

# Translating US Pharmacopoeia Methods to Sub-2 Micron and Solid Core Using the New USP <621> General Chapter Guidelines

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## 1. Introduction – Monograph Testing

- Ensures the **safety** and **quality** of pharmaceutical products and can include an LC test typically for assay or purity
- Many monographs use legacy column formats (e.g. 250 x 4.6 mm, 10 µm)
- Advances in column technology (sub 2-micron fully porous and solid core particles) and small column formats (e.g. 50 x 3.0 mm) allow **substantial improvements** in productivity and large cost savings
- However, allowable changes in column formats specified within monographs have previously been tightly restricted
- For isocratic methods, the **revised USP <621>** (general chapter on chromatography) now provides improved flexibility to the chromatographer to use modern column technology as allowable changes to the LC method
- This poster **summarises the recent changes** and demonstrates how to **achieve productivity and cost savings** using both HPLC and UHPLC technology

## 4. Translating Isocratic Methods and L/d<sub>p</sub> Approach

New USP <621> guidelines allow **two options** for changing the particle size (d<sub>p</sub>) and column length (L):

- Keep L/d<sub>p</sub> constant or within **-25% to +50%** of the original method
- Keep N constant or within **-25% to +25%** of the original method

This work explores the use of **option 1**.

For **successful translation** of isocratic LC methods, the following principles are applied:

### Translation of flow rate:

Scaled to new column i.d. (d<sub>c</sub>) to **maintain linear velocity**

$$F_2 = F_1 \times \left( \frac{d_{c2}^2}{d_{c1}^2} \right)$$

or scaled to new column i.d. and smaller d<sub>p</sub>

$$F_2 = F_1 \times \left( \frac{d_{c2}^2 \times d_{p1}}{d_{c1}^2 \times d_{p2}} \right)$$

### Scaling injection volume:

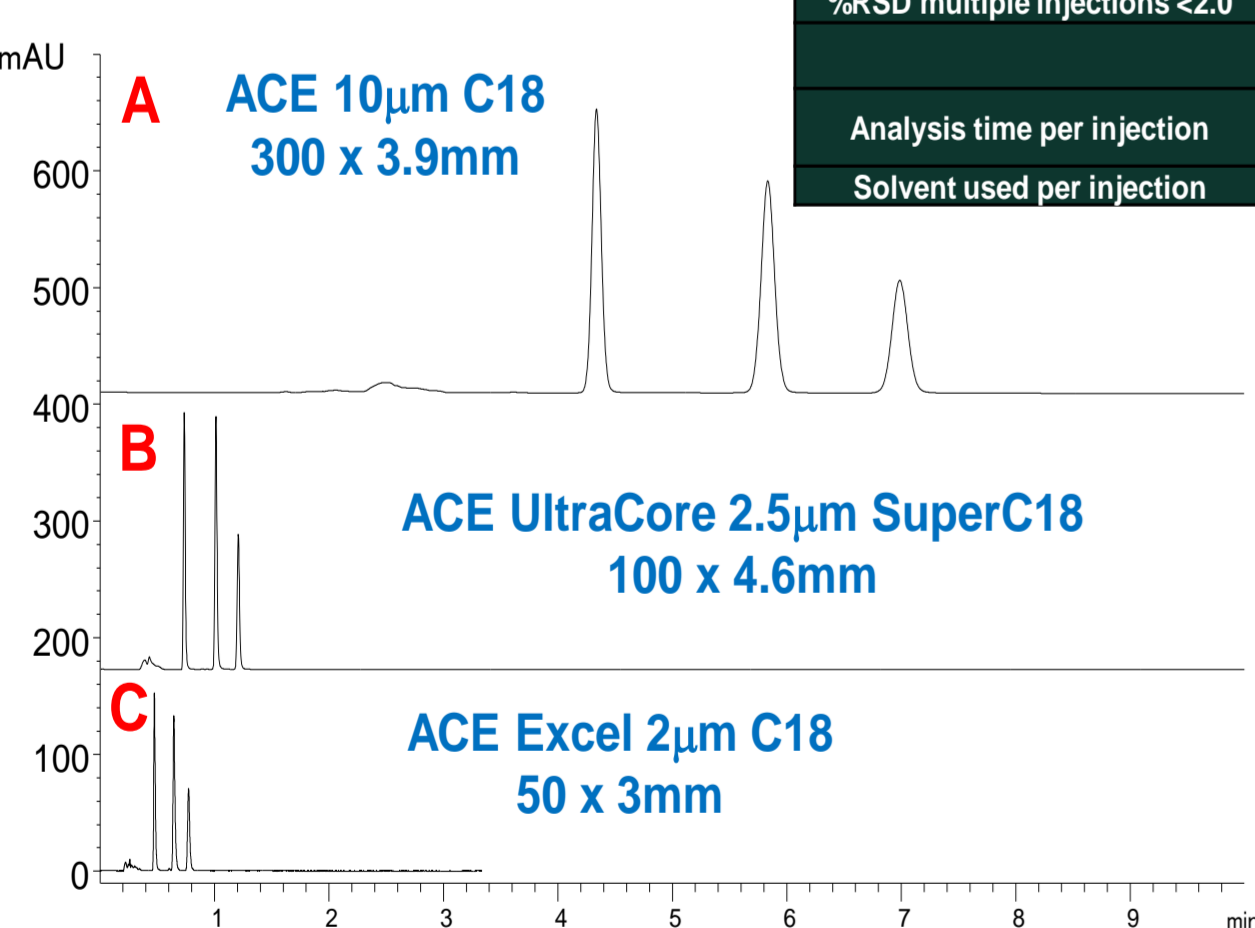
Injection volume is **scaled to new column volume (V<sub>M</sub>)**

