### Modernizing USP Methods According to <621> with Superficially Porous Particle Columns

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## Outline

- Review allowable changes with USP <621>
- Understanding the impact and benefits of using smaller I.D. analytical columns

• Case studies

• Summary

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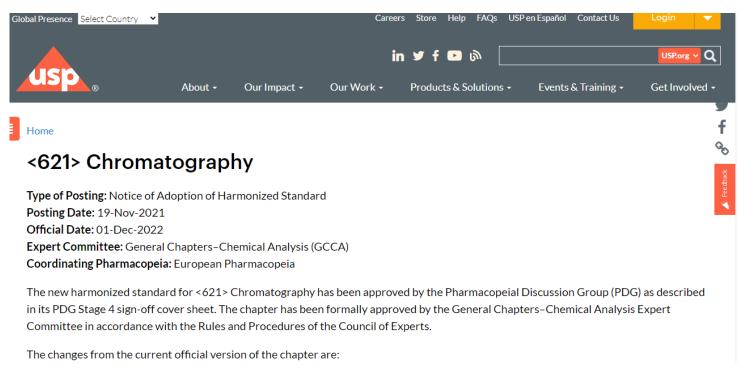
FAI

USP Chapter <621> Guidelines for Changing Isocratic and Gradient Methods

#### Pharmacopeia Changes

## HALO

• For United States Pharmacopeia (USP), changes went into effect December 1, 2022



• For British Pharmacopoeia (BP), European Pharmacopoeia (EP), and Japanese Pharmacopoeia (JP), changes went into effect January 2023



#### Allowable Changes to USP Methods

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Parameter	Isocratic Methods	Gradient Methods
Stationary Phase	Must keep same L category Change from totally porous particle (TPP) to superficially porous particle (SPP) is allowed	Same as isocratic
Column Dimensions	Particle size and/or length of the column may be modified, provided that ratio of the column length (L) to the particle size $(d_p)$ remains constant or in the range between -25% to +50% of the prescribed L/d <sub>p</sub> ratio*	Same as isocratic <sup>^</sup>
Internal Diameter	Change allowed	Change allowed
Flow Rate	$F_2 = F_1 \times \left[ (dc_2^2 \times dp_1) \div (dc_1^2 \times dp_2) \right]$	Same as isocratic

\*For changes from TPP to SPP, other combinations of L and dp can be used, provided that the plate number (N) is within –25% to +50%, relative to the prescribed column. These changes are acceptable, provided that system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent

^For changes from TPP to SPP, other combinations of L and dp can be used provided that the ratio  $(t_R/W_h)^2$  is within-25% to +50%, relative to the prescribed column for all the peaks used to determine the system suitability parameters. These changes are acceptable provided system suitability criteria are fulfilled, and selectivity and elution order of the specified impurities to be controlled are demonstrated to be equivalent.

## Allowable Changes to USP Methods - continued HALO

Parameter	Isocratic Methods	Gradient Methods
Detector Wavelength	No change allowed	No change allowed
Mobile Phase Composition	Amount of the minor components of the mobile phase can be adjusted by ±30% relative. However, the change in any component cannot exceed ±10% absolute	Gradient time adjusted by equation $t_{G2} = t_{G1} \times (F_1/F_2)[(L_2 \times d_{C2}^2)/(L_1 \times d_{C1}^2)]$
рН	±0.2 pH units, unless otherwise prescribed	Same as isocratic
Buffer	±10%	Same as isocratic
Injection Volume	$V_{inj2} = V_{inj1} \times (L_2 d_{c2}^2) / (L_1 d_{c1}^2)$	Same as isocratic
Column Temperature	± 10°C	± 5° C



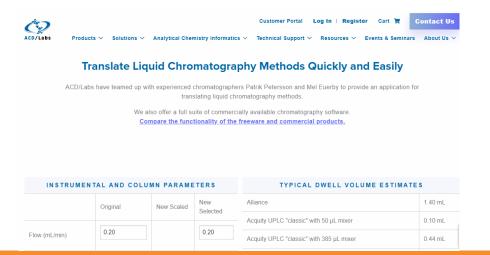
#### **Method Translators**

## HALO

<u>https://ispso.unige.ch/labs/fanal/hplc\_calculator:en</u>

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> HPLC Calculator					
- HPLC calculator: software for chromatographic performance evaluation and HPLC method transfer					
6	HPLC o	alculator v3.	1 🕜	NEWS *	
		transfer method			
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	Chromatographic	performance in iso			

