Trouble-free Food Testing

Technologies to improve uptime and productivity

Chemistries & Supplies

Mark Powell
Applications Engineer

Overview

- Needs in Food Testing Today
  - Considerations and Tips for LC and LCMS analysis
  - A Quick Look at Sample Prep Options
- Important Method Development Fundamentals
- A Food Analysis Workflow using LC/MS
  - Botanical insecticide in fish
- Tools to Improve Your Food Analyses

Where is Food Testing Today?

- Increasing complexity in food sourcing and supply
  - Globalization of the food supply has made it necessary for detailed analysis of nutritional value, composition, and contaminants
  - Concerns over food safety and the effects on human health have increased regulation and requirements associated with food testing
  - Older methodologies for food testing were time consuming and not amenable to modern needs for higher throughput food analyses
- Samples come in all types
- Appropriate food analysis cannot be achieved without the proper LC and sample prep methodology

Chromatographic Techniques for Food testing

- Gas Chromatography
  - Typically used for:
  - Non-polar, volatile, oils, etc
  - Can require derivatization of compounds

- Liquid Chromatography
  - Typically used for:
  - Polar, thermally labile
  - No need for derivatization of many compounds
  - Often has less time consuming sample prep

Challenges in Food Testing

- New method development
- Finding ways to cut costs
- Doing more with less – increasing lab productivity
- Keeping up with the latest food safety changes

LC Method Development on Food Testing

What's different in LC analysis for food analysis?

- Sample matrix complexity
- Multiple residues of interest
- Often very polar analytes in the sample of interest

Modern column technologies make food testing faster and easier

New Column Technologies for Method Development

Columns for high resolution and high speed analysis

- Sub-2 µm columns for ultra-high pressure operation
- Sub-3 µm superficially porous columns

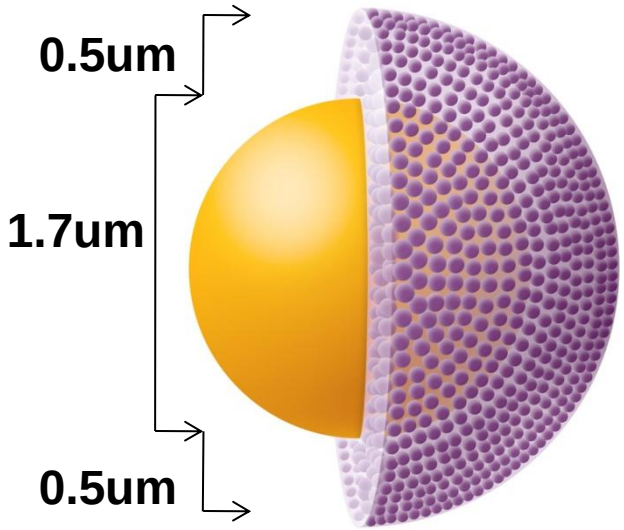
Considerations when developing methods on new column technologies

- Particle size (< 3 µm)
- Column pressure limits >400 bar (600-1200 bar typical)
- Other factors remain same as for legacy, 5 µm columns

New Superficially Porous Column Technologies

Poroshell 120 columns:

- Efficiency ≈ 90% of sub-2 µm
- Pressure ≈ 40-50% of sub-2 µm
- N ≈ 2X 3.5 µm (totally porous)
- d_p = 2.7µm
- 2 µm frit to reduce clogging
- P_{limit} = 600 bar for HPLC or UHPLC
- Particles
  - 1.7 µm solid core
  - 0.5 µm diffusion path
  - 2.7 µm total diameter



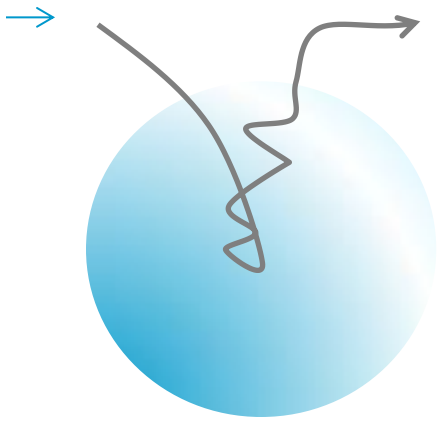
Comparing Efficiency and Pressure with Different Types of Columns

Particle Size/Type	Pressure	Efficiency	LC Compatibility
5µm Totally Porous	80 bar	5,000	All 400 bar instruments
3.5µm Totally Porous	123 bar	7,800	All 400 bar instruments
2.7µm Poroshell 120	180 bar	12,000	All LCs/UHPLCs (up to 600 bar)
1.8µm Totally Porous	285 bar	12,500	All LCs/UHPLCs (up to 1200 bar)

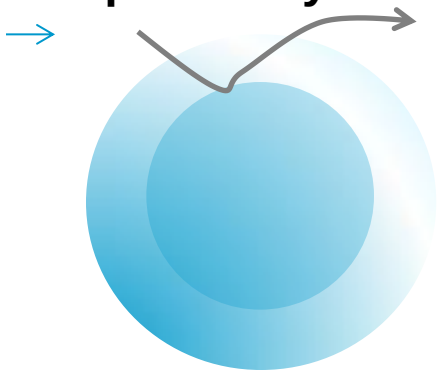
Columns: 4.6 x 50mm, Mobile Phase: 60% ACN:40% Water Flow Rate: 2 mL/min

Analyte Mass Transfer Improvements through Lower Diffusion

Totally Porous



Superficially Porous



- **Totally porous particles**
  - **diffusion throughout particle**
- **Poroshell 120**
  - **diffusion limited to outer shell**

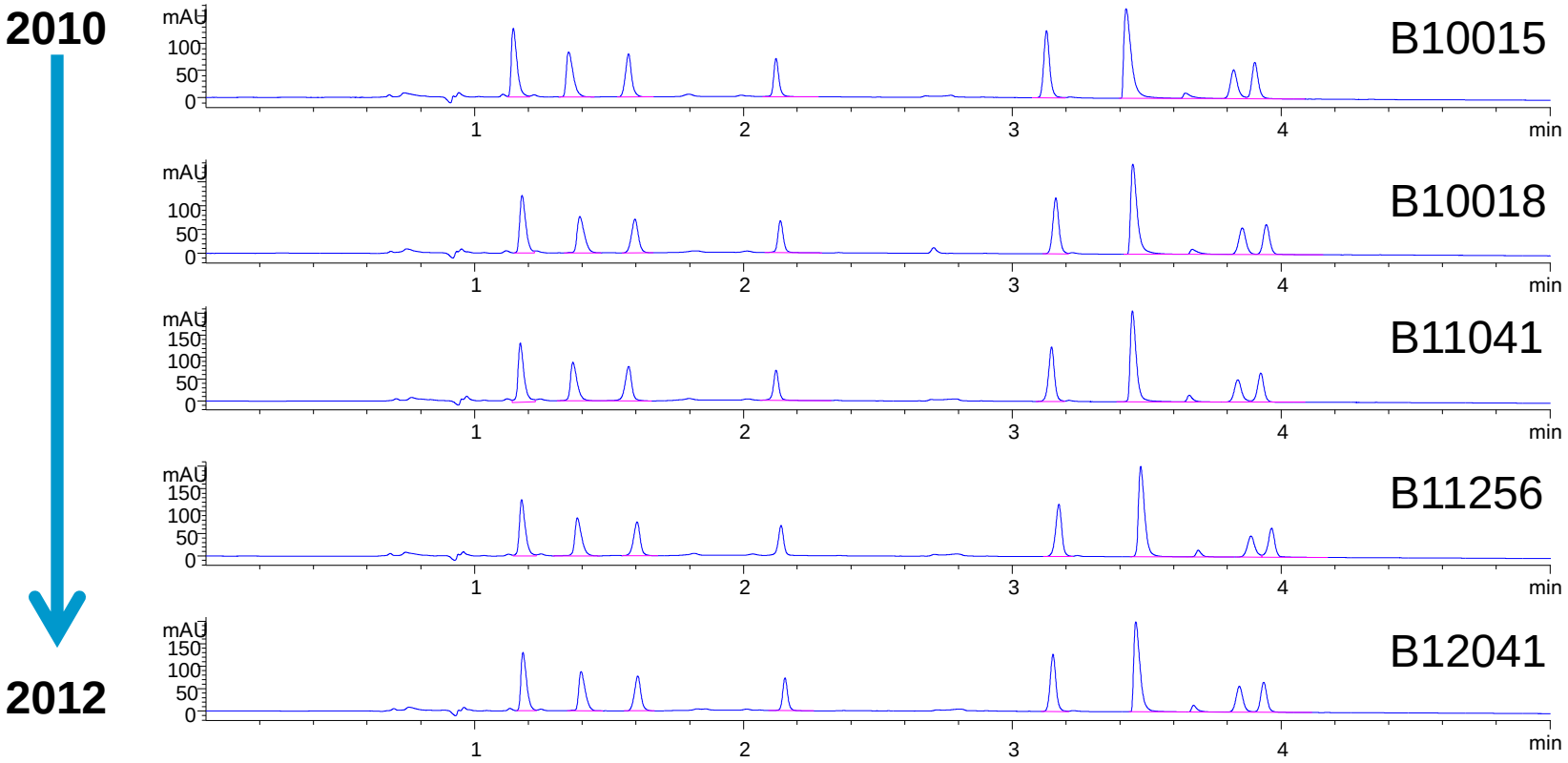
van Deemter equation:

$$h = A + B/\nu + C \cdot \nu$$

- **Results:**
  - **Lower C term**
  - **Higher efficiency**
- **And**
  - **Higher flow rate with**
  - **Minimal impact on efficiency**

Other Considerations when Selecting a Column

- **Robustness and batch-to-batch reproducibility of Poroshell 120 columns**



Beverage Additives

General Steps to a robust LC method for Food Analysis

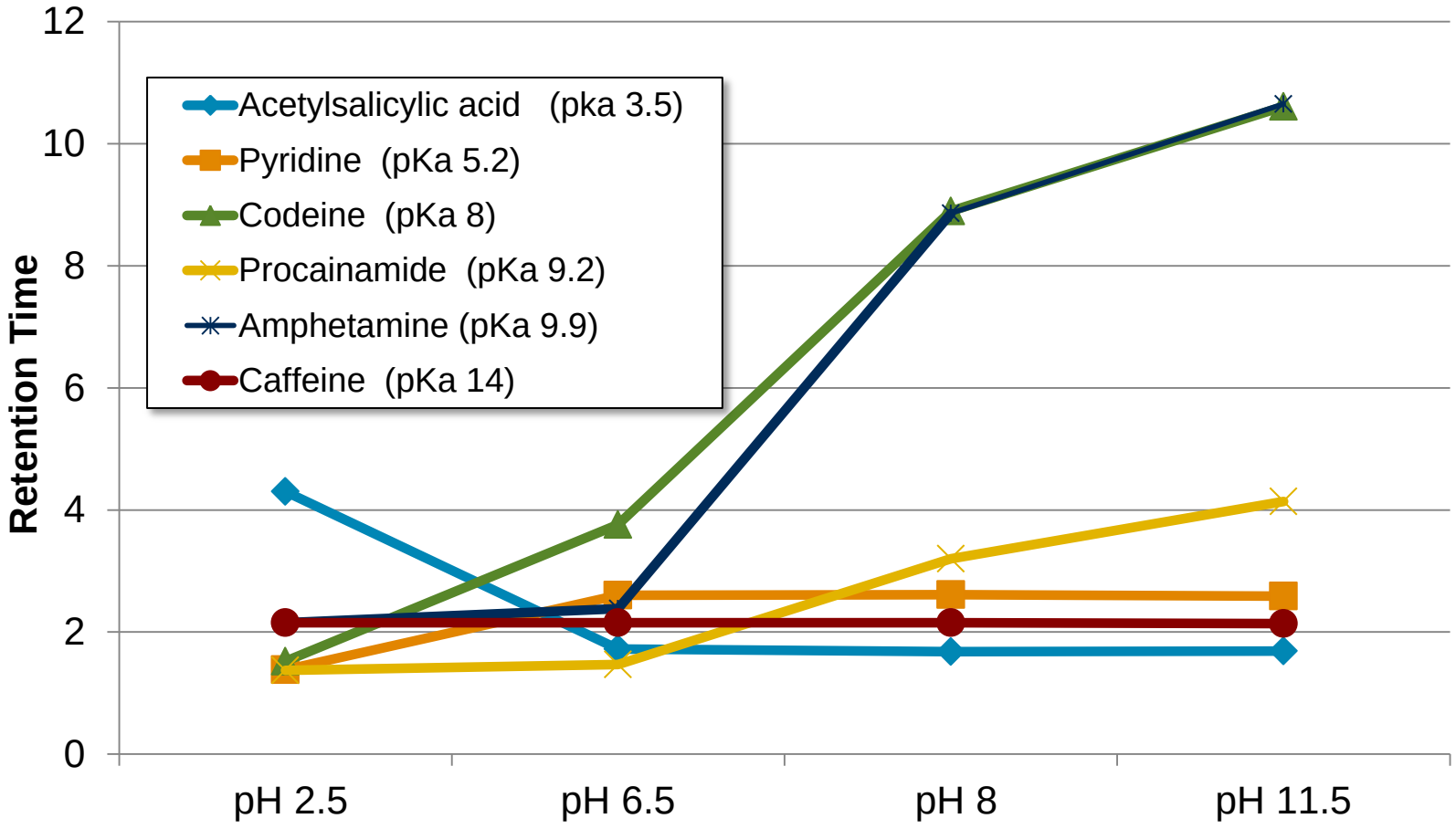
- Choose the type of chromatography – often Reversed Phase (C18, polar embedded, etc…) or HILIC phase
- Choose the appropriate starting and ending gradient conditions
- Adjust pH to shift analyte retention
- Change the bonded phase to adjust for proper peak spacing and resolution

Change in Retention with pH for Ionizable Compounds is Key to Method Development

- Non-charged analytes have better retention (i.e. acids at low pH and bases at high pH)
- Silanols on silica ionize at mid-pH, increasing retention of basic analytes (i.e. possible ion-exchange interactions)
- Choose mobile phase pH to optimize retention and selectivity during method development
- Poroshell 120 EC-C18 can be used over a wide pH range
- Other choices exist for high pH

Change in Retention with pH for Ionizable Compounds is Compound-Dependent

More retention for non-charged analytes (i.e. acids at low pH and bases at high pH)



Mobile Phase: 45% MeOH, 55% 20 mM Phosphate Buffer

Why Change the Bonded Phase?

