Method Makeover

Updating older HPLC methods

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Agilent
Trusted Answers

Why Update?

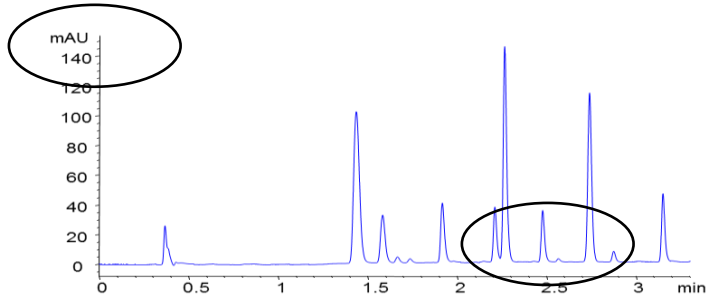
Do I need to update? What are my goals?

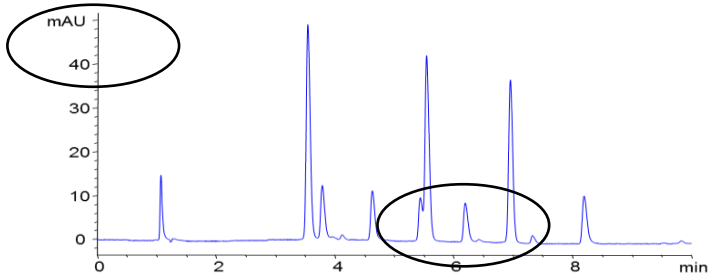
- Analysis time
  - Productivity (freeing up time for other tasks)
  - More analyses (freeing up instrument time)
  - More samples (higher throughput)
- Robustness and ruggedness
  - Improve resolution
  - Improve sensitivity
  - Better lot-to-lot reproducibility
- Improve sensitivity
- Cost
  - Solvent use
  - Solvent disposal
  - Sometimes older columns are more expensive
  - Newer columns may have longer life

What Are the Column Options?

- Smaller particle size
  - Higher efficiency -> shorter column -> faster method
  - Increase the resolution
  - Better sensitivity
  - Consider the pressure limit of your instrument
- Smaller diameter
  - Solvent savings
  - Depends on instrument configuration and plumbing
- Bonded phase
  - Match USP designation
  - More robust column life

HPLC Column Particle Evolution

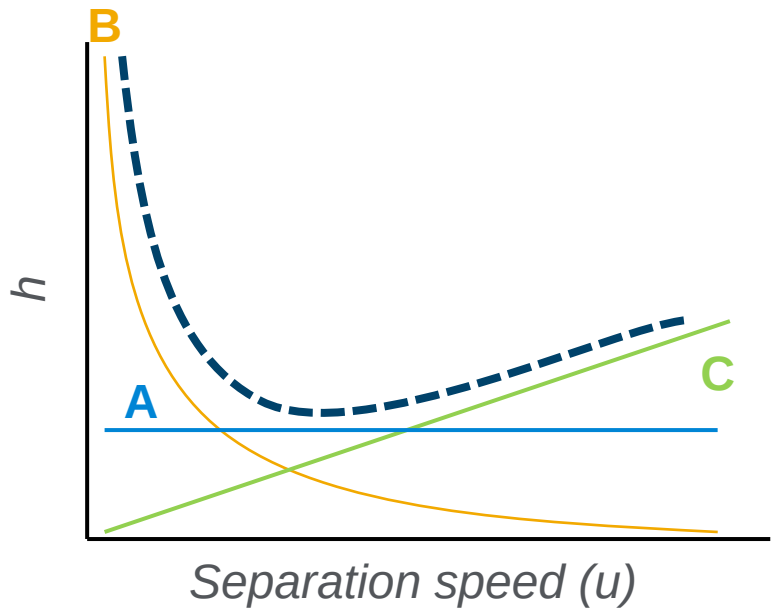




Van Deemter Equation

$$h = L/N$$

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

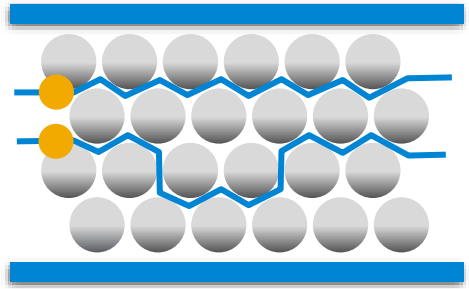


- **A term: eddy diffusion and flow distribution**
  - **Particle size and packing quality are important**
  - **Narrow particle size distribution**
- **B term: longitudinal diffusion**
  - **Diffusion in the mobile phase**
- **C term: mass transfer**
  - **Shorter diffusion paths**
  - **Better with superficially porous particles**
  - **Greater effect on large molecules**
- ***u*: linear velocity**
  - **velocity of mobile phase through the column**
  - **$u = L/t_0$ in cm/s**

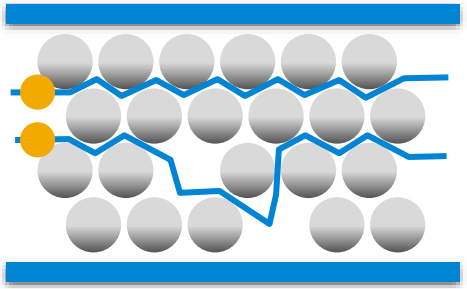
Lower *h (reduced plate height)* = higher efficiency

Van Deemter Equation
Eddy diffusion

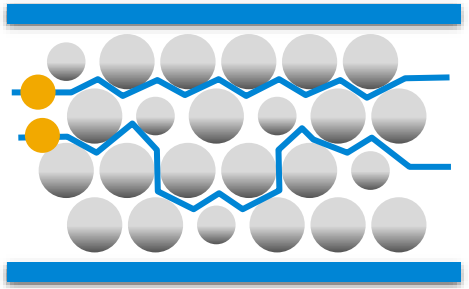
Differences in diffusion paths are due to:



Different paths



Poor column packing



Broad particle size distribution

- Some methods use columns that were introduced decades ago
  - Using irregularly shaped silica particles
  - Not packed as efficiently

The Agilent Small Molecule LC Columns Portfolio

When to choose which product family

InfinityLab Poroshell 120

HPLC	UHPLC	LD-UHPLC
4 µm	2.7 µm	1.9 µm

Features

Modern column technology that offers higher performance at a similar backpressure to STM

Or comparable performance at a reduced backpressure

Designed in with Agilent LC instruments and supplies

Universal column platform with offerings for all separation modes (RP, NP, HILIC, SFC, as well as chiral LC)

Modern, high-performance HPLC and UHPLC columns, designed in for state-of-the-art instruments

ZORBAX

HPLC	UHPLC	LD-UHPLC
5 µm, 3.5 µm	1.8 µm (RRHT)	1.8 µm (RRHD)

Features

Traditional, reliable columns that offer a vast amount of unique chemistries

Higher overall retention, especially for early eluters, accepts larger amounts of strong solvent during injection

Scalable phases that range from UHPLC and HPLC to research scale prep

Scalable from UHPLC and HPLC to preparative LC with higher retention

Special Phases

HPLC	UHPLC	LD-UHPLC
5 µm, 3 µm	---	---

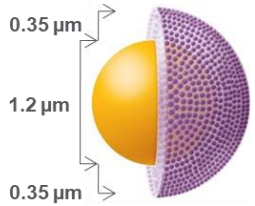
Features	Phases
High carbon load columns	Pursuit XRs, Pursuit XRs Ultra
Analytical to prep	Pursuit, Polaris
Alternative selectivity for polar and nonpolar	Polaris C18-Ether, C18 Amide, NH_2

Unique chemistries that help to solve nonstandard applications from HPLC to prep

Benefits of Transferring to Agilent InfinityLab 120 Poroshell

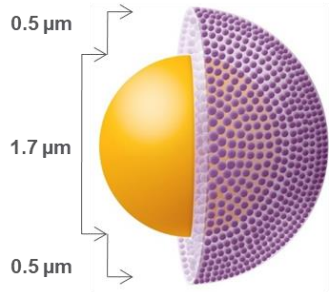
- Superficially porous particles
  - Faster methods without sacrificing resolution
  - Existing instruments can turn over more samples per hour
  - Boosts the sample throughput on low-pressure instruments
  - 30 to 50% increase in efficiency near the backpressure of a 3.5 or 5.0 µm particle

- Short column lengths (or narrow column diameters)
  - Cuts solvent flow by 50 to 80%
  - Reduces solvent consumption
  - Cuts purchase and disposal costs



