Understanding the Proposed Changes to USP General Chapter <621>

Finland UPLC Users Meeting
Mai, 22th

Frederic Forini
Waters European Headquarters
What is the USP?

- The United States Pharmacopeia (USP) is an independent, official public standards-setting authority
  - Prescription and OTC medicines
  - Healthcare products
  - Food ingredients
  - Dietary supplements

- USP standards recognized and used in >130 countries

- Headquarters and offices
What is the USP-NF?

- The United States Pharmacopeia – National Foundry (USP-NF) is a book of pharmacopeial standards
  - Drugs substances & preparations monographs: USP
  - Dietary supplements & ingredients monographs: USP
  - Excipient monographs: NF
  - More than 4500 monographs

- The USP-NF is the official authority – FDA-enforceable standards
  - Enforcement of USP standards is the responsibility of FDA and other government authorities in the U.S. and elsewhere
  - USP has no role in enforcement

The U.S. Federal Food, Drug, and Cosmetics Act designates the USP-NF as the official compendia for drugs marketed in the United States
What is the Pharmacopeial Forum (PF)

- USP standards are established and maintained through public participation
  1. Sponsors provide draft standards and supporting data
  2. USP scientific staff and volunteer experts review, test and forward new/revised monograph to PF
  3. Monograph is refined and finalized through public review and comment in PF

- PF is FREE bimonthly online journal where public review and comment takes place
Question From CURRENT USP-NF Online FAQs¹

- **Q.** How much can I modify a chromatographic procedure and still be in compliance? Can column length, internal diameter, mobile phase composition be modified?

- **A.** Chromatography <621> contains a list of *allowed adjustments* to chromatographic systems. However, the user should verify the suitability of the method under the new conditions by assessing the relevant analytical performance characteristics potentially affected by the change (see section *System Suitability* under *Chromatography <621*>).

¹http://www.uspnf.com/uspnf/scienceFAQ.html#q4
Back in August 2007: ACQUITY UPLC® Columns included in USP LC columns “L” listings
- Waters was instrumental in changing minimum particle size

<table>
<thead>
<tr>
<th>BRAND NAME</th>
<th>MANUFACTURER</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE C16-300</td>
<td>Advanced Chrom. Technol.</td>
</tr>
<tr>
<td>ACE AQ</td>
<td>Advanced Chrom. Technol.</td>
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<td>ACE C18</td>
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<td>ACE C18-AR</td>
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<td>Waters Corp.</td>
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<td>ACQUITY UPLC HSS C18</td>
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<td>ACQUITY UPLC HSS C18 SB</td>
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<tr>
<td>ACQUITY UPLC OST C18</td>
<td>Waters Corp.</td>
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</tbody>
</table>
## Current Regulation Allowed Adjustments

<table>
<thead>
<tr>
<th>Allowed HPLC Adjustment</th>
<th>USP (Ref: General Chapter &lt;621&gt;)</th>
<th>EP (Ref: General Chapter 2.2.46)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column Length</strong></td>
<td>±70%</td>
<td>±70%</td>
</tr>
<tr>
<td><strong>Internal Diameter</strong></td>
<td>Can be adjusted if linear velocity is kept constant</td>
<td>±25%</td>
</tr>
<tr>
<td><strong>Particle Size</strong></td>
<td>Reduction of 50%, no increase</td>
<td>Reduction of 50%, no increase</td>
</tr>
<tr>
<td><strong>Flow Rate</strong></td>
<td>±50% or more as long as the linear velocity is kept constant</td>
<td>±50%</td>
</tr>
<tr>
<td><strong>Column Temperature</strong></td>
<td>±10°C</td>
<td>±10% Max 60°C</td>
</tr>
<tr>
<td><strong>Injection Volume</strong></td>
<td>Change allowed as long as SST criteria are met</td>
<td>May be decreased (if LOD and repeatability ok)</td>
</tr>
<tr>
<td><strong>PH</strong></td>
<td>±0.2 Units</td>
<td>±0.2 Units (±1% for neutral substances)</td>
</tr>
<tr>
<td><strong>UV wavelength</strong></td>
<td>&lt;±3 nm</td>
<td>&lt;±3 nm</td>
</tr>
<tr>
<td><strong>Conc. Salts in Buffer</strong></td>
<td>±10%</td>
<td>±10%</td>
</tr>
<tr>
<td><strong>Composition of mobile phase</strong></td>
<td>Minor Components (&lt;50%) ±30% or ±10% absolute whichever is smaller</td>
<td>Minor Components ±30% or ±2% absolute whichever is larger</td>
</tr>
</tbody>
</table>
Because Waters is THE acknowledged leader in separation science, we were asked *(by a customer)* to help modernize LC column selection in General Chapter <621> Chromatography.

