Method Development Today

New Tools for Established Methods

ZORBAX
High performance column, superior reproducibility and long-term stability

www.agilent.com/chem/zorbax

Rita Steed
September 17, 2013
How is Method Development Evolving?

Greater need for faster & LC/MS-compatible methods
• Newer column choices – superficially porous or sub-2 μm
• Volatile mobile phases – formic acid, acetic acid

Greater robustness expected
• Emphasis on Quality by Design
• More automated method development
• Faster with new column technologies

Minimizing sample prep when possible
• “Just enough” sample clean-up, i.e. QuEChERS for food samples,
• Protein precipitation / lipid removal (Captiva and Captiva ND Lipids)
Examples of Common Separation Goals and Method Performance Criteria

Good System Suitability Parameters

- Resolution: \( \geq 2 \)
- Peak shape: USP \( T_f \) close to 1 (<2)
- Injection Repeatability: areas, \( T_f \), etc. (RSD 0.1 - 0.25%)
- Absolute retention factors: \( 1 < k < 10 \)
- Relative Retention: \( \alpha \) or \( k_2/k_1 \)
- Signal-to-Noise Ratio: >10

Method Performance Criteria

- Accuracy
- Precision
  - Ruggedness
  - Robustness
- Selectivity/Specificity
- Linearity
- Range
- Quantitation Limit (LOQ, 10x S/N)
- Detection Limit (LOD, 3x S/N)

AVOID THESE for System Suitability Criteria:

Column efficiency (theoretical plates)

Absolute retention time

These may prevent the ability to speed up your method in the future!
What are Some Method Development Practices?

1. **Follow MD scheme:** Do “hands-on” method development
   - Based on selectivity, changing parameters
     - pH, column bonded phase, mobile phase type

2. **Implement Quality by Design; Use method development software to determine most robust method**

3. **Evaluate multiple columns & mobile phases - manually or automated**
   - Determine best results
     - Often used in conjunction with QbD approach

➢ **Practice #1 used by many - incorporates “hands-on” learning**
   - Requires nothing special
   - Review practice in light of today’s faster & more rigorous MD environment
Good Starting Point for “Hands-on” Method Development?

1. **Choose a C18 column** – available in many particle types
   a) Short columns - Minimize method development time
   b) Smaller particle size (1.8, 2.7 µm - Provide resolution needed in a short time
   c) Column choices changing/improving – **consider newer options**

2. **Choose a simple mobile phase** – reliable, works with many samples
   a) TFA, formic acid, acetate, etc. in aqueous portion
   b) Acetonitrile or methanol as organic modifier

3. **Adjust mobile phase to get desired retention and resolution**
   a) Adequate resolution of all peaks, Rs ≥ 2.0
   b) Retention of first peak at least k=1
   c) Analysis time as short as desired

**Newer column choices; Short columns, smaller particle sizes**
- More efficiency and resolution
- Quicker
- Speed up method development
Typical Parameters Selectivity, Efficiency, Retention Effects on Resolution

Selectivity Impacts Resolution Most
- Change bonded phase
- Change mobile phase

Plates are easiest to increase

\[ R_s = \frac{N^{\frac{1}{2}}}{4} \cdot \frac{(\alpha - 1)}{\alpha} \cdot \frac{k'}{(k' + 1)} \]

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<thead>
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<th>Plates</th>
<th>5000</th>
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Beyond the Start
What Process Will Method Development Follow?

