New Perspectives in Pesticide Analysis

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Overview

Background & Objectives

Challenges We've Heard

Sample Preparation Techniques

Developments in GC/LC Column Technology

Background

Pesticide analysis has historically driven by regulatory requirements

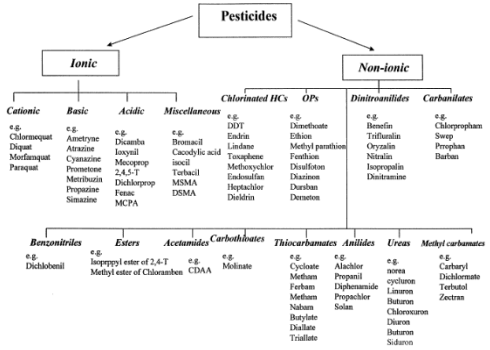
Traditional approaches have been sufficient, but technological and intellectual advances offer the opportunity to improve upon older methods

Today's Goal

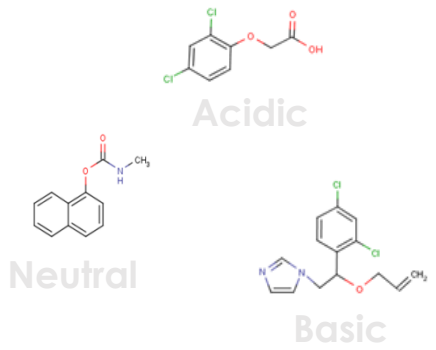
- Present an overview of options in sample prep, GC, and LC analysis that may provide significant increases in productivity and/or improvements in data quality

Common Challenges

Variety of Compounds

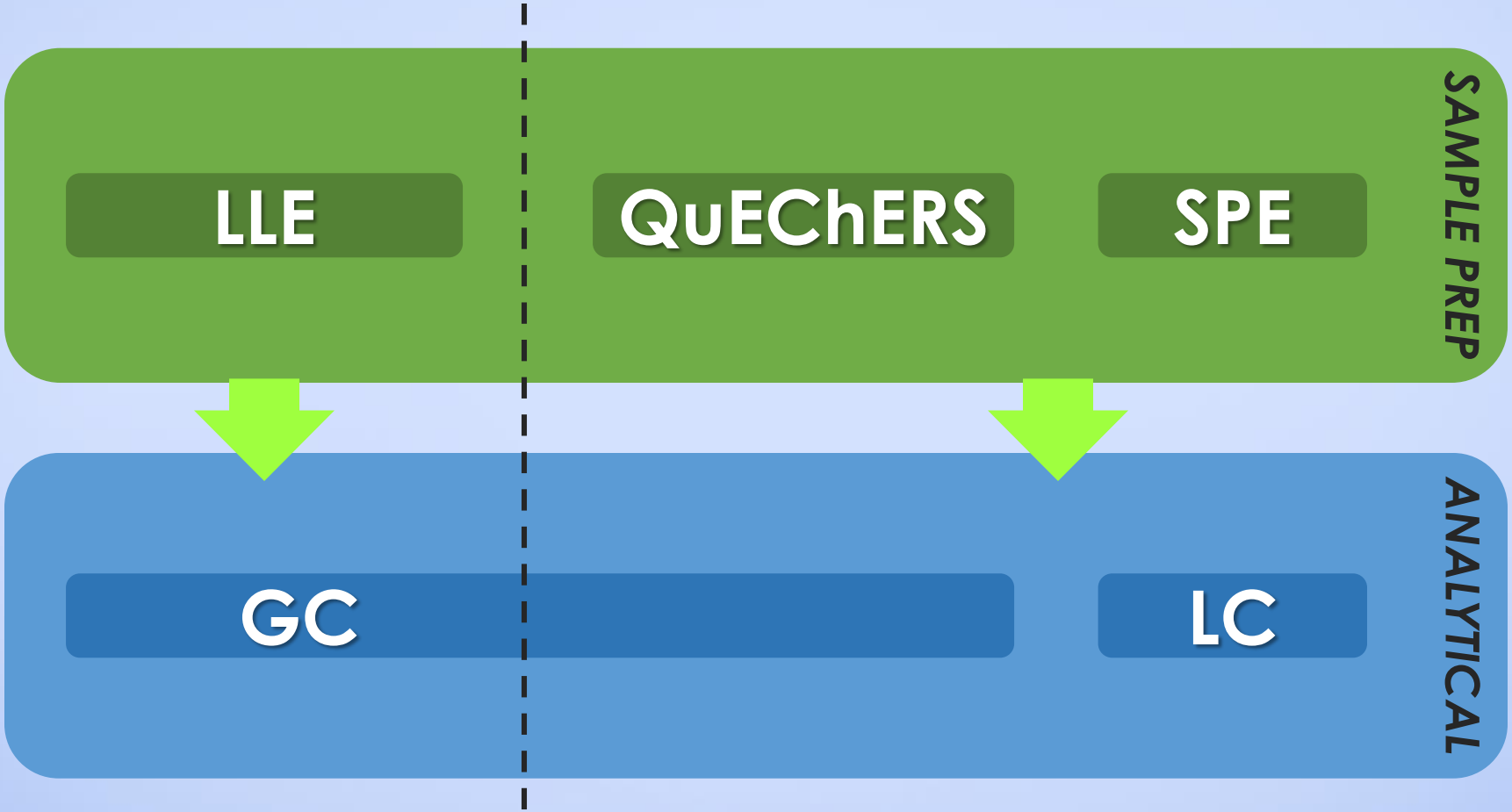


Wide Range of Chemical Properties



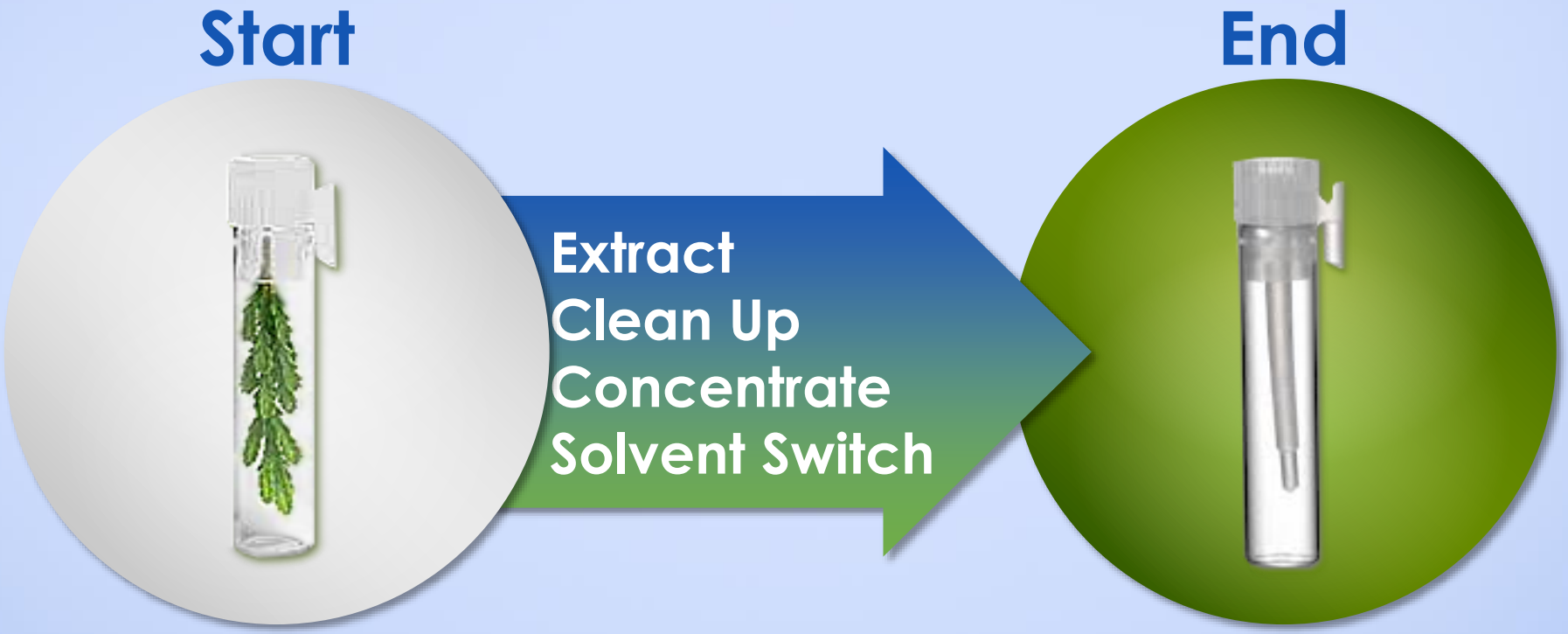
Wide Range of Sample Matrices

Analytical Options



Sample Preparation Techniques

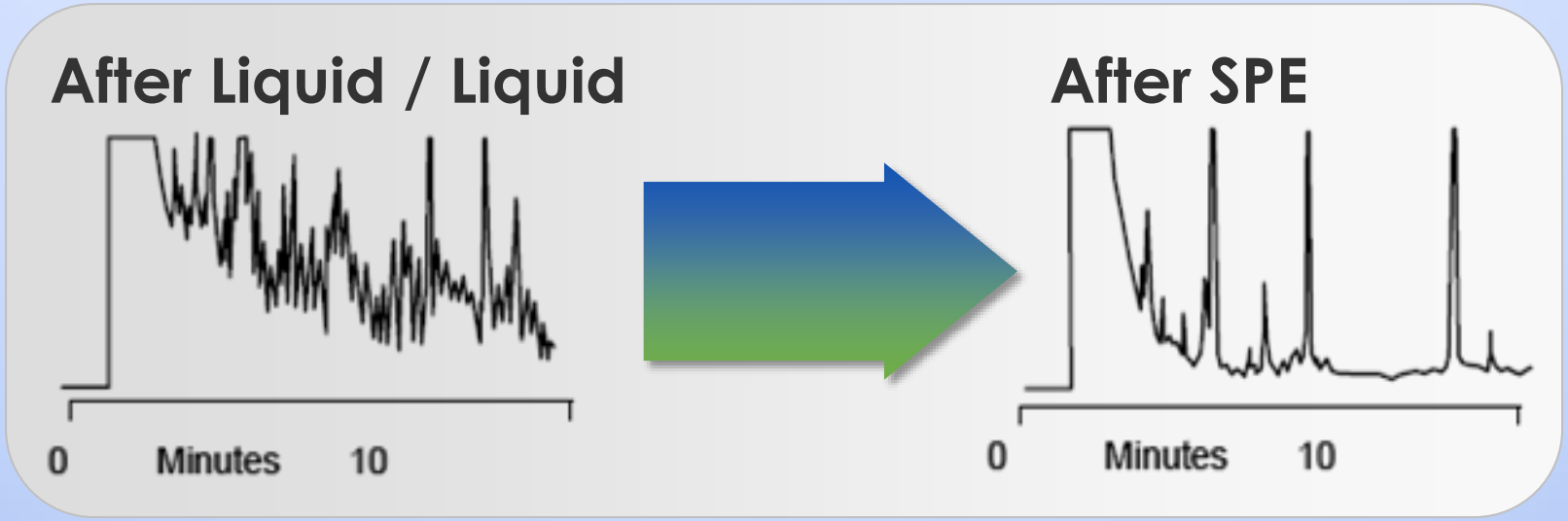
Sample Preparation Goals



The Importance of Selectivity

The ability to target specific analytes from any given sample matrix for extraction, concentration and clean up

More selective sample preparation can lead to better analytical methods



Selectivity In Sample Preparation

Higher analytical performance can be attained with more selective, intricate sample prep techniques

Solid Phase Extraction (SPE)
QuEChERS
Liquid / Liquid Extraction (LLE)
Filtration
Centrifugation
Settling and Decanting
Homogenization
Dilution

Liquid-Liquid Extraction (LLE)

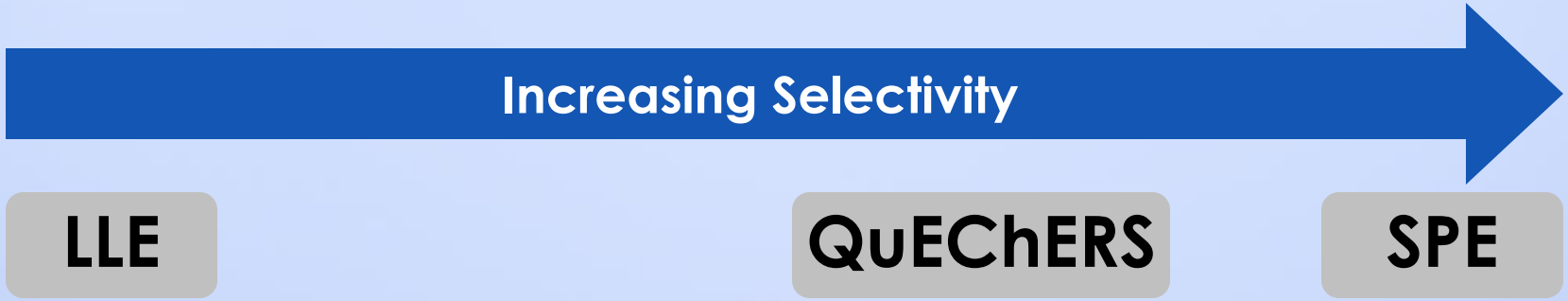
Advantages
- Cheap
- Relatively quick (may still require blow-down)
- Simple
- No special equipment needed

Disadvantages
- Non-specific and non-selective
  - Co-extraction of interferences of similar chemistry (log P)
- Hazardous solvents
- Large volumes of waste

Improvements Over LLE

There has been an increase adoption of alternative sample prep techniques that addresses some of the drawbacks or deficiencies of LLE

This includes QuEChERS and SPE



We All Know QuEChERS!

