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應用手冊

The Effects of SFC Preparative Scale-up on Throughput, Purity, and Recovery of an Impurity in an API Mixture

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Abstract

In this application note, a method is converted from analytical ACQUITY (UPC^2) to preparative scale for the isolation of impurities in an API mixture.

- Accurate method scale-up for the isolation of the low-level impurity from analytical scale (ACQUITY UPC^2) to preparative scale (Prep SFC 150 Mgm) was achieved using geometric scale-up calculations.
- Low level impurities (i.e. 0.1%) were effectively isolated using the ChromScope v2.0 Software. The Prep SFC 150 Mgm provided accurate recovery of compounds with peak widths seconds in length.
- When selecting a purification column diameter, time savings (i.e. throughput), is an important consideration. Usage requirements associated with materials, such as CO_2 and co-solvent to produce a desired amount of isolate, does not change with column diameter.
- Purity and throughput of the isolate increases with each purification cycle, because the starting concentration of the target compound, when compared to other components in the mixture, is greater.

Benefits

- The Waters Prep SFC 150 Mgm System can be used for high throughput, semiprep to preparative scale purifications of low level impurities within an API mixture.
- The Waters ChromScope v2.0 Chromatography Data Software provides real-time detection and accurate isolation of compounds at preparative scale with peak widths only seconds in length.
- The Waters Prep SFC 150 Mgm System stacked injection module facilitates increased preparative throughput, via sequential stacked injection, for rapid and efficient target compound isolation.
- The Waters ACQUITY UPLC H-Class provides highly sensitive, orthogonal analysis of SFC isolates by reversedphase chromatography.

Introduction

To minimize the consumption of sample and solvents, there is a benefit in developing separation methods on a small scale and transferring them to a larger scale. The analytical scale method is converted to preparative scale in order to isolate milligram (mg) to gram (g) quantities of purified material. The ability to achieve comparable chromatography between scales when using SFC depends upon several important factors. In this application note, a method is converted from analytical ACQUITY (UPC^2) to preparative scale (Prep SFC 150 Mgm) for isolation of an impurity at 0.1% (4-nitrophenol), as it relates to an API (acetaminophen). A cost and time analysis is provided to demonstrate the relationship between column size and throughput when purifying compounds at 19 mm and 30 mm column diameters via SFC.

Experimental

Analytical SFC UPC2 Method Conditions

Column:	Waters, Torus 2-PIC, 130 Å, 5 µm, 4.6 × 100 mm

	(p/n 186008551)
Injection mode:	Mixed-stream
Flow rate:	3.5 mL/min
Co-solvent:	Methanol

Composition

Isocratic:	80:20 CO_2/Co-Solvent
Temp.:	Ambient
ACQUITY PDA:	247 nm, 306 nm
Injection volume:	5 µL
Software:	Empower 3 Chromatography Data System

Prep SFC Method Conditions

Column:	Waters, Torus 2-PIC, 130 Å, 5 µm, 19 × 100 mm (p/n 186008586)
Flow rate:	Refer to scale-up calculations (Equation 1 and 2)
Injection mode:	Modifier-stream
Injection volume:	500 µL
Temp.:	35 °C

2489 UV/Vis Detector:	247 nm, 306 nm
ABPR pressure setting:	120 bar
Software:	ChromScope v2.0

UPLC

Phase column:	ACQUITY CORTECS C_{18} Column, 130 Å, 1.7 µm, 2.1 mm × 150 mm (p/n 186005298)
Flow rate:	0.50 mL/min
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Acetonitrile
Gradient:	Starting conditions at 20% mobile phase B with a 1 minute hold time, linear increase to 80% mobile phase B over 5 minutes
Column temp:	40 °C
PDA detector:	Wavelength 247 nm and 306 nm at 4.8 nm resolution, 3D data scan range 200–400 nm
Injection volume:	5 µL
Software:	Empower 3 Chromatography Data System

Solvents, standards and samples

A sample solution of API (acetaminophen, Sigma-Aldrich, p/n A3035) and associated impurities, 4-

chloroacetanilide and 4-nitrophenol (Sigma-Aldrich, p/n 158631 and p/n 241326) at 0.1% was prepared in a volumetric flask. Solids were fully dissolved in methanol to equal 60 mg/mL (API) and 0.06 mg/mL (impurities), respectively.

