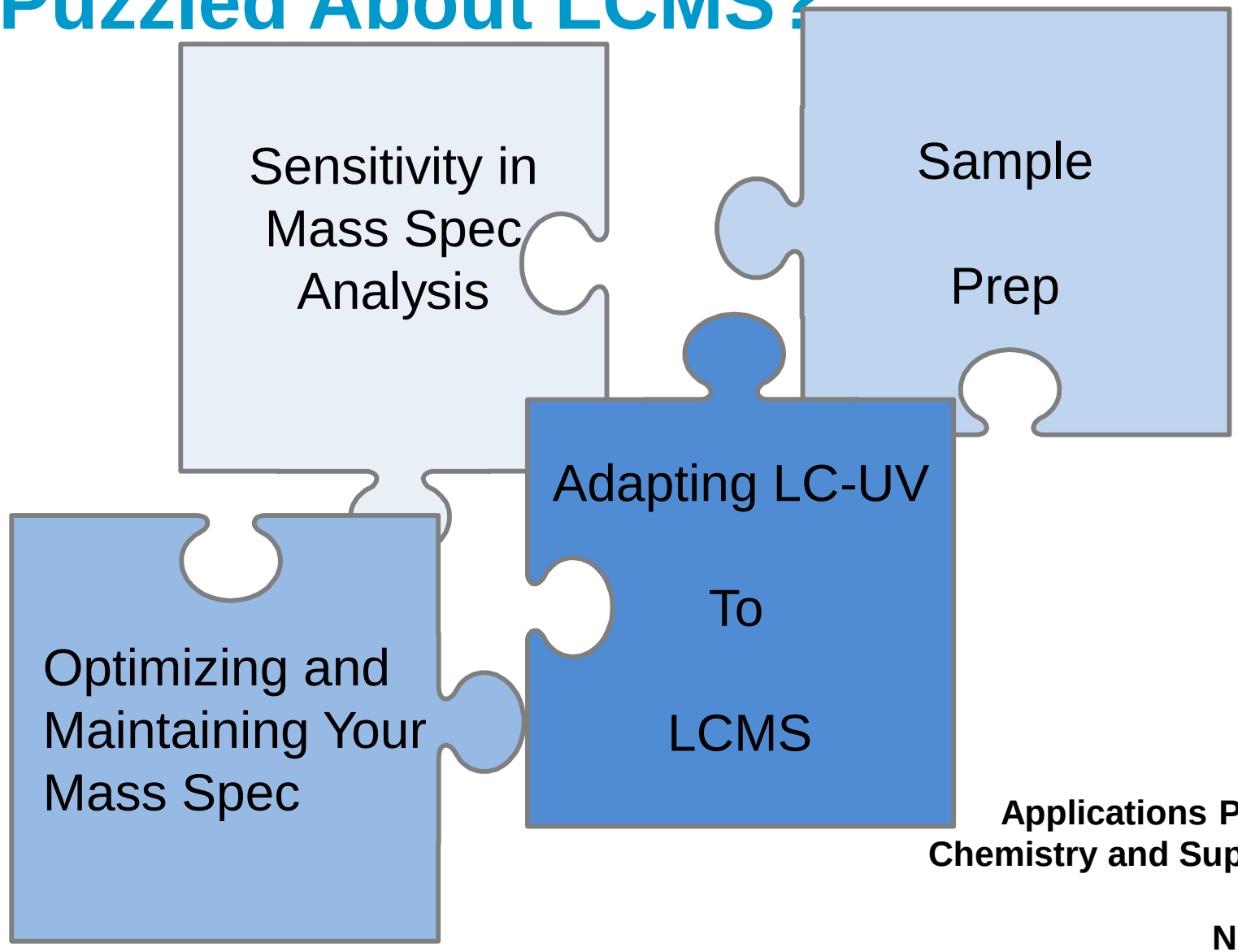
Puzzled About LCMS?



Paul Altiero
Alex Ucci

Applications Phone Support
Chemistry and Supplies Division

November 2017

Sensitivity in Mass Spec Analysis

Signal to Noise

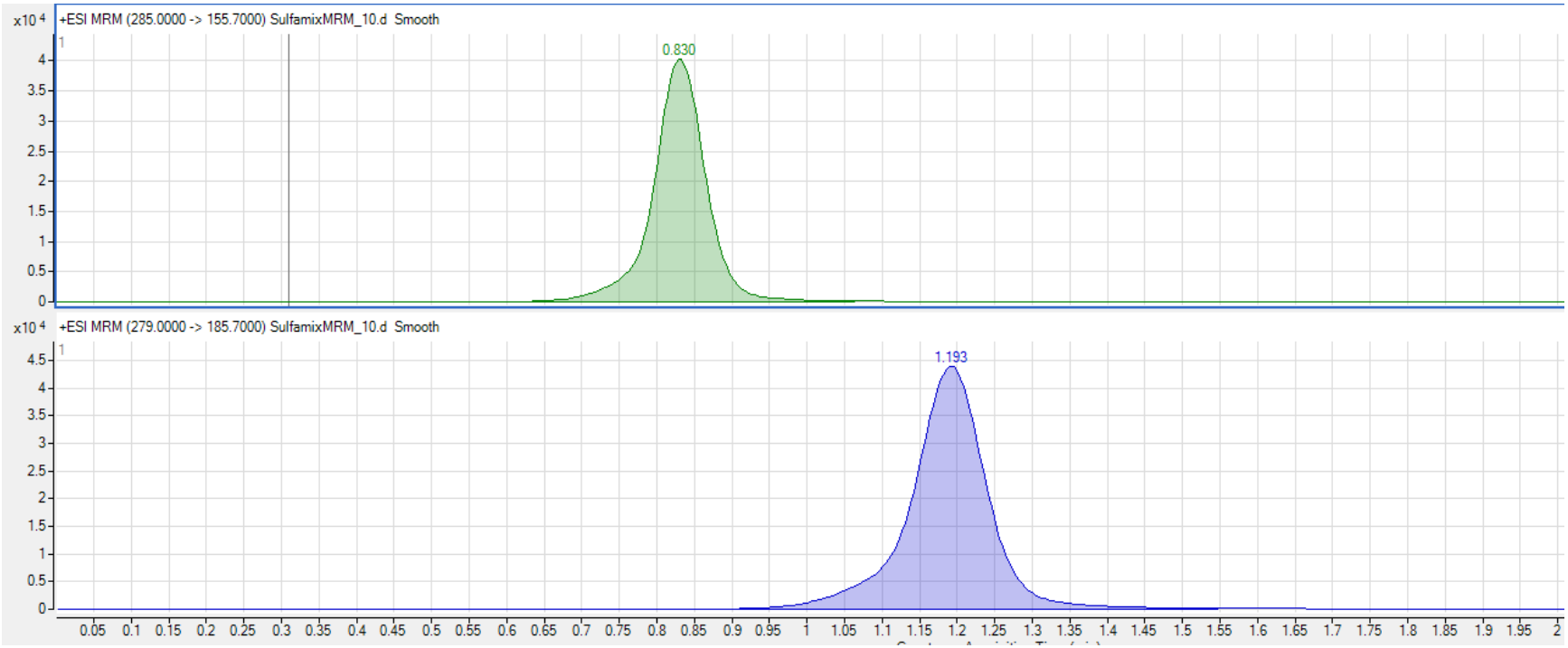
- Signal to Noise
- Solvent Composition and Drying Effects

analytical sensitivity. The concentration at which the mean response is statistically beyond the noise limits of the signal at zero concentration. Analytical sensitivity is the ability of a test to detect a target analyte

