SFC Purification System Performance Evaluation of Purity and Recovery

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INTRODUCTION

Purity and recovery are impacted by not only the quality of the chromatographic separation, but the attributes of the instrument design and implementation. Reliable system and column performance during a long purification run (i.e. 24 hrs) plays a critical role in generation of consistent isolate purity and recovery results. In this poster, techniques for optimizing system performance as it relates to the purity and recovery of a sample test mixture, will be demonstrated. Additionally, a cost comparison is performed to reveal the influences of peak resolution versus throughput after system optimization for a representative sample preparation.

METHODS

SFC System:	Waters Prep SFC 150 Mgm System w/5 mL sample loop
Detector:	Waters 2489 UV/Visible, 215 nm
Columns:	Torus 2-PIC 130Å, 5µm, OBD, 19 x 50 mm (186008585)
	Torus 2-PIC 130Å, 5µm, OBD, 19 x 100 mm (186008586)
	Torus 2-PIC 130Å, 5µm, OBD, 19 x 150 mm (186008587)
Solvent / Co-solvent:	CO_2, / Methanol
Flow Rates:	125 ml/min total flow, 8% co-solvent (10.0 mL/min) 125 ml/min total flow, 4% co-solvent (5.0 mL/min)
Sample Solution:	8 mg/mL Compound 1 (ketoprofen), 6 mg/mL Compound 2 (warfarin) in methanol
Injection Volume:	4.0 mL
GLS Make-up Solvent:	Methanol
Software:	Chromscope™ 2.0 with Windows 10

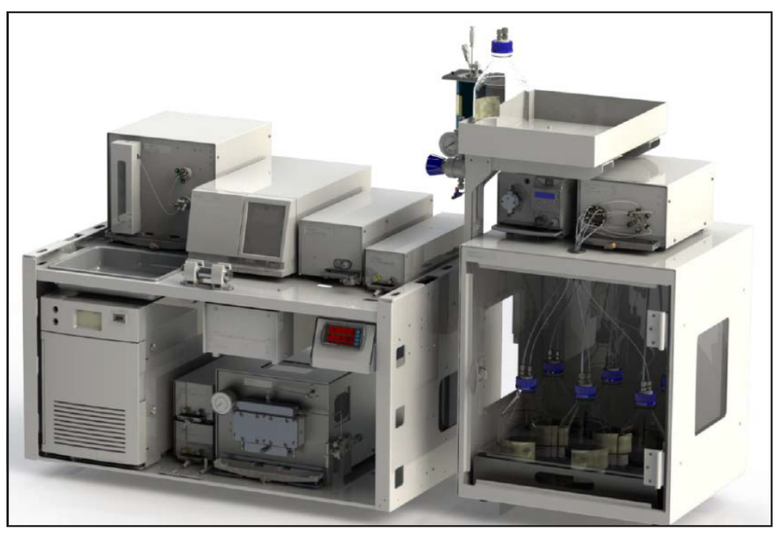

Figure 1: *Waters Prep SFC 150 Mgm*

DISCUSSION

Injection Repeatability

Consecutive injections of the sample solution were performed over 24 hours. The injection sequence was divided into 6 sample sets containing 48 stacked injections, each resulting in a total of 288 injections.

Using the Torus 2-PIC 130Å, 5µm, OBD column, the %RSD was less than 1% for area and peak height at 0.3% and 0.9%, respectively. The retention time of four representative injections from each sample set were graphed in Figure 2. The linear regression, R^2= 1.00000, showed no change in retention during the 24 hour run. The column and the Prep SFC 150 Mgm system displayed robust operation.

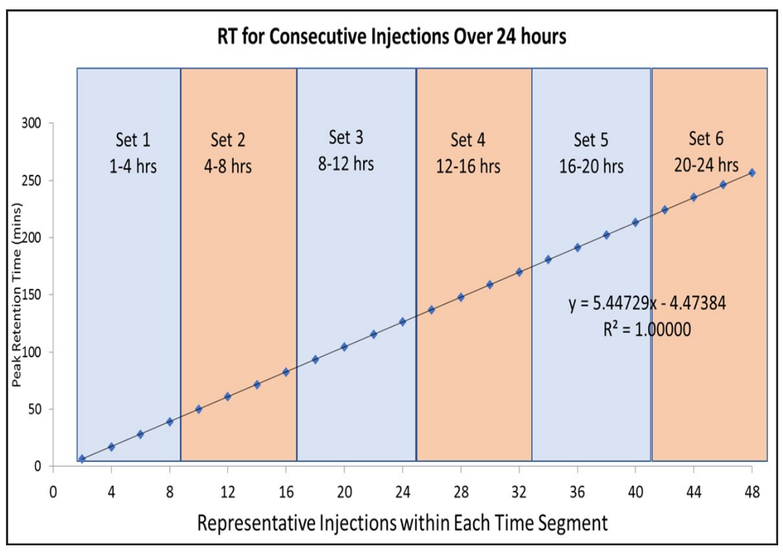

Figure 2: *Linear regression plot of retention time for four representative injections in each time segment during a 24 hr run.*