Change selectivity to improve resolution with key parameters – mobile phase and bonded phase

Mobile phase (1st choice to change because it’s easy)

- Organic modifier (ACN, MeOH etc.)
- pH – over a wide pH range

Bonded phase - optimize for a robust method

- New column technologies
  - Poroshell 120 EC-C18, Eclipse Plus
    - Best peak shape performance
- May need to look beyond C18
  - C8, Phenyl, Phenyl-Hexyl, Polar-embedded, CN
Column Choices for Method Development

1. Shorter columns with smaller particle sizes

- Poroshell 120, 2.7µm superficially porous or RRHD/RRHT 1.8µm totally porous particles
  - 50-100mm lengths
- Many bonded phase choices
  - P120 Selectivity MD Kit; 2.1 x 50mm, Poroshell 120 EC-C18, Phenyl-hexyl, BonusRP
    - PN 5190-6155
  - RRHD Eclipse Plus MD Kit; 2.1 x 50mm, Eclipse Plus C18, C8, Phe-Hex
    - PN 5190-6153

2. Improved bonded phases for superior performance

- Better peak shape and efficiency result in more resolution
- Critical for best methods
- Poroshell 120 EC-C18 and Eclipse Plus deliver excellent peak shape
Superficially Porous Column Technology

Poroshell 120 columns:
- Efficiency ≈ 90% of sub-2 μm
- Pressure ≈ 40-50% of sub-2 μm
- \( N \approx 2 \times 3.5 \) μm (totally porous)
- \( d_p = 2.7 \) μm
- 2 μm frit to reduce clogging
- \( P_{\text{limit}} = 600 \) bar for HPLC or UHPLC
- Particle
  - 1.7 μm solid core
  - 0.5 μm diffusion path
  - 2.7 μm total diameter
ZORBAX Eclipse Plus columns:

- Use ZORBAX silica – improved with patented, proprietary treatments for better peak shape
- Use improved bonding reagents and processes for better peak shape
- Have tighter specifications for superior lot-to-lot reproducibility and more reliable performance
Consistent Column Selectivity
As a Function of Particle Size


Consistent Column Selectivity
As a Function of Particle Size


Consistent Column Selectivity
As a Function of Particle Size

Implementing New Column Choices in the Method Development Process?

- Use a method development process or tool based on simple strategies
- Optimize selectivity and resolution with a variety of reliable column choices

3 key parameters for method development optimization are:

- mobile phase
- mobile phase pH
- bonded phase
  - Method development kits
    - Aqueous
    - pH
    - Selectivity
Method Development Scheme – First Steps

**STEP 1**
- Choose Poroshell 120 EC-C18 or Eclipse Plus C18
  - Low pH
  - Adjust %ACN/MeOH for 0.5 < k < 20

**STEP 2**
- Change % organic

**STEP 3**
- Change organic modifier
  - Adjust % organic for 0.5 < k < 20

**STEP 4**
- Change bonded phase
  - Eclipse Plus/Poroshell 120 Phenyl-Hexyl, Bonus-RP, CN, SB-C18

- Select a high quality (Poroshell 120 EC or Eclipse Plus) C18 bonded phase first for good retention and resolution with typical acidic, basic and neutral samples.
- Optimize the organic component of the mobile phase to change selectivity.
- Choose alternate bonded phases to completely optimize method if needed.
- Evaluate other bonded phases and conditions for most robust method.

- With new superficially porous or sub-2µm columns, steps can be done quickly
  - Develop a robust method fast
Separation Conditions for D2/D3 on Poroshell 120

**HPLC:** binary pump, well plate sampler, thermostatic column compartment

**Column:** Poroshell 120 EC-C18, 2.1 x 50mm, 2.7 µm

**Column temperature:** 50°C

**Injection volume:** 10 µL

**Auto sampler temp:** 5°C

**Needle wash:** flush port (50:25:25, IPA:MeOH:H2O) 5 seconds

**Mobile phase:**
- A = H₂O + 0.1% Formic Acid
- B = MeOH + 0.1% Formic Acid

**Flow rate:** 0.5 mL/min

**Isocratic Analysis:**
- A = 20%
- B = 80%

**Analysis Time:** 5.0 min
Isocratic Optimization of D2/D3 on Poroshell 120

Column: Poroshell 120 EC-C18, 2.1 x 50mm, 2.7 µm  Mobile Phase: A:0.1% Formic Acid

