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

ACE®
UHPLC and HPLC Columns

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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

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

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

3. New USP <621> Guidelines: Operating Conditions

	USP 36 / NF31 <621>	USP 37 / NF 32 <621>
Method		
Flow rate	Isocratic & gradient: - $F_2 = F_1 \times \left(\frac{{d_{c_2}}^2}{{d_{c_1}}^2}\right)$ (Where d_c = column diameter and F = flow rate) - Or, flow rate may change ±50%	Isocratic: - If particle size has changed use following equation for similar performance: $F_2 = F_1 \times \left(\frac{{d_{c_2}}^2 \times d_{p_1}}{{d_{c_1}}^2 \times d_{p_2}}\right)$ (Where d_c = column diameter, F = flow rate and d_p = particle size) - Additional increase in flow allowed provided column efficiency does not drop below 20%. - Or, flow rate may change ±50% Gradient: - No changes
Injection volume	Isocratic & gradient: - Any reduction	Isocratic & gradient: Any change as long as peak repeatability is satisfactory
Temperature	Isocratic & gradient: - ±10°C when temperature is listed	Isocratic & gradient: ±10°C when temperature is listed
Detection wavelength	Isocratic & gradient: - No change permitted. ±3 nm between detectors	Isocratic & gradient: 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).

4. Translating Isocratic Methods and L/d_p Approach

New USP <621> guidelines allow two options for changing the particle size (d_p) and column length (L):

1. Keep L/d_p constant or within -25% to +50% of the original method
2. 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_c) to maintain linear velocity

$$F_2 = F_1 \times \left(\frac{{d_{c_2}}^2}{{d_{c_1}}^2}\right)$$

or scaled to new column i.d. and smaller d_p

$$F_2 = F_1 \times \left(\frac{{d_{c_2}}^2 \times d_{p_1}}{{d_{c_1}}^2 \times d_{p_2}}\right)$$

Scaling injection volume:
Injection volume is scaled to new column volume (V_M)

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

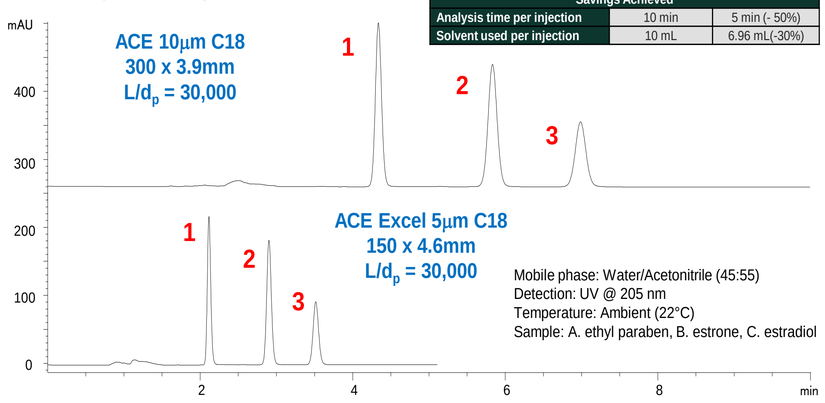
5. Example 1: USP Estradiol Assay

Translating method from 10 µm to 5 µm

Column dimensions scaled (maintain L/d_p)

- Flow rate scaled (constant linear velocity)
- Inj. Volume scaled to V_M
- HPLC system compatible

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



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6. Exploring L/d_p: Estradiol

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

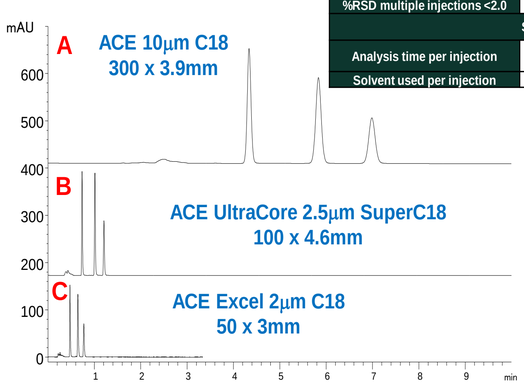
Particle Size (µm) \ Column Length (mm)	50	75	100	125	150	250	300
1.7	29,412	44,118	58,824				
1.8	27,778	41,667	55,556				
1.9	26,316	39,474	52,632				
2	25,000	37,500	50,000	62,500	75,000		
2.5	20,000	30,000	40,000	50,000	60,000	100,000	
2.6	19,231	28,846	38,462	48,077	57,692	96,154	
2.7	18,519	27,778	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_p options (and column formats) are available within the range -25% to +50% for use with HPLC and UHPLC instruments (highlighted green).

