

Investigation and Application of “Superoptimal” Flow Rates with Preparative Supercritical Fluid Chromatography

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Abstract

Supercritical fluid chromatography (SFC) utilizes liquid CO₂, allowing higher flow rates compared to liquid chromatography. The use of a large-scale SFC preparative device makes it possible to achieve greater flow rates (up to 400 mL/min total flow) compared to a lower flow (70 mL/min) model. “Superoptimal” flow rates (>100 mL/min) with typical 250 x 21 mm, 5 μm columns have been achieved and are beneficial for decreasing the cycle time of purifications. Two commercial compounds were analyzed to generate Van Deemter plots to utilize a large-scale SFC at an optimal flow rate. In addition, Van Deemter plots were generated for both a 70 mL/min device and an analytical SFC. Purification cases and Van Deemter analyses data will be presented.

Introduction

Supercritical fluid chromatography (SFC) is an advantageous technique for purification of samples due to its inherent benefits. The liquid CO₂ is removed during the process, leaving behind just the organic modifier (including acidic and/or basic additives); these volumes are usually quite less than reverse or normal phase. Because of the low viscosity and high diffusivity of liquid CO₂ in its mobile phase, flow rates are achieved for separations that are much higher than typical reverse or normal phase liquid chromatographic conditions.

“Superoptimal” flow rates can be used to purify samples via typical 21 x 250 mm, 5 μm columns. Flow rates greater than 100 mL/min on the aforementioned columns are considered superoptimal. Though sample amounts per injection are less than that expected when utilizing a column of larger dimensions, the dramatic reduction in cycle time increases throughput. Samples of poor solubility are handled nicely by injecting less but more frequently. In addition, no modification of sample loop size or purchase of the larger columns is necessary to do this.

It is useful to understand the viable flow rates for a separation. An acceptable purification flow rate can be determined through observation; peak shape and separation are noted. A more thorough method involves a systematic analysis and generation of a Van Deemter plot. Ideally, one would want to achieve a flow rate that will decrease cycle time but not at the expense of theoretical plate height, which could deleteriously affect a separation. In this poster, a Van Deemter study is shown along with examples of superoptimal purifications. Overall, it was observed (using trans-stilbene oxide (TSO)) that the plate height varied little between 70 and 180 mL/min.

Experimental

Materials

The SFC-grade carbon dioxide was obtained from BOC Gases (Murray Hill, NJ, USA). Methanol (MeOH) was HPLC-grade from Mallinckrodt Baker (Muskegon, MI, USA). Diethylamine (DEA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Trans-stilbene oxide (TSO) was purchased from TCI (Portland, OR, USA).

Analytical SFC instrumentation, columns and analysis methods

The analytical SFC instrument used was a Berger SFC unit (Mettler-Toledo Autochem, Newark, DE, USA) with an FCM1200 flow control module, a dual pump control module, a TCM2100 thermal column module, a column selection valve capable of switching between six columns, and a solvent control valve for up to six modifiers to be selected. The SFC was equipped with an Agilent 1100 photodiode array detector with a high-pressure flow cell (Agilent Technologies, Palo Alto, CA, USA). The autosampler/injector was a CTC LC Mini PAL from Leap Technologies (Carrboro, NC, USA). A Waters (Milford, MA, USA) ZQ benchtop single quadrupole mass spectrometer with an atmospheric pressure chemical ionization (APCI) source was coupled to the SFC. The software used in the analyses were Berger MassWare™ v. 4.01 and MassLynx™ v. 4.0 SP1.

The Chiral Technologies (Exton, PA, USA) column utilized was the Chiralpak AD-H. The dimension of the column is 4.6 x 250 mm with 5 μm particle size.

The analysis method for the Van Deemter study was isocratic, and the mobile phase was comprised of 88% liquid CO₂ and 12% methanol. Flow rate was varied between 1.0 and 6.0 mL/min. Oven and nozzle temperature were set to 35 °C, and the outlet pressure was 100 bar. Injections consisted of 10 μL of a TSO solution with a concentration of 2.0 mg/mL.

Preparative SFC instrumentation, columns, and methods

MultiGram II

The preparative SFC was a Berger MultiGram™ II from Mettler-Toledo Autochem (Newark, DE, USA). The components were the Separator Control Module (SCM)-2500, Electronics Control Module (ECM-2500), ventilated collection cabinet, UV variable wavelength detector, and a waste containment vessel. Organic modifier was delivered via a Varian (Walnut Creek, CA, USA) SD-1 Pump. CO₂ was delivered using a modified Varian SD-1 pump. The injector was a Modular Digital Pump (Model XL3000) from Cavro Scientific Instruments Inc. (Sunnyvale, CA, USA). CO₂ was cooled using a Julabo (Allentown, PA, USA) chiller. The software used in the purification was Berger SFC ProNTo™ v. 1.5.305.15.

