

The Use of Sub-2 μm Particles in SFC

4X the Speed and 1/4th the Pressure Drop

Terry A. Berger, Aurora SFC Systems, Inc.

INTRODUCTION

There is an amazing scarcity of publications on the use of sub-2 μm particles in supercritical fluid chromatography (SFC). The performance of a 1.8 μm Zorbax RX-Sil (3 x 100 mm) column packed with spherical silica will be demonstrated using SFC.

Pressure studies indicated that a modifier of 65% methanol at a 1.5 mL/min flow rate and 50% methanol at 2 mL/min could be used before the column head pressure reached 400 bar. At 22.5% methanol, the flow rate could be increased to over 3 mL/min before reaching 400 bar, with pressure drops not exceeding 240 bar. Efficiency was generally good, exceeding 24,000 plates. Pseudo Van Deemter curves are presented showing the optimum flow rate reduction as modifier concentrations are increased. The optimum flow rate was found to be between 1.5 and 2 mL/min.

Compared to the results reported for ultra-high performance liquid chromatography (UHPLC) for columns with similar dimensions, the optimum performance was obtained at 4 times the linear velocity and with 1/4th the pressure drop.

These small particle columns appear suitable for routine use when using common 400 bar HPLC equipment modified to perform SFC. However, HPLCs capable of 600 bar with higher detector speeds and lower dispersion can be more versatile while also providing superior performance.

A diverse range of solute families including sulfonamides, steroids, xanthenes, profens, and nucleic acids were each separated in less than 1 minute with high efficiency. Ten injections of the steroid mix yielded retention time RSDs of <0.2%, with area RSDs of <0.5%. UV detector noise was 0.01-0.04 mAU at a filter setting of >0.01 min.

INSTRUMENTATION AND EXPERIMENTAL CONDITIONS

- The chromatograph consisted of an Aurora SFC Fusion™ A5 from Aurora SFC Systems, Inc., Redwood City, CA, USA, and a Model 1200SL HPLC from Agilent Technologies, Waldbronn, Germany. The resulting system is a very low dispersion SFC.
- The 1200SL system included a binary pump, solvent cabinet, thermal control compartment, model 1200C diode array detector (DAD), model 1100 degasser and autosampler.
- The DAD employed a 1.7 μL , 6 mm, 400 bar flow cell. The electronic filter was generally set to >0.01 min. In some of the faster separations, the filter was set to >0.005 min. At 30% modifier, a setting of <0.0025 min (80 Hz) was required.
- The tubing from the injection valve to the detector was 1/16" OD, 0.005" (0.125 mm) ID stainless steel tubing (~70 cm total).
- All operating parameters such as flow rate, modifier concentration, column temperature, injection and detector settings were controlled by the Agilent ChemStation software. The Fusion A5 was controlled by the Aurora control application which is a ChemStation software add-on.
- Mobile Phase: Standard-grade CO₂ with methanol as a modifier.
- The Fusion A5 pre-compressed the CO₂ before it reached the HPLC. The compressibility compensation for side A of the pump was set to zero and was used for CO₂ metering. The compressibility compensation for side B was set to 130 x 10⁻⁴/bar and was used to pump degassed methanol.
- Column: Zorbax RX-Sil 1.8 μm (3 x 100 mm) unless otherwise indicated.
- Column temperature: 50 °C.