7. Estradiol Optimisation: Fully Porous or Solid Core

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

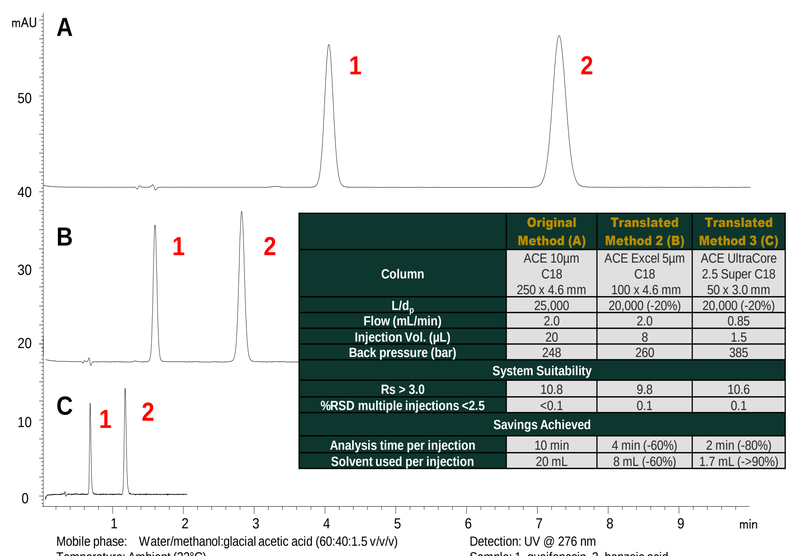
	Original Method (A)	Translated Method 1 (B)	Translated Method 2 (C)
Column	ACE 10 µm C18 300 x 3.9 mm	ACE UltraCore 2.5µmSuperC18 100 x 4.6 mm	ACE Excel 2 µm C18 50 x 3.0 mm
L/d_p	30,000	40,000 (+33.3%)	25,000 (-16.7%)
Flow (mL/min)	1.0	1.39	0.59
Injection Vol. (µL)	25	10.1	2.5
Back pressure (bar)	79	145	222
System Suitability			
Rs between B and C > 2.0	4.8	5.1	4.0
%RSD multiple injections <2.0	0.1	<0.1	0.2
Savings Achieved			
Analysis time per injection	10 min	3.3min (67% reduction)	1.7min (83% reduction)
Solvent used per injection	10 mL	4.6mL (-54%)	1.0mL (-90%)



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

8. Example 2: Guaifenesin Tablets Assay

- When excess resolution is obtained, L/d_p can be reduced (allowable change)



	Original Method (A)	Translated Method 2 (B)	Translated Method 3 (C)
Column	ACE 10µm C18 250 x 4.6 mm	ACE Excel 5µm C18 100 x 4.6 mm	ACE UltraCore 2.5 Super C18 50 x 3.0 mm
L/d_p	25,000	20,000 (-20%)	20,000 (-20%)
Flow (mL/min)	2.0	2.0	0.85
Injection Vol. (µL)	20	8	1.5
Back pressure (bar)	248	260	385
System Suitability			
Rs > 3.0	10.8	9.8	10.6
%RSD multiple injections <2.5	<0.1	0.1	0.1
Savings Achieved			
Analysis time per injection	10 min	4 min (-60%)	2 min (-80%)
Solvent used per injection	20 mL	8 mL (-60%)	1.7 mL (->90%)

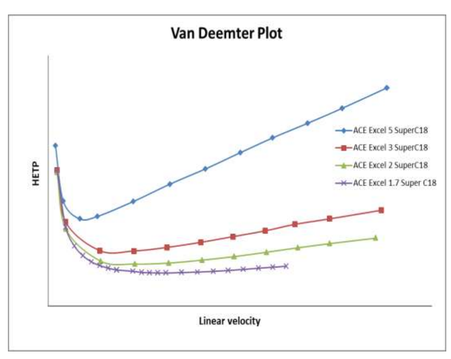
Mobile phase: Water/methanol:glacial acetic acid (60:40:1.5 v/v/v)
Temperature: Ambient (22°C)
Detection: UV @ 276 nm
Sample: 1. guaifenesin, 2. benzoic acid

9. L/d_p 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.

$$F_2 = F_1 \times \left(\frac{{d_{c_2}}^2 \times d_{p_1}}{{d_{c_1}}^2 \times d_{p_2}}\right)$$

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



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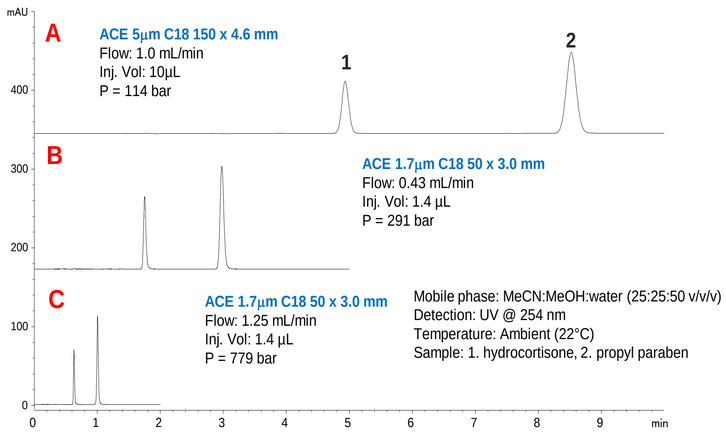
10. UHPLC Example: Hydrocortisone

- 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)



11. UHPLC Example – Hydrocortisone assay

	Original Method (A)	Translated Method 1 (B)	Translated Method 2 (C)
Column	ACE 5 µm C18 150 x 4.6 mm	ACE Excel 1.7 µm C18 50 x 3.0 mm	ACE Excel 1.7 µm C18 50 x 3.0 mm
L/d_p	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
System Suitability			
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
Savings Achieved			
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.

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_p 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.**