MultiGram III

The preparative SFC was a Berger MultiGram™ III from Mettler-Toledo Autochem (Newark, DE, USA). The components were the Separator Control Module, Electronics Control Module (ECM-2500), and a UV variable wavelength detector. Organic modifier was delivered via a Varian (Walnut Creek, CA, USA) SD-1 Pump. CO₂ was delivered using a modified Varian SD-1 pump. The injector was a Modular Digital Pump (Model XL3000) from Cavro Scientific Instruments Inc. (Sunnyvale, CA, USA). CO₂ was cooled using a Huber (Offenburg, Germany) Unichiller chiller. Effluent is collected in a ventilated cabinet. The cabinet also stores the organic solvent and is outfitted with a CO₂ detector. Software used in the purification was Berger SFC ProNTo™ v. 1.5.305.15.

Columns

Chiral Technologies (Exton, PA, USA) columns utilized were the Chiralpak AD-H and Chiralcel OD-H (21 x 250 mm with 5 μm particle size), as well as the Chiralpak AD-H (30 x 250 mm with 5 μm particle size). The Princeton Chromatography (Cranbury, NJ, USA) column used was the Princeton PAA (21.2 x 250 mm with 5 μm particle size).

Methods

Van Deemter study

Preparative SFC methods utilized a mobile phase consisting of 88% liquid CO₂ and 12% methanol. Flow rate was varied between 10 mL/min and 70 mL/min for the MultiGram II; the flow rate was varied between 10 mL/min and 180 mL/min for the MultiGram III. Oven temperature was set at 35 °C. Outlet pressure was 100 bar. Injections consisted of 0.5 mL of a 2.0 mg/mL TSO solution for the 30 x 250 mm column and 0.5 mL of a 1.0 mg/mL TSO solution for the 21 x 250 mm column.

Throughput study

Preparative SFC methods utilized a mobile phase consisting of 88% liquid CO₂ and 12% methanol. The flow rates used were 70 and 140 mL/min. Oven temperature was set at 35 °C. Outlet pressure was 100 bar. Each injection consisted of 1.5 mL of a 50 mg/mL solution of TSO.

