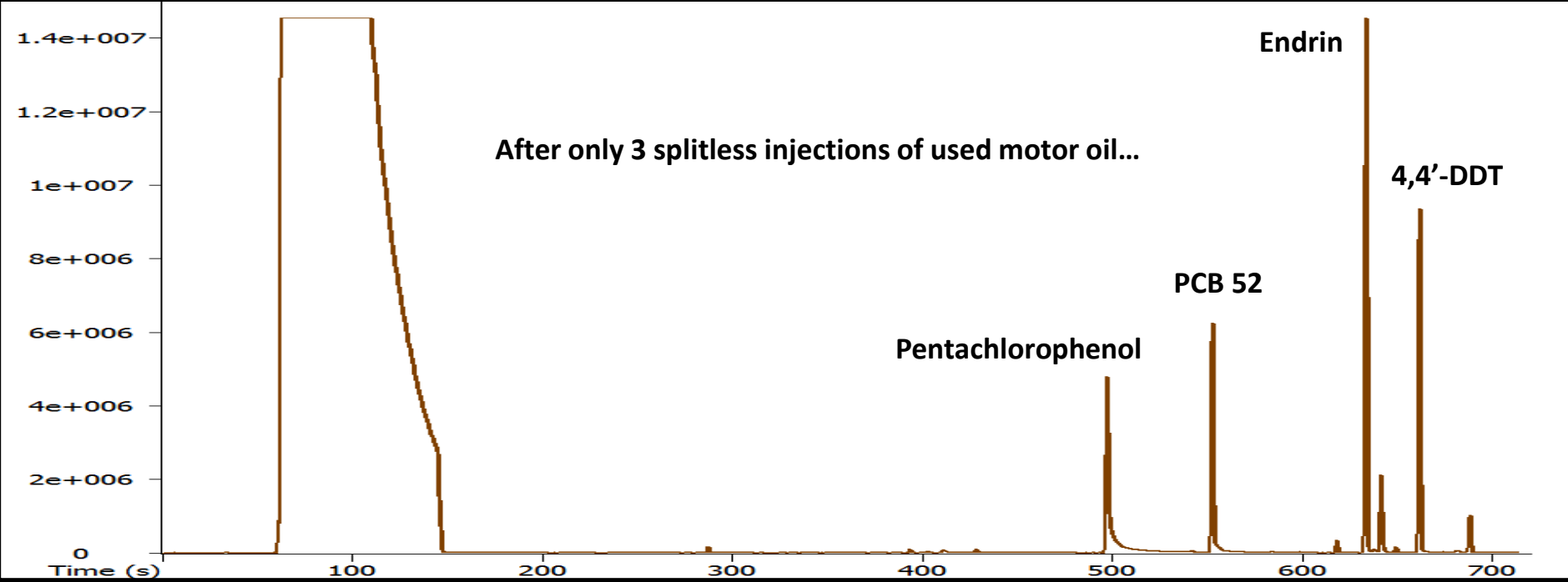
After only 3 splitless injections of used motor oil...

Response Factor = 1.08

Tailing Factor = 2.5



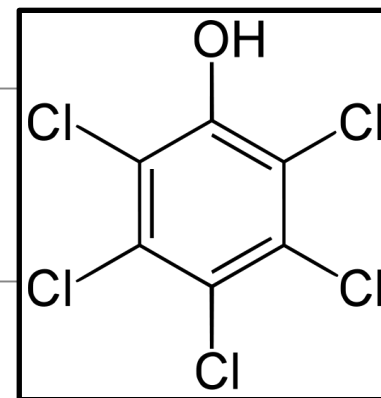




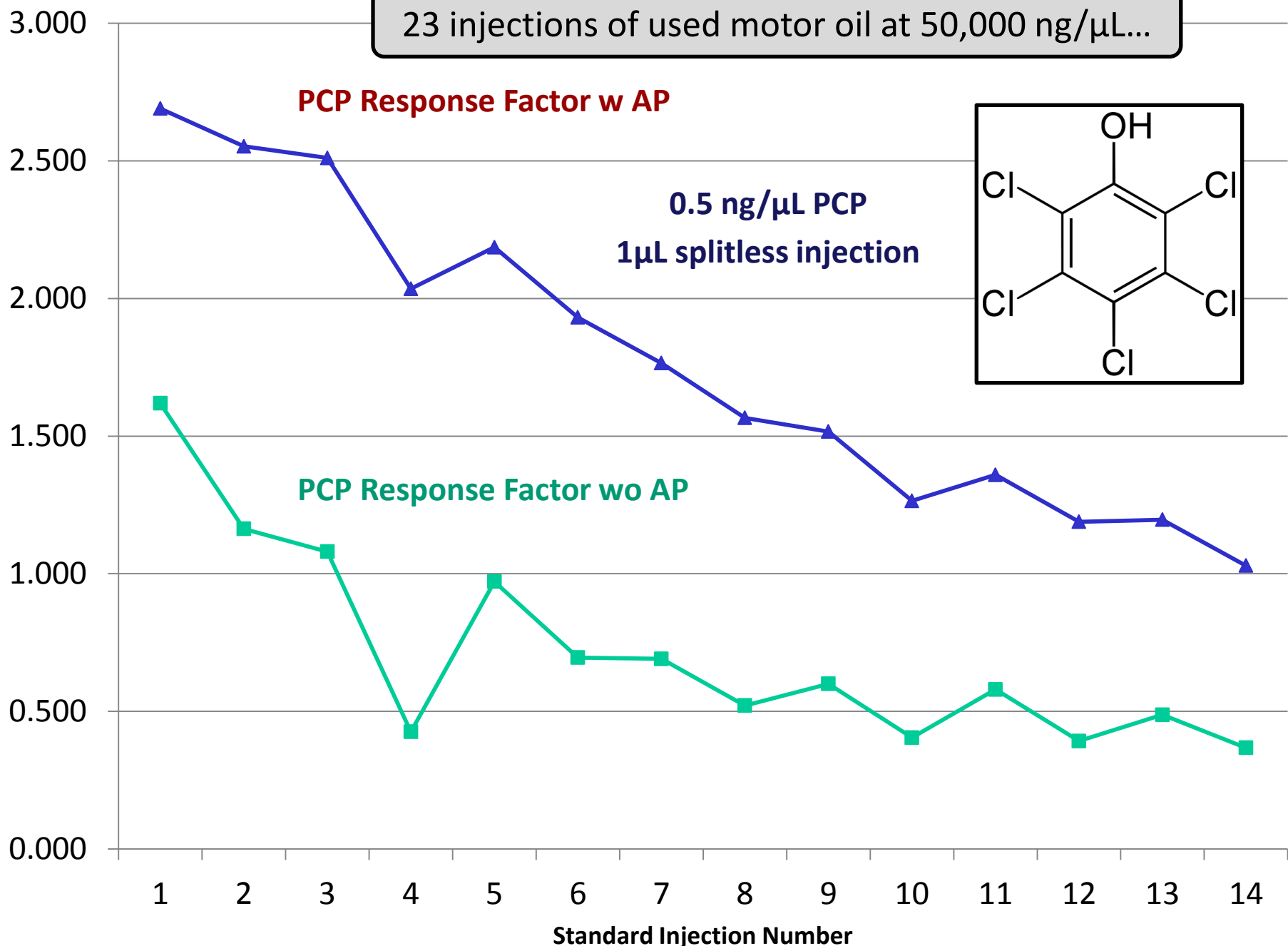
23 injections of used motor oil at 50,000 ng/ μ L...

PCP Response Factor w AP

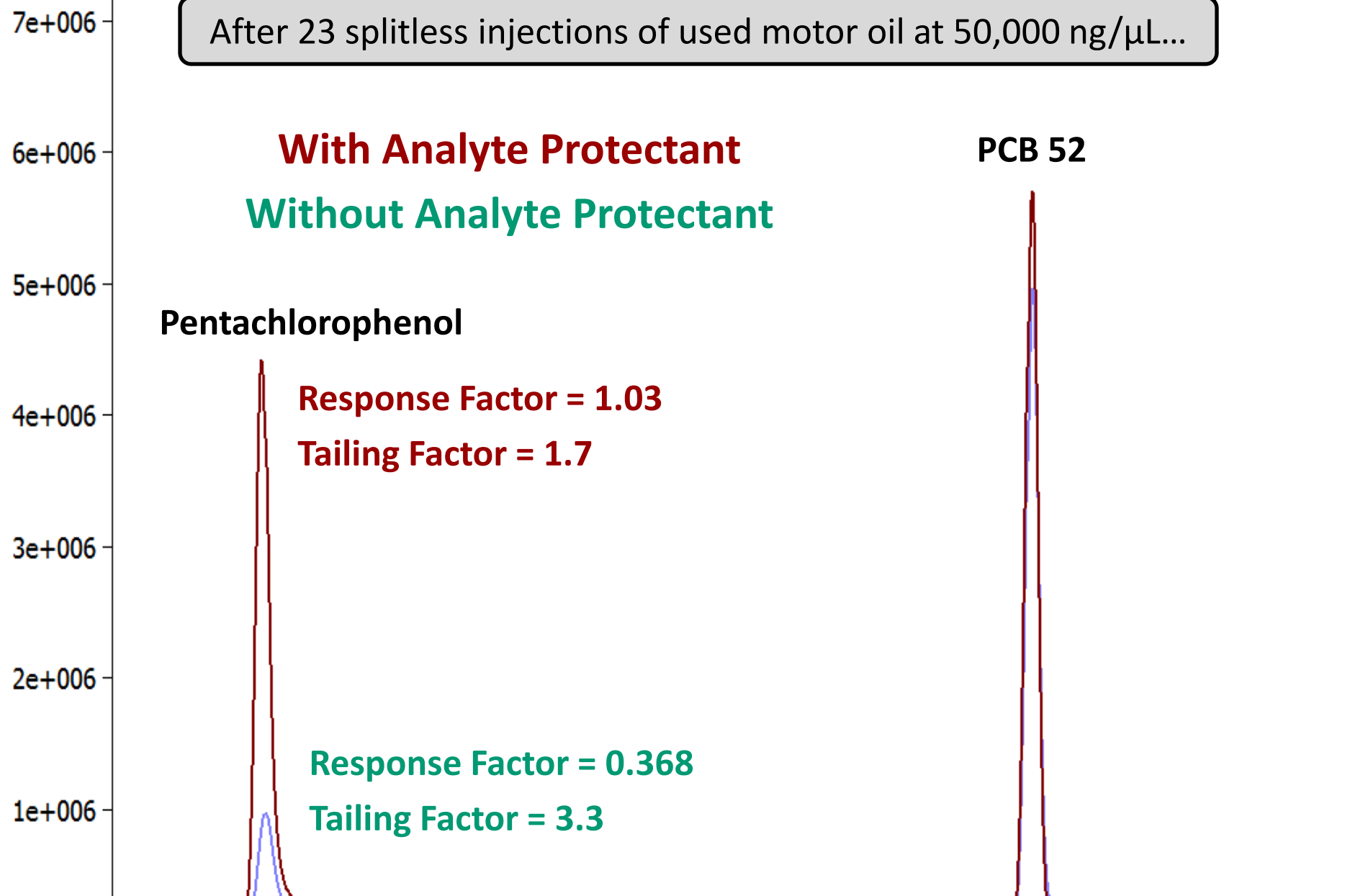
**0.5 ng/ μ L PCP
1 μ L splitless injection**



PCP Response Factor wo AP



After 23 splitless injections of used motor oil at 50,000 ng/ μ L...



With Analyte Protectant

Without Analyte Protectant

PCB 52

Pentachlorophenol

Response Factor = 1.03

Tailing Factor = 1.7

Response Factor = 0.368

Tailing Factor = 3.3

Analyte Protectants Summary for Splitless Injection GC

- Approach shows promise for better GC of active compounds, including pesticides
 - Increased response factors and less peak tailing
- Less successful for reactive compounds
 - Endrin and DDT still subject to degradation
- Additional analyte protectants need testing
 - GC-MS will increase protectant choices

Splitless Injection GC Issues

- Active/polar/thermal sensitive analytes
- Dirty samples
- Matrix enhanced/degraded responses
- Limited transfer from GC inlet to column
- Poor detectability
- Significant quantification bias
- Frequent GC inlet and column maintenance
- Offline cleanup

Pesticide Analysis with GC

- Active/polar/thermal sensitive analytes
- Dirty samples
- Matrix enhanced/degraded responses
- Limited transfer from GC inlet to column
- Poor detectability
- Significant quantification bias
- Frequent GC inlet and column maintenance
- Offline cleanup

Pesticide Analysis with GC

Split injection

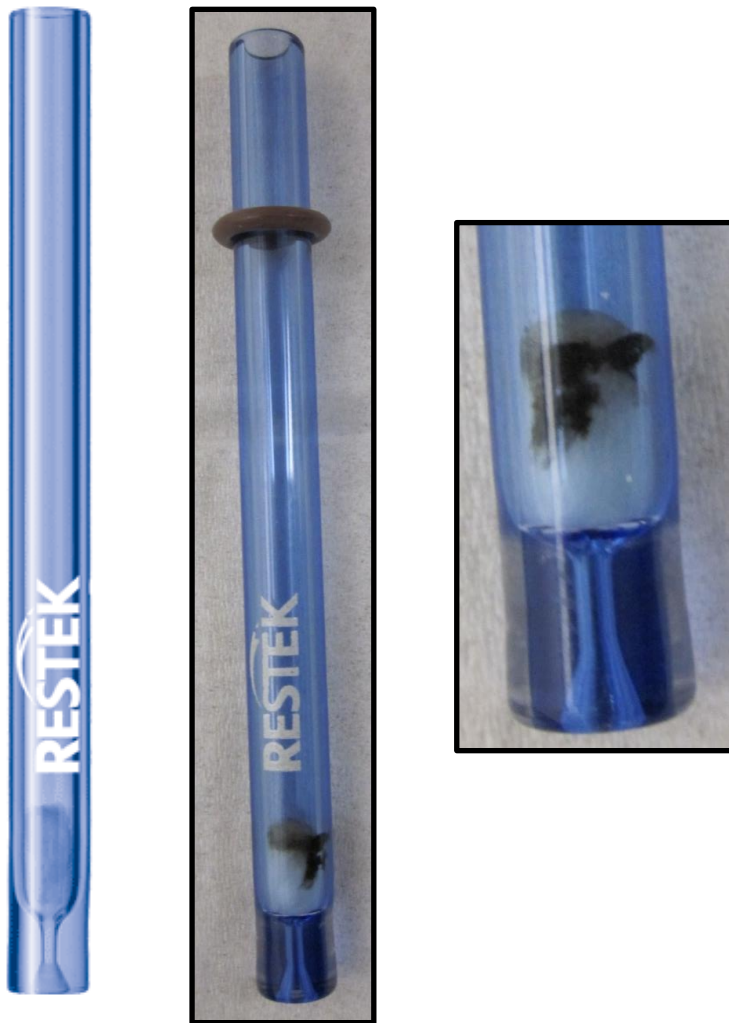
Matrix *effect* diluted to the point where it doesn't negatively impact *GC inlet* efficiency

Shoot-and-Dilute GC

Relies on detector sensitivity and selectivity improvements, e.g. MS/MS

- Frequent GC inlet and column maintenance
- Offline cleanup

Sky® 4mm ID Single Taper
Inlet Liner with Quartz Wool
Splitless Injections



Sky® 4mm ID Precision®
Inlet Liner with Quartz Wool
Split Injections



- Goal is for part of sample to make it to GC column
 - Based on split ratio
- Wool wipes needle and helps homogenize sample
 - Excellent for repeatability
 - High split ratio reduces wool and “dirt” impact on compounds prone to breakdown or sorption losses



1

QuEChERS Performance Standards Kit

- Designed for use in all QuEChERS methods for pesticides in fruits and vegetables, including the original unbuffered method, AOAC 2007.01, and EN15662.
- Kit contains organochlorine, organonitrogen, organophosphorus, and carbamate pesticides commonly used on fruits and vegetables.
- Volatile, polar, active, base-sensitive, and nonvolatile compounds are included to allow comprehensive evaluation of QuEChERS extraction and cleanup efficiencies, and optimization of GC and LC instrumental conditions.
- Ideal for initial method evaluations and ongoing method performance validations.
- Analytes are divided into three ampuls based on compatibility for maximum stability and shelf life.*
- Precise formulations improve data quality and operational efficiency; spend more time running samples and less time sourcing and preparing standards.
- Quantitatively analyzed to confirm the composition and stability of each mixture.
- Produced and tested in accordance with ISO Guide 34 and 17025 accreditation.