$$V_{inj2} = V_{inj1} \times \left( \frac{V_{M2}}{V_{M1}} \right)$$

## 7. Estradiol Optimisation: Fully Porous or Solid Core

- Solid core and fully porous options
- Compatible with **standard HPLC instrumentation** (400 bar system pressure)

|                                | Original Method (A)           | Translated Method 1 (B)                      | Translated Method 2 (C)           |
|--------------------------------|-------------------------------|--|-----------------------------------|
| Column                         | ACE 10 µm C18<br>300 x 3.9 mm | ACE UltraCore 2.5µm SuperC18<br>100 x 4.6 mm | ACE Excel 2 µm C18<br>50 x 3.0 mm |
| L/d <sub>p</sub>               | 30,000                        | 40,000 (+33.3%)                              | 25,000 (-16.7%)                   |
| Flow (mL/min)                  | 1.0                           | 1.39   | 0.59                              |
| Injection Vol. (µL)            | 25                            | 10.1   | 2.5                               |
| Back pressure (bar)            | 79                            | 145  | 222                               |
| <b>System Suitability</b>      |                               |  |                                   |
| Rs between B and C > 2.0       | 4.8                           | 5.1  | 4.0                               |
| %RSD multiple injections < 2.0 | 0.1                           | <0.1   | 0.2                               |
| <b>Savings Achieved</b>        |                               |  |                                   |
| Analysis time per injection    | 10 min                        | 3.3 min (67% reduction)                      | 1.7 min (83% reduction)           |
| Solvent used per injection     | 10 mL                         | 4.6 mL (-54%)                                | 1.0 mL (-90%)                     |