- *medical-dictionary.thefreedictionary.com/analytical+sensitivity*

Sensitivity in Mass Spec Analysis

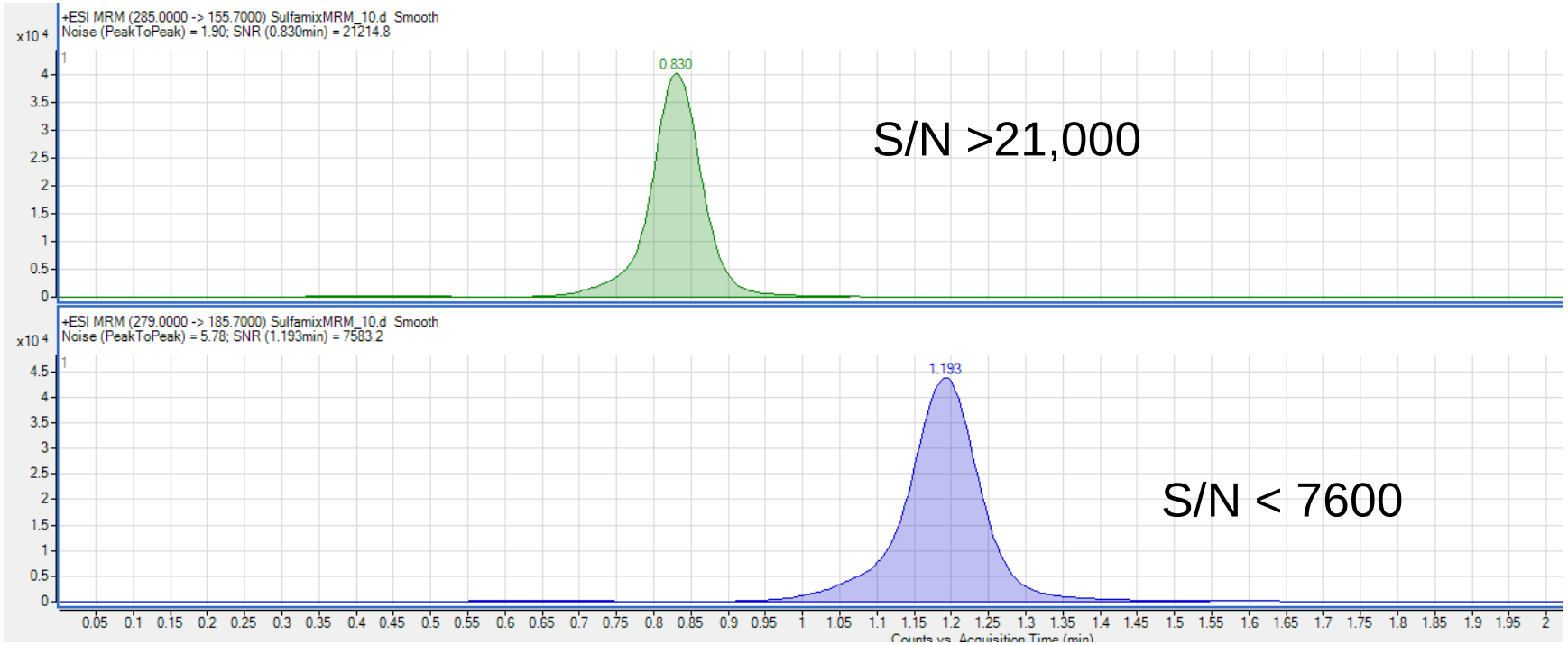
2 Analytes at the Same Concentration



Agilent Technologies

Sensitivity in Mass Spec Analysis

Does the higher response mean better sensitivity?



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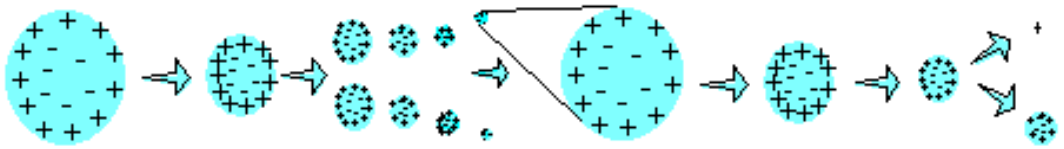
Sensitivity in Mass Spec Analysis

Solvent Composition and Drying Effects

- Signal to Noise
- Solvent Composition and Drying Effects

Ion Evaporation Model



Evaporation **Coulomb Explosion**

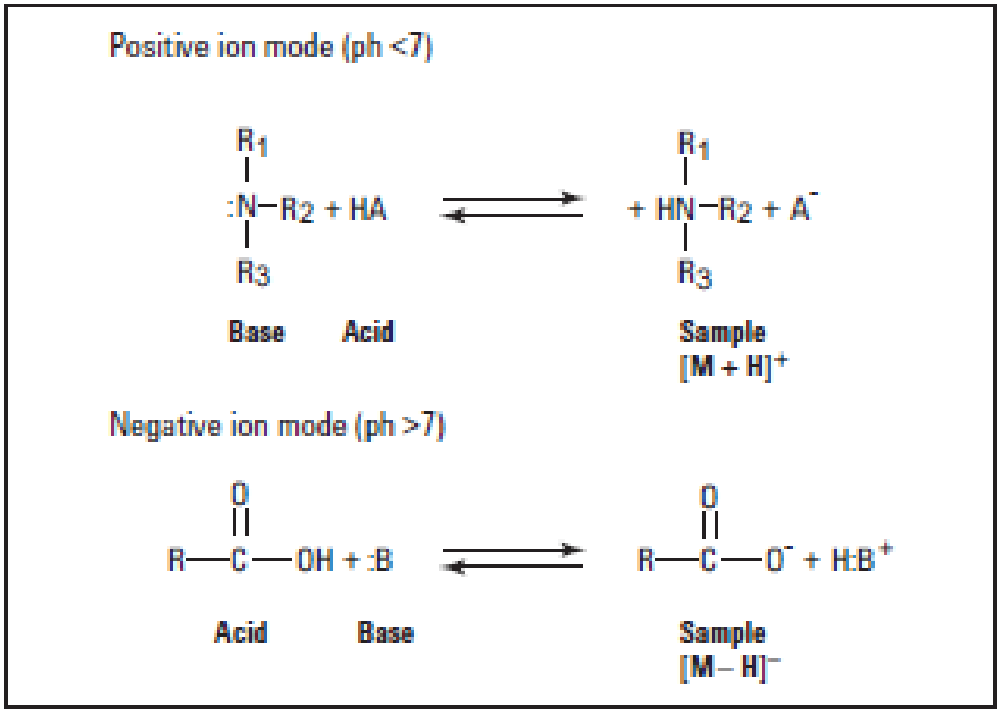
Rayleigh Limit

Analyte Ion Is ejected from the surface of the droplet due to field strength at the surface

Sensitivity in Mass Spec Analysis

Solvent Composition and Drying Effects

- Signal to Noise
- Solvent Composition and Drying Effects



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Sensitivity in Mass Spec Analysis

Solvent Composition and Drying Effects

- Signal to Noise
- Solvent Composition and Drying Effects

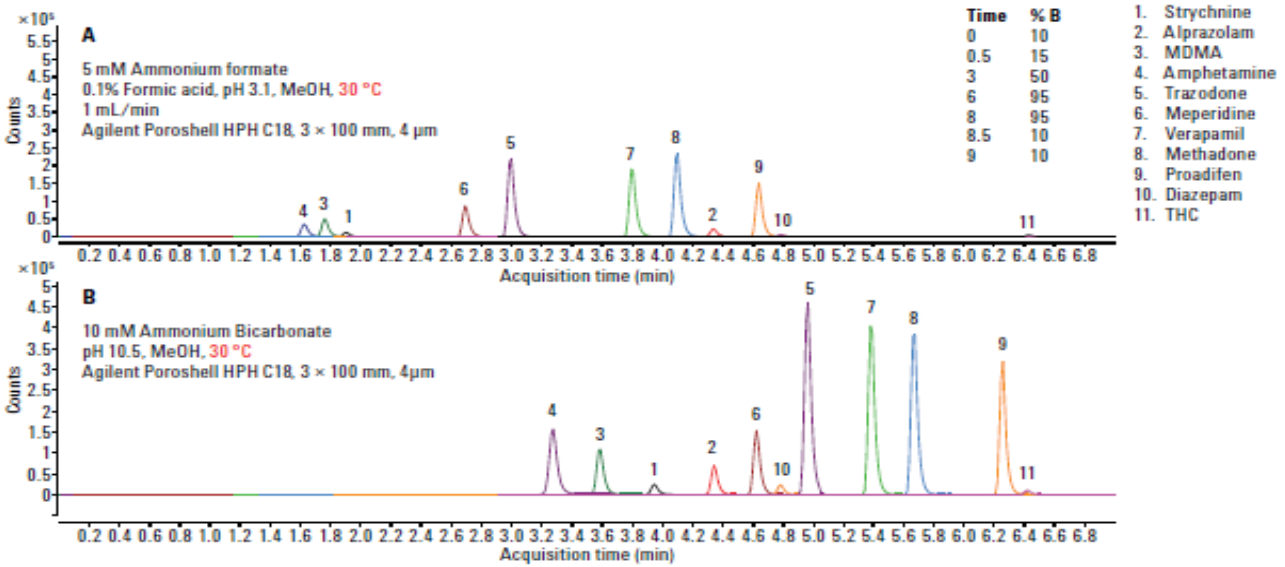

Figure 1. Separation at Low and High pH using an Agilent Poroshell HPH C18, 4 µm column.

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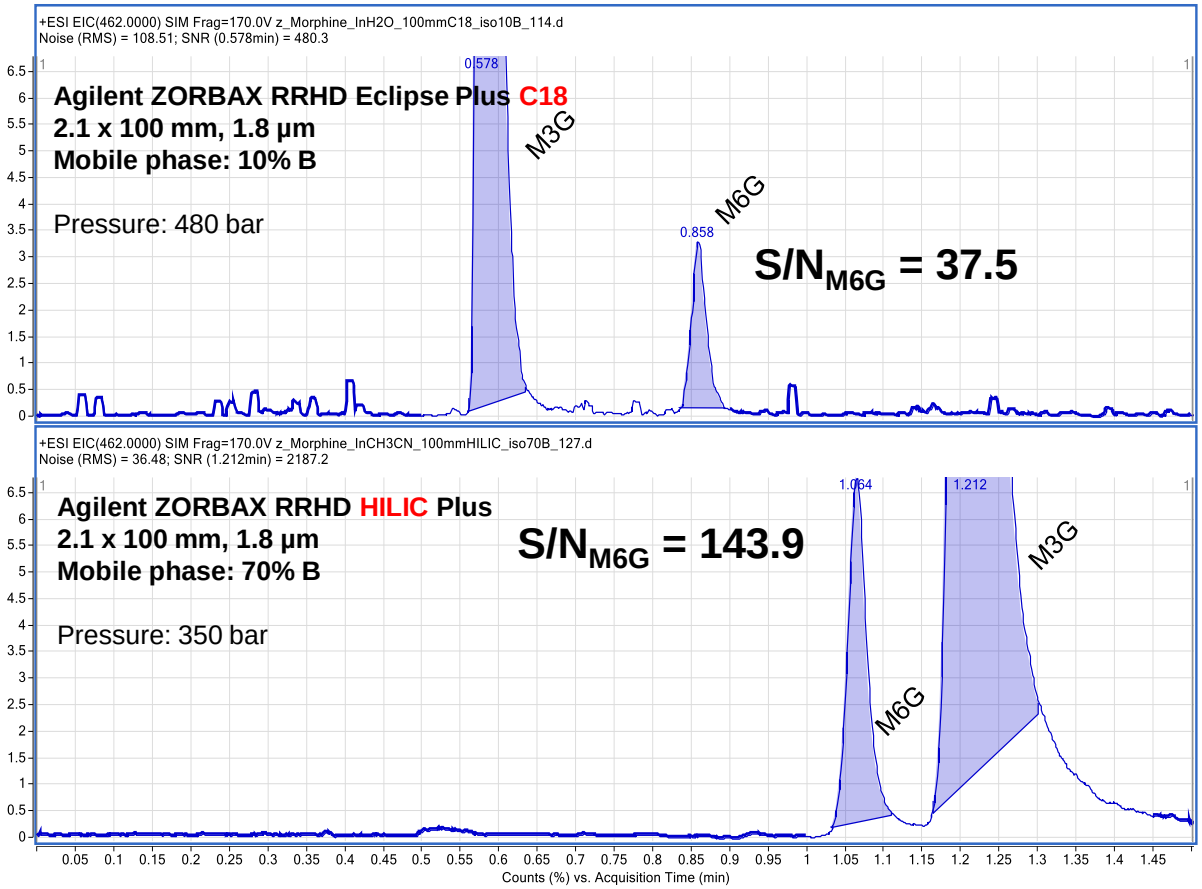
Sensitivity in Mass Spec Analysis