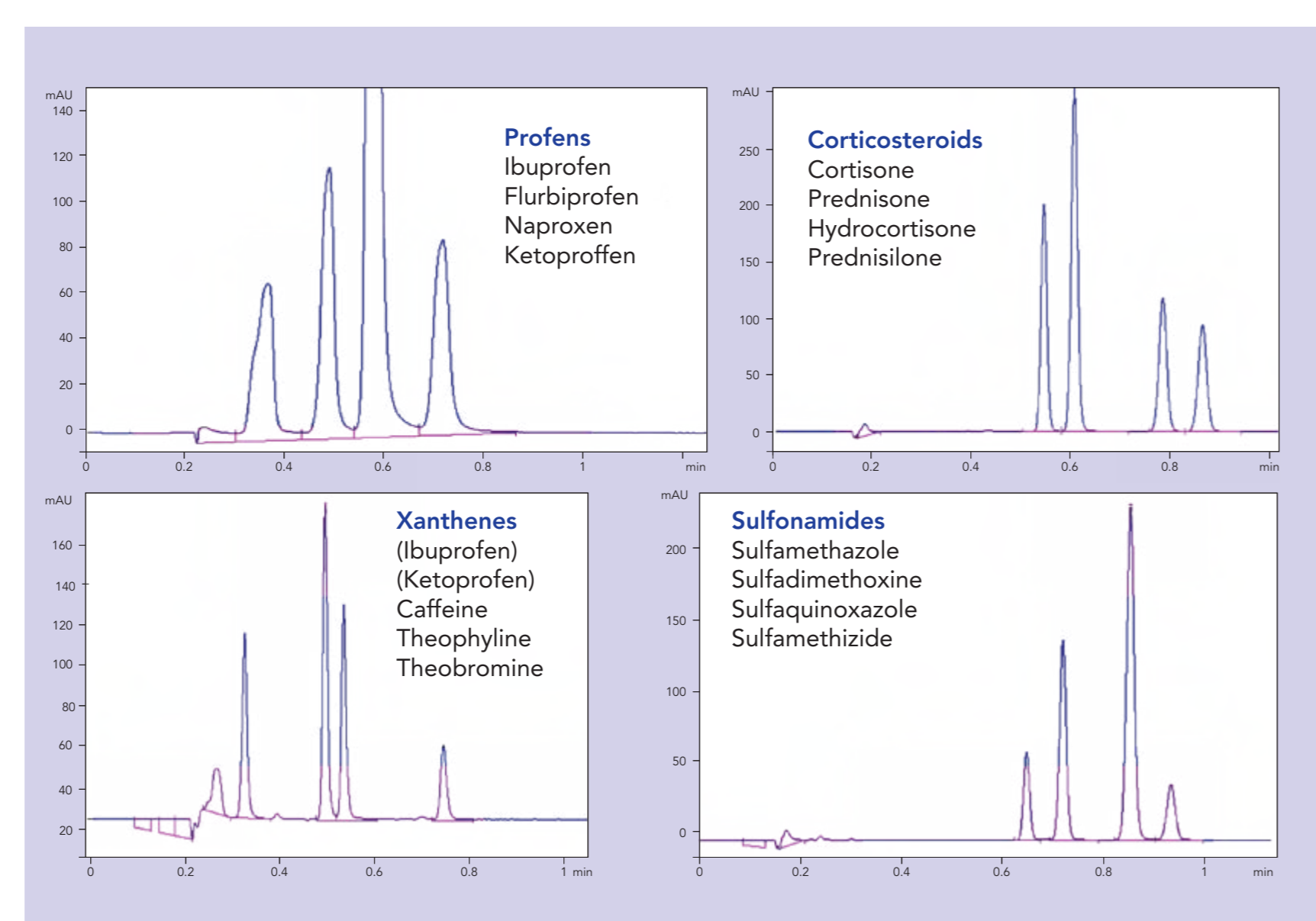


FIGURE 1. Separation of four solute families. Mobile phase: CO₂ with MeOH at varying percentages, no additives, outlet pressure: 150 bar.

SOLUTE ANALYSIS

A broad group of compounds corresponding to various polarity ranges were run to determine the performance of the Zorbax RX-Sil column with SFC. Figure 1 demonstrates analysis of profens, corticosteroids, xanthenes and sulfonamides. Each run time was less than 1 minute.

It is worthy to note that this configuration of instrumentation is capable of very fast, high resolution separations using only a silica column and very simple CO₂/MeOH mobile phases. Because low detector noise levels can also be achieved, analytical SFC becomes a viable complement to UHPLC separations – adding another dimension of information for critical analyses.