• <u>https://www.acdlabs.com/resources/freeware/translator/index.php</u>





# Understanding the Impact and Benefits of Using Smaller I.D. Analytical Columns

## Words of Caution for Moving to Smaller Particle Sizes HAI and Smaller Column I.D.s

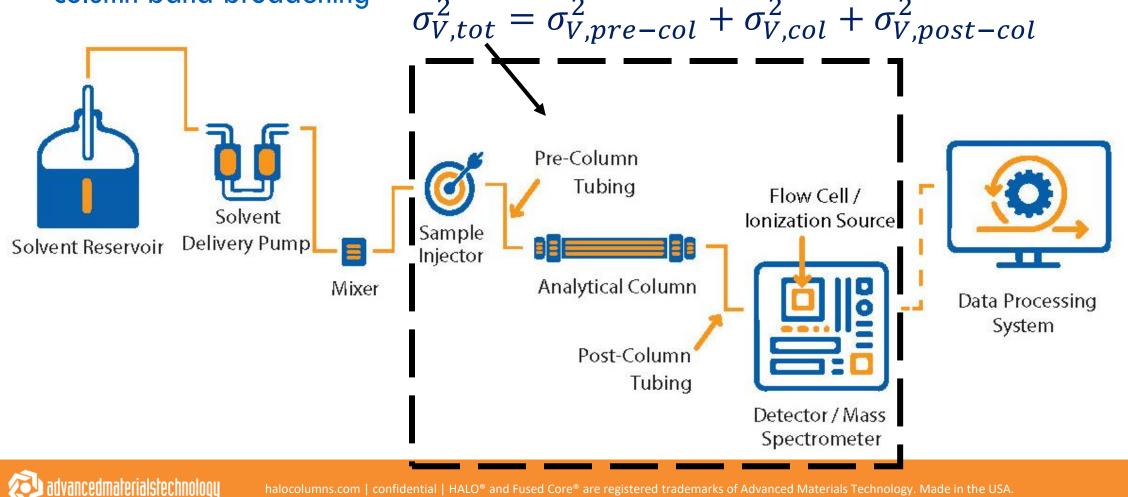
 Smaller particle sizes and smaller column I.D.s are more susceptible to extracolumn band broadening



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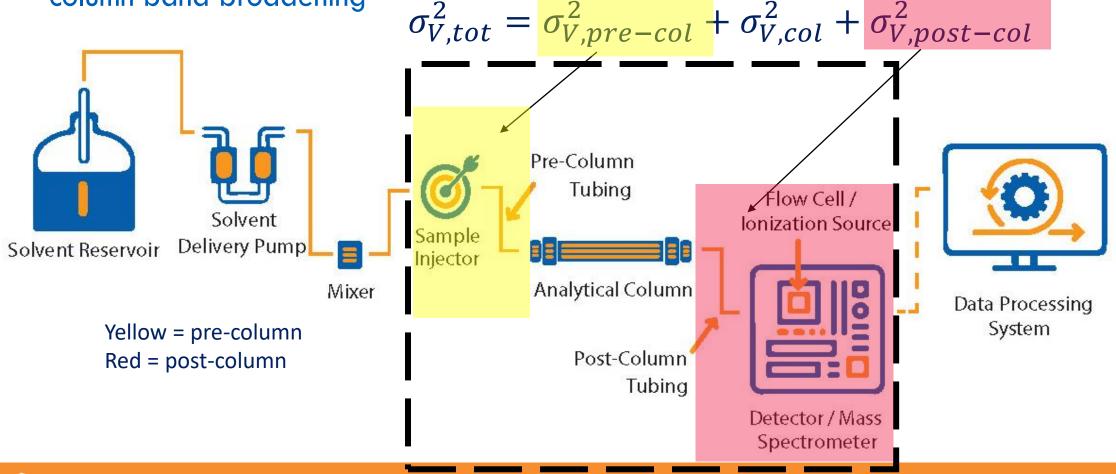
#### Words of Caution for Moving to Smaller Particle Sizes and Smaller Column I.D.s