Organochlorine
 Organonitrogen
 Organophosphorus
 Carbamate
 Pesticides

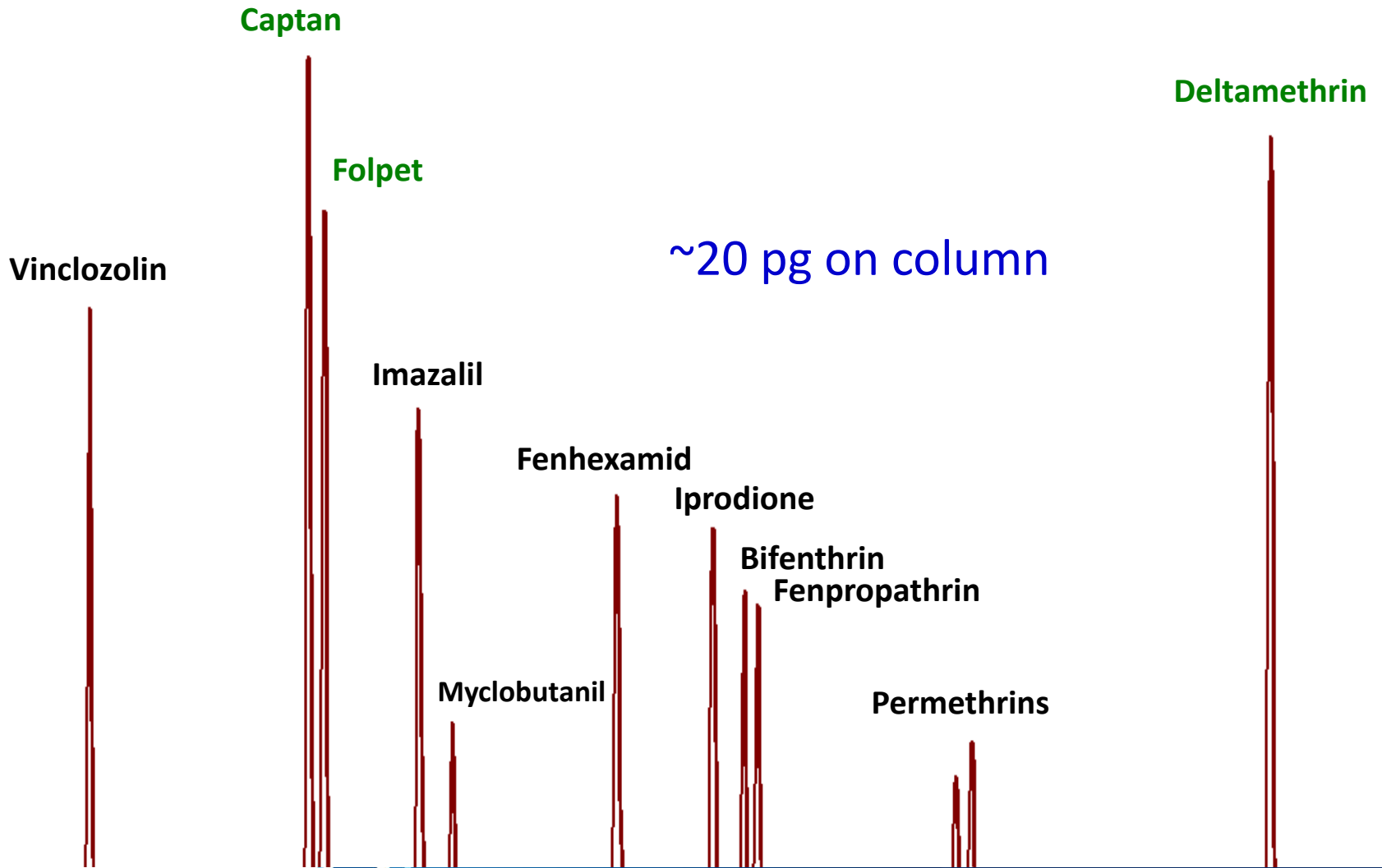
Restek Offers a Full
 Line of Certified
 Reference Materials



If your lab must use certified reference materials (CRMs), please be sure to tell us when ordering so we can help you meet your regulatory requirements as we transition our inventory.

Standard C
 contains difficult
 pesticides such as
 Captan, Folpet,
 Deltamethrin...

QuEChERS Performance Standard C – 200 pg/ μ L – 1 μ L – Split Ratio 10
GC-ECD – 15m x 0.25mm x 0.25 μ m Rxi-5ms column



Splitless and Split Injections of Strawberry Extracts



- EN QuEChERS organic strawberry extracts
 - dSPE cleaned with 50 mg per mL PSA and C18
 - Spiked to 100 pg/ μ L each pesticide
- GC-ECD
 - 15m x 0.25mm x 0.25 μ m Rxi-5ms column
- Splitless injection at 250°C
 - 4mm ID single taper liner with wool
- Split injection at 250°C, split ratio 10
 - 4mm ID Precision split liner with wool



Inlet Ruggedness GC-ECD

- Sample queue
- Two 100 pg/ μ L solvent-only standards
- Two 100 pg/ μ L spiked strawberry extracts
- Repeat
- 40 analyses each of standards and extracts



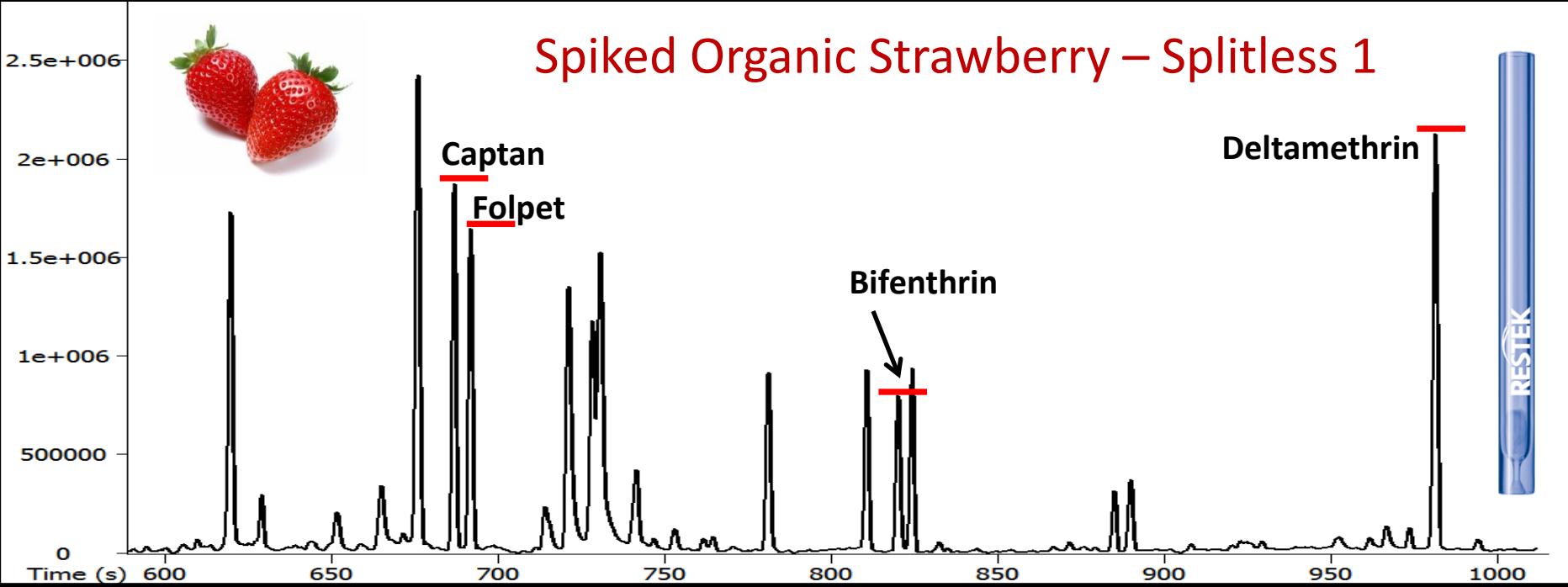
Splitless Injection GC-ECD Method

- 4mm single taper liner with quartz wool
 - 1 μ L, 250°C, 1.4 min splitless valve time
- 15m x 0.25mm x 0.25 μ m Rxi-5ms column
 - Constant flow He, 1.4 mL/min
 - 70°C (1.4 min), 15.2°C/min to 330°C (1.5 min)
 - Total run time: 20 min
- Electron capture detector
 - 350°C, 20 Hz
 - Make-up (nitrogen) + column flow 50 mL/min

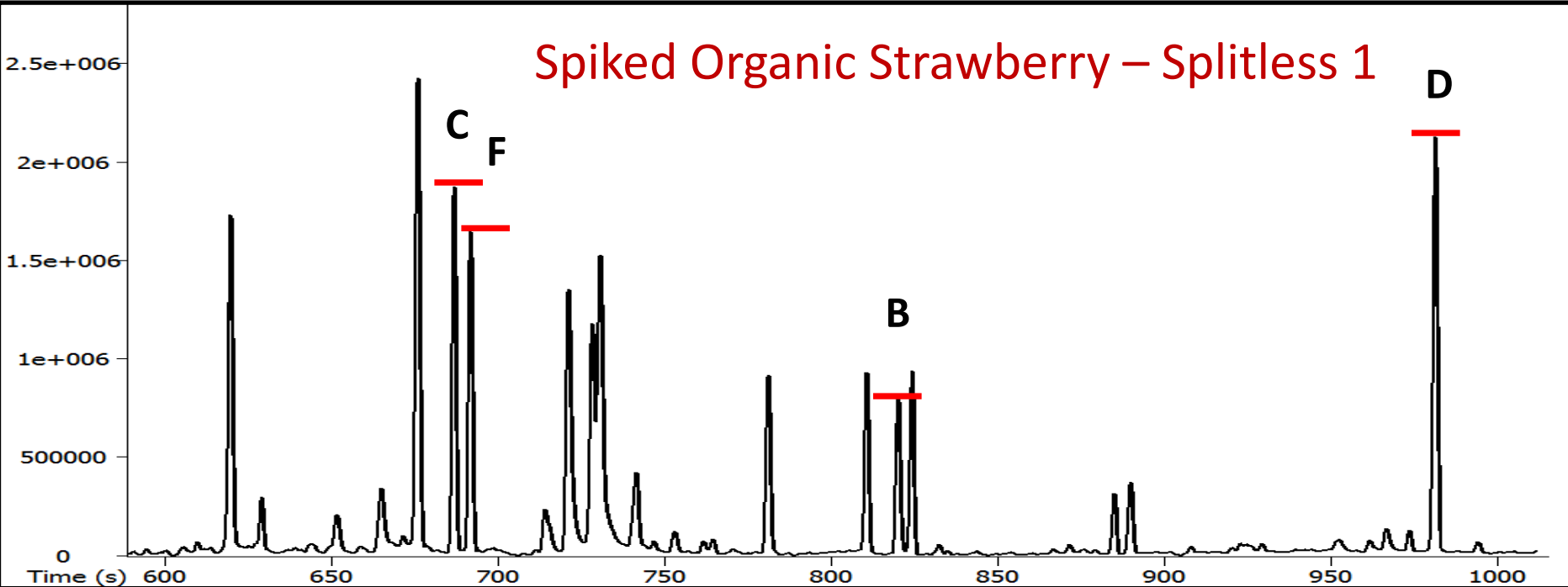




Spiked Organic Strawberry – Splitless 1

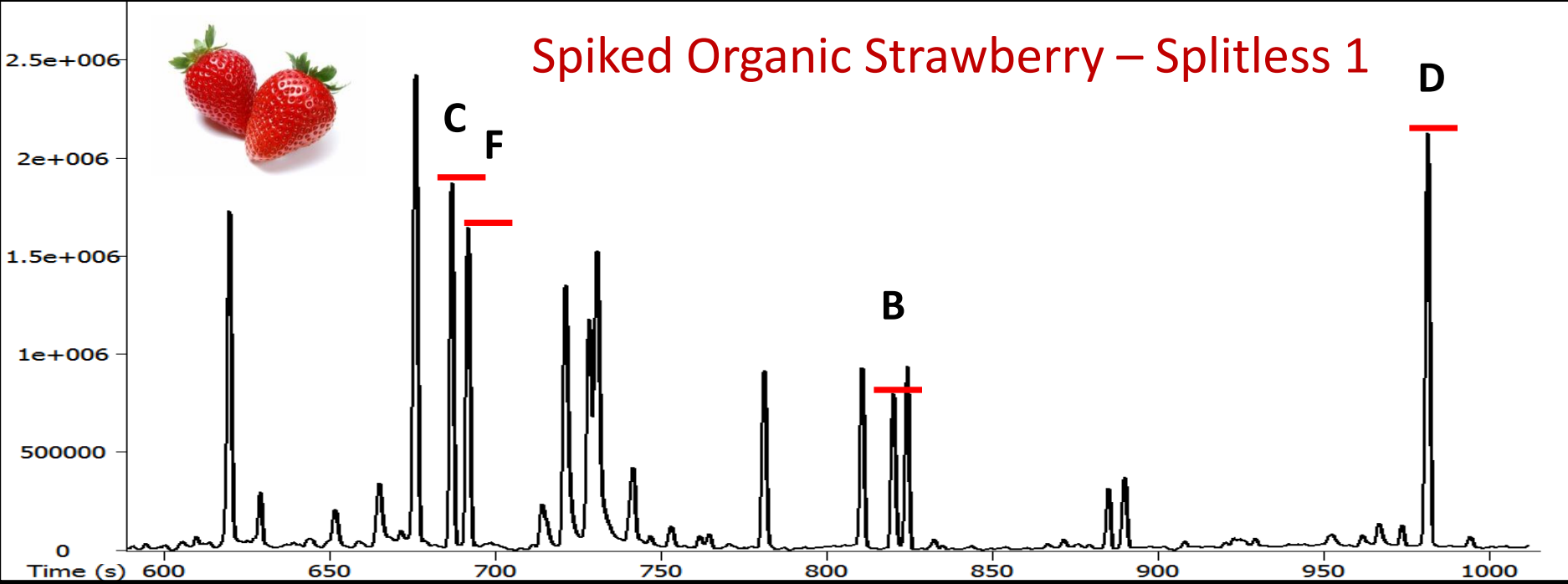


Spiked Organic Strawberry – Splitless 1

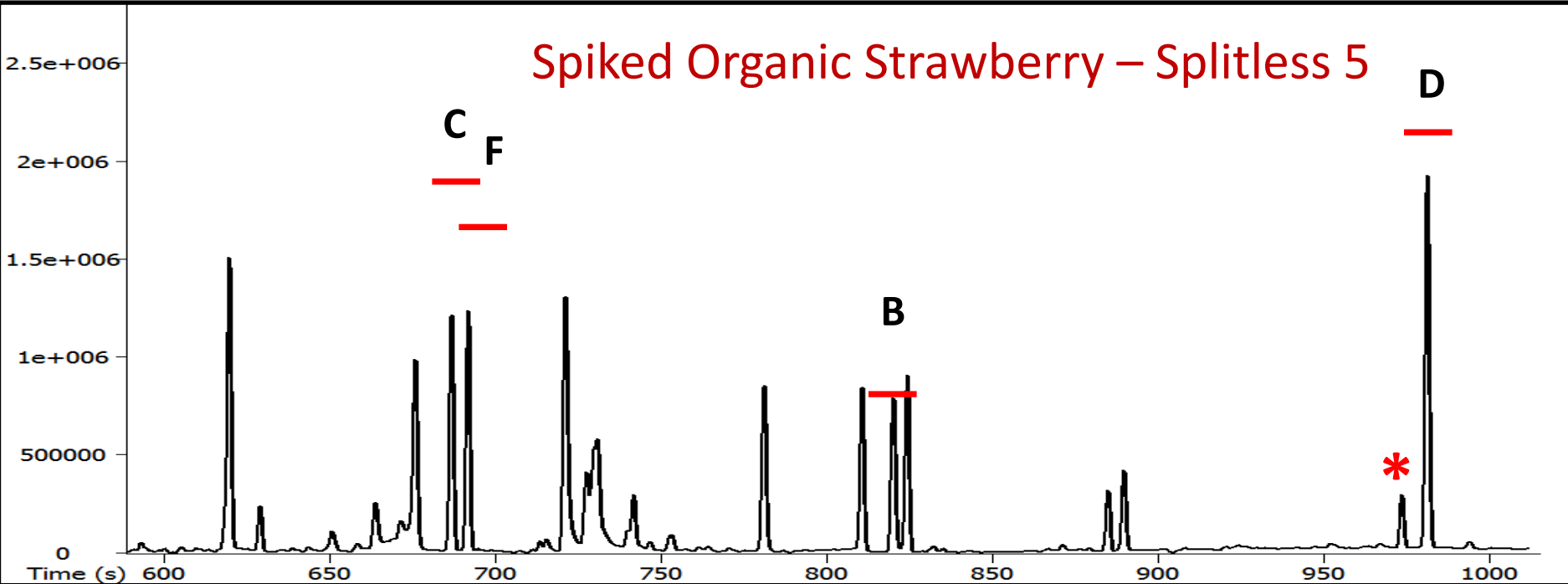




Spiked Organic Strawberry – Splitless 1



Spiked Organic Strawberry – Splitless 5



Split Injection GC-ECD Method

- 4mm focus-type split liner with quartz wool
 - 1 μL , 250°C, split ratio 10
- 15m x 0.25mm x 0.25 μm 5ms column
 - Constant flow He, 1.4 mL/min
 - 70°C (0.1 min), 15.2°C/min to 330°C (0.8 min)
 - Total run time: 18 min
- Electron capture detector
 - 350°C, 20 Hz
 - Make-up (nitrogen) + column flow 50 mL/min