In the summer of 2009 Dr. Uwe Neue *et. al.* wrote a paper (article) that basically describes UPLC technology and method transfer.

- Dr. Neue’s calculations are also the basis of the ACQUITY UPLC Columns Calculator.
**ABSTRACT** This *Stimuli* article contains proposals to help the analyst adjust HPLC column length and particle size to achieve separation power at least equivalent to that used in the original procedure, markedly increasing the range of options currently allowed in *Chromatography* (621). The article presents the scientific rationale for application of these proposals to isocratic procedures and follows with gradient procedures.
What Does This Stimuli Article Propose?

<table>
<thead>
<tr>
<th>Variable</th>
<th>Allowable Changes</th>
<th>Current Regulation</th>
<th>Stimuli Article Proposal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Particle Size</td>
<td>-50%</td>
<td></td>
<td>L/dp = Cst (±25%)</td>
</tr>
<tr>
<td>Column Length</td>
<td>±70%</td>
<td></td>
<td>±70%</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>±50%</td>
<td></td>
<td>Accordingly to L, dp and column ID</td>
</tr>
<tr>
<td>Column ID</td>
<td>Any allowed</td>
<td></td>
<td>Any allowed</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>Any reduction</td>
<td></td>
<td>Any allowed</td>
</tr>
<tr>
<td>Column Temperature</td>
<td>±10%</td>
<td></td>
<td>±10%</td>
</tr>
<tr>
<td>Mobile Phase pH</td>
<td>±0.2 unit</td>
<td></td>
<td>±0.2 unit</td>
</tr>
</tbody>
</table>

Be aware that selectivity is not affected! This is only about geometrical transfer.

If *Stimuli Article PF 35(6) [Nov-Dec 2009]* Officially Becomes Part of Chromatography <621>, Analysts Will Have More Flexibility to Utilize Modern Chromatographic Techniques Such as UPLC Technology.
USP-NF Development Process

- Sponsor submits Request for Revision (RR) to USP
- Scientific Liaison forwards RR for publication in Pharmacopeial Forum
- Public comments received on RR from Pharmacopeial Forum (90 days)
- Expert Committee reviews comments and accepts or rejects them, and possibly alters RR text as it deems appropriate

**Nov-Dec 2009**
Waters Stimuli Submission
PF 35(6)

**2nd May, 2011**
Amended Chromatography
<621> Posted in PF 37(3)

**1st March, 2012**
Revised Proposal
Posted in PF 38(2)

**29th Dec, 2011**
Chromatography
<621> System Suitability
Deferred*

*Deferrals are items that have been proposed in Pharmacopeial Forum (PF) but are not yet approved by an Expert Committee.
What Does This Revision Status Change Mean?