Smaller particle sizes and smaller column I.D.s are more susceptible to extracolumn band broadening



## Words of Caution for Moving to Smaller Particle Sizes HA and Smaller Column I.D.s

 Smaller particle sizes and smaller column I.D.s are more susceptible to extracolumn band broadening



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#### Ways to Reduce Extra-Column Band Broadening HALO

• Use the shortest and smallest I.D. connecting tubing between instrument components

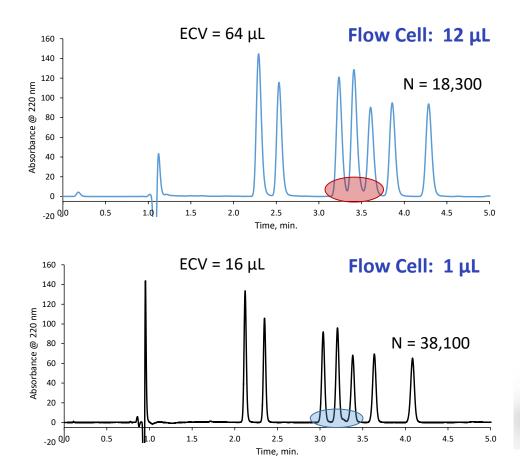


- Use a smaller volume detector flow cell ( $\leq 2.5 \ \mu$ L)
- Increase the data acquisition rate ( $\geq 20$  Hz)
- Decrease the injection volume (more critical for isocratic than gradient separations)



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#### Extra-Column Band Broadening Impact on Resolution and Efficiency



50% average increase in plates is observed by reducing the excess volume in the system!



Isocratic Separations with HALO 90 Å C18, 2 µm, 3.0 x 150 mm

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#### Strategy for Modernizing Methods

- Keep the same USP classification
- Calculate -25 to + 50% of the L/dp ratio for original column
- If current method uses a FPP column:
  - Use a smaller particle size HALO<sup>®</sup> column in smaller ID and shorter column dimension
- If current method uses a SPP column:
  - Can either use a similar size HALO<sup>®</sup> column or move to a smaller particle, smaller ID, and shorter column dimension



## **Case Studies**

#### While Making Changes, Take Notice!

 Adjustment of conditions with gradient elution (HPLC) ...is more critical than with isocratic (HPLC) ...elution, since it may shift some peaks to a different step of the gradient ..., potentially causing partial or complete coelution of adjacent peaks or peak inversion, and, thus leading to the incorrect assignment of peaks and to the masking of peaks or a shift such that elution occurs beyond the prescribed elution time.