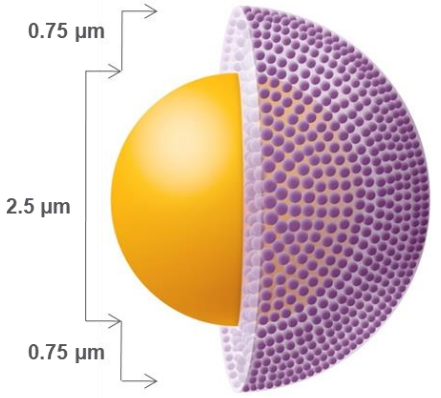
InfinityLab Poroshell 120
1.9 µm

Highest UHPLC performance



InfinityLab Poroshell 120
2.7 µm

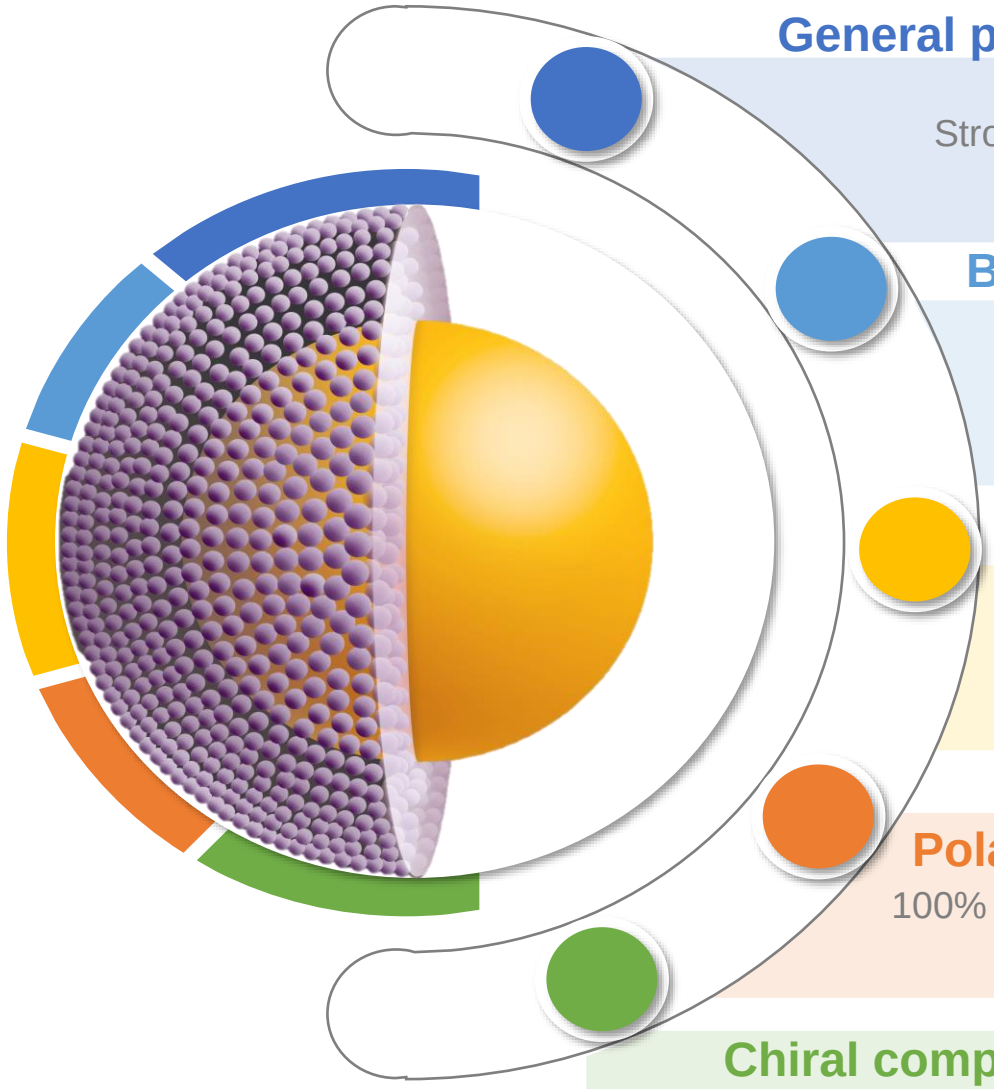
UHPLC performance at lower pressure



InfinityLab Poroshell 120
4 µm

Improved HPLC performance

Poroshell 120 Selection Guide



		Yes	No	Alternative selectivity
General purpose	Strong retention?	EC-C18 1.9 µm, 2.7 µm, 4 µm	EC-C8 1.9 µm, 2.7 µm, 4 µm	Bonus-RP 2.7 µm Phenyl-Hexyl 1.9 µm, 2.7 µm, 4 µm Aromatic interaction
Basic compounds (pKa>6)	High pH mobile phases?	HPH-C18 1.9 µm, 2.7 µm, 4 µm HPH-C8 2.7 µm, 4 µm		CS-C18 2.7 µm
Acidic compounds (pKa<3)	Low pH mobile phases?	SB-C18 1.9 µm, 2.7 µm, 4 µm SB-C8 2.7 µm		CS-C18 2.7 µm AQ-C18 2.7 µm
Polar compounds	100% aqueous mobile phase?	AQ-C18 2.7 µm SB-Aq 1.9 µm, 2.7 µm, 4 µm	HILIC-Z 1.9 µm, 2.7 µm, 4 µm	HILIC-OH5 2.7 µm
Chiral compounds		Chiral-V 2.7 µm		Chiral-T Chiral-CF Chiral-CD 2.7 µm

Chemistries with Unique Selectivity: Zorbax

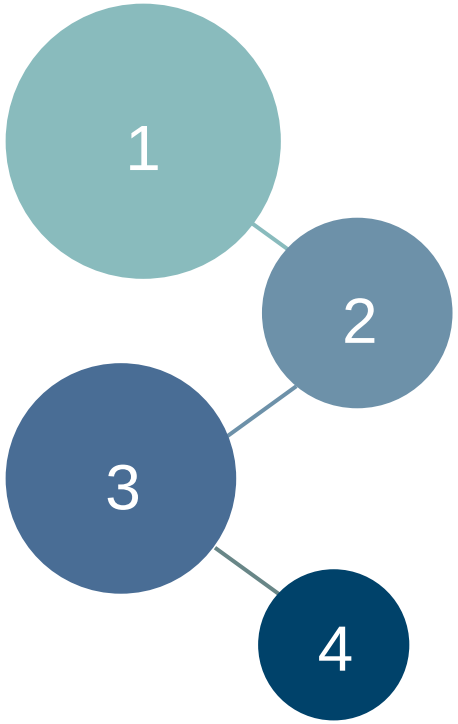
Reliable totally porous columns for highest sample capacity and resistance to sample solvents

Best All Around	Best for Low pH Mobile Phases	Best for High pH Mobile Phases	Best for Alternative Selectivity	Best for More Polar Analytes
Eclipse Plus C18 / C8 1.8 / 3.5 / 5 µm	**SB-C18** 1.8 / 3.5 / 5 / 7 µm	**Extend-C18** 1.8 / 3.5 / 5 µm	**Bonus-RP** 1.8 / 3.5 / 5 / 7 µm	**SB-Aq** 1.8 / 3.5 / 5 / 7 µm
Eclipse XDB C18 / C8 1.8 / 3.5 / 5 / 7 µm	**SB-C8** 1.8 / 3.5 / 5 / 7 µm		**SB-Phenyl** 1.8 / 3.5 / 5 / 7 µm	**SB-CN** 1.8 / 3.5 / 5 / 7 µm
Eclipse Plus Phenyl-Hexyl 1.8 / 3.5 / 5 µm	**SB-C3** 1.8 / 3.5 / 5 / 7 µm		**Eclipse PAH** 1.8 / 3.5 / 5 µm	**HILIC Plus** 1.8 / 3.5 µm
Eclipse XDB Phenyl 1.8 / 3.5 / 5 / 7 µm				**Eclipse XDB-CN** 1.8 / 3.5 / 5 / 7 µm
Rx-C18 3.5 / 5 / 7 µm	**Rx-C8** 3.5 / 5 µm			**Rx-Sil/ NH2** 5 / 7 µm

Adjusting Compendial Methods

USP Compendial Methods

- What are compendial methods
- How are USP methods used



Adjusting Compendial Methods

- Changes can be made to USP methods
- Save time with newer column technology

Examples of Adjustments

- Isocratic adjustment (TPP and SPP)
- Gradient adjustment (TPP and SPP)

Conclusions

- Tips and tools
- References

The USP and Compendial Methods

- *USP* develops written (USP-NF, United States Pharmacopeia–National Formulary) and reference standards. These standards are used by regulatory agencies and manufacturers to ensure that products are of the appropriate identity, as well as strength, purity, and consistency.
- Compendial methods are standardized methods and specification testing for pharmaceutical raw materials and finished products.
- Each compound has a monograph. The monograph will be divided into sections and will typically include a section for assaying the raw material, and each type of dosage form. It will include conformational identification tests.
- USP methods are currently being actively modernized. As such only the most recent update is considered legal.
- Validated methods such as compendial methods may be adjusted within <621> guidelines and can be used after verification.

What Is USP General Chapter <621>?

- The general chapter <621> is one of the most important USP general chapters.
- This chapter describes general procedures, definitions, and calculations of common parameters as well as general applicable requirements for system suitability. On December 1, 2022, the newly harmonized general chapter <621> (Chromatography) of the USP became official.

Major changes:

- New definitions are introduced, including plate height and plate number, as well as definitions for size-exclusion chromatography, including total mobile phase time and distribution constant.
- Formulas for **plate number** (previously referred to as plate count) and **resolution** have been modified to use half-height. Tangent width no longer appears in the updated USP <621>.
- **Tailing factor** has been renamed as symmetry factor.
- **Signal-to-noise** is now calculated using a range of noise five times the width at the half-height of the peak.
- **Changes allowed to gradient chromatographic conditions.**

Allowable Adjustments per USP General Chapter <621> After December 1, 2022

Parameters	Method Transfer from Totally Porous Particle to Totally Porous Particle Column	Method transfer from Totally Porous Particle to Superficially Porous Particle Column	Method Transfer from Totally Porous Particle to Totally Porous Particle Column	Method Transfer from Totally Porous Particle to Superficially Porous Particle Column
	Isocratic Mode		Gradient Mode	
Stationary phase	No changes allowed	No changes allowed	No changes allowed	No changes allowed
Column dimensions (Particle size, length)	L/dp: -25% to +50%	N: -25% to +50%	L/dp: -25% to +50%	$\left(\frac{tR}{Wh}\right)^2$: -25% to +50%
Column id	Flexible	Flexible	Flexible	Flexible
Gradient time			Adjust each segment of the gradient $t_{G2} = t_{G1} \times \left(\frac{F_1}{F_2}\right) \times \frac{[L_2 \times d_2^2]}{[L_1 \times d_1^2]}$	
Flow rate	Based on column id and particle size: $F_2=F_1\times[(dp_1\times d_2^2)/(dp_2\times d_1^2)]$ An additional change in flow rate of ±50% is permitted.		Based on column id and particle size: $F_2=F_1\times[(dp_1\times d_2^2)/(dp_2\times d_1^2)]$	
Injection volume	Based on column dimensions: $V_2=V_1\times[(l_2\times d_2^2)/(l_1\times d_1^2)]$	Based on column dimensions: $V_2=V_1\times[(l_2\times d_2^2)/(l_1\times d_1^2)]$	Based on column dimensions: $V_2=V_1\times[(l_2\times d_2^2)/(l_1\times d_1^2)]$	Based on column dimensions: $V_2=V_1\times[(l_2\times d_2^2)/(l_1\times d_1^2)]$
Column temperature	±10 °C		±5°C	
Mobile phase pH	±0.2 units	±0.2 units	±0.2 units	±0.2 units
Salt concentration	Within ±10%, if the permitted pH variation is met			
Ratio of components in mobile phase	Minor component (≤50%): ±30% relative, but cannot exceed ±10% absolute; may only adjust one minor component in ternary mixtures		The principal peaks elute within ±15% of the retention time(or times) obtained with the original conditions; this requirement does not apply when the column dimensions are changed. The composition of the mobile phase and the gradient are such that the first peaks are sufficiently retained, and the last peaks are eluted	
Wavelength of UV-VIS detector	No changes allowed	No changes allowed	No changes allowed	No changes allowed

Plate number $N = 5.54(t_R / W_h)^2$

The USP Designation for HPLC Column Phases

HPLC column L-numbers for many Agilent Zorbax and Poroshell phases

USP Designation	ZORBAX	Poroshell 120
L1	Eclipse Plus C18 Eclipse XDB-C18 Eclipse Plus PAH SB-C18 Extend C18 Rx-C18	EC-C18 Aq-C18 SB-C18 HPH-C18 CS-C18
L3	HILIC Plus Rx-Sil	HILIC
L7	Eclipse Plus C8 Eclipse XDB-C8 SB-C8 Rx-C8	EC-C8 SB-C8 HPH-C8
L10	Eclipse XDB CN SB-CN	EC-CN
L11	Eclipse Plus Phenyl-Hexyl Eclipse XDB Phenyl SB Phenyl	Phenyl-Hexyl
L43		PFP
L45		Chiral-CD
L56	SB-C3	
L60	Bonus-RP	Bonus-RP
L63		Chiral-T
L86		HILIC-OH5
L88		Chiral-V
L96	SB-Aq	SB-Aq
L114		HILIC-Z

Important to know

! Switching the stationary phase in USP methods requires a full validation

Scenarios for Adjustment

- Isocratic
  - Totally porous to totally porous particle (TPP)
  - Totally porous to superficially porous particle (SPP)
- Gradient
  - Totally porous to totally porous particle (TPP)
  - Totally porous to superficially porous particle (SPP)