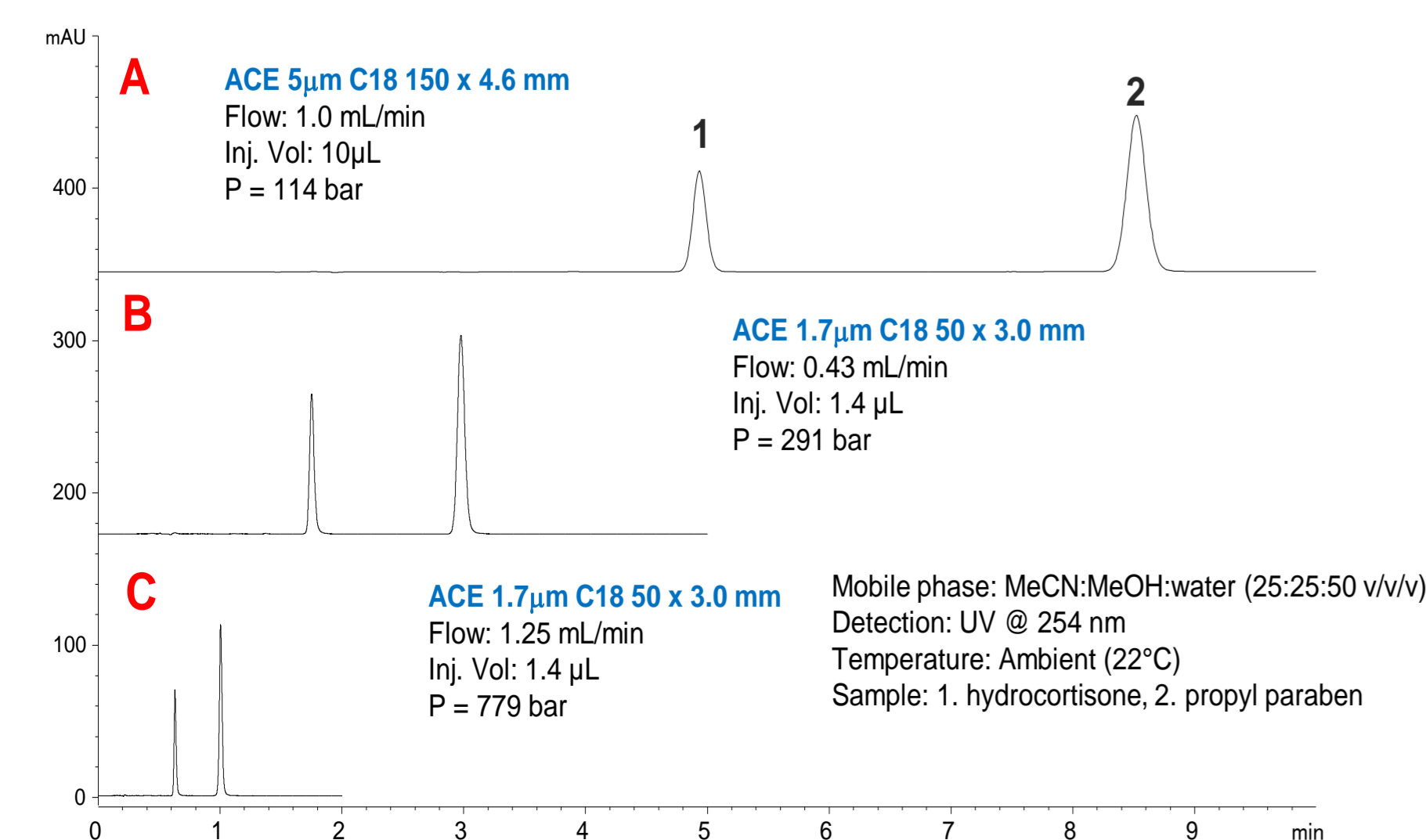


- Reduction in analysis time up to **83%**
- Solvent consumption reduced up to **90%**

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## 10. UHPLC Example: Hydrocortisone

- Translate method from **5 µm L1, 150 x 4.6 mm** to **1.7 µm L1, 50 x 3.0 mm**
- Approach 1: scale flow to maintain **constant linear velocity** (0.43 mL/min)
- Approach 2: scale flow to **reduced particle size** (1.25 mL/min)



