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SFC Analytical to Preparative Scale Up of Similar Compounds with Torus DIOL 1.7 µm and 5 µm Columns: Separation of Benzoic Acid and Derivatives

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GOAL

Demonstrate the analytical to preparative scale up of an SFC separation using matched average column pressure (ACP) with Torus™ Columns.

BACKGROUND

For supercritical fluid chromatography (SFC) separations, the scaling strategy between columns of different geometries and/or particle sizes differ from that used for reversed phase liquid chromatography (RP LC). This is due to the compressibility of SFC mobile phases, which typically contain carbon dioxide and an organic co-solvent like methanol. When scaling between columns of different geometries and/or particle sizes, the column pressure changes, which affects the density of the SFC mobile phase. This alters the mobile phase elution strength which, if not compensated for, can make SFC method scaling difficult. This is especially true for challenging separations such as that of the benzoic acids mixture shown in Figure 1.

SFC analytical methods can be easily scaled to prep with Torus 1.7 µm and 5 µm Columns by matching the average column pressure.

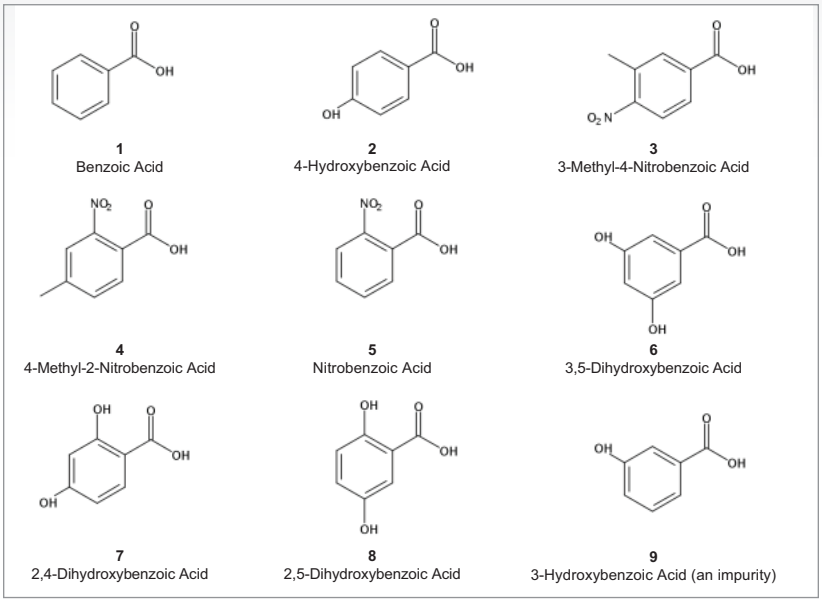

Figure 1. The chemical structures of benzoic acid and derivatives used in this study.

THE SOLUTION

Torus Column stationary phases are available in 1.7 µm and 5 µm particle sizes. The 1.7 µm material is useful for rapid screening and analytical method development, whereas the 5 µm sorbent is necessary for preparative (prep) work. Matching the mobile phase density when changing column dimensions and/or particle sizes is very important to achieve similar chromatographic results and a successful method transfer.[1-3] A simple and effective way to attain the same average mobile phase density is to match the average column pressure (ACP) between methods. The ACP is the average of the pressure measured by the pump head and the active backpressure regulator (ABPR) setting.

Figure 2 shows the scale up of the separation of the benzoic acids mixture on Torus 1.7 µm and 5 µm analytical and preparative columns. An ACQUITY UPC²® System with photodiode array (PDA) detection was used with a Torus DIOL, 1.7 µm, 3.0 x 50 mm Column (p/n: 186007609), and a Torus DIOL, 5 µm, 3.0 x 50 mm Column (p/n: 186008555). A Prep 100q SFC System with PDA detection was used with a Torus DIOL OBD™ Prep, 5 µm, 19 x 150 mm Column (p/n: 186008604). The co-solvent was 20 mM ammonium hydroxide in 1:1 v/v isopropanol:methanol at a flow rate of 1.60 mL/min for the analytical columns and 100 mL/min for the preparative column. ABPR settings of 1800 psi (1.7 µm, 3.0 x 50 mm), 2600 psi (5 µm, 3.0 x 50 mm), and 1740 psi (5 µm, 19 x 150 mm) were used in order to match the ACP to 3073 + 10 psi for all three columns. The columns' temperature was set at 30 °C and UV data was acquired at 220 nm.

Use of matched ACP allows easy scaling of this separation from 1.7 µm to 5 µm columns of the same geometry and from 1.7 µm to 5 µm columns with greatly different geometries. This method scaling also provides separation of a small impurity, 3-hydroxybenzoic acid (X), present in the sample. With the Prep 100q SFC System, all components, including this impurity, could be isolated by triggering fraction collection using the UV absorbance at 220 nm as displayed in Figure 3.