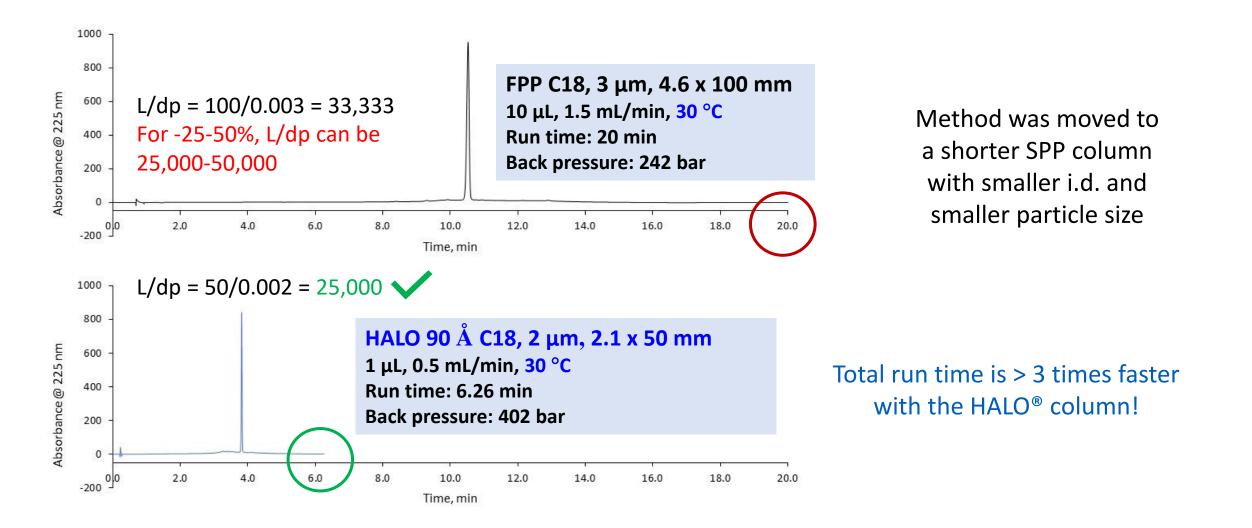


• For some parameters, the adjustments are explicitly defined in the monograph to ensure the system suitability.



#### USP Monograph for Itraconazole – Modified

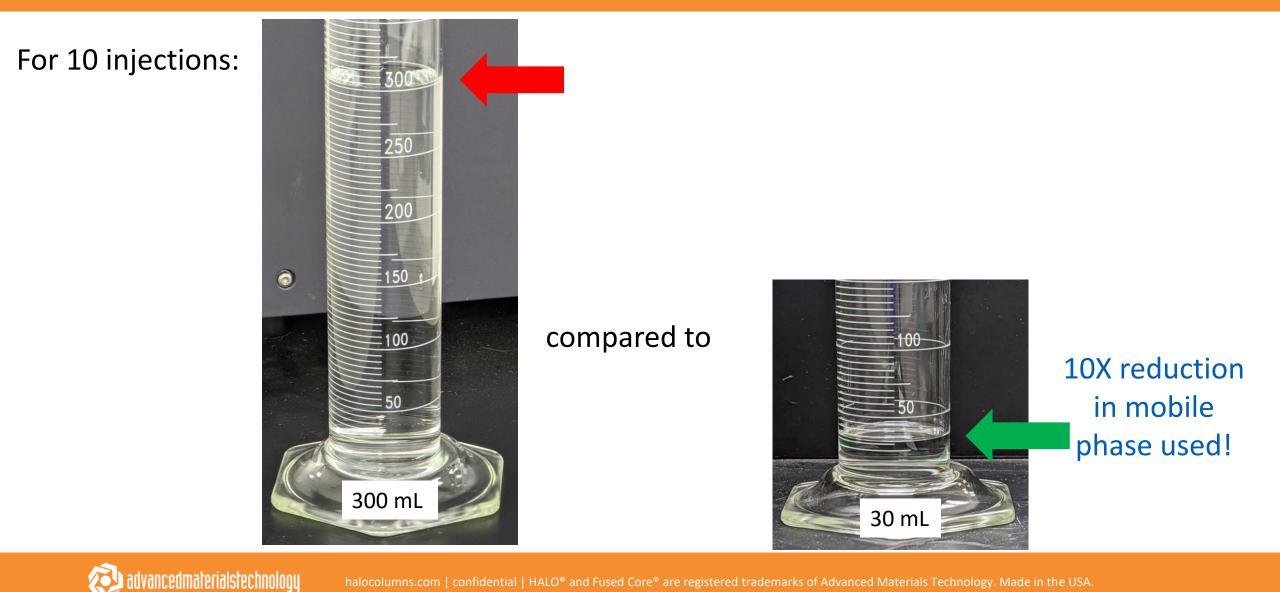
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#### USP Monograph for Itraconazole: **Mobile Phase Reduction**