The primary goal is to reduce matrix interferences in a sample

- Typically is **qualitative** screening rather than quantitation

Most applicable to multi-class screening

- Analytes with widely varying chemistry; thus more selective choices like SPE are not as appropriate
- Goal is to remove as much **matrix interference** as possible

QuEChERS Technique

Quick Easy Cheap Effective Rugged Safe

For matrix removal and extraction of pesticides

Pros

- Screening for wide range of pesticides
- Applicable to many sample matrices
- Reduce loss of key analytes
- Quick and simple procedure

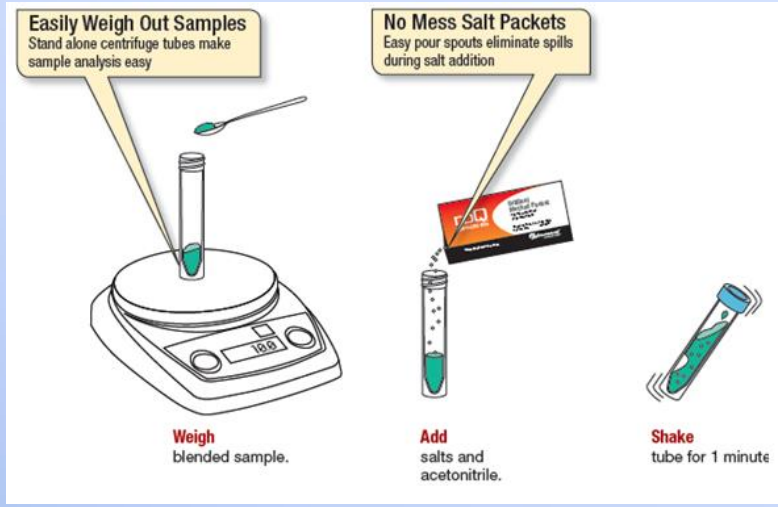
Cons

- Not the most selective sample prep technique
- Kits needed (logistical headache if not available)
- Method development is still required
- Qualitative vs. quantitative

The QuEChERS Process

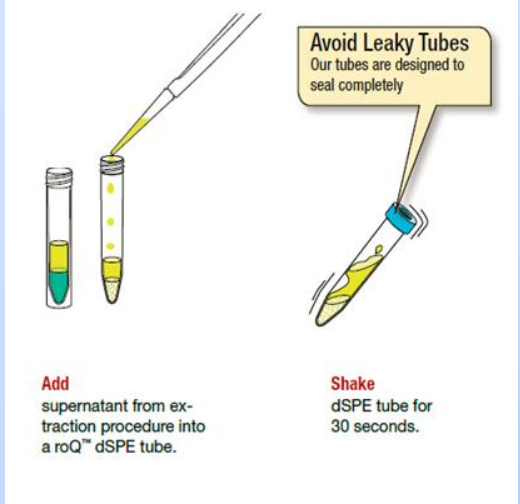
Step 1: Liquid Extraction

- Sample is homogenized
- Add organic solvent + salts to extract target analytes
- Centrifuge to pellet the homogenate



Step 2: Dispersive SPE

- Supernatant is combined with loose sorbent
- Interferences adhere to the sorbent
- Spin to pellet sorbent, decant and analyze supernatant



Pesticides in Spinach by LC/MS/MS

Liquid nitrogen / dry ice homogenization

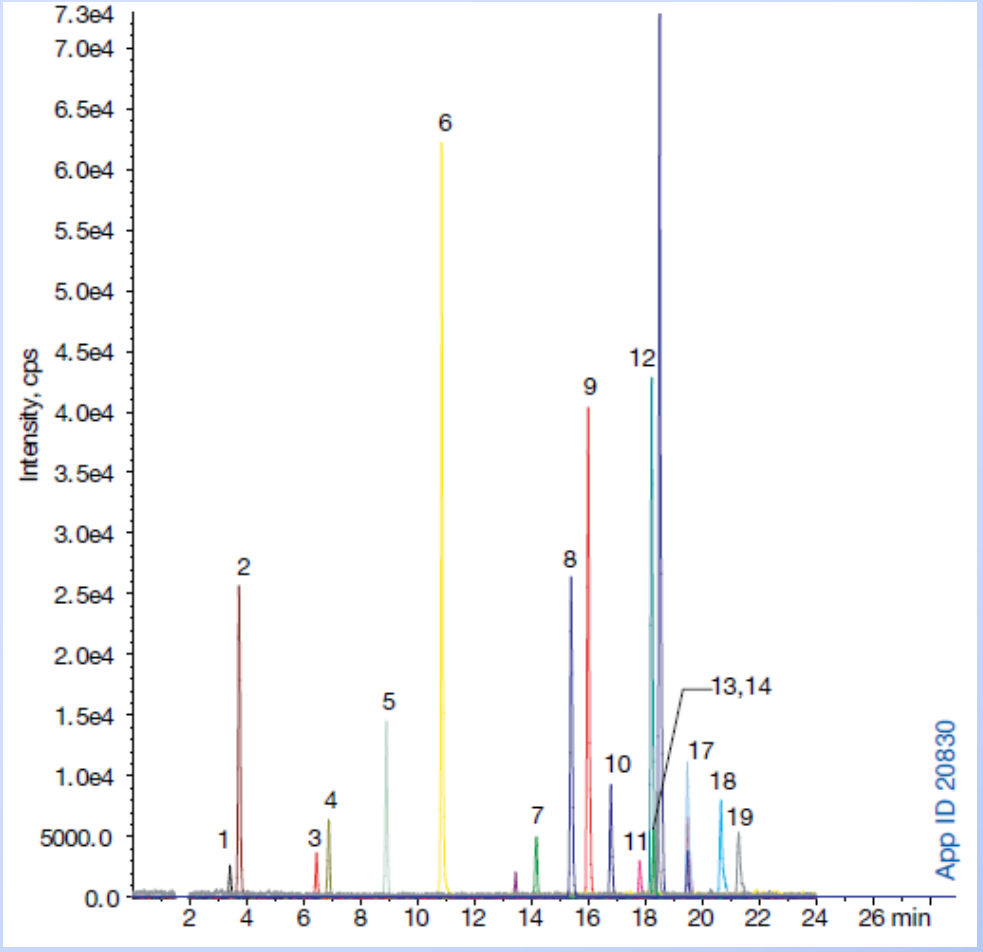
Liquid Extraction / dSPE Steps

Pesticides in Spinach by LC/MS/MS

After Liquid Extraction

After dSPE

MRM chromatogram of spinach extract spiked at 200 ng/g



QuEChERS vs. Solid Phase Extraction

QuEChERS Technique

- Target analytes in solution are extracted with ACN and salts
- Organic layer is transferred to a separate tube containing loose SPE sorbent
- **Matrix interferences bind to the sorbent**, rather than the analytes
- Supernatant containing the analytes is removed and analyzed

Solid Phase Extraction (SPE)

- Liquid sample is applied to cartridge containing chemically-modified sorbent
- Target analytes bind to sorbent through chemical interaction
- **Matrix interferences are washed away** using organic or salts
- Clean analytes are eluted and analyzed

Quick Review: Solid Phase Extraction

In SPE, a support particle is modified with different functional groups

- Silica or polymeric particles
- Wide range of functional groups (RP, IEX, NP)

Target analytes bind to the media

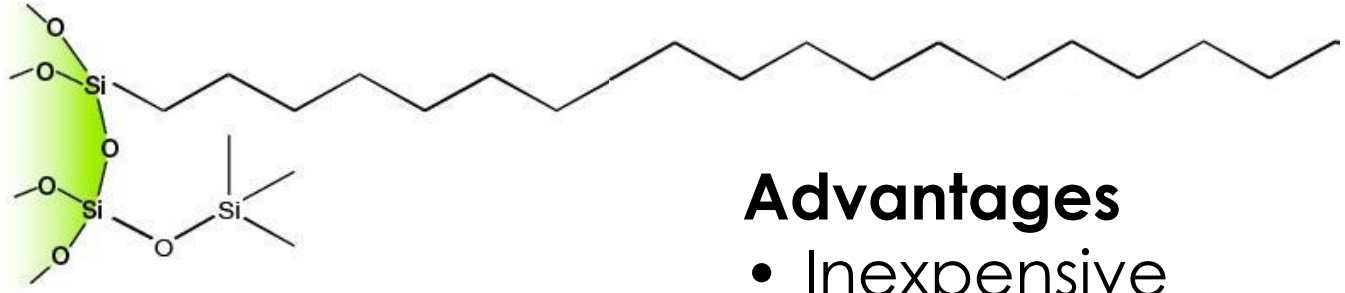
- Matrix interferences are washed away using different washing protocols

The key distinction is that you optimize your method to target & recover your analytes

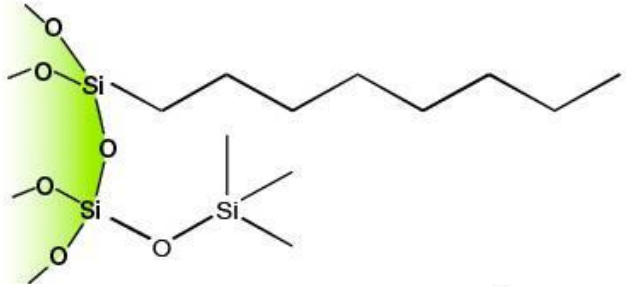
- More selective than QuEChERS

Traditional Silica-Based SPE Media

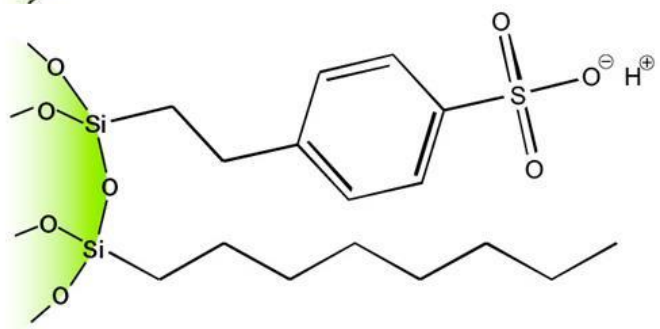
Strata
- C18



Strata
- C8



Strata
- Screen-C



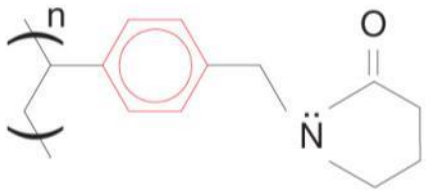
Advantages
- Inexpensive
- Available for normal phase

Disadvantages
- Limited pH stability (2-7)
- **Deconditioning!**

Polymeric SPE Media

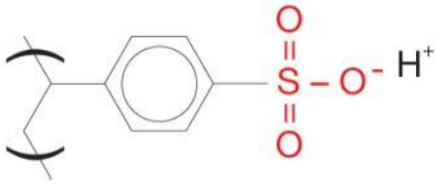
Strata-X

- Reversed-phase



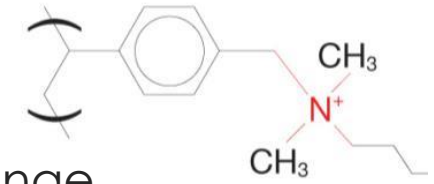
Strata-X-C

- Strong Cation Exchange



Strata-X-A

- Strong Anion Exchange



Advantages

- Resists deconditioning!
- Larger capacity
- pH range 1-14

Disadvantages

- **More costly** than Si-based

Why SPE?