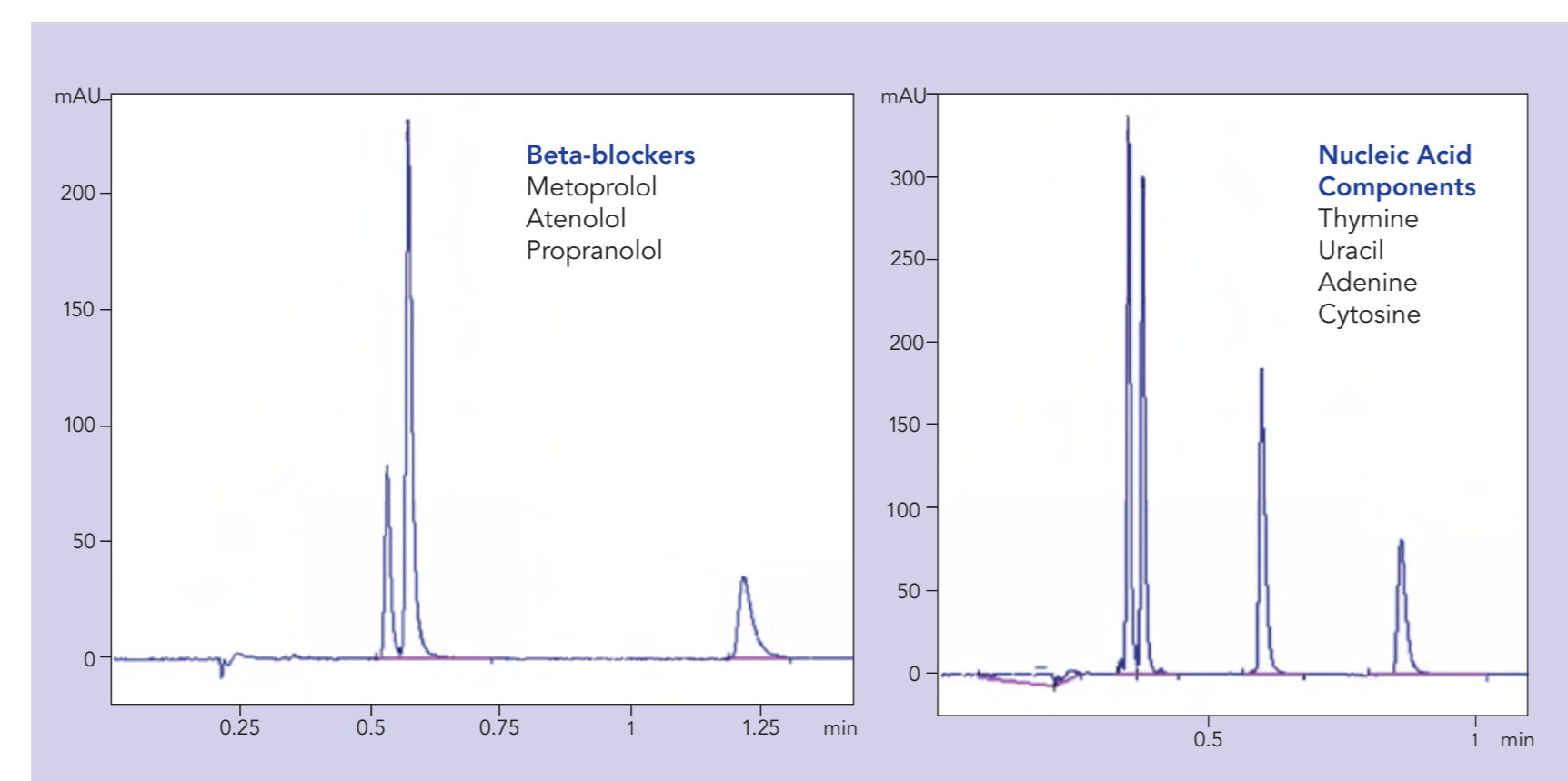


FIGURE 2. Analysis of beta-blockers and nucleic acid components. Mobile phase: 30% MeOH and 0.2% TEA in CO₂, flow rate: 2 mL/min, outlet pressure: 150 bar.

The range of polarity that SFC methods may be applied to is wider than simple chiral separations – or niche preparative work. As shown in Figure 2, beta-blocker and nucleic acid components were also analyzed with run times of approximately 1 minute.

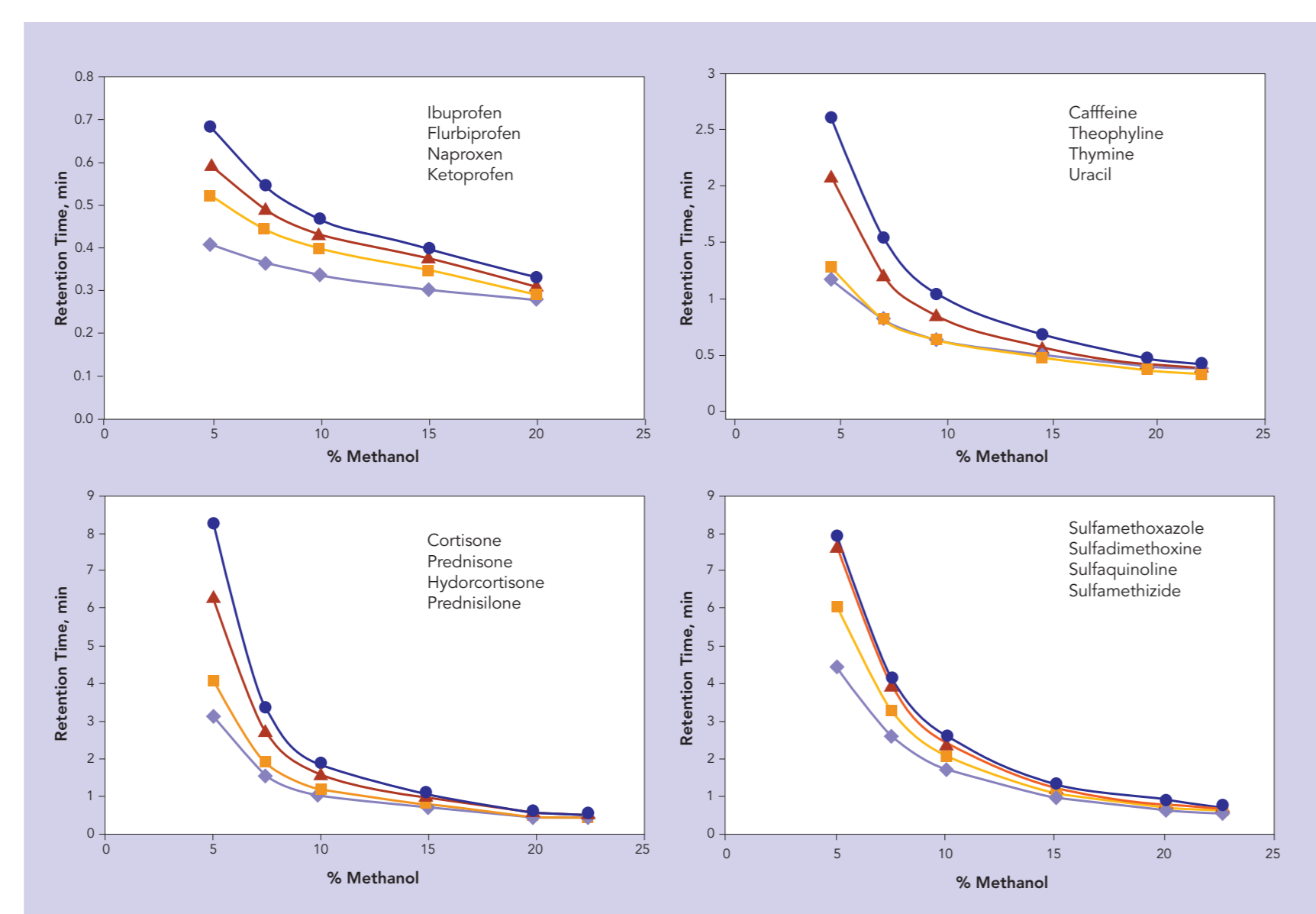


FIGURE 3. Retention time of different solute families at various modifier concentrations. Mobile phase: MeOH in CO₂, flow rate: 2 mL/min, outlet pressure: 150 bar.

The retention times of various solute families with increasing MeOH concentrations were also studied. Note that the different shapes of the individual lines generated (Figure 3) permit useful method development protocols to capitalize on retention changes. The data in Figure 3 indicate an expected general trend in reduced retention with increasing modifier strength, with minor changes in selectivity.