## 2. New USP <621> Guidelines: Mobile and Stationary Phase

|                     | USP 36 / NF31 <621>  | USP 37 / NF 32 <621>   |
|---------------------|--|--|
| <b>Mobile phase</b> |  |  |
| Composition         | Isocratic & gradient:<br>- Minor components can be changed by ±30% relative or ±10% absolute | Isocratic:<br>- Minor components can be changed by ±30% relative or ±10% absolute  |
| pH                  | Isocratic & gradient:<br>- ±0.2 units (1% for neutrals)                                      | Isocratic & gradient:<br>±0.2 units  |
| Ionic strength      | Isocratic & gradient:<br>- ±10% if the permitted pH variation is met                         | Isocratic & gradient:<br>- ±10% if the permitted pH variation is met   |
| <b>Column</b>       |  |  |
| Length              | Isocratic & gradient:<br>- ±70%  | Isocratic:<br>- Particle size (d <sub>p</sub> ) and length (L) may be changed if a) L/d <sub>p</sub> is constant or varies -25% to +50% OR b) number of plates (N) is -25% to +50% |
| Particle size       | Isocratic & gradient:<br>-50%  | Isocratic:<br>- No changes   |
| Internal diameter   | Isocratic & gradient:<br>- Any changes if linear velocity kept constant<br>- ±25%            | Isocratic:<br>- Any changes if linear velocity kept constant<br>- No changes   |

## 5. Example 1: USP Estradiol Assay

Translating method from **10 µm to 5 µm**

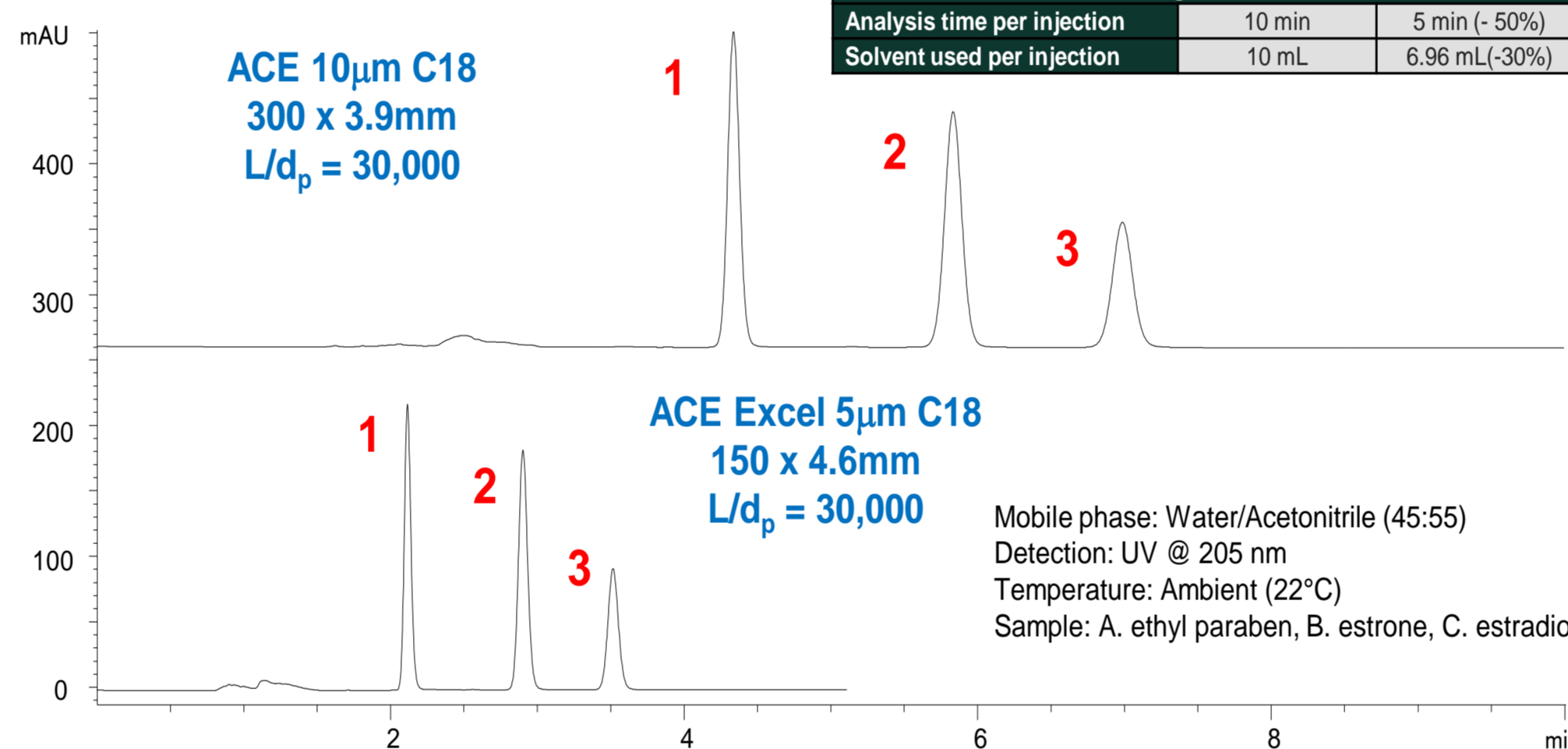
Column dimensions scaled (maintain L/d<sub>p</sub>)