- If superficially porous particles are involved in any part of the adjustment, follow the rules dictating transfer to an SPP particle.
  - Adjustment from a SPP to a TPP
  - Adjustment from a SPP to a SPP of a different particle size

Updates to Isocratic Methods

Adjustment for isocratic elution

According to the method adjustment in USP <621>, the change in conditions for isocratic elution requires four steps:

1. Adjust the column length and particle size according to the L/d_p ratio for TPP to TPP or N for TPP to SPP. The ratio remains constant or in the range between −25% to +50% of the prescribed. Plate number: $N = 5.54(t_R / W_h)^2$

2. Adjust the flow rate for changes in particle size and column diameter. The flow rate is adjusted using the following equation:

$F_2=F_1\times[(dp_1\times dc_2^2)/(dp_2\times dc_1^2)]$

F_1= flow rate indicated in the monograph (mL/min)
F_2= adjusted flow rate (mL/min)
dc_1= internal diameter of the column indicated in the monograph
dc_2= internal diameter of the column used (mm)
dp_1= particle size indicated in the monograph (µm)
dp_2= particle size of the column used (µm)

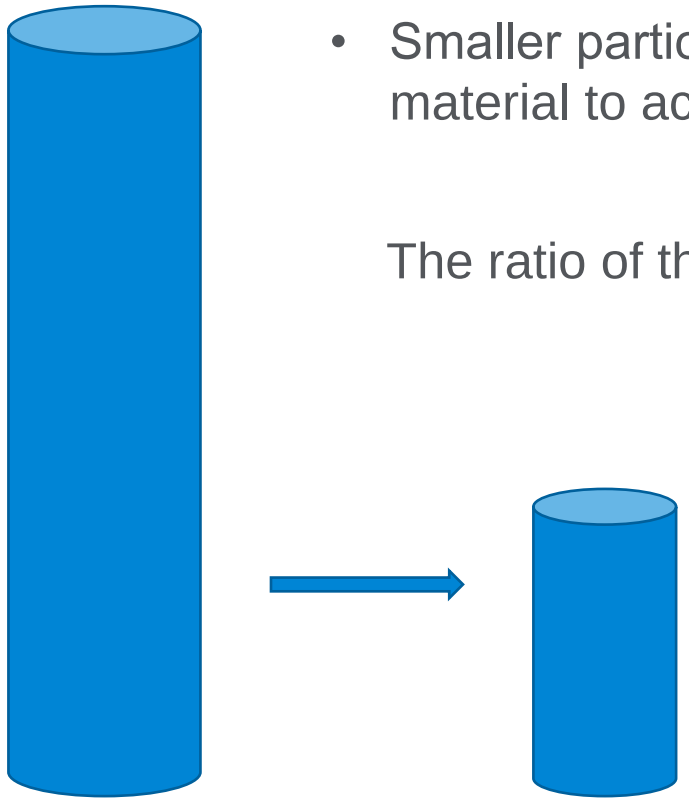
3. Adjust the injection volume using the following equation:

$V_2=V_1\times[(l_2\times dc_2^2)/(l_1\times dc_1^2)]$

V_1= injection volume indicated in the monograph (µL)
V_2= adjusted injection volume (µL)
l_1= column length indicated in the monograph (cm)
l_2= new column length (cm)
dc_1= column internal diameter indicated in the monograph (mm)
dc_2= new column internal diameter (mm)

4. Run the adjusted method and check the system suitability results. Other adjustments in analytical, procedure conditions, including mobile phase, temperature, and pH, may be required (within the permitted ranges described under system suitability).

Injection Volume Adjustments



- Adjust the injection volume to avoid saturating the detector or overloading the column.
- Smaller particles lead to sharper peaks, so we don't need to inject as much material to achieve the same or better sensitivity.

The ratio of the volume of two cylinders is proportional to the ratio of injection volumes

$$\frac{Inj\ Vol\ 2}{Inj\ Vol\ 1} = \frac{L_2\ \pi\ \left(\frac{D_2}{2}\right)^2}{L_1\ \pi\ \left(\frac{D_1}{2}\right)^2} = \frac{L_2\pi(r_1)^2}{L_1\pi(r_2)^2}$$

Where L_1 is the length of column 1

Where L_2 is the length of column 2

Where D_1 is the diameter of column 1

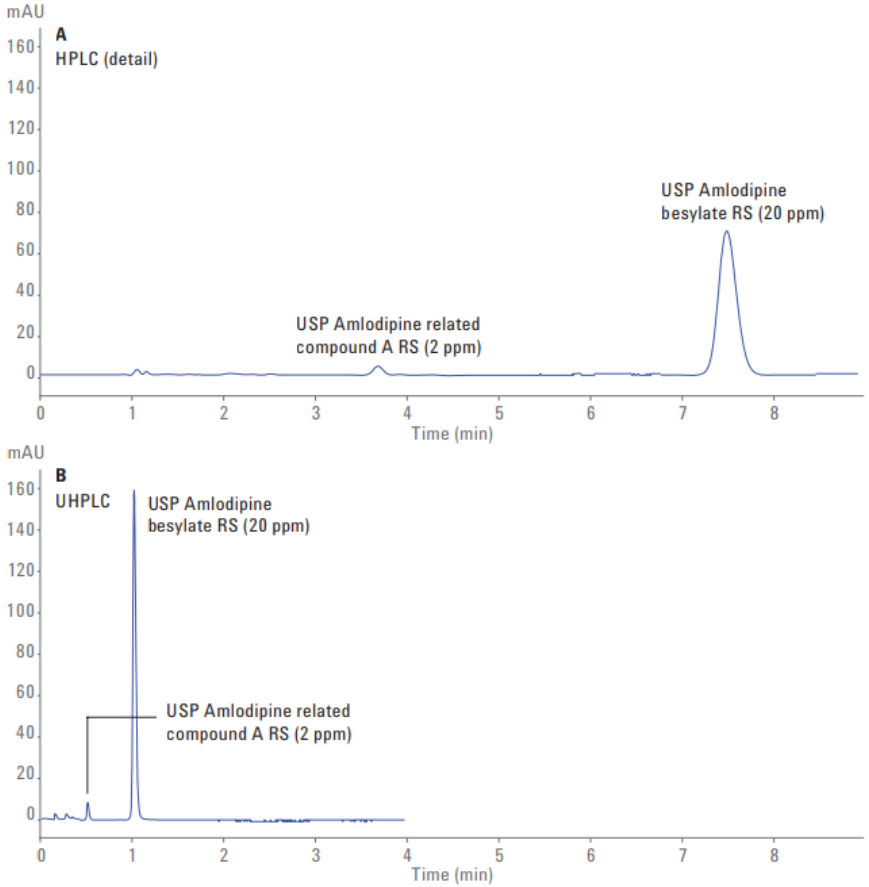
Where D_2 the diameter of column 2

Isocratic Method Transfer from TPP to TPP

USP assay method of amlodipine besylate

	Original in USP	Using HPLC	Using UHPLC
Column	Packing L1 3.9 × 150 mm, 5 µm	Agilent ZORBAX Eclipse Plus C18, 3 × 150 mm, 5 µm (p/n 959993-302)	Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
L/dp ratio	30,000 (-25% to 50%)	30,000	27,778 (-7.4%)
Mobile phase	Methanol/acetonitrile/buffer 35/15/50, isocratic		
Flow rate	1 mL/min	0.6 mL/min	0.8 mL/min
Temperature	Not indicated	30 °C	30 °C
Injection volume*	50 µL	20 µL	10 µL
Detection	UV 237 nm	DAD signal 237/4 nm, ref off 5 Hz	DAD signal 237/4 nm, ref off 20 Hz
Analysis time	Approximately three times the retention of amlodipine	23 minutes	3.1 minutes

*Injection volume: Can be adjusted if it is consistent with the accepted precision, linearity, and detection limits.

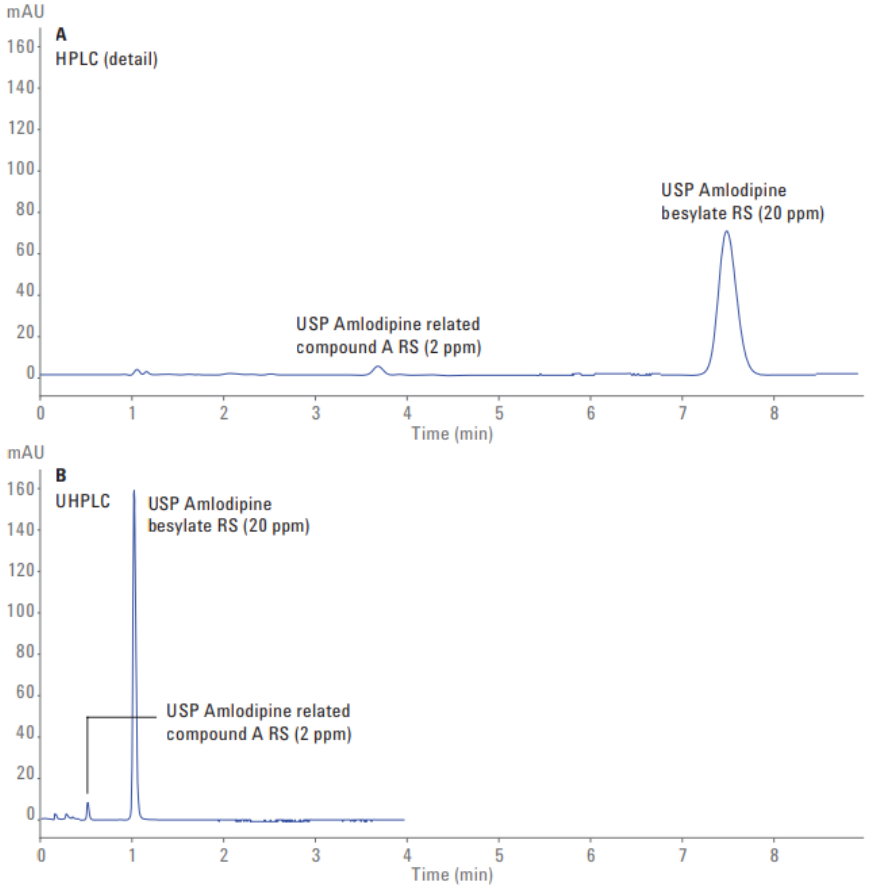


Isocratic Method Transfer from TPP to TPP

USP assay method of amlodipine besylate

	USP requirements	Using HPLC	Using UHPLC (Modernized Methods)
Resolution	NLT 8.5	13.3	9.7
Tailing factor at 5% height	NMT 2.0	1.14	1.31
Area %RSD amlodipine (n = 6)	NMT 1.0 %	0.03	0.02
Area %RSD amlodipine related compound A (n = 6)	NMT 5.0 %	0.29	0.33
Retention time	–	7.52 minutes	1.02 minutes
Analysis time	–	23.00 minutes	3.10 minutes
Mobile phase consumption	–	13.8 mL	2.5 mL

- Analysis time was reduced over 85%, and mobile phase consumption for UHPLC was less than 20% of the consumption in the original HPLC method.
- Laboratory productivity and sample throughput was enhanced.