Results and Discussion

Chromatographic method development was performed at analytical scale using ACQUITY UPC^2 to separate the API from the impurities. The API (acetaminophen) and impurity (4-chloroacetanilide) were detectable at 247 nm, while the 0.1% impurity of interest (4-nitrophenol) was not adequately detectable at this wavelength. After PDA full spectrum analysis (210–400nm), two wavelengths were selected to provide detection of all compounds present in the mixture.

The flow rate ACQUITY (UPC^2) utilized at analytical scale (4.6 mm) was directly scaled to preparative scale (19 mm) using the geometric scale-up formulas.1 Method scale-up was simplified by selecting analytical and preparative columns of the same packing material, length, and particle size. By holding these geometries constant, the column length to particle size ratio (L/dp), important for retention time accuracy, was maintained.

In the UPC^2 System, the pump uses volume based flow control (mL/min) for delivering CO_2, while in preparative systems, the CO_2 pump meters CO_2 by mass (g/min). As a result, the actual amount of CO_2 delivered differs between the analytical and preparative pumps without compensation for mass flow. These system flow control metering differences must be accounted for by utilizing the density of CO_2 (approximately 0.936 mg/mL) and is highly dependent upon system pressure and temperature. The ACQUITY UPC^2 analytical flow rate volume flow units were converted to mass flow units (Equation 1) to provide accurate total flow computation at preparative scale (Equation 2).

$$3.5\ mL/min\ _{Analytical\ Total\ Flow}$$

$$3.5\ mL/min\ _{Analytical\ Total\ Flow} \times (80/100)\ _{Analytical\ Ratio\ CO_2} = 2.80\ mL/min\ _{Analytical\ CO_2\ Volumetric\ Flow}$$

$$2.80\ mL/min\ _{Analytical\ CO_2\ Volumetric\ Flow} \times 0.936\ g/mL\ _{Density\ of\ CO_2} = 2.62\ g/min\ _{Analytical\ CO_2\ Mass\ Flow}$$

$$3.5\ mL/min\ _{Analytical\ Total\ Flow} \times (20/100)\ _{Analytical\ Ratio\ Co\text{-}solvent} = 0.7\ mL/min\ _{Analytical\ Co\text{-}solvent\ Flow}$$

Equation 1: ACQUITY UPC² CO_2 (g/min) and co-solvent (mL/min) determination from total flow.

$$\left(\frac{Column\ Diameter\ _{Prep}}{Column\ Diameter\ _{Analytical}}\right)^2 \times Flow\ Rate\ _{Analytical} = Flow\ Rate\ _{Prep}$$

Equation 2: The Prep SFC 150 Mgm (19 mm) flow rate determination from analytical scale UPC² (4.6 mm) using the geometric scale-up formula.

$$\left(\frac{19\ mm}{4.6\ mm}\right)^2 \times 2.62\ g/min\ _{Analytical\ CO_2\ Mass\ Flow} = 45\ g/min\ _{Prep\ CO_2\ Mass\ Flow}$$

Step 1: CO Flow Rate Scale-Up

$$\left(\frac{19\ mm}{4.6\ mm}\right)^2 \times 0.7\ mL/min\ _{Analytical\ Co\text{-}solvent\ Flow} = 12\ mL/min\ _{Prep\ Co\text{-}solvent\ Flow}$$

Step 2: Co-solvent Flow Rate Scale-Up

$$45\ g/min\ _{Prep\ CO_2\ Mass\ Flow} \times 12\ mL/min\ _{Prep\ Co\text{-}solvent\ Flow} = 57\ _{Prep\ Total\ Flow}$$

Step 3: Preparative System Total Flow

$$\frac{12\ mL/min\ _{Prep\ Co\text{-}solvent\ Flow}}{57\ _{Prep\ Total\ Flow}} \times 100 = 21.1\%\ _{Prep\ Co\text{-}solvent\ in\ Total\ Flow}$$

$$\frac{45\ g/min\ _{Prep\ Co\text{-}solvent\ Flow}}{57\ _{Prep\ Total\ Flow}} \times 100 = 78.9\%\ _{Prep\ Percent\ CO_2\ in\ Total\ Flow}$$

Step 4: Preparative System % Co-solvent and CO_2

SFC system pressure (run density profile) has an impact on chromatographic separation therefore density profiles must be identical between systems to achieve comparable chromatographic resolution. The system run density profiles were 167 bar at analytical scale and 129 bar at preparative scale (Equation 3).