- Different interactions for polar and non-polar compounds.
- Exploit other interactions with bonded phase (e.g., pi-pi)
- These all change with bonded phase!
- Changing the bonded phase can improve selectivity/resolution, reduce analysis time
- **When you use Poroshell 120 columns the comparison of bonded phases can be done quickly!**
- Easy with multiple column choices plus high speed technologies

Poroshell 120 Column Chemistries

Poroshell 120 EC-C18 and C8

- Robust endcapped C18 for best peak shape at pH 2-9

Poroshell 120 StableBond C18 and C8

- Robust chemistries for pH<2

Poroshell 120 Phenyl-Hexyl

- Same Eclipse Plus bonding process as ZORBAX Eclipse Plus Phenyl-Hexyl
- Excellent choice for pi-pi interactions
- Alternative selectivity to EC-C18 or SB-C18
- Selectivity similar to phenyl, diphenyl, or other phenyl-hexyl columns

Poroshell 120 SB-Aq

- Proprietary bonding phase is an excellent choice for polar analytes

Poroshell 120 Bonus-RP

- Embedded polar group provides unique selectivity for polar compounds

Poroshell 120 EC-CN

- Flexible endcapped CN chemistry with Normal and Reversed Phase character

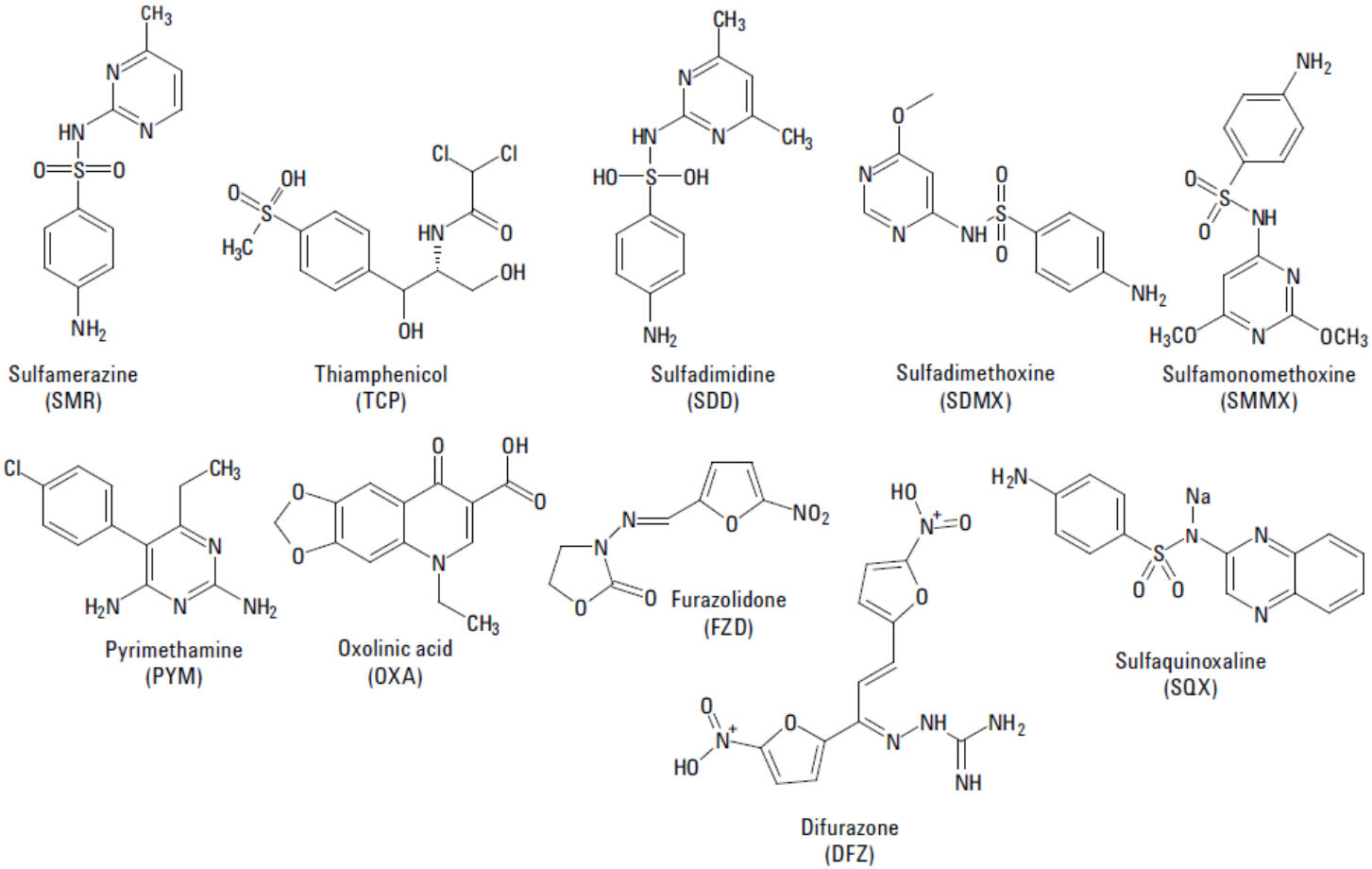
Poroshell 120 HILIC

- Bare silica HILIC for use in Hydrophilic interaction chromatography of polar molecules

Considerations for LCMS Method Development

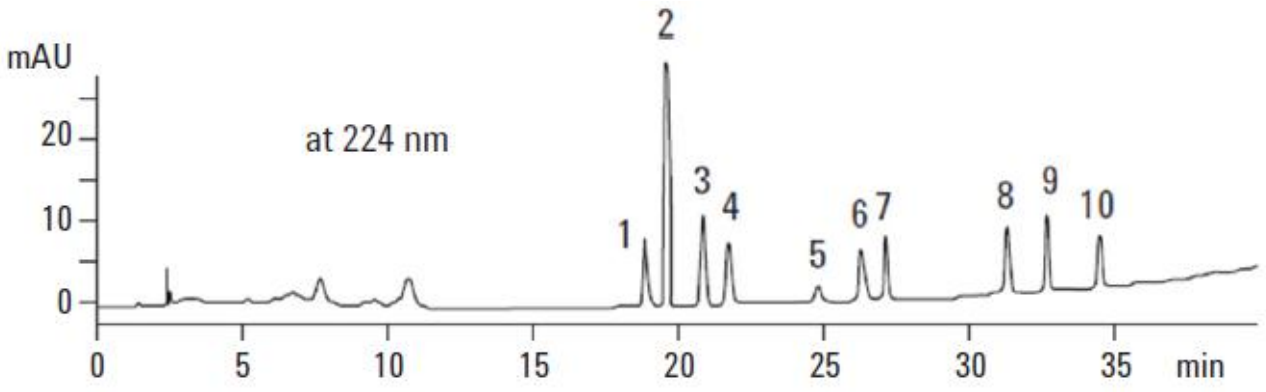
- Mobile Phase Selection
  - Gradients often preferred due to the number of analytes and differences in retention characteristics
- Buffer Choice
  - Choose a volatile buffer (formic acid, acetic acid, etc)
- Column dimensions for higher sensitivity and throughput
  - Often choose narrow columns for higher sensitivity and shorter columns for faster analysis

