

# 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  $\mu\text{m}$  columns for ultra-high pressure operation
- Sub-3  $\mu\text{m}$  superficially porous columns

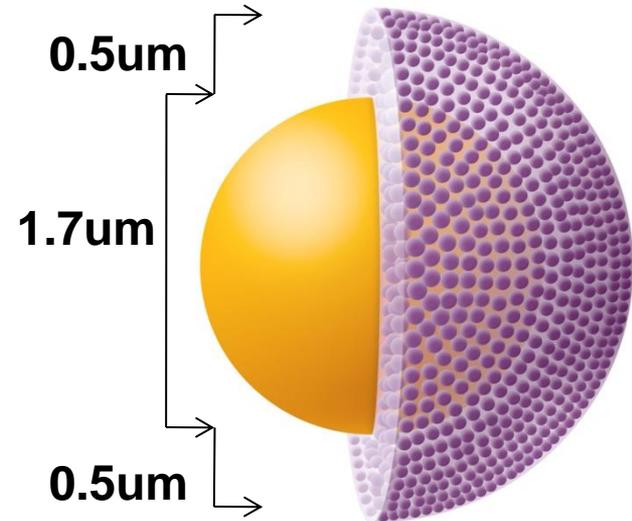
## Considerations when developing methods on new column technologies

- Particle size ( $< 3 \mu\text{m}$ )
- Column pressure limits  $>400$  bar (600-1200 bar typical)
- Other factors remain same as for legacy, 5  $\mu\text{m}$  columns

# New Superficially Porous Column Technologies

## Poroshell 120 columns:

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



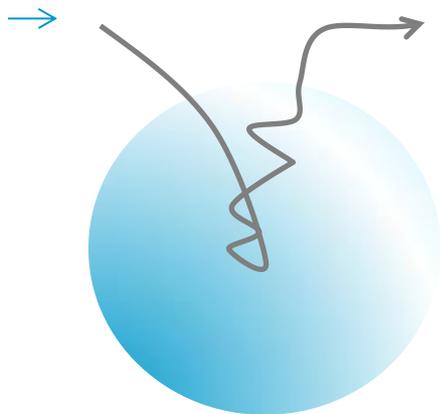
# Comparing Efficiency and Pressure with Different Types of Columns

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

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



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

van Deemter equation:

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

Superficially Porous

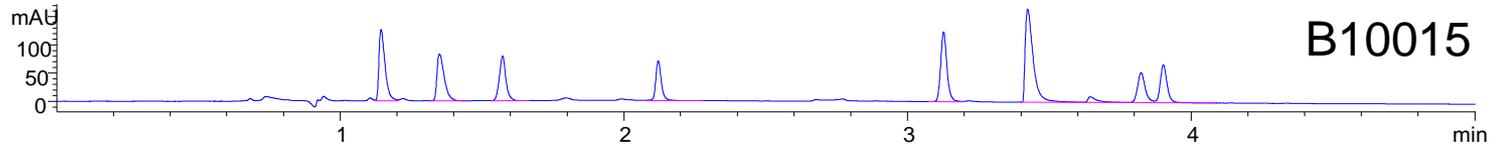


- **Results:**
  - Lower C term
  - Higher efficiency
- **And**
  - Higher flow rate with
  - Minimal impact on efficiency

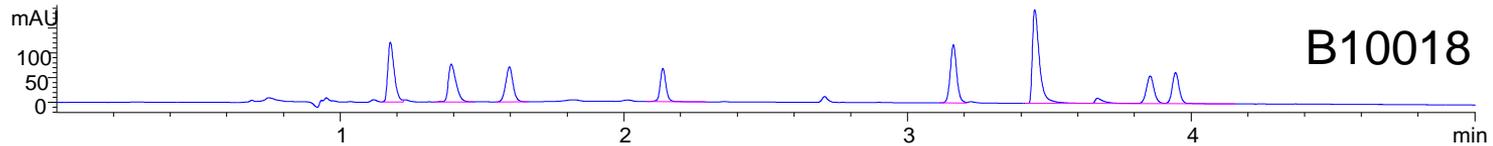
# Other Considerations when Selecting a Column