Isocratic Method Transfer from TPP to SPP

Update method from conventional ZORBAX to SPP Poroshell columns to improve productivity and quality of data

A typical example for the method transfers from TPP columns to SPP columns for isocratic elution is the USP method for diphenhydramine HCl impurities.

Follow the same steps as case study 1 except:

- Change the column length and particle size using combinations of L and dp, provided that the plate number (N) is within the −25 to +50% range.

This rule is for the adjustment of TPP columns to SPP columns for isocratic elution.

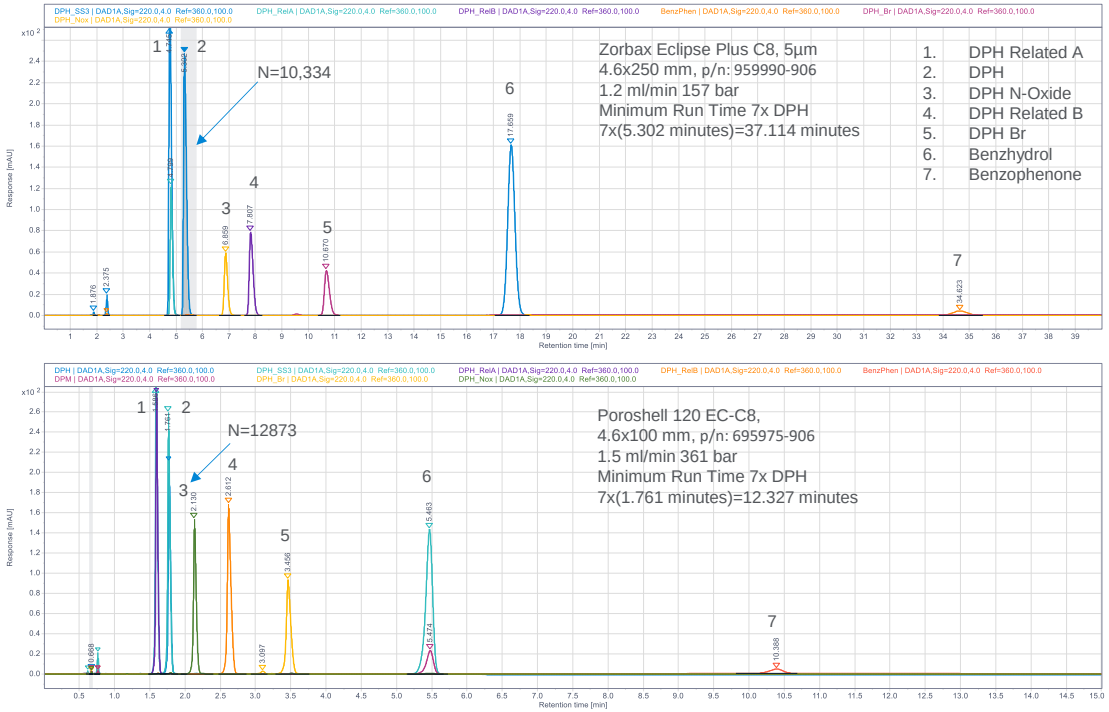
Note: Previously, superficially porous columns could be adjusted using the L/dp rule, but under new guidance, only the N (plate number) rule is acceptable.

Isocratic Method Transfer from TPP to SPP

USP method for diphenhydramine HCl impurities

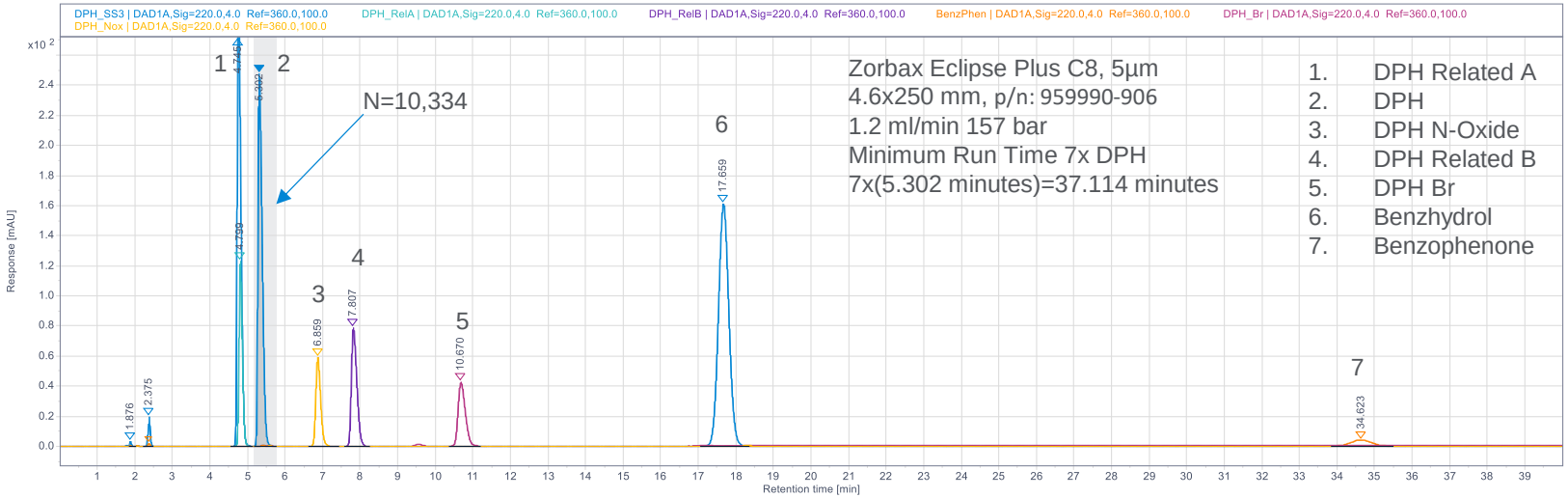
	Original in USP	Using HPLC	Using UHPLC
Column	Packing L7 4.6 × 250 mm, 5 µm	Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm (p/n 959990-906)	Agilent Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm (p/n 695975-906)
No. of plates of diphenhydramine	–25% to +50% range of the original method	10,334	12,873 (+24.6%)
Mobile phase	Premix buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0. Acetonitrile and buffer (35:65)		
Flow rate	1.2 mL/min	1.2 mL/min	1.5 mL/min
Temperature	Not indicated	25 °C	25 °C
Injection volume*	10 µL	10 µL	4 µL
Detection	UV 220 nm	DAD signal 220, ref off 5 Hz	DAD signal 220 nm, ref off 40 Hz
Run time	Not less than seven times retention of diphenhydramine	37.1 minutes	12.3 minutes

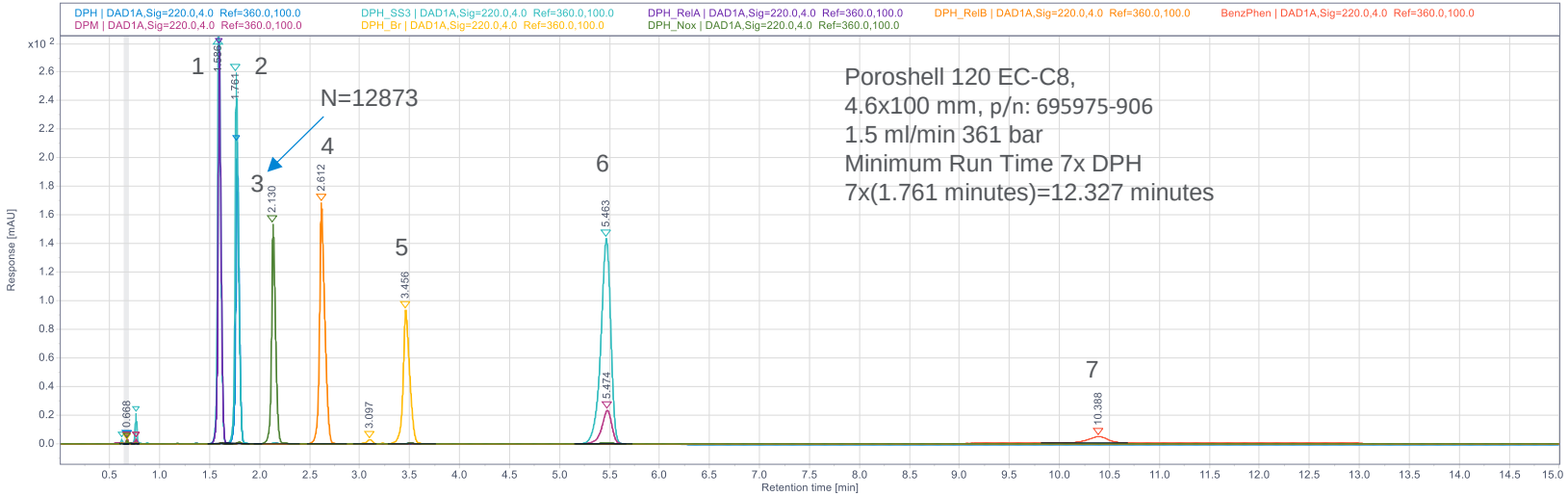
*Injection volume: Can be adjusted if it is consistent with the accepted precision, linearity, and detection limits.



Isocratic Method Transfer from TPP to SPP

USP method for diphenhydramine HCl impurities

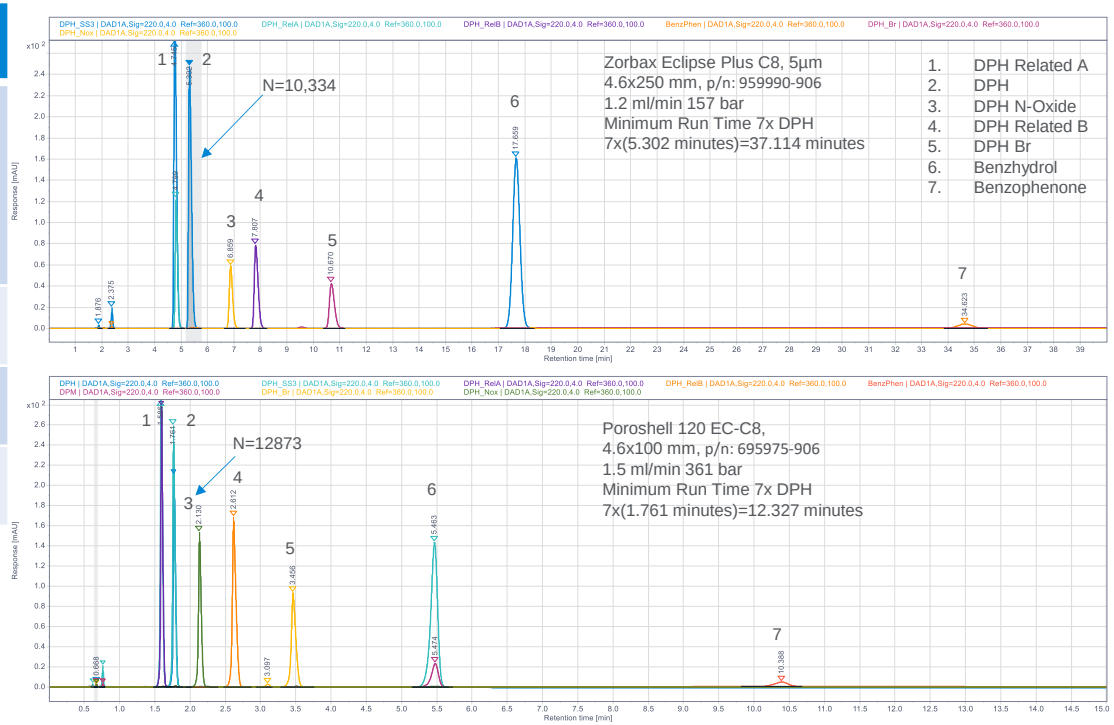




Isocratic Method Transfer from TPP to SPP

USP method for diphenhydramine HCl impurities

	USP requirements	Using HPLC	Using UHPLC (Modernized methods)
NLT 2.0 between diphenhydramine-related compounds A and diphenhydramine	NLT 2.0	2.85	3.02
Retention time of diphenhydramine	–	5.30 minutes	1.76 minutes
Run time	–	37.1 minutes	12.3 minutes (- 66%)
Mobile phase consumption	–	13.8 mL	2.5 mL (- 82%)



- The USP method for diphenhydramine HCl impurities was transferred from a 5 µm TPP column to a 2.7 µm SPP column and no additional method validation was required.
- System suitability criteria were evaluated and reached with both methods.
- Analysis time was reduced by 66%, and mobile phase consumption in UHPLC was 18% of the consumption in the original HPLC method.
- Increased laboratory productivity and reduction in cost-per-sample can be achieved using the described approach.