A) ACQUITY UPC² run density profile (1 psi = 0.069 bar):

$$Run\ Density\ Profile = \frac{Solvent\ Manager\ Pressure\ (bar) + Convergence\ Manager\ ABPR\ Backpressure\ (bar)}{2}$$

B) Prep SFC 150 Mgm run density profile (bar):

$$Run\ Density\ Profile = \frac{Observed\ CO_2\ Pump\ Pressure\ (bar) + ABPR\ Pressure\ Method\ Setting\ (bar)}{2}$$

Equation 3: System average pressure A) ACQUITY UPC² and B) Prep SFC 150 Mgm system.

To account for the run density profile differences between the systems during scale-up, modifications to the preparative method were made in order to generate a comparable chromatographic separation. The ABPR Pressure Setting can be adjusted on the Prep SFC 150 Mgm in ChromScope from 120 bar to 158 bar to match the run density profile of ACQUITY UPC2. A complementary approach was employed in this application note. The column temperature used for UPC² method development was set for ambient temperature, while the CO_2 and co-solvent heaters were operated at 35 °C on the Prep SFC 150 Mgm. The increased preparative CO_2 temperature resulted in a decrease in the density of CO_2 and in the viscosity of the mobile phase. This effect caused a shift in the chromatography, to adequately maintain separation of the target compound and the API without adjustment of the ChromScope software system pressure setting. With this approach, resolution and retention times for the impurity of interest (4-nitrophenol) and the peak eluting prior, were nearly identical when

comparing the ACQUITY UPC^2 and Prep SFC 150 Mgm separations, as shown in Figure 1.