Transfer of Existing Method for Antibiotics



5990-6238EN

Transfer of Existing Method for Antibiotics

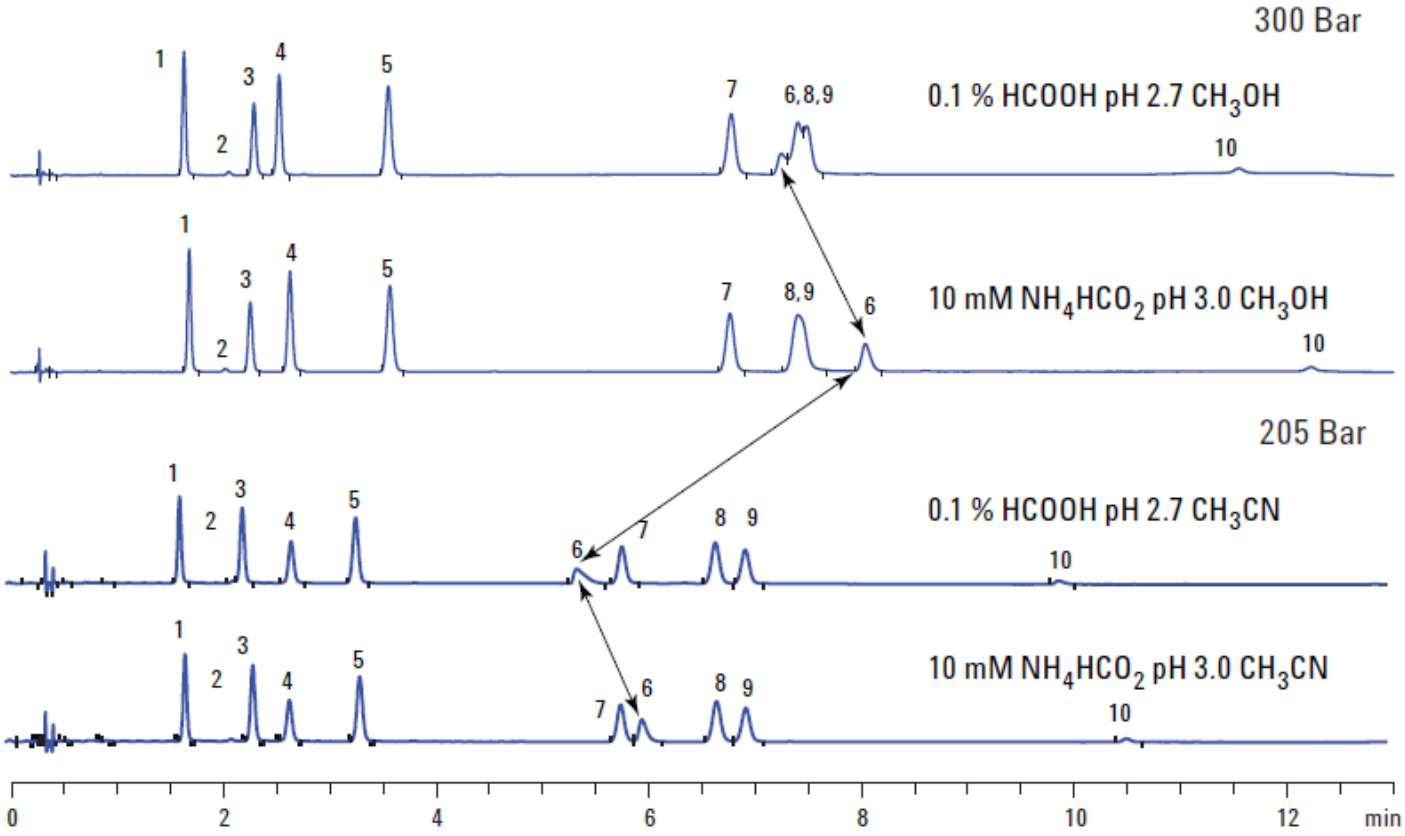


1	SMR	6	SMMX
2	PYM	7	DFZ
3	TCP	8	SDMX
4	SDD	9	SQX
5	FZD	10	OXA

Instrument:	Agilent 1100 Series HPLC
Column:	250 mm × 4 mm id, RP-18 Purospher, 5 µm, p/n 79925PU-584
Mobile phase:	A = 0.7% Phosphoric acid, B = CH_3CN
Gradient:	0.0 min 5% B; 10.0 min 5% B; 40.0 min 65% B; 45.0 min 65% B; Post Time 7.0 min 5% B
Flow rate:	1.0 mL/min
Temperature:	40 °C
Injection volume:	20 µL

5988-7135EN

Transfer of Existing Method for Antibiotics



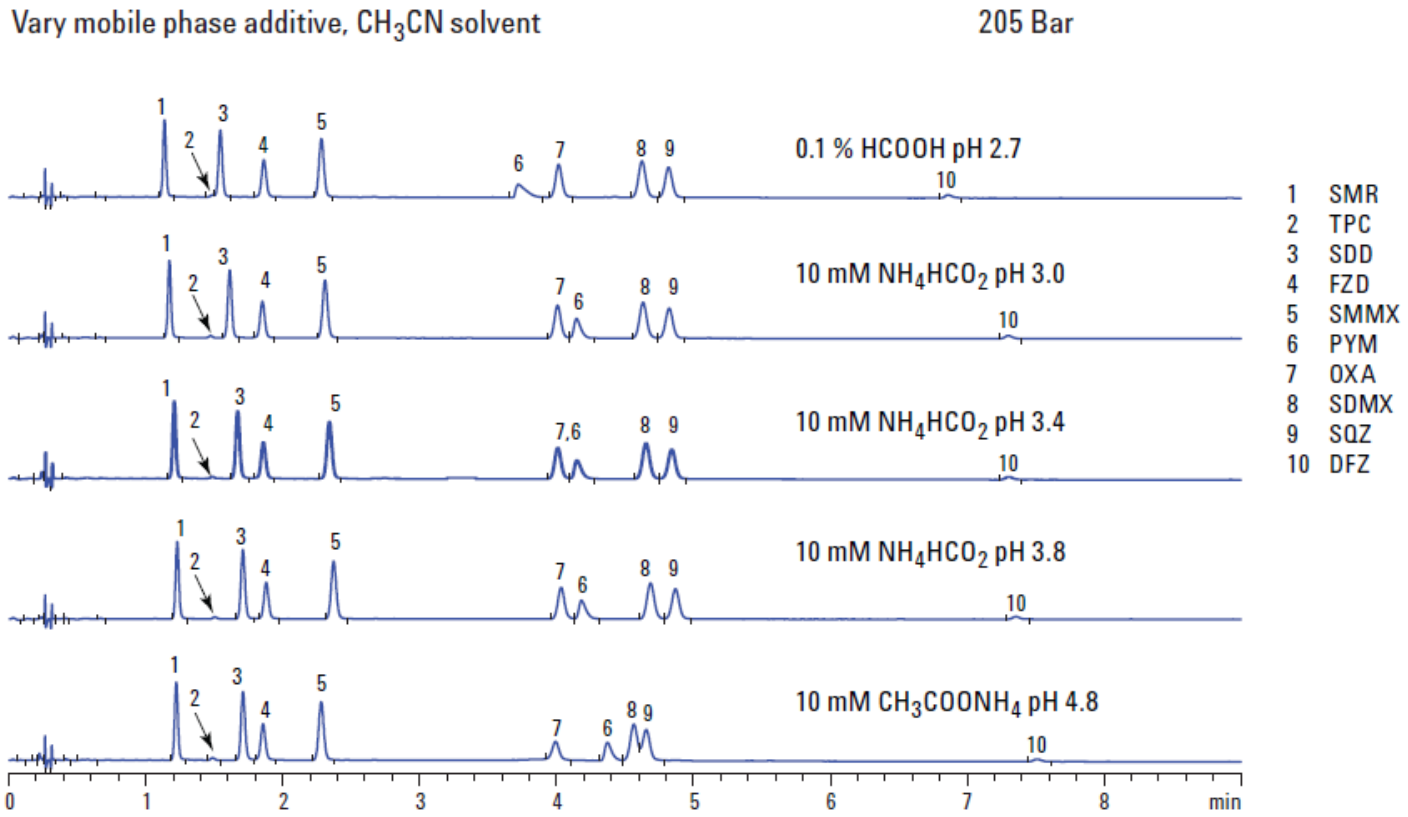
1 SMR
2 TPC
3 SDD
4 FZD
5 SMMX
6 PYM
7 OXA
8 SDMX
9 SQZ
10 DFZ