Results

Van Deemter Plots

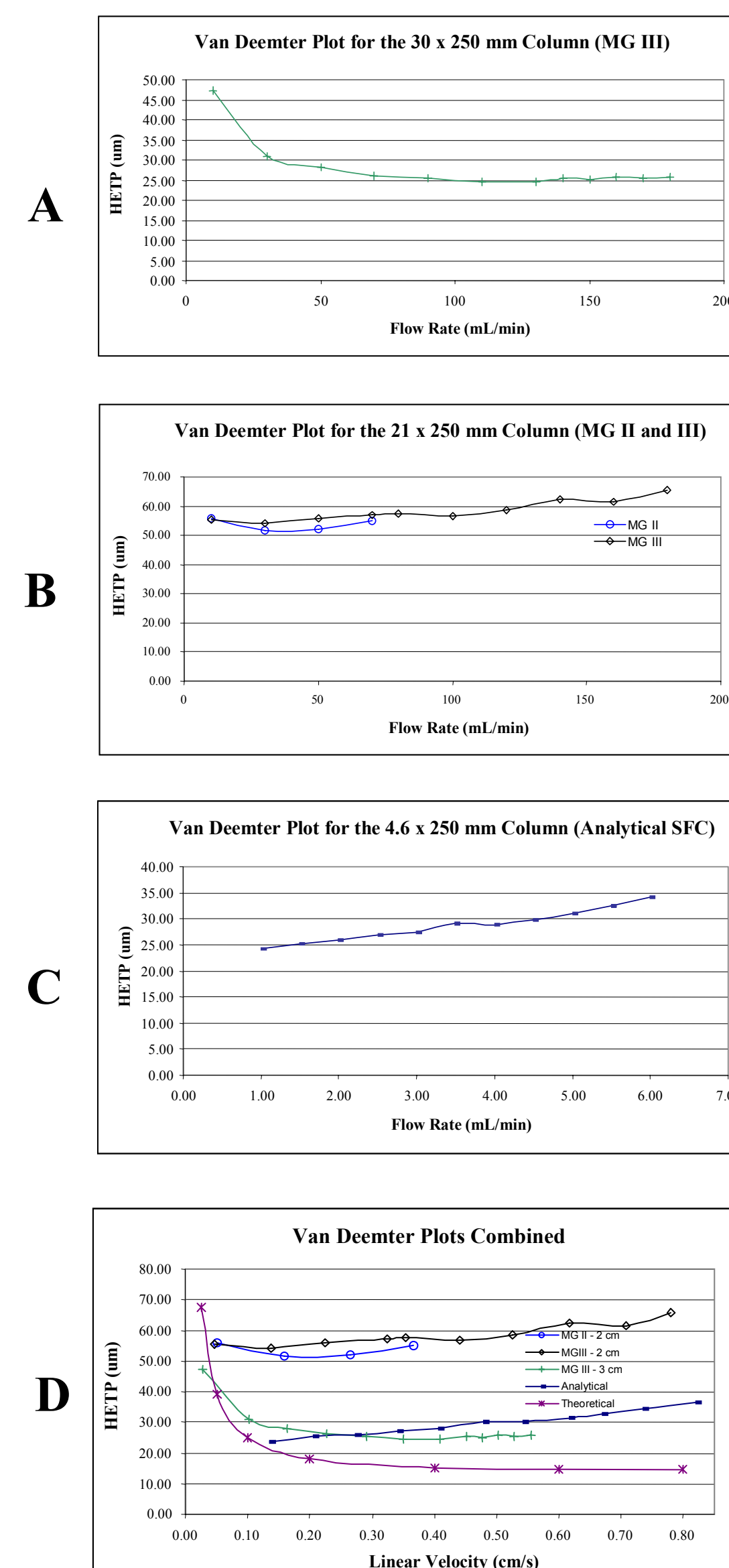


Figure 1. Modified Van Deemter plots for 30 mm (A) (MultiGram III), 21 mm (B) (MultiGram II and III) and 4.6 mm (C) (analytical SFC) diameter columns, as well as a combined Van Deemter plot (D) for all columns and systems (including theoretical). TSO was utilized in these analyses.