When selectivity for target analytes is preferred

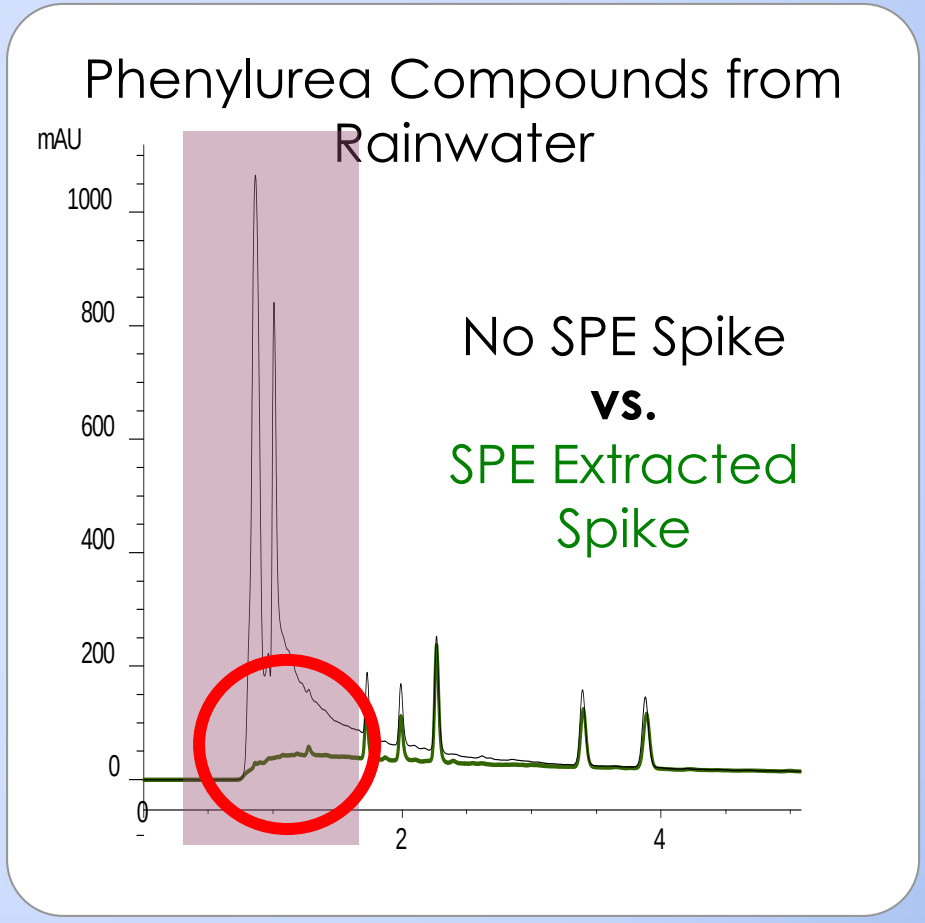
- Most selective sample prep technique

Higher recoveries of target analytes

Concentration of key analytes

Can be automated

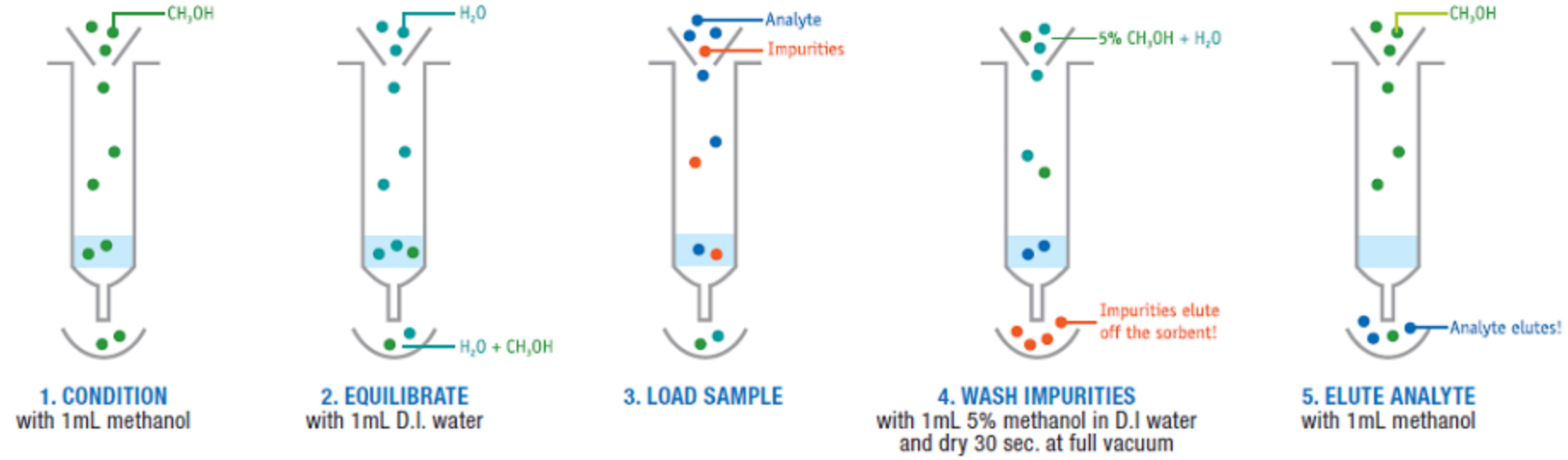
Elimination of emulsions



General SPE Screening Method

Example for reversed phase procedure

(Volumes shown are for 30 mg sorbent mass)



Paraquat/Diquat Extraction From Water

- Condition with Methanol
- Equilibrate with Water

→

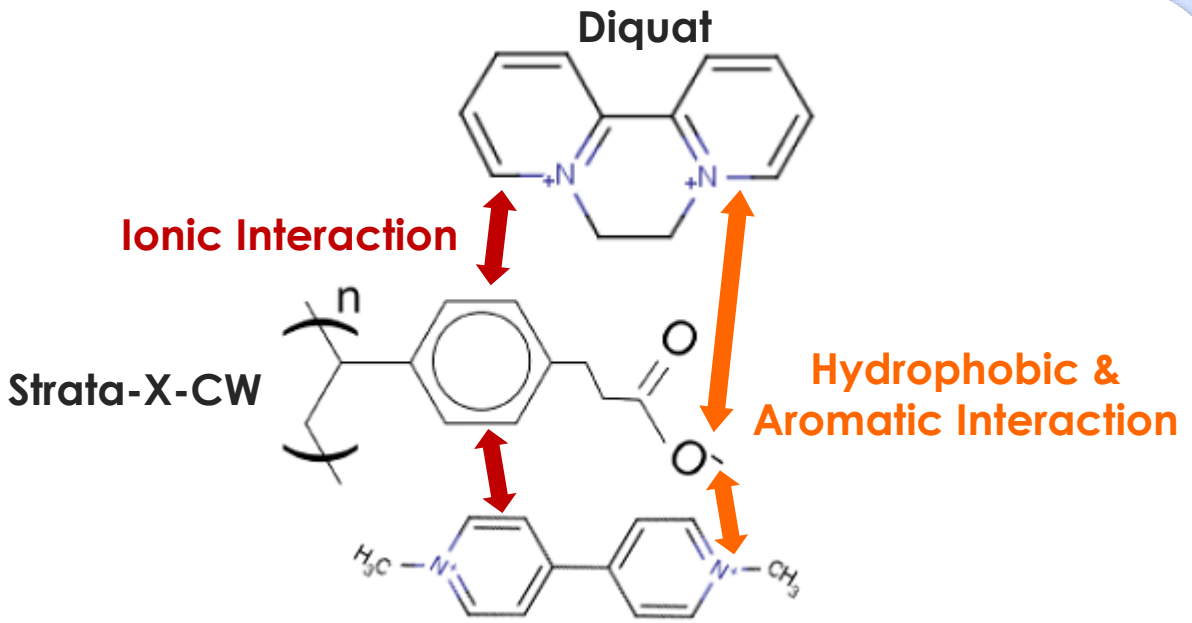
- Load Water Sample Slowly

→

- 1st Wash: Water
- 2nd Wash: Methanol
- Dry Cartridge On Vacuum

→

- Elute with 5% Formic Acid in Methanol



Sample Prep Summary

Effective sample preparation is an essential component of most analytical methods

Choice of sample prep technique is dependent on your analytical goals – what is most important?

Advantages of QuEChERS and SPE include

- Decreased down time
- Better selectivity for specific pesticides
- Cleaner samples / better analytical starting point

GC Columns: Beyond the 5% Phenyl

Traditionally Used GC Columns

Choice commonly guided by government regulation (EPA, USDA, EN, etc.)

Manufactured by every GC column producer

ZB-1	ZB-5	ZB-5ms	ZB-35	ZB-50	ZB-1701	ZB-XLB
DB®-1	DB-5	DB-5ms	DB-35	DB-17	DB-1701	DB-XLB
Rtx®-1	Rtx-5	Rtx-5ms	Rtx-35	Rtx-17	Rtx-1701	Rtx-XLB

Overcoming Common GC Productivity Thieves

Instrument downtime related to column lifetime

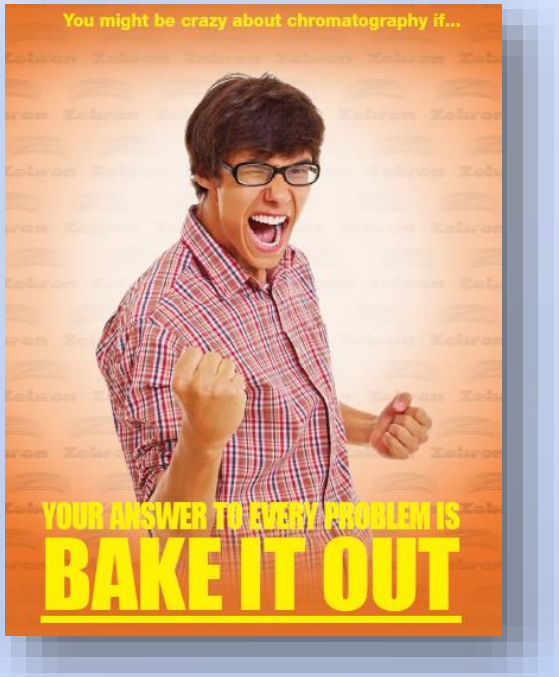
Potential remedy: contaminant removal

High temperature stable column

Coelutions of isomers or structurally similar compounds

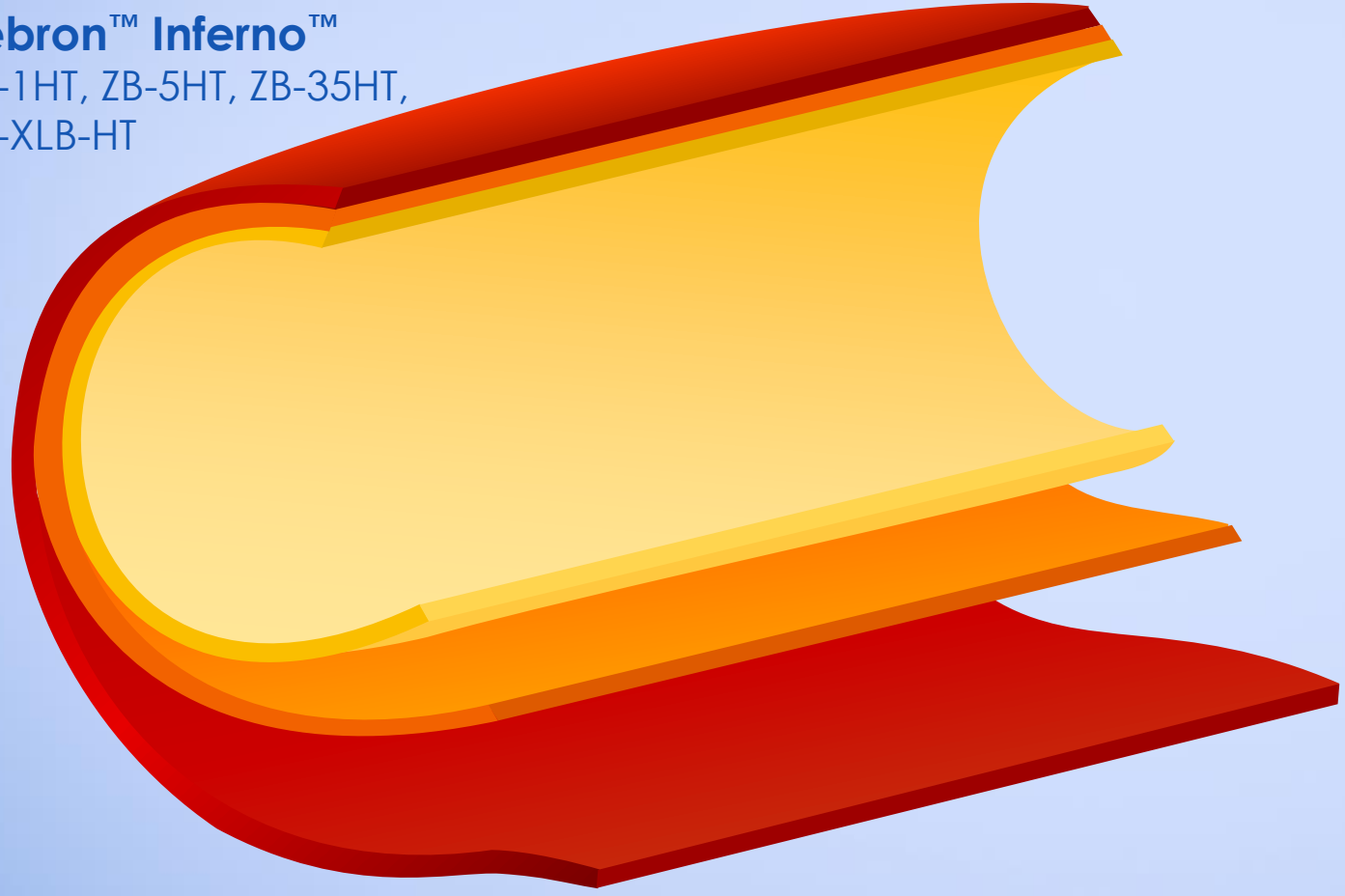
Active compound breakdown (Endrin, DDT, etc.)