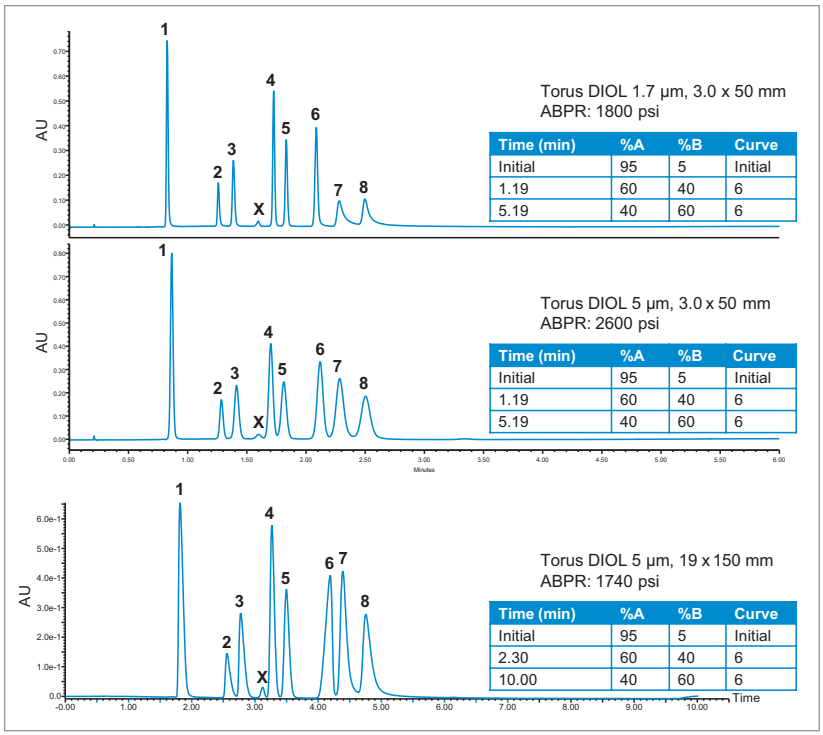

Figure 2. Chromatograms showing the separation of a benzoic acids mixture, scaled from analytical to prep using Torus DIOL Columns.

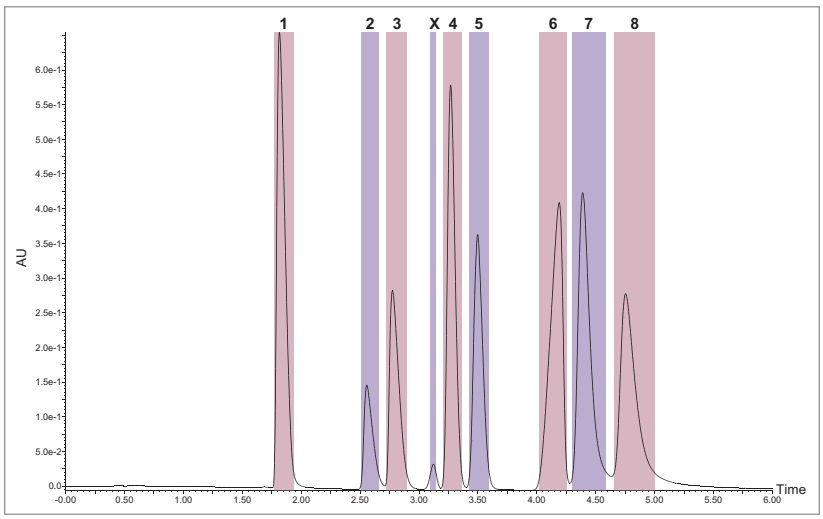

Figure 3. Fraction collection display for the separation of eight benzoic acids and an impurity on a Torus DIOL Prep Column.

SUMMARY

Torus 1.7 µm and 5 µm Columns deliver scalable analytical to preparative SFC method transfers. By matching the average column pressure, a method separating structurally similar benzoic acid derivatives was transferred from a small particle analytical column to large particle analytical and preparative columns.

References

1. Hudalla, C. J., Tarafder, A., Jablonski, J., and Fountain, K. J. UPC² Strategy for Scaling from Analytical to Preparative SFC Separations. Waters Application Note 720004818EN.
2. Hudalla, C. J., Tarafder, A., Jablonski, J., Roshchin, R., Fountain, K. J., and Patel, M. A. UPC² Strategy for Scaling SFC Methods: Applications for Preparative Chromatography. Waters Application Note 720005064EN.
3. Tarafder, A., and Hill, J., "Scaling rule in SFC. II. A practical rule for isocratic systems" *J. Chromatogr. A*, 1482 (2017) 65–75.

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