Separation of TSO– 70 mL/min vs 140 mL/min

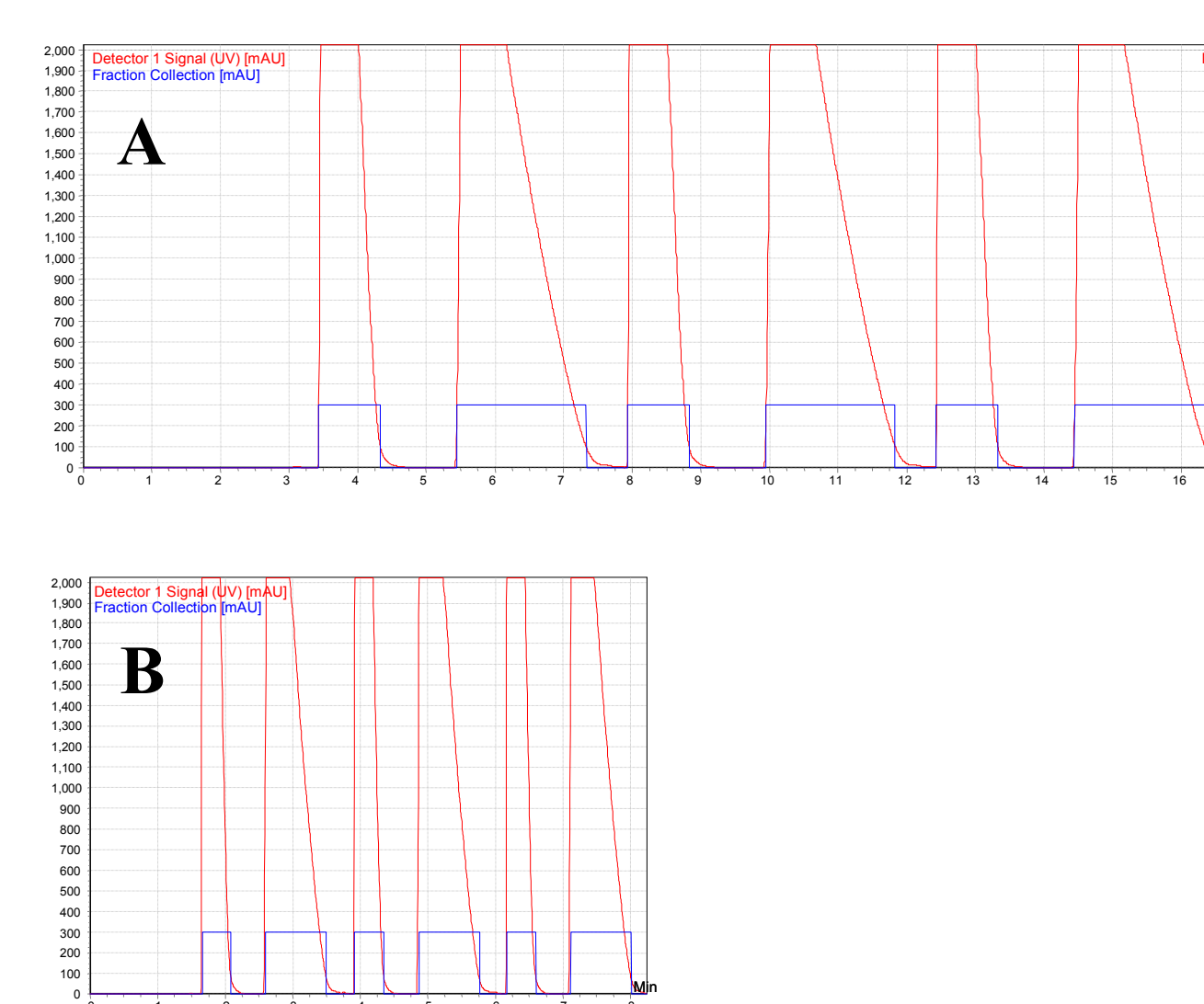


Figure 2. Preparative SFC chromatograms showing the separation of TSO (50 mg/mL) enantiomers at 70 mL/min (A) and 140 mL/min (B). Column: Chiralpak AD-H (21 x 250 mm, 5 μm) Mobile phase: 12% methanol (isocratic).

Table 1. Comparison of throughputs between the 70 and 140 mL/min flow rate separations of TSO.

Comparison of 70 vs 140 mL/min			
Flow Rate (mL/min)	Cycle Time (min)	Throughput (mg/hr)	Resolution
70	4.50	900	1.56
140	2.25	1800	1.53

Example #1 – Sample A – purification of a “poorly” soluble, achiral sample using a “superoptimal” flow rate