Solvent Composition and Drying Effects

- Signal to Noise
- Solvent Composition and Drying Effects



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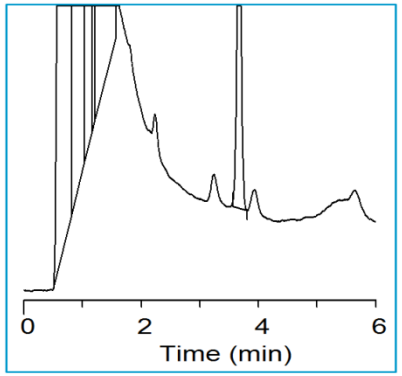
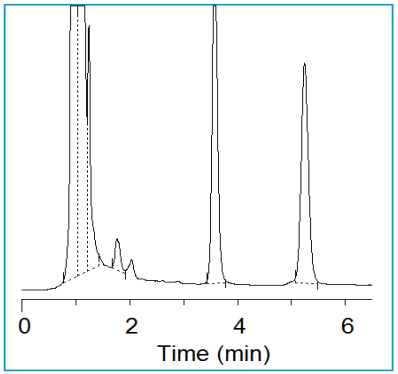
Why Perform Sample Clean-Up?

- To acquire desired sensitivity/selectivity
- To reduce contamination/carryover issues
- Use of sensitive and expensive instruments: *Protect your investment!!!*

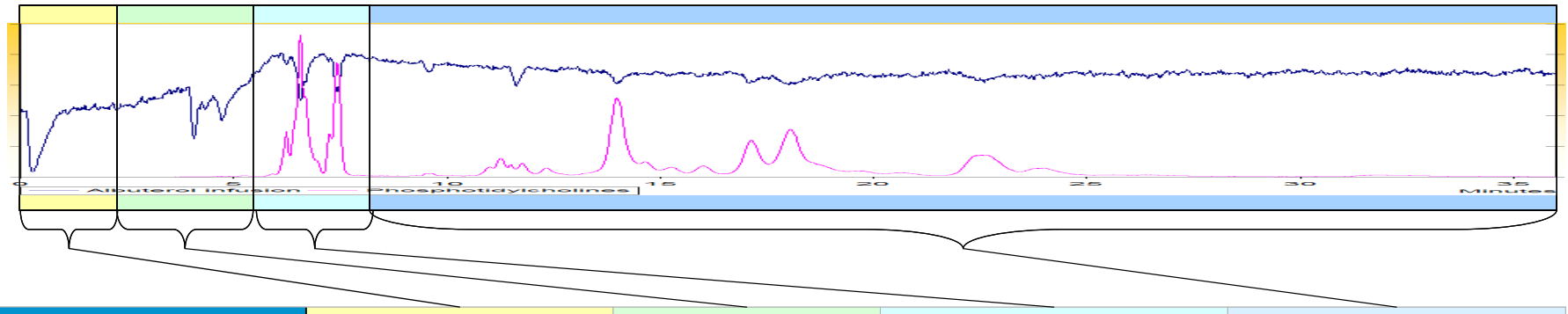
Pesticides in Avocado *without* SP



Pesticides in Avocado with SP



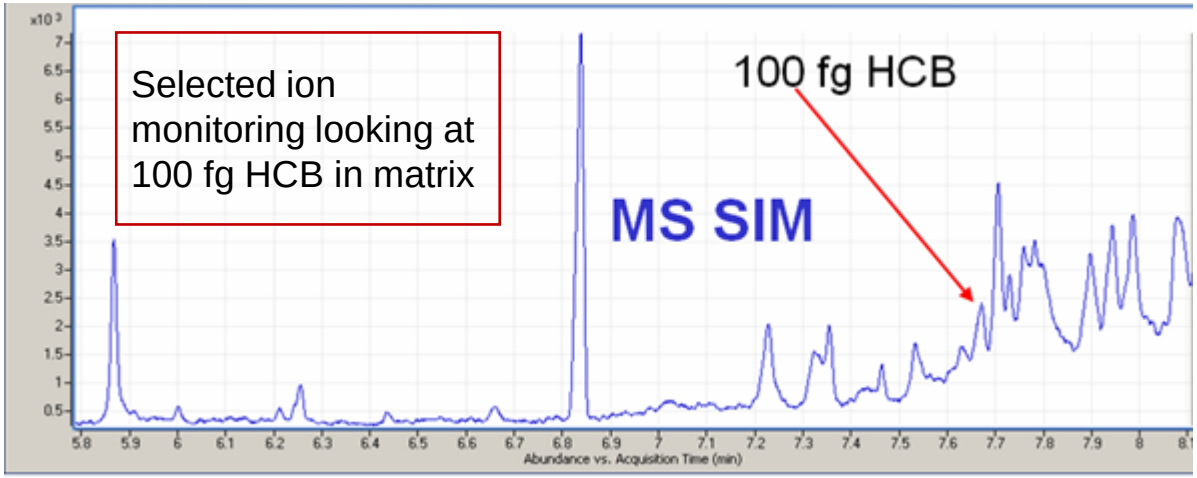
Ion Suppression: What Can Dirty Samples Do?

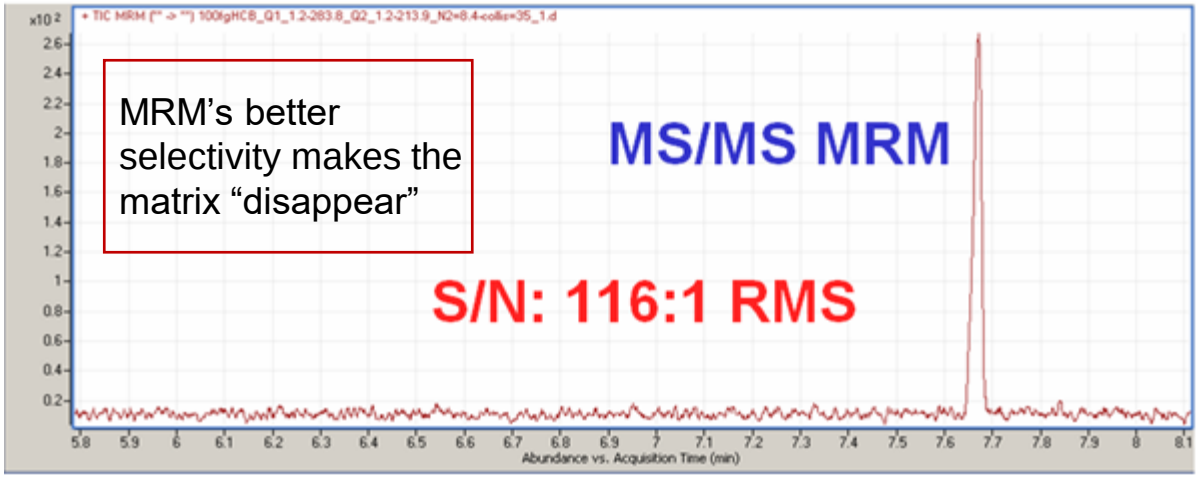


Interference type	Salt/Polar ionics	Proteins/ Peptides	Lyso-phosphatidylcholines	Lipids and other hydrophobics
Typical Elution Conditions (C18 column)	At or near void with < 20% organic	10's of column volumes at 40% - 70% organic	10's of column volumes at 70% - 90% organic	10's to 100's of column volumes at > 90% organic
Short term effect (single injection)	Significant ion-suppression	Significant ion-suppression	Significant ion-suppression	Some ion suppression, however, usually retained on LC column)
Long term effect (multiple injections)	Unknown	Unknown	Decreased sensitivity, Increased variability	Decreased sensitivity, Increased variability
Likely long term causes	Ion source contamination	Ion source contamination	Ion source contamination, Some column build-up	Ion source contamination, Column build-up

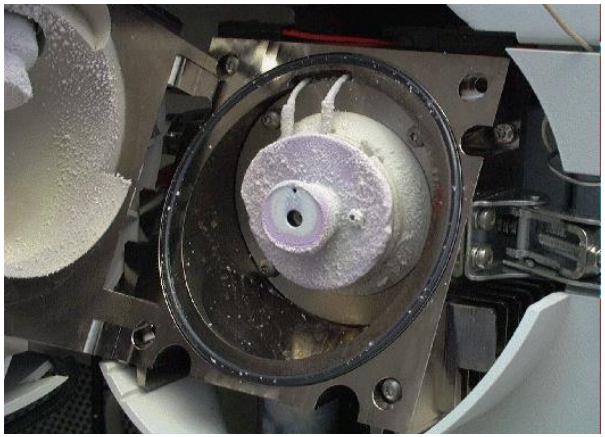
Need to remove salts, proteins/peptides, and lipids!