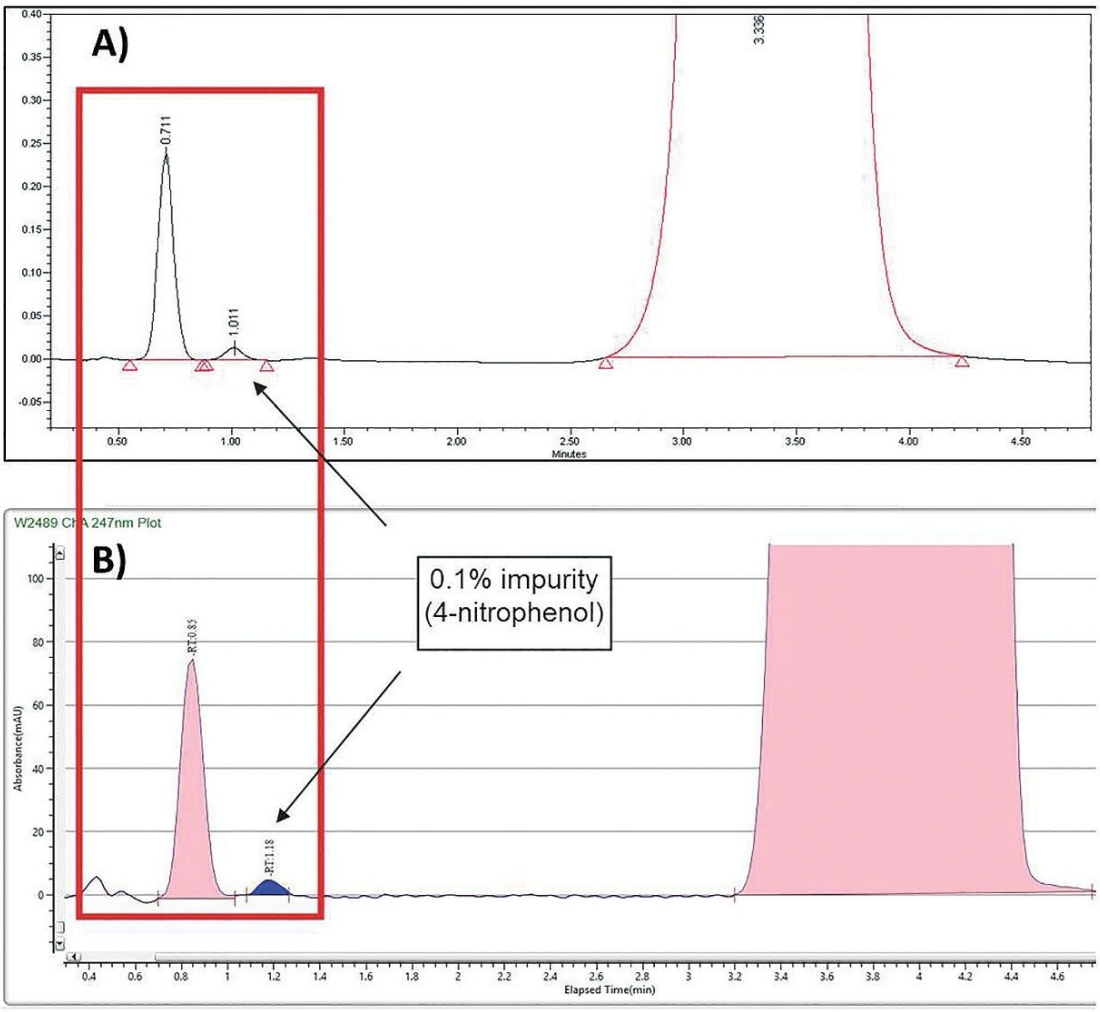

Figure 1. Red box compares the 0.1% impurity retention time and the peak eluting prior by (A) ACQUITY UPC^2 using a 4.6 mm column by Empower and (B) Prep SFC 150 Mgm with a 19 mm column via ChromScope v2.0 at 247 nm.

The ACQUITY UPC^2 standard configuration employs a mixed-stream injection mode in which the overall mobile phase composition (CO_2 plus co-solvent) carries the sample to the column. This mode is not employed for the Prep SFC 150 Mgm (i.e. preparative scale) because strong, polar sample diluents, when injected into the flow path at high volume, can affect the strength of the overall mobile phase. The effect may result in peak distortion

and/or a retention time shift. Instead, the Prep SFC 150 Mgm utilizes a patented modifier-stream injection configuration in which the strong, polar sample diluent is mixed with the co-solvent prior to the addition of CO_2. This configuration improves peak shape and resolution when using high volume, preparative scale injections.[1]

For separations intended for scale-up, injection loading studies are sometimes performed at analytical scale to conserve valuable, or volume limited, sample material. The injection load is then mathematically converted to preparative scale via geometric loading calculations.1 With this technique, loading studies at analytical scale require the conversion of the ACQUITY UPC^2 System from a mixed-mode to a modifier-stream injection configuration, to result in comparable modes of sample introduction into the flow path.[2] In this application note, the sample was not limited or of high value and preparative method development efficiency was highest priority. As a result, injection load determination was performed at the preparative scale via injection of 0.2 mL, 0.5 mL, 1 mL, 1.5 mL, and 1.75 mL. Chromatographic resolution of the target compound was evaluated at each injection volume (data not shown). Because loading studies were performed at preparative scale rather than analytical scale, conversion of the ACQUITY UPC^2 from mixed stream injection mode to modified stream injection mode was not required.

Twenty-five stacked injections of the optimal injection load (0.5 mL) were performed in triplicate on the 19 mm preparative SFC column and collected (Figure 2). Isolates were quantitatively transferred to a 100 mL volumetric flask and diluted to volume with methanol.