In Figure 4, a 17-component mix representing a wide range of polarity was analyzed using 17% modifier with a run time of just over 1 minute.

The approximate elution order of compounds is shown in Table 1.

Peak Number	Component
1	Flurbiprofen
2	Naproxen
3	Ketoprofen
4	Warfarin
5	Theophylline
6	Caffeine
7	Uracil
8	Thymine
9	Cortisone
10	Prednisone
11	Aceamidophenol
12	Sulfamethoxazole
13	Sulfadimethoxine
14	Hydrocortisone
15	Prednisolone
16	Sulfaquinazoline
17	Sulfamethizide

TABLE 1. Elution order of 17-component mix using 17% modifier.

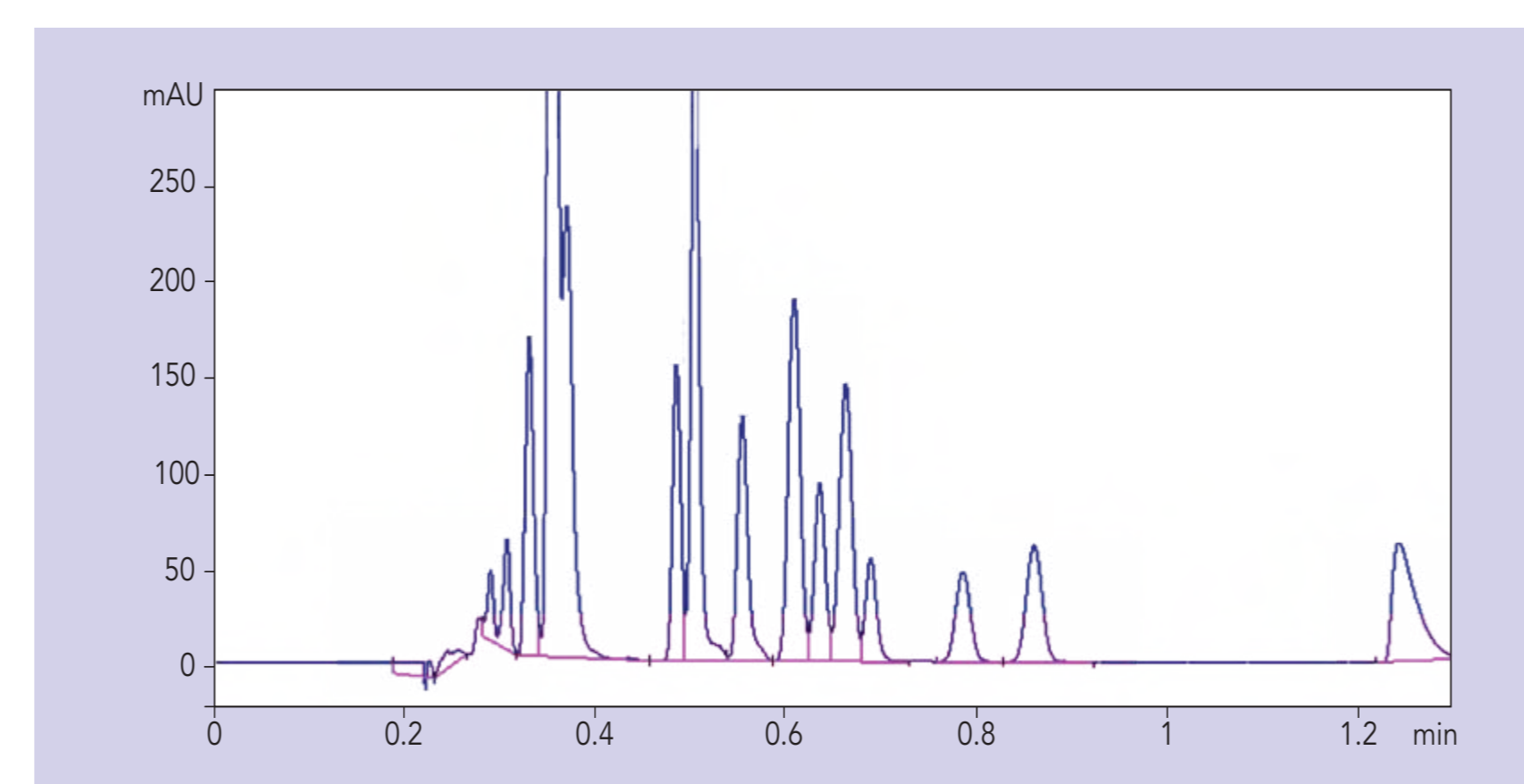


FIGURE 4. Analysis of a 17-component mix. Mobile phase: 17% MeOH in CO₂, flow rate: 2.0 mL/min, outlet pressure: 150 bar.

Sixteen of the Table 1 components were individually analyzed at various modifier concentrations. Each solute family responds differently to changes in modifier concentration (Figure 5). This implies that method optimization could yield useful results for this broad range of solutes in real world samples.