10% to 40% B/12 min at 2 mL/min
Agilent Poroshell 120 EC-C18 4.6 mm × 50 mm, 2.7 µm

Column Temperature: 25°C; Detection: DAD 270 nm, 8 nm, ref off 3 mm, 2 uL micro flow cell; Peak width >0.05 min. (40Hz)

5990-6238EN

Transfer of Existing Method for Antibiotics

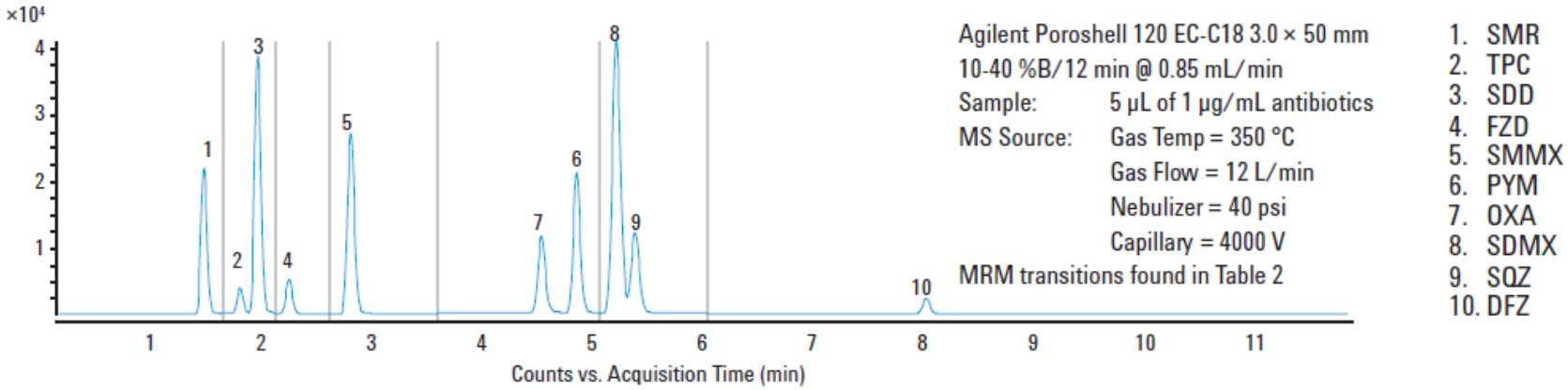


10% to 40% B/12 min at 2 mL/min
Agilent Poroshell 120 EC-C18 4.6 mm × 50 mm, 2.7 µm

Column Temperature: 25°C; Detection: DAD 270 nm, 8 nm, ref off 3 mm, 2 uL micro flow cell; Peak width >0.05 min. (40Hz)

5990-6238EN

Transfer of Existing Method for Antibiotics



A = 10 mM NH_4HCO_2 pH 3.8
B = acetonitrile

- Conditions were scaled for a 3.0 x 50 mm column
- Shows that 3.0 mm can easily be used for conventional UV and MS detection

5990-6238EN

In the Food Analysis Workflow, what trips you up the most?

- Selecting the ‘right’ sample prep method for my food sample
- Sample preparation takes too long
- Column selection / refining methods
- Columns clog up

Sample Prep in Food Analysis

SPE

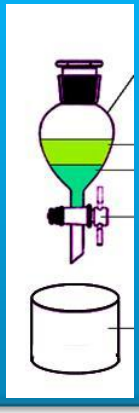
- Detection limits
- Automation

QuEChERS

- Easy-to-use
- Ideal for food samples

Liquid-liquid extraction

- Phase separation
- Often time consuming

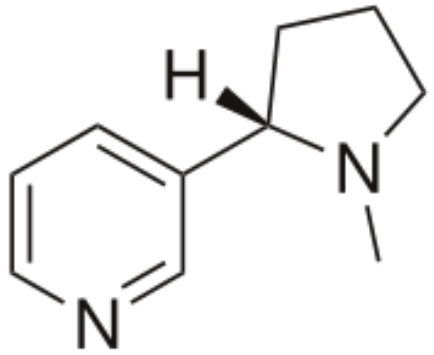


Filtration

- Particulate removal
- Lipid removal

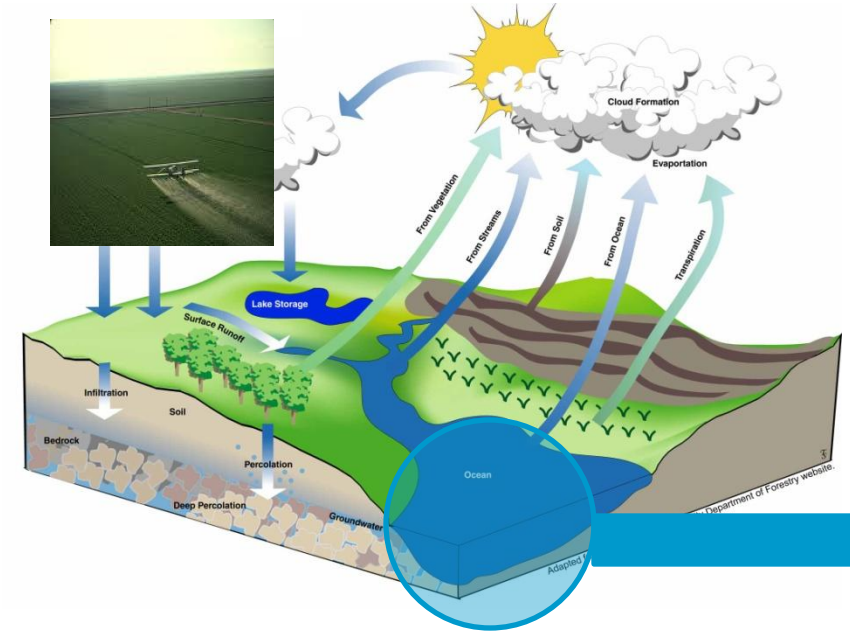
A close look at a food-testing workflow:
Botanical insecticides in fish

- Nicotine is used as a botanical insecticide in the US and Canada
- Banned as an insecticide in EU and will be banned in the US beginning 2014
- Oily liquid at room temperature
- Water miscible



A close look at a food-testing workflow:
Botanical insecticides in fish

- How nicotine end up in fish or other aquatic organisms?
  - Insecticides used on the farm → Rain → River → Ocean



A close look at a food-testing workflow:
Botanical insecticides in fish

- How to prepare real fish sample?

QuEChERS

– If the sample matrix is solid, especially food, consider QuEChERS first.

– QuEChERS is the most common sample preparation method used in many food testing labs.

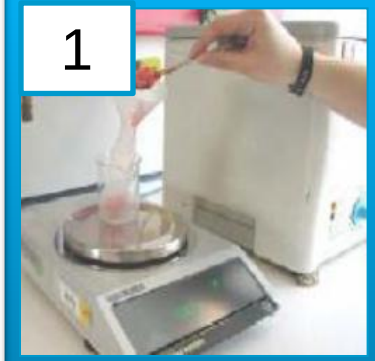
– **Qu**ick, **E**asy, **Ch**eap, **E**ffective, **R**ugged, **S**afe.