Gas-liquid Separator (GLS) Peak Integrity

The sample was separated using 50 mm, 100 mm and 150 mm columns with 125 mL/min total flow and 8% co-solvent (10 mL/min). Resolution (Rs) (Figure 3), recovery, and purity (data not shown) of the two compounds in the sample mixture were determined for each separation.

The increase in column length yielded greater resolution between compound 1 and compound 2 resulting in incrementally greater recovery of compound 1, while purity was high for all separations. Since the 50 mm column showed the least resolution between the sample compounds, this column was employed for further system optimization studies.

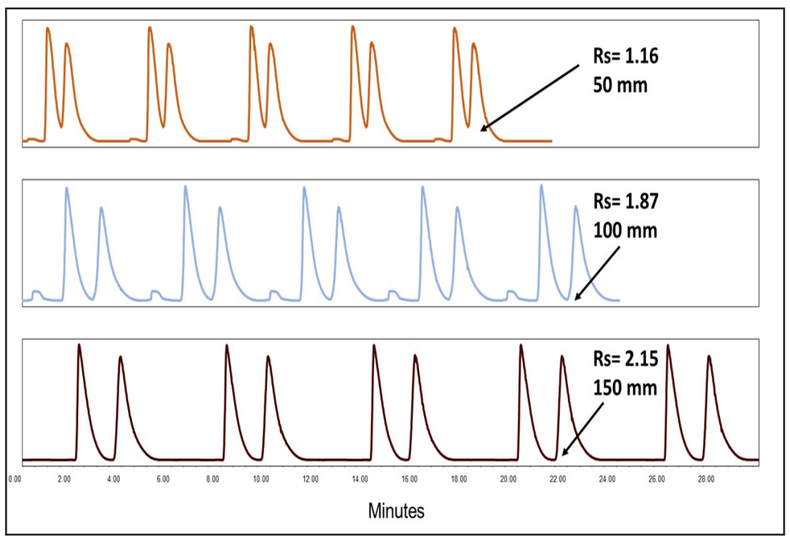

Figure 3: *Resolution obtained by the 50 mm, 100 mm and 150 mm columns under identical chromatographic conditions.*

Twelve fractions (two sets of six) were collected from the 50 mm column separation as shown in Figure 4. Additionally, the two main peaks in the sample mixture were isolated as individual fractions from a separate injection.

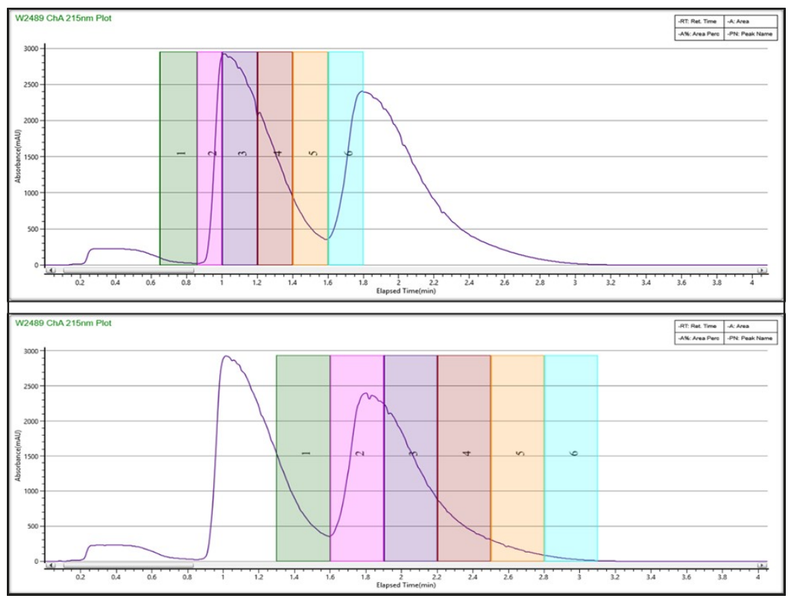

Figure 4: *Twelve fractions (two individual sets of six) were collected using the 50 mm column, Rs = 1.16.*

The recovery obtained in each of the twelve fractions was plotted per unit time. The peak profile was directly comparable to the peak resolution and retention time observed in the UV trace (Figure 5), therefore resolution and peak integrity were maintained as fractions traveled through the GLS at the tested flow rates. The observed peak tailing is typical of preparative scale column loading.