Tandem Mass Spectrometry and "The Case of the Disappearing Matrix"

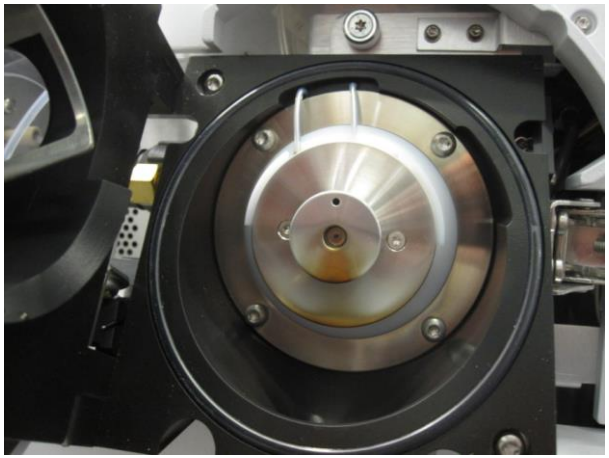




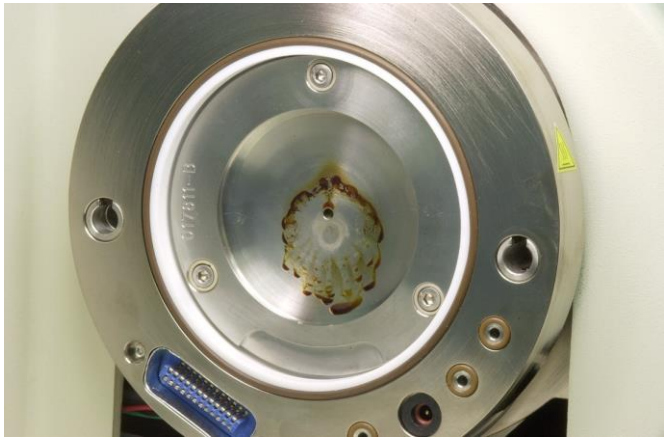
Instrument Contamination



Salt build-up in LC-MS ion source from unextracted salts



ESI Ion Source contamination after 3000x Urine Dilute/Shoot Injections



Curtain plate after injection of 25 samples with extractions from raisins without cleanup

Sample Preparation Techniques for Today's Discussion

Solid Phase Extraction (SPE)

Multi-step approach for highest level of sample cleanup

QuEChERS (dSPE)

Sample cleanup by extraction of bulk interferences

Captiva EMR-Lipids (PPT and lipid removal)

Removes precipitated proteins by in-well protein precipitation and also removes lipids

Filtration

Simple and fast removal of particulates

Functionalized Filtration: Captiva EMR-Lipid

- Captiva EMR-Lipid: effective lipid and protein removal
  - 99% lipid removal
- Available in 96-well plate, 1 mL, 3 and 6 mL cartridge formats
  Pass-through/clean-up format
- Solvent retention frit in 96-well plate and 1mL cartridge formats allows for in-well or in-cartridge protein precipitation
- 3 and 6mL cartridge formats do not contain solvent retention frit, which allows for gravity elution
- High analyte recovery
- One step sample addition and activation (20% water is needed to achieve optimum lipid removal)
  - 20% water provided in 3:1-5:1 crash solution for protein precipitation workflow

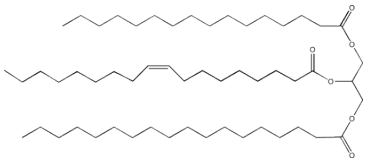
Key Application Notes

5991-8006EN	Efficiency of Biological Fluid Matrix Removal using Agilent Captiva EMR-Lipid Cleanup 5991-8006EN
5991-8007EN	Quantitative LC/MS/MS Analysis of Drugs in Human Serum with Agilent Captiva EMR-Lipid Cleanup
5991-7956EN	Vitamin D Metabolite Analysis in Biological Samples Using Agilent Captiva EMR-Lipid 5991-7956EN

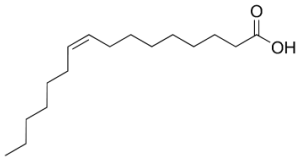
Enhanced Matrix Removal: EMR-Lipid

When "activated" by water…

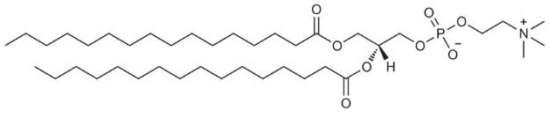
- The materials selective hydrophobic interactions increase.
- Suspension of particles with high surface area.
- Rapidly interacts with straight chain, "lipid-like" functional groups.
- Does not retain analytes



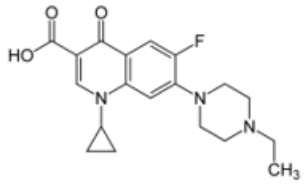
Triglycerides



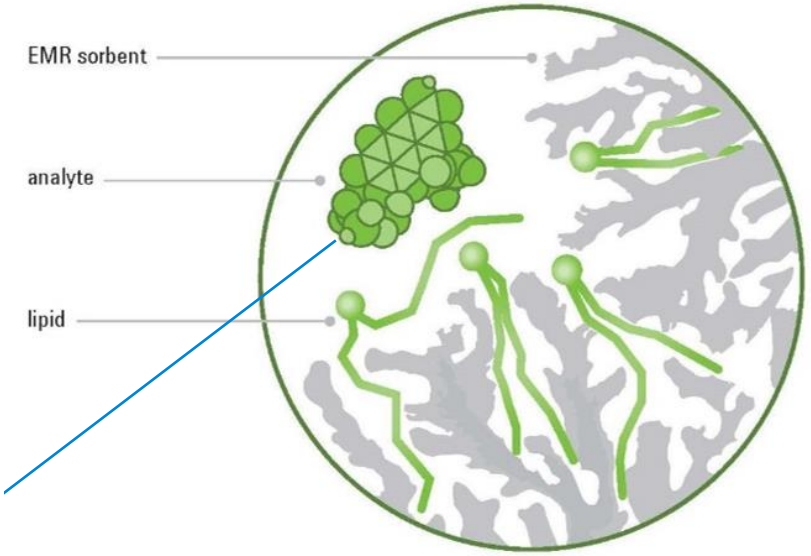
Free Fatty Acids



Phospholipids



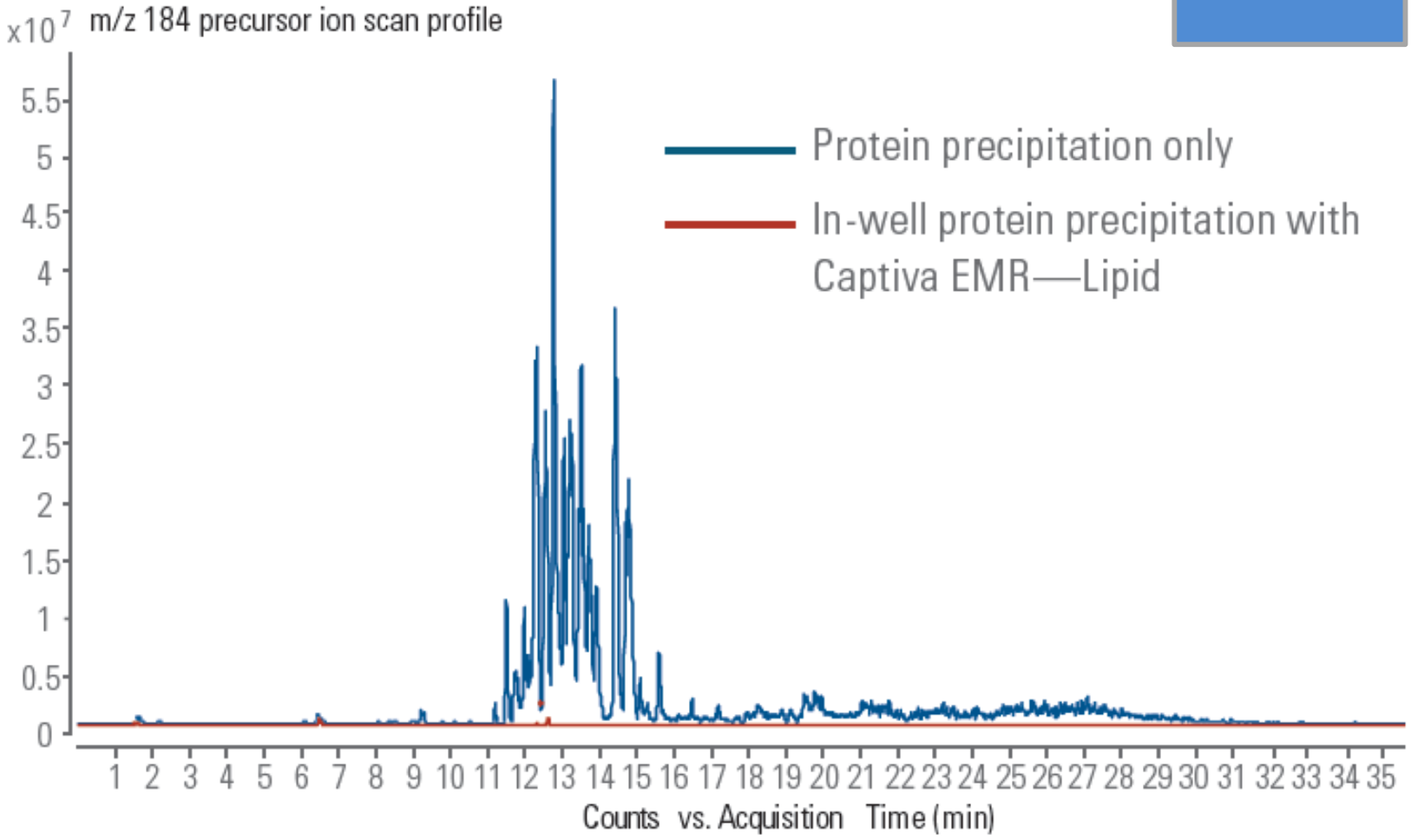
Fluoroquinolones



EMR-Lipid Mechanism –

Combined size exclusion and hydrophobic interaction.