- Vary Conditions
- Make either 5% or 10% changes in organic to optimize

• Compare speed of separation versus chromatographic resolution

90% MeOH 3 min

80% MeOH 5 min

85% MeOH 3 Min
Let’s Look at Method Development of These Compounds

propranolol

pindolol

dipyridamole

β-blocker

Anti-arrhythmic

Vasodilator

Ca+ channel blocker
Start at Low pH, Adjust Organic - Acetonitrile

Column: ZORBAX RRHT Eclipse Plus C18, 4.6 x 50 mm, 1.8 μm
Mobile Phase: A: 25 mM NaH₂PO₄, pH 3.0  B: ACN
Flow Rate: 2.0 mL/min  Temperature: 30°C  Detection: UV 240 nm

40% ACN

30% ACN

20% ACN

- Good resolution
- Fast analysis
- No time wasted

To get this k on a 25cm column at 2 mL/min would require 1.5 hours run time!!

k= 44!!

Using RRHT Eclipse Plus C18, optimize % organic in mobile phase fast
Why We Recommend Changing Organic Modifier Before Changing Bonded Phase

- Easy
- Acetonitrile and methanol are readily available
- Works on any bonded phase - A tool to optimize your separation no matter the column choice
- Selectivity changes
Same Sample, Same Column, Same Conditions - *Except* Change Organic Modifier - **Methanol**

**Column:** ZORBAX RRHT Eclipse Plus C18, 4.6 x 50 mm, 1.8 µm  
**Mobile Phase:** A: 25 mM NaH₂PO₄, pH 3.0  B: MeOH  
**Flow Rate:** 2.0 mL/min  **Temperature:** 30°C  **Detection:** UV 240 nm  
**Sample:** Cardiac Drugs  1. Pindolol  2. Diisopyridamide  3. Propranolol  4. Diltiazem  5. Dipyridamole

- Adjust MeOH; retention vs. ACN
- MeOH changes selectivity & gives longer analysis time for these analytes
- Compare solvents quickly with short, RRHT/RRHD columns

\[ k = 49!! \]
Method Development - Change Organic Modifier

Comparison of Acetonitrile and MeOH

**Column:** ZORBAX RRHT Eclipse Plus C18, 4.6 x 50 mm, 1.8 μm

**Mobile Phase:** A: 25 mM NaH₂PO₄, pH 3.0  B: organic

**Flow Rate:** 2.0 mL/min  **Temperature:** 30°C  **Detection:** UV 240 nm

**Sample:** Cardiac Drugs 1. Pindolol 2. Disopyridamide 3. Propranolol 4. Diltiazem 5. Dipyridamole

**Resolution of pairs**

(2,3), (3,4), (4,5) is better in MeOH.

**Peak 5 selectivity shift**

Resolution of critical pair (1,2), is better in ACN

50% MeOH

30% ACN
Why Is Changing the Bonded Phase Effective?

- Differences in interactions between polar and non-polar compounds.
- Other types of interactions with a bonded phase can be exploited (pi-pi interactions etc.)

*These all change with bonded phase!*

- Changing the bonded phase can
- Improve selectivity/resolution, reduce analysis time

**Compare bonded phases quickly – Use short, smaller particle size columns like Poroshell 120 or RRHD/T (1.8um)**

- Multiple column choices available with different high speed technologies make this easy
Why Is Changing the Bonded Phase Effective?

- Differences in interactions between polar and non-polar compounds.
- Other types of interactions with a bonded phase can be exploited (pi-pi interactions etc.)