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

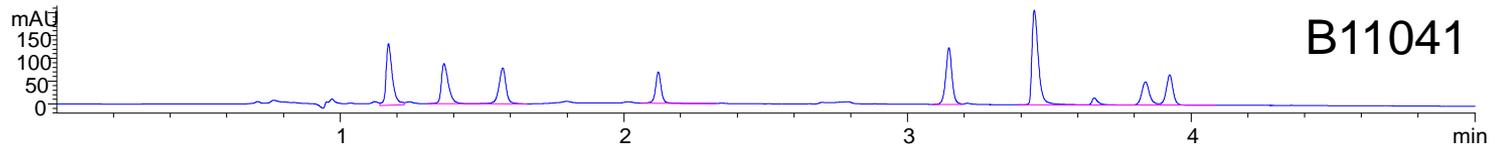
2010



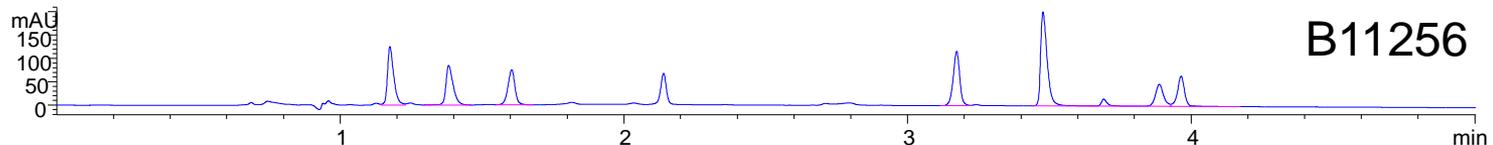
B10015



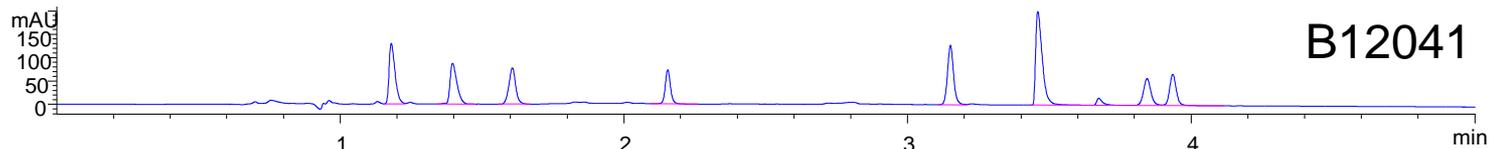
B10018



B11041



B11256



B12041

2012

**Beverage Additives**

# General Steps to a robust LC method for Food Analysis

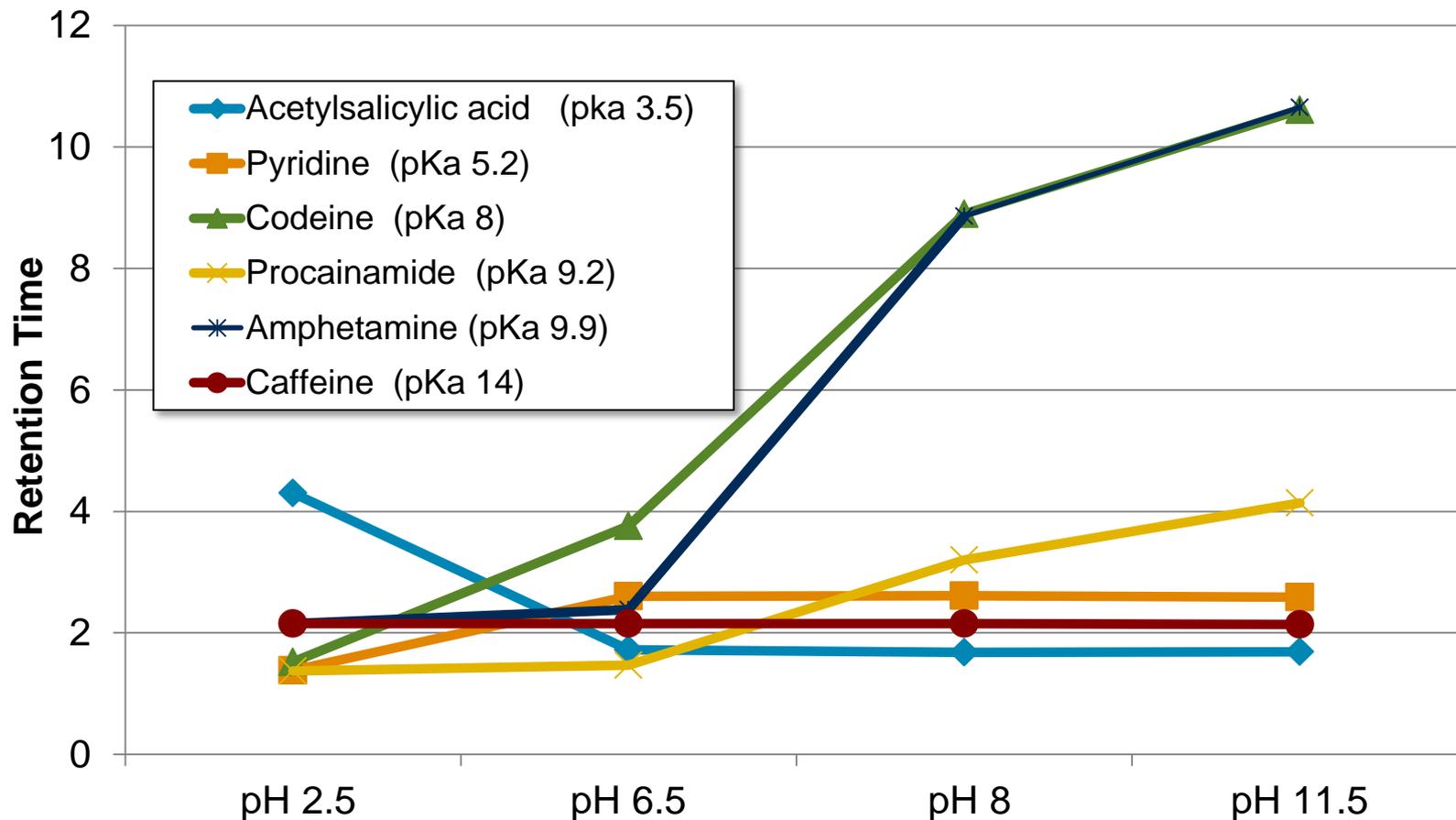
- Choose the type of chromatography – often Reversed Phase (C18, polar embedded, etc...) or HILIC phase
- Choose the appropriate starting and ending gradient conditions
- Adjust pH to shift analyte retention
- Change the bonded phase to adjust for proper peak spacing and resolution

# 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

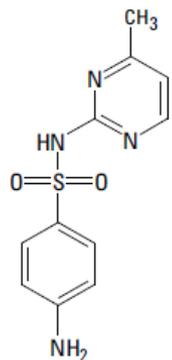


# Considerations for LCMS Method Development

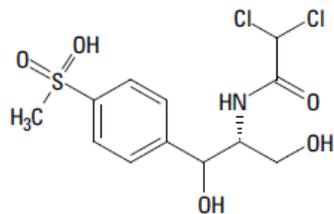
- Mobile Phase Selection
  - Gradients often preferred due to the number of analytes and differences in retention characteristics
- Buffer Choice
  - Choose a volatile buffer (formic acid, acetic acid, etc)
- Column dimensions for higher sensitivity and throughput
  - Often choose narrow columns for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



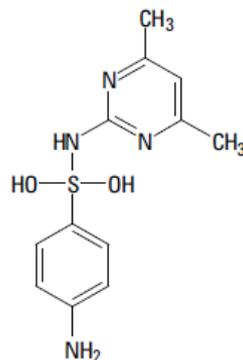
# Transfer of Existing Method for Antibiotics



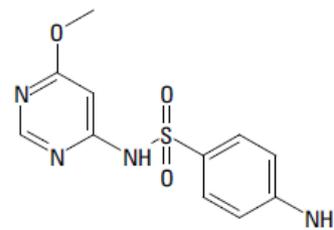
Sulfamerazine  
(SMR)



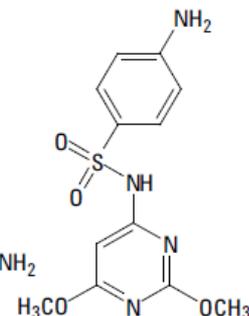
Thiamphenicol  
(TCP)



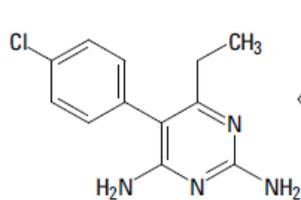
Sulfadimidine  
(SDD)



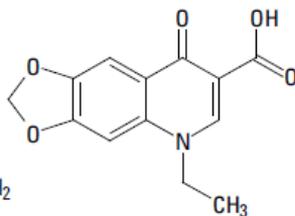
Sulfadimethoxine  
(SDMX)



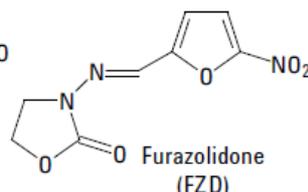
Sulfamonomethoxine  
(SMMX)



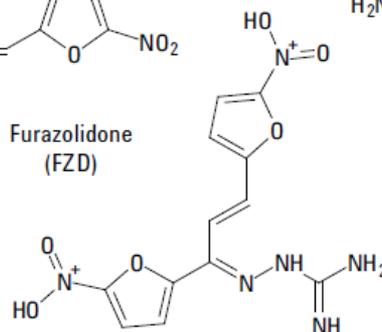
Pyrimethamine  
(PYM)



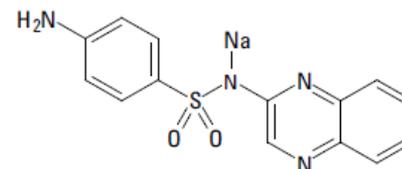
Oxolinic acid  
(OXA)



Furazolidone  
(FZD)