Splitless

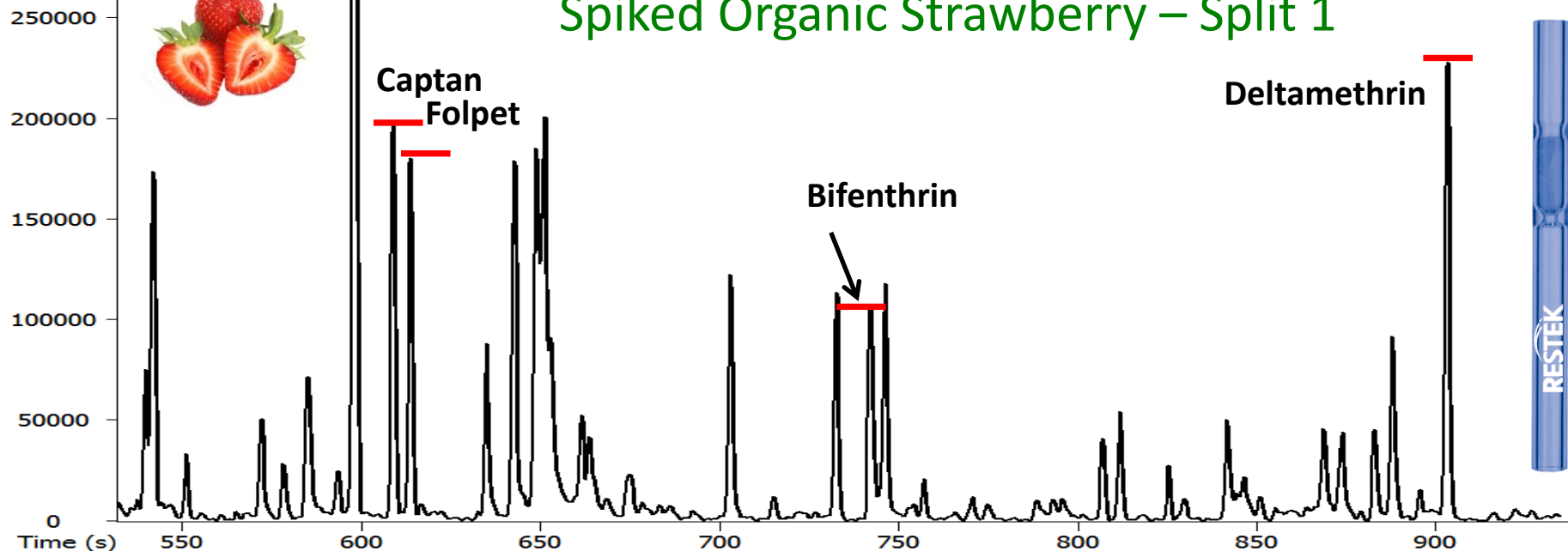


Split

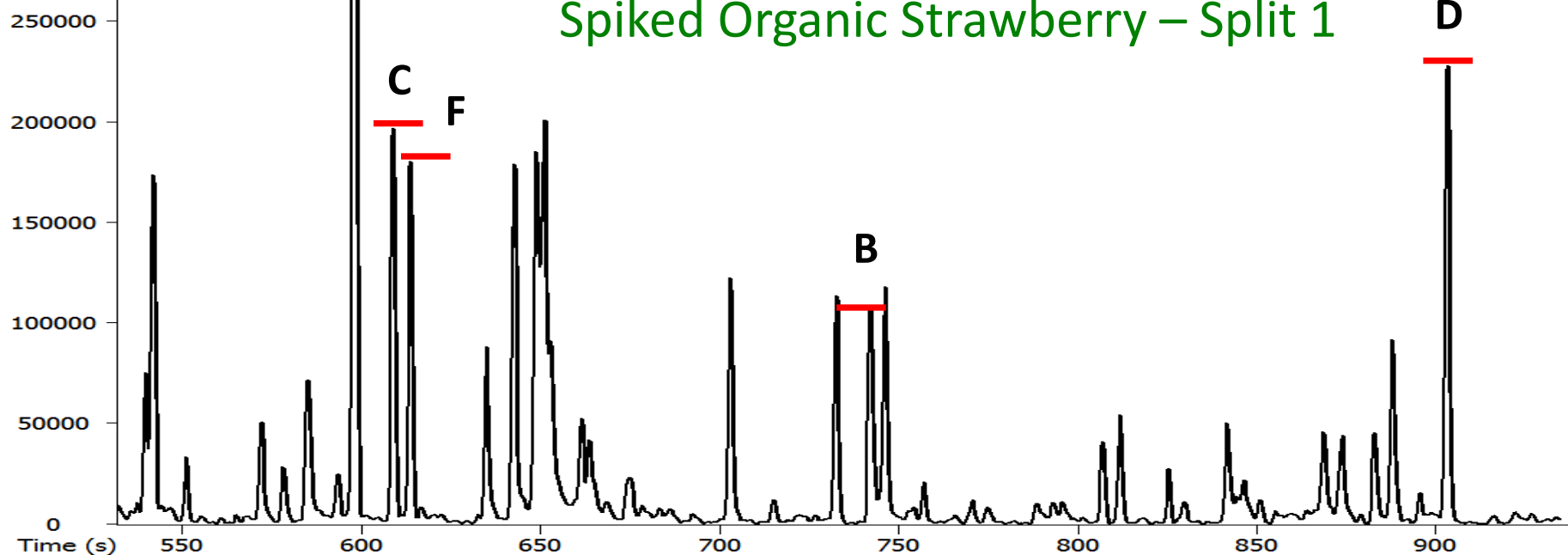




Spiked Organic Strawberry – Split 1

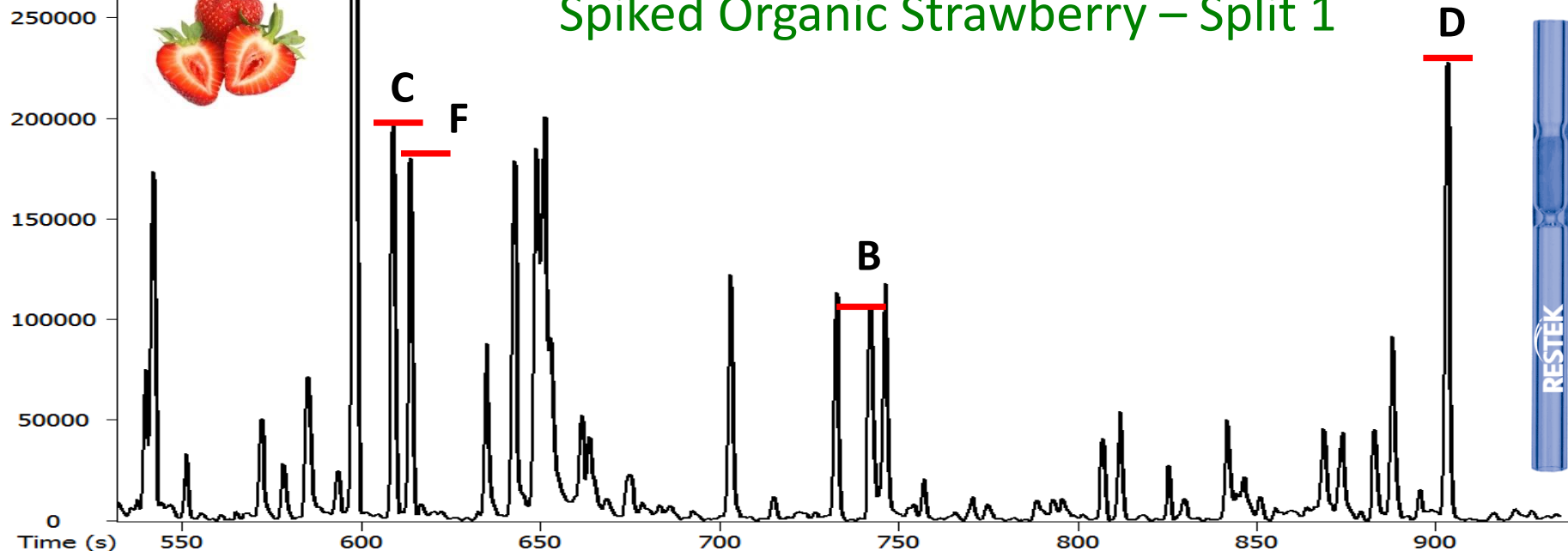


Spiked Organic Strawberry – Split 1

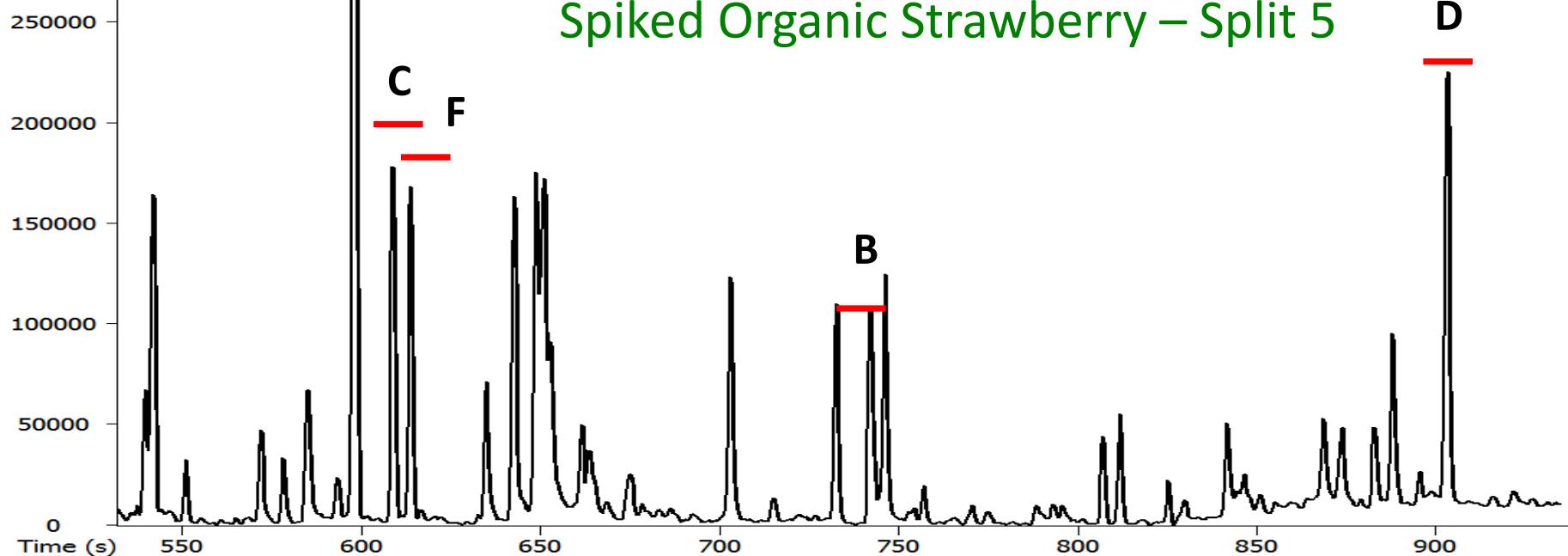




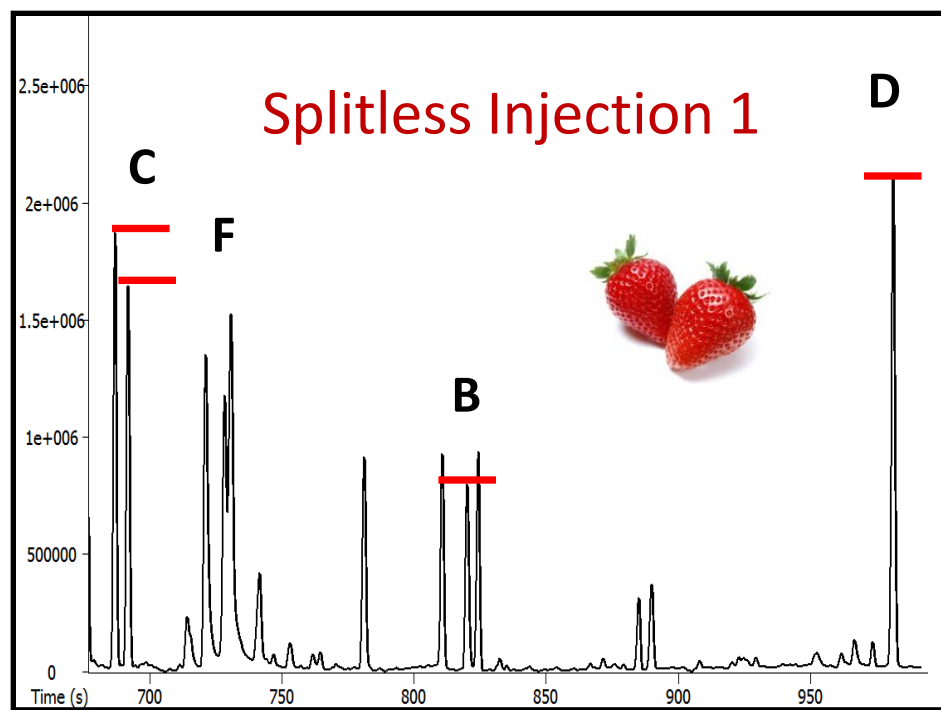
Spiked Organic Strawberry – Split 1



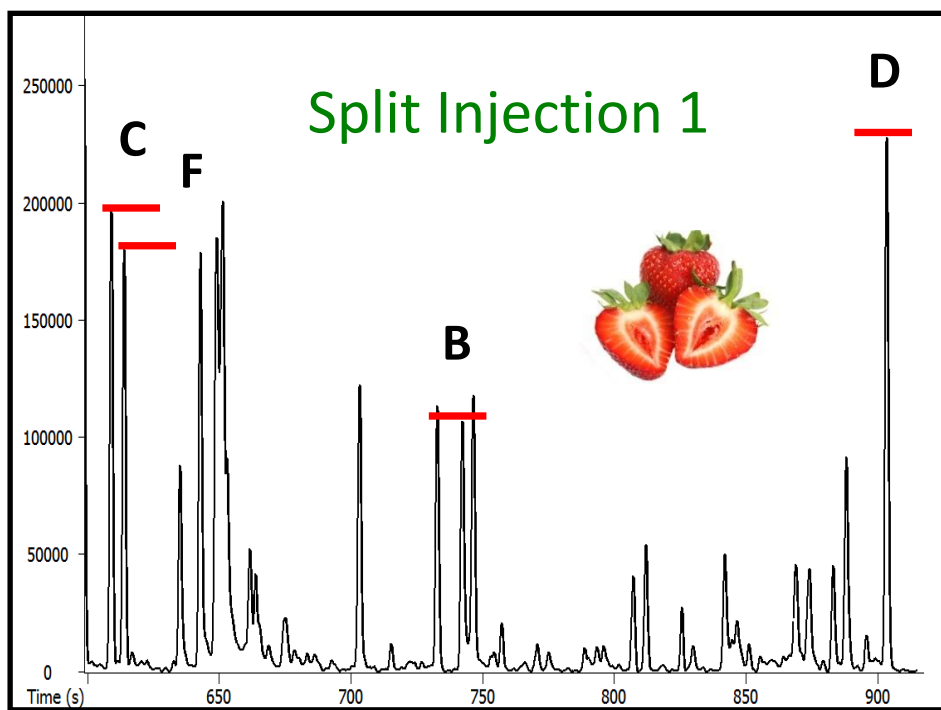
Spiked Organic Strawberry – Split 5



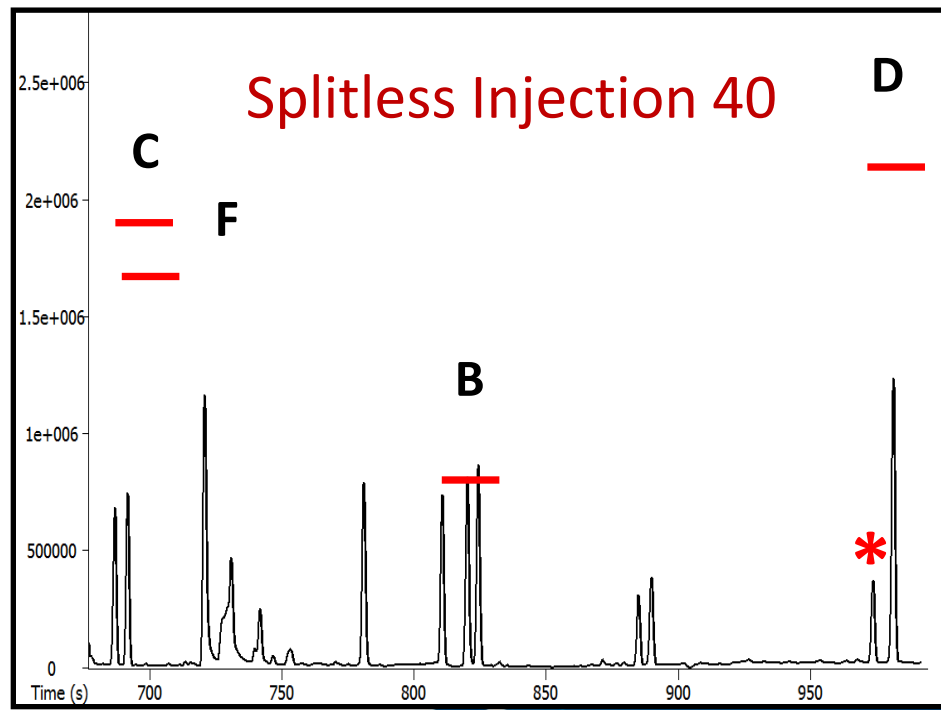
Splitless Injection 1



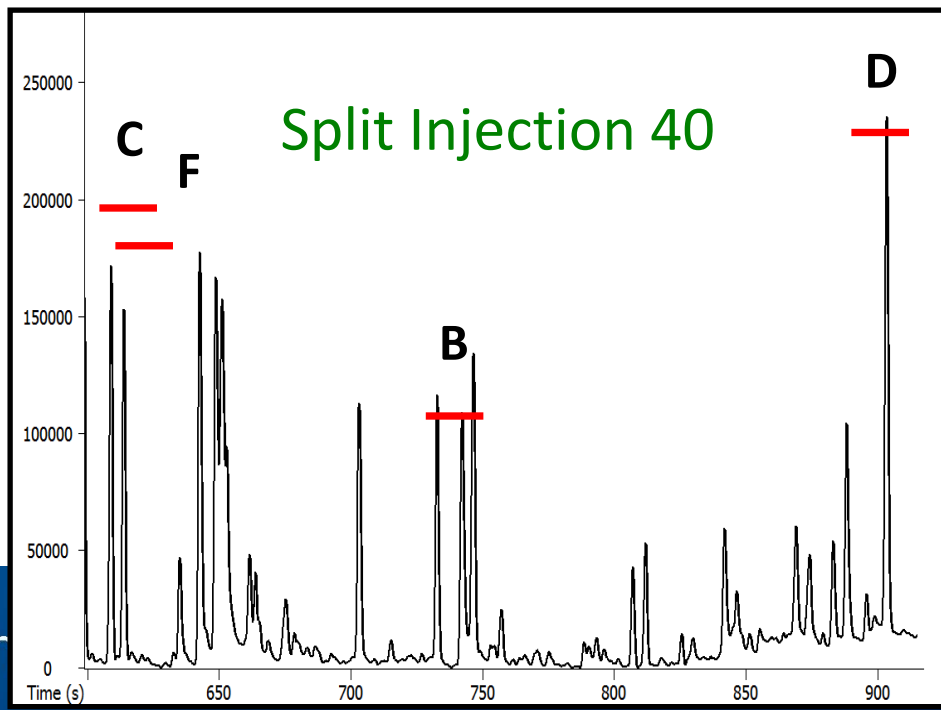
Split Injection 1



Splitless Injection 40



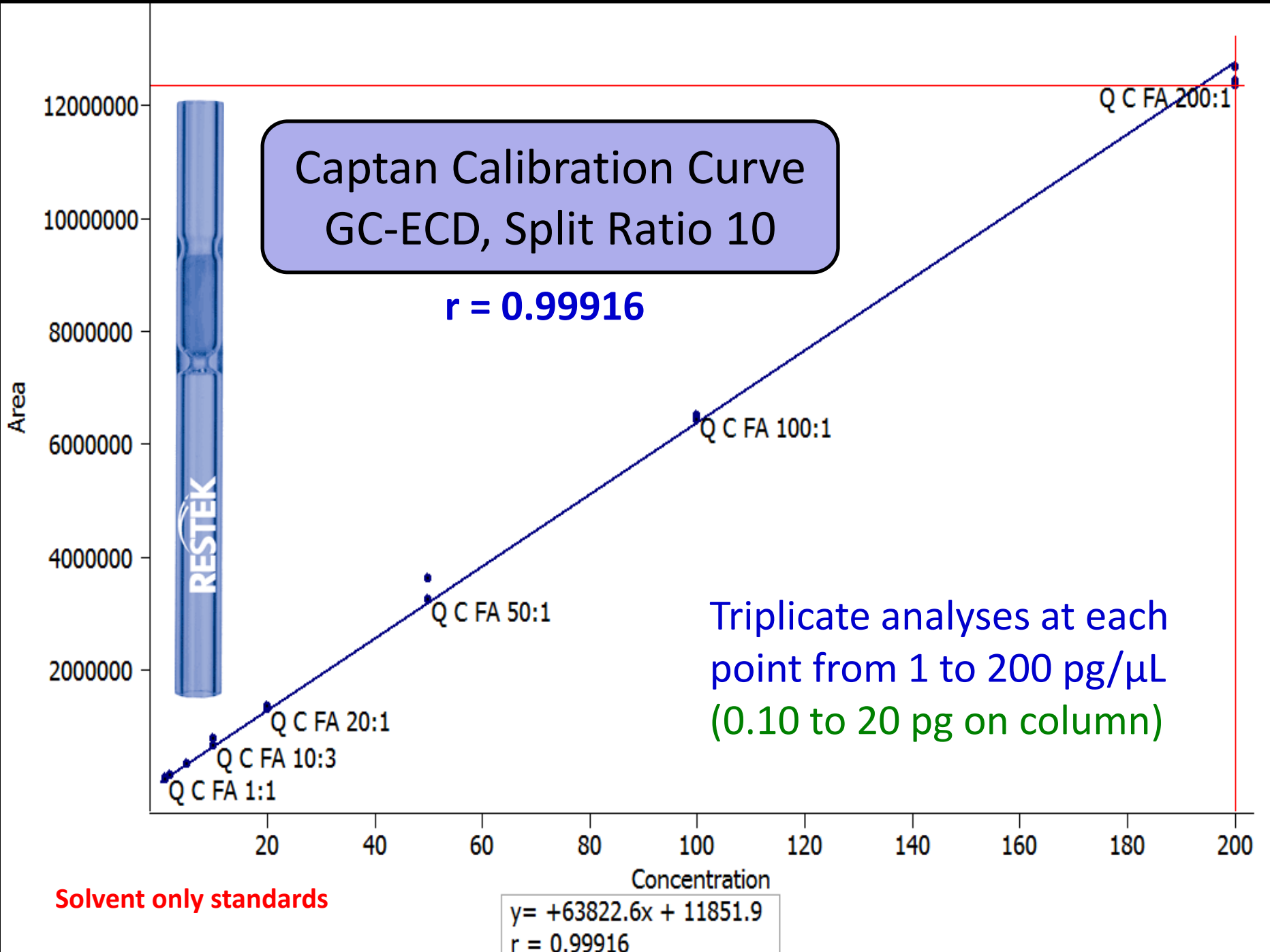
Split Injection 40

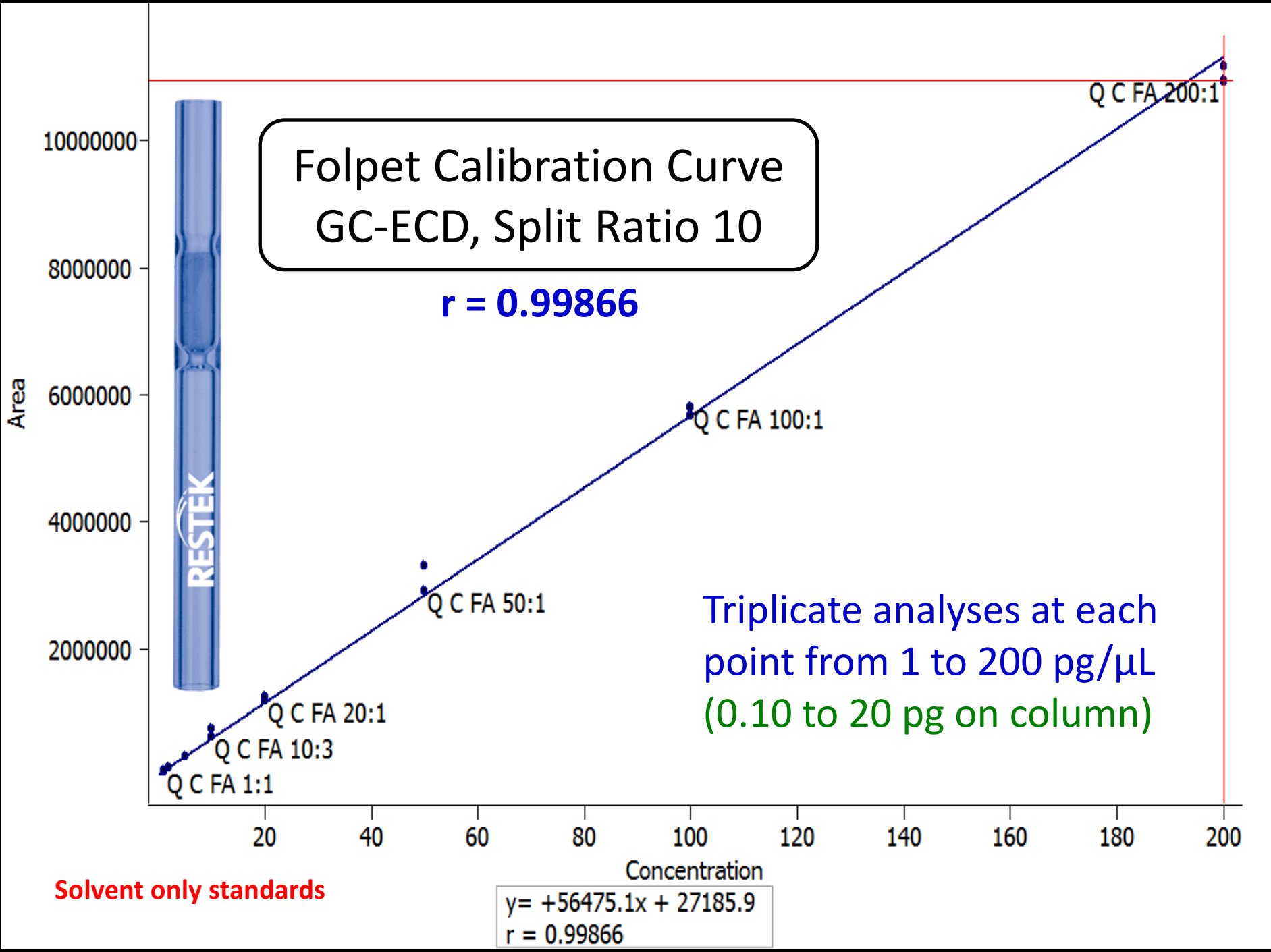


Comparing Average Response Factor (RF) and % RSD RF for Splitless and Split Injection

Pesticide	Splitless Avg RF	Split Avg RF		Splitless % RSD RF	Split % RSD RF
Captan	2480	4550		31	5.6
Folpet	2540	4200		26	5.7
Myclobutanil	3240	2810		24	7.4
Fenhexamid	2460	3820		8.4	8.4
Iprodione	2220	3180		6.2	3.3
Bifenthrin	2360	3490		1.9	3.0
Fenpropathrin	2420	4180		3.1	7.6