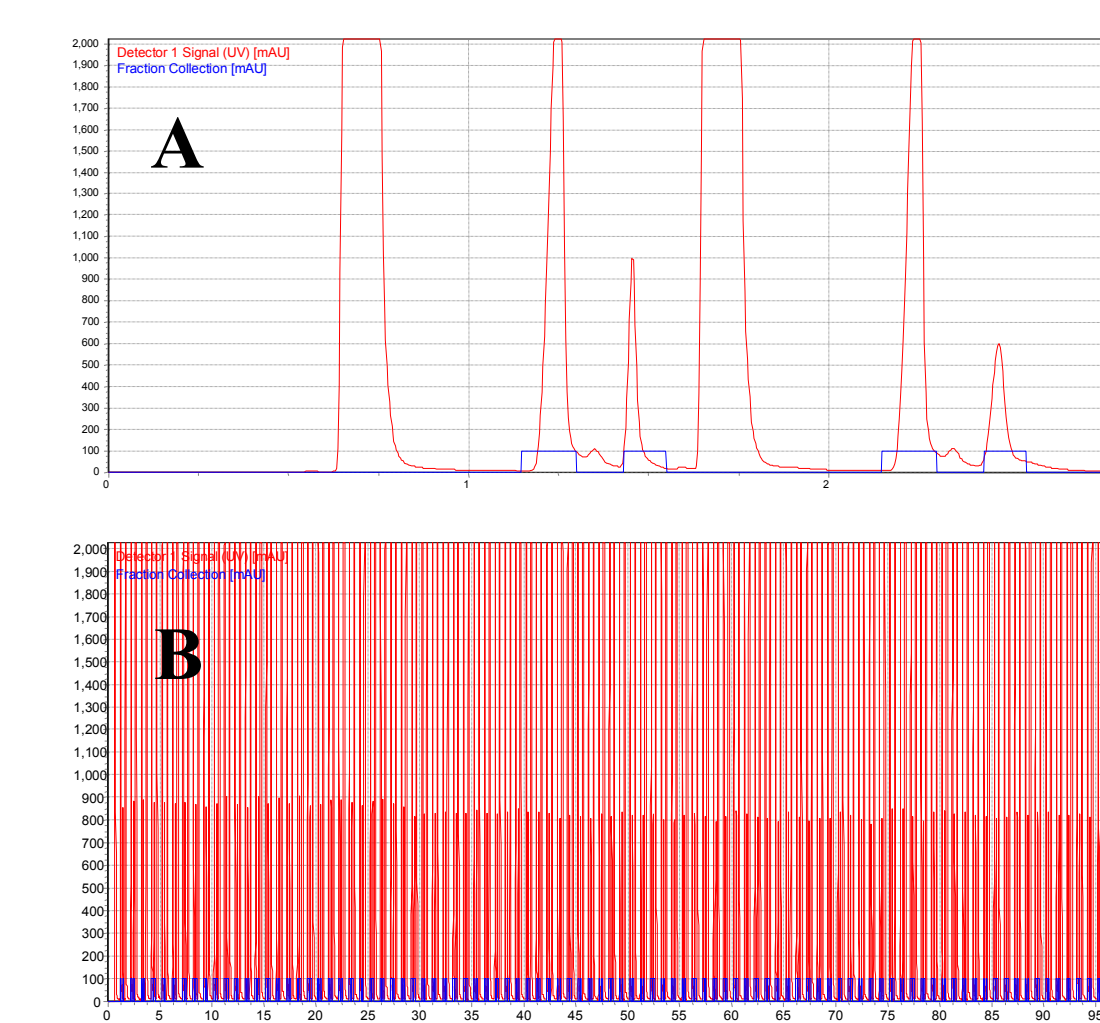


Figure 3. Example preparative SFC chromatogram showing the purification of sample A. The compound was soluble at 3 mg/mL. 4.5 mg of sample was used per injection. A: Test injection. B: Stacked injections. Column: Princeton PAA (21 x 250 mm, 5 μm). Mobile phase: 16% methanol (0.2% DEA) (isocratic). Flow rate: 140 mL/min.

Example #2 – Sample B – purification of a chiral sample using a “superoptimal” flow rate