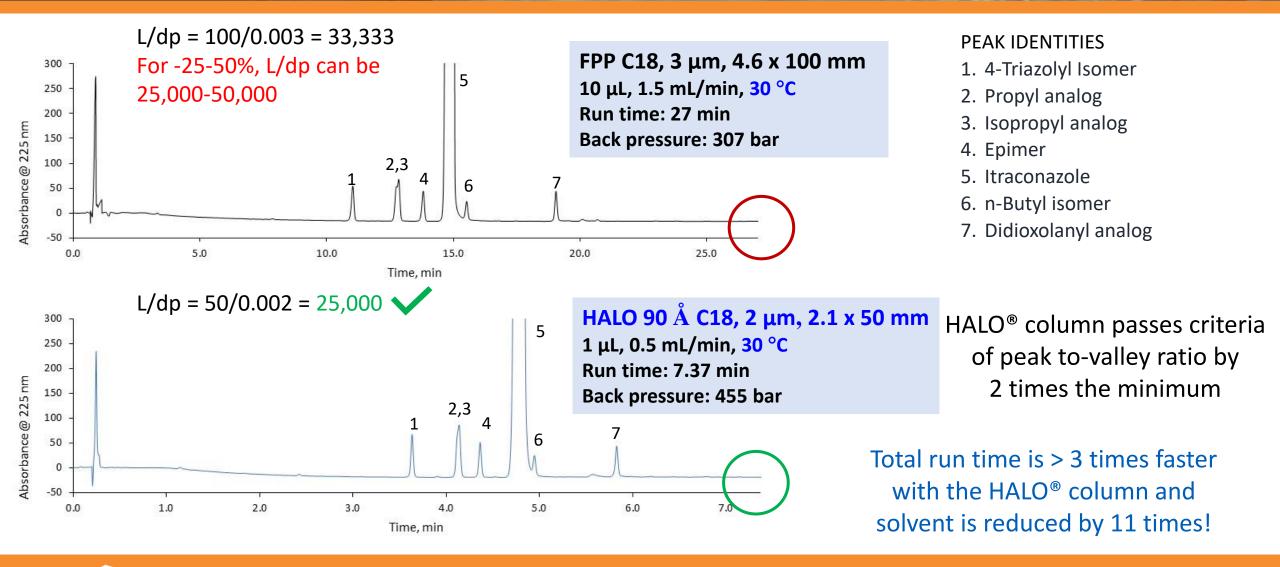
## HALO



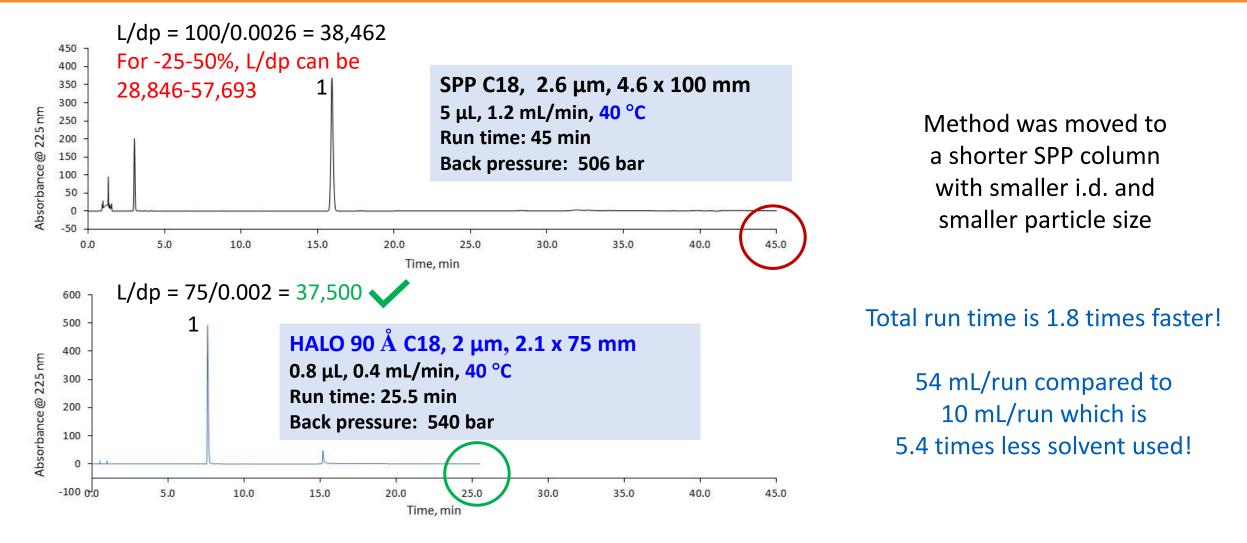
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#### USP Monograph for Itraconazole System Suitability – Modified