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  - improve selectivity/resolution
  - reduce analysis time

**Compare bonded phases quickly – Use short, smaller particle size columns like Poroshell 120 or RRHD/T (1.8µm)**
Evaluate Bonded Phase Selectivity Quickly
Use High Efficiency Short Columns

Columns: 4.6 x 50mm, 1.8um Mobile Phase 40 % ACN 60 % 25 mM Sodium Phosphate Buffer pH 2.4 Flow Rate: 1.5 ml/min  UV 210 nm 2µl Elution order for Eclipse Plus Phenyl Hexyl: (1) Piroxicam, (2) Sulindac,(3) Tolmetin, (4) Naproxen, (5) Ibuprofen, (6) Diclofenac, (7) Celebrex (equal portions of approximately 1 mg/ml solutions

Elution order reversal between Phenyl-Hexyl and Alkyl chains

Eclipse Plus Phenyl Hexyl

Eclipse Plus C8

Eclipse Plus C18
Bonded Phase Comparison - 8 Steroids

Best Resolution of all analytes with Poroshell 120 Phenyl-Hexyl

Poroshell 120 EC-C18

Poroshell 120 SB-C18

Poroshell 120 Phenyl Hexyl

Poroshell 120 Bonus RP

Bonded Phase Comparison - Beta Blockers

1. Atenolol
2. Pindolol
3. Naldolol
4. Metoprolol
5. Acebutolol
6. Propranolol
7. Alprenolol
Selectivity: Comparison of Sulfa Drugs
Different Phases with 0.1 % Formic Acid:ACN

1. Sulfadiazine
2. Sulfathiazole
3. Sulfapyridine
4. Sulfamerazine
5. Sulfamethazine
6. Sulfamethizole
7. Sulfamethoxypyridazine
8. Sulfachloropyridazine
9. Sulfamethoxazole
10. Sulfamethoxine

Eclipse Plus C18
3 x 100 mm

Eclipse Plus Phenyl Hexyl
3 x 100 mm

Bonus RP
3 x 100 mm

Method Development Today
Agilent Restricted
9/17/2013
Use pH and Mobile Phase to Adjust Peak Spacing

- pH sensitive compounds
- pH can affect retention and resolution
- How pH influences method development strategy?
- How does pH influence column choice?

Learn more about the new Agilent Electrochemistry family at www.agilent.com/chem/Agilentph
When Does pH Affect Resolution?
Compound Type Comparison

- **ionizable** compounds – acids and bases can change retention and selectivity most with changes in pH
Key to Method Development
Change in Retention with pH for Ionizable Compounds

• 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 and Eclipse Plus 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
Method Development Scheme – Evaluating Mid pH

From low pH

STEP 5
- Poroshell 120 EC-C18 or Eclipse Plus C18
  - pH 7 (6-9) acetate or other buffer,
  - Adjust % ACN for 0.5 < k < 20

More resolution needed

STEP 6
- Change % organic

More resolution needed

STEP 7
- Change organic modifier (MeOH)
  - Adjust % organic for 0.5 < k < 20
  - Restart at STEP 6

More resolution needed

STEP 8
- Try Eclipse Plus Phenyl-Hexyl, Bonus-RP or Poroshell 120 EC-C8
  - Restart at STEP 5

- Mid pH can provide better selectivity
- May be more compatible with sample
- Process for investigating mid pH is the same as for low pH
- Poroshell 120 EC and Eclipse Plus, good choices at mid pH - outstanding peak shape and lifetime
- Alternate bonded phases should also be considered if improved selectivity is desired
Eclipse Plus Wide pH Range (pH 2 – 9) Optimize Selectivity

pH 2.7 0.1% formic acid/ACN

pH 4.8 NH₄OAc/ACN

pH 7 NaPO₄/ACN

Selectivity and resolution can change with pH

Eclipse Plus can be used with many mobile phases and pHs

Conditions: Column: Eclipse Plus C18 Gradient: 10 – 90% in 10 minutes Detection: UV 254 nm

1. procainamide
2. buspirone
3. pioglitazone
4. eletriptan
5. dipyridamole
6. diltiazem,
7. furosemide
Selectivity Differences at Mid pH - Key to $R_s$