- Flow rate scaled (**constant linear velocity**)

- Inj. Volume scaled to V<sub>M</sub>

- HPLC system compatible**

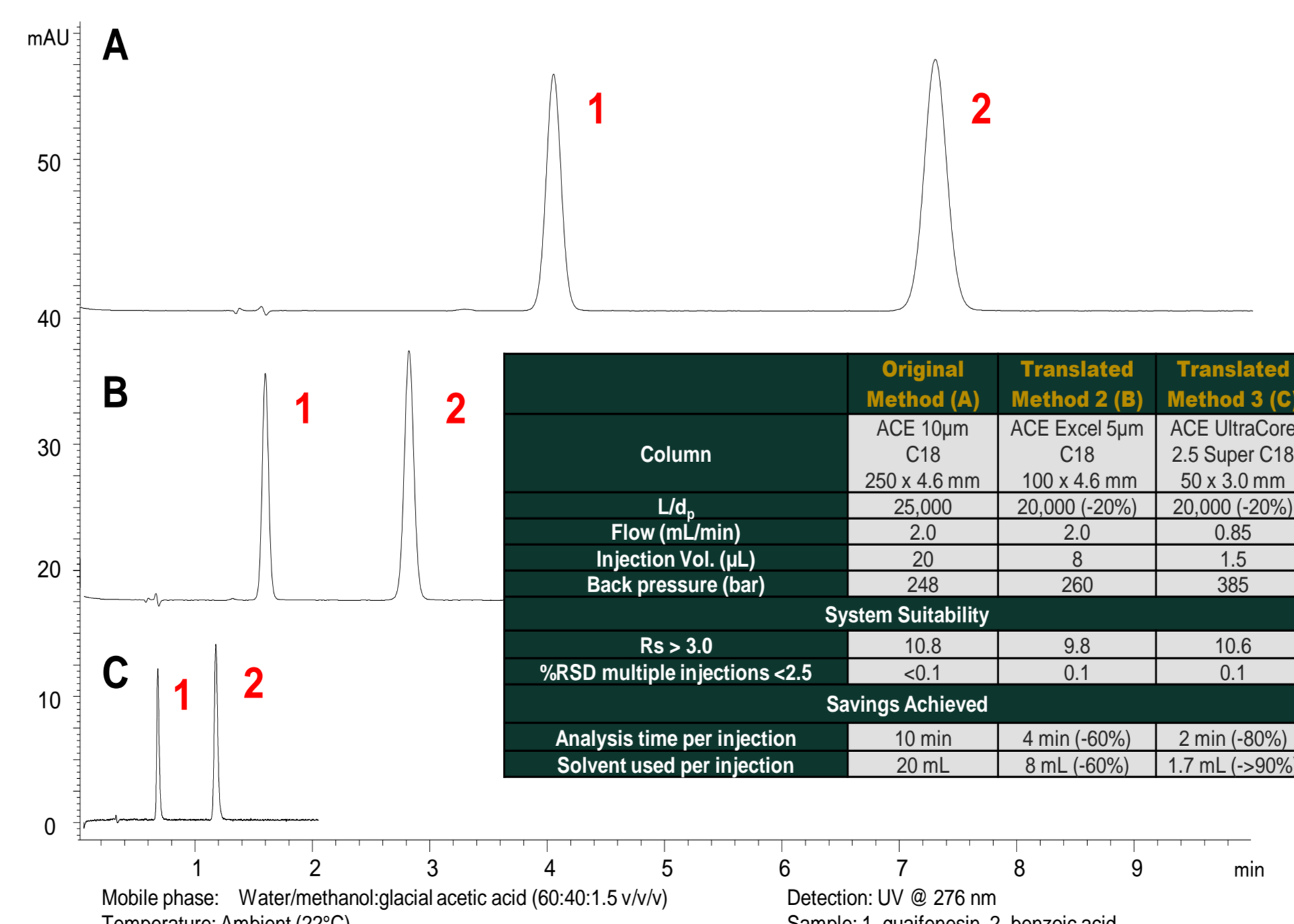
|                                | Original Method               | Translated Method                  |
|--------------------------------|-------------------------------|------------------------------------|
| Column                         | ACE 10 µm C18<br>300 x 3.9 mm | ACE Excel 5 µm C18<br>150 x 4.6 mm |
| L/d <sub>p</sub>               | 30,000                        | 30,000                             |
| Flow (mL/min)                  | 1.0                           | 1.39                               |
| Injection Vol. (µL)            | 25                            | 17.4                               |
| <b>System Suitability</b>      |                               |                                    |
| Rs between B and C > 2.0       | 4.8                           | 4.0                                |
| %RSD multiple injections < 2.0 | 0.1                           | 0.1                                |
| <b>Savings Achieved</b>        |                               |                                    |
| Analysis time per injection    | 10 min                        | 5 min (-50%)                       |
| Solvent used per injection     | 10 mL                         | 6.96 mL (-30%)                     |