- How do you behave concerning revalidation?
  - Do you NEVER revalidate (never change methods) OR
  - Do you ALWAYS revalidate (even though changes were within USP allowable adjustments – audit fears) OR
  - Do you SOMETIMES revalidate (when changes are outside of USP allowable adjustments)

- It means:
  - Sufficient changes to revised General Chapter <621> Chromatography were made that require public comment (again)
  - Comment period will end 01-June-2012 according to PF 38(2)
  - Changes to <621> may occur late 2012 or 2013

- Depending of what is finally accepted, this will ease or remove the revalidation steps when moving to UPLC/UHPLC technologies
  - Assuming changes are allowed and system suitability requirements are met
Let's take a concrete example.

Original HPLC method: L/dp = 30'000

Geometrical transfer allowed with current regulation: L/dp = 28'571

What are we doing until the finalized USP Chapter <621> revision?
Industry Trends: The Market has Changed

Transitioning from HPLC to UPLC Technology

- Increasing number of organizations have realized the business and scientific advantages of UPLC Technology

- Increased availability of UHPLC instruments and sub 3 µm chemistries provides vendor choice

- Technology shift has led companies to evaluate how to best utilize their existing HPLC instruments as they continue to invest in, and transition to, newer UPLC systems

During this transition, a number of challenges have arisen that need to be addressed
Introducing The Waters 2.5 µm eXtended Performance Column Family
Packed in ultra-low dispersion hardware to minimize band spreading

Designed to withstand high pressure
- 4.6 mm ID capable of 9,000 PSI
- 2.1 and 3.0 mm IDs compatible with UPLC pressures

Flexibility in configurations
- 2.1, 3.0 and 4.6 mm ID (2.1 and 3.0 mm incorporating eCord™ technology)
- 30, 50, 75 and 100 mm lengths
- 14 scalable stationary phases

Packed with XBridge [BEH] and XSelect [CSH and HSS] 2.5 µm particles and chemistries
- **BEH** $C_{18}$, Shield RP18, $C_8$, Phenyl, HILIC and Amide
- **CSH** $C_{18}$, Phenyl-Hexyl and Fluoro-Phenyl
- **HSS** $C_{18}$, T3, $C_{18}$ SB, Cyano and PFP
Based Upon Two Fully-Scalable LC Column Platforms

**XBridge Columns**

Family designed and optimized for *pH stability*

-most *MS-compatible* HPLC columns on the market

**XSelect Columns**

Family designed and optimized for *selectivity*

-Multiple particle substrates to solve *multiple* chromatographic problems

-2.5 µm XP eXtended Performance Columns

-1.7 [UPLC], 2.5, 3.5, 5 and 10 µm

-New

-1.7 [UPLC], 2.5, 3.5 and 5 µm CSH
-1.8 [UPLC], 2.5, 3.5 and 5 µm HSS
Two compendial (USP) method transfer examples

1. **Isocratic Assay:** Levonorgestrel and Ethinyl Estradiol
   - Compare original HPLC method with:
     - XP column methods on Alliance HPLC system
     - XP column method on ACQUITY UPLC H-Class system
     - ACQUITY UPLC column method on ACQUITY UPLC H-Class system

2. **Gradient Impurities:** Abacavir
   - Transfer HPLC method to XP columns run on Alliance HPLC and ACQUITY UPLC H-Class systems
   - Observe the effects of system band spread on separation and resolution
## Positioning the Right Column with the Right System

<table>
<thead>
<tr>
<th>System</th>
<th>HPLC</th>
<th>UPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Particle Size</strong></td>
<td>3.5 μm, 5 μm</td>
<td>1.7/1.8 μm UPLC, 2.5 um XP, 3.5 μm, 5 μm</td>
</tr>
<tr>
<td><strong>Routine Pressure</strong></td>
<td>&lt; 4000 psi</td>
<td>&lt; 15000 psi (H-Class)</td>
</tr>
<tr>
<td><strong>Column ID</strong></td>
<td>4.6 mm</td>
<td>2.1 &amp; 3.0 mm</td>
</tr>
<tr>
<td><strong>Column Length</strong></td>
<td>≤ 250 mm</td>
<td>≤ 150 mm</td>
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</table>
Original Isocratic USP Assay: 4.6 x 150 mm, 5 µm (Alliance HPLC)