Poor peak shapes



High Temperature Science

Zebron™ Inferno™
ZB-1HT, ZB-5HT, ZB-35HT,
ZB-XLB-HT

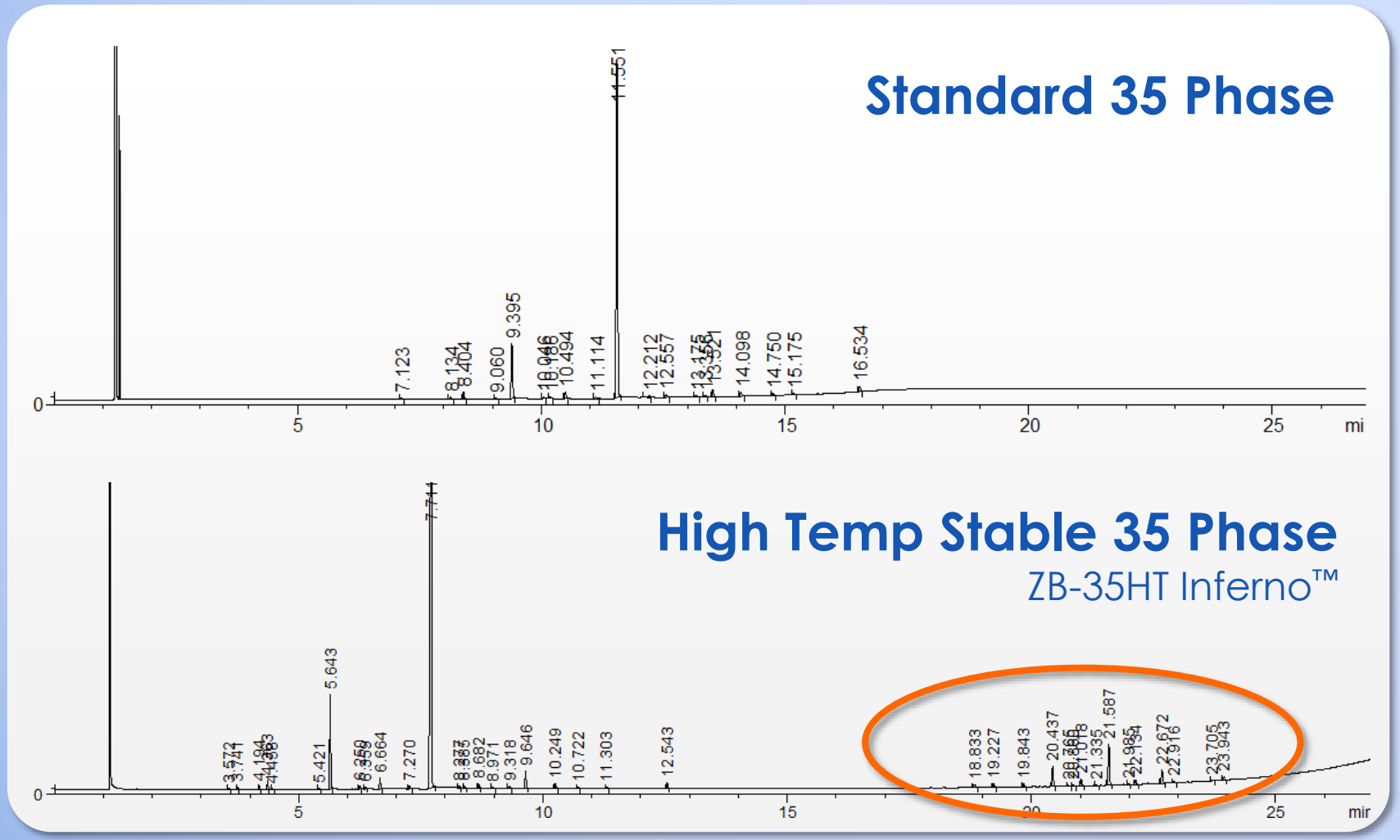


Low Bleed Stationary Phase

Deactivated Fused Silica

High Temperature Polyimide
Resists pitting, brittleness, and breakage up to 430 °C

High Temperature Benefits



Remove contaminants and see high boilers you may be missing

Overcoming Common GC Productivity Thieves

Instrument downtime related to column lifetime

Coelutions of isomers or structurally similar compounds

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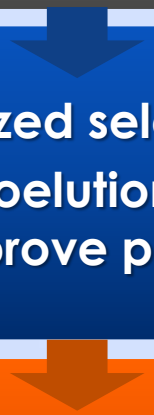
Poor peak shapes

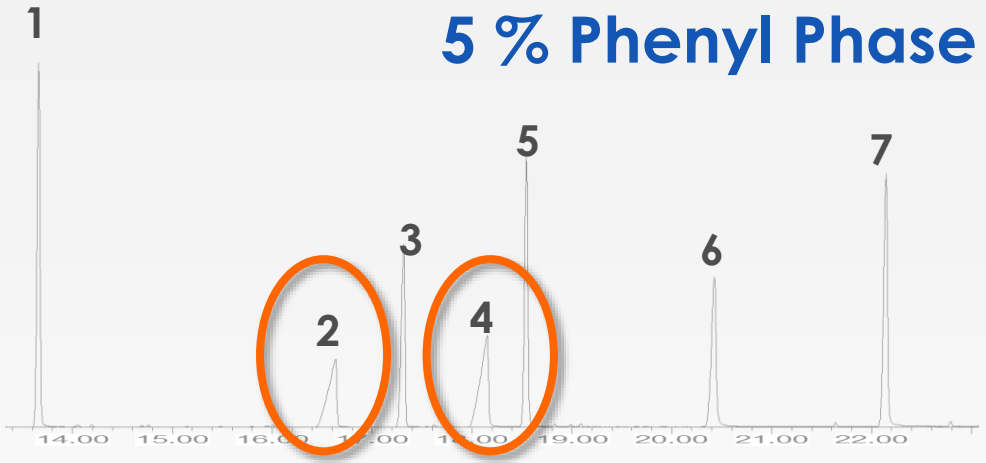


Optimized selectivity (resolve coelutions; resolve isomers; improve peak shapes)

Application-Specific Column

Application-Specific GC Phases

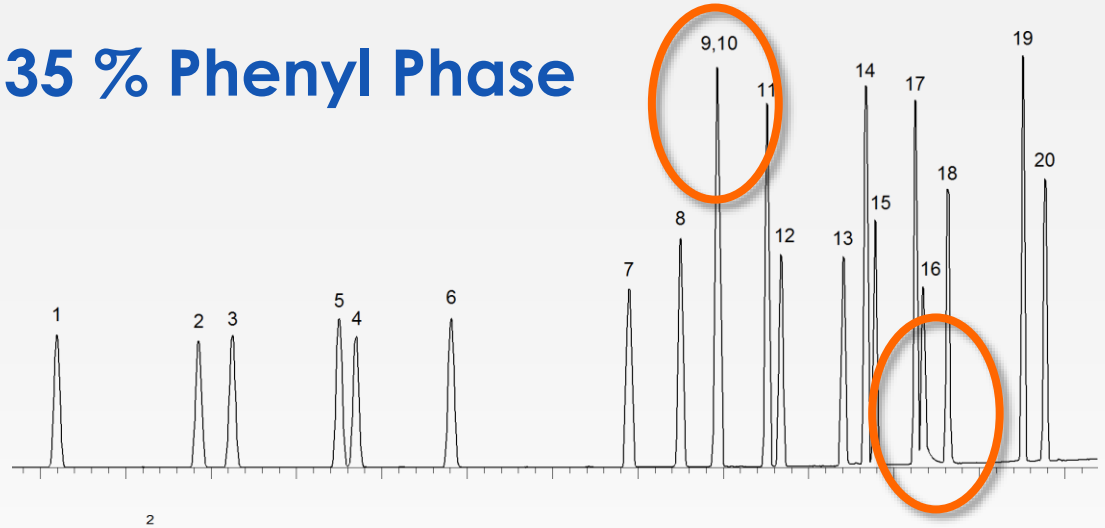
5 % Phenyl Phase



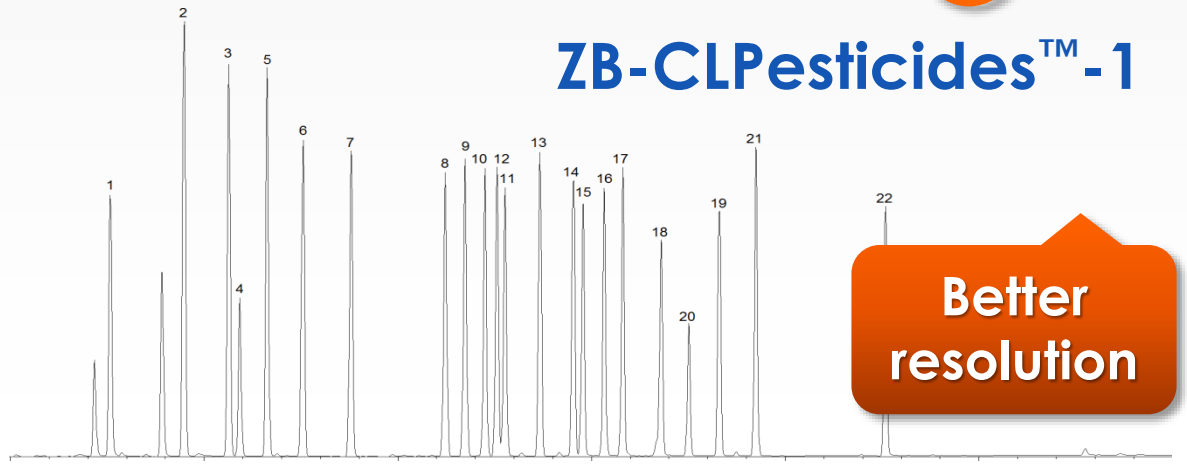
ZB-MultiResidue™-1

Better peak shapes

35 % Phenyl Phase



ZB-CLPesticides™-1



Better resolution

Comparative separations may not be representative of all applications.

Overcoming Common GC Productivity Thieves

Instrument downtime related to column lifetime

Coelutions of isomers or structurally similar compounds

Active compound breakdown (Endrin, DDT, etc.)

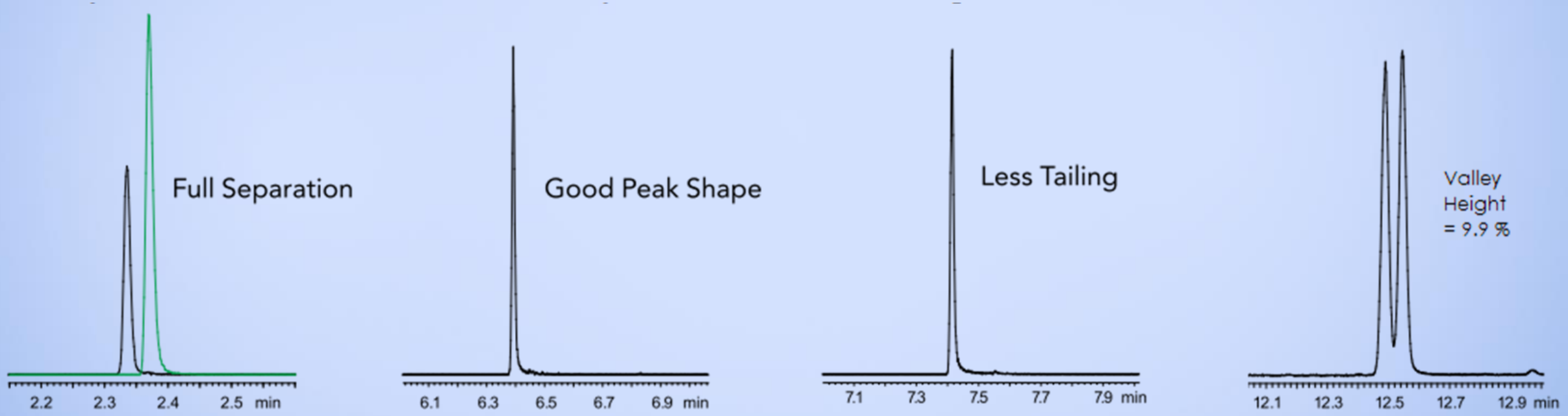
Poor peak shapes

Reduce activity contributed by the column

Highly Inert, Well-Deactivated Column

What You Want

Reduced tailing, resolution, good detection limits



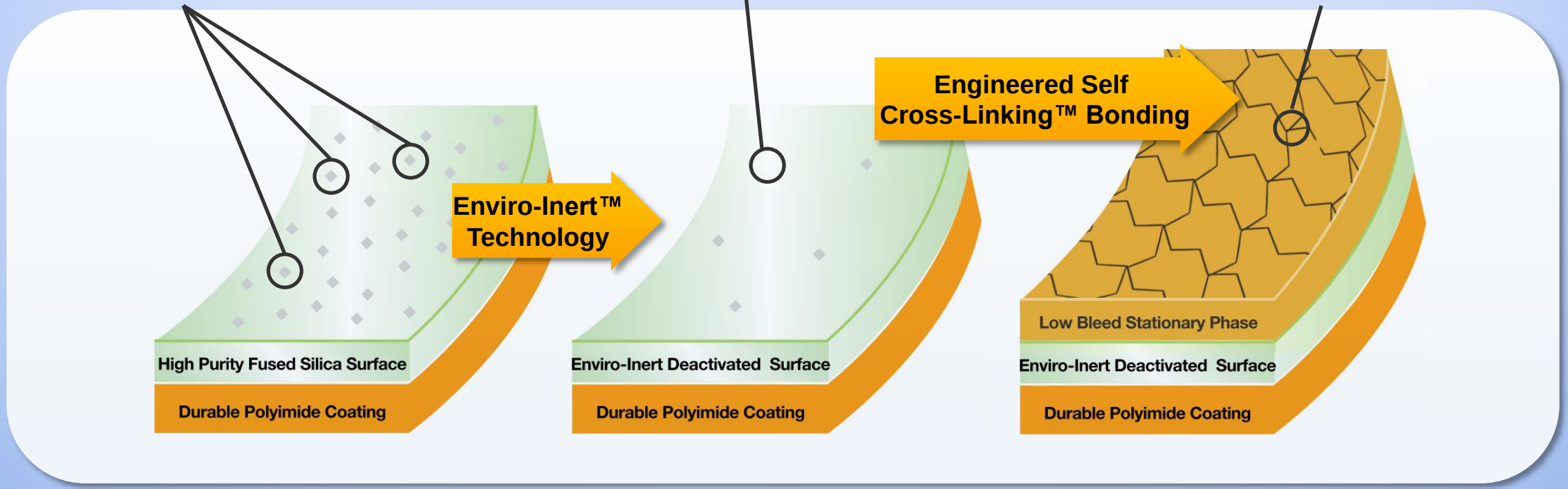
How You Get It – Manufacturing

High purity fused silica glass is used – but even the purest has inherent silanol activity