Effective phospholipid removal



QuEChERS

Screening of pesticide residues in fruit and vegetables

- Developed to make sample cleanup of food faster, simpler, less expensive, and greener

Now used with other matrices and compound classes as well

QuEChERS: Quick Easy Cheap Effective Rugged Safe

Commercially available kits allow for ease of use and convenience leading to increased throughput

Consists of two steps, and thus **2 kits**:

Step 1: Liquid Extraction

Step 2: Dispersive SPE / Interference Removal

Agilent Dispersive Kits

Dispersive kit contains:

Centrifuge tubes containing pre-weighed SPE sorbents such as:

C18: removes residual proteins and lipids

PSA: 'primary/secondary amine' for removal of organic acids and sugars

GCB: graphitized carbon black, removes pigments

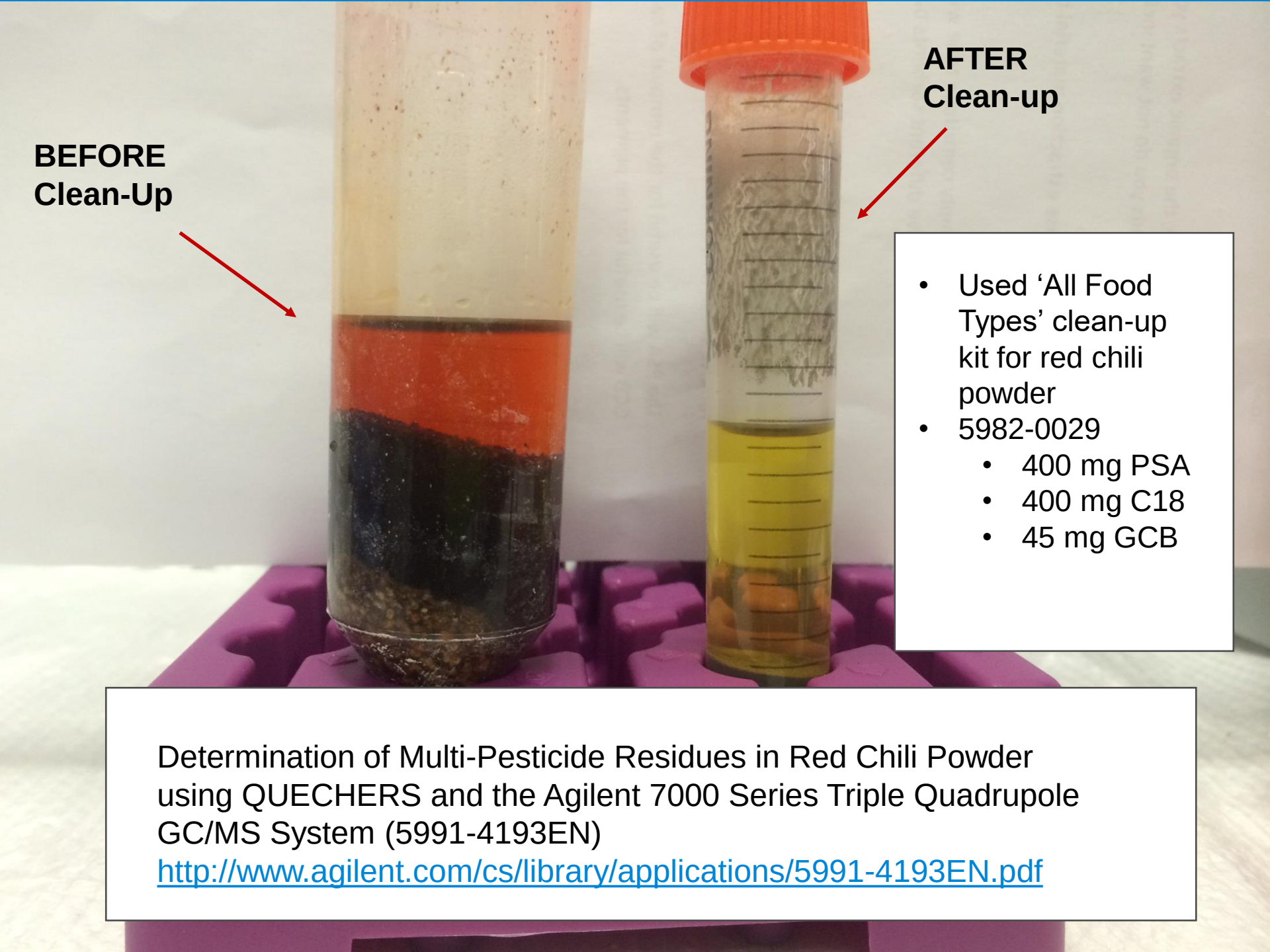
EMR-Lipid: selectively removes fats/lipids

Kits available for different food types

For both AOAC (US) method and EN (Europe)

QUECHERS is a non-selective technique, does not remove ALL the matrix, but just enough

SPE sorbent also available as bulk material



- Used ‘All Food Types’ clean-up kit for red chili powder
- 5982-0029
  - 400 mg PSA
  - 400 mg C18
  - 45 mg GCB

Determination of Multi-Pesticide Residues in Red Chili Powder using QUECHERS and the Agilent 7000 Series Triple Quadrupole GC/MS System (5991-4193EN)
http://www.agilent.com/cs/library/applications/5991-4193EN.pdf

What is Solid Phase Extraction?

- Uses a plastic disposable cartridge packed with varying amounts of sorbent between two frits
- Sorbent can be silica/polymeric based and involve a variety of phases
  - chosen based on the chemistry of the analyte of interest and interferences
- Uses of SPE: removal of interferences, concentration/enrichment, desalting, solvent exchange

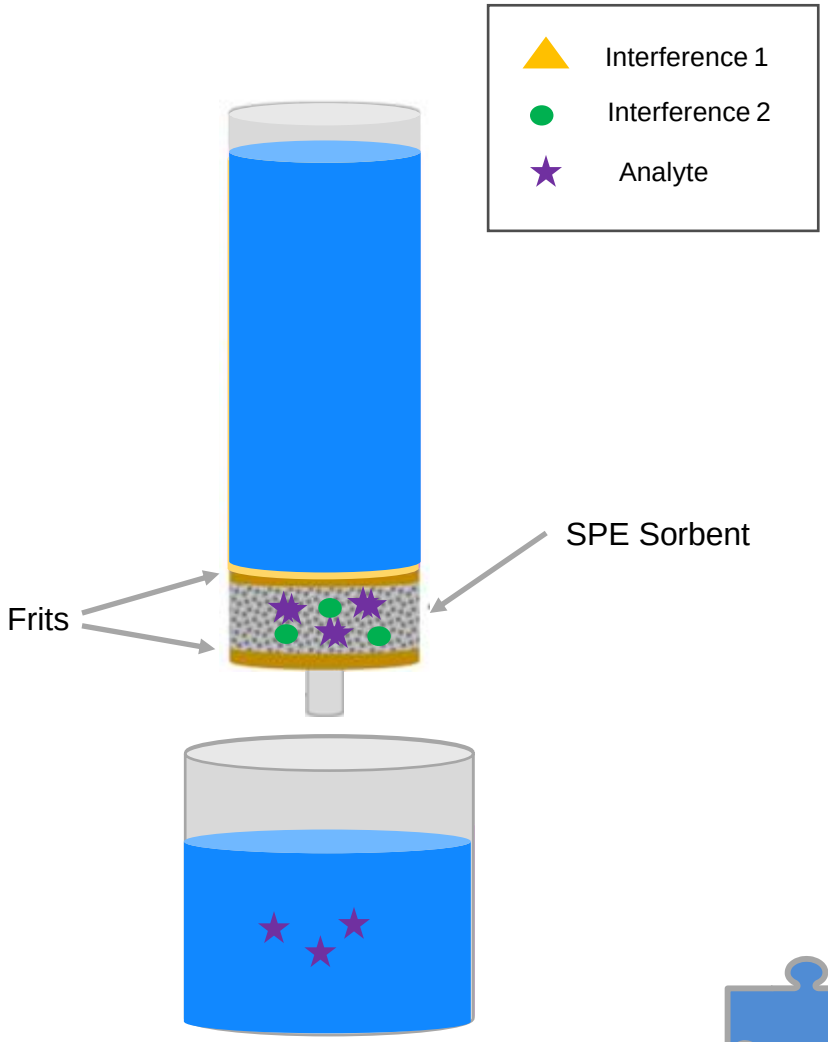
Typical SPE Sequence

Step 1: Condition the cartridge

Step 2: Apply sample

Step 3: First wash of the cartridge (interference removal)

Step 4: Apply solvent to elute



Why Choose SPE?