Flow Rate: 1.00 mL/min  
Pressure: 1400 psi  
Run time: 8.00 min

USP Res: 6.9

System Suitability Requirements
USP Resolution: NLT 2.5  
Peak Area Precision: NMT 2.0% RSD

L/dp = 30000  
N = 1X  
Rs = 1X  
Run time = 1X  
Pressure = 1X
## Compiled Status – Isocratic Method

Criteria USP Res>2.5

<table>
<thead>
<tr>
<th>LC Mode</th>
<th>Column ID (mm)</th>
<th>Column Length (mm)</th>
<th>Particle Size (µm)</th>
<th>Ratio L/dp</th>
<th>Flow Rate (ml/min)</th>
<th>Pressure (PSI)</th>
<th>Retention Time (min)</th>
<th>USP Res.</th>
<th>Instrument</th>
<th>Comments</th>
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<td>4.6</td>
<td>150</td>
<td>5</td>
<td>30’000</td>
<td>1</td>
<td>1400</td>
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<td>6.9</td>
<td>Alliance</td>
<td>Original HPLC Method</td>
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</tbody>
</table>
Isocratic Method Transferred to 4.6 mm XP Columns (Alliance HPLC)

4.6 x 75 mm XP 2.5 µm
Flow Rate: 1.00 mL/min
Pressure: 2600 psi
Run time: 4.00 min
USP Res: 6.5

Could not run at SCALED flow rate due to system pressure limitations

Results:
Rs = 0.9X
Run time = 0.5X
Pressure = 2X

4.6 x 50 mm XP 2.5 µm
Flow Rate: 2.00 mL/min
Pressure: 3600 psi
Run time: 1.30 min
USP Res: 5.0

LOWER L/dp column (20000) to reduce pressure:

Results:
Rs = 0.7X
Run time = 0.16X
Pressure = 2.9X

> 50% flow rate change not compliant ❗
## Compiled Status – Isocratic Method

Criteria USP Res > 2.5

<table>
<thead>
<tr>
<th>LC Mode</th>
<th>Column ID (mm)</th>
<th>Column Length (mm)</th>
<th>Particle Size (µm)</th>
<th>Ratio L/dp</th>
<th>Flow Rate (ml/min)</th>
<th>Pressure (PSI)</th>
<th>Retention Time (min)</th>
<th>USP Res.</th>
<th>Instrument</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>4.6</td>
<td>150</td>
<td>5</td>
<td>30’000</td>
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<td>1400</td>
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<td>Alliance</td>
<td>Original HPLC Method</td>
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<td>HPLC</td>
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<td>HPLC</td>
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<td>3600</td>
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</tbody>
</table>
Isocratic Method Transferred to 4.6 mm XP Columns (Alliance HPLC)

4.6 x 75 mm XP 2.5 µm
Flow Rate: **1.00 mL/min**
Pressure: **2600 psi**
Run time: **4.00 min**
USP Res: **6.5**

Could not run at SCALED flow rate due to system pressure limitations
Results:
Rs = 0.9X
Run time = 0.5X
Pressure = 2X

4.6 x 50 mm XP 2.5 µm
Flow Rate: **1.50 mL/min**
Pressure: **3050 psi**
Run time: **1.60 min**
USP Res: **5.2**

LOWER L/dp column (20000) to reduce pressure:
Results:
Rs = 0.8X
Run time = 0.2X
Pressure = 2.2X

Both flow rates are <621> compliant
### Compiled Status – Isocratic Method Criteria USP Res > 2.5

<table>
<thead>
<tr>
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<th>Column ID (mm)</th>
<th>Column Length (mm)</th>
<th>Particle Size (µm)</th>
<th>Ratio L/dp</th>
<th>Flow Rate (ml/min)</th>
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<td>5.2</td>
<td>Alliance</td>
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</tbody>
</table>
## LC Systems and Column Positioning: Transfer to ACQUITY UPLC H-Class System