Increasing the modifier concentration makes SFC more like HPLC in terms of pressure drops and separation performance as seen by the

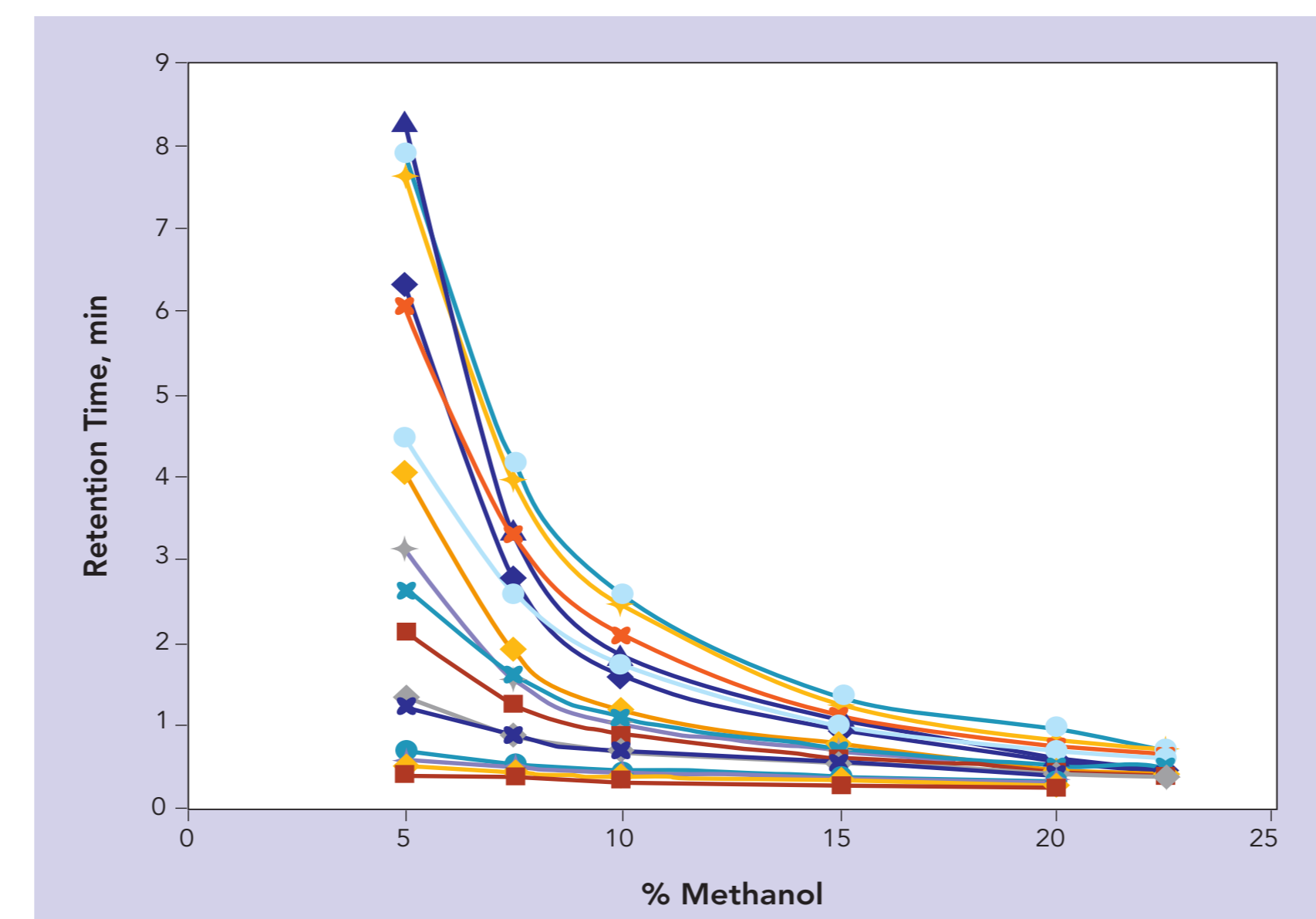


FIGURE 5. Individual analysis of solute components. Mobile phase: MeOH in CO₂, outlet pressure: 150 bar.

reduced plate heights in Figure 6. It is usually better to use lower modifier concentrations, if possible. Remember that the linear velocity in the column is actually higher because the temperature of the mobile phase at the pump is at ambient, while the column temperature is set to 50 °C, making the mobile phase 20-25% less dense.