Eclipse Plus C18 4.6 x 75 mm 3.5 µm
20 mM phosphate buffer, pH 3.0

1. Famotidine
2. Cimetidine
3. Pirenzipine

N=7900
TF: 1.05

N=8000
TF: 0.99

Eclipse Plus C18 4.6 x 75 mm 3.5 µm
20 mM phosphate buffer, pH 7.0

N=5700
TF: 1.28

Resolution is not possible at pH 3, but is at pH 7
Eclipse Plus was used to experiment at both pH’s

Columns: as listed
Mobile Phase: 20% MeOH, 80%
20 mM phosphate, pH as listed
Flow Rate: 1 mL/min.
Detection: UV 230 semi micro flow cell

Agilent Technologies
Method Development at High pH – a Third Choice

From Mid pH

STEP 9
• ZORBAX Extend-C18
  • pH 10.5 (9-12) 5 mM ammonia, or TEA, or 10 – 50 mM organic or borate buffers
  • T = 25°C (ambient – 40°C)
  • Adjust MeOH for 0.5 < k < 20

More resolution needed

STEP 10
• Change organic modifier (ACN or THF)
  • Adjust for 0.5 < k<20

Try different HPLC mode - HILIC

Reasons to Consider High pH

➤ Increase retention of basic compounds by analyzing them in non-charged form
➤ Improve selectivity

Reasons *Not* to Choose High pH

➤ Fewer good additives for LC/MS
➤ Compromises column lifetime
➤ Harsher on LC system
Robust Method Develop Using Quality by Design Approach

Screen to evaluate variables initially to determine which ones impact the method.

• Make list of potential method variables and prepare to test them
• Automatic “MD system” can be most efficient way to test different conditions

Determine any interactions/limitations between key variables (i.e. pH, resolution and column lifetime)

• May require more testing

Optimize – develop model to find useful operating conditions

• Will likely have sufficient data from the evaluation stage to complete this
• Easiest if a software solution, like ChromSword can be used

e-seminars

➢ Demystifying the Chromatographic Process
➢ Systematic Approach vs. Random Walk

What LC Method Parameters are Usually Screened and Evaluated

<table>
<thead>
<tr>
<th>General Parameter</th>
<th>Some Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Selectivity choices – must be wide range and evaluate for resolution of critical components to meet method goals</td>
</tr>
<tr>
<td></td>
<td>Column lot – consider under robustness testing</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>pH, buffer strength, organic (choice, range, etc.)</td>
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<tr>
<td>Mobile Phase Gradient</td>
<td>Test 2 very different gradient times to test sensitivity to gradient steepness</td>
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<td>Column Temperature</td>
<td>Working within a reasonable range for column</td>
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<tr>
<td>Injection Volume</td>
<td>Peak shape, tailing factor changes with sample load</td>
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<tr>
<td></td>
<td>Sensitivity adequate for method goals</td>
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<tr>
<td>Detection Wavelength</td>
<td>Evaluate for sensitivity and appropriateness of choice to deliver consistent peak height, area</td>
</tr>
<tr>
<td>Dwell volume</td>
<td>Must measure and should check for retention/resolution changes with change in dwell volume</td>
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<tr>
<td>Sample Preparation</td>
<td>Impact on method performance, selectivity, specificity</td>
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</table>
What Does Agilent have to Assist in QbD?

Wide range of reliable LC/UHPLC columns

Method development approach to make the development process effective

– Approach we just went through – considering pH, organic solvents, and bonded phases

Method Development LC

– Allows for method scouting and automated testing of all the experimental method variables.