<table>
<thead>
<tr>
<th>System</th>
<th>HPLC</th>
<th>UPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Particle Size</strong></td>
<td>3.5 µm, 5 µm</td>
<td>2.5 µm XP, 2.5 um XP, 3.5 µm, 5 µm</td>
</tr>
<tr>
<td><strong>Routine Pressure</strong></td>
<td>&lt; 4000 psi</td>
<td>&lt; 4000 psi</td>
</tr>
<tr>
<td><strong>Column ID</strong></td>
<td>4.6 mm</td>
<td>4.6 mm</td>
</tr>
<tr>
<td><strong>Column Length</strong></td>
<td>≤ 250 mm</td>
<td>≤ 75 mm</td>
</tr>
</tbody>
</table>
Isocratic Method Transferred to ACQUITY UPLC H-Class System

**2.1 x 75 mm XP, 2.5 µm**
- Flow Rate: 0.5 mL/min
- Pressure: 5200 psi
- Run Time: 1.80 min
- USP Res: 7.9

**Results:**
Rs = 1.1X
Run time = 0.25X
Pressure = 3.1X

**Maximum benefits obtained on ACQUITY UPLC System**

**2.1 x 50 mm UPLC, 1.7 µm**
- Flow Rate: 0.61 mL/min
- Pressure: 7700 psi
- Run Time: 0.90 min.
- USP Res: 7.0

**Results:**
Rs = 1X
Run time = 0.1X
Pressure = 5.5X
<table>
<thead>
<tr>
<th>LC Mode</th>
<th>Column ID (mm)</th>
<th>Column Length (mm)</th>
<th>Particle Size (µm)</th>
<th>Ratio L/dp</th>
<th>Flow Rate (ml/min)</th>
<th>Pressure (PSI)</th>
<th>Retention Time (min)</th>
<th>USP Res.</th>
<th>Instrument</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>4.6</td>
<td>150</td>
<td>5</td>
<td>30’000</td>
<td>1</td>
<td>1400</td>
<td>8</td>
<td>6.9</td>
<td>Alliance</td>
<td>Original HPLC Method</td>
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<tr>
<td>HPLC</td>
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<td>75</td>
<td>2.5</td>
<td>30’000</td>
<td>1</td>
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</tr>
<tr>
<td>HPLC</td>
<td>4.6</td>
<td>50</td>
<td>2.5</td>
<td>20’000</td>
<td>2</td>
<td>3600</td>
<td>1.3</td>
<td>5</td>
<td>Alliance</td>
<td>Not USP Compliant w/ Current Guidelines</td>
</tr>
<tr>
<td>HPLC</td>
<td>4.6</td>
<td>50</td>
<td>2.5</td>
<td>20’000</td>
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<td>5.2</td>
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<td>USP Compliant w/ Current Guidelines</td>
</tr>
<tr>
<td>HPLC</td>
<td>2.1</td>
<td>75</td>
<td>2.5</td>
<td>30’000</td>
<td>0.5</td>
<td>5200</td>
<td>1.8</td>
<td>7.9</td>
<td>ACQUITY H-Class</td>
<td>USP Compliant w/ Current Guidelines</td>
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<tr>
<td>UPLC</td>
<td>2.1</td>
<td>50</td>
<td>1.7</td>
<td>29’400</td>
<td>0.61</td>
<td>7700</td>
<td>0.9</td>
<td>7</td>
<td>ACQUITY H-Class</td>
<td>Not USP Compliant w/ Current Guidelines</td>
</tr>
</tbody>
</table>
Two compendial (USP) method transfers

1. **Isocratic Assay:** Levonorgestrel and Ethinyl Estradiol
   - Compare original HPLC method with:
     - XP column methods on Alliance HPLC system
     - XP column method on ACQUITY UPLC H-Class system
     - ACQUITY UPLC column method on ACQUITY UPLC H-Class system

