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Considerations and Impact of Extra-Column Volume on the Analysis and Scaling of a HPLC Separation

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PURPOSE

With changes to regulatory guidance[1], scaling of HPLC methods to sub 2-µm columns can increase throughput and reduce solvent consumption, improving sustainability. However, when scaling methods to lower particle columns on a system capable of housing 250 mm columns, instrument characteristics, such as extra-column volume, can impact sensitivity and peak width[2]. By optimizing the pre- and post-column tubing, increases in performance can be realized for not only sub-2µm but also HPLC columns.

OBJECTIVE(S)

- Evaluate the impact of pre- and post-column tubing ID on gradient separations for HPLC (4.6 x 250 mm) and UPLC™/UHPLC (2.1 x 100 mm) Columns.
- Demonstrate scaling of HPLC separations (3.5 and 5 µm) to UPLC/UHPLC sub-2µm columns maintaining separation efficiency on a single system, by controlling extra-column volume.

METHOD(S)

System Configuration:

ACQUITY™ UPLC H-Class PLUS System with CM-30S and PDA Detector
Tubing kits: 0.004” ID (p/n 205002335)

Orange Extract Gradient Separation Conditions:

Column: CORTECS™ C18+, 4.6 x 150 mm, 2. 7um Column (HPLC)
CORTECS C18+ 2.1 x 100 mm, 1.6 um Column (UHPLC)
Flow rate: 1.0 mL/min (HPLC), 0.352 mL/min (UHPLC)
Column temperature : 30 °C
Wavelength: 315 nm at 20 points/sec
Injection volume: 10 µL(HPLC) , 1.4 µL (UHPLC)
Mobile phase A: 0.1% Formic acid in water
Mobile phase B: 0.1% Formic acid in acetonitrile
Gradient: 5-90% B in 23 min (HPLC), 5-90% B in 9.08 min (UHPLC)

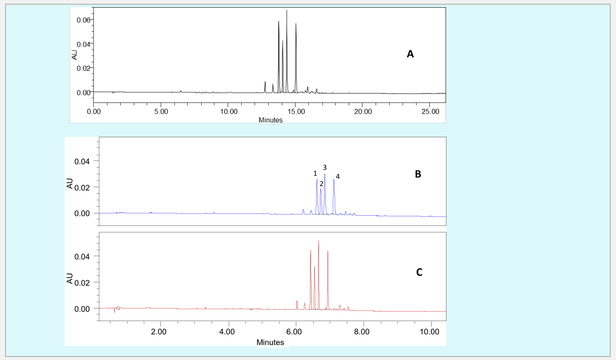
USP Quetiapine Assay Monograph[3]:

Column: Xbridge™ C8, 4.6 x 250 mm, 5 µm Column (HPLC)
ACQUITY BEH™ C8 2.1 x 100 mm, 1.7 um Column (UHPLC)
Flow rate: 1.3 mL/min (4.6 mm), 0.35 mL/min (2.1 mm)
Injection volume: 50 µL (HPLC), 4.2 µL (UHPLC)
Wavelength: 230 nm at 20 points/sec
Buffer: 2.6 g/L of dibasic ammonium phosphate. Adjust with phosphoric acid to a pH of 6.5.
Mobile phase: Methanol: acetonitrile: buffer (54:7:39)

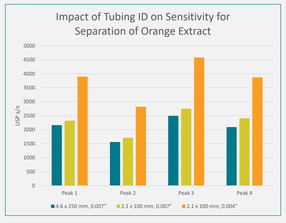
RESULT(S)

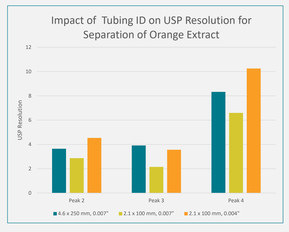
A UPLC System was configured with a column compartment capable of housing up to 8 300mm columns. To accommodate the larger volume columns the system was configured with tubing compatible with HPLC columns. The bandpread was found to be 36.2 µL at 4σ with 0.007” ID pre- and post-column tubing. By changing the tubing to 0.004” ID but maintain the tubing length, the extra column dispersion was reduced to 10.8 µL at 4σ. The impact of the extra-column dispersion on the separation can vary based on method conditions and column dimensions.

In this study a separation of flavonoids in orange extract was conducted on an HPLC column. The method was scaled to a UHPLC column, keeping L/dp ratio within guidelines of -25% to +50% (55,000 to 62,500), and accounting for both column dimensions and particle size. The resulting scaled method reduced the run time by 47% from 23 min to under 10 min. When the pre- and post-column tubing was optimized for the UHPLC column, sensitivity was significantly higher than observed with either the HPLC column or the UHPLC column with larger bore tubing.



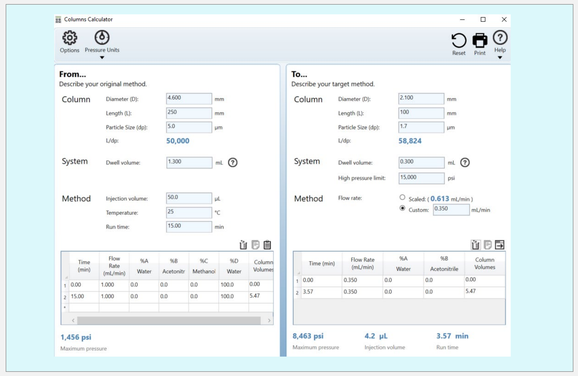
Separation of flavonoids in orange extract and the impact of tubing inner diameter (ID) on separations .
A. Separation on an HPLC column on a UHPLC system with a column heater configured with 0.007" pre- and post-column tubing.
B. Scaled method 2.1 x 100 mm UHPLC column keeping tubing ID the same.
C. Scaled method on UHPLC with 0.004" pre- and post-column tubing.