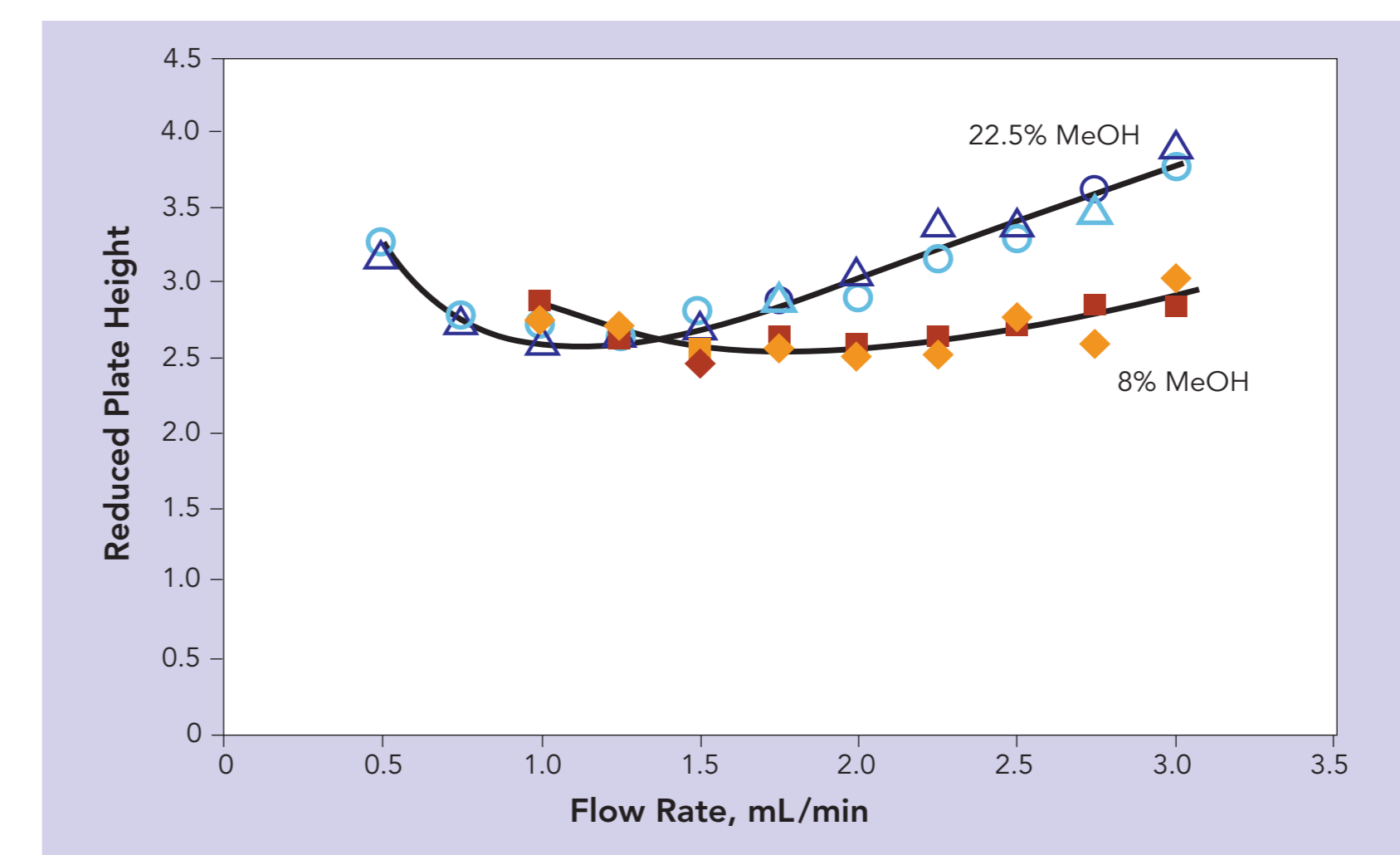


FIGURE 6. Effect of modifier concentration on reduced plate height at various flow rates. Mobile phase: MeOH in CO₂, outlet pressure: 150 bar.

PRESSURE CONSIDERATIONS

The pressure drop across the column and system tubing for flow rates of 1.5 and 2.0 mL/min at various modifier concentrations is shown in Figure 7. This clearly demonstrates the impact of increasing modifier concentration on column head pressure.