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## 8. Example 2: Guaifenesin Tablets Assay

- When **excess resolution** is obtained, L/d<sub>p</sub> can be reduced (**allowable change**)



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## 11. UHPLC Example – Hydrocortisone assay

|                                | Original Method (A)          | Translated Method 1 (B)             | Translated Method 2 (C)             |
|--------------------------------|------------------------------|-------------------------------------|-------------------------------------|
| Column                         | ACE 5 µm C18<br>150 x 4.6 mm | ACE Excel 1.7 µm C18<br>50 x 3.0 mm | ACE Excel 1.7 µm C18<br>50 x 3.0 mm |
| L/d <sub>p</sub>               | 30,000                       | 29,412                              | 29,412                              |
| Flow (mL/min)                  | 1.0                          | 0.43                                | 1.25                                |
| Injection Vol. (µL)            | 10                           | 1.4                                 | 1.4                                 |
| Back pressure (bar)            | 114                          | 293                                 | 779                                 |
| <b>System Suitability</b>      |                              |                                     |                                     |
| Rs > 9.0                       | 14.1                         | 13.4                                | 10.1                                |
| N > 3,000 for hydrocortisone   | 9,167                        | 9,887                               | 6,441                               |
| Tailing factor < 1.2           | ✓                            | ✓                                   | ✓                                   |
| %RSD multiple injections < 2.0 | 0.1                          | <0.1                                | <0.1                                |
| <b>Savings Achieved</b>        |                              |                                     |                                     |
| Analysis time per injection    | 10 min                       | 3.3 min (-66%)                      | 1.2 (-88%)                          |
| Solvent used per injection     | 10 mL                        | 1.4 mL (-86%)                       | 1.5 mL (-85%)                       |

- Alpha decreases by **~8%**...possibly due to **pressure effects**
- 66% reduction in run time** and **86% reduction in solvent use** when scaling flow to maintain **constant linear velocity**.
- 88% reduction in run time** and **85% reduction in solvent use** when scaling flow to **reduced particle size**.