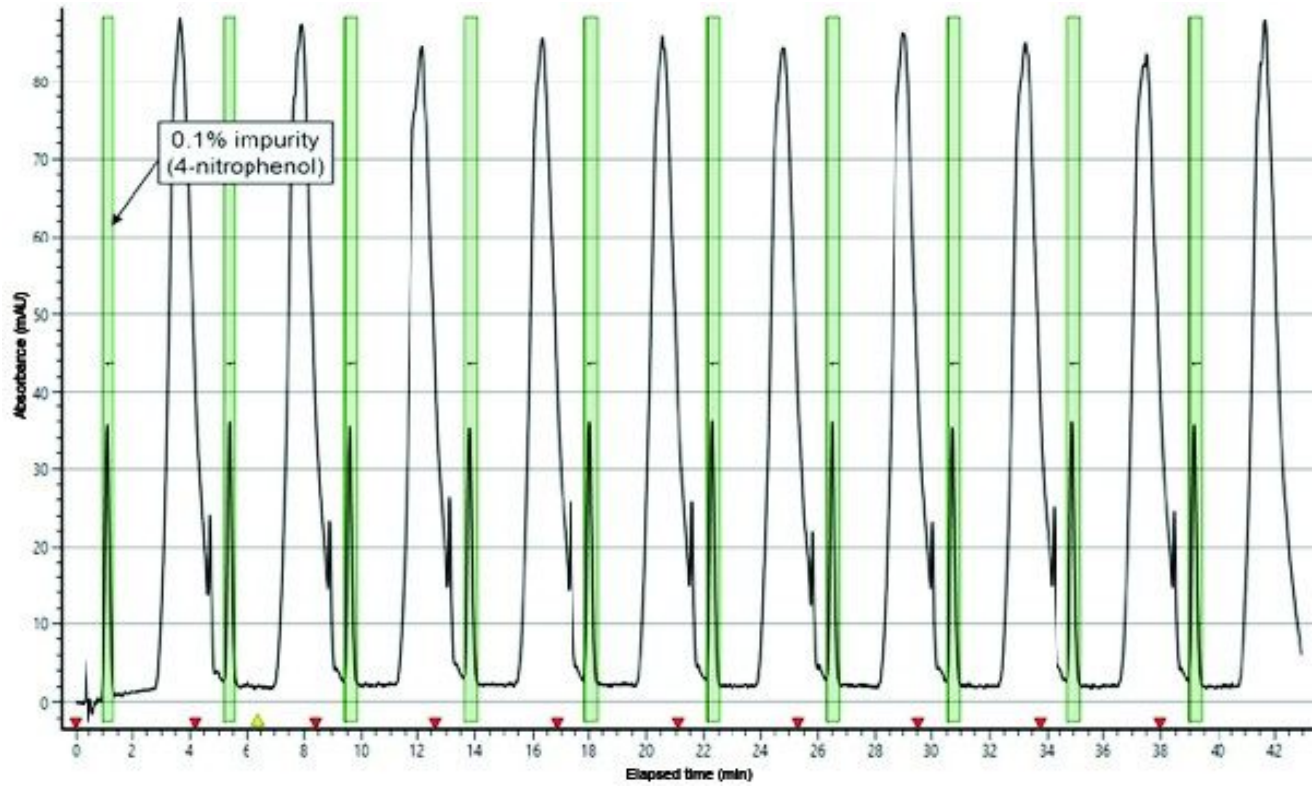

Figure 2. Example ChromScope v2.0 chromatogram of 10 stacked injections at 306 nm to show maximum detection of 4-nitrophenol. The peak of interest is highlighted in green with a collection time of 21 seconds.

Highly concentrated solutions containing low-level target compounds typically require a multi-cycle purification scheme. One approach is to initially isolate the most concentrated component (i.e. API), from a pooled mixture of the low level components (i.e. impurities). A second purification cycle can then be performed on the pooled impurity mixture to further isolate the target compound.

An alternative purification scheme was employed in this application note. Only the target compound was isolated from the sample mixture in the first cycle, followed by a subsequent round of purification to increase the purity of target compound.

Recovery and purity of the isolates were determined individually by reversed-phase chromatography due to the technique's high sensitivity and ability to separate compounds orthogonally to SFC. After the initial purification cycle, the average target compound recovery was 92%, and the average purity was 52%. Although the target compound was chromatographically resolved, rapid stacked injections of the concentrated sample solution

resulted in low-level cross contamination caused by tailing of the API. This phenomenon is not uncharacteristic when employing rapid preparative scale chromatography for isolation of low-level components from large volume injections of exceedingly concentrated primary sample components, as in the case presented here for the 60 mg/mL API sample solution.

The isolates from the initial purification cycle were dried under nitrogen flow, followed by reconstitution in 10 mL of methanol. They were re-purified via a second cycle using identical parameters as the initial cycle, via triplicate runs of 10 stacked injections. Reversed-phase revealed an increase in purity of the isolate to 99% (Table 1), while the average recovery was maintained at approximately 90%.