Deltamethrin	4380	6620		15	2.4

QuEChERS strawberry extracts spiked with 100 pg/ μ L pesticide standards.
Avg RF calculated from 40 analyses of spiked strawberry extracts.





Folpet Calibration Curve
GC-ECD, Split Ratio 10

$r = 0.99866$



Area

Concentration

Solvent only standards

$y = +56475.1x + 27185.9$
 $r = 0.99866$

Triplicate analyses at each point from 1 to 200 $\mu\text{g}/\mu\text{L}$ (0.10 to 20 μg on column)

Q C FA 200:1

Q C FA 100:1

Q C FA 50:1

Q C FA 20:1

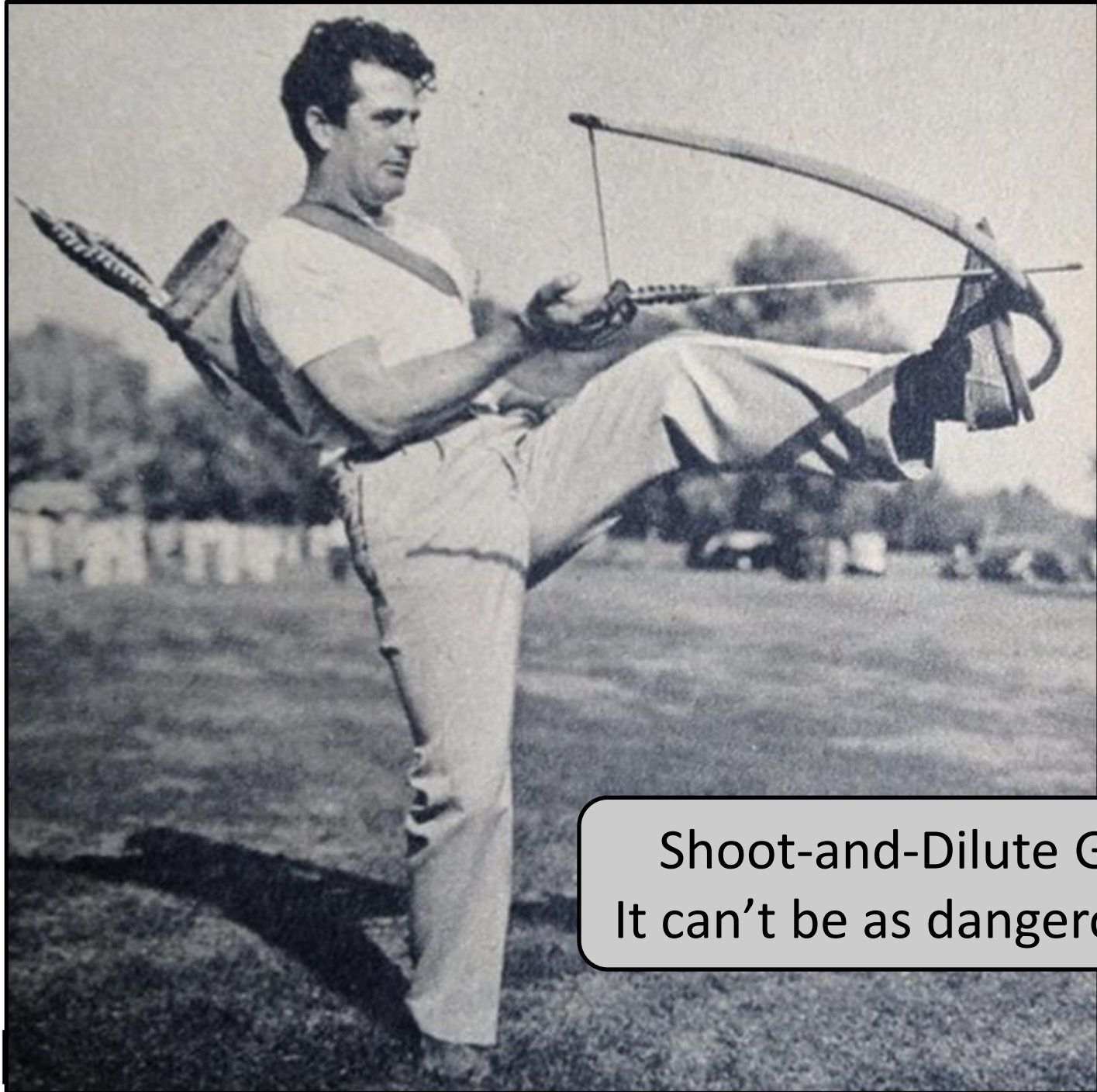
Q C FA 10:3

Q C FA 1:1

Conclusions

Shoot-and-Dilute GC-ECD

- **Splitless injection** for sensitive pesticides like Captan, Folpet, and Deltamethrin **is not robust**
 - Response factors quickly fall off when analyzing QuEChERS extracts of strawberries
- **Split injection** for these same pesticides **shows better repeatability** for response factors
 - Higher flow through inlet leads to decreased pesticide degradation and sorption losses



Shoot-and-Dilute GC-MS/MS
It can't be as dangerous as this...