Every column is specially deactivated to reduce activity

Low bleed 5% phenyl-arylene phase is applied

No retention time shifts from other 5 % phenyl-arylenes



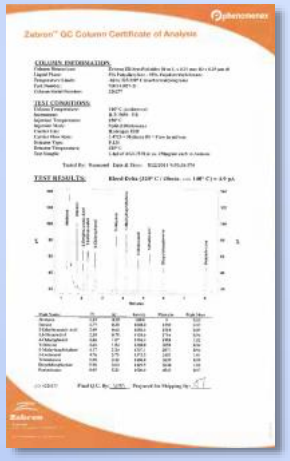
How You Get It – Quality Control

2 tests to ensure SVOC success

1

Traditional Test Mix

Efficiency, Polarity, Bleed, Activity



2

EPA 8270D Test Mix

- Is the DFTPP Tune Mix with pyridine addition
- Better measure of activity

Test Probe	Measure	EPA Requirement	ZB-SemiVolatiles Requirement
Pyridine	Peak Response	Not Specified	≥ 0.6
Pentachlorophenol	Peak Skew	≤ 2.0	≤ 2.0
	Peak Response	Not Specified	≥ 0.3
Benzidine	Peak Skew	≤ 2.0	≤ 2.0
DDT	Breakdown	< 20 %	< 20 %

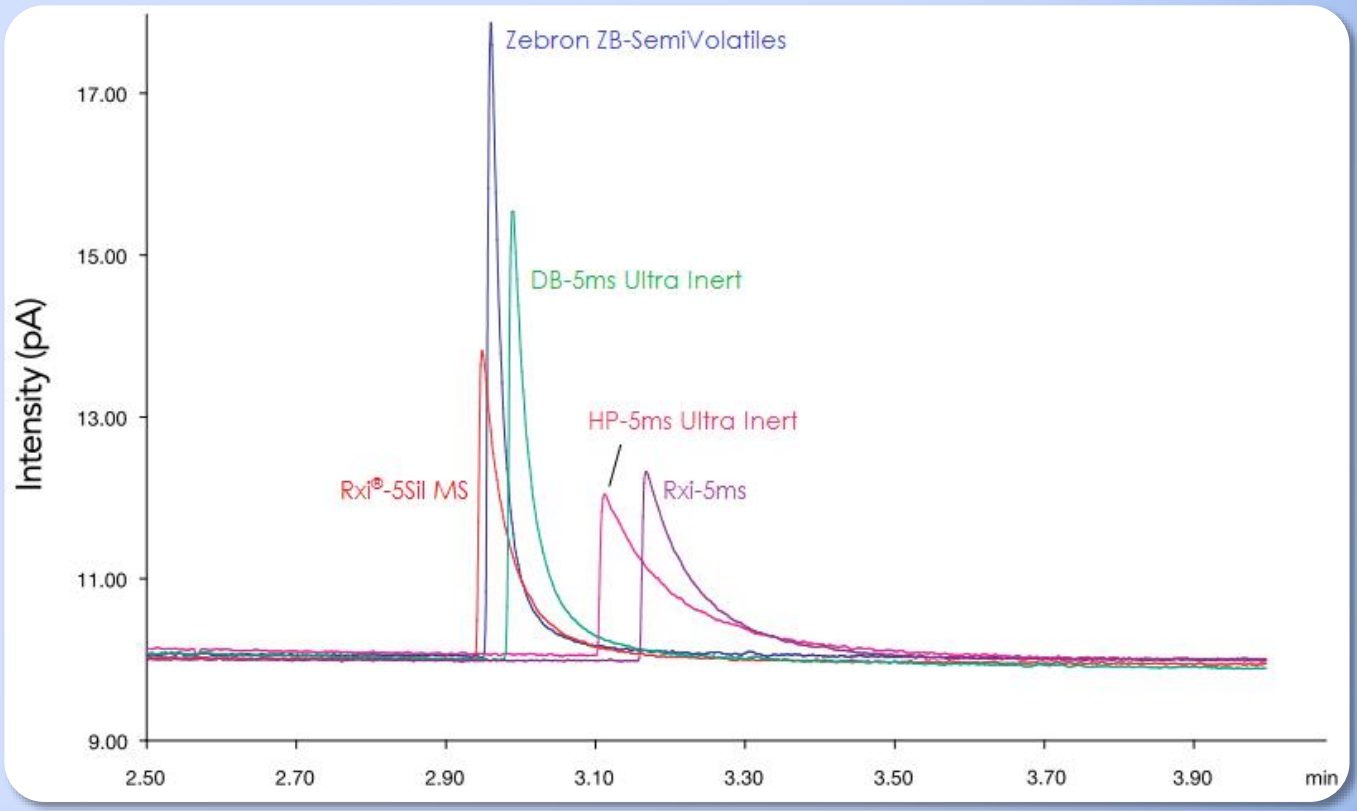
Why is Pyridine Important?

Good gauge of column lifetime

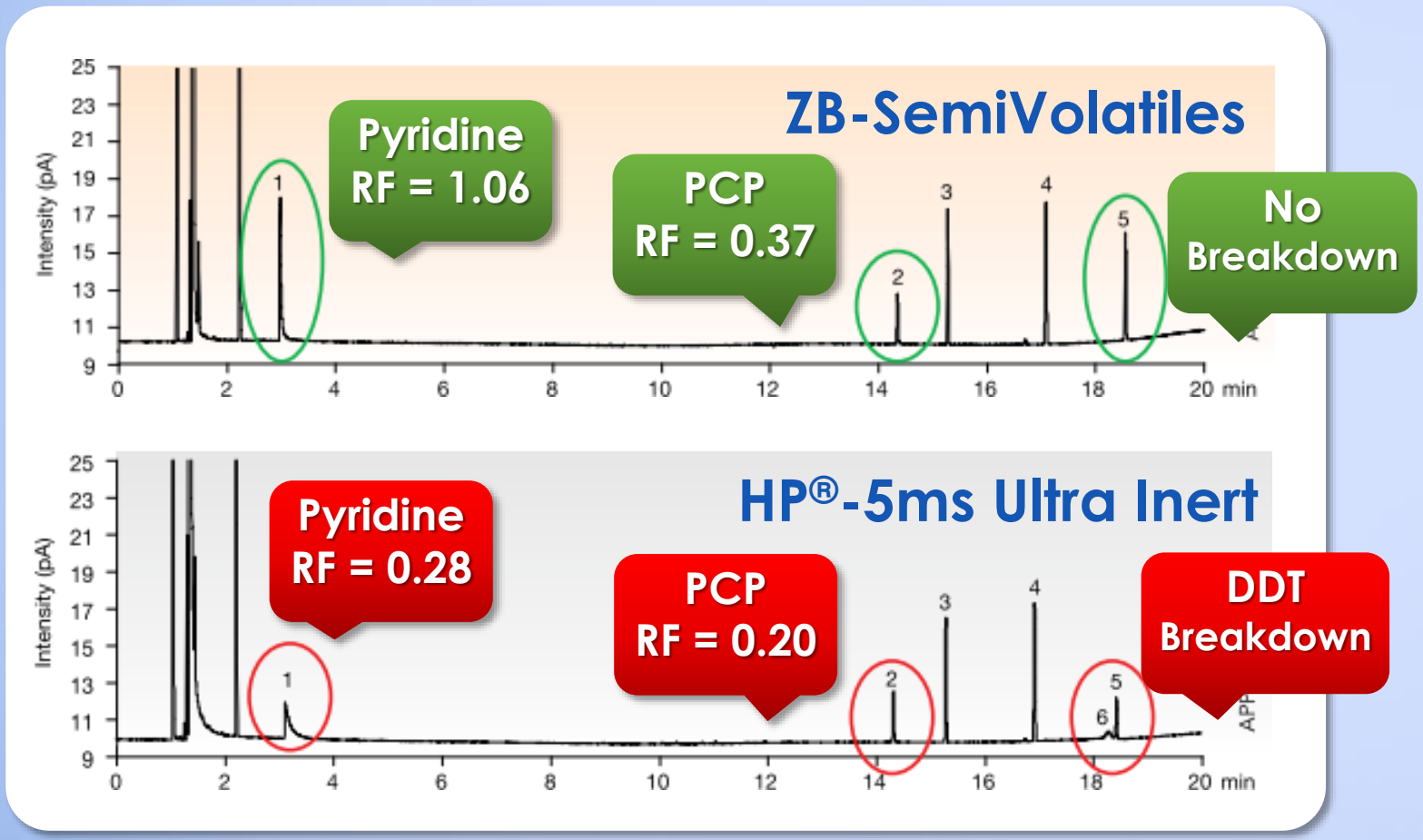
Indicator of column activity

"Pyridine may perform poorly … Therefore, if pyridine is to be determined in addition to other target analytes, it may be necessary to perform separate analyses."

--US EPA 8270D



Reduced Activity On A 5ms



Not all 5ms columns are equal

Productivity Advantage

Improved peak shapes and responses for active compounds out-of-the-box

- Improved RSD values when calibrating instrument
- Eliminates downtime caused by new columns failing Tune Mix requirements

Stands up to contamination better to give longer lifetime

- Improved quantitation for active compounds across all concentrations
- Low concentrations – stronger response
- All concentrations – improved peak shape allows easier and more consistent integration

Overcomes SVOC productivity thieves to increase productivity

GC Summary

Instrument downtime related to column lifetime	Active compound breakdown (Endrin, DDT, etc.) Poor peak shapes	Coelutions of isomers or structurally similar compounds
↓	↓	↓
High temperature stable column	Highly Inert, Well-Deactivated Column	Optimized Column Selectivity