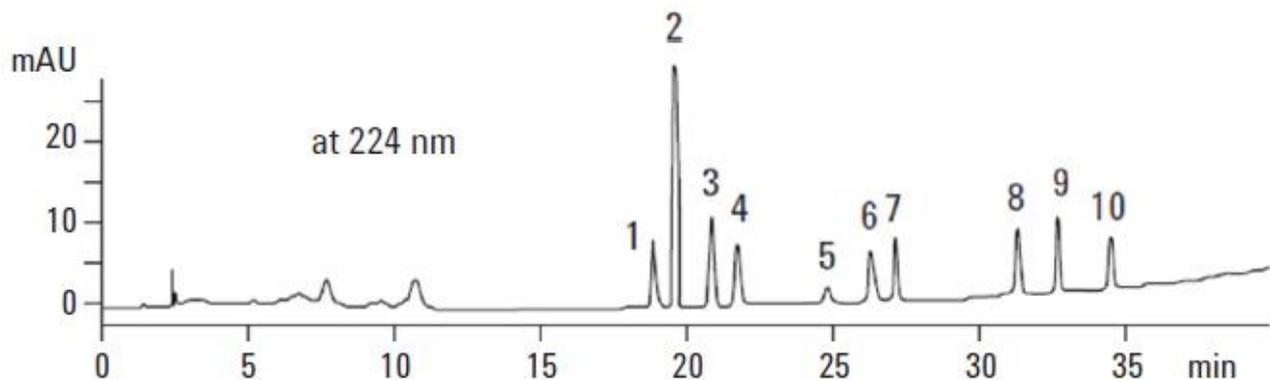


Difurazone  
(DFZ)



Sulfaquinoxaline  
(SQX)

# Transfer of Existing Method for Antibiotics

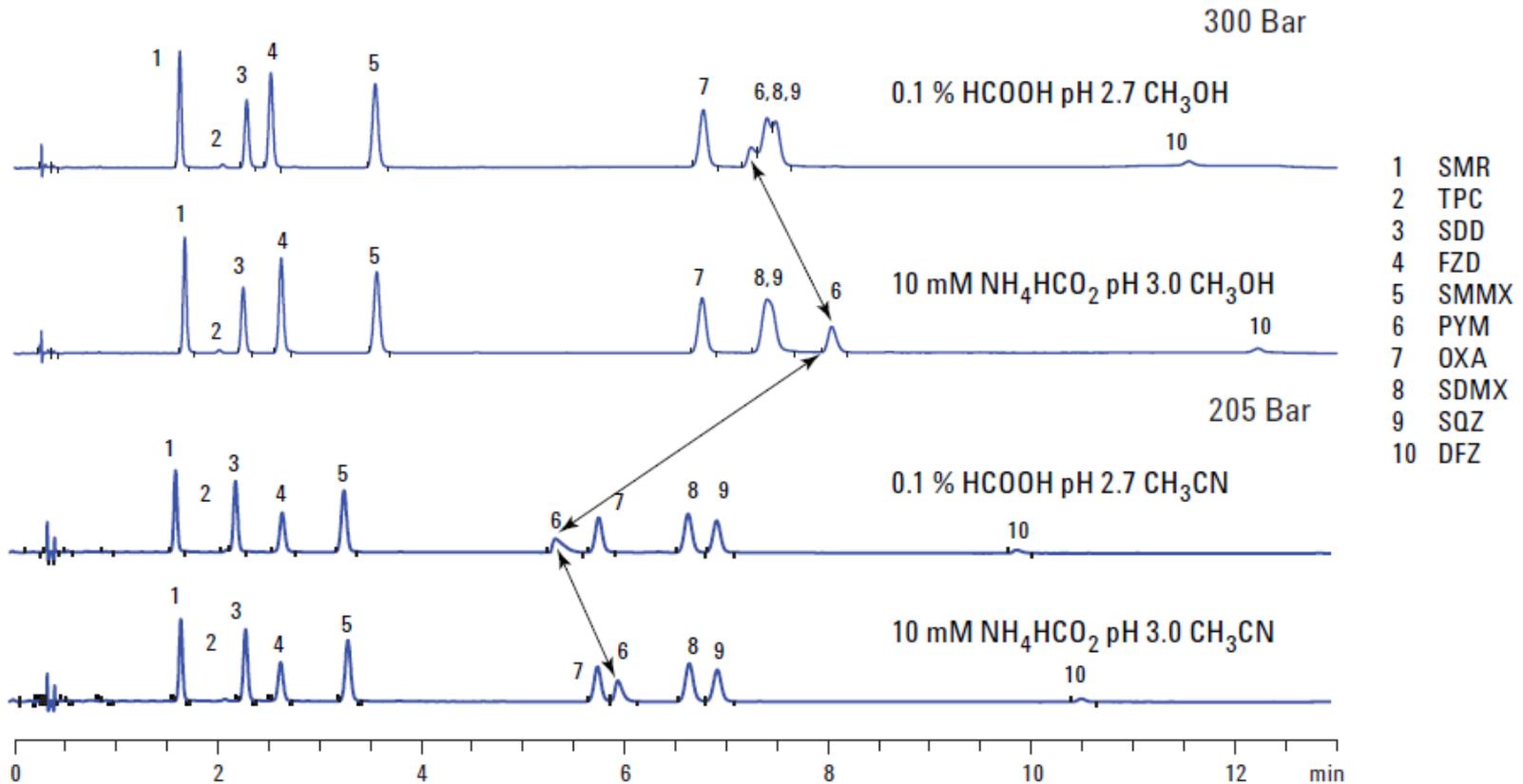


1	SMR	6	SMMX
2	PYM	7	DFZ
3	TCP	8	SDMX
4	SDD	9	SQX
5	FZD	10	OXA

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<sub>3</sub>CN  
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



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

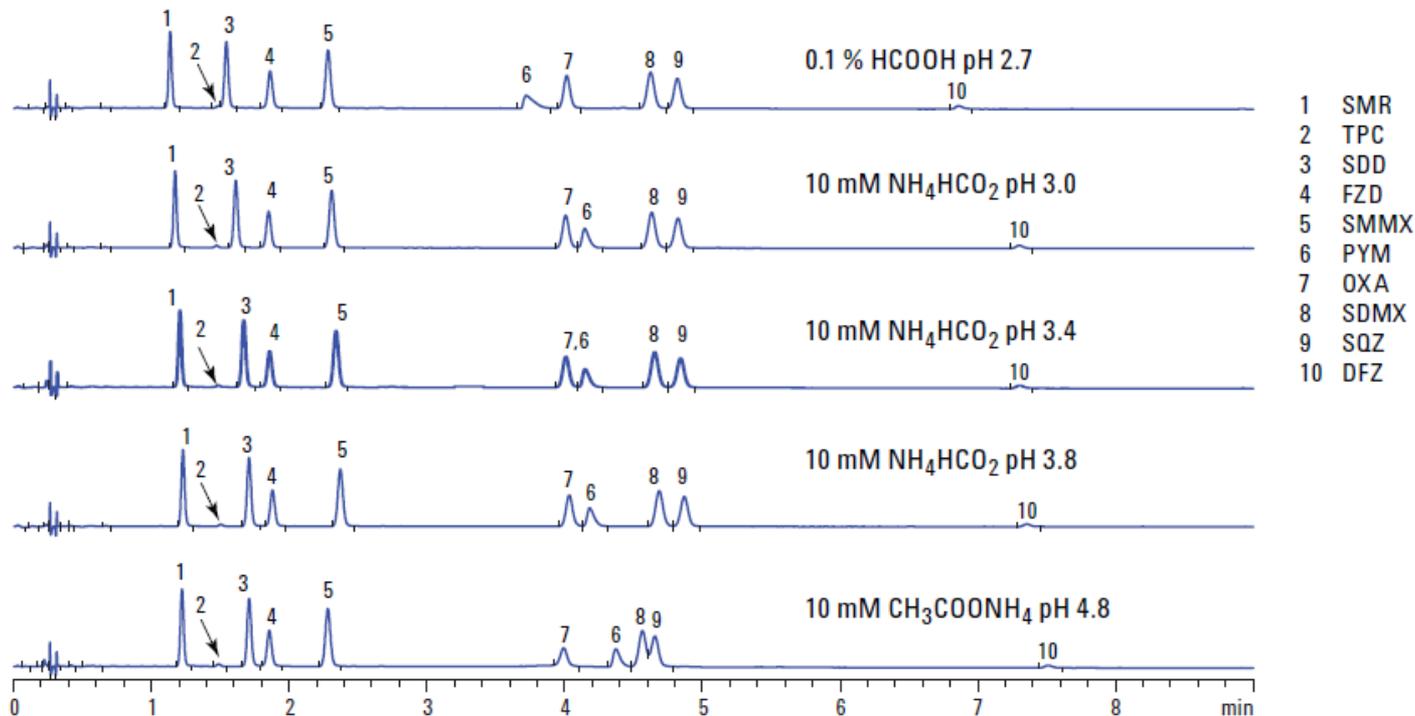
Column Temperature: 25°C; Detection: DAD 270 nm, 8 nm, ref off 3 mm, 2 uL micro flow cell; Peak width >0.05 min. (40Hz)