Shoot-and-Dilute GC-MS/MS

Fruit and Vegetable



Celery
Low pigment
“Easy”



Orange
Low pH
“Hard”



Green pepper
Medium pigment
“Moderate”

All of the above are high water content...





Cat.# 32562

1

GC Multiresidue Pesticide Kit

- Accurately identify and quantify pesticide residues by GC-MS/MS in fruits, vegetables, botanicals, and herbals like tea, ginseng, ginger, Echinacea, and dietary supplements.
- Comprehensive 203-compound kit covers food safety lists by the FDA, USDA, and other global governmental agencies; individual ampuls also sold separately.
- Formulated and grouped for maximum long-term stability*.
- Quantitatively tested to confirm composition; detailed support documentation provided.
- Optimized multiresidue pesticide method is offered free of charge; [downloadable XLS file](#) includes conditions and transition tables.
- Certified reference material (CRM) manufactured and QC-tested in Restek's ISO-accredited labs satisfies your ISO requirements.

Restek Offers a Full
Line of Certified
Reference Materials



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If your lab must use certified reference materials (CRMs), please be sure to tell us when ordering so we can help you meet your regulatory requirements as we transition our inventory.

9 stable mixes containing 203 GC-able pesticides

Organophosphorus (3), organochlorine (1),
organonitrogen (3), synthetic pyrethroid (1),
herbicide methyl ester (1) compounds

Downloadable transitions table for TSQ 8000

EN QuEChERS Extraction

Homogenize

10 g + 10 mL MeCN

Shake 1 min

Add EN salts

Shake 1 min

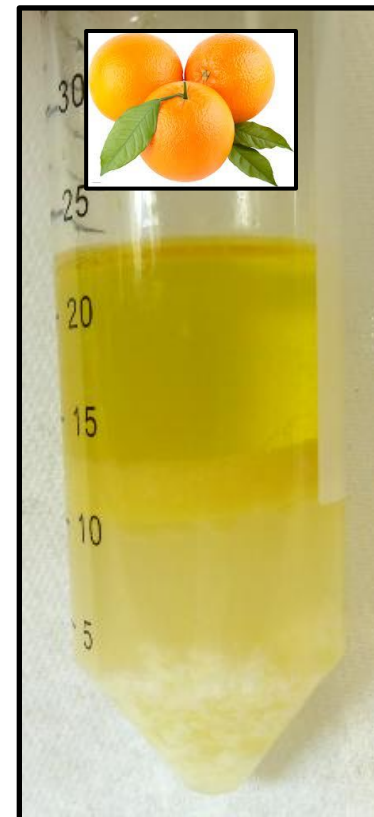
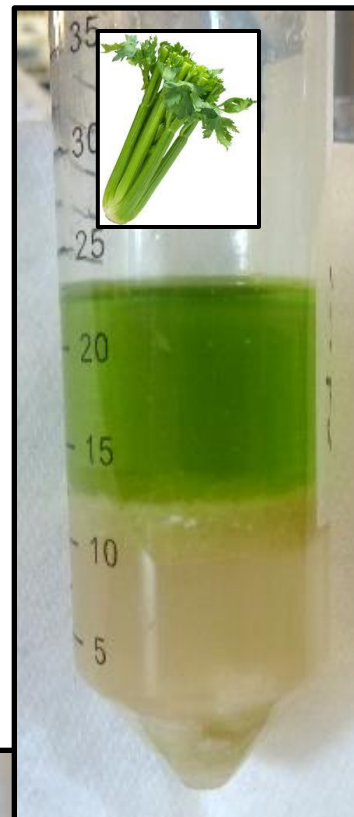
Centrifuge 5 min

dSPE Cleanup

Shake 30 sec

Centrifuge 5 min

Formic acid



mL Extract

150 mg MgSO₄

50 mg PSA

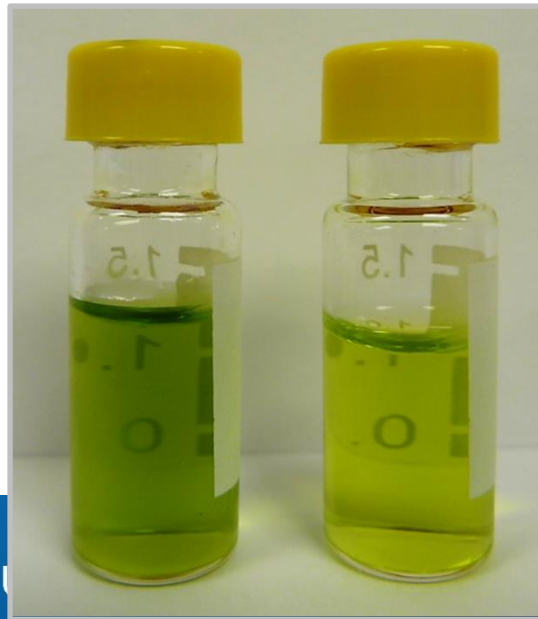
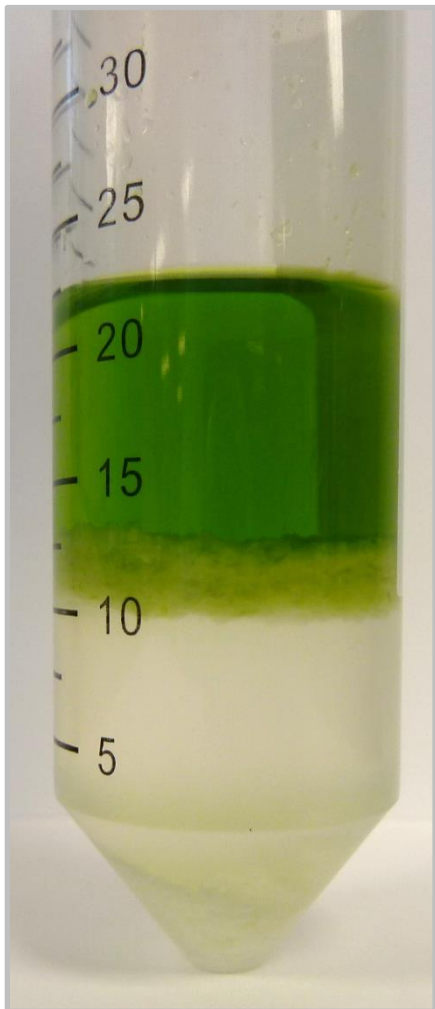
50 mg C18

7.5 mg GCB



mL Extract
150 mg MgSO₄
50 mg PSA
50 mg C18

We want it slightly dirty for our ruggedness experiment (and for planar pesticide recovery)...



Shoot-and-Dilute GC-MS/MS for Multiresidue Pesticide Analysis?



Detectability



Ruggedness

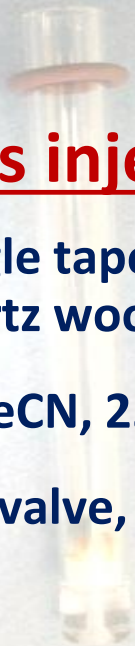


Splitless injection

4mm single taper liner
with quartz wool

1 μ L MeCN, 250°C

Splitless valve, 1.7 min

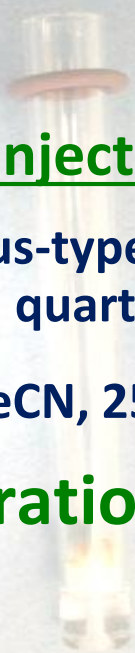


Split injection

4mm focus-type split
liner with quartz wool

1 μ L MeCN, 250°C

Split ratio 10



GC Conditions

30m x 0.25mm x 0.25 μ m
5ms-type column

Constant flow helium,
1.4 mL/min

GC oven program:
90°C (1.7 min), 8.9°C/min
to 330°C (hold 5 min)

Thermo Scientific TSQ 8000 GC-MS/MS

Transfer line: 290°C

Source temperature: 325°C

Electron ionization: 70 eV

Stored mass range: 2 SRMs per

Minimum Dwell: 26 msec

Shoot-and-Dilute GC-MS/MS for Multiresidue Pesticide Analysis?



Detectability

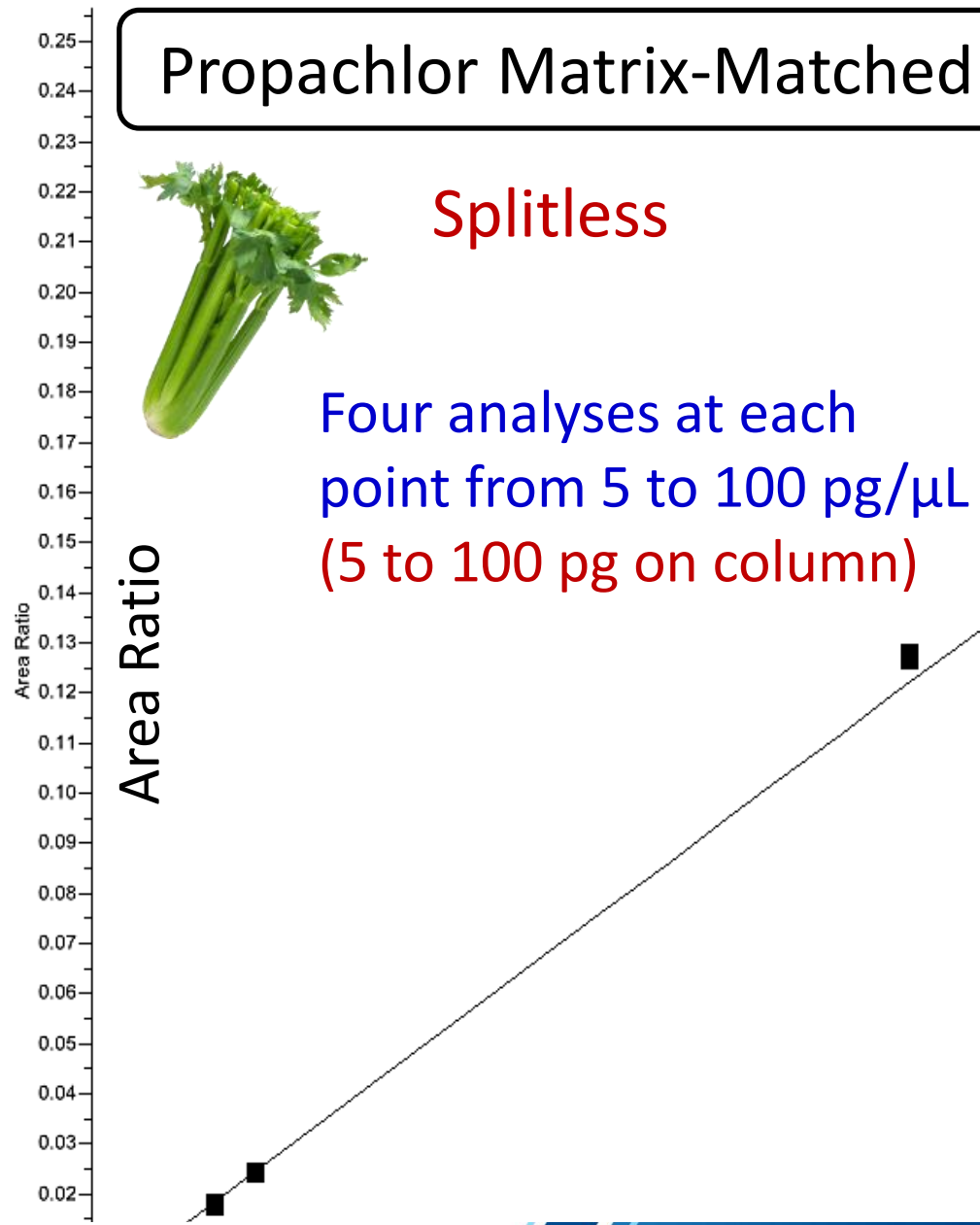
Propachlor Matrix-Matched Calibration Curve



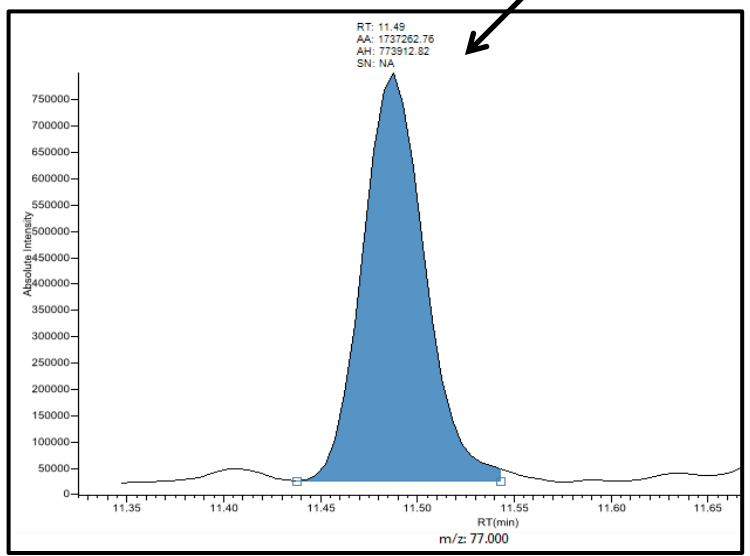
Splitless

$R^2 = 0.9987$

Four analyses at each point from 5 to 100 $\mu\text{g}/\mu\text{L}$
(5 to 100 μg on column)