2. **Gradient Impurities:** Abacavir
   - Transfer HPLC method to XP columns run on Alliance HPLC and ACQUITY UPLC H-Class systems
   - Observe the effects of system band spread on separation and resolution
Gradient USP Impurities Assay: Abacavir Method Transfer

<table>
<thead>
<tr>
<th>System</th>
<th>HPLC</th>
<th>UPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Particle Size</strong></td>
<td>3.5 µm, 5 µm</td>
<td>1.7/1.8 µm UPLC, 2.5 um XP, 3.5 µm, 5 µm</td>
</tr>
<tr>
<td><strong>Routine Pressure</strong></td>
<td>&lt; 4000 psi</td>
<td>&lt; 15000 psi (H-Class)</td>
</tr>
<tr>
<td><strong>Column ID</strong></td>
<td>4.6 mm</td>
<td>2.1 &amp; 3.0 mm</td>
</tr>
<tr>
<td><strong>Column Length</strong></td>
<td>≤ 250 mm</td>
<td>≤ 150 mm</td>
</tr>
</tbody>
</table>
Original Gradient USP Impurities: 4.6 x 150 mm 5 µm (Alliance HPLC)

Flow Rate: 1.00 mL/min
Max Pressure: 2800 psi
Gradient time: 35 min

USP Res: 3.1

System Suitability Requirements
USP Res: NLT 1.5
## Compiled Status – Gradient Method

Criteria USP Res>1.5

<table>
<thead>
<tr>
<th>LC Mode</th>
<th>Column ID (mm)</th>
<th>Column Length (mm)</th>
<th>Particle Size (µm)</th>
<th>Ratio L/dp</th>
<th>Flow Rate (ml/min)</th>
<th>Pressure (PSI)</th>
<th>Retention Time (min)</th>
<th>USP Res.</th>
<th>Instrument</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>4.6</td>
<td>150</td>
<td>5</td>
<td>30’000</td>
<td>1</td>
<td>2800</td>
<td>35</td>
<td>3.1</td>
<td>Alliance</td>
<td>Original HPLC Method</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

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Gradient USP Impurities Method Transferred to 2.1 mm ID Columns (UPLC H-Class)

2.1 x 75 mm XP, 2.5 µm
Flow Rate: 0.5 mL/min
Max Pressure: 8630 psi
Gradient time: 7.30 min

USP Res: 2.9 (0.94X)

Scaled (same) linear velocity as original HPLC separation

What if I ran this 2.1 mm ID XP column on an Alliance HPLC system?
## Compiled Status – Gradient Method
### Criteria USP Res>1.5

<table>
<thead>
<tr>
<th>LC Mode</th>
<th>Column ID (mm)</th>
<th>Column Length (mm)</th>
<th>Particle Size (µm)</th>
<th>Ratio L/dp</th>
<th>Flow Rate (ml/min)</th>
<th>Pressure (PSI)</th>
<th>Retention Time (min)</th>
<th>USP Res.</th>
<th>Instrument</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPLC</td>
<td>4.6</td>
<td>150</td>
<td>5</td>
<td></td>
<td>30’000</td>
<td>1</td>
<td>2800</td>
<td>35</td>
<td>Alliance</td>
<td>Original HPLC Method</td>
</tr>
<tr>
<td>HPLC</td>
<td>2.1</td>
<td>75</td>
<td>2.5</td>
<td></td>
<td>30’000</td>
<td>0.5</td>
<td>8630</td>
<td>7.3</td>
<td>ACQUITY UPLC H-Class</td>
<td>USP Compliant w/ Current Guidelines</td>
</tr>
</tbody>
</table>
Running a 2.1 x 75 mm XP Column: Alliance vs. ACQUITY UPLC H-Class

ACQUITY UPLC H-Class
Flow Rate: 0.21 mL/min
Max Pressure: 3600 psi
Gradient Time: 17.0 min
USP Res: 3.3 (1.1X)

Alliance HPLC
Flow Rate: 0.21 mL/min
Max Pressure: 3500 psi
Gradient Time: 17.0 min
USP Res: ?