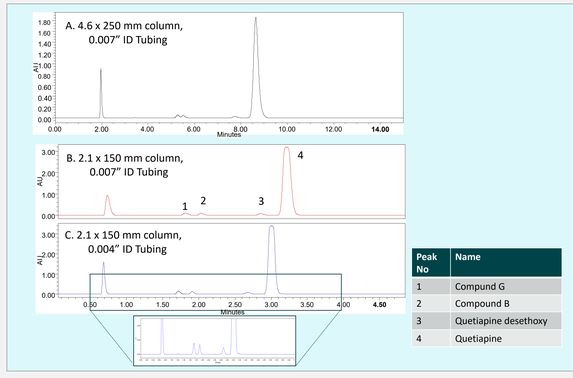


Impact of Tubing ID on USP s/n and USP Resolution for Separation of Orange Extract.
***Sensitivity Graph (left)**: Lower bore ID tubing (0.004" ID) increased sensitivity on a UHPLC column approximately 2x compared with the higher bore tubing (0.007" ID) with same column. Results on an HPLC column with larger bore tubing for USP s/n were lower than a UHPLC column with either tubing.*
***Resolution Graph (right):** UHPLC column with lower bore tubing (0.004") produced highest USP resolution. HPLC column produced higher resolution values than UHPLC column with larger bore tubing. Changing tubing for UHPLC column from larger bore to smaller bore increased USP resolution approximately 50%.*

The same approach was applied to the USP monograph for quetiapine assay, an isocratic method. As specified in the USP monograph the assay prescribes a 4.6 x 250 mm column. Within 621 guidelines, scaling of isocratic methods across different column dimensions is allowable, keeping column L/dp within -25% to +50%. Scaling also requires adjusting both flow rate and injection volume for particle size and column volume respectively. When these steps were performed the method runtime was reduced by over 50% and solvent consumption by 80%.

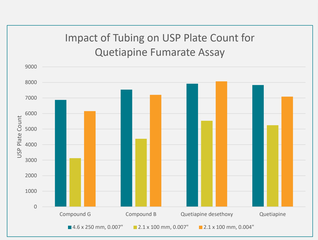


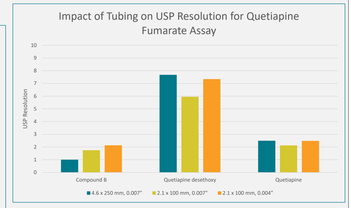
Scaling from a 4.6 x 250 mm, 5 um column to a 2.1 x 100mm, 1.7 um column. Waters Columns Calculator shows that the L/dp ratio is within -25% to +50% as specified in USP ,621. For the UHPLC column, the flow rate, injection volume and run time were all geometrically scaled for the smaller column dimensions.



Peak No	Name
1	Compund G
2	Compound B
3	Quetiapine desethoxy
4	Quetiapine

USP Assay of Queitipaine Fumarate, System Suitability Standard.
A. Separation on an HPLC 4.6 x 250 mm column
B. Scaling of the method to a UHPLC 2.1 x 100 mm column reduced run time from 14 to 4.5 min.
C. Optimization of tubing pre and post column for 2.1 mm ID column, produced sharper peaks and slightly earlier retention times.





Impact of Tubing ID on System Suitability of Quetiapine Fumarate Assay.
***USP Plate Count (upper left):** Plate count values were lowest for UHPLC column with larger bore tubing. Changing pre- and post-column tubing to be compatible with UHPLC column preserved efficiency compare to HPLC column.*
***USP Resolution (upper right):** For critical pair of compound G and compound B resolution was lowest for HPLC column, all other resolution values were lowest for UHPLC column with larger bore (0.007" ID) tubing. Resolution improvements observed when tubing changed to 0.004" for UHPLC column. All results met system suitability criteria of NLT 1.5 for USP resolution of quetiapine (quetiapine desethoxy/quetiapine)*

CONCLUSION(S)

Scaling of HPLC methods to sub 2-µm columns can improve throughput, reduce solvent consumption and improved sustainability. However, performing HPLC and UHPLC separations on a single LC system can negatively impact the performance of UHPLC columns, particularly if the system is configured for HPLC columns ≥150mm, as these systems may have excessive tubing for column connections. This lower-than-expected performance of UHPLC columns is often due to the LC system’s extra-column volume, which may use slightly larger bore tubing (>0.005” ID). By optimizing the pre- and post-column tubing, UHPLC column efficiency gains can be achieved, resulting in system suitability results comparable or better than that of an HPLC column. By adjusting the tubing for each column dimensions, a single system can be used for both HPLC and UHPLC methods, allowing for optimum performance from both column dimensions.

REFERENCE

1. <621> CHROMATOGRAPHY. In *United States Pharmacopeia and National Formulary (USP 43 NF38).* , United Book Press, Inc.: Baltimore, MD, 2020; Vol. , p 6853.
2. Hong, P., and McConville, Patricia R. Dwell Volume and Extra-Column Volume: What Are They and How Do They Impact Method Transfer *Application Note* [Online], 2016.
3. USP, Quetiapine Fumarate. *United States Pharmacopeia and National Formulary (USP 43-NF38)* 2020**,** (GUID-DBEED03E-7C75-4167-BD21-4E30BA2EFF2B_2_en-US), 3800.

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