5990-6238EN

# Transfer of Existing Method for Antibiotics

Vary mobile phase additive, CH<sub>3</sub>CN solvent

205 Bar

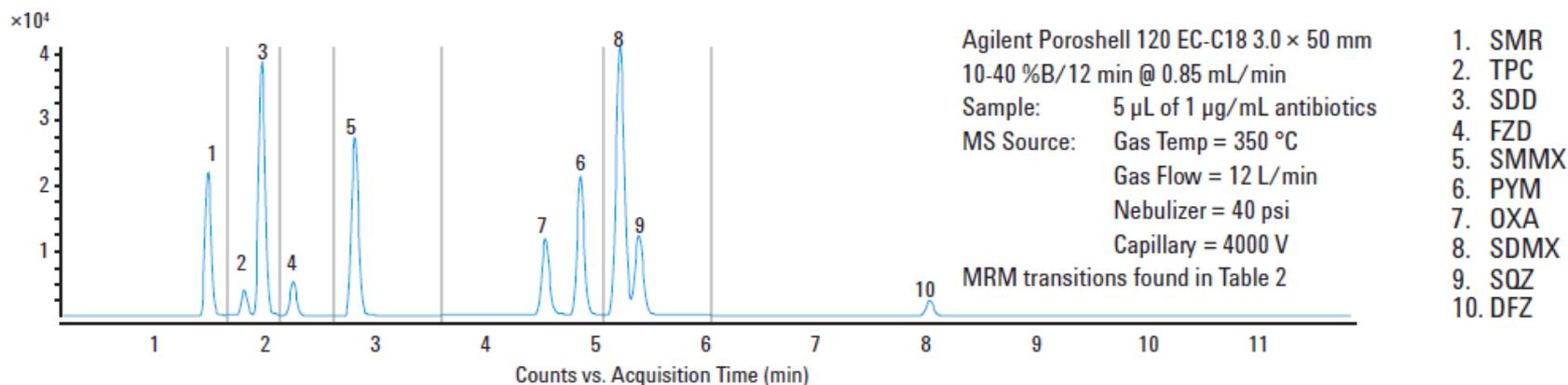


10% to 40% B/12 min at 2 mL/min  
Agilent Poroshell 120 EC-C18 4.6 mm × 50 mm, 2.7 μm

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<sub>4</sub>HCO<sub>2</sub> 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



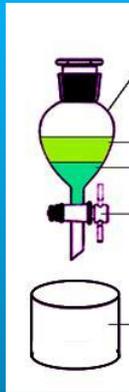
## QuEChERS

- Easy-to-use
- Ideal for food samples



## Liquid-liquid extraction

- Phase separation
- Often time consuming



## Filtration

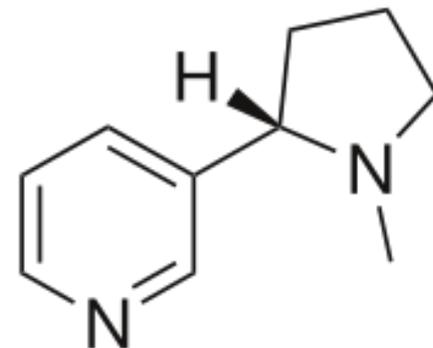
- Particulate removal
- Lipid removal



# A close look at a food-testing workflow:

## Botanical insecticides in fish

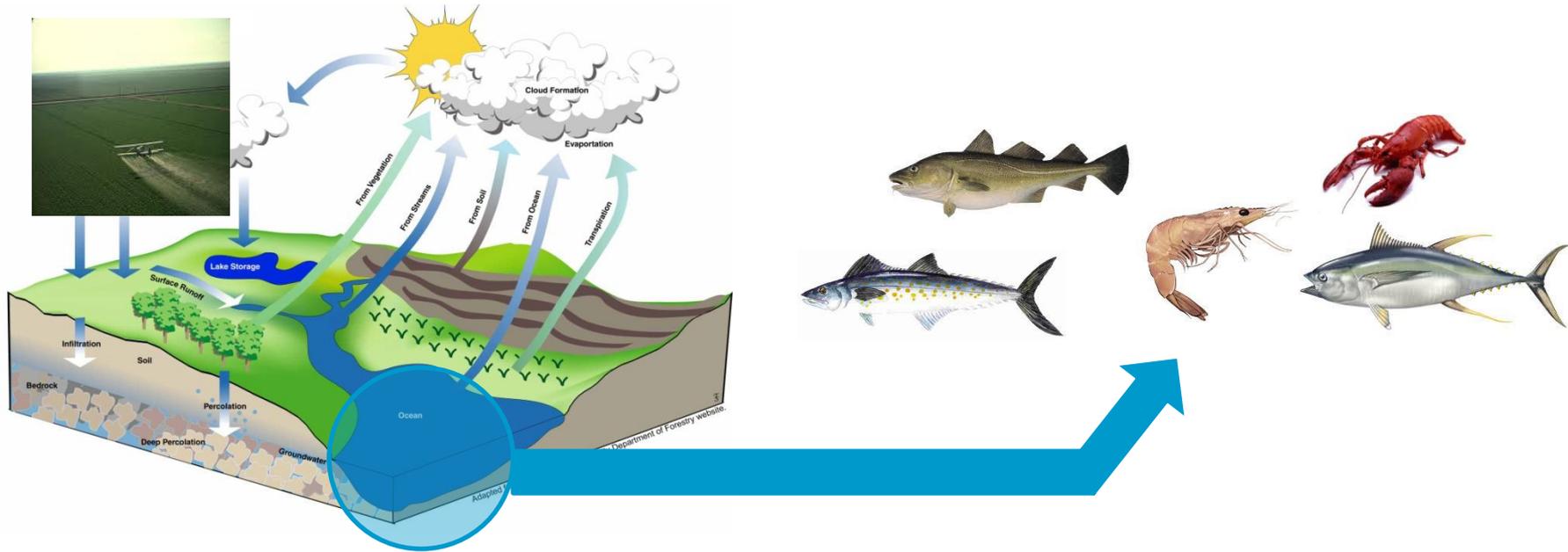
- Nicotine is used as a botanical insecticide in the US and Canada
- Banned as an insecticide in EU and will be banned in the US beginning 2014
- Oily liquid at room temperature
- Water miscible