## 3. New USP <621> Guidelines: Operating Conditions

| Method               | USP 36 / NF31 <621>   | USP 37 / NF 32 <621>  |
|----------------------|---|---|
| Flow rate            | Isocratic & gradient:<br>- $F_2 = F_1 \times \left( \frac{d_{c2}^2}{d_{c1}^2} \right)$<br>(Where d <sub>c</sub> = column diameter and F = flow rate)<br>- Or, flow rate may change ±50% | Isocratic:<br>- If particle size has changed use following equation for similar performance:<br>$F_2 = F_1 \times \left( \frac{d_{c2}^2 \times d_{p1}}{d_{c1}^2 \times d_{p2}} \right)$<br>(Where d <sub>c</sub> = column diameter, F = flow rate and d <sub>p</sub> = particle size)<br>- Additional increase in flow allowed provided column efficiency does not drop below 20%.<br>- Or, flow rate may change ±50% |
| Injection volume     | Isocratic & gradient:<br>- Any reduction  | Isocratic & gradient: Any change as long as peak repeatability is satisfactory  |
| Temperature          | Isocratic & gradient:<br>- ±10°C when temperature is listed   | Isocratic & gradient:<br>±10°C when temperature is listed   |
| Detection wavelength | Isocratic & gradient:<br>- No change permitted. ±3 nm between detectors   | Isocratic & gradient:<br>No change permitted. ±3 nm between detectors   |

A. P. McKeown, Chromatography Today (2015) 32-36  
United States Pharmacopoeia General Chapter <621> "Chromatography" First Supplement to USP 37-NF 32 (United States Pharmacopoeial Convention, Rockville, MD, USA).

## 6. Exploring L/d<sub>p</sub>: Estradiol

- For **isocratic methods**, reducing column dimensions whilst **maintaining column length (L) to particle size (d<sub>p</sub>) ratio** results in **similar performance**
- USP <621> now permits L/d<sub>p</sub> **-25% to +50%**
- E.g. for 10 µm 300 x 3.9 mm (= 30,000)

| Particle size (µm) | Column Length (mm) |        |        |        |        |         |        |
|--------------------|--------------------|--------|--------|--------|--------|---------|--------|
|                    | 50                 | 75     | 100    | 125    | 150    | 250     | 300    |
| 1.7                | 29,412             | 44,118 | 58,824 |        |        |         |        |
| 1.8                | 27,778             | 41,667 | 55,556 |        |        |         |        |
| 1.9                | 26,316             | 39,474 | 52,632 |        |        |         |        |
| 2                  | 25,000             | 37,500 | 50,000 | 62,500 | 75,000 |         |        |
| 2.5                | 20,000             | 30,000 | 40,000 | 50,000 | 60,000 | 100,000 |        |
| 2.6                | 19,231             | 28,846 | 38,462 | 48,077 | 57,692 | 96,154  |        |
| 2.7                | 18,519             | 27,778 | 37,037 | 46,296 | 55,556 | 92,593  |        |
| 3                  | 16,667             | 25,000 | 33,333 | 41,667 | 50,000 | 83,333  |        |
| 5                  | 10,000             | 15,000 | 20,000 | 25,000 | 30,000 | 50,000  |        |
| 10                 | 5,000              | 7,500  | 10,000 | 12,500 | 15,000 | 25,000  | 30,000 |