Why the HUGE difference in performance?

Lower flow rate based upon HPLC system pressure limit
What is Band Spreading?

- Any component of the chromatographic system [*instrument and column*] that contributes to the distortion and broadening of a chromatographic band
  - **Wide chromatographic band**
    - Increased peak width
    - Dilution effect decreases peak height and sensitivity
  - **Narrow chromatographic band**
    - Decreased peak width
    - High efficiency, sensitivity and resolution

Minimize band spreading to improve chromatographic performance
Waters Disruptive Technology is the Providing a Complete (Holistic) Solutions for Meaningful Impact of Achieving Theoretical Promise of Separation

\[
\sigma_{v,\text{total}}^2 = \sigma_{v,\text{injection}}^2 + \sigma_{v,\text{pre-column}}^2 + \sigma_{v,\text{column}}^2 + \sigma_{v,\text{post-column}}^2 + \sigma_{v,\text{detector}}^2 + \tau_{\text{detector}}^2 \cdot F^2
\]

- All developments are focused on the separation integrity through system
  - Expanding and optimizing particle synthesis and packing of columns
  - Injector design, injector volume, fittings, flow path, sealing surfaces
  - Reduced tubing volumes
  - Optimized detectors to minimize dispersion and increasing signal while minimizing noise
## Summary: Positioning Column Configurations with LC Systems

<table>
<thead>
<tr>
<th>Chromatographic System</th>
<th>Band Spread*</th>
<th>Recommended Column ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shimadzu Prominence UFLC</td>
<td>41 µL</td>
<td>Primary: 4.6 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secondary: 3.0 mm</td>
</tr>
<tr>
<td>Alliance 2695 HPLC</td>
<td>29 µL</td>
<td></td>
</tr>
<tr>
<td>Agilent 1260 UHPLC (600 bar)</td>
<td>28 µL</td>
<td>Primary: 3.0 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secondary: 2.1 mm</td>
</tr>
<tr>
<td>Thermo Accela UHPLC</td>
<td>21 µL</td>
<td></td>
</tr>
<tr>
<td>Agilent 1290 UHPLC (1200 bar)</td>
<td>17 µL</td>
<td>Primary: 2.1 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secondary: 3.0 mm</td>
</tr>
<tr>
<td>ACQUITY UPLC</td>
<td>12 µL</td>
<td></td>
</tr>
<tr>
<td>ACQUITY UPLC H-Class w/Column Manager</td>
<td>12 µL</td>
<td>Primary: 2.1 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secondary: 3.0 mm</td>
</tr>
<tr>
<td>ACQUITY UPLC H-Class</td>
<td>9 µL</td>
<td></td>
</tr>
<tr>
<td>ACQUITY UPLC I-Class (FTN)</td>
<td>7.5 µL</td>
<td>Primary: 2.1 mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secondary: 1.0 mm</td>
</tr>
<tr>
<td>ACQUITY UPLC I-Class (FL)</td>
<td>5.5 µL</td>
<td></td>
</tr>
</tbody>
</table>

(*) – measured by System Marketing Laboratory
## Compiled Status – Gradient Method
### Criteria USP Res>1.5