# 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.
- **Quick, Easy, Cheap, Effective, Rugged, Safe.**

# 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
    - 1<sup>st</sup> extraction, 2<sup>nd</sup> dispersive SPE
  - Little or no method development is required
    - 1<sup>st</sup> extraction: Simply choose AOAC, EN, or original method
    - 2<sup>nd</sup> dispersive SPE: Choose one of our dispersive packets
  - Customizable to your needs

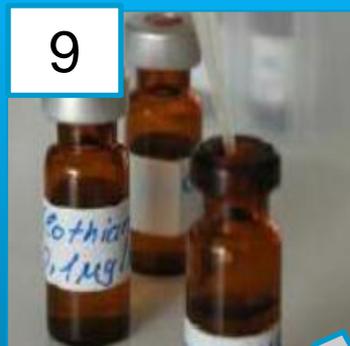
# A close look at a food-testing workflow: Botanical insecticides in fish

## 1<sup>st</sup>: Extraction



Repeat 3

## 2<sup>nd</sup>: Dispersive SPE



Repeat 5

# 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 <i>REQUIRED</i>
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  $\mu\text{m}$ , 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

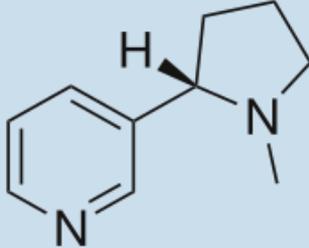
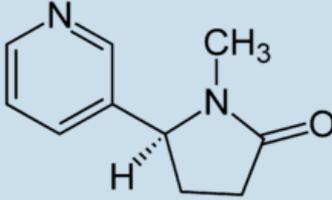
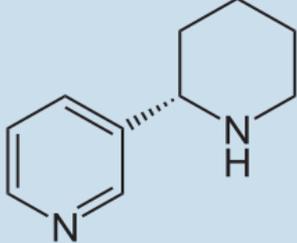
- Gradient:

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

# A close look at a food-testing workflow:

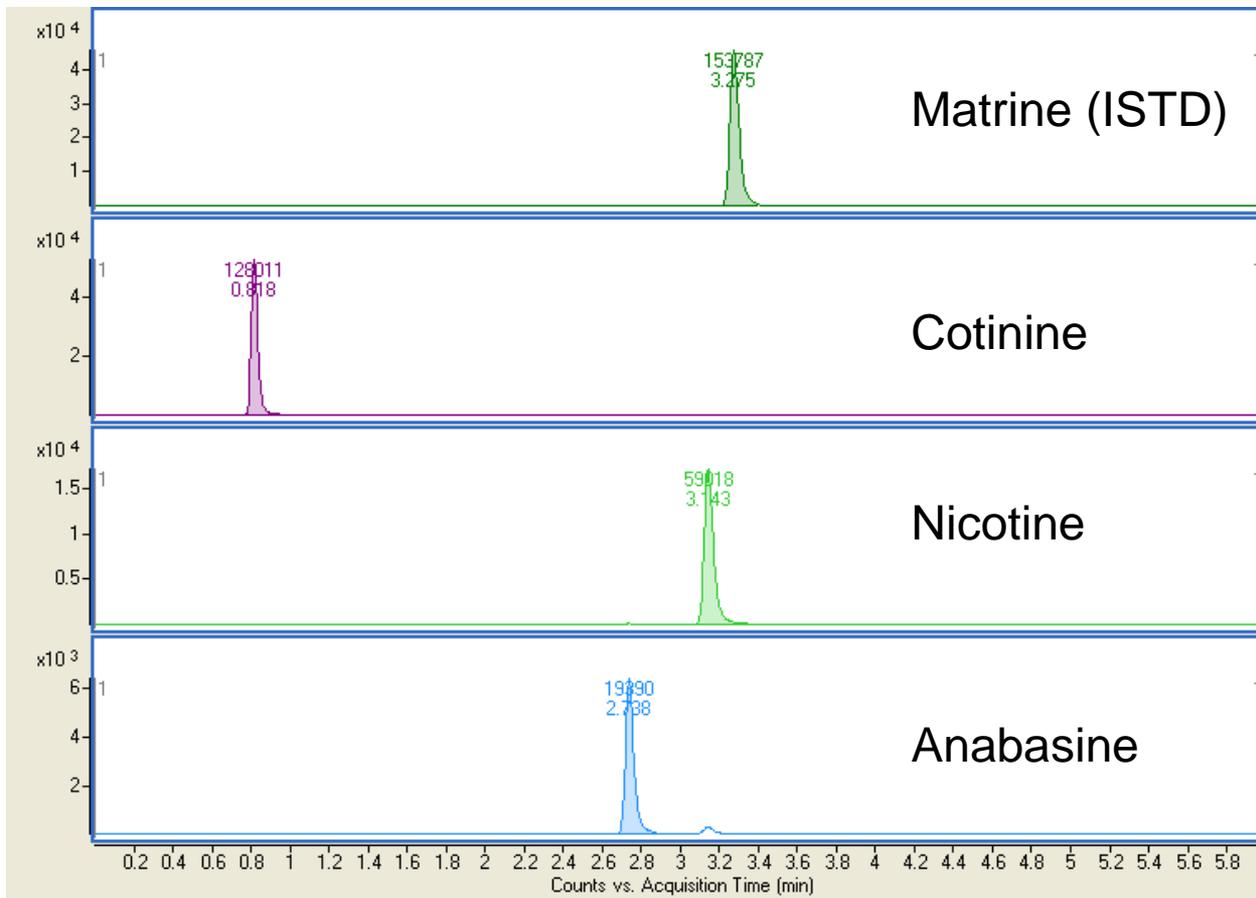
## Botanical insecticides in fish

- 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

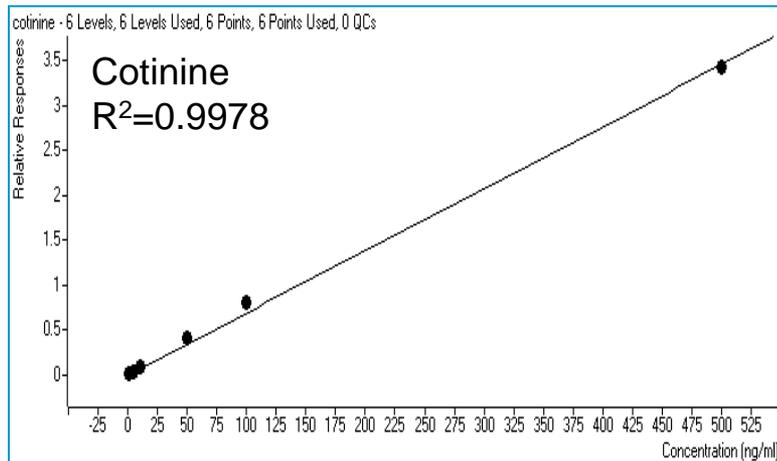
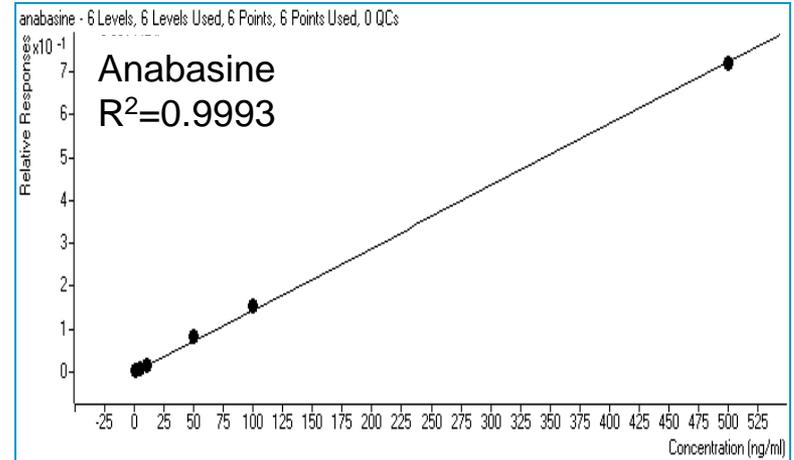
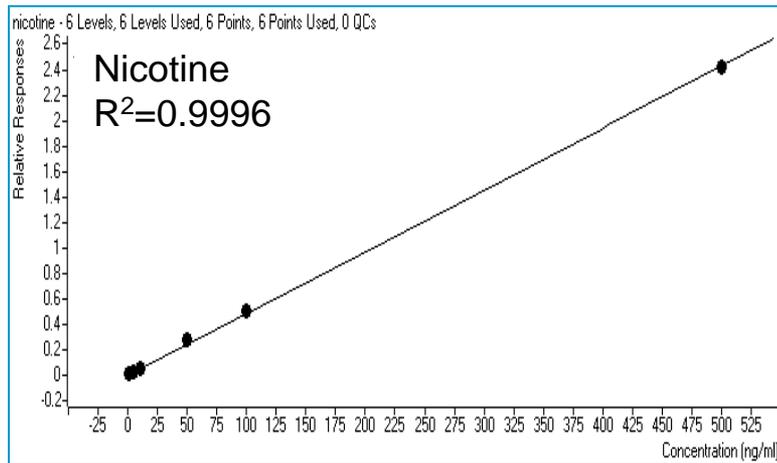
# A close look at a food-testing workflow: Botanical insecticides in fish

- 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?

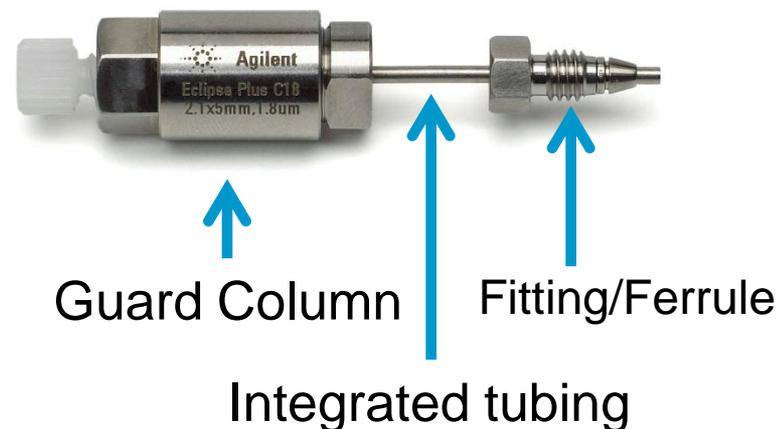
- Instrument maintenance tips
  - Add a guard column, in-line filter to protect your system.
  - Filter samples → Guarantee removal of particulates
  - **Avoid** high salt concentration in HILIC

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

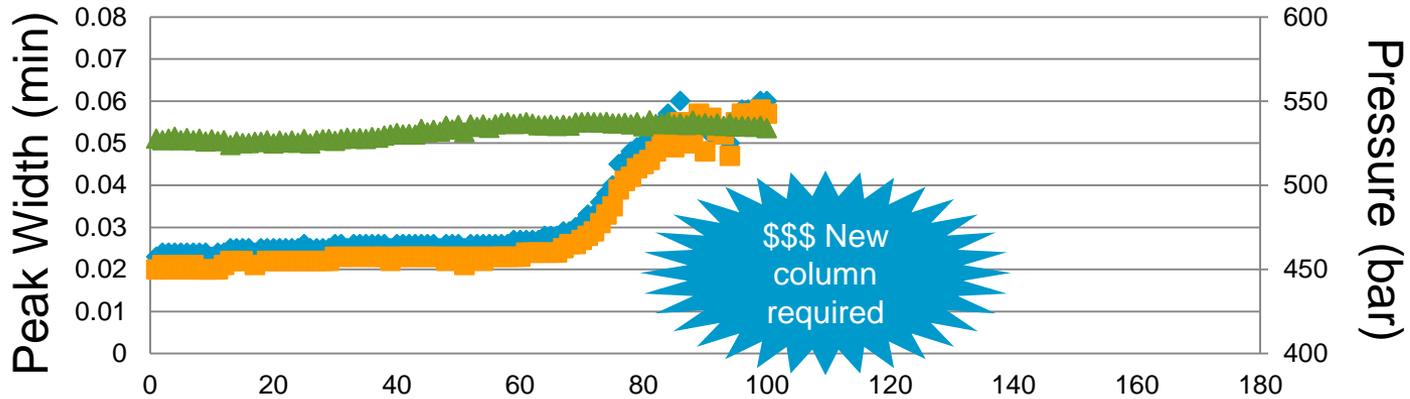
Poroshell 120  
Fast Guard for UHPLC



RRLC In-line filter

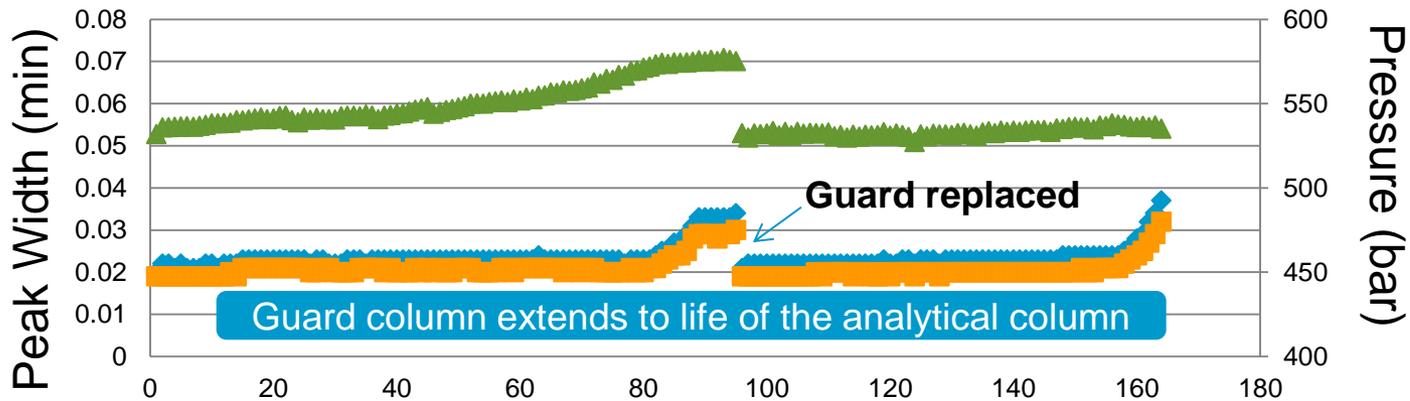
# Benefits of Installing a Fast Guard for UHPLC