10 μg



Concentration ($\mu\text{g}/\mu\text{L}$)
Pure Chromatography

www.restek.com

Propachlor Matrix-Matched Calibration Curve

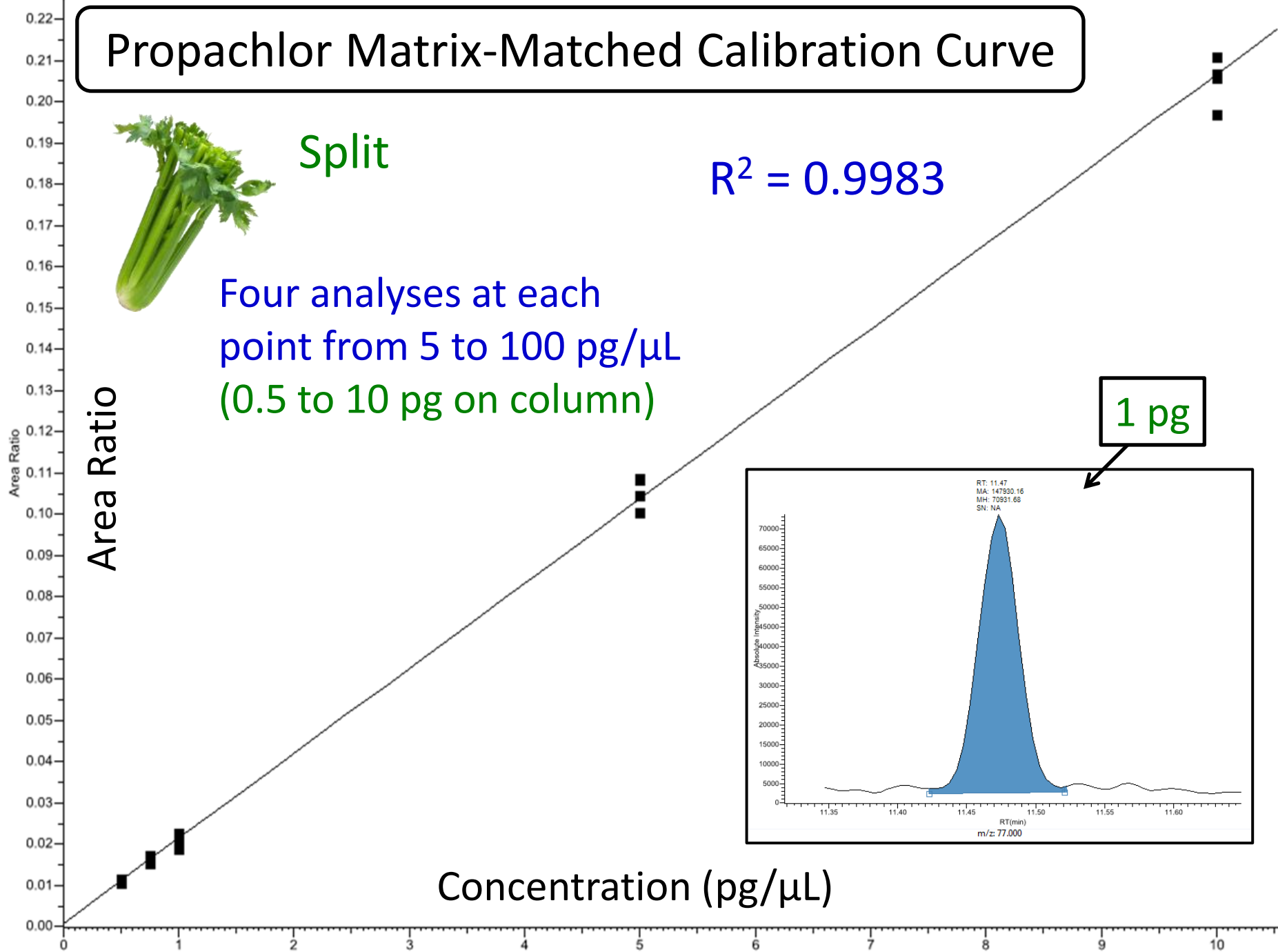


Split

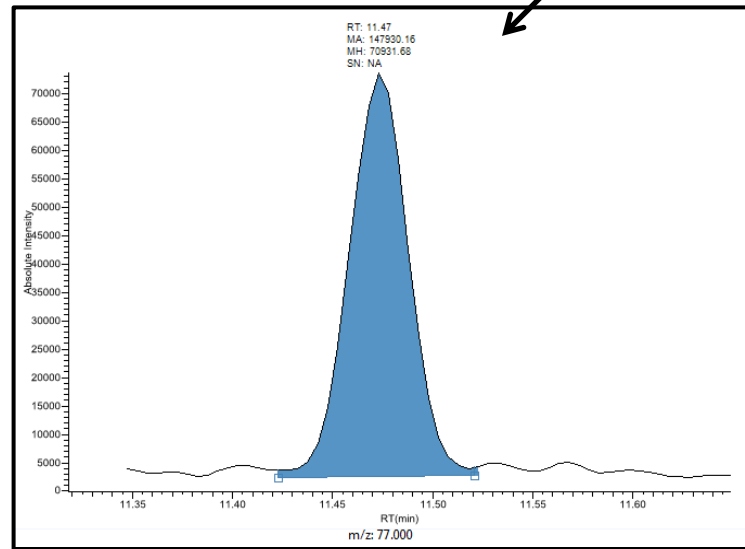
$R^2 = 0.9983$

Four analyses at each point from 5 to 100 $\text{pg}/\mu\text{L}$ (0.5 to 10 pg on column)

Area Ratio



1 pg



Concentration ($\text{pg}/\mu\text{L}$)

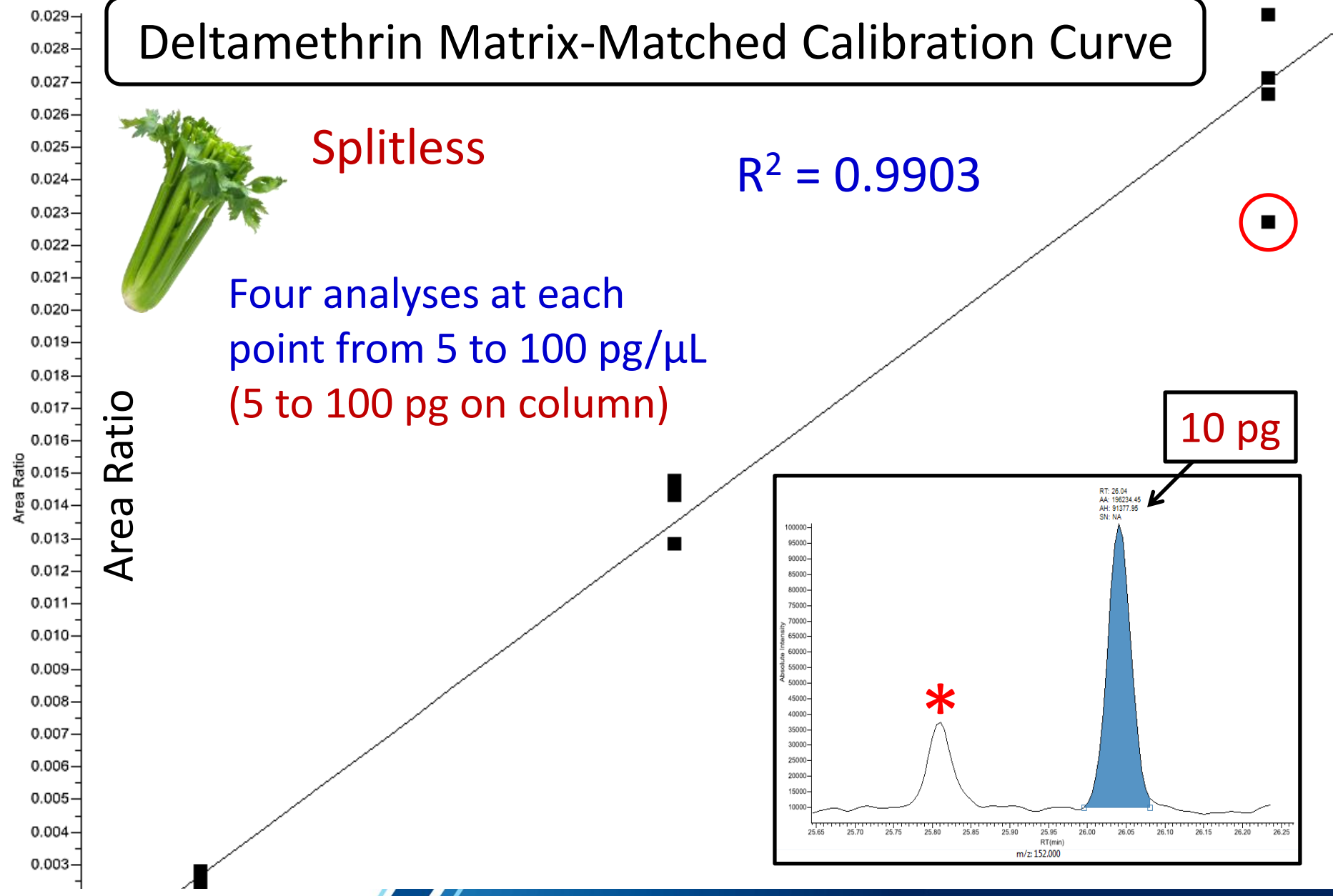
Deltamethrin Matrix-Matched Calibration Curve



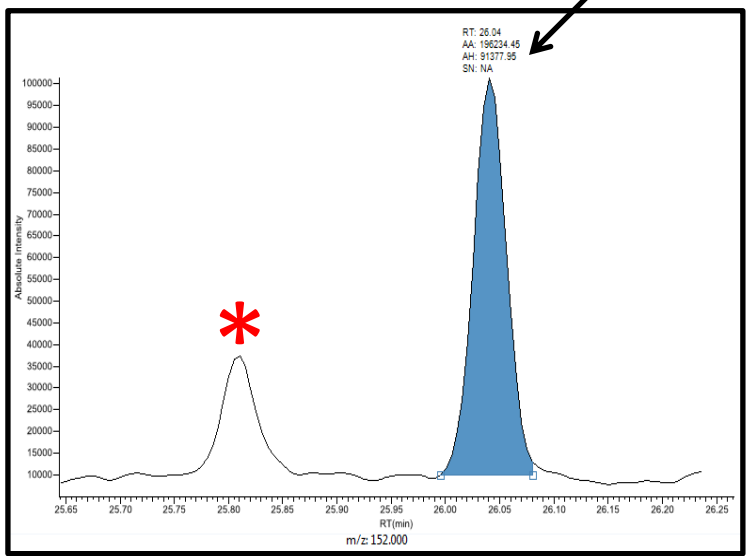
Splitless

$R^2 = 0.9903$

Four analyses at each point from 5 to 100 $\mu\text{g}/\mu\text{L}$ (5 to 100 μg on column)



10 μg



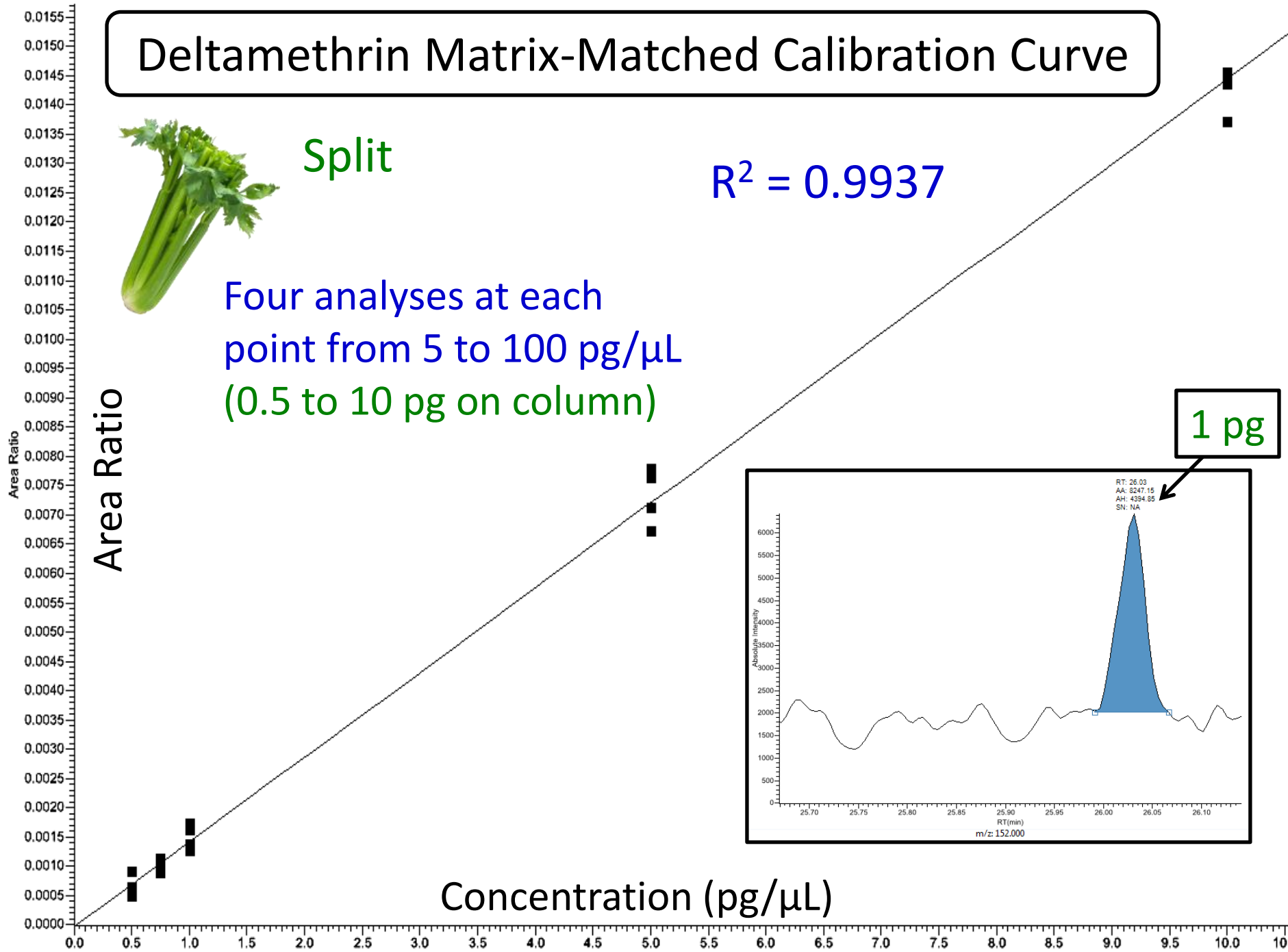
Deltamethrin Matrix-Matched Calibration Curve



Split

$R^2 = 0.9937$

Four analyses at each point from 5 to 100 $\text{pg}/\mu\text{L}$ (0.5 to 10 pg on column)



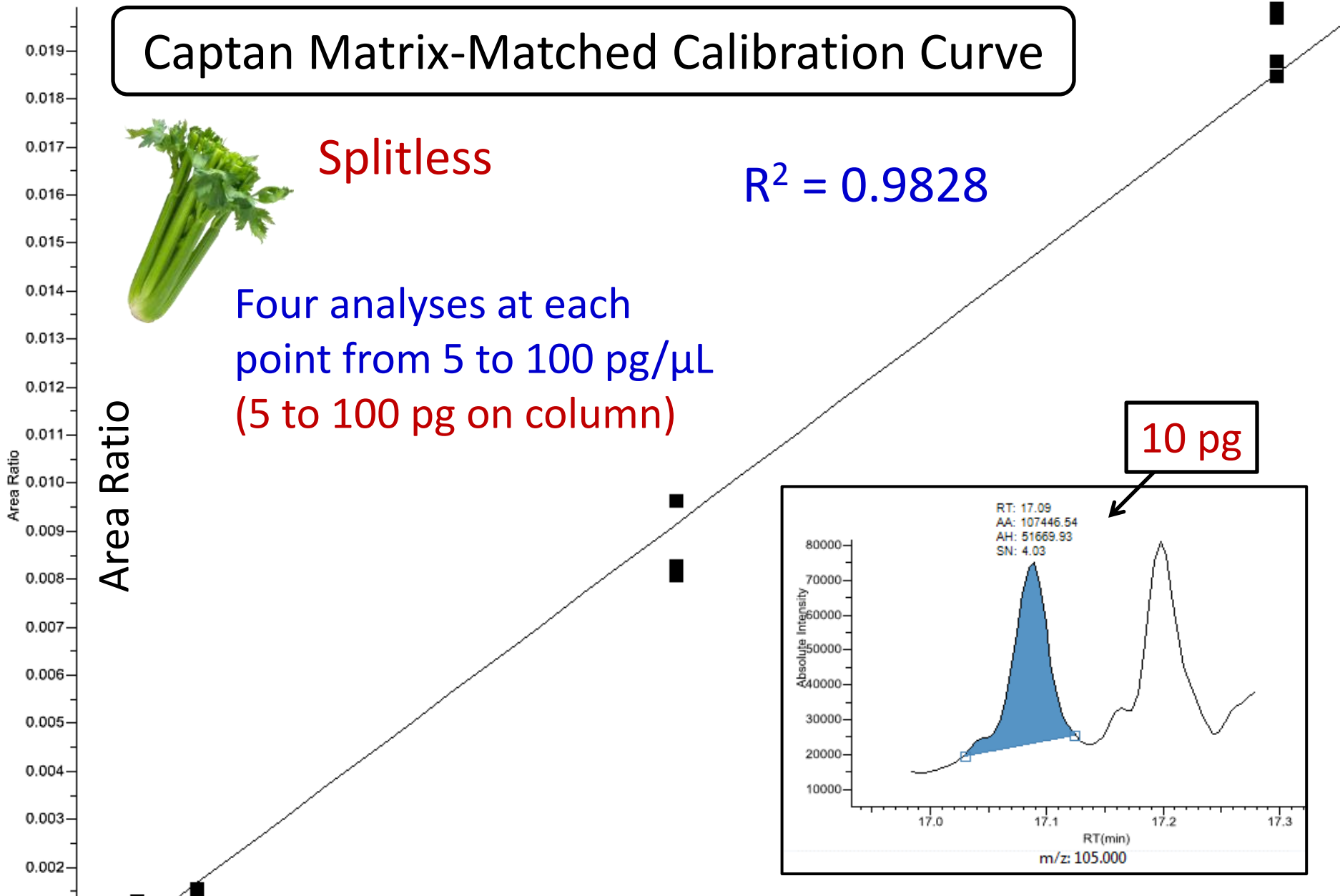
Captan Matrix-Matched Calibration Curve



Splitless

$R^2 = 0.9828$

Four analyses at each point from 5 to 100 pg/ μ L (5 to 100 pg on column)