A close look at a food-testing workflow:
Botanical insecticides in fish

- Benefits of QuEChERS in nicotine analysis in fish
  - QuEChERS is a combination of two steps
    - 1st extraction, 2nd dispersive SPE
  - Little or no method development is required
    - 1st extraction: Simply choose AOAC, EN, or original method
    - 2nd dispersive SPE: Choose one of our dispersive packets
  - Customizable to your needs

A close look at a food-testing workflow:
Botanical insecticides in fish

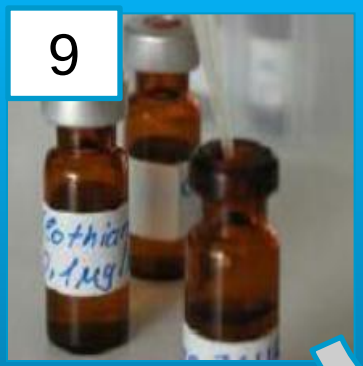
1st: Extraction



Repeat 3

Repeat 5

2nd: Dispersive SPE



A close look at a food-testing workflow:
Botanical insecticides in fish

- Which column to choose for analysis of nicotine? → HILIC

Benefits of HILIC over Reversed Phase		
	HILIC	Reversed Phase
Retention Time	Nicotine & metabolites will retain and separate	Most of nicotine and its metabolites will elute too early
Sample Solvent	No evaporation & reconstitution	•Sample solvent switch or •Dilution is *REQUIRED*
Initial Mobile Phase	90% ACN	Extremely low organic mobile phase or 100% aqueous
MS Sensitivity	High	Lower
Backpressure	Low → Wider flow rate range	High → Limited flow rate rang.

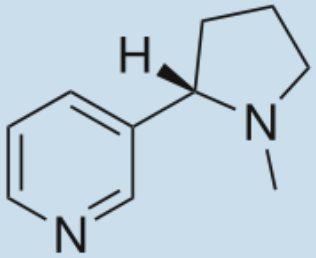
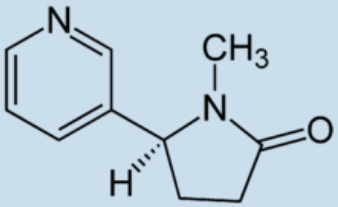
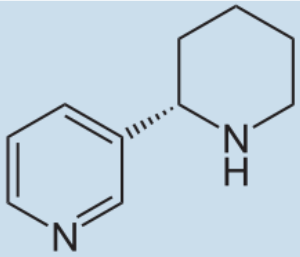
A close look at a food-testing workflow:
Botanical insecticides in fish

- LC-MS/MS Conditions
  - Column: Poroshell 120 HILIC 2.7 µm, 2.1 X 100 mm
  - LC: Agilent Infinity 1290 UHPLC
  - MS: Agilent 6460 with JetStream and ESI+
  - Mobile phase A: 10 mM ammonium formate (pH=3.0)
  - Mobile phase B: ACN
  - Flow rate: 0.7 mL/min
  - Gradient:

Time (min)	%B
0	90
4	70
4.5	70
4.6	90
6	90

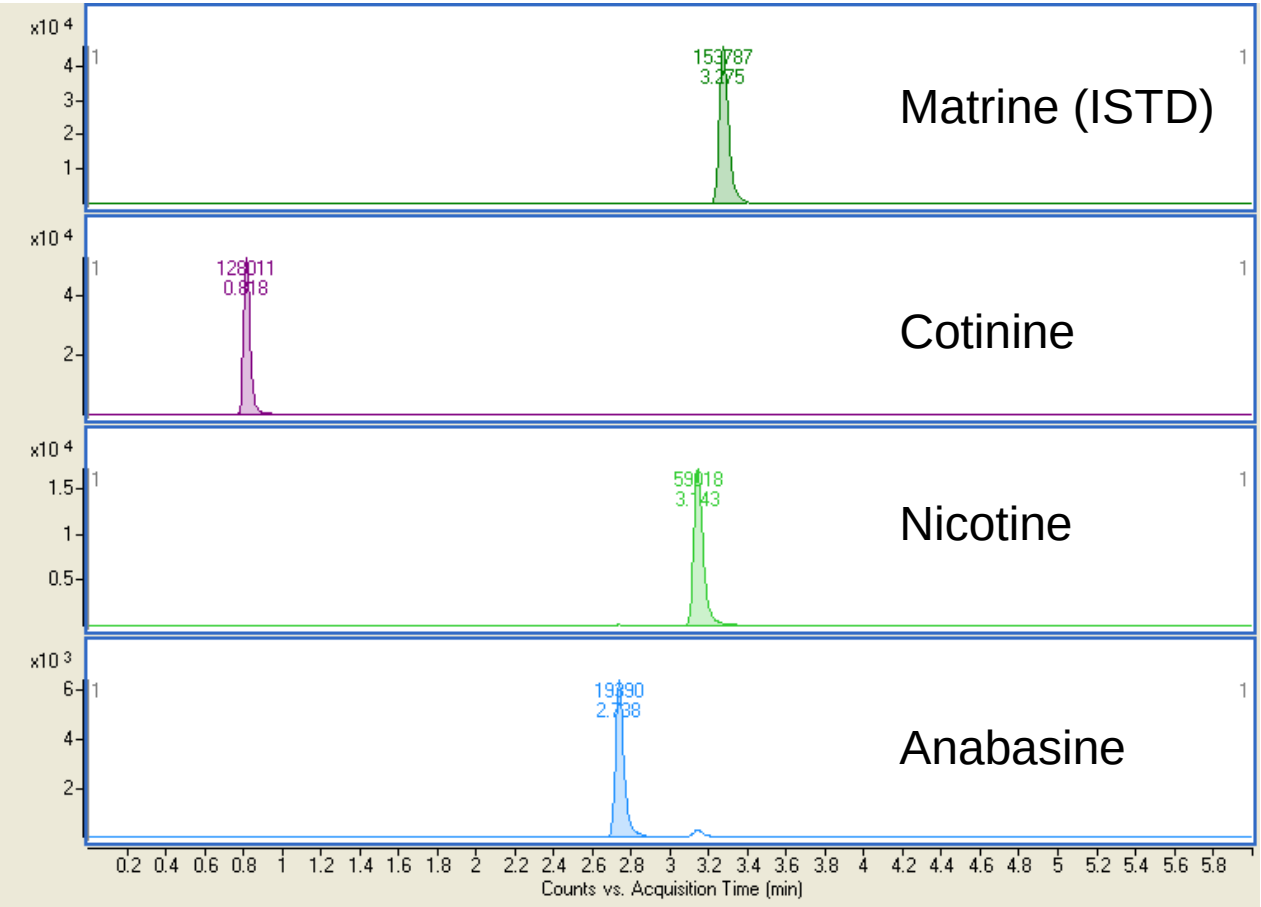
A close look at a food-testing workflow:
Botanical insecticides in fish

- Nicotine and its metabolites

	Nicotine	Cotinine	Anabasine
			
log P	1.20	0.07	1.25
pKa	8.02	8.8	11.0
MRM	163.1→132.1	177.1→80.1	163.1→118.1
Collision E	82	112	92