Updates to Gradient Methods

Adjustment for gradient elution was not allowed under USP37-NF32S1 (official Aug 1, 2014), but is allowed under USP Stage 4 Harmonization, official December 1, 2022.

According to method adjustment in USP <621>, the change in conditions for gradient elution requires the following steps:

1. Adjust the column length and particle size according to the L/dp ratio for TPP to TPP or $(t_R/W_h)^2$ for TPP to SPP. The ratio remains constant or in the range between −25% to +50%.

2. Adjust the flow rate for changes in particle size and column diameter. The flow rate is adjusted using the following equation:

$$F_2=F_1\times[(dp_1\times dc_2^2)/(dp_2\times dc_1^2)]$$

3. Adjust the injection volume using following equation:

$$V_2=V_1\times[(l_2\times dc_2^2)/(l_1\times dc_1^2)]$$

4. Adjust the gradient time of each segment for changes in column length, diameter, and flow rate using the following equation:

$$t_{G2}= t_{G1}\times (F_1/ F_2) [(L_2\times dc_2^2)/ (L_1\times dc_1^2)]$$

t_{G1} = Gradient volume or gradient time (initial)

t_{G2} = New gradient time

F = Flow rate

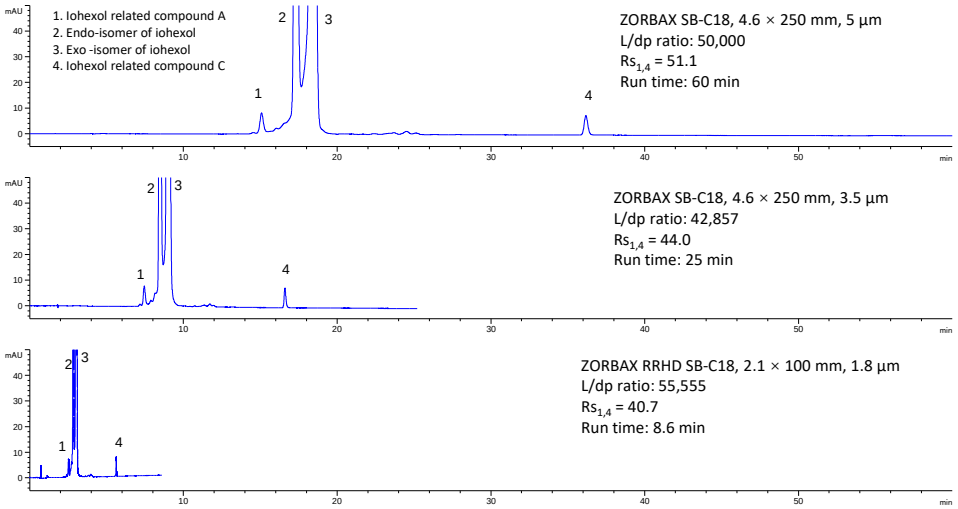
$L\times dc^2$ = The gradient time for each gradient segment needs to be adjusted to maintain a constant ratio of the gradient volume to the column volume

5. Run the adjusted method and check the system suitability results. Other adjustments in analytical procedure conditions, including mobile phase, temperature, and pH, may be required (within the permitted ranges described under system suitability).

Gradient Method Transfer from TPP to TPP

USP method for Iohexol

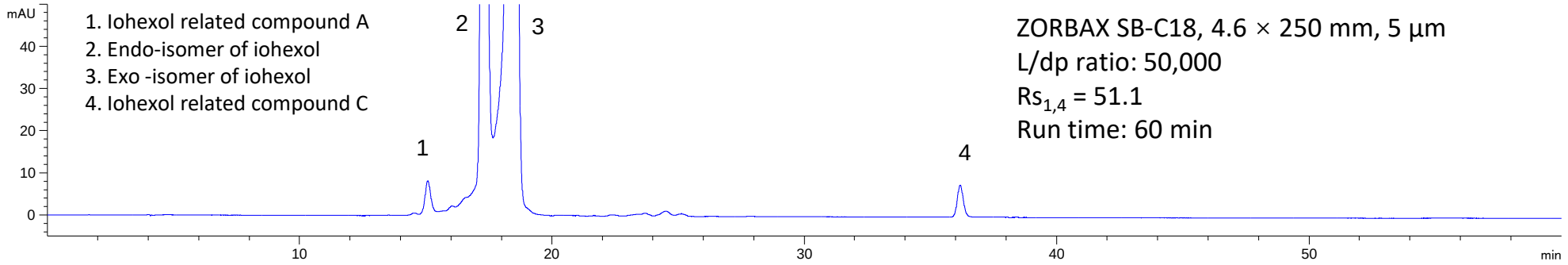
	Original in USP	Using HPLC	Using UHPLC	Using UHPLC
Column	Packing L1 4.6 × 250 mm, 5 µm	Agilent ZORBAX SB-C18, 4.6 × 250 mm, 5 µm (p/n 880975-902)	Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm (p/n 863954-302)	Agilent ZORBAX RRHD SB-C18, 2.1 × 100 mm, 1.8 µm (pen 858700-902)
L/dp ratio	50,000 (-25% to 50%)	50,000	42,857 (-14.3%)	55,555 (+11.1%)
Mobile phase	A: water/ B: acetonitrile			
Flow rate	1 mL/min	1.0 mL/min	0.6 mL/min	0.58 mL/min
Gradient	Time (min) B% 0 1 60 13	Time (min) B% 0 1 60 13 Postrun: 6 min	Time (min) B% 0 1 25 13 Postrun: 4 min	Time (min) B% 0 1 8.6 13 Postrun: 2 min
Temperature	Not indicated	25 °C	25 °C	25 °C
Injection volume*	10 µL	10 µL	3 µL	1 µL
Detection	UV 254 nm	DAD signal 254/4 nm, ref off 5 Hz	DAD signal 254/4 nm, ref off 10 Hz	DAD signal 237/4 nm, ref off 40 Hz
Analysis time	60 min	60 min	25 min	6 min



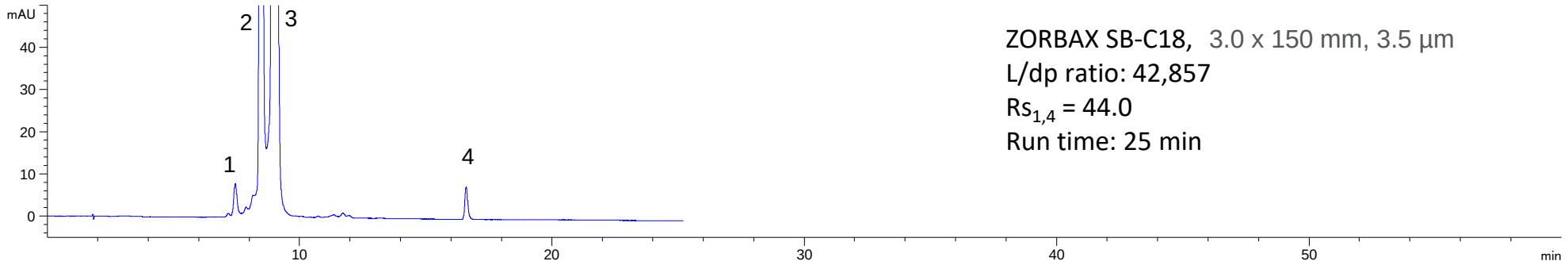
*Injection volume: Can be adjusted if it is consistent with the accepted precision, linearity, and detection limits.

Gradient Method Transfer from TPP to TPP

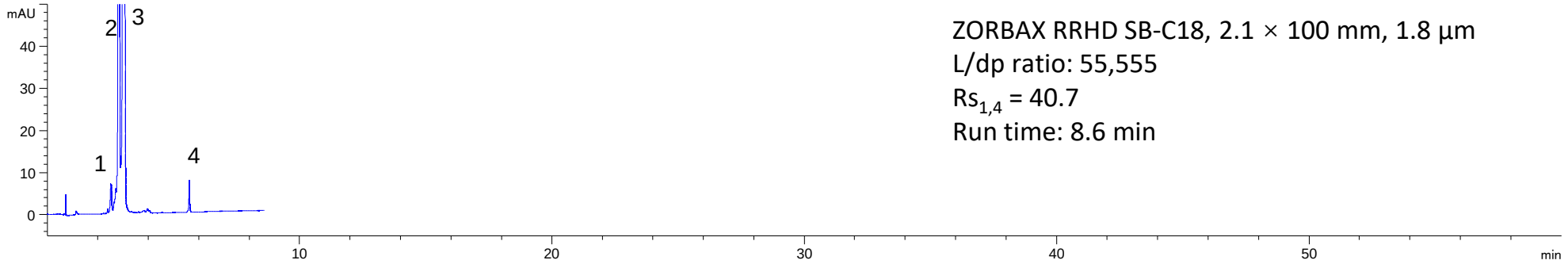
USP method for Iohexol



ZORBAX SB-C18, 4.6 × 250 mm, 5 µm
L/dp ratio: 50,000
$Rs_{1,4}$ = 51.1
Run time: 60 min



ZORBAX SB-C18, 3.0 x 150 mm, 3.5 µm
L/dp ratio: 42,857
$Rs_{1,4}$ = 44.0
Run time: 25 min