- Flexible - match a broad spectrum of sample and target compound types to different sorbents and forms
- Wide array of formats and sorbents for lower detection limits and longer instrument uptime from cleaner extracts
- Agilent has over 40 sorbent materials/phases available!
- Increase sample throughput with automation-friendly formats
- Easy adoption of methods due to high number of publications and applications
- Get the right answer the first time with highest accuracy and confidence
- Best balance of sample cleanliness, accuracy of results, and cost-per-sample

6410 QQQ Sensitivity Results

Dilute/Shoot (1/10 dilution) versus SPE Sample Preparation

Opiates/Opioids

Compound	D/Shoot LLOQ (ng/ml)	SPE LLOQ (ng/ml)	ULOQ (ng/ml)
6-monoacetyl morphine	10	<1	1000
buprenorphine	10	1	1000
codeine	25	<1	1000
dihydrododeine	25	<1	1000
EDDP	10	<1	1000
fentanyl	1	<1	1000
heroin	10	<1	1000
hydrocodone	10	<1	1000
hydromorphone	5	<1	1000
meperidine	5	<1	1000
methadone	10	<1	1000
morphine	5	<1	1000
naloxone	5	<1	1000
naltrexone	10	<1	1000
N-desmethyltramadol	10	1	1000
norbuprenorphine	25	3	1000
norfentanyl	1	<1	1000
normeperidine	5	<1	1000
norpropoxyphene	5	<1	1000
o-desmethyltramadol	5	<1	1000
oxycodone	10	<1	1000
oxymorphone	5	<1	1000
propoxyphene	5	<1	1000
tapentadol	5	<1	1000
tramadol	1	<1	1000
trazodone	1	<1	1000

Sedatives/hypnotics

Compound	D/Shoot LLOQ (ng/ml)	SPE LLOQ (ng/ml)	ULOQ (ng/ml)
2-OH-ethylflurazepam	200	5	1000
7-aminoclonazepam	10	<1	1000
7-aminoflunitrazepam	5	<1	1000
alpha-OH-midazolam	10	<1	1000
alprazolam	10	<1	1000
a-OH-alprazolam	20	<1	1000
a-OH-triazolam	50	<1	1000
chlordiazepoxide	10	<1	1000
clonazepam	25 to 50	<1	1000
desalkylflurazepam	20	1	1000
diazepam	10	<1	1000
flunitrazepam	10	1	500
flurazepam	5	1	1000
lorazepam	50	20	1000
midazolam	10	<1	1000
nitrazepam	25	5	1000
nordiazepam	25	<1	1000
oxazepam	50	25	1000
temazepam	25	<1	1000
triazolam	5	<1	1000
zolpidem	5	<1	1000

Processing 96-Well Plates and Cartridges

Captiva Vacuum Collar

Vac-20 vacuum manifolds

96 well plate vacuum manifold

Positive Pressure Manifolds

Productivity Benefits with Sample Preparation

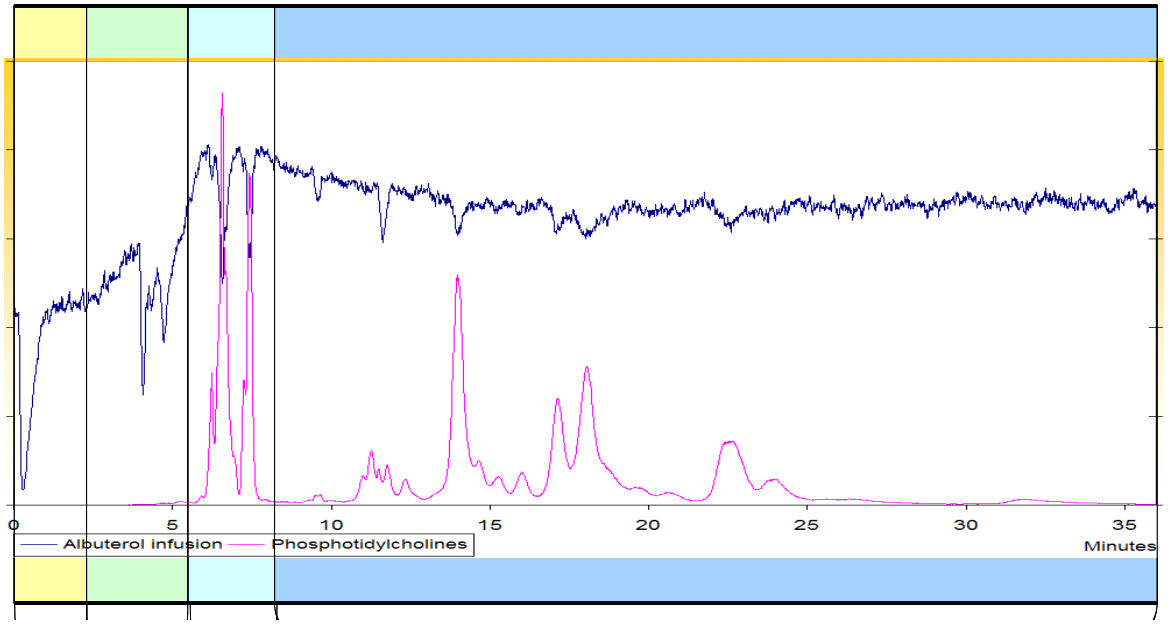
More Matrix Removal = Less Matrix Entering System = Time and Cost Savings!

- ✓ Less matrix build-up
  - Less interferences
  - Improved S/N
  - Better reproducibility
- ✓ Better chromatography
  - Less time spent on data analysis/manual integration
  - Less time spent on re-runs/recalibrations
- ✓ Less maintenance
  - Less instrument down-time
  - Saves $$ on consumables/services
- ✓ Less troubleshooting
  - “Is it my column or my MS”?
  - Less instrument down-time

Optimizing and Maintaining Your Mass Spec

Matrix, Modifiers and Suppression

- Matrix, Modifiers and Suppression
- Instrument settings and data collection



Optimizing and Maintaining Your Mass Spec

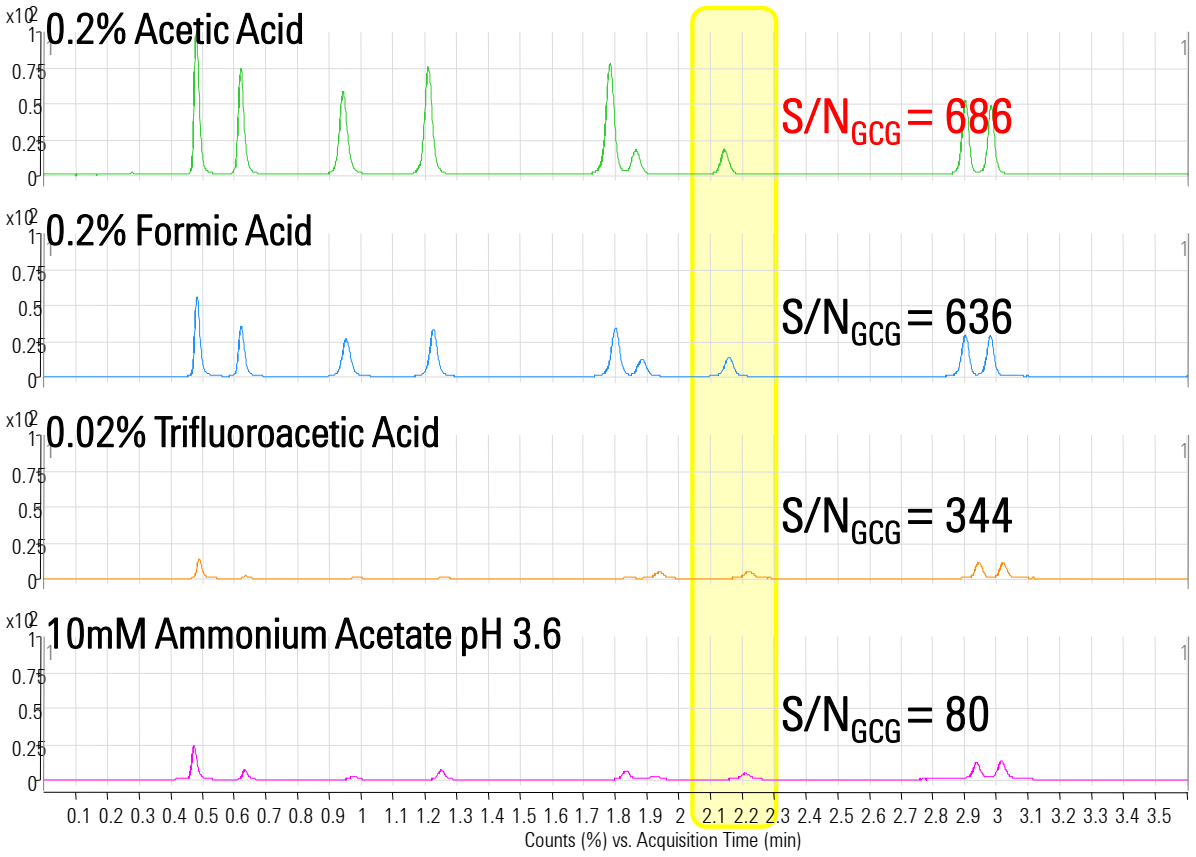
Matrix, Modifiers and Suppression

- Matrix, Modifiers and Suppression
- Instrument settings and data collection

A: Acid in H_2O
B: CH_3CN
0.729 mL/min

Time	0.00	1.43
	2.86	
%B	10	15
	27	

40 °C
Agilent Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm
Source: 350 °C, 10 L/min, 50 psi, -3500 V
Acquisition: SIM Neg (169, 305, 193**, 289, 457, 441), **caffeine is not detected in Neg mode