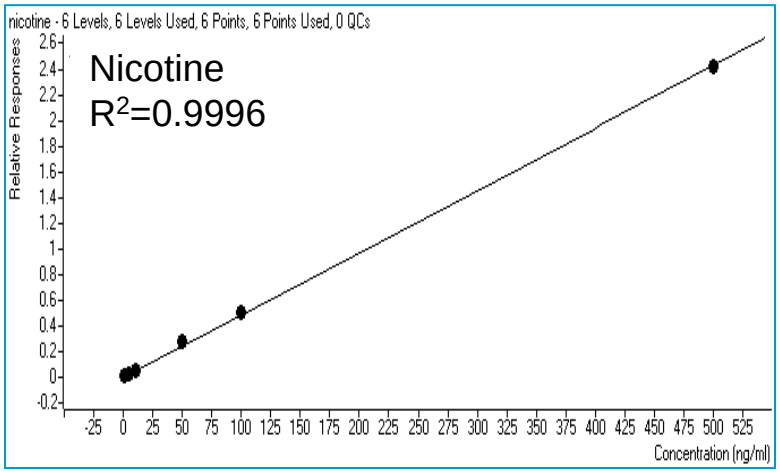
A close look at a food-testing workflow:
Botanical insecticides in fish

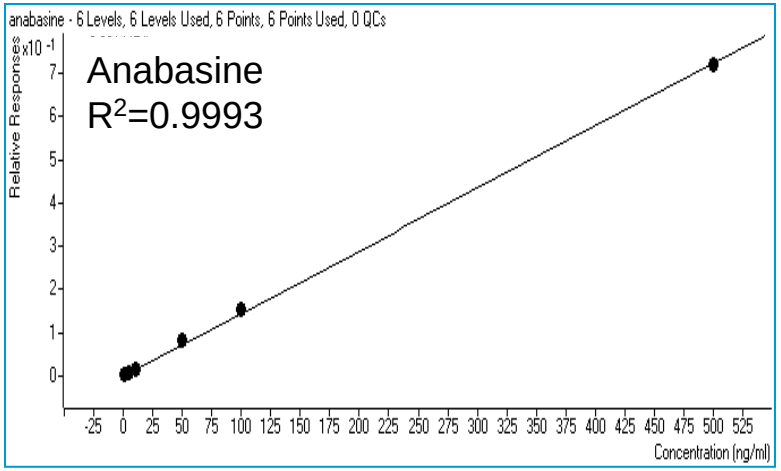
- Results – MS Chromatogram

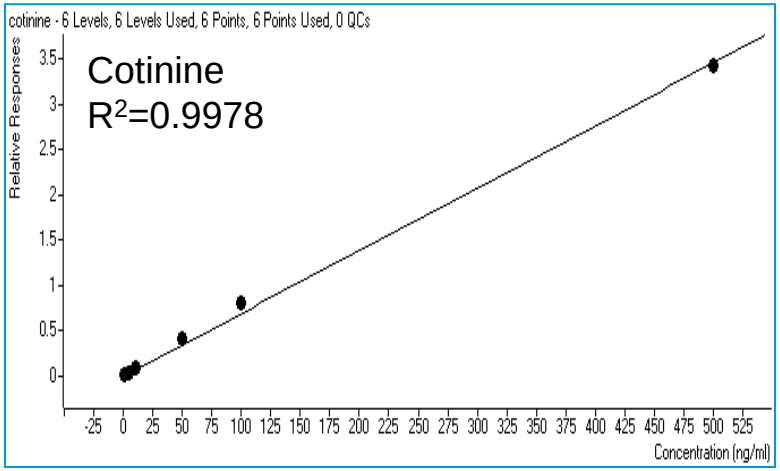


A close look at a food-testing workflow:
Botanical insecticides in fish

- Results – Calibration curves & detection limits







Calibration range 1 – 500 ng/g fish.

Great linearity.

LOD: 1 ng/g

LOQ: 5 ng/g

A close look at a food-testing workflow:
Botanical insecticides in fish

- Results – Precision & accuracy (n=8)

	Nicotine			Anabasine			Cotinine		
	Low (10 ng/g)	Mid (100 ng/g)	High (500 ng/g)	Low (10 ng/g)	Mid (100 ng/g)	High (500 ng/g)	Low (10 ng/g)	Mid (100 ng/g)	High (500 ng/g)
Average	11.4	97.7	450.2	9.2	86.4	389.1	12.8	117.0	466.9
Recovery	113.7 %	97.7 %	90.0 %	91.8 %	86.4 %	77.8 %	127.9 %	117.0 %	93.4 %
%RSD	6.4 %	1.6 %	2.1 %	6.2 %	1.6 %	2.5 %	5.4 %	2.2 %	2.1 %

Where can we make improvements?

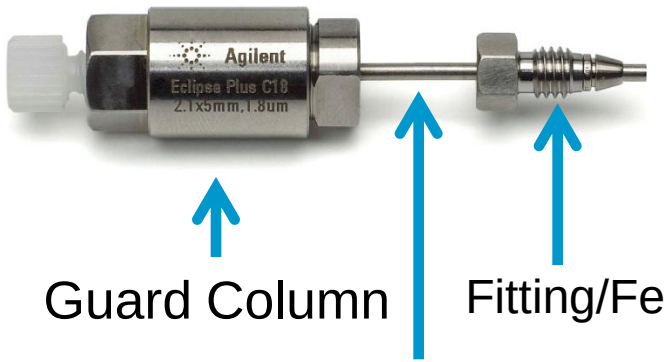
- Instrument maintenance tips
  - Add a guard column, in-line filter to protect your system.
  - Filter samples → Guarantee removal of particulates
  - ***Avoid*** high salt concentration in HILIC

    Start with 10 mM ammonium formate (pH=3.0)

Use of Guard Columns and Inline Filters

- Inline filters and guard columns extend the life of HPLC columns by preventing particulates and impurities from clogging and potentially irreversibly sticking to the analytical column
- Column lifetime is extended
- $$$ savings from fewer analytical columns purchased
- Minimal, if any, impact to the chromatography