Trends

Multi-residue

Complex matrices

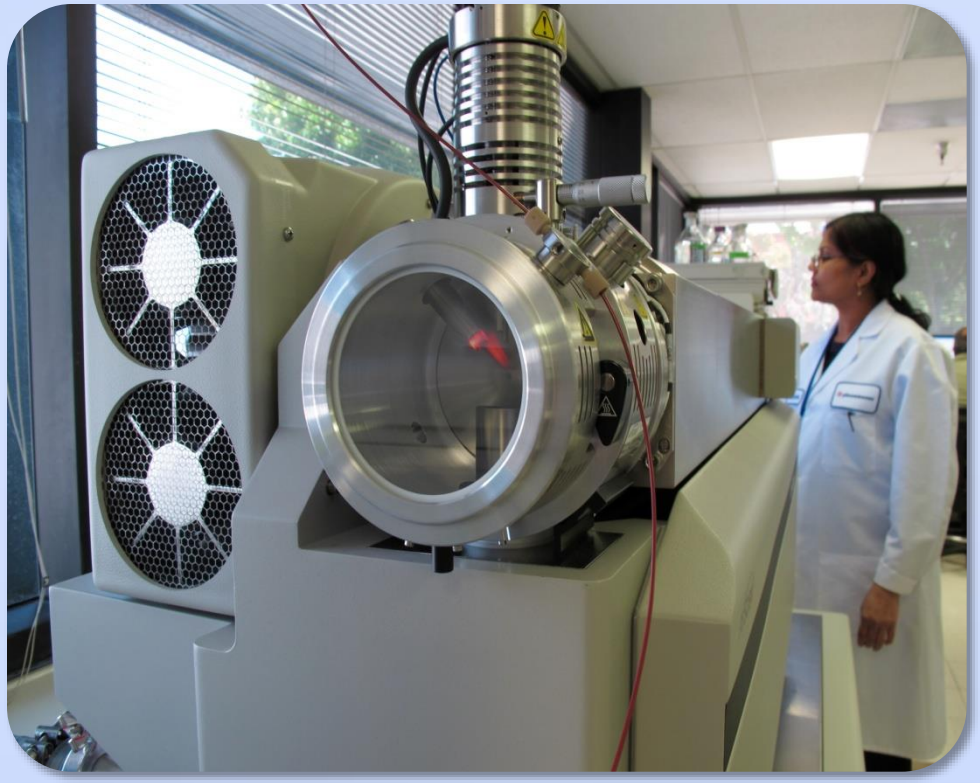
Updated older methods

Lower limits

Larger lists

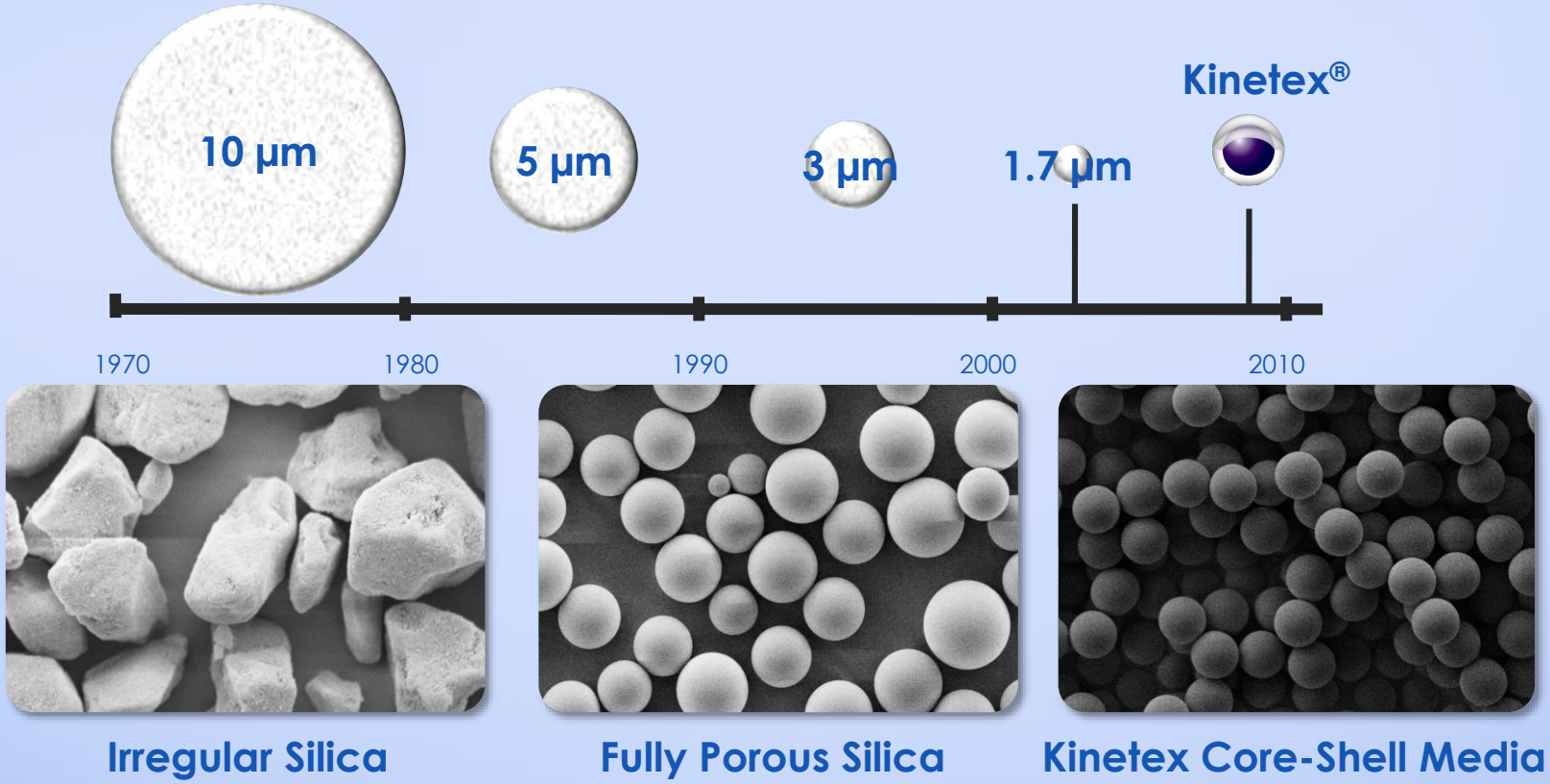
Improved instrumentation

Developments in LC Column Technology



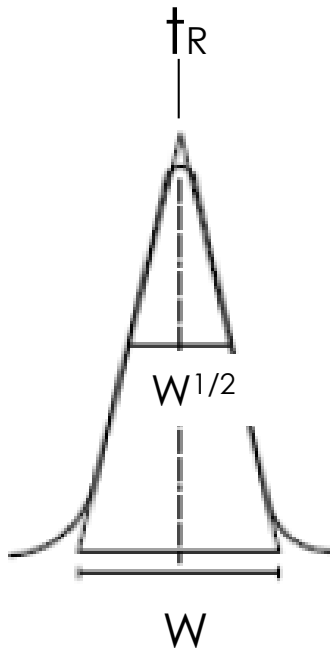
The Evolution of LC Media

The primary trend in LC media development has been the movement towards particles with increasingly higher efficiency



Column Efficiency (N)

$$N = 5.54 \left(\frac{\text{Retention time}}{W_{\text{half height}}} \right)^2$$

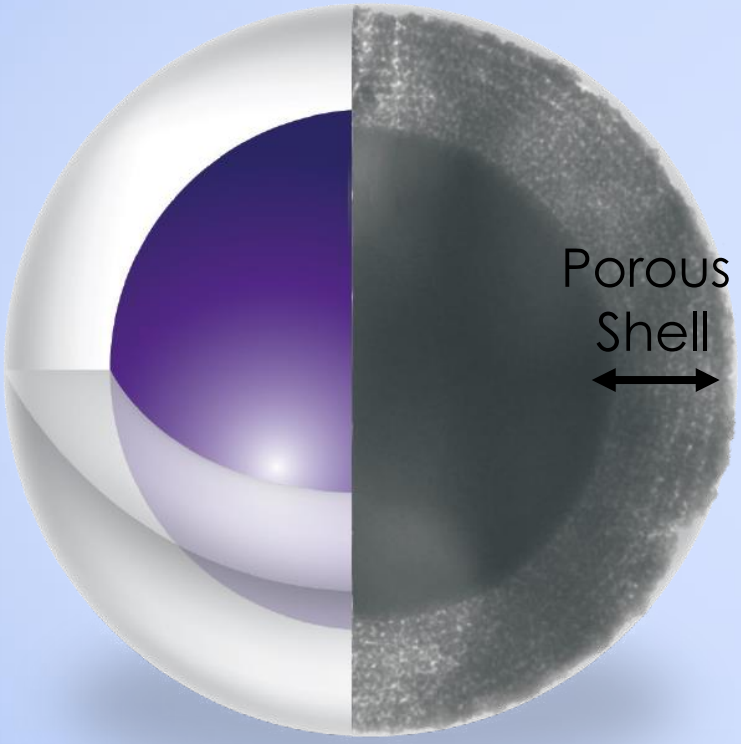


The efficiency of a column is a function of the amount of band broadening

- Columns that cause a lot of peak broadening have low efficiency
- Columns that produce very narrow peaks have high efficiency

Narrower peaks = closely-eluting peaks are easier to separate!

Kinetex® Core-Shell Technology



Monodispersed silica particles consisting of an impermeable silica core surrounded by a layer of fully porous silica

What is the core-shell advantage?

- Kinetex core-shell columns provide significantly greater efficiency than columns packed with fully porous media
- Can deliver UHPLC-equivalent performance on conventional HPLC systems

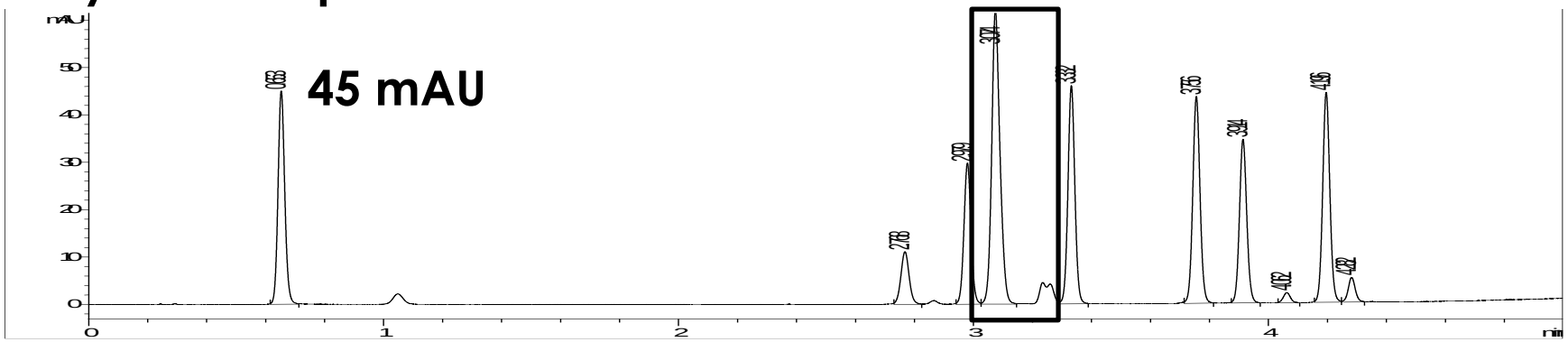
What are the benefits?