Software solutions for QbD Robustness Testing
A **1290 Infinity TCC Cluster** is the heart of the Agilent Multi-method solution for automated column selection for Method Development and Multi-Method Applications.
Experimental Strategy and Details

4 Columns
- SB-C18
- Eclipse Plus C18
- Bonus RP
- Eclipse Plus Phenyl Hexyl

6 Buffers
- 0.1 % TFA (pH 1.8)
- 0.1 % Formic Acid (pH 2.8)
- 0.1 % Acetic Acid (pH 3.8)
- 10 mm CH₃COONH₄ (pH 4.8)
- 10 mM CH₃COONH₄ (pH 6.5)
- Water

24 Scouting Experiments
- 2ml/min 4.6 x 50 mm RRHT 25 C
- 1200 Method Development Solution
- Acetonitrile
- 3 min scouting gradient
Quality by Design – ChromSword AutoRobust

- AutoRobust controls the HPLC system and performs the ROBUSTNESS experiments fully automatically.

- Designing experiments for the evaluation of HPLC method robustness is easily programmable with the AutoRobust Wizard.

- Results of experiments can be rapidly browsed and a report is generated automatically with Report Viewer.

- AutoRobust can be used as stand-alone program or add-on to the ChromSword® Auto software
Quick Summary of Families and Bonded Phases for Method Development

**Poroshell 120**
- Start with Poroshell 120 EC-C18
- Additional phases: EC-C18, SB-C18, Phenyl-Hexyl, SB-AQ, SB-C8, Bonus-RP, HILIC, EC-CN

**Eclipse Plus**
- Start with Eclipse Plus C18
- Additional phases: C8, Phenyl-Hexyl, PAH, HILIC

**StableBond**
- Choice for pH 1-2, alternate selectivity
- Phases: SB-C18, SB-C8, SB-Phenyl, SB-CN, SB-AQ

**Eclipse XDB**
- Alternate Selectivity choices with a C18, C8, Phenyl, CN

**Bonus-RP**
- For changes in selectivity, particularly with acids and bases

**Extend-C18**
- Choice for high pH methods
New Method Development Kits

Choose from a variety of kits to suit your needs

- **Poroshell 120 L1, L7, and L10 USP Kits** make it easier to improve speed and sample throughput – without sacrificing resolution – by transferring your 5μm USP methods to Poroshell 120 columns

- **Poroshell 120 Selectivity Kits** provide a variety of column chemistries to help quickly adjust your analyte retention & selectivity

- **ZORBAX RRHD and Poroshell 120 Aqueous Method Development Kits** are ideal for polar compounds and 100% aqueous conditions, so you can achieve greater analyte retention without the phase collapse that can occur with C18 chemistries

- **ZORBAX RRHD Eclipse Plus Kits** help you achieve outstanding performance, peak shape, and method development flexibility for pH 2-9

- **ZORBAX RRHD pH Method Kits** give you more options when performing separations at varying pH levels

- **Other standard analytical method development kits also available**

<table>
<thead>
<tr>
<th>Method Development Kits</th>
<th>Description (One of each)</th>
<th>Dimension</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poroshell 120 Selectivity</td>
<td>EC-C18, Phenyl-Hexyl, Bonus-RP</td>
<td>2.1 x 50 mm</td>
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<td>Poroshell 120 Selectivity</td>
<td>EC-C18, Phenyl-Hexyl, Bonus-RP</td>
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<td>Poroshell 120 Aqueous</td>
<td>SB-Aq, Phenyl-Hexyl, Bonus-RP</td>
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<td>Poroshell 120 Aqueous</td>
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<td>Poroshell 120 L1, L7, and L10 USP</td>
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<td>Poroshell 120 L1, L7, and L10 USP</td>
<td>EC-C18, EC-C8, EC-CN</td>
<td>3.0 x 100 mm</td>
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<td>ZORBAX RRHD pH</td>
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<td>ZORBAX RRHD Eclipse Plus</td>
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Validation Kits Available

Same column type, dimensions, bonded phase, particle size from different manufacturing lots

<table>
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<tr>
<th>Method Development Today</th>
<th>Agilent Restricted</th>
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### Agilent ZORBAX Rapid Resolution High Definition (RRHD) Method Validation Kits