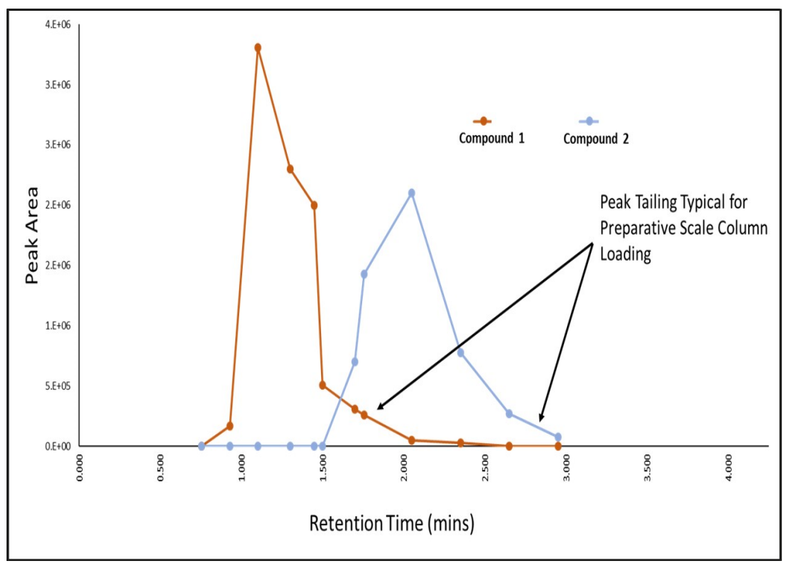

Figure 5: *Plot of post GLS peak integrity.*

Elution Order vs. Recovery and Purity

The purity and recovery of compound 1 were determined for method co-solvent concentrations of 8% (10.0 mL/min) for each column, and 4% (5.0 mL/min). Isolation of compound 1 began as the area increased from the baseline and terminated in the valley between the two peaks, while compound 2 was isolated from the valley between the peaks to the point where the trace met the baseline (Figure 6,A).

The most significant difference in purity and recovery results, as they relate to peak elution order, were observed at low method co-solvent i.e. 4% (5.0 mL/min). The post GLS resolution and peak shape observed in Figure 5 were directly reflected in the data observed in Figure 6,B. Due to the resolution, the tail of compound 1 was included in the isolated fraction of compound 2 (Figure 6,A), which resulted in lower recovery (95%) of compound 1, and lower purity (95%) for compound 2 (Figure 6,B).

In contrast, high purity (99%) was observed for compound 1 and high recovery (98%) observed for compound 2. The results indicate that elution order can influence purity and recovery results for low resolution separations due to peak tailing,a common phenomenon evident at preparative scale..

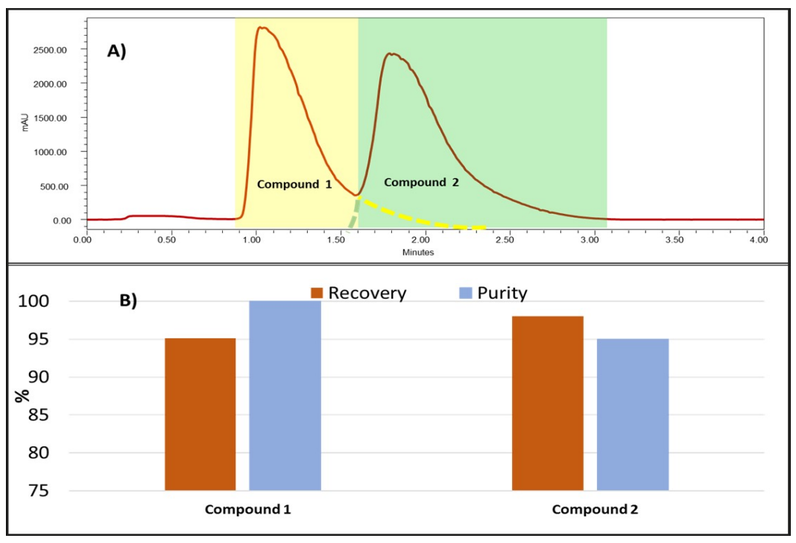

Figure 6: A) *Chromatogram showing isolation of compound 1 and compound 2 (Rs = 1.16). The dotted line represents typical preparative scale peak shape* ***B)*** *Recovery and purity of each fraction based on elution order.*