#### USP Monograph for Cobamamide – Modified



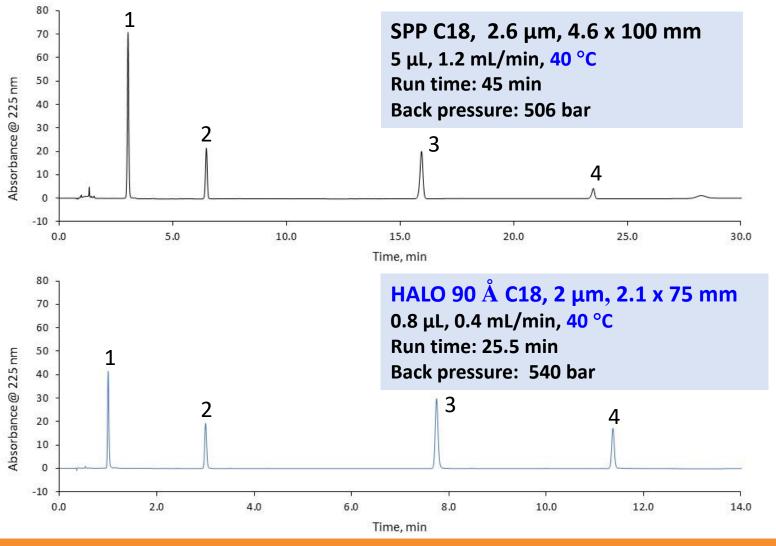
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#### USP Monograph for Cobamamide – Modified System Suitability

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**Peak Identities** 

- 1. hydroxocobalamin chloride
- 2. cyanocobalamin
- 3. cobamamide
- 4. methylcobalamin

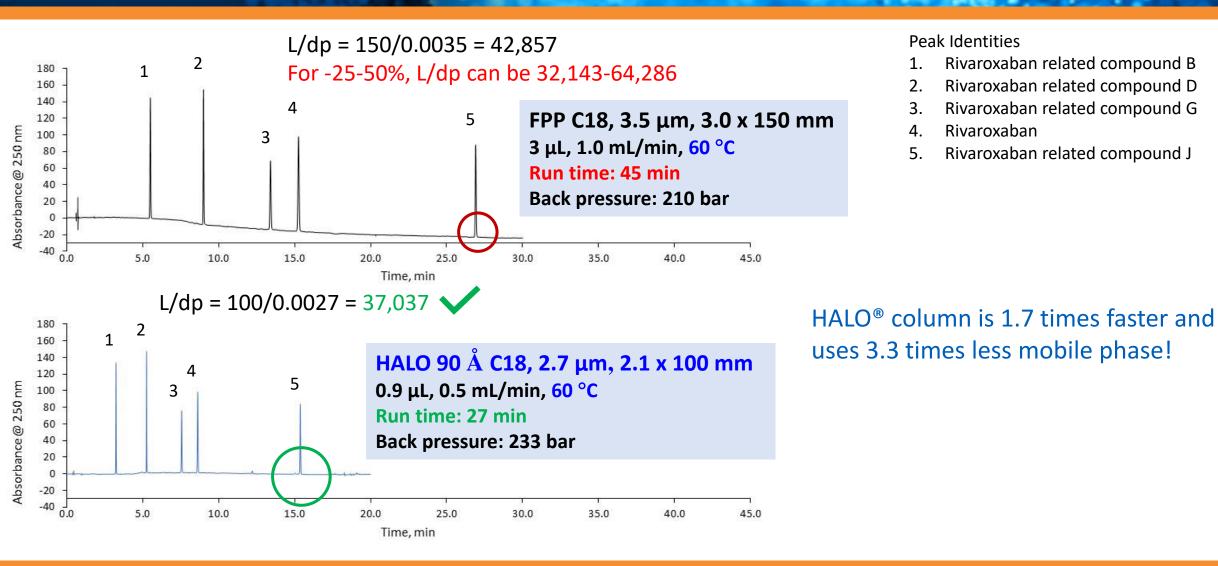
## Elution order is identical on both columns