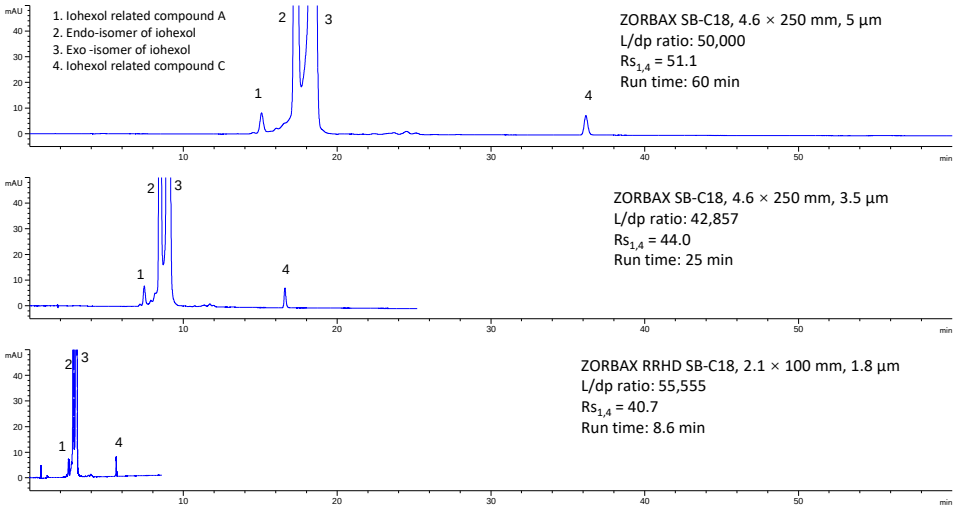


ZORBAX RRHD SB-C18, 2.1 × 100 mm, 1.8 µm
L/dp ratio: 55,555
$Rs_{1,4}$ = 40.7
Run time: 8.6 min

Gradient Method Transfer from TPP to TPP

USP method for Iohexol

	USP Requirements	Using HPLC	Using UHPLC (Modernized Methods)	Using UHPLC (Modernized Methods)
Resolution between related compound A and C	NLT 20	51.1	44.0	40.7
Analysis time	–	60 minutes	25 minutes (- 58%)	8.6 minutes (- 86%)
Mobile phase consumption	–	60.0 mL	15 mL (- 75%)	5.0 mL (-92%)



- USP method for related compounds analysis of iohexol was transferred from a 5 µm ZORBAX SB-C18 column to 3.5 µm and 1.8 µm ZORBAX SB-C18 columns without additional method validation.
- System suitability criteria were evaluated and in compliance with all methods.
- Analysis time was reduced by 58% with a 3.5 µm column and 86% with a 1.8 µm column. Mobile phase consumption was also dramatically reduced by 75% with a 3.5 µm column and 92% with a 1.8 µm column.
- Laboratory productivity and sample throughput can be enhanced using this approach.

Gradient Method Transfer from TPP to SPP

USP assay method for diphenhydramine HCl

	Original in USP	Using HPLC	Using UHPLC
Column	Packing L7 4.6 × 250 mm, 5 µm	Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm (p/n 959990-906)	Agilent Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm (p/n 695975-906)
$(t_R/W_h)^2$ of DPH	–25 to +50% range of original method	2,410 (1,805 to 3,615)	2,778 (+15.3%)
Mobile phase	Mobile phase A: Buffer: 5.4 g/L of monobasic potassium phosphate. Adjust with phosphoric acid to a pH of 3.0. Mobile phase B: Acetonitrile Diluent: Acetonitrile and buffer (35:65)		
Flow rate	1.2 mL/min	1.2 mL/min	1.8 mL/min
Gradient	Time (min) B% 0 35 4 35 7 80 9 35 13 35	Time (min) B% 0 35 4 35 7 80 9 35 13 35	Time (min) B% 0 35 1.1 35 1.9 80 2.4 35 3.5 35
Temperature	Not indicated	25 °C	25 °C
Injection volume*	10 µL	10 µL	4 µL
Detection	UV 220 nm	DAD signal 220, ref off 5 Hz	DAD signal 220 nm, ref off 40 Hz
Run time	13 minutes	13 minutes	3.5 minutes

*Injection volume: Can be adjusted if it is consistent with the accepted precision, linearity, and detection limits.

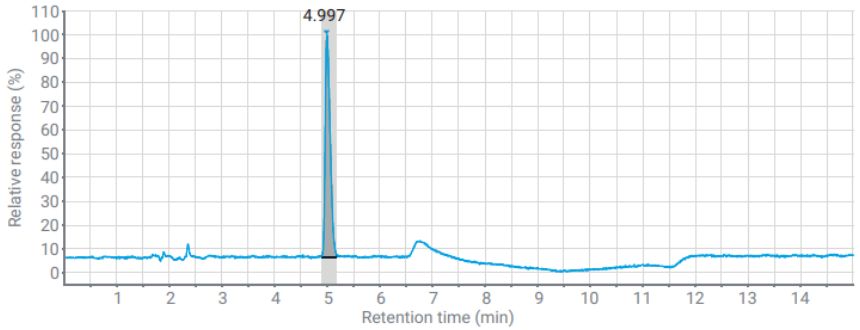


Figure 2. Diphenhydramine assay 1.2 mL/min Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm.

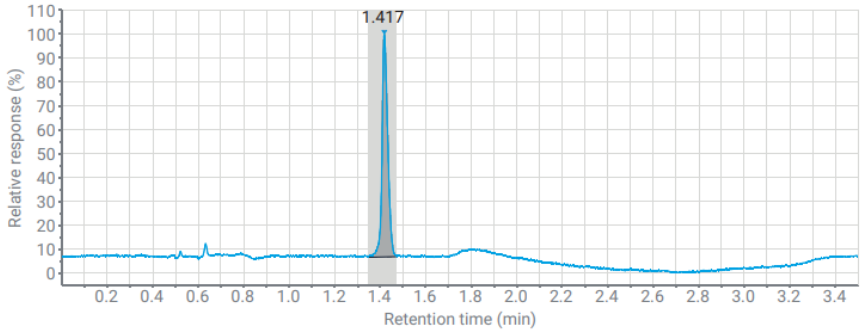


Figure 3. Diphenhydramine assay 1.8 mL/min Agilent Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm.

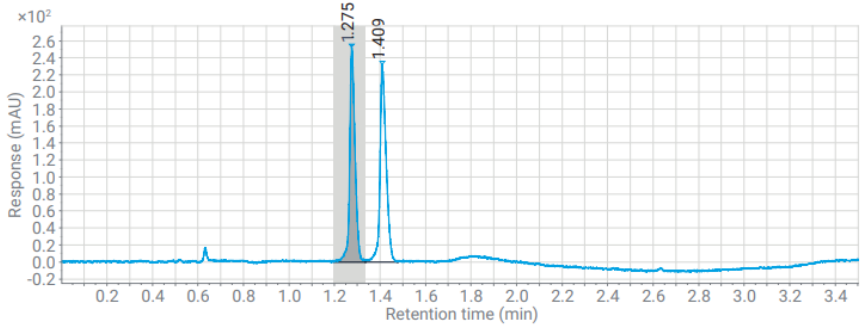


Figure 4. Diphenhydramine assay 1.8 mL/min Agilent Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm system suitability sample.

Flow Rate Optimization

Column	Flow Rate (mL/min)	t_R (min)	(w_h) (min)	$(t_R/(w_h))^2$	Acceptable Range
Eclipse Plus C8 4.6 x 250, 5 µm	1.2	5.069	0.103	2410	1805 to 3615
Poroshell 120 EC-C8 4.6 x 100, 2.7 µm	1.2	2.015	0.037	2897	Meets
	1.4	1.755	0.0323	2863	Meets
	1.6	1.563	0.0309	2804	Meets
	1.8	1.411	0.0268	2778	Meets

Gradient Method Transfer from TPP to SPP

USP assay method for diphenhydramine HCl

	USP Requirements	Using HPLC	Using UHPLC (Modernized Method)
Resolution between diphenhydramine related compound A and diphenhydramine	NLT 1.5	-	2.96
RSD for standard solution	NMT 0.85	-	0.68
Tailing factor	NMT 1.8	-	1.11
Run time	–	13 minutes	3.5 minutes (-73%)
Mobile phase consumption	–	15.6 mL	6.3 mL (-60%)

- USP assay method for diphenhydramine HCl was transferred from 5 µm TPP column to 2.7 µm SPP column.
- System suitability criteria were evaluated and in compliance with both methods.
- Analysis time and mobile phase consumption were reduced by 73% and 60%, respectively, compared to the original HPLC method.
- Laboratory productivity and sample throughput can be enhanced using the described approach.

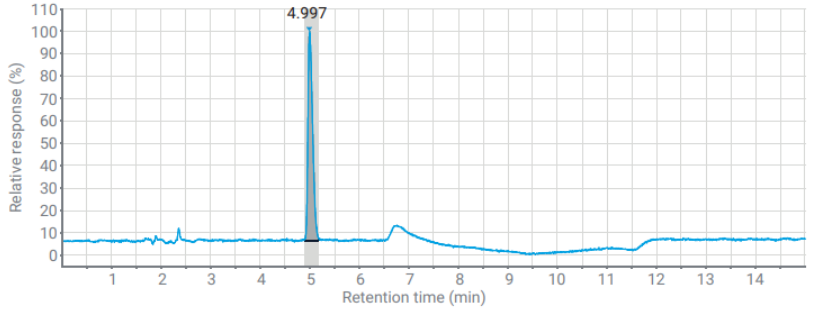


Figure 2. Diphenhydramine assay 1.2 mL/min Agilent ZORBAX Eclipse Plus C8, 4.6 × 250 mm, 5 µm.

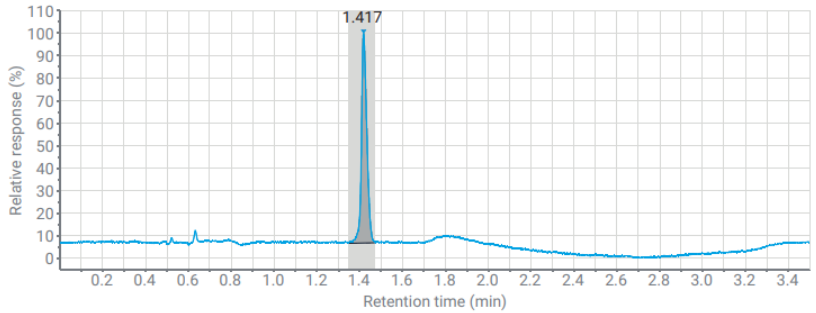


Figure 3. Diphenhydramine assay 1.8 mL/min Agilent Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm.

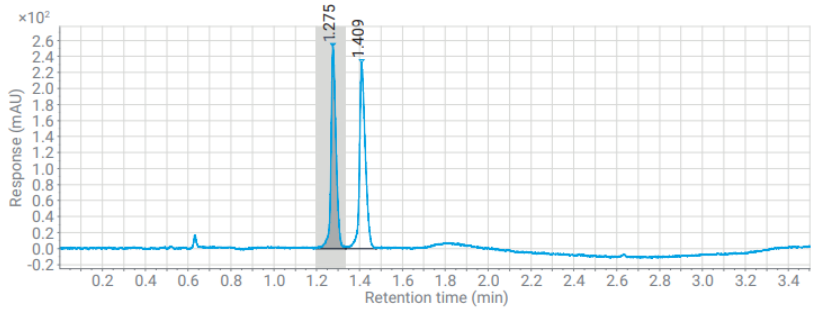


Figure 4. Diphenhydramine assay 1.8 mL/min Agilent Poroshell 120 EC-C8, 4.6 × 100 mm, 2.7 µm system suitability sample.