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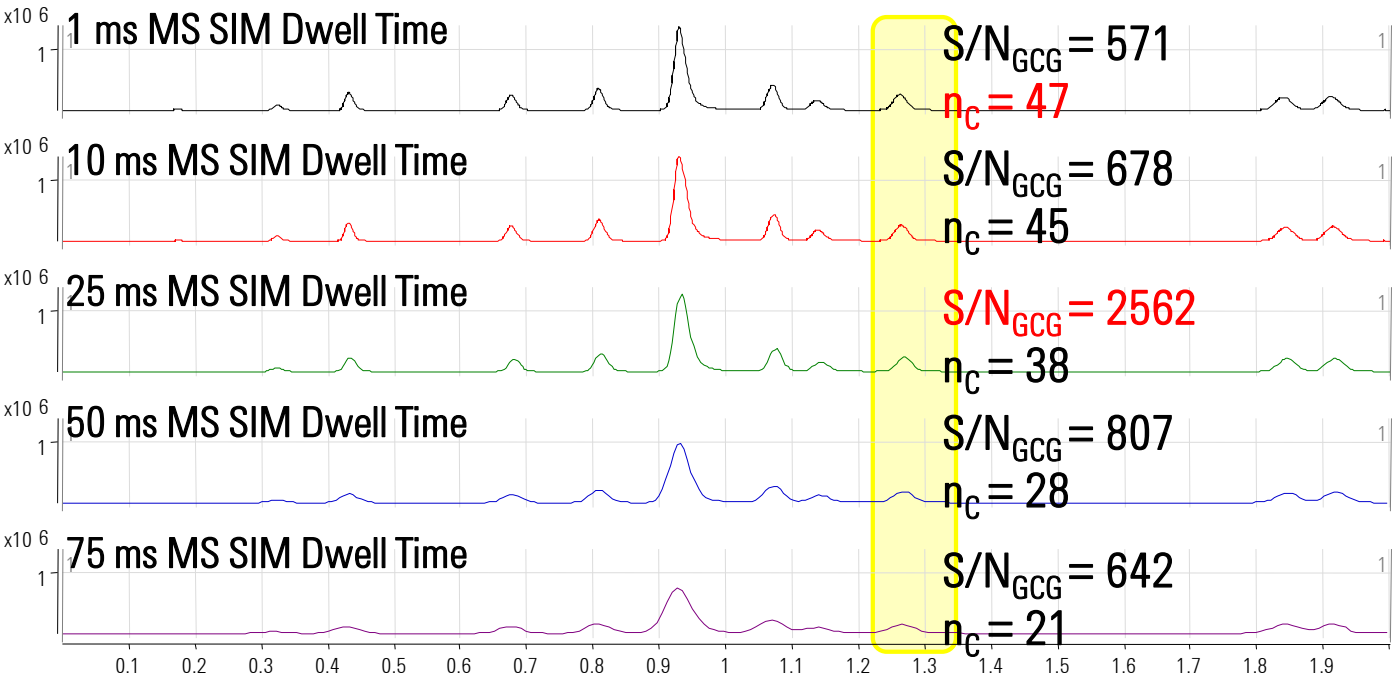
Optimizing and Maintaining Your Mass Spec

Data Collection

- Matrix, Modifiers and Suppression
- Instrument settings and data collection



Optimizing and Maintaining Your LCMS

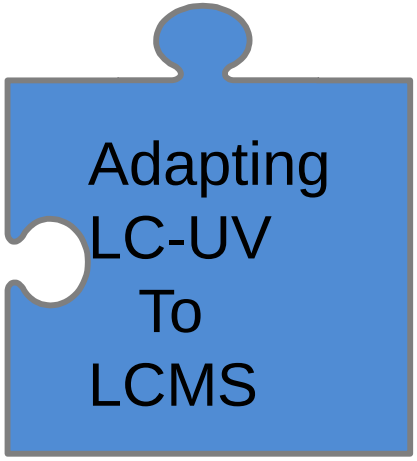
Checking instrument settings and performance

- Matrix, Modifiers and Suppression
- Instrument settings and data collection

Inject a known standard and check response – ideally v. SQC chart

Run a blank – zero volume injection

Run check tune and compare to previous tunes



Changing Column Size

Smaller columns reduce solvent

Require smaller injection volumes

Keep linear velocity constant by reducing flowrate

- Changing column size
  - Injection Volumes
  - Flowrates
- Trading chromatographic resolution for speed

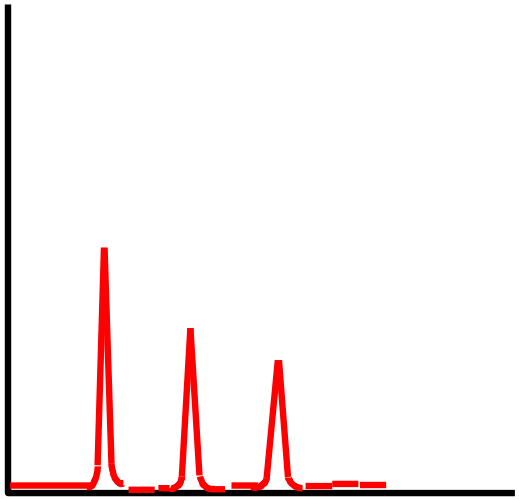
Column ID	Column Volume	Typical Injection	Typical Inj Range	Typical Flowrate
4.6 mm	1500µl	15µl	5 - 50µl	1 ml/min
3.0 mm	600µl	5µl	3 - 30µl	400 µl/min
2.1 mm	300µl	2µl	0.5 - 10µl	200 µl/min
1.0 mm	70µl	0.5µl	0.1 - 2.5µl	50 µl/min

Adapting LC-UV To LCMS

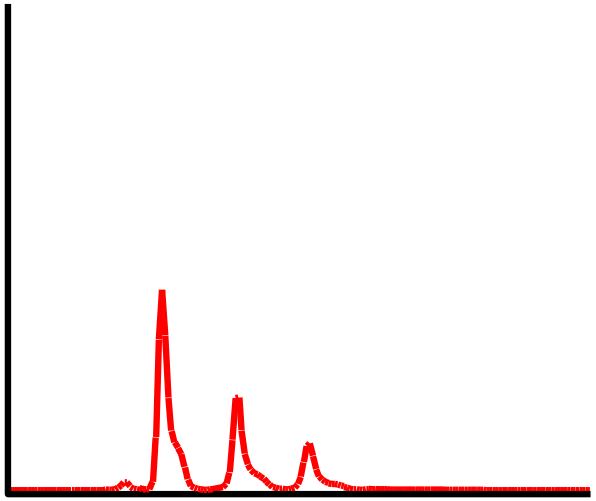
Changing Column Size

Keep injection volumes small and organic composition of sample at or below starting gradient conditions

- Changing column size
  - Injection Volumes
  - Flowrates
- Trading chromatographic resolution for speed



Sample in Mobile Phase

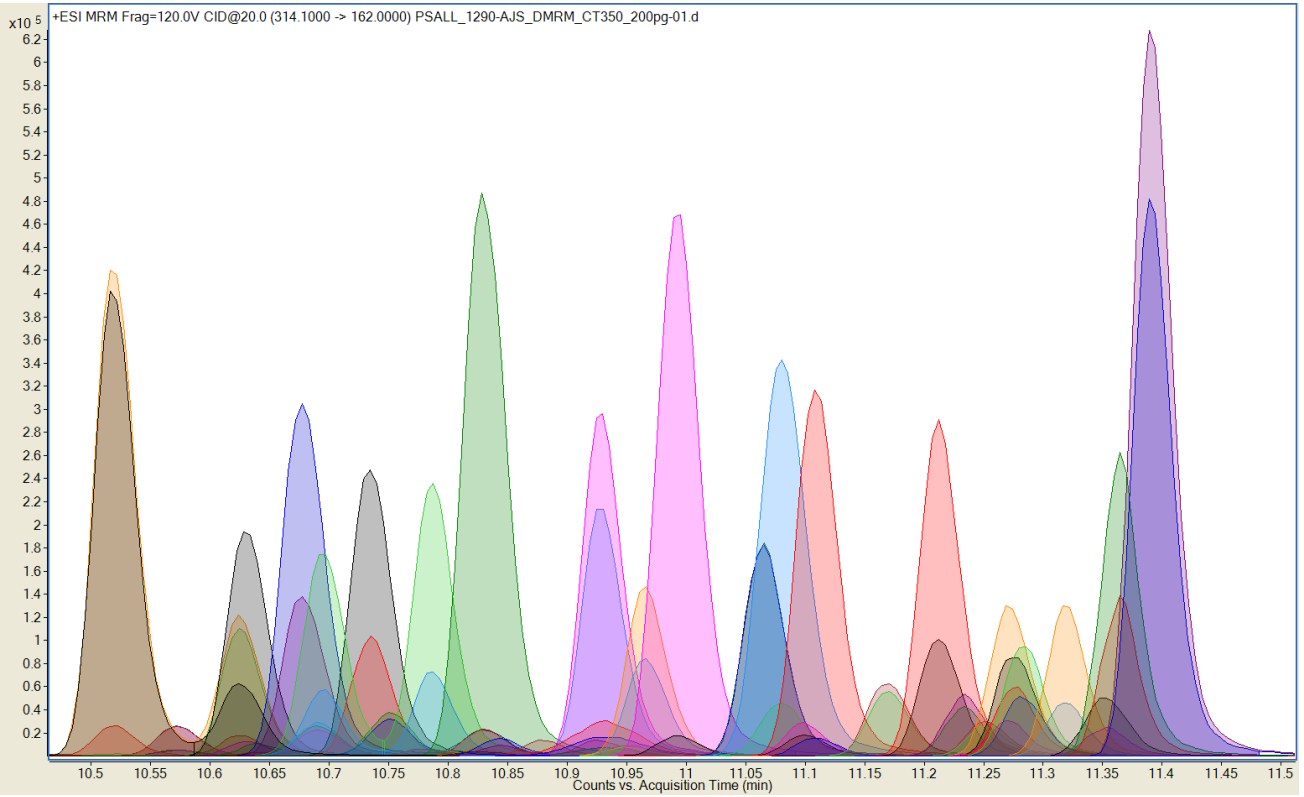


Sample in Stronger Solvent

Adapting LC-UV To LCMS

Trading Chromatographic Resolution for Speed

- Changing column size
  - Injection Volumes
  - Flowrates
- Trading chromatographic resolution for speed