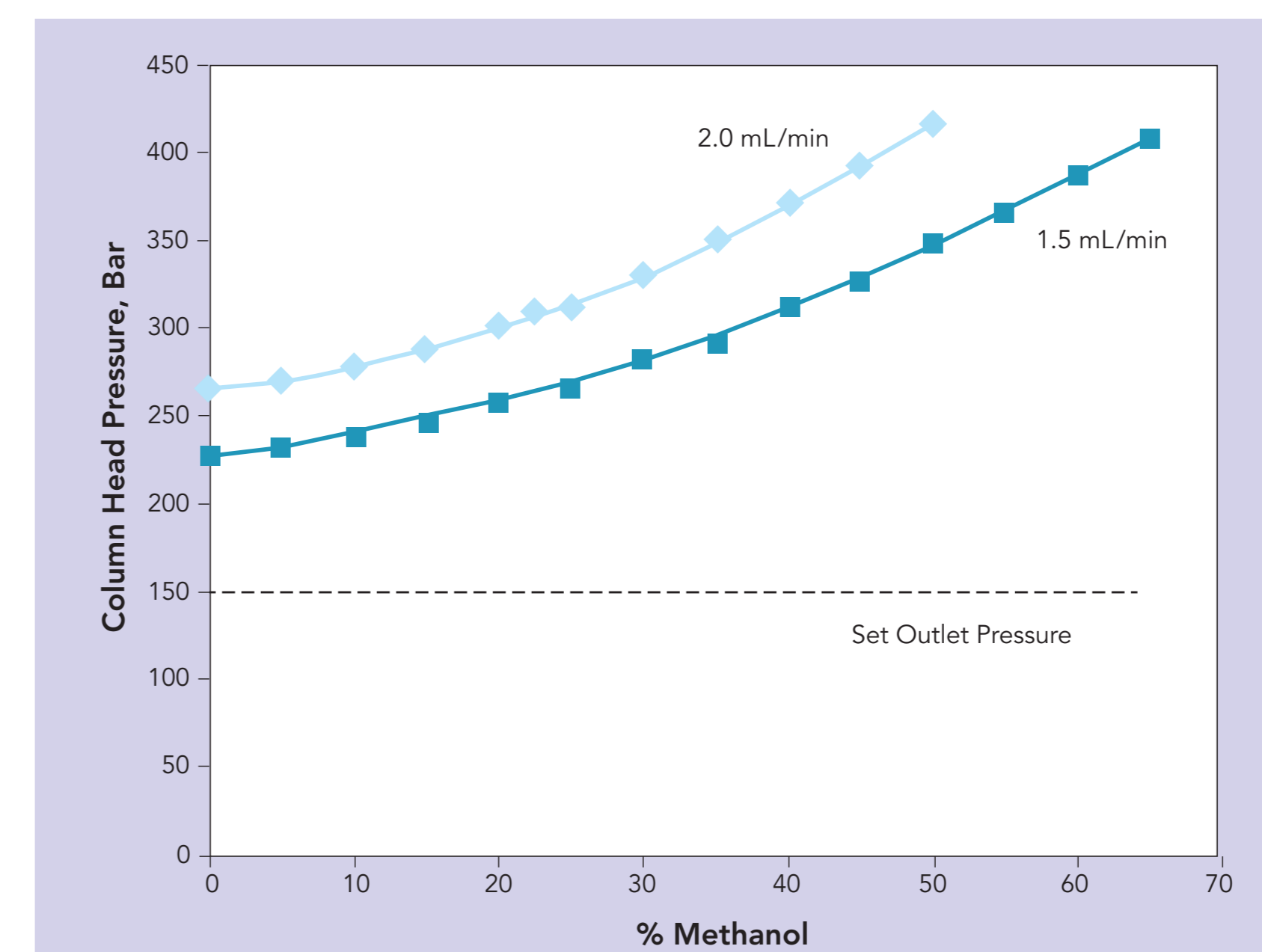


FIGURE 7. Column pressure vs modifier concentration at two flow rates. Mobile phase: MeOH in CO₂, outlet pressure: 150 bar.

In Figure 8, the modifier concentration was held constant and the flow rate was varied in order to determine the maximum allowable flow rate on the Zorbax RX-Sil column. The data clearly identify that flow rates below approximately 2.5 mL/min can be used without exceeding 400 bar, even at modest modifier conditions.

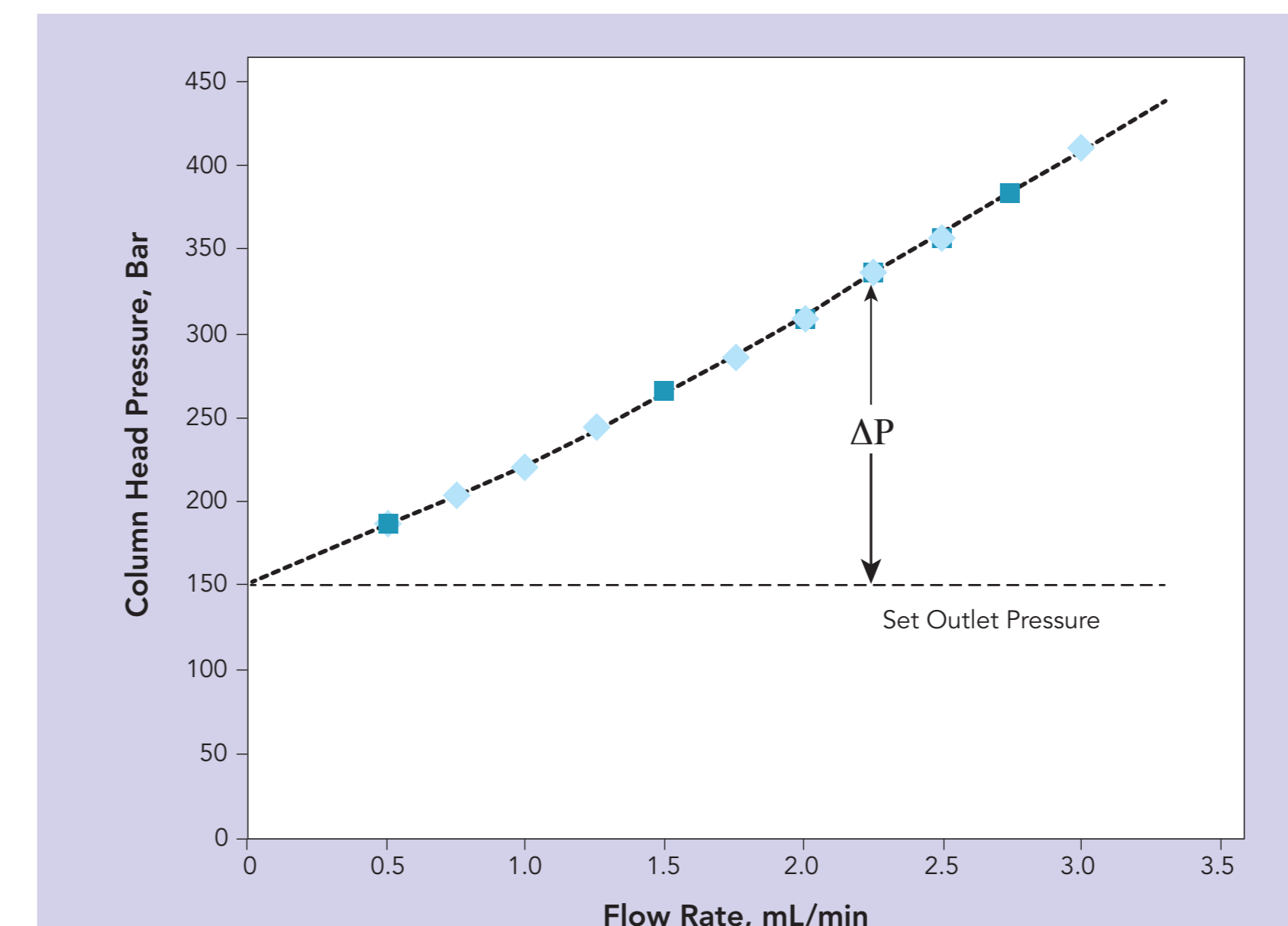


FIGURE 8. Column pressure vs flow rate, Mobile phase: 22.5% MeOH in CO₂, outlet pressure: 150 bar.