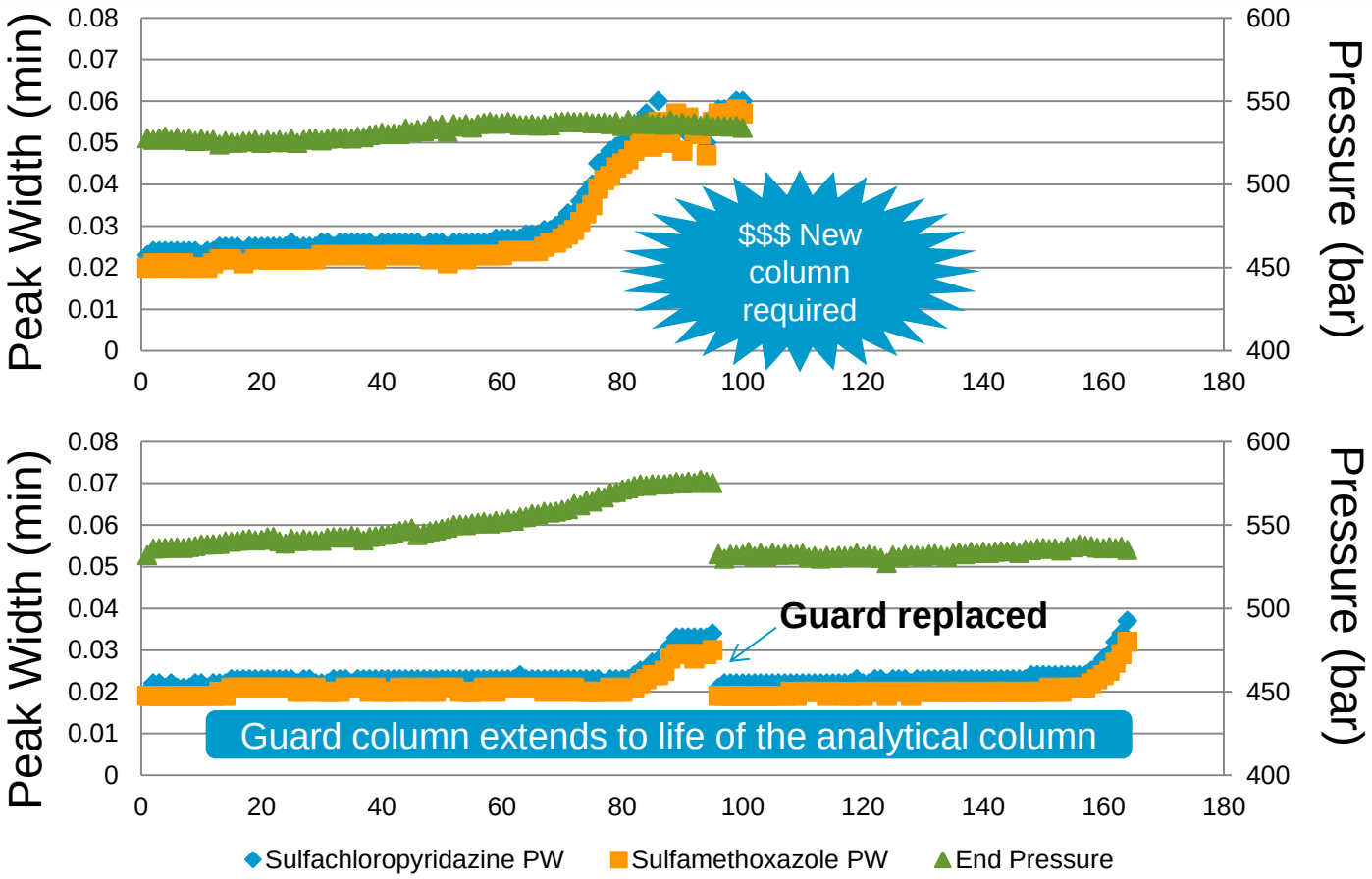
Poroshell 120
Fast Guard for UHPLC



RRLC In-line filter

Benefits of Installing a Fast Guard for UHPLC

Method: **Accelerated Lifetime Test** - Similac sample (milk substitute diluted 300:1) containing 2 sulfa drugs; Peak width change indicating column failure



No Guard

Column failure; new column required

With Guard

Guard failure; guard replaced;
same column used throughout analysis

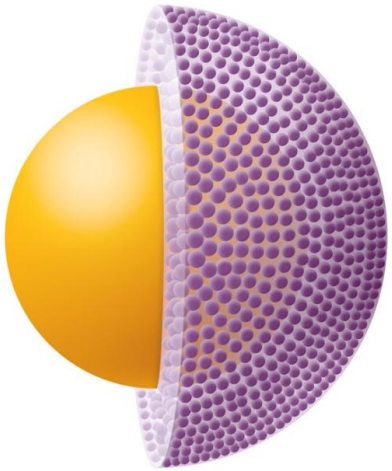
By installing a guard column when using dirtier samples, one can extend the life of their column, and utilize more inexpensive guard columns rather than column replacements

Summary—Getting to *your* trouble-free solution

- Find the best tools for you
  - Choices include traditional SPE, liquid-liquid extraction or time & cost saving newer methods such as QuEChERS
  - QuEChERS is ideal for MRL (maximum residue level) detections for many food samples and traditional SPE is good for lower level detections when needed
  - Sample filtration and guard columns to protect your LC system

Benefits of Poroshell 120 Column technology

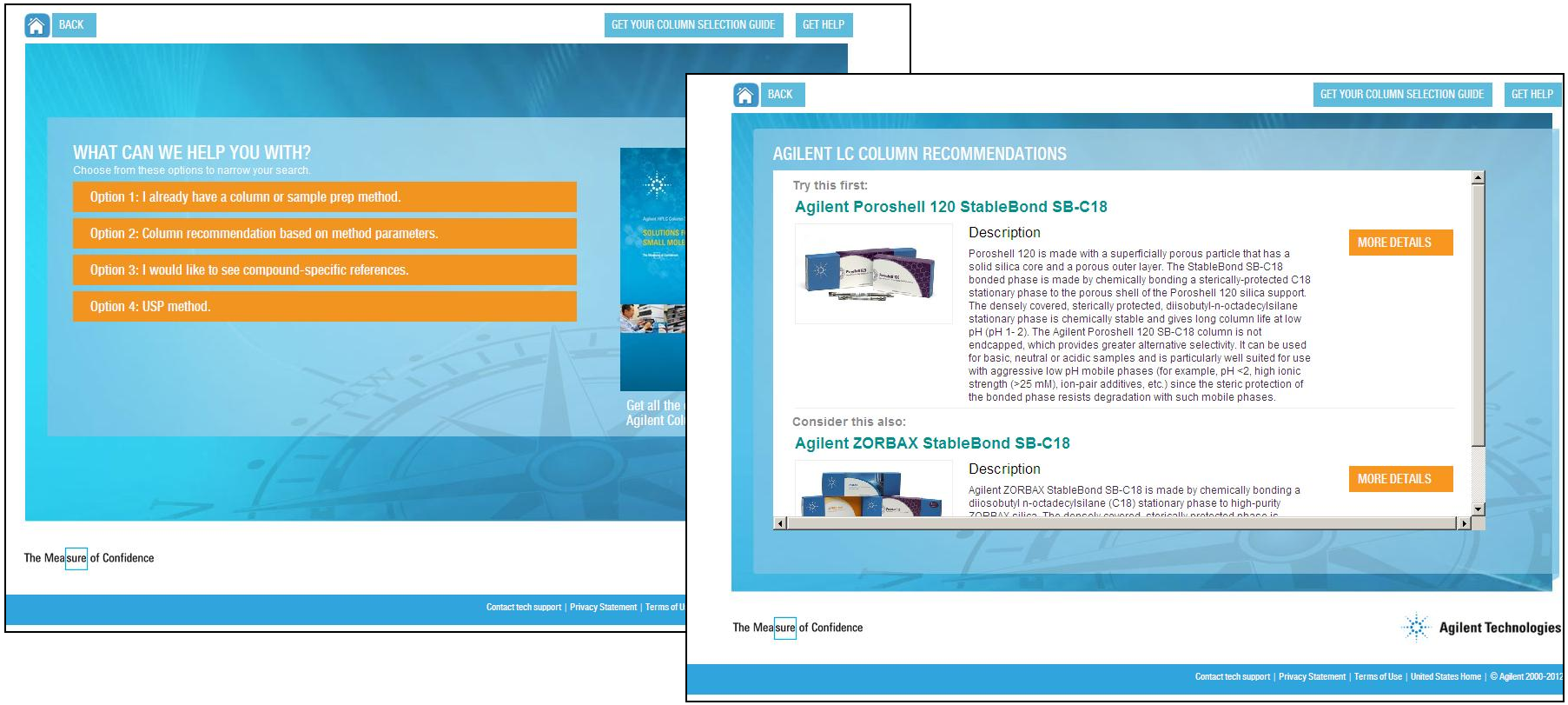
- Increased separation speeds (reduce your analysis time)
- Use with any instrument (HPLC or UHPLC)
- Reduced solvent & waste costs
- Poroshell 120
  - Scalability
  - Ease of method transfer
  - Similar phases to
    totally porous ZORBAX columns



ATool for Method Development

The Column and Sample Prep NAVIGATOR:

http://www.agilent.com/chem/navigator



Making Column and Sample Prep choices easier!

Agilent is Here to Help

See www.agilent.com/chem/cstechsupport