Captan Matrix-Matched Calibration Curve



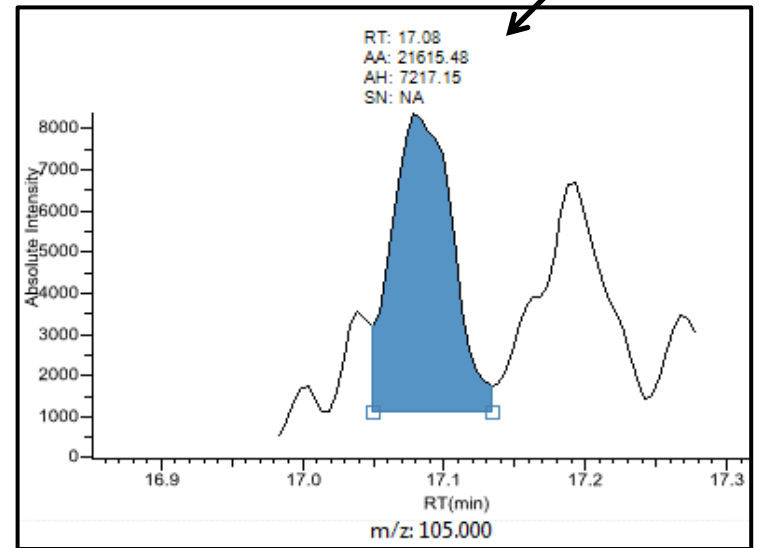
Split

$R^2 = 0.9319$

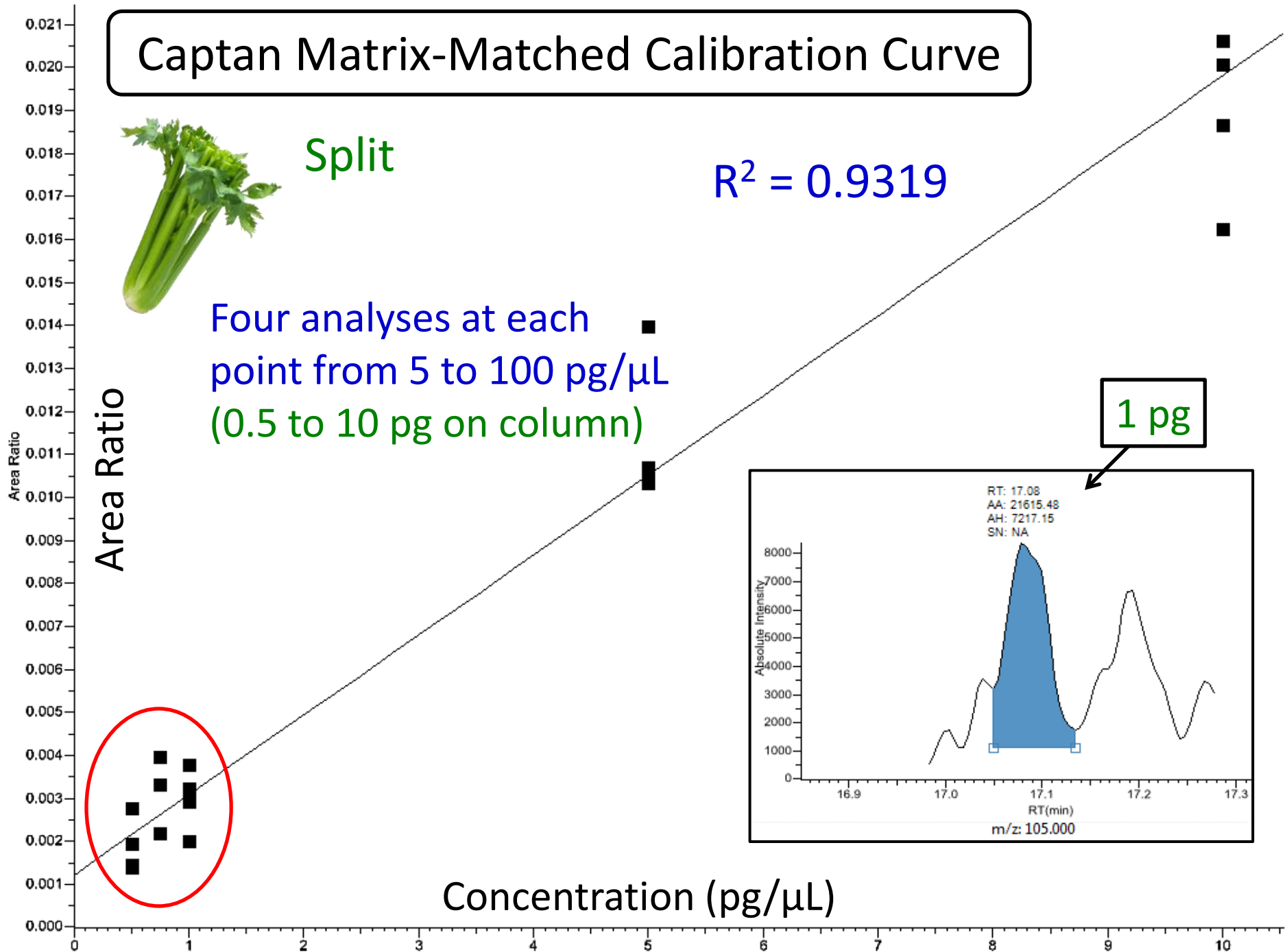
Four analyses at each point from 5 to 100 $\text{pg}/\mu\text{L}$ (0.5 to 10 pg on column)

Area Ratio

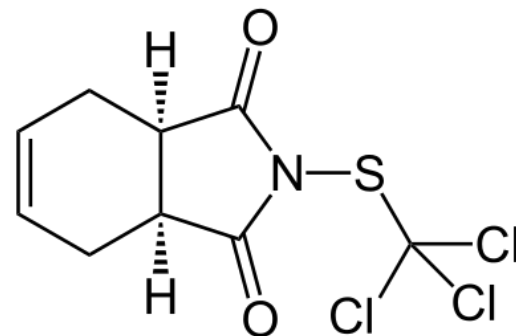
1 pg



Concentration ($\text{pg}/\mu\text{L}$)

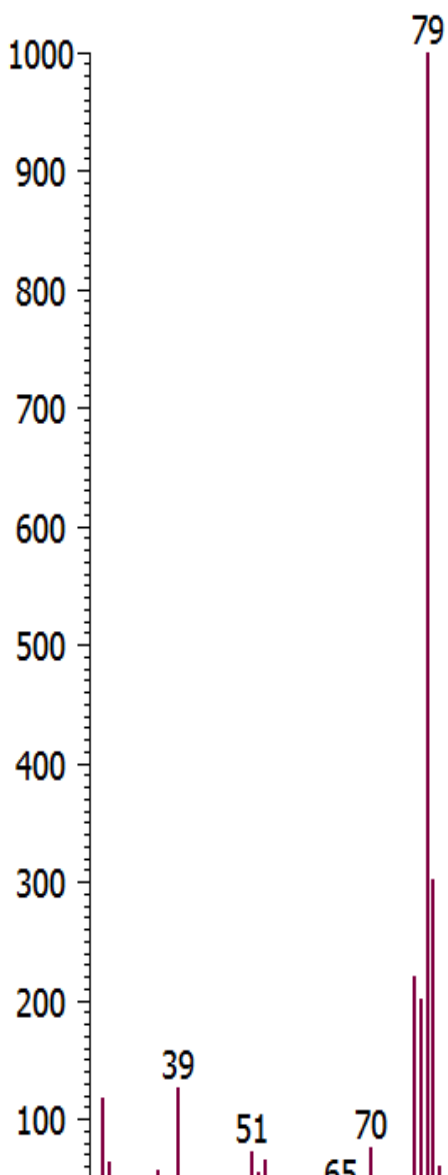


Captan



Quant SRM, 149 to 105

Qual SRM, 149 to 79





Detectability Summary



- 203 pesticides in celery and orange extracts
 - ~180 pesticides detected < 1 pg on column
 - ~195 pesticides detected 1 pg on column
- Captan and Folpet detected > 1 pg on column
 - Low m/z precursor and product ions
- Isomeric pesticides detected > 1 pg on column
 - Cyfluthrins and cypermethrins, e.g., split response

Calibration Summary

Splitless and Split Injection



- 203 pesticides in celery and orange extracts
 - Matrix-matched standards
 - 5, 7.5, 10, 50, 100 pg/ μ L – 4 replicates each
- Calibration curve correlation coefficients
 - Large majority of pesticides > 0.99
- Calibration curve Average RF RSD% values
 - Large majority of pesticides < 20%

Calibration Summary

Splitless and Split Injection



- **Splitless**
- Detectability advantage
- “Generic” data processing
- Quicker review
- **Split**
- Detectability reasonable
- “Manual” data processing
- Lengthy review

Does the possibility of longer GC system uptime and more stable response factors from split injection offset some detectability and data processing issues?

Ruggedness

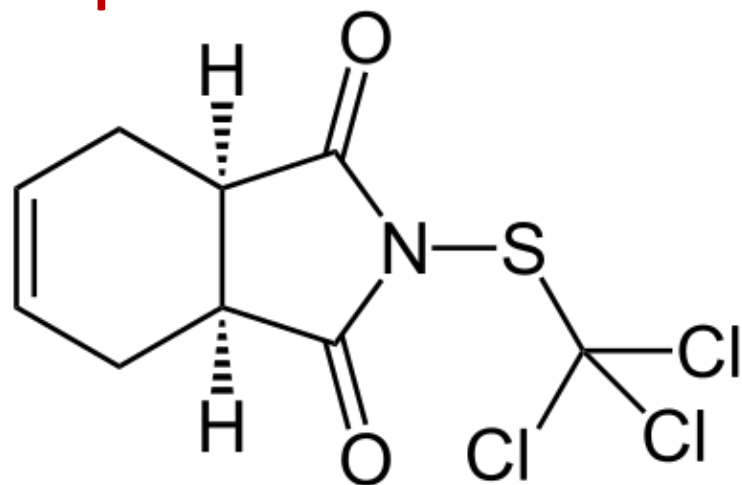


Does the split injection GC system stay up longer?

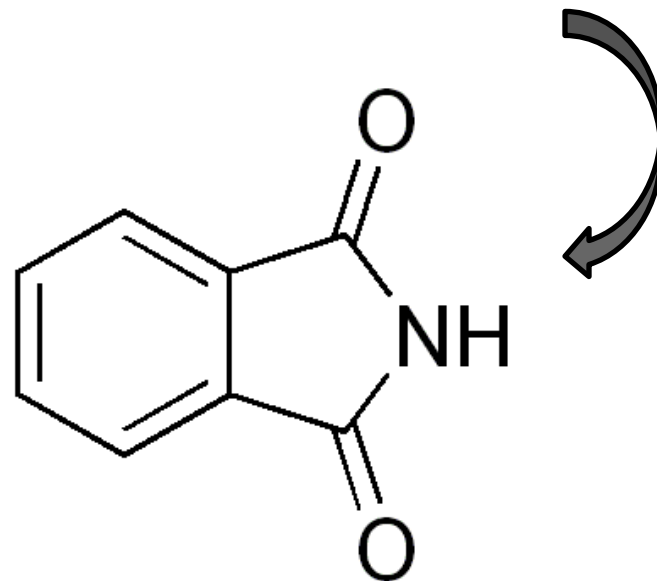
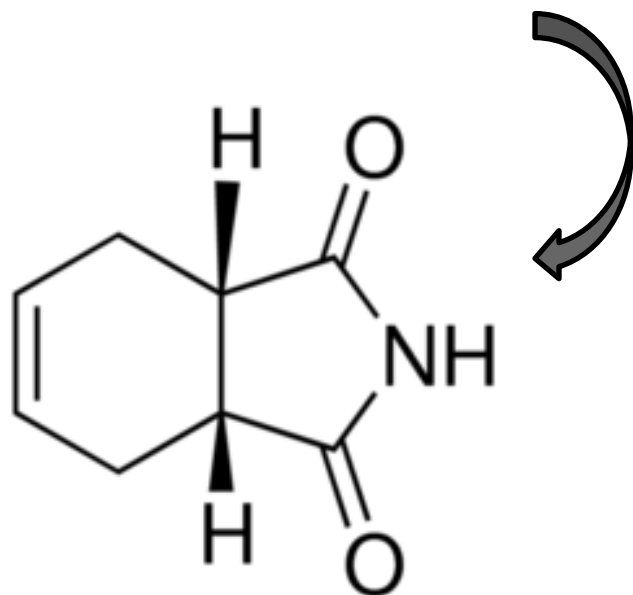
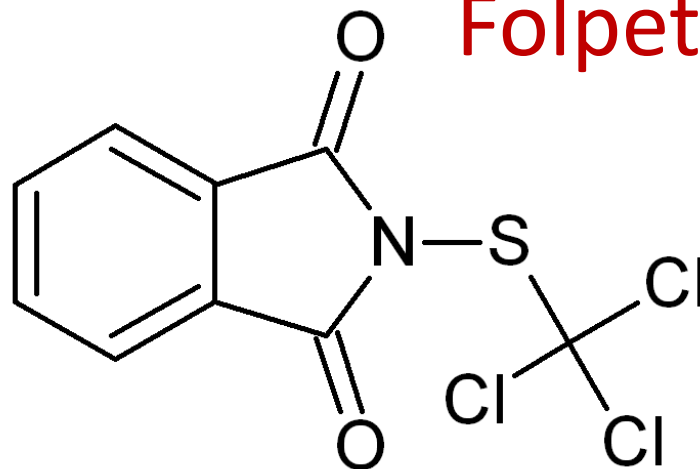
RES

m

Captan



Folpet



Tetrahydrophthalimide (THPI)

Phthalimide

Splitless

Split

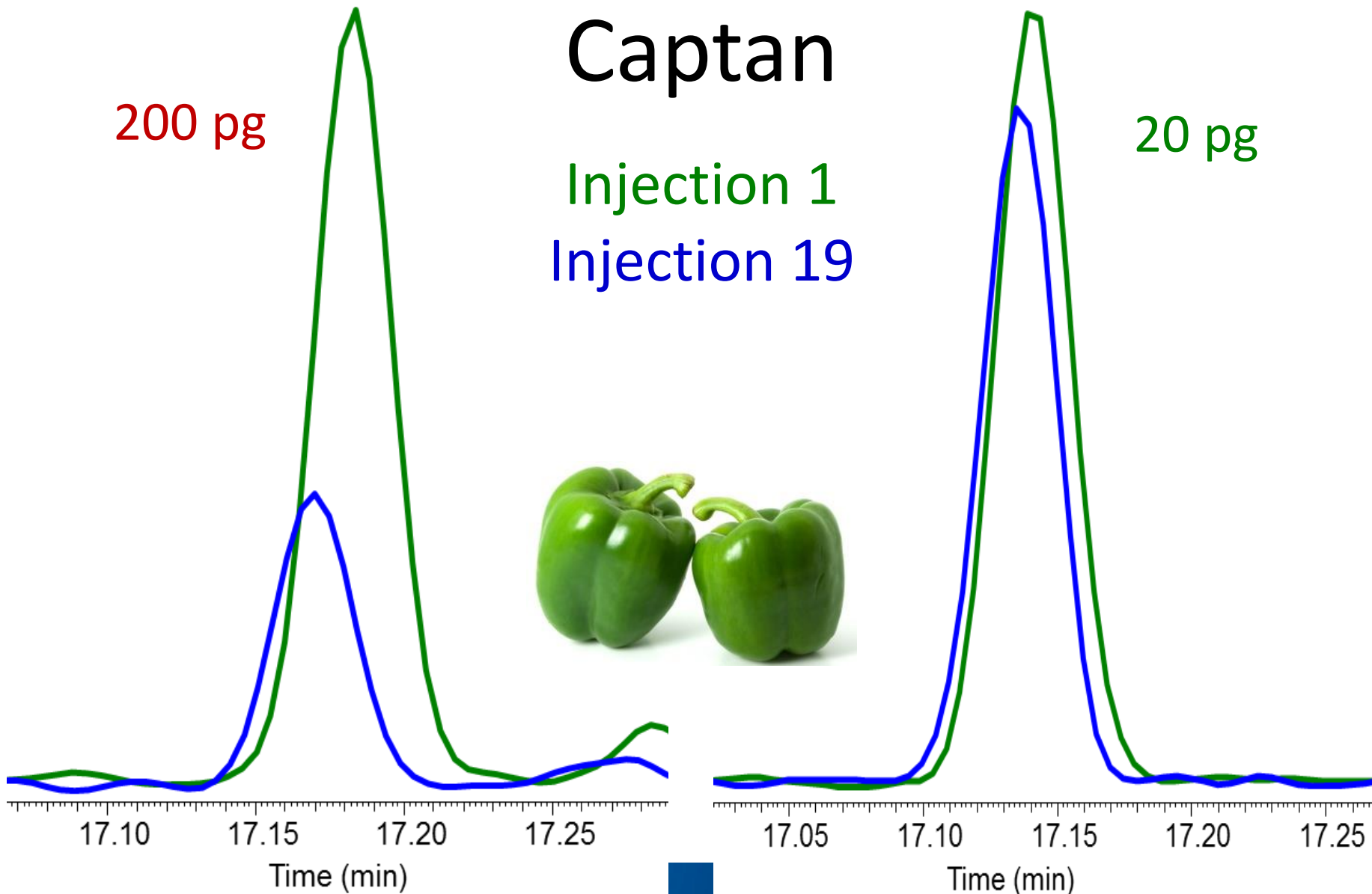
Captan

200 pg

20 pg

Injection 1

Injection 19



Splitless

Split

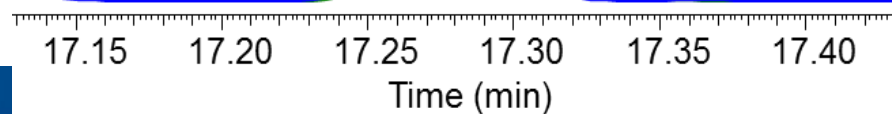
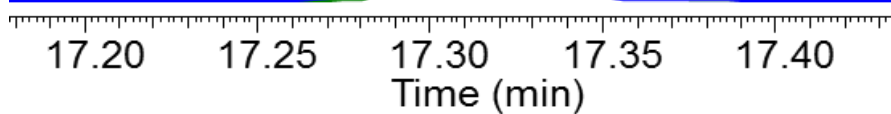
Folpet