GLS Make-up Flow Optimization

The ChromScope 2.0 software contains a solvent saving feature that provides additional liquid flow to the GLS, only at times required by the isolation, via the make-up pump. The most significant impact was observed at low method co-solvent, i.e. 4% (5.0 mL/min). At this flow rate, isolate recovery was approximately 93% for compound 1. When optimized liquid flow was added to the GLS via the make-up pump, recovery increased to 99%, while purity remained stable (Figure 7).

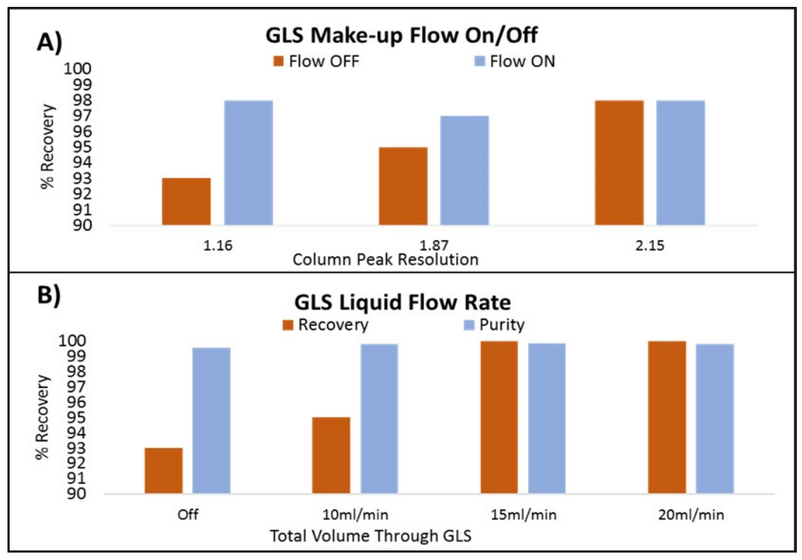

Figure 7: *Make-up pump at a system flow rate of 5 mL/min recovery and purity* ***A)*** *for each Rs and* ***B)*** *at different make-up flow rates for Rs=1.16.*

Cost Comparison

A cost comparison was prepared for isolation of compound 1 using a 4% (5 mL/min) system co-solvent flow rate. Throughput (mg/hr), while employing the 50 mm column, was approximately two fold greater when compared to the longer, more expensive columns, and co-solvent and CO_2 costs were two fold less. Recovery increased to 99% and purity remained above 99% after optimized addition of make-up flow to the GLS. The 50 mm column was the most economical for isolation of several milligrams of high purity isolate after GLS liquid flow optimization.

Table 2: *Purification cost summary comparing column resolution*

Column Dimensions	Compound Resolution	Est. Column Retail Cost	Injections /Hr	Throughput	Cost Co-solvent/mg*	Cost CO_2/mg	% Recovery	% Purity	Total Purification Cost	Total Purification Time
19 x 50 mm	1.16	$1740	50	392 mg/hr	$0.06 /mg	$0.10/mg	98	99.7	Lowest	Shortest
19 x 100 mm	1.18	$2870	25	194 mg/hr	$0.12/mg	$0.20/mg	97	99.9	Mid	Mid
19 x 150 mm	1.21	$3425	20	160 mg/hr	$0.15/mg	$0.24/mg	98	99.6	Highest	Longest

** Does not include GLS make-up solvent.*

CONCLUSIONS

- Chromatographic retention time drift, changes in peak area or peak height were not observed for consecutive sample injections over 24 hours. The Prep SFC 150 Mgm, combined with the Torus 2-PIC 130Å, 5µm, OBD column, is a stable preparative system.
- Resolution integrity was maintained by the gas-liquid separator (GLS) at system and make-up pump flow rates investigated for the sample test mixture.
- The addition of static or compensated GLS make-up flow increased recovery of the sample test mixture at low system method solvent flow rates (i.e. 5 mL/min), while high isolate purity was maintained.
- Although chromatographic resolution can be optimized using longer, more expensive preparative columns, gains in chromatographic resolution may not have a measureable impact on the recovery and purity of the overall final product within a purification campaign.

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