- Improved resolution
- Increased sensitivity
- Increase productivity

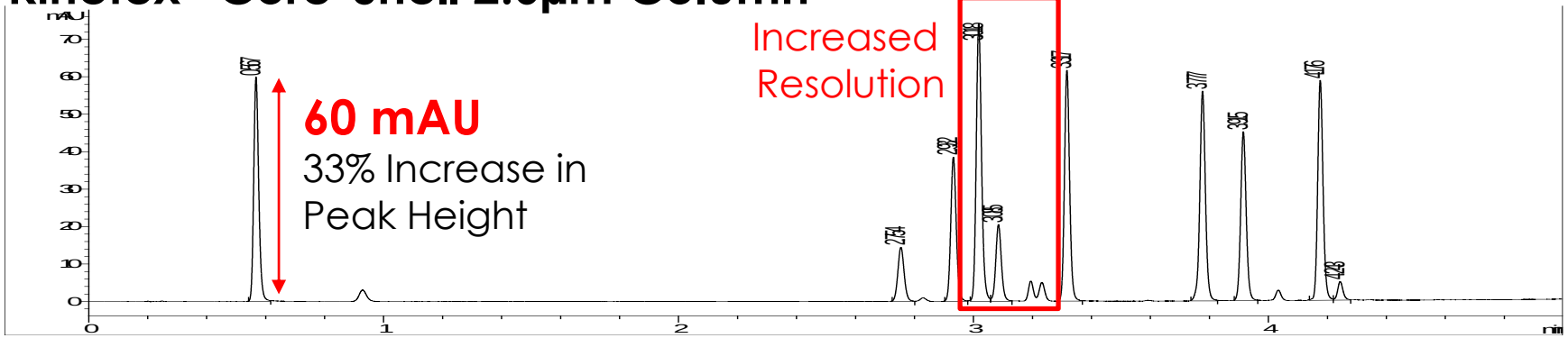
The Core-Shell Advantage

More Efficiency → Increased Sensitivity
More Efficiency → Improved Resolution

Fully Porous 3µm Column



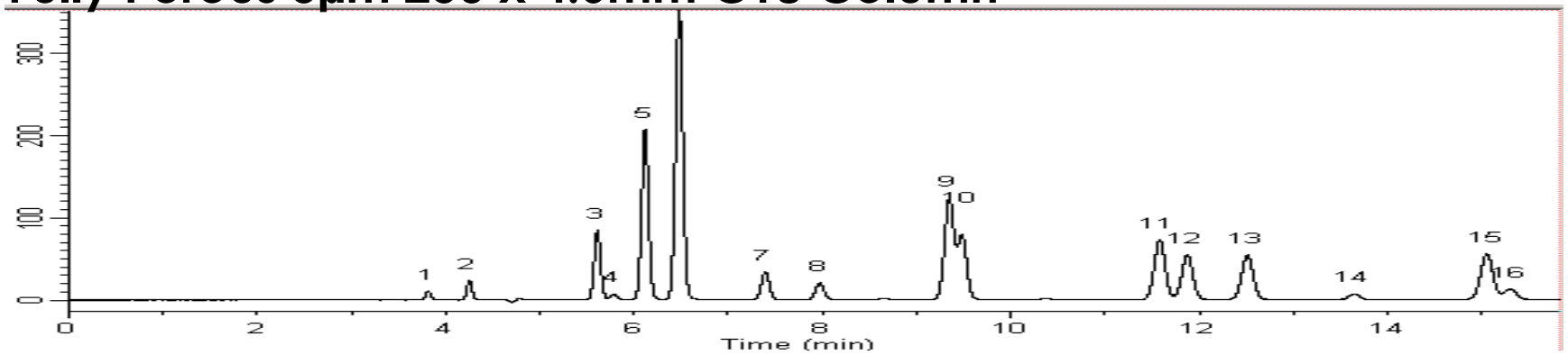
Kinetex® Core-Shell 2.6µm Column



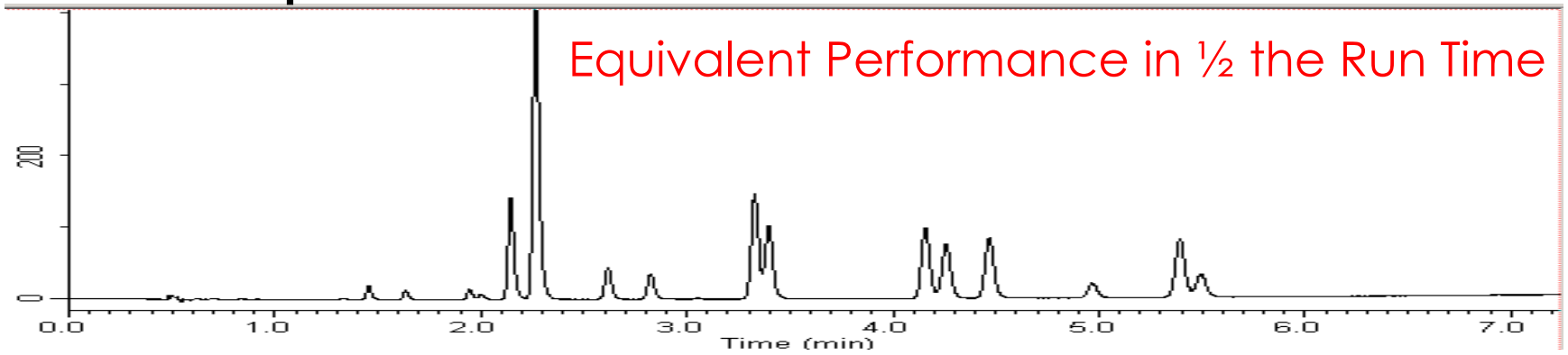
The Core-Shell Advantage

More Efficiency → Increased Productivity

Fully Porous 5µm 250 x 4.6mm C18 Column



Kinetex® 2.6µm 100 x 4.6mm C18 Column



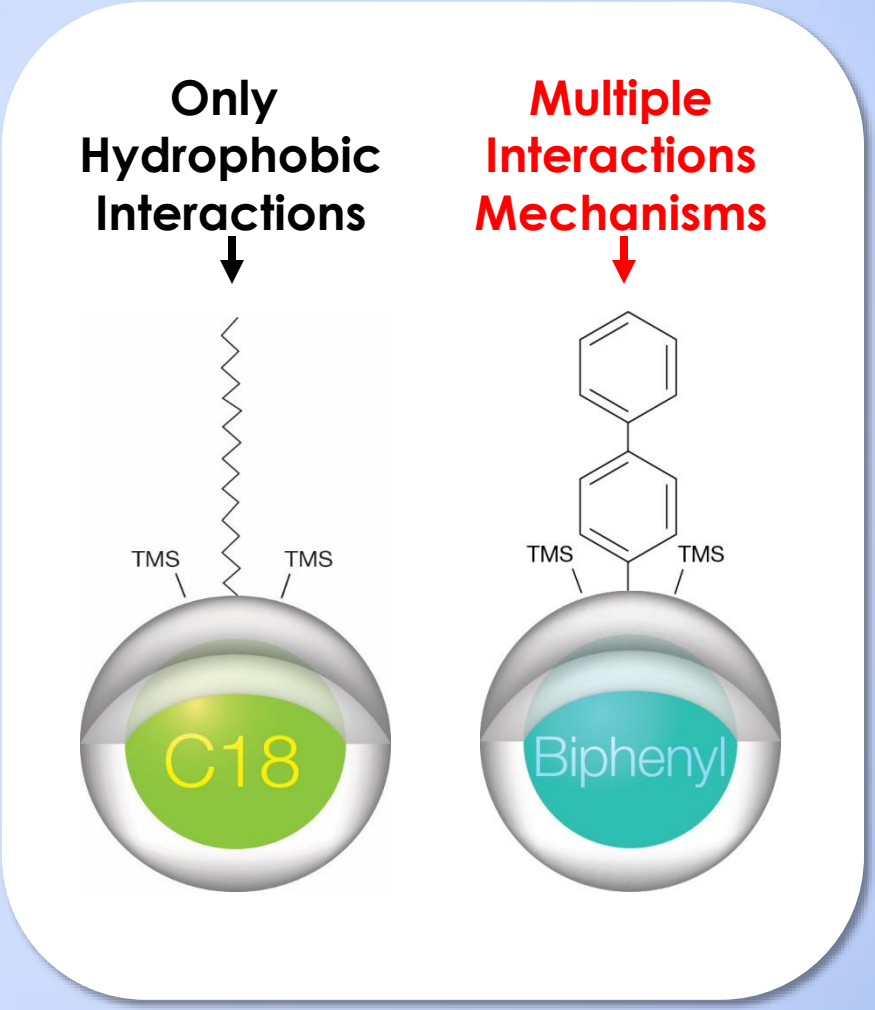
Beyond A Standard C18

Kinetex® Biphenyl

- Unique stationary phase chemistry
- High degree of selectivity for polar & basic analytes
- Delivers the efficiency benefits of the Kinetex core-shell particle

The selectivity of Kinetex Biphenyl is very distinct (orthogonal) from the typical C18 phases

- Use Kinetex Biphenyl when a standard C18 doesn't have the right selectivity
- Start your method development with Kinetex Biphenyl when you have polar, basic analytes that don't have enough polar retention on a standard alkyl-bonded phase



Biphenyl Selectivity

Stationary phase based upon:

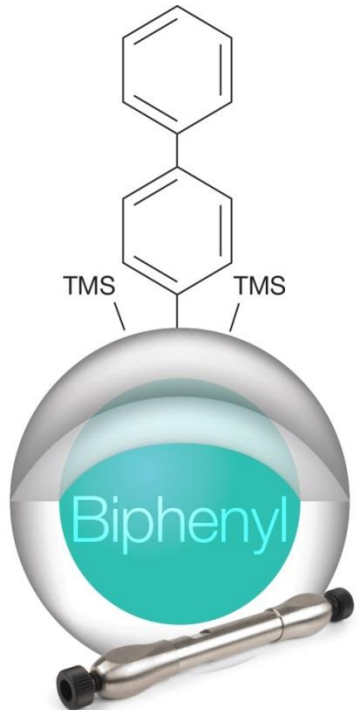
Aromatic pi-pi interactions – between aromatic rings and pi electrons of target molecules and the double aromatic rings of the Kinetex® Biphenyl ligand

Hydrophobic interactions – between carbon skeleton of ligand and target analytes

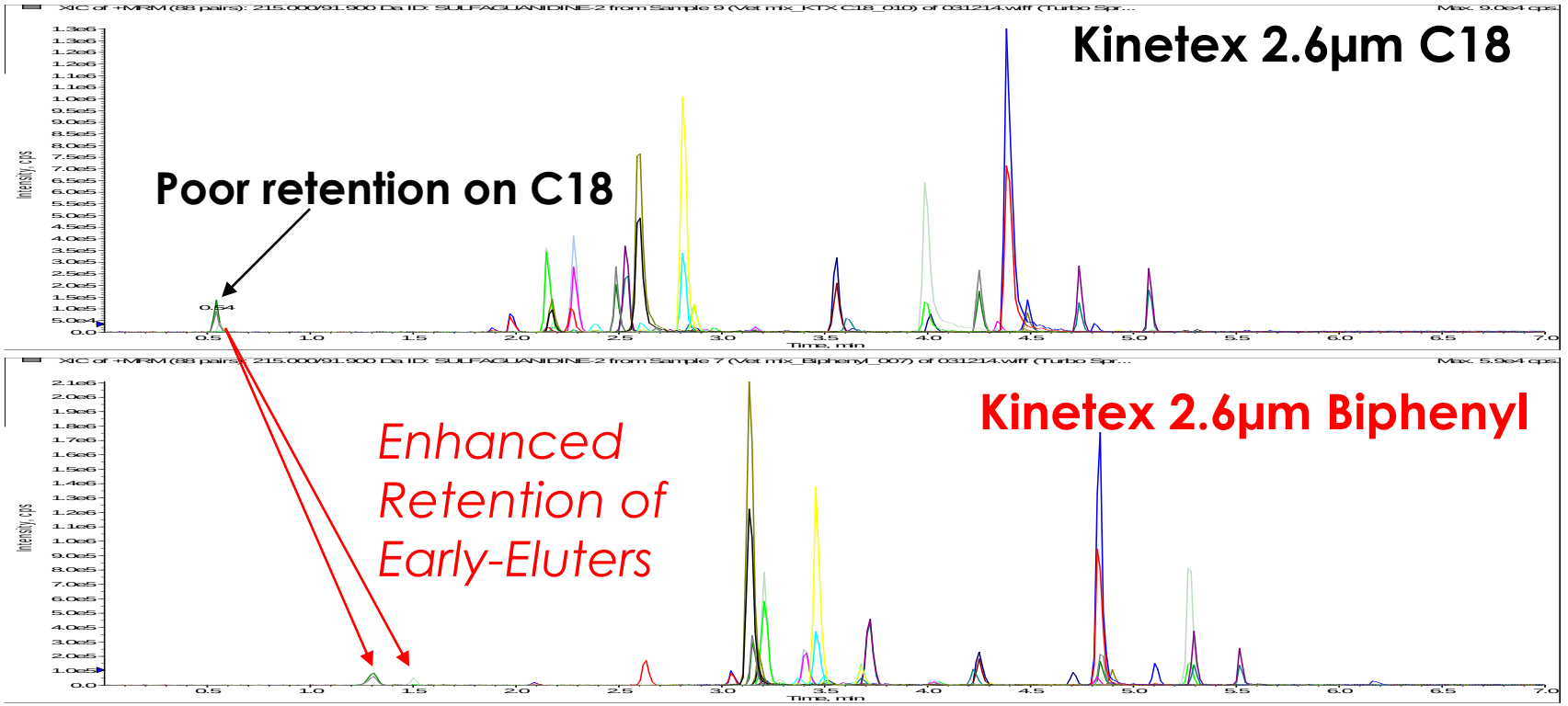
- Hydrophobicity of Kinetex Biphenyl (i.e. retention of analytes based primarily on hydrophobic interactions) is less than C18

Weak ionic or dipole interactions with the phenyl rings

- High electron density
- Behaves almost as a weak cation exchanger, giving enhanced retention of many basic analytes



Polar Retention of Kinetex® Biphenyl



Pesticide Screening

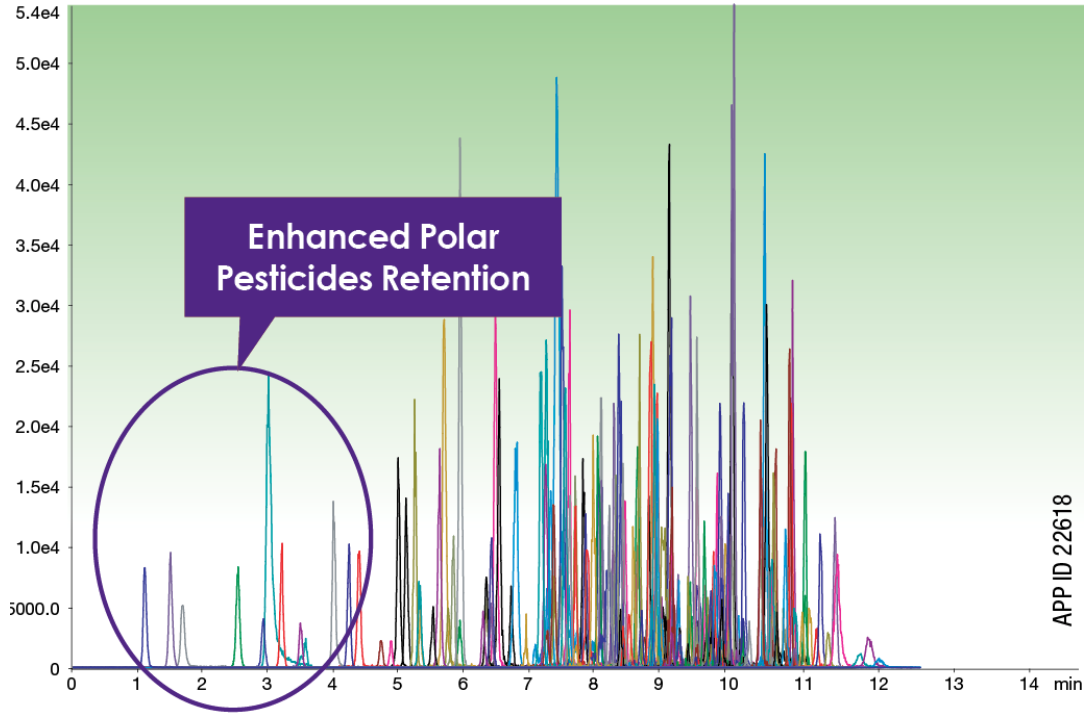
Better separation of early elutors

Column:	Kinetex® Biphenyl 5µm	
Dimensions:	100 x 2.1 mm	
Mobile Phase:	A:	5 mM Ammonium Formate in H2O
	B:	5 mM Ammonium Formate in MeOH