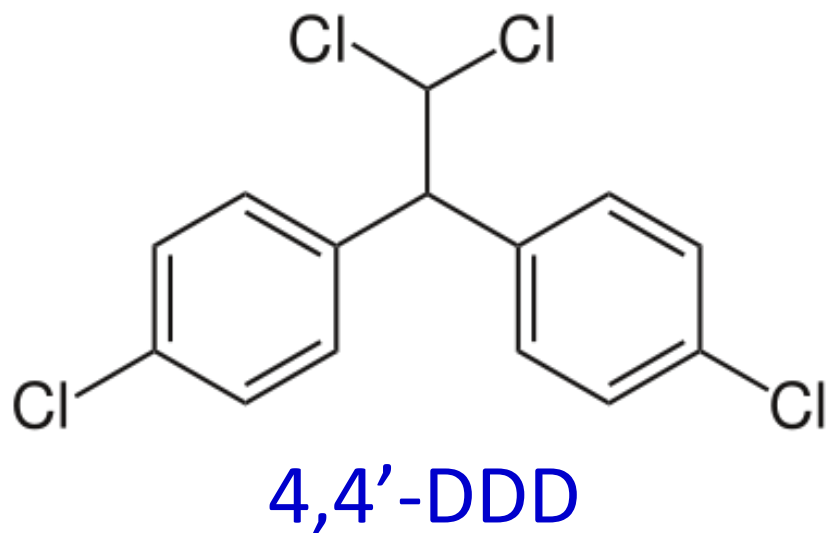
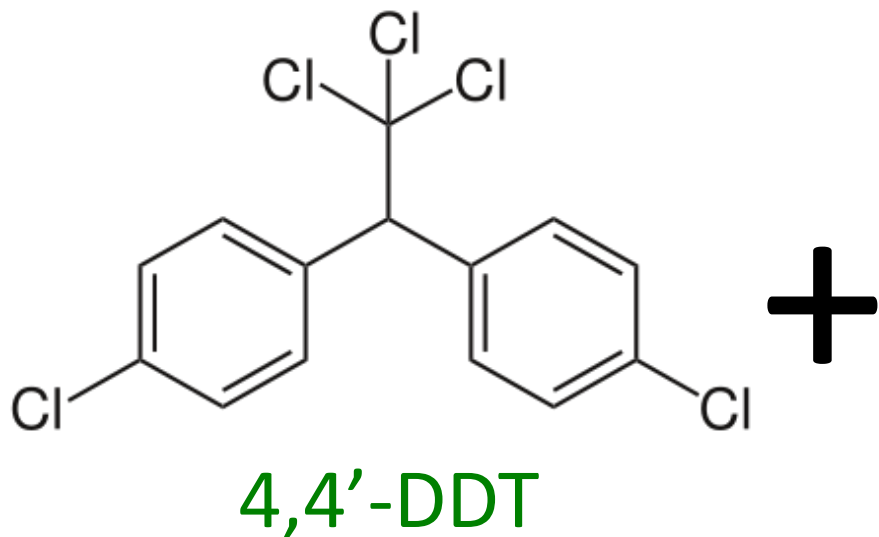
200 pg

20 pg

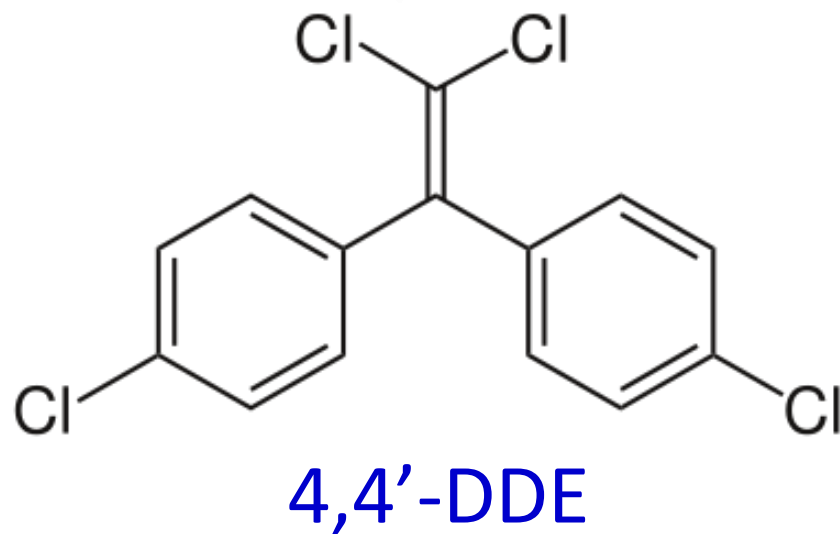
Injection 1

Injection 19





+



4,4'-DDD

Splitless

200 pg

4,4'-DDT

Injection 1

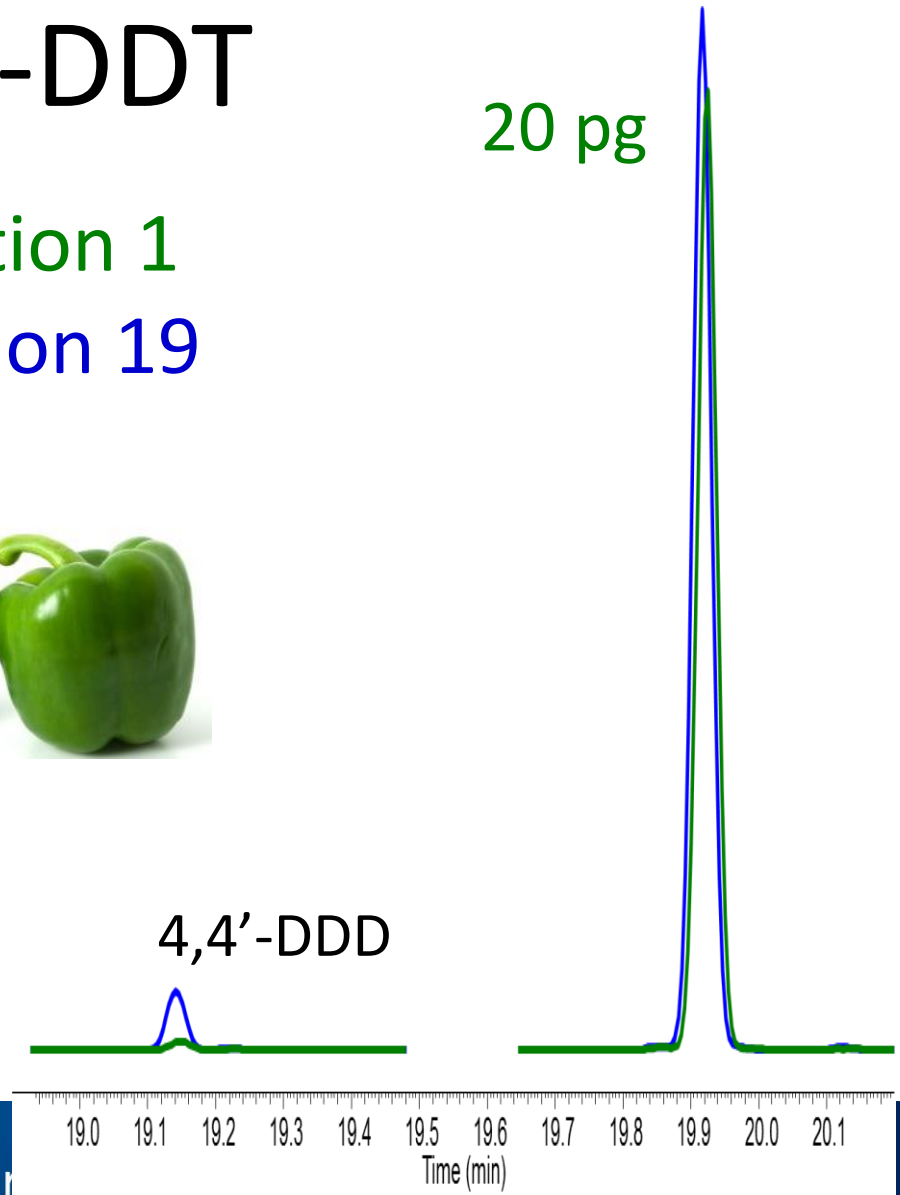
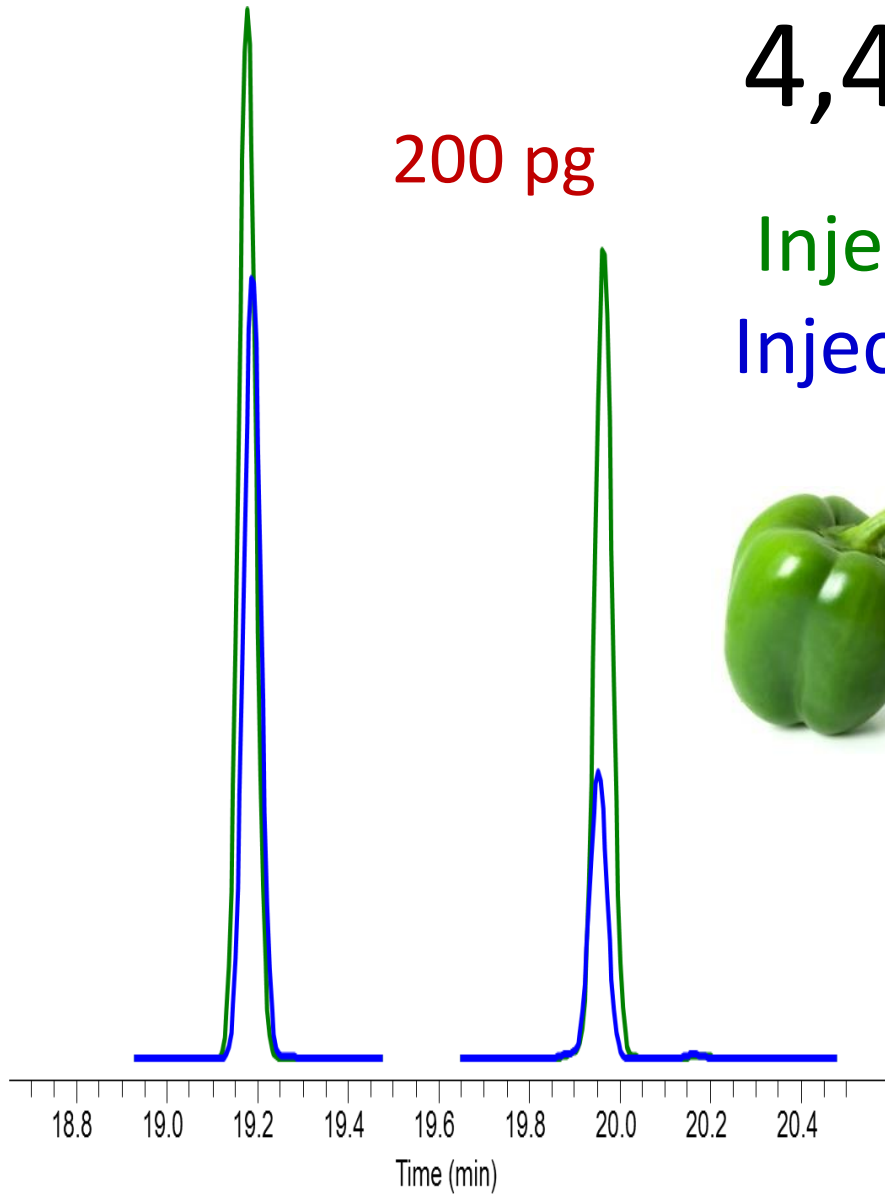
Injection 19



Split

20 pg

4,4'-DDD



Comparing Average Response Factor (RF) and % RSD RF for Splitless and Split Injection

Pesticide	Splitless Avg RF	Split Avg RF		Splitless % RSD RF	Split % RSD RF
gamma-BHC	0.131	0.199		7.6	3.2
Chlorothalonil	0.056	0.070		7.6	3.2
Carbaryl	0.078	0.129		7.7	2.2
Methiocarb	0.254	0.302		6.8	2.1
Dichlofluanid	0.381	0.444		4.9	1.6
Captan	0.004	0.034		25	9.3
4,4'-DDT	0.117	0.480		30	3.6
Deltamethrin	0.073	0.051		14	4.9

QuEChERS green pepper extracts spiked with 200 pg/ μ L pesticide standards. Avg RF calculated from 19 analyses of spiked green pepper extracts.

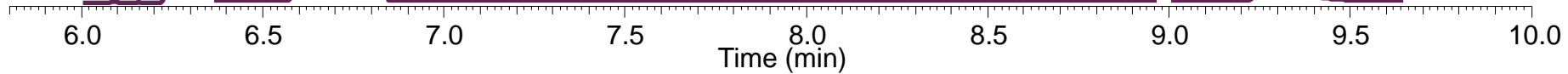
Splitless

Methamidophos

Dichlorvos

Acephate

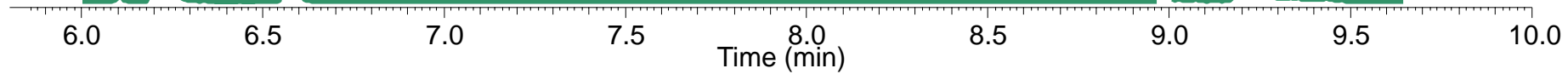
200 pg/ μ L
1 μ L MeCN



Split

200 pg/ μ L
1 μ L MeCN

Split ratio 10
0.1 μ L MeCN
20 pg



Conclusions

Shoot-and-Dilute GC-MS/MS

- Split injection shows promise for GC-MS/MS
 - Ruggedness is superior to splitless injection
- Need better detectability for some pesticides
 - Further optimization of MS/MS parameters
- Data review is somewhat lengthy when LODs and LOQs are approached
 - Better choice of auto integration settings

Other Points

- Food matrix quantification bias (theoretically) the same for splitless and split injections
 - Quantification accuracy defined by split LODs
- Possibility of avoiding dSPE cleanup
 - Split injection keeps the system up longer
 - Better base-sensitive pesticide recoveries (no PSA)
 - Better planar pesticide recoveries (no GCB)
- Possibility of using solvent-only standards instead of matrix-matched standards for split injection
 - High inlet flow encourages good pesticide transfer

Recent Success of Shoot-and-Dilute GC

- Split injection GC-MS (NCI) approach for straightforward analysis of problematic phthalimide fungicides and chlorothalonil
 - K. Lichtmanegger, J. Cochran, H. Unterluggauer, F. Steemann, S. Masselter
 - Analytical and Bioanalytical Chemistry (submitted)
- QuEChERS extracts of apple, fish, oilseed, feed, kidney fat, and honey
- 3x reduction in GC-MS run time
- 3x increase in GC-MS up time

Thank
you!