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

◆ Sulfachloropyridazine PW    ■ Sulfamethoxazole PW    ▲ End Pressure

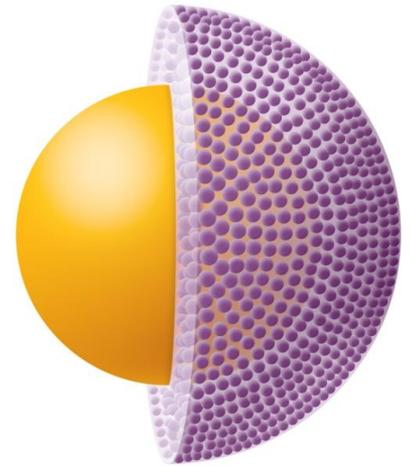
By installing a guard column when using dirtier samples, one can extend the life of their column, and utilize more inexpensive guard columns rather than column replacements

# 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

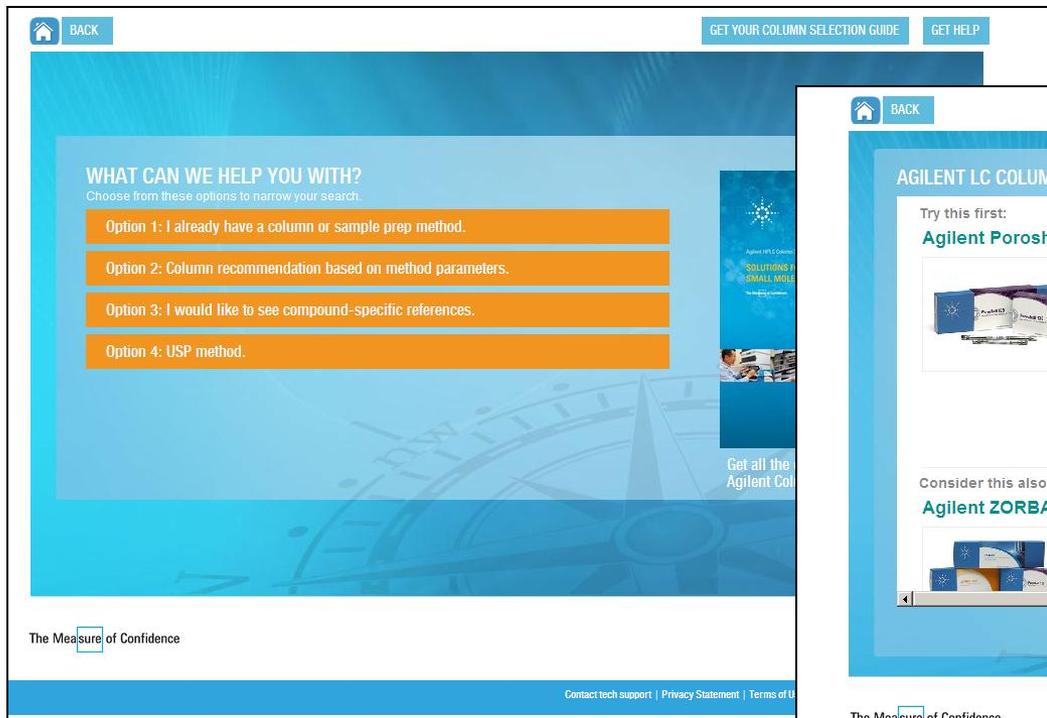
- Increased separation speeds (reduce your analysis time)
- Use with any instrument (HPLC or UHPLC)
- Reduced solvent & waste costs
- Poroshell 120
  - Scalability
  - Ease of method transfer
  - Similar phases to totally porous ZORBAX columns



# A Tool for Method Development

## The Column and Sample Prep NAVIGATOR:

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



Home BACK GET YOUR COLUMN SELECTION GUIDE GET HELP

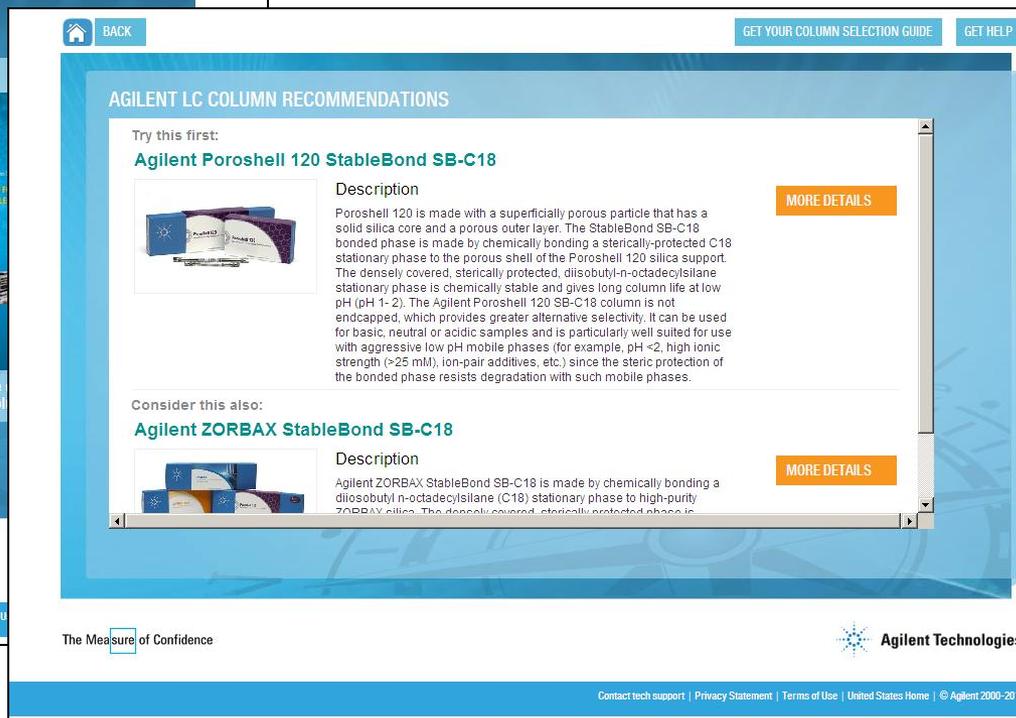
**WHAT CAN WE HELP YOU WITH?**  
Choose from these options to narrow your search.

- Option 1: I already have a column or sample prep method.
- Option 2: Column recommendation based on method parameters.
- Option 3: I would like to see compound-specific references.
- Option 4: USP method.

Get all the Agilent Col

The Measure of Confidence

Contact tech support | Privacy Statement | Terms of U



Home BACK GET YOUR COLUMN SELECTION GUIDE GET HELP

**AGILENT LC COLUMN RECOMMENDATIONS**

Try this first:

**Agilent Poroshell 120 StableBond SB-C18**

**Description**

Poroshell 120 is made with a superficially porous particle that has a solid silica core and a porous outer layer. The StableBond SB-C18 bonded phase is made by chemically bonding a sterically-protected C18 stationary phase to the porous shell of the Poroshell 120 silica support. The densely covered, sterically protected, diisobutyl-n-octadecylsilane stationary phase is chemically stable and gives long column life at low pH (pH 1-2). The Agilent Poroshell 120 SB-C18 column is not endcapped, which provides greater alternative selectivity. It can be used for basic, neutral or acidic samples and is particularly well suited for use with aggressive low pH mobile phases (for example, pH <2, high ionic strength (>25 mM), ion-pair additives, etc.) since the steric protection of the bonded phase resists degradation with such mobile phases.