Additional Considerations for <621> Adjustments

- In USP <621>: “Caution is necessary when the adjustment results in smaller peak volumes due to a smaller particle size or smaller internal column diameter, a situation that may require adjustments to minimize extra-column band broadening by factors such as instrument connections, detector cell volume and sampling rate, and injection volume.”
- Agilent recommends a risk assessment to assess the cumulative effect of multiple adjustments during modernization and method transfers across column platforms.
- System suitability is the primary verification for compliance of satisfactory performance.
- **Considering dwell and dispersion volumes is another major criterion for method transfers:**
  - Monographs preferably include an isocratic step before the start of the gradient program so that an adaptation can be made to the gradient time points to take account of differences in dwell volume between the system that was used for analytical development and the LC system actually used.
  - The revised USP<621> shows how to calculate dwell volume as well.

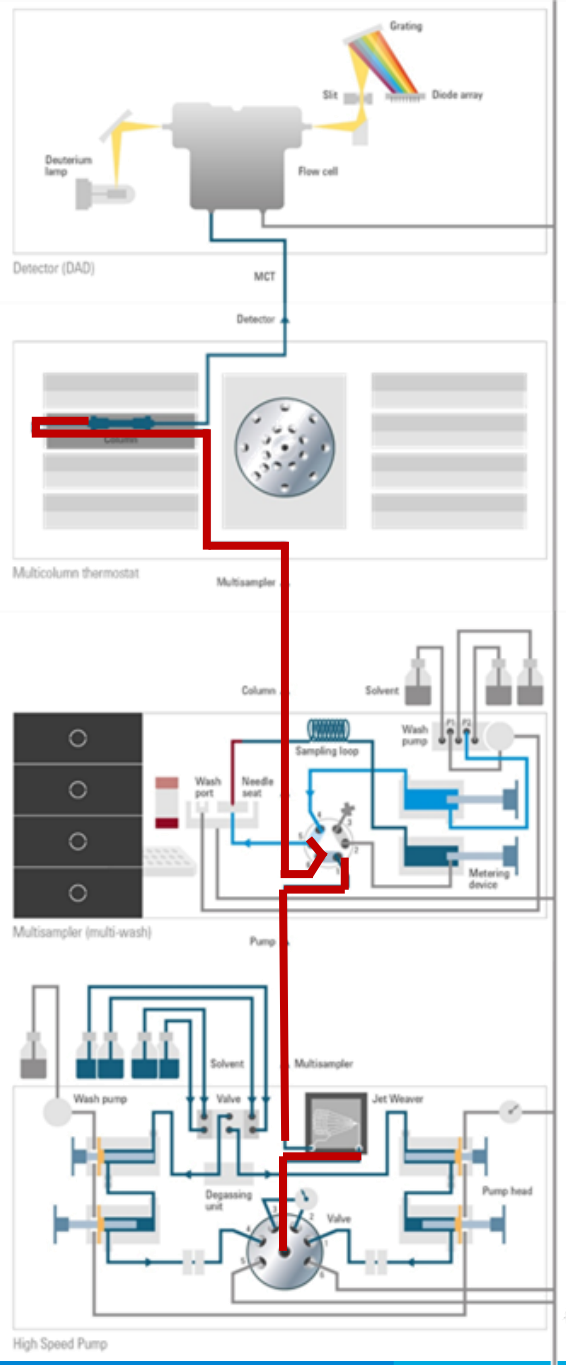
System Considerations

Dwell volume: from the formation of the gradient to the top of the column

- Important to consider when transferring a method between two different instruments.

Can affect:

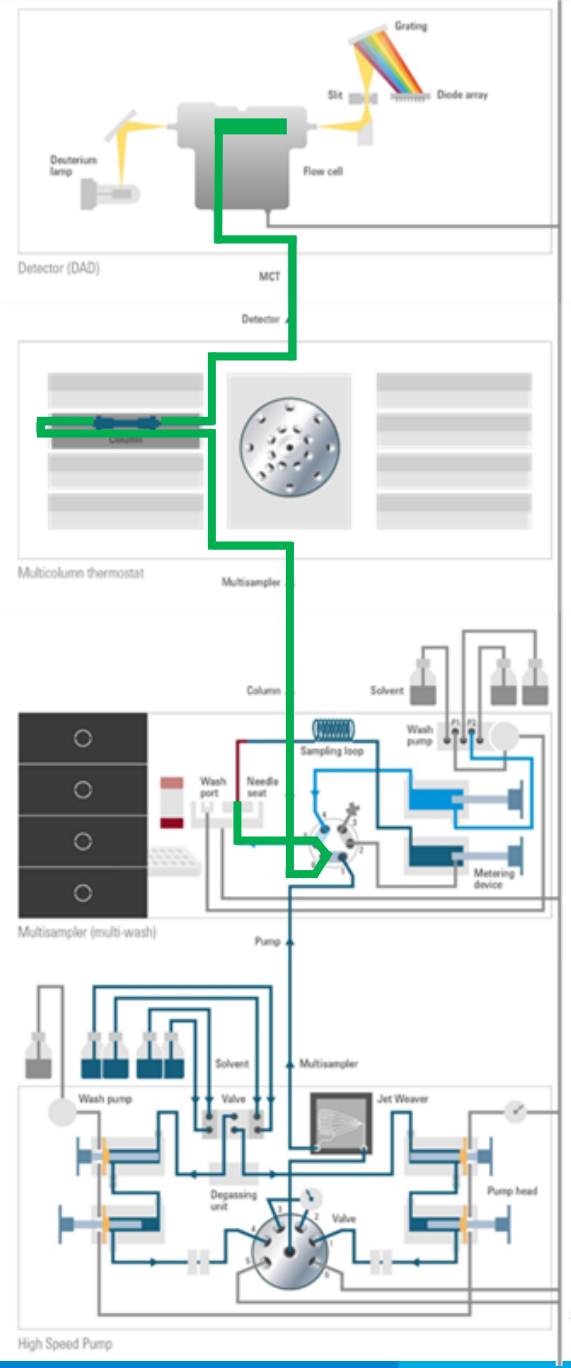
- Retention time
- Resolution
- Peak width



System Considerations

Extra-column volume (dispersion): from the point of injection to the detector (flow cell) outside of the column

- Minimize to reduce band broadening, for sharper peaks and better resolution



Resources

Find resources at:

[Revisions per USP 621 | Agilent]

Understanding the Latest Revisions to USP <621>

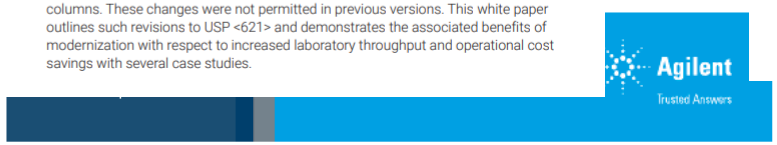
Adoption of the revised guidance for analytical method transfers and modernization of LC methods

Authors

Rongjie Fu, Manu Grover, Rob Freeman, and William Long
Agilent Technologies, Inc.

Abstract

Modernization of LC methods is key in lifecycle management of analytical procedures. United States Pharmacopeia (USP) General Chapter <621> allows method adjustments and transfers, making it easier for labs to modernize original USP methods. The revised version of USP <621>, which became effective in December 2022, has been updated to meet industry needs. The USP <621> revisions allow a change in gradient methods, as well as a change from totally porous silica-based analytical columns to superficially porous particle-based columns. These changes were not permitted in previous versions. This white paper outlines such revisions to USP <621> and demonstrates the associated benefits of modernization with respect to increased laboratory throughput and operational cost savings with several case studies.



Gradient Method Transfer of the Iohexol USP Monograph HPLC Method for Related Compounds to Smaller Particle Size ZORBAX Columns

Author

Rongjie Fu
Agilent Technologies (Shanghai) Co., Ltd

Abstract

The original USP monograph HPLC method of related-compounds analysis for iohexol was transferred to smaller particle size 3.5 and 1.8 µm Agilent ZORBAX columns following the newly revised U.S. Pharmacopeia (USP) General Chapter <621> guidelines. The original method uses a gradient separation with a 4.6 × 250 mm, 5 µm column and requires 60 minutes for the analysis. The analysis time was reduced from 60 to 25 minutes when the method was transferred to the Agilent ZORBAX SB-C18, 3.0 × 150 mm, 3.5 µm column (58% reduction in analysis time and 75% reduction in solvent consumption). Furthermore, analysis time was reduced from 60 to 8.6 minutes when the method was transferred to the Agilent ZORBAX RRHD SB-C18 column, 2.1 × 100 mm, 1.8 µm (86% reduction in analysis time and 92% reduction in solvent consumption), without method revalidation. All system suitability requirements were met while achieving significant reductions in both analysis time and solvent consumption.

A Simple Conversion of the USP Method for Diphenhydramine HCl Impurities to the Agilent InfinityLab Poroshell 120 EC-C8 Column

Author

William J. Long
Agilent Technologies, Inc.

Abstract

The transfer of the USP Impurities method for diphenhydramine hydrochloride is demonstrated using Agilent ZORBAX Eclipse Plus C8 and Agilent InfinityLab Poroshell 120 EC-C8 columns. The initial method uses a 5 µm 4.6 × 250 mm column and requires 40 minutes for the analysis. When InfinityLab Poroshell 120 EC-C8 columns (4.6 × 100 mm, 2.7 µm) are used, analysis time is reduced from 40 to 33% of the original method time, without need for revalidation using the InfinityLab Poroshell 120 EC-C8 column. Pressure is monitored and considered a factor in instrument transfer. This transfer is consistent with allowed adjustments under USP37-NF32S1 (official August 1, 2014), and USP Stage 4 Harmonization, to be official December 1, 2022.

A Simple Conversion of the USP Assay Method for Diphenhydramine HCl to the Agilent InfinityLab Poroshell 120 Column EC-C8

Author

William J. Long
Agilent Technologies, Inc.

Abstract

The transfer of the USP Assay method for diphenhydramine hydrochloride is demonstrated using Agilent ZORBAX Eclipse Plus C8 and Agilent InfinityLab Poroshell 120 EC-C8 columns. The initial method uses a 4.6 × 250 mm, 5 µm column and requires 13 minutes for the analysis. When InfinityLab Poroshell 120 EC-C8 columns (4.6 × 100 mm, 2.7 µm) are used, analysis time is reduced from 13 to 3.5 minutes (27%) of the original method time, without need for revalidation using the InfinityLab Poroshell 120 EC-C8 column. Pressure is monitored and considered as a factor in instrument transfer. This transfer is not allowed under USP37-NF32S1 (official August 1, 2014), but will be allowed under USP Stage 4 Harmonization, to be official December 1, 2022.