<table>
<thead>
<tr>
<th>LC Mode</th>
<th>Column ID (mm)</th>
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<tr>
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<td>150</td>
<td>5</td>
<td>30’000</td>
<td>1</td>
<td>2800</td>
<td>35</td>
<td>3.1</td>
<td>Alliance</td>
<td>Original HPLC Method</td>
</tr>
<tr>
<td>HPLC</td>
<td>2.1</td>
<td>75</td>
<td>2.5</td>
<td>30’000</td>
<td>0.5</td>
<td>8630</td>
<td>7.3</td>
<td>2.9</td>
<td>ACQUITY UPLC H-Class</td>
<td>USP Compliant w/ Current Guidelines</td>
</tr>
<tr>
<td>HPLC</td>
<td>2.1</td>
<td>75</td>
<td>2.5</td>
<td>30’000</td>
<td>0.21</td>
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<td>17</td>
<td>3.3</td>
<td>ACQUITY UPLC H-Class</td>
<td>Not USP Compliant w/ Current Guidelines</td>
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<tr>
<td>HPLC</td>
<td>2.1</td>
<td>75</td>
<td>2.5</td>
<td>30’000</td>
<td>0.21</td>
<td>3500</td>
<td>17</td>
<td>NA</td>
<td>Alliance</td>
<td>NA</td>
</tr>
</tbody>
</table>
Methods can be transferred to **4.6 mm ID** XP columns on **HPLC systems**
- May need to reduce flow rate when using >50mm length columns
- If column length and particle size are not scaled, resolution values will be different than original

Improved performance for **2.1 mm ID** XP and UPLC columns obtained on lower dispersion, higher-pressure-tolerant **ACQUITY UPLC systems**
- Properly scaled column lengths and flow rates can be utilized

Maximum performance benefits with complete ACQUITY UPLC system solution
Summary

- Encouraging sign from regulatory agencies to facilitate adoption of new technology

- Waters will continue to work with USP and other regulatory bodies worldwide
  - We are committed to advance and modernize separation science

- USP Method Transfer Project gives us a keen understanding of challenges associated with running drug final formulations

- 2.5 µm XP particles is a ready solution while USP Chapter is being updated
  - XP columns bridge the gap between HPLC and UPLC
  - Positioned with our UPLC system, productivity could be significantly improved today
  - Methods can be modernized NOW within existing compendial guidelines
  - It offers scalability to real UPLC
  - H-Class is definitively the best LC for today and tomorrow since new R&D project are being developed on 1,7 µm particles
Concrete Summary Example

XSelect CSH C_{18}  
4.6 x 100 mm, 3.5 µm  
HPLC System  
1ml/min

3.5 µm

XSelect CSH C_{18}XP  
2.1 x 75 mm, 2.5 µm  
UPLC System  
With UPLC Conditions  
Opt. Flow Rate: 0.54mL/min

2.5 µm XP

ACQUITY UPLC CSH C_{18}  
2.1 x 50 mm, 1.7 µm  
UPLC System  
0.8ml/min

9X Faster than 3.5 µm HPLC  
5X Faster than 2.5 µm HPLC

1.7 µm
For how many years do you envisage the system that you are purchasing remaining “fit for purpose”
State of System LifeCycle

Year 2012
Run existing legacy HPLC methods

Year 2016
Technology in hand fully optimized

Year 2020
State of the art technology fully deployed
ACQUITY UPLC I-Class
- New chemical entities, research, impurity analysis
- 1.7/1.8 µm UPLC columns

ACQUITY UPLC H-Class
- Method development, method transfer, QA/QC
- Changing priorities from small – to – large molecules
- 1.7/1.8 µm UPLC columns as well as 2.5 µm XP, 3.5 and 5 µm HPLC columns

Alliance 2695
- Faster HPLC assays
- 2.5 µm XP columns

- Traditional HPLC assays
  - 3.5 / 5 µm HPLC columns

Future-Proofing [Long-Term Vision/Strategy]
Thank You For Your Time and Attention

Questions?
What Happen at the European Level

■ Will EurP follow USP?
  - USP and EurP are harmonizing both chapter <621> and 2.2.46 to the extent possible

■ What about the EurP Chapter 2.2.46?
  - Draft 2.2.46 chapter has already been written

■ When will this be released?
  - EurP expect to release the new 2.2.46 chapter at the same time as the USP <621> or with a short delay