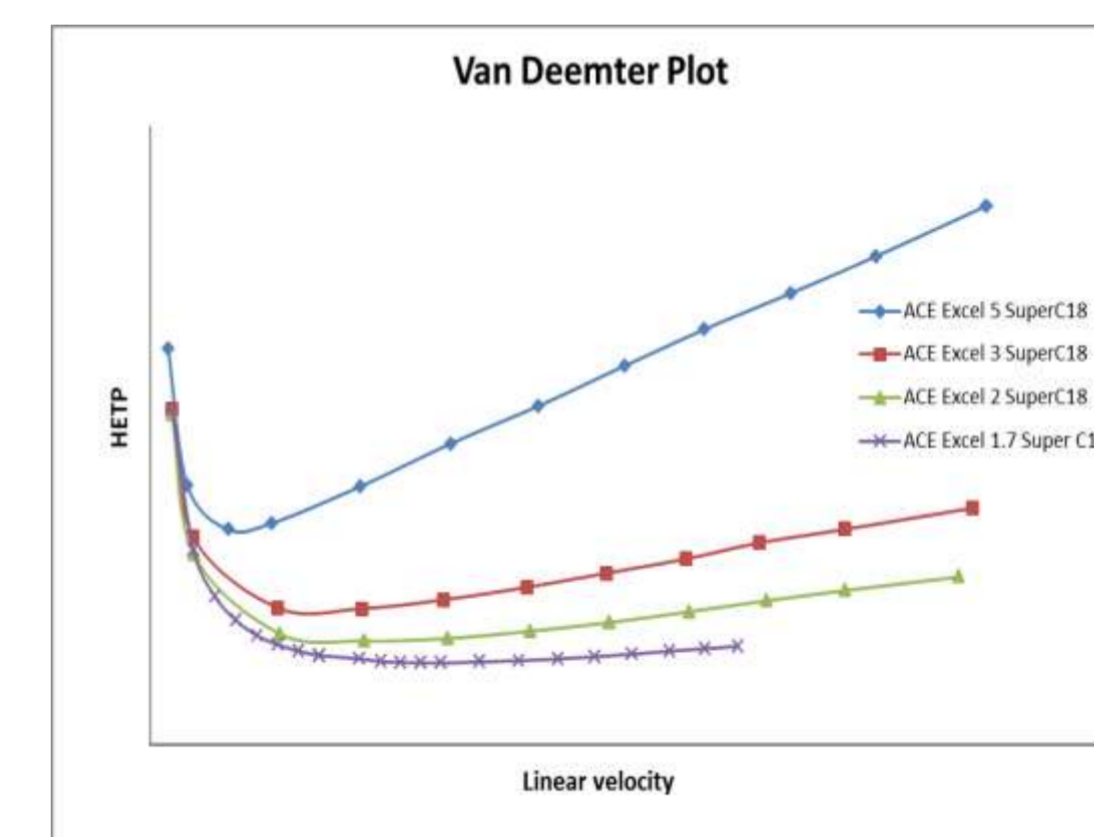
- Various L/d<sub>p</sub> options (and column formats) are available within the range **-25% to +50%** for use with HPLC and UHPLC instruments (**highlighted green**).

## 9. L/d<sub>p</sub> and Flow Adjustment: UHPLC

- USP <621> also allows translation of the flow rate to a **higher linear velocity** to take advantage of **high efficiencies** achievable with **small particles**.

$$F_2 = F_1 \times \left( \frac{d_{c2}^2 \times d_{p1}}{d_{c1}^2 \times d_{p2}} \right)$$

- i.e. allows chromatographer to **fully exploit sub 2 micron particles** and operate under **UHPLC conditions**



## 12. Summary and Conclusions

- The changes for isocratic methods in the new USP <621> provide **considerable flexibility** to the chromatographer. Reading the full text is **highly recommended** for detailed explanations.
- Use of **small particles** and **solid core technology** is now accommodated, allowing **significant increases in productivity** and **reduced cost per analysis**.
- This work successfully demonstrates how the **L/d<sub>p</sub> approach** can be applied to take advantage of the **latest column technology** using both **HPLC and UHPLC**.
- 80% reduction in run time** and **72% reduction in solvent use** for estradiol on an HPLC system.
- 80% reduction in run time** and **>90% reduction in solvent use** for guaifenesin on an optimised HPLC system.
- 88% reduction in run time** and **85% reduction in solvent use** for hydrocortisone on a UHPLC system.

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