Pressure drops across various column formats and particle sizes were measured with the contributions of the 70 cm of system tubing subtracted (Figure 9). This indicates that a diverse range of columns and particle sizes are entirely appropriate for consideration to optimize run times or separation capabilities.

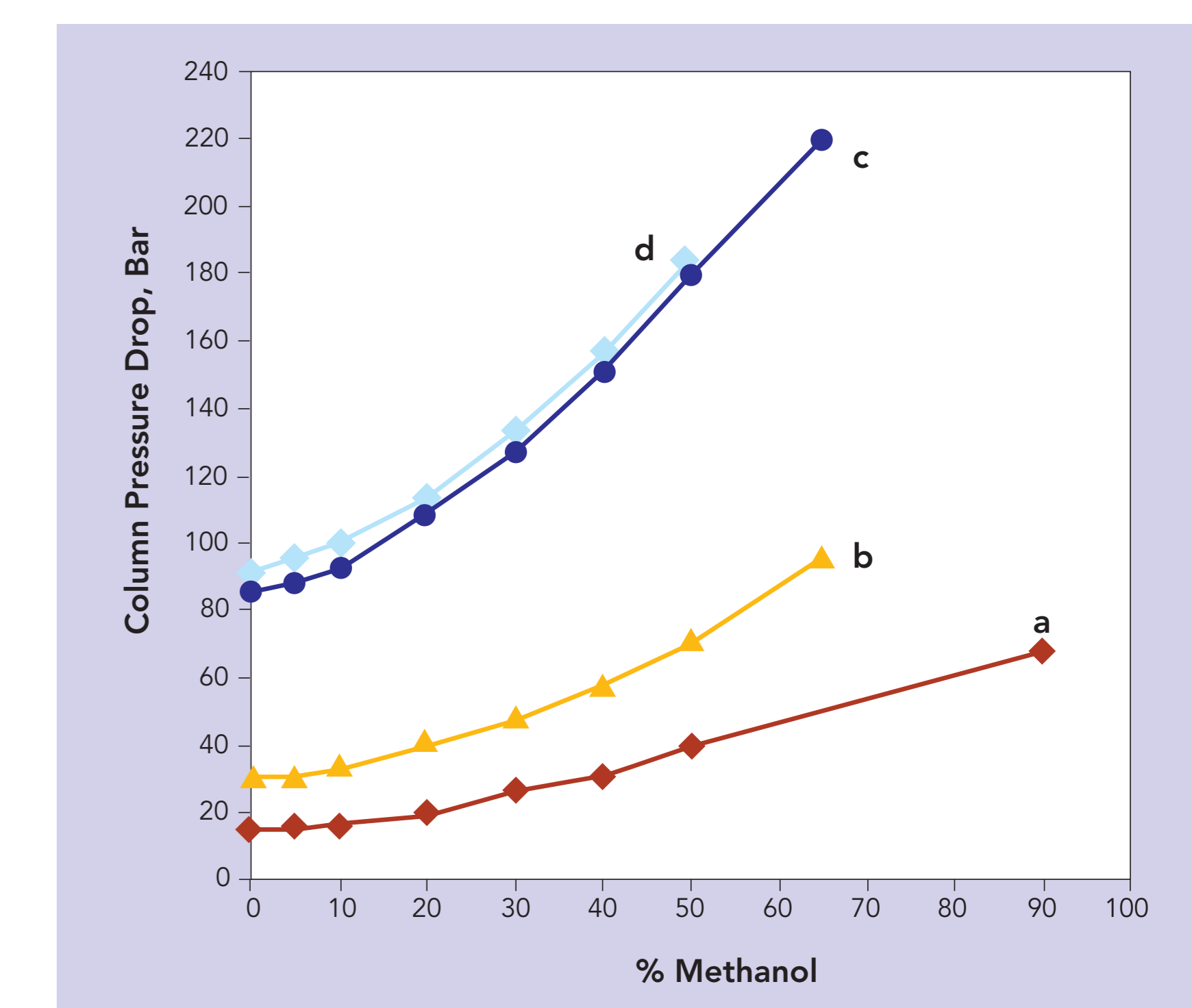


FIGURE 9. Pressure drops across various Zorbax RX-Sil columns with different modifier concentrations. Mobile phase: MeOH in CO₂. a) 5 μm (4.6 x 250 mm), flow rate: 2 mL/min, outlet pressure: 150 bar. b) 3.5 μm (4.6 x 150 mm), flow rate: 3 mL/min, outlet pressure: 150 bar. c) 1.8 μm (4.6 x 50 mm), flow rate: 5 mL/min, outlet pressure: 150 bar. d) 1.8 μm (4.6 x 50 mm), flow rate: 5 mL/min, outlet pressure: 100 bar.

CONCLUSIONS

- A large number of compounds can be eluted rapidly using the bare silica 1.8 μm Zorbax RX-Sil column. Peaks were symmetrical and an additive was not required.
- Efficiency is high and pressure drops are moderate allowing good results under diverse flow rate conditions.
- The 3 x 100 mm columns with 1.8 μm particles can be used with 400 bar HPLC systems. Newer systems with higher pressure capability and lower dispersion can be used when higher resolution and speed are required.
- Sub-2 μm particles are surprisingly easy to use in SFC. There appears to be nothing to prevent their routine use.
- With the comparatively low operating pressures that CO₂/methanol mobile phases offer, small particle size columns may be used with available instrumentation. Analytical SFC becomes a complimentary technique to UHPLC for situations where orthogonal identification and quantitation is important.