Gradient:

Time	%B
0.01	10
1.00	10
10.00	90
15.00	90
15.10	10
20.00	10

Flow Rate:	0.5 mL/min
Column Temp:	35 °C
Detector:	AB SCIEX 4500 QTRAP®



200+ pesticides with great sensitivity for polar pesticides in under 20 minutes

Neonicotinoids by LC/MS/MS

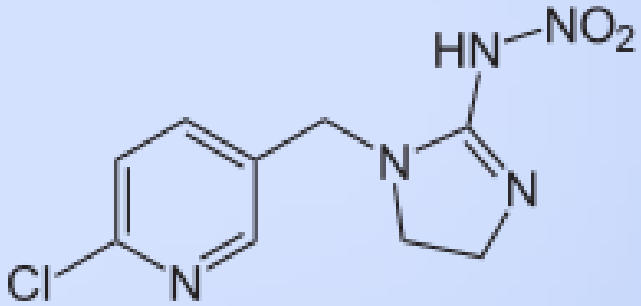
Nicotine-related neurotoxic insecticides

Less toxic than organophosphate and carbamate pesticides

May be related to honey bee die-offs

System:	API 4500 MS + Shimadzu LC-30 UFLC
Mobile Phase:	Water with 0.1% formic acid
	ACN with 0.1% formic acid
Gradient:	5-70%B in 3min
Flow rate:	600 µL/min
Temp:	40C
Sample:	

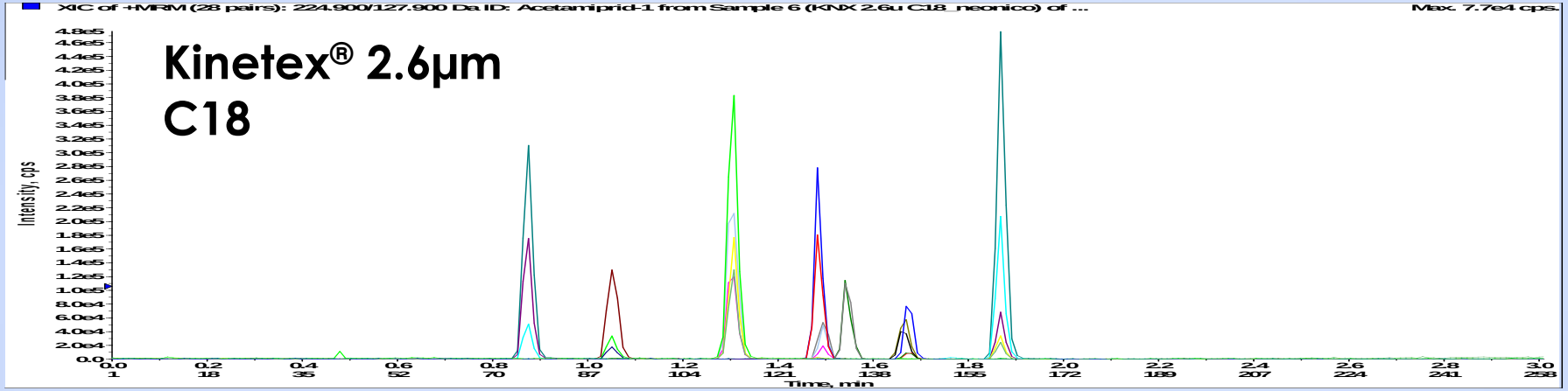
1. Dinotefuran
2. Nitenpyram
3. Clothianidin
4. Acetamiprid
5. Thiacloprid
6. Imidacloprid
7. Thiamethoxam

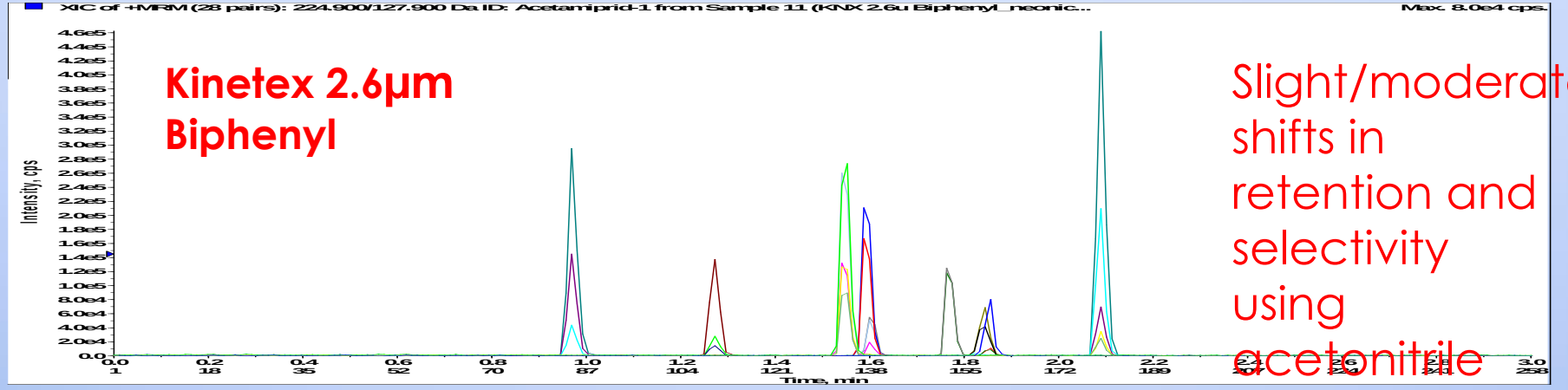


Imidacloprid

Enhance Selectivity: Solvent Choice

Neonicotinoids using acetonitrile

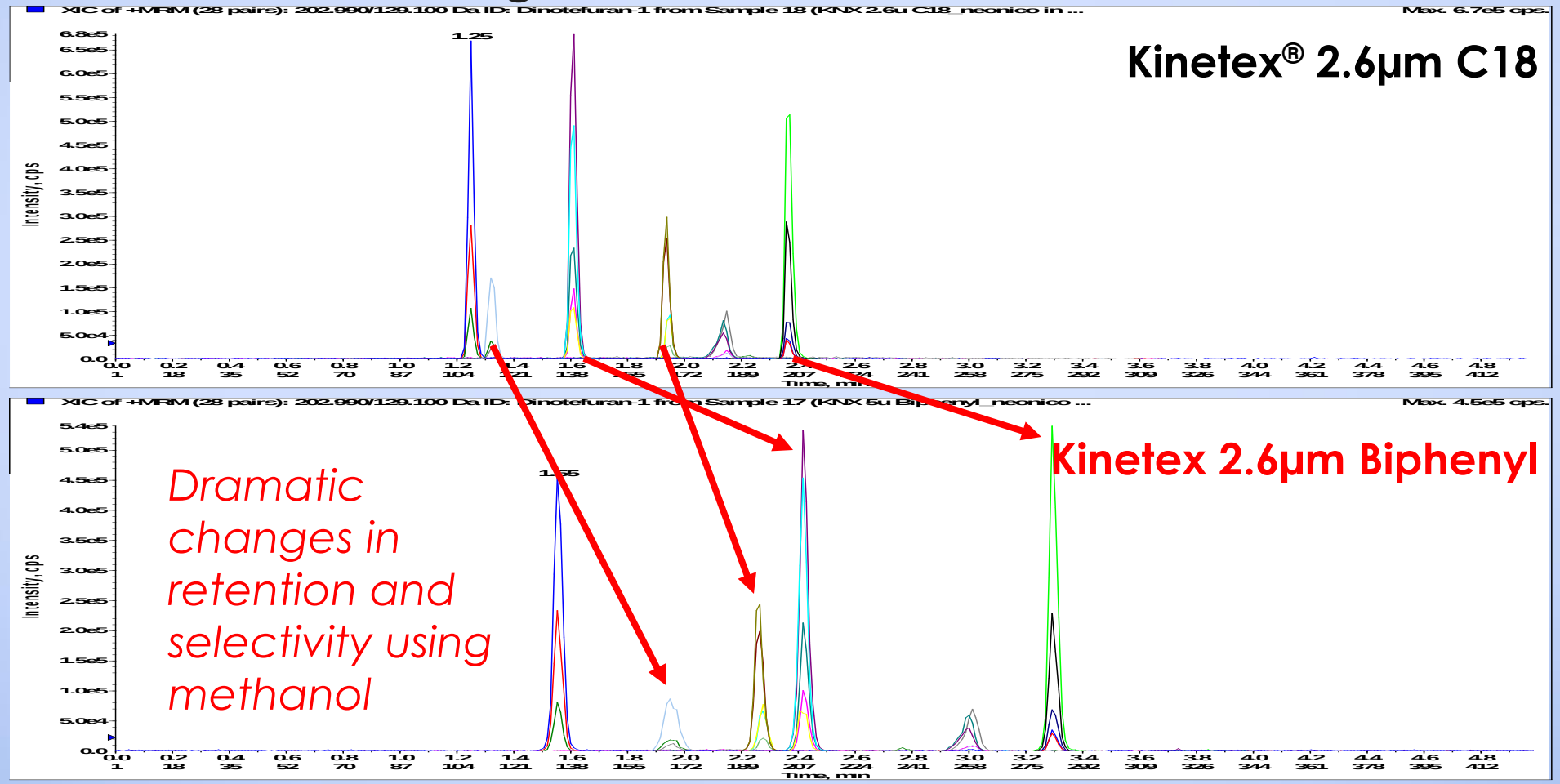




Slight/moderate shifts in retention and selectivity using acetonitrile

Methanol to Enhance Selectivity

Neonicotinoids using methanol



Summary: LC Columns

Columns packed with core-shell media are able to provide significantly greater efficiency than columns packed with fully porous media

- Improved resolution
- Improved sensitivity
- Improved productivity

Stationary phase selectivity is also crucial to the success of any method

- Phases that complement standard C18 phases, such as the Biphenyl, can provide the necessary to resolve mixtures that are difficult to chromatograph on the go-to C18s
- Every lab that has a Kinetex C18 should have a Kinetex Biphenyl

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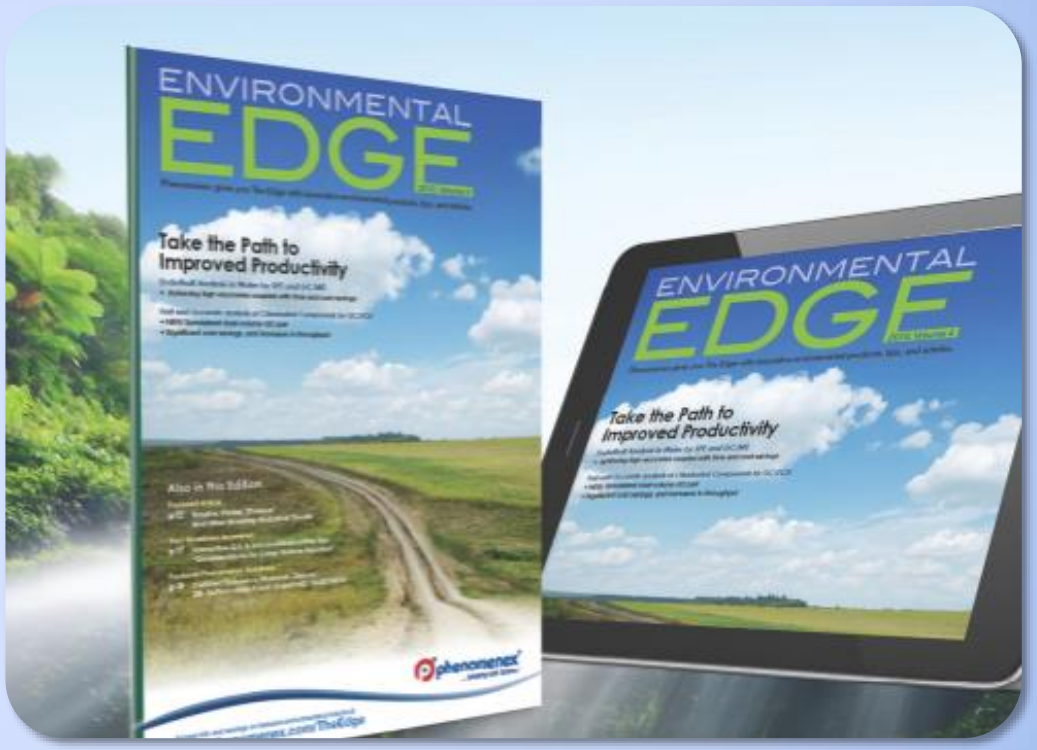

Thank You! Questions?