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<th>Size (mm)</th>
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<th>Eclipse Plus C18</th>
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### Agilent ZORBAX Method Validation Kits

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</table>
Conclusions

- New column choices like Poroshell 120 and Eclipse Plus RRHD/RRHT can speed up method development
  - Provide fast, high resolution
  - Allow more options to be considered quickly

- Retention and selectivity of many compounds are affected by pH – a key element in method development

- Method development schemes, automated and manual should include the use of different bonded phases

- 3rd key aspect of method development is mobile phase organic modifier – an effective tool for many separations

- Agilent supports the QbD approach to method development with columns, MD LC and software tools

- Contact Tech Support
  - 1-800-227-9770 or
  - lc-column-support@agilent.com
Appendix
The Method Development Poster Recommends Poroshell 120 and Eclipse Plus, 5989-7282EN

ZORBAX gives you total confidence in your HPLC Method Development. Whether you're developing a new method or improving an existing one, ZORBAX Poroshell 120 and Poroshell Eclipse Plus LC columns help you boost your productivity by ensuring that you're generating method robustness across a range of applications and conditions. ZORBAX Poroshell Eclipse Plus and Poroshell 120 EC-C18 LC columns are the best choice for HPLC method development. These columns were engineered to obtain superior performance by:

- Forming smaller particles with the efficiency of a sub-2.0 μm column
- Providing improved stability to rapidly transfer methods without revalidation
- Longer lifetimes and superior重现性 compared to other ZORBAX columns
- Providing high pressure stability with high windowing limits compared to other ZORBAX columns
- Offering superior column efficiency compared to other ZORBAX columns

From simple analyses to complex method development, Agilent ZORBAX and Poroshell Eclipse Plus columns ensure confidence in your results every time.

Poroshell 120: High efficiency and resolution with lower backpressure.

The ZORBAX family: High performance columns, superior reproducibility, long-term stability, including Eclipse Plus with special treatment for demanding reversed phase applications.

ZORBAX RSLC 1.8 μm, suitable for UHPLC, available in Eclipse Plus and more than two other phases.

Agilent Technologies

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9/17/2013
Agilent LC Columns – Method Development

Productivity Enhancers

• **Educational tools** to help all chromatographers, including **videos, selection tools, seminars and primers** and on-site support.

• **On-line searchable application notes** developed for your methods to increase productivity.

• **LC and GC mobile apps** to measure pressure and flow, anywhere and anytime.

• Local and worldwide **expert resources** to help you.
Agilent Has Three Types of Columns for UHPLC Method Development

RRHD, 1.8um
Speed and Resolution

Poroshell 120
Speed and Resolution

RRHT 1.8um
Speed
600 bar with 4.6 mm ID
UHPLC Column Technology Pairs with UHPLC

1290, 1200 bar

RRHD, 1200 bar

Poroshell 120, 600 bar

1260, 600 bar
## UHPLC Choices for Every Separation with Poroshell 120, RRHD, and RRHT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RRHD 1.8um, 1200 bar</th>
<th>RRHT 1.8um, 600bar</th>
<th>Poroshell 120 2.7um 600 bar</th>
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<td>&gt;140</td>
<td>&gt;80</td>
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<tr>
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<td>120Å</td>
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<tr>
<td>Scalability to other particle sizes</td>
<td>Yes – 3.5, 5, 7um</td>
<td>Yes – 3.5, 5, 7um</td>
<td>Nearly identical selectivity to 3.5, 5µm</td>
</tr>
<tr>
<td>Recommended Choice for LC</td>
<td>Eclipse Plus C18, 3.0 x 100, 1.8um 959758-302</td>
<td>Eclipse Plus C18, 4.6 x 50, 1.8um 959941-902</td>
<td>Poroshell 120 EC-C18 4.6 x 100mm, 2.7um 695975-902</td>
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<td>Eclipse Plus C18 2.1 x 50mm, 1.8um 959741-902</td>
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