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

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INTRODUCTION

Scale-up of SFC analytical methods to preparative scale allows laboratories to generate purified bulk quantities of target compounds. In some laboratories, users are provided with an analytical scale method from which an isolate of a specified purity and quality must be generated within strict timelines. The success of achieving this task depends directly upon the accuracy of the scale-up procedure. In this poster we will describe the preparative scale-up of an analytical scale method for isolation of milligram (mg) to gram (g) quantities (per run) for a mixture of an API and its associated impurities. A cost and time analysis is provided after scale-up to demonstrate the relationship between column size and throughput.

METHODS

Instrumentation

Analytical SFC:	Waters ACQUITY UPC^2 System
Preparative SFC:	Waters Prep SFC 150 Mgm System
Analytical Reversed-Phase:	Waters ACQUITY H-Class UPLC System

Analytical SFC UPC^2 Method Conditions:

Column:	Waters, Torus 2-PIC, 4.6 x 100mm, 5µm, pn:186008551
Injection mode:	Mixed-stream
Flow rate:	3.5mL/min
Co-solvent:	80:20 Methanol: Acetonitrile
Composition Isocratic:	80:20 CO_2 / Co-Solvent
Temperature:	Ambient
ACQUITY PDA:	247nm, 306nm
Injection volume:	5µL
Binary Solvent manager pressure:	2860psi
Convergence manager ABPR:	2000psi
Avg. system operating pressure:	(2860psi + 2000psi backpressure) / 2 = 2430psi = 167bar
Software:	ChromScope™ 2.0

Prep SFC Method Conditions:

Columns:	Waters, Torus 2-PIC, 19 x 100mm, 5µm, pn:186008586
Injection mode:	Modifier-stream
Injection volume:	500µL
Temperature:	35ºC
2489 UV Detector:	247nm, 306nm
CO2 pump pressure:	137bar
ABPR pressure setting:	120bar
Avg. system operating pressure:	(137bar + 120bar backpressure)/2 = 129bar
Software:	ChromScope™ 2.0

Analytical Reversed-Phase Orthogonal Fraction Analysis Conditions:

UPLC Reversed Phase Column:	ACQUITY CORTECS C_{18} Column, 2.1 mm x 150 mm 1.7µm, pn: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:	40ºC
PDA Detector:	Wavelength 247nm and 306nm at 4.8 nm resolution, 3D data scan range 200-400nm
Injection Vol:	5µL
Pressure:	9000psi
Software:	Empower® 3 Chromatography Data System
Sample Solution :	Mixture of API (acetaminophen, Sigma-Aldrich, pn:A3035) and 0.1% impurities (4-chloroacetanilide, Sigma-Aldrich, pn:158631 and 4-nitrophenol, Sigma-Aldrich, pn:241326) were prepared in methanol at 60mg/mL and 0.06mg/mL, respectively.

RESULTS AND DISCUSSION

Chromatographic method development was performed at analytical scale using the UPC^2 mixed stream injection system configuration. The API (acetaminophen) and impurity (4-chloroacetanilide), were detectable at 247nm, while the 0.1% impurity of interest, 4-nitrophenol, showed low visibility at this wavelength. Dual wavelengths (247nm, 306nm) were monitored using the Prep SFC 150 Mgm UV/Vis detector 2489 during collection to provide visibility of all compounds in the mixture.

The analytical flow rate from which the method separation was developed was directly scaled from analytical 4.6mm to preparative 19mm using the geometric, flow rate scale-up formulas[1]. Scale-up was simplified by choosing analytical and preparative columns of the same packing material, length, and particle size. By holding these parameters constant, the column length to particle size ratio (L/dp), important for retention time accuracy, was maintained.

UPC^2 total flow:	3.5mL/min
UPC^2 CO_2 volumetric flow:	3.5mL/min x 0.80% = 2.80 mL/min
UPC^2 CO_2 mass flow:	2.8mL/min x 0.936g/mL density of CO_2 = 2.62g/min
Co-solvent flow:	3.5mL/min total flow x 20% = 0.7mL

The co-solvent flow rate was calculated from the UPC^2 method % mobile phase B.

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

$$\left(\frac{19\text{mm}}{4.6\text{mm}}\right)^2 \times 2.62\ \text{g/min}_{\text{CO2 Mass Flow Analytical}} = 45\ \text{g/min}_{\text{CO2 Prep}}$$

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

$$45\ \text{g/min}_{\text{CO2 Prep}} + 12\ \text{mL/min}_{\text{Cosolvent Flow Prep}} = 57\text{g/min}_{\text{Total Flow Prep}}$$

$$\frac{12\ \text{mL/min}_{\text{Cosolvent Flow Prep}}}{57\ \text{g/min}_{\text{Total Flow}}} \times 100 = 21.1\%_{\text{Cosolvent Prep}}$$

Analytical and preparative SFC systems that operate at different average pressures can result in different run density profiles, which may impact the chromatography. The average system pressure was compared between the UPC^2 and Prep SFC 150 Mgm.