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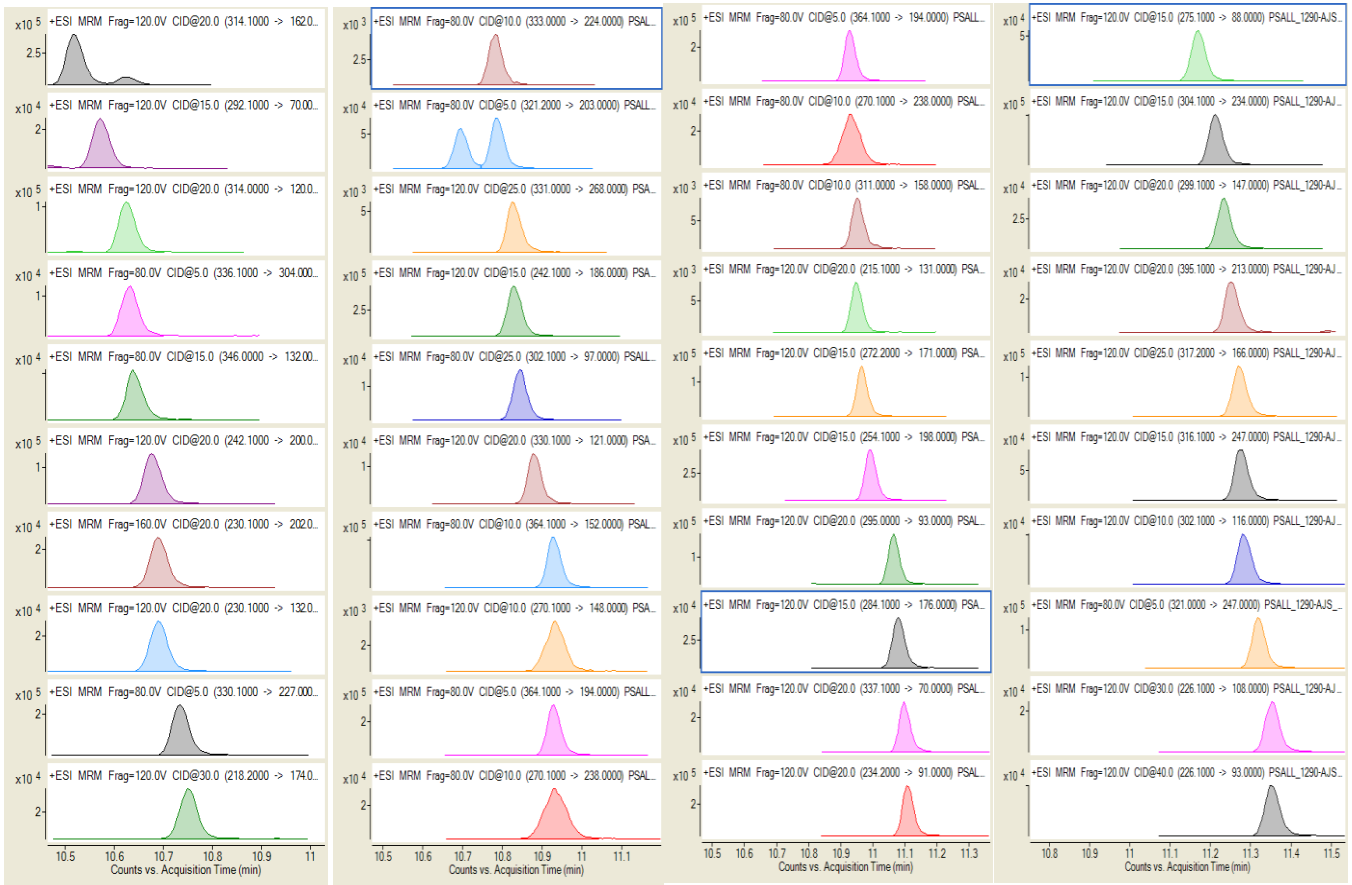
Adapting LC-UV To LCMS

Trading Chromatographic Resolution for Speed

- Changing column size
  - Injection Volumes
  - Flowrates
- Trading chromatographic resolution for speed



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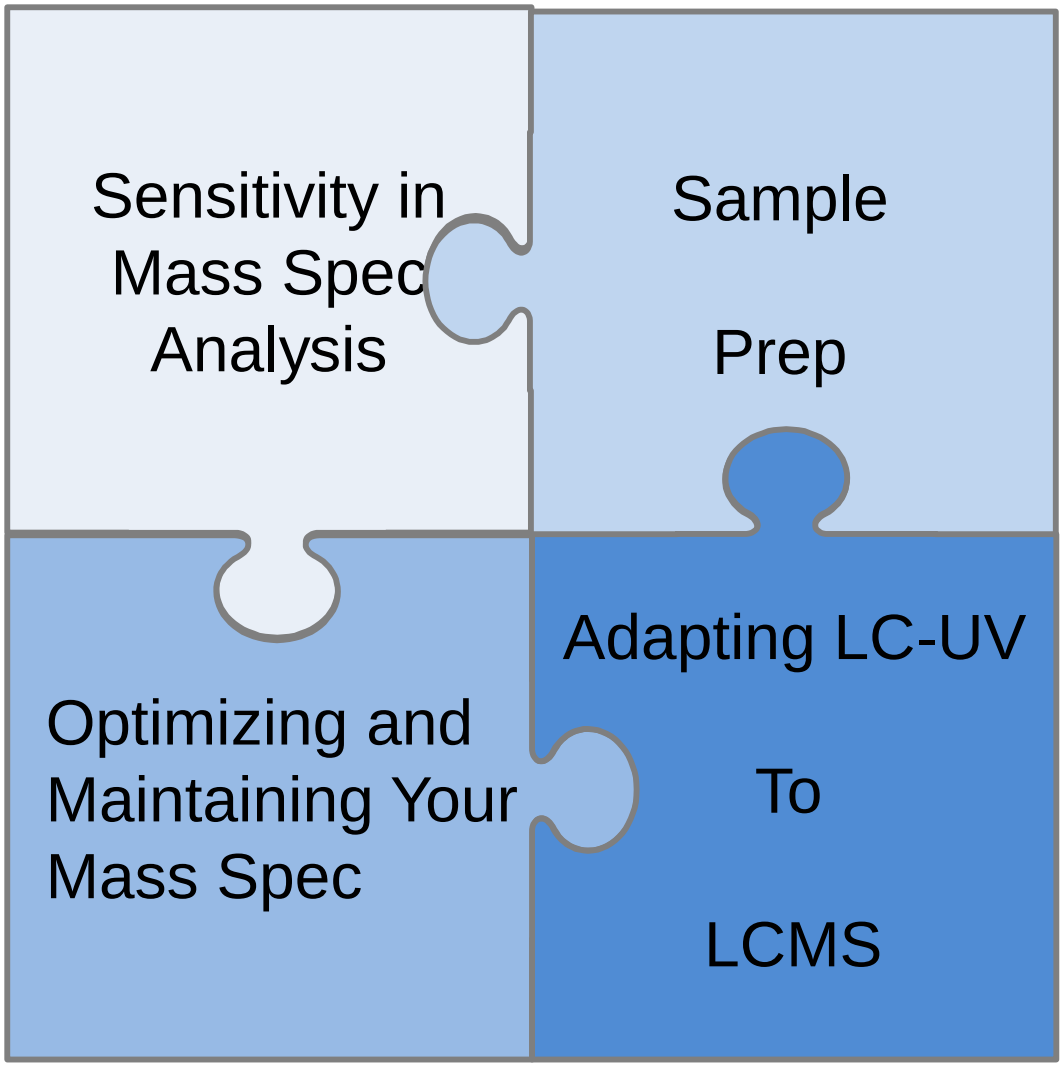
Piecing it all together



Contact Agilent Chemistries and Supplies Technical Support

1-800-227-9770 Option 3, Option 3:

Option 1 for GC/GCMS Columns and Supplies

Option 2 for LC/LCMS Columns and Supplies

Option 3 for Sample Preparation, Filtration and QuEChERS

Option 4 for Spectroscopy Supplies

Available in the USA & Canada 8-5 all time zones

gc-column-support@Agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

Questions?