**MORE DETAILS**

Consider this also:

**Agilent ZORBAX StableBond SB-C18**

**Description**

Agilent ZORBAX StableBond SB-C18 is made by chemically bonding a diisobutyl n-octadecylsilane (C18) stationary phase to high-purity ZORBAX silica. The densely covered, sterically-protected phase is

**MORE DETAILS**

The Measure of Confidence

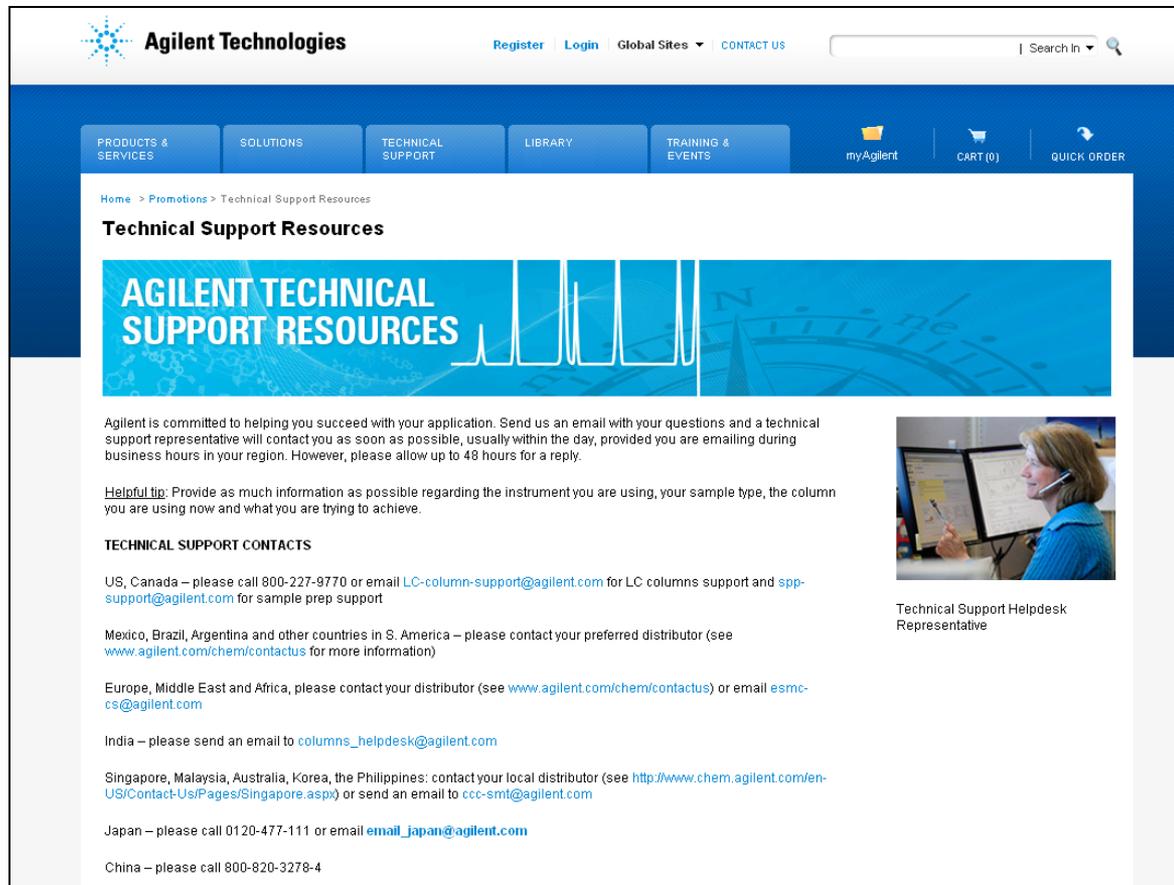
Agilent Technologies

Contact tech support | Privacy Statement | Terms of Use | United States Home | © Agilent 2000-2012

*Making Column and Sample Prep choices easier!*

# Agilent is Here to Help

See [www.agilent.com/chem/cstechsupport](http://www.agilent.com/chem/cstechsupport)



The screenshot shows the Agilent Technologies website's "Technical Support Resources" page. The header includes the Agilent logo, navigation links for Register, Login, Global Sites, and CONTACT US, and a search bar. A blue navigation bar contains links for PRODUCTS & SERVICES, SOLUTIONS, TECHNICAL SUPPORT, LIBRARY, and TRAINING & EVENTS, along with icons for myAgilent, CART (0), and QUICK ORDER. The main content area features a breadcrumb trail (Home > Promotions > Technical Support Resources) and a large blue banner with the text "AGILENT TECHNICAL SUPPORT RESOURCES" and a chromatogram. Below the banner, a paragraph states Agilent's commitment to helping users succeed with their applications. A "Helpful tip" section provides advice on providing detailed information when contacting support. The "TECHNICAL SUPPORT CONTACTS" section lists contact information for various regions: US/Canada, Mexico/Brazil/Argentina, Europe/Middle East/Africa, India, Singapore/Malaysia/Australia/Korea/Philippines, Japan, and China. A photograph of a technical support representative is shown on the right side of the page.

**Agilent Technologies** Register | Login | Global Sites | CONTACT US | Search In

PRODUCTS & SERVICES | SOLUTIONS | TECHNICAL SUPPORT | LIBRARY | TRAINING & EVENTS | myAgilent | CART (0) | QUICK ORDER

Home > Promotions > Technical Support Resources

## Technical Support Resources

### AGILENT TECHNICAL SUPPORT RESOURCES

Agilent is committed to helping you succeed with your application. Send us an email with your questions and a technical support representative will contact you as soon as possible, usually within the day, provided you are emailing during business hours in your region. However, please allow up to 48 hours for a reply.

**Helpful tip:** Provide as much information as possible regarding the instrument you are using, your sample type, the column you are using now and what you are trying to achieve.

#### TECHNICAL SUPPORT CONTACTS

US, Canada – please call 800-227-9770 or email [LC-column-support@agilent.com](mailto:LC-column-support@agilent.com) for LC columns support and [spp-support@agilent.com](mailto:spp-support@agilent.com) for sample prep support

Mexico, Brazil, Argentina and other countries in S. America – please contact your preferred distributor (see [www.agilent.com/chem/contactus](http://www.agilent.com/chem/contactus) for more information)

Europe, Middle East and Africa, please contact your distributor (see [www.agilent.com/chem/contactus](http://www.agilent.com/chem/contactus)) or email [esmc-cs@agilent.com](mailto:esmc-cs@agilent.com)

India – please send an email to [columns\\_helpdesk@agilent.com](mailto:columns_helpdesk@agilent.com)

Singapore, Malaysia, Australia, Korea, the Philippines: contact your local distributor (see <http://www.chem.agilent.com/en-US/Contact-Us/Pages/Singapore.aspx>) or send an email to [ccc-smt@agilent.com](mailto:ccc-smt@agilent.com)

Japan – please call 0120-477-111 or email [email\\_japan@agilent.com](mailto:email_japan@agilent.com)

China – please call 800-820-3278-4

Technical Support Helpdesk Representative