UPC^2 (PSI converted to bar):

$$\text{Average Pressure} = \frac{\text{Binary Solvent Manager Pressure (Bar)} + \text{Convergence Manager Pressure (Bar)}}{2}$$

Prep SFC 150 Mgm System (bar):

$$\text{Average Pressure} = \frac{\text{CO2 Pump pressure (Bar)} + \text{ABPR Instrument Method Pressure Setting (Bar)}}{2}$$

The average pressures were 167bar and 129bar respectively. There are several ways to account for the difference in average pressure. One option is to adjust the ABPR instrument method setting from 120bar to 158bar to account for the 38bar difference pressure between the systems. An alternative method is to increase the preparative CO_2 temperature to decrease viscosity of the mobile phase. The later technique was employed by increasing the CO_2 and co-solvent temperatures to 35ºC. Resolution and retention times for the impurity of interest (4-nitrophenol) and the peak eluting prior were nearly identical when comparing the 4.6mm UPC^2 and the 19mm Prep SFC 150 Mgm separation using this technique, as shown in Figure 1.

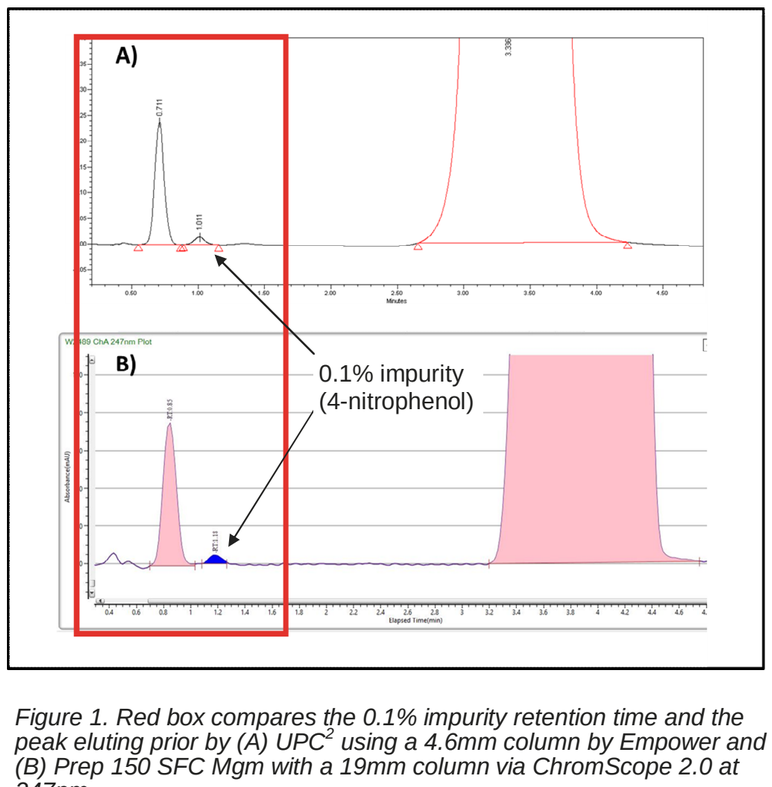

Figure 1. Red box compares the 0.1% impurity retention time and the peak eluting prior by (A) UPC^2 using a 4.6mm column by Empower and (B) Prep 150 SFC Mgm with a 19mm column via ChromScope 2.0 at 247nm.

The Prep SFC 150 Mgm utilizes a patented modifier stream injection configuration. Typically, to conserve sample, column capacity studies are performed at analytical scale after conversion of the UPC^2 system to a modifier stream configuration. In this case, sample volume was not limited. As a result, capacity determination was accomplished at the preparative scale without conversion of the UPC^2 to the modifier stream injection mode by injecting 0.2mL, 0.5mL, 1mL, 1.5mL and 1.75mL at preparative scale while monitoring the resolution of the impurity of interest. An injection volume of 0.5mL successfully accomplished this criteria.