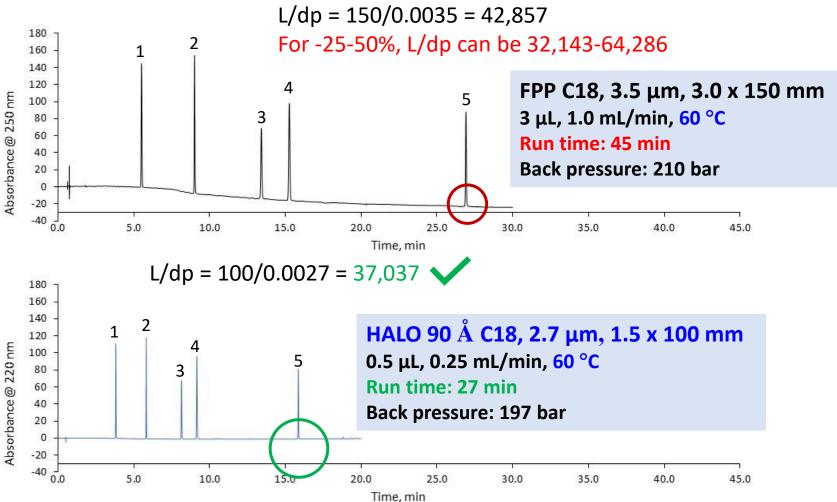
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#### Modified USP Method for Rivaroxaban

## HALO



## Modified USP Method for Rivaroxaban using 1.5 mm ID HALO

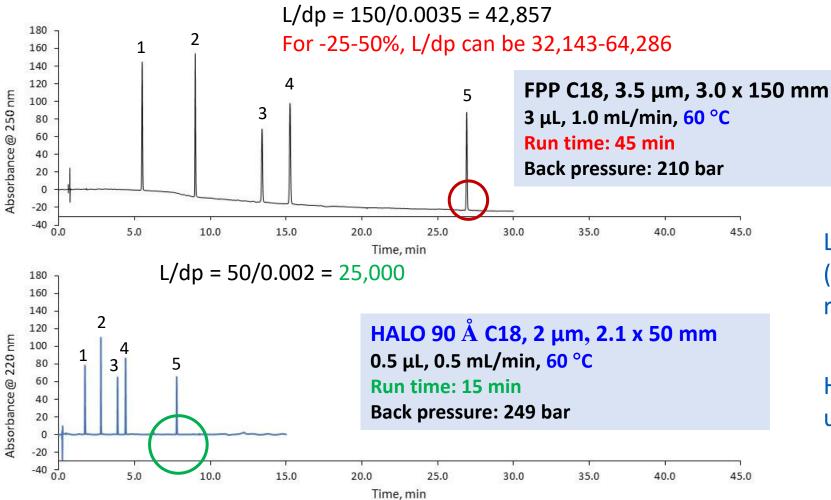


Peak Identities

- 1. Rivaroxaban related compound B
- 2. Rivaroxaban related compound D
- 3. Rivaroxaban related compound G
- 4. Rivaroxaban
- 5. Rivaroxaban related compound J

HALO<sup>®</sup> column is 1.7 times faster and uses 6.7 times less mobile phase!

## Modified USP Method for Rivaroxaban using 2 µm HALO



Peak Identities

- 1. Rivaroxaban related compound B
- 2. Rivaroxaban related compound D
- 3. Rivaroxaban related compound G
- 4. Rivaroxaban
- 5. Rivaroxaban related compound J

L/dp criteria is not met, but the ratio  $(t_R/W_h)^2$  is within -25% to +50%, relative to the prescribed column

HALO<sup>®</sup> column is 3 times faster and uses 6 times less mobile phase!

#### A Few Words About L1 USP Classification

- L1 = Octadecyl silane chemically bonded to porous or nonporous silica particles or superficially porous particles or ceramic micro-particles, 1.5 to 10 μm in diameter, or a monolithic rod.
- There are >1100 columns listed in the L1 category of USP
- All L1 stationary phases are not equivalent
- <u>https://www.uspchromcolumns.com/chrom/display</u> can be used to find which column was used to validate the USP monograph



#### A Few Words About L1 USP Classification - continued

• Doxapram Hydrochloride

#### DOXAPRAM HYDROCHLORIDE

PF	LGS#	Column Brand	Type Of Test	Comments
41(4)	<u>L1</u>	XSelect CSH C18	Assay and Organic Impurities	4.6 mm x 5 cm, 2.5 μm. Manufacturer: Waters Corp.