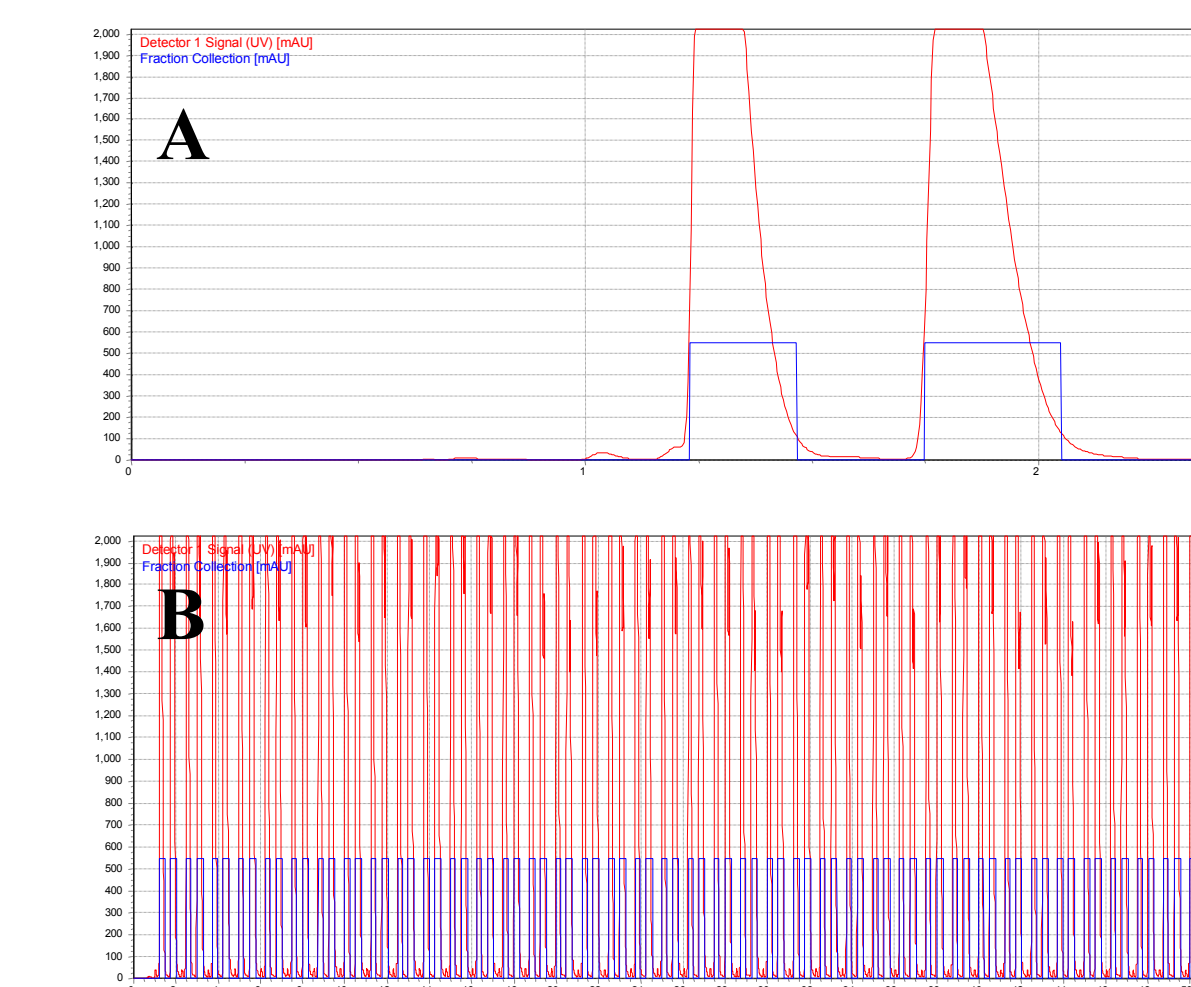


Figure 4. Example preparative SFC chromatogram showing the purification of Sample B. 39 mg of material was injected each cycle. A: Test injection. B: Stacked injections. Column: Chiralcel OD-H (21 x 250 mm, 5 μm). Mobile phase: 26% methanol (isocratic). Flow rate: 110 mL/min.

Discussion

The Van Deemter curves (Figure 1) clearly show that the separation of TSO was achievable at flow rates up to 180 mL/min on a 21 x 250 mm, 5 μm column without sacrificing plate height. Plate height changed little between the 70 mL/min and 180 mL/min flow rates. Such a wide range of allowable flow rates would not be expected for reverse or normal phase separations. Plate height was also stable over a wide range of flow rates for the 30 x 250 mm, 5 μm column. The 4.6 x 250 mm, 5 μm column on the analytical SFC showed a gradual increase in plate height over the range of investigated flow rates. The empirical plate heights for all columns and systems were greater than theoretical calculations.

Separation of TSO enantiomers (see Figure 2) utilizing a “superoptimal” flow rate doubled the throughput (see Table 1) without requiring a change of column or injection loop size. Doubling the flow rate reduced the cycle time by half (4.5 min to 2.25 min). A factor that may limit the flow rate allowed for a separation requiring higher organic content is inlet pressure. The methanol pump on the preparative SFC is limited to 279 bar.

Figures 3 and 4 show the application of “superoptimal” flow rates for the purification of actual samples. The use of a 140 mL/min flow rate for the separation of Sample A shows how a sample of low solubility (3 mg/mL) was purified in a timely manner by using a “superoptimal” flow rate. Injecting a small amount (4.5 mg) of sample every minute allowed over 200 mg to be purified each hour. The entire sample was purified in half the time it would have required by using a more standard 70 mL/min flow rate for the purification. Figure 4 shows the purification of Sample B at a flow rate of 110 mL/min. Again, simply increasing the flow rate decreased the time required to complete the purification of the sample.

Conclusions

Utilizing “superoptimal” flow rates (flow rates greater than 100 mL/min for a 21 x 250 mm column) with SFC is an excellent way of increasing throughput without modifying column or injection loop size. High flow rates are achieved without sacrificing plate height. This practice is now utilized regularly in our labs to decrease turnaround times for achiral and chiral purifications. It would be expected that using superoptimal flow rates with larger columns would further increase throughput.

The limitation of viable flow rates (over the ranges that were studied) lies in the pressure threshold (279 bar for the instruments of interest) allowed by the instrument. A higher organic content in the mobile phase will cause an increase in system pressure, thus decreasing the maximum allowable flow rate.

Lastly, throughput can be increased via a number of method parameter modifications (e.g., sample loading, column choice, etc...). However, simply increasing the flow rate to improve sample throughput is a simple but effective option.

Key References

Jennifer Van Anda (2005) “MultiGram III Purification Objectives and Design Essentials.” Berger West Coast User’s Meeting.

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