Size: 19 mm	Concentration of sample stock solution	Purity of sample stock solution	Injection volume	No. of Injections per hour	Expected throughput	Observed purity	Observed throughput	Cost co-solvent per mg	Cost CO_2 per mg
Cycle 1	0.06 mg/mL	0.01%	0.5 mL	13	0.39 mg/hour	52%	0.37 mg/hr	$105/mg	$40/mg
Cycle 2	0.25 mg/mL	52%	0.5 mL	40	5.0 mg/hour	99%	4.5 mg/hr	$9/mg	$3/mg

Table 1. Observed throughput and mobile phase cost per mg when using the 19 mm column. Co-solvent was 80% MeOH/20% ACN where MeOH= $37/L or $0.04/mL and ACN = $110/L or $0.11/mL, and 20 lb tank of CO_2 = $50 or $2.5/lb.

Efficiency of the purification described in this application note was increased by utilizing the ChromScope v2.0 software stacking mode for preparative sample injection. This injection mode employs rapid delivery of the sample to the injection loop, in one continuous sample run to efficiently generate the desired isolate. The ChromScope stacked injection mode is especially useful when employed to increase purification efficiency of less concentrated sample solutions, especially those prepared at low concentrations to preserve analyte solubility.

From the recovery and purity of 4-nitrophenol generated using the 19 mm column, geometric scaling and mass load calculations were employed to determine the theoretical cost and purification time for 10 mg using a 30 mm column (Table 2) (Table 3). The cost (CO_2 and co-solvent) did not change with column diameter, but the total purification time for 10 mg (throughput) achieved by the larger diameter column (30 mm) showed a significant decrease from 29 hours to 12 hours, when compared to the 19 mm column.

Size: 30 mm	Concentration of sample stock solution	Purity of sample stock solution	Injection volume	No. of Injections per hour	Expected throughput	Observed purity	Observed throughput	Cost co-solvent per mg	Cost CO_2 per mg
Cycle 1	0.06 mg/mL	0.01%	1.25 mL	13	0.98 mg/hour	NA	NA	$99/mg	$38/mg
Cycle 2	0.25 mg/mL	52%	1.25 mL	40	12.5 mg/hour	NA	NA	$8/mg	$3/mg

Table 2. Throughput and mobile phase cost per mg when using the 30 mm column calculated using geometric scaling equations.[1]

Column size and internal volume	Estimated column cost	Desired Amount	Desired final purity	Concentration of initial sample solution	Cost co-solvent per 10 mg	Cost CO_2 per 10 mg	Total mobile phase cost per 10 mg	Overall total cost per 10 mg (including column cost)	Throughput for 10 mg
19 mm 28 mL	$2870	10 mg	99%	0.01%	$1050	$400	$1450	$4230	26 hours $_{cycle\ 1}$ + 2 hours $_{cycle\ 2}$ = 28 hours
				52%	$90	$30	$120	$2990	2 hours
30 mm 71 mL	$7175			0.01%	$990	$380	$1370	$8545	10 hours $_{cycle\ 1}$ + 1 hours $_{cycle\ 2}$ = 11 hours
				52%	$80	$30	$110	$7285	1 hour

Table 3. Cost to isolate 10 mg of the impurity 4-nitrophenol with 99% purity from the API mixture.

Conclusion

- Accurate method scale-up for the isolation of the low level impurity from analytical scale (ACQUITY UPC2) to preparative scale (Prep SFC 150 Mgm) was achieved using geometric scale-up calculations.
- Low level impurities (i.e. 0.1%) were effectively isolated using the ChromScope v2.0 Software. The Prep SFC 150 Mgm provided accurate recovery of compounds with peak widths seconds in length.
- When selecting a purification column diameter, time savings (i.e. throughput), is an important consideration. Usage requirements associated with materials, such as CO2 and co-solvent to produce a desired amount of isolate, does not change with column diameter.
- Purity and throughput of the isolate increases with each purification cycle, because the starting concentration of the target compound, when compared to other components in the mixture, is greater.

References

1. Runco, Jacquelyn. *Beginners Guide to Preparative Chromatography*. Library of Congress 2017933625, Waters Corporation, www.waters.com, 2017.
2. Runco, Jacquelyn and Aubin, Andrew J. "Practical Strategies for Successful Scaling from UPC^2 to Preparative SFC". *Chromatography Today*. September 13, 2018.

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