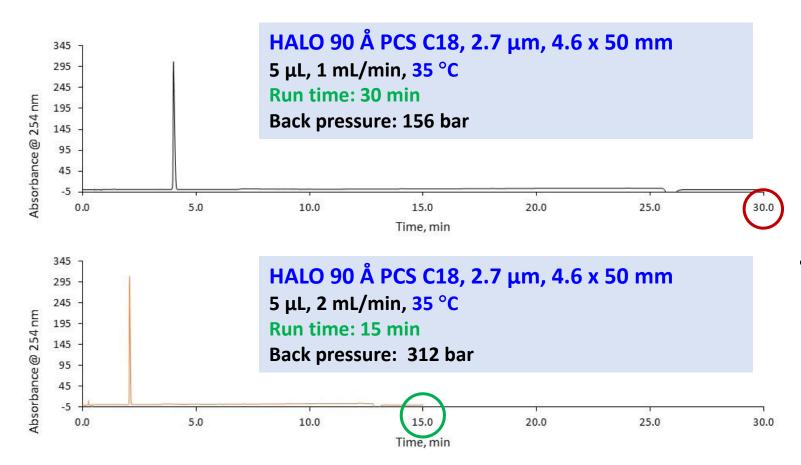
- XSelect CSH C18 is a charged surface hybrid stationary phase
  - Closest HALO equivalent is the HALO 90 Å PCS C18 a positively charged surface stationary phase

XSelect: 4.6 x 50 mm, 2.5 μm L/dp = 50/0.0025 = 20,000 For -25-50%, L/dp can be 15,000-30,000 HALO: 4.6 x 50 mm, 2.7 μm L/dp = 50/0.0027 = 18,518



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#### USP Method for Doxapram HCI: Original & Modified



- Method was moved to an SPP column using double the flow rate
- HALO<sup>®</sup> column delivers 50% time savings and meets the system suitability for tailing NMT 2 and RSD NMT 0.73%



#### Summary

## HALO

- HALO<sup>®</sup> columns are well suited for USP method improvements.
- Making an assessment whether to modernize a USP method should be based on monograph column availability and the goals for time and solvent savings.
- Following new USP guidelines for gradient method modernization enables both FPP and SPP methods to be improved for speed and mobile phase savings using HALO<sup>®</sup> Fused-Core<sup>®</sup> column technology without the need for revalidation.
- For those looking to improve their sustainability, the HALO<sup>®</sup> 1.5 mm ID columns are a great option for those with low dispersion UHPLC systems.



#### Acknowledgements

## HALO

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#### Questions

## HALO

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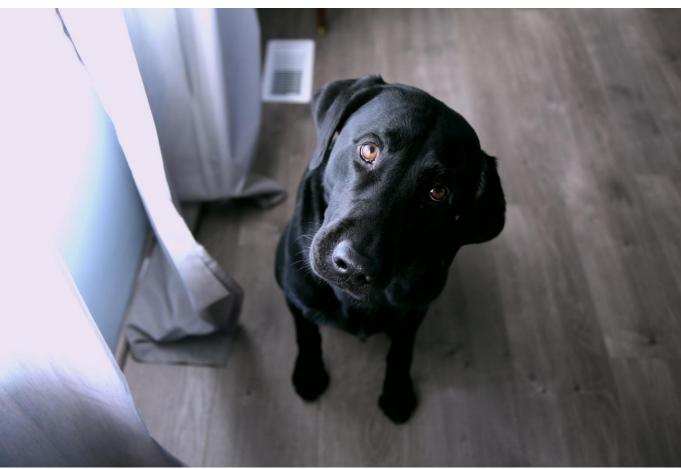


Photo by <u>Alexander Grey</u> on <u>Unsplash</u>



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