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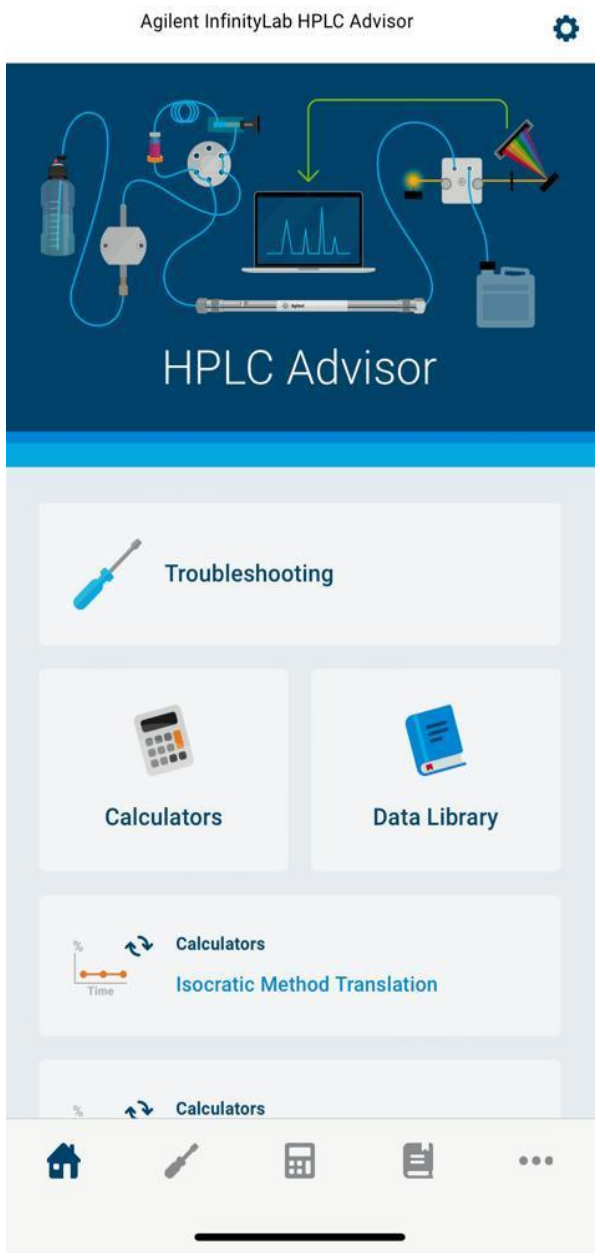

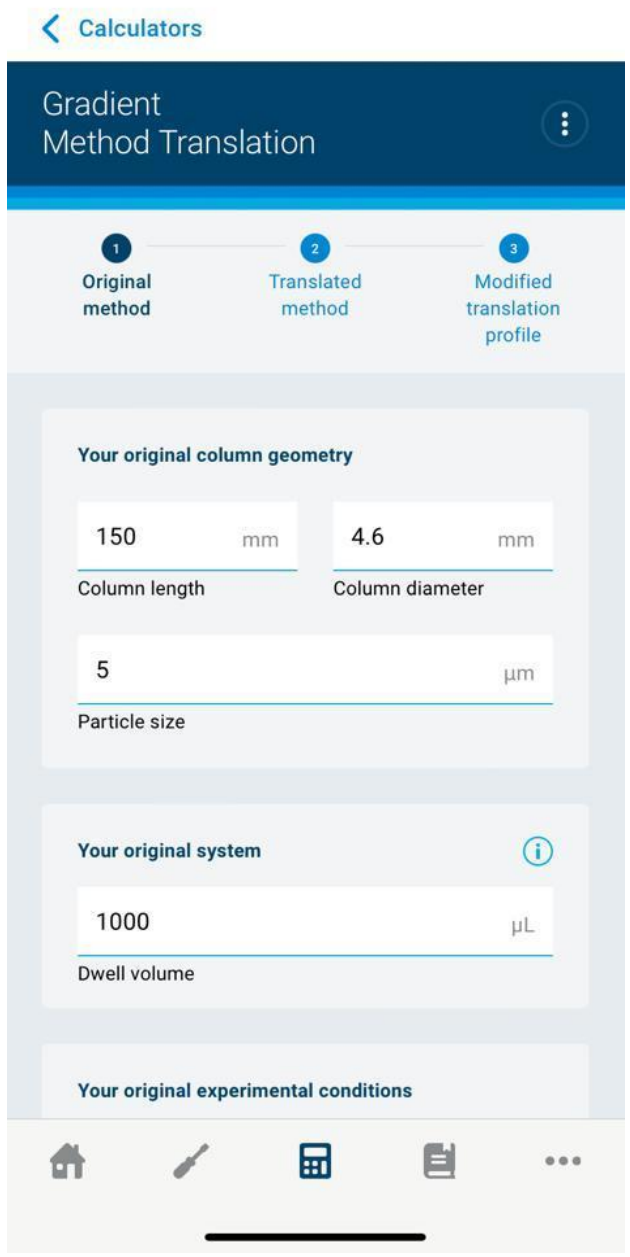

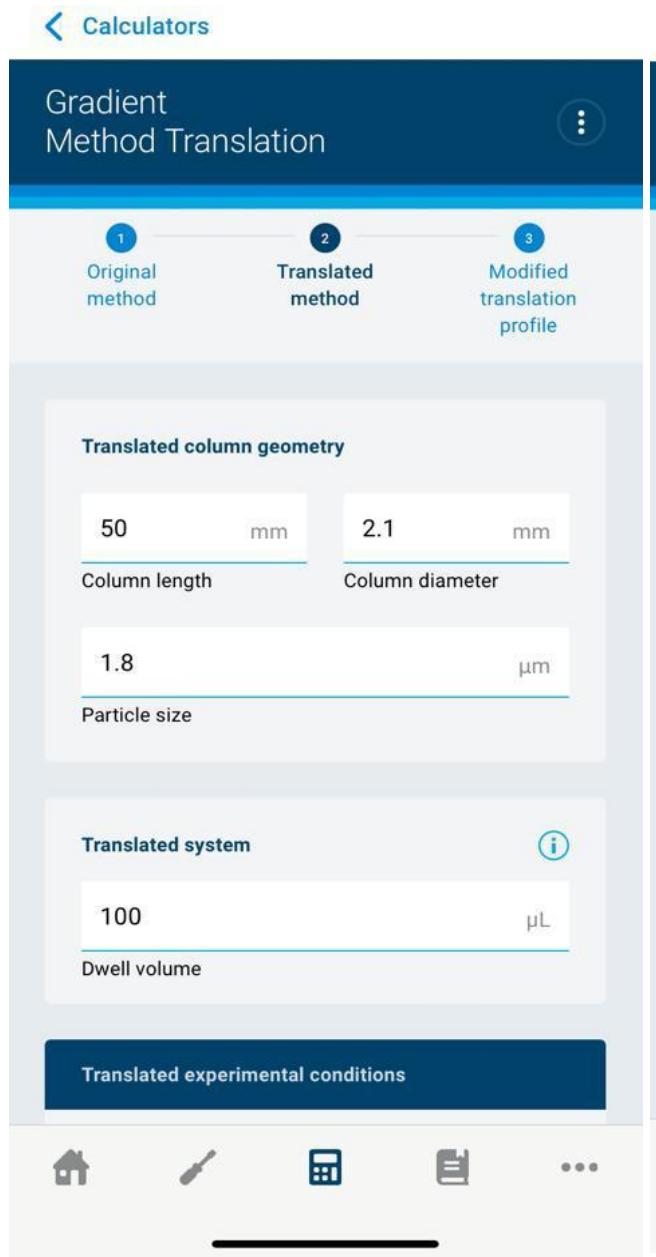

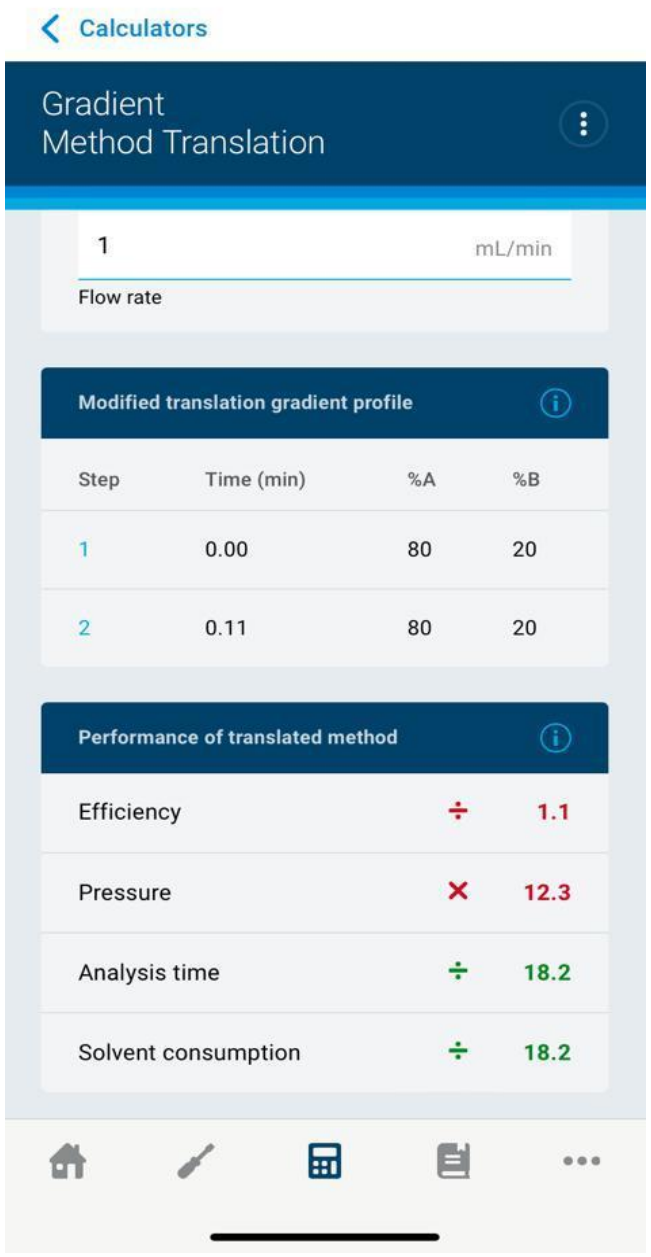

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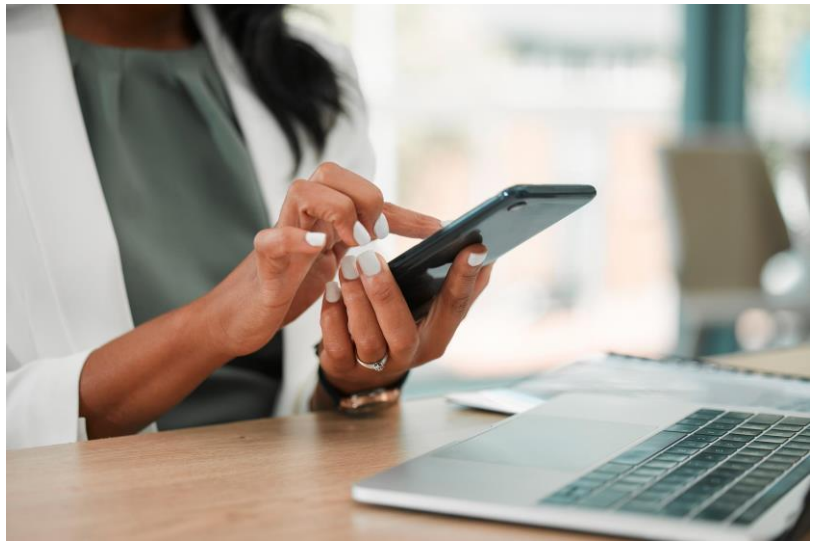

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