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

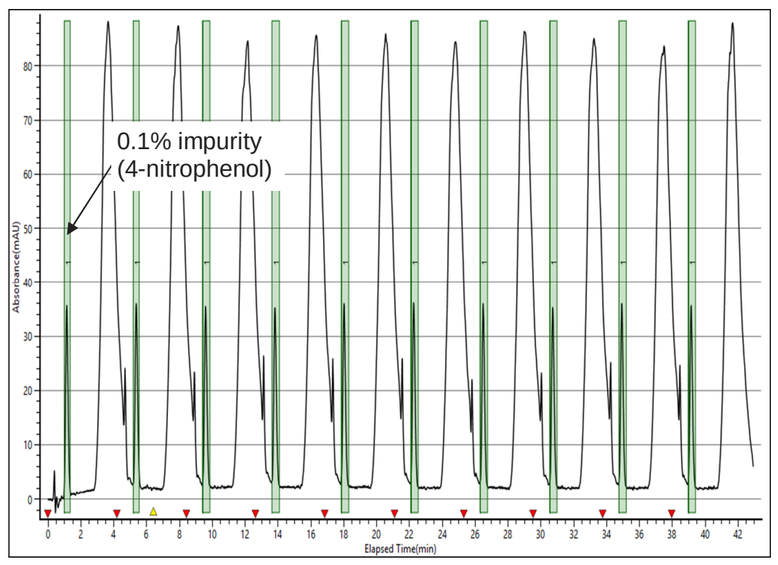

Figure 2. Example ChromScope 2.0 chromatogram of stacked injections at 306nm to show maximum detection of 4-nitrophenol. The peak of interest is highlighted in green with a collection time of 21 seconds.

Recovery and purity of the isolate were determined orthogonally by reversed phase. Recovery of the 0.1% impurity, 4-nitrophenol, generated from the first purification cycle averaged 92% (n=3), while purity averaged 52%, primarily due to carryover of the highly concentrated API (60 mg/mL) from the stock sample solution. While the stock sample solution was prepared at 60mg/mL to facilitate detection of the 0.1% impurity of interest, the high concentration of the API lead to inherent system carry-over resulting in adequate recovery, but low purity, of the impurity 4-nitrophenol from the first purification cycle.

The isolates from the first purification cycle were dried under nitrogen flow and reconstituted to equal a final volume of approximately 10mL. The isolates were re-purified using the same parameters as the first cycle, via 10 injections. Reversed-phase revealed an increase in purity of 4-nitrophenol to 99% after the second purification cycle by further removing the API, while the recovery of 4-nitrophenol averaged 89% (Table 1).

From the recovery and purity of 4-nitrophenol generated using the 19mm column, geometric scaling and mass load calculations were employed to determine the theoretical cost and purification time for a 30mm column, for comparison purposes (Table 2). Total time and cost of purification for 10mg were compared for the 19mm and 30mm columns (Table 3).

Table 1:Observed throughput and mobile phase cost per mg when using the 19mm 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 20lb tank of CO_2 = $50 or $2.5/lb.

Size: 19mm	Conc. of Sample Stock Soln.	Purity of Sample Stock Solution	Inj. Vo.	# Inj. per hour	Expected Through-put	Observed Purity	Observed Through-put	Cost Co-solvent* per mg	Cost CO_2 per mg
Cycle 1	0.06 mg/mL	0.01 %	0.5 mL	13	0.39mg/ hour	52%	0.37 mg/ hour	$105 / mg	$40 / mg
Cycle 2	0.25 mg/mL	52 %	0.5 mL	40	5.0 mg/ hour	99%	4.5 mg/ hour	$9 /mg	$3 / mg

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

Size: 30mm	Conc. of Sample Stock Soln.	Purity of Sample Stock Soln.	Inj. Vol.	# Inj. per hour	Expected Through-put	Observed Purity	Observed Through-put	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 3: Cost to isolate 10mg of the impurity 4-nitrophenol with 99% purity from the API mixture.

Column Size and Internal Volume	Est. Column Cost	De-sired Amou nt	De-sired Final Purity	Conc. of Initial Sample Solution	Cost Co-solvent Per 10mg	Cost CO_2 Per 10mg	Total Mobile Phase Cost Per 10mg	Overall Total Cost Per 10mg (including column cost)	Throughput for 10mg
19mm 28mL	$2870	10 mg	99%	0.01 %	$1050	$400	$1450	$4320	26 Hours $_{\text{cycle 1}}$ + 2 hours $_{\text{cycle 2}}$ = 28 Hrs
				52 %	$90	$30	$120	$2990	2 hours
30mm 71mL	$7175			0.01 %	$990	$380	$1370	$8545	10 Hours $_{\text{cycle 1}}$ + 1 hour $_{\text{cycle 2}}$ = 11 Hrs
				52 %	$80	$30	$110	$7285	1 hour

CONCLUSIONS

- Low level impurities (i.e. 0.1%) can be effectively isolated using the ChromScope 2.0 software. The Prep SFC 150 Mgm provides accurate recovery of compounds with peak widths seconds in length.
- Column volume and sample load capacity increases by 2.5 times when moving from the 19mm to the 30mm column due to the geometric scaling calculations. As a result, the time required to generate a desired amount of purified isolate is reduced by 2.5 times.
- When selecting a purification column diameter, the time savings (i.e. throughput) accomplished by utilizing larger diameters 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 can be increased via a second purification cycle. Throughput of the isolate is much higher in the second cycle because the concentration of the peak of interest, compared to other compounds in the mixture, is much greater.

References

1. "Runco, J. "Beginners Guide to Preparative Chromatography". Library of Congress 2017933625, Waters Corporation, www.waters.com, 2017.

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