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Mind the pressure in preparative up-scaling – effects of particle size and solvent composition on back pressure

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SUMMARY

The back pressure generated by an HPLC column is an important factor to consider during method development for larger scale purification methods. Preparative pumps with higher flow rates often have a lower maximum pressure than analytical HPLC pumps what can cause problems during the scale up process. Similar issues with pressure limitations can occur with other system components like switching valves.

Here, the impact of flow rate, particle size, and solvent composition on column back pressure was investigated. These findings provide practical guidance for method development at the analytical scale and can be readily translated to larger-scale operations

INTRODUCTION

The back pressure of a chromatographic system is generated by the system components, the capillaries, the column, the solvent, and the applied flow rate. Each system has a maximum pressure which it can sustain, and therefore the pressure must be monitored as it is a limiting factor during method development.

The back pressure generated by a column is dependent on the particle size and its length. The development of a preparative method is ideally performed on an analytical scale and after optimization the method is then scaled up to preparative scale. If a linear scale-up is performed the back pressure should not change

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according to the following Darcy law equation (ΔP – back pressure, L – column length, dp – diameter particle):

$$\Delta P_2 = \Delta P_1 * \frac{L_2}{L_1} * \frac{{dp_1}^3}{{dp_2}^3}$$

As preparative HPLC systems normally have lower maximum pressures, it is important to keep the generated pressure in the range of the later preparative system.
The larger the particles of the column material are, the lower will be the generated back pressure. With increasing particle size, the resolution is decreasing at the same time. Depending on the separation task, the resolution can be a critical point, and it is important to find the right compromise between resolution and backpressure. Additionally, larger particles are significantly less price intensive, which is another factor to be considered especially for large scale purifications.
Often solvent mixtures of water with acetonitrile, methanol or ethanol are used for reverse phased methods. Depending on the organic solvent composition, the generated back pressure differs significantly which could be reduced by changing the solvent composition slightly or the organic solvent if possible. The back pressure generated by the solvent will not or only slightly change during the up-scaling process.
Here, the influence of particle size, flow rate and solvent composition on the back pressure was evaluated. Further, a practical scale-up was performed to evaluate the values obtained on analytical scale to those in larger scale.

RESULTS

Determining back pressure in analytical HPLC
The first set of experiments were conducted with a standard analytical KNAUER HPLC system. The back pressure of four C18 150 mm x 4.6 mm columns with particle sizes of 5 µm, 10 µm, 15 µm and 20/45 µm was measured with linear gradients from 5% - 100% of water/acetonitrile, water/ethanol, and water/methanol. Further, three different flow rates which are typically applied for this column inner diameter were tested: 1.0 ml/min, 1.2 ml/min and 1.4 ml/min.
The measured back pressure is the sum of the pressure generate by the column, the system components and the capillaries. Therefore, it is important to measure the system back pressure without the column and subtract that from the total back pressure to obtain the column back pressure. For each condition, the maximum pressure was determined and the system pressure subtracted.

Back pressure profiles of acetonitrile/water gradients
The back pressure curves of the tested columns showed a similar shape under all tested conditions with an acetonitrile/water gradient. The pressure remained nearly constant until a maximum at 35% of acetonitrile was reached. Then the pressure dropped continuously until reaching 100% acetonitrile. The pressure was approx. 50% lower than its maximum pressure at 35% **(Fig.1** a-c).
As expected, the highest back pressure was measured with the smallest used particles (5 µm) at 1.4 ml/min and the lowest with the largest used particles (20/45 µm) at 1.0 ml/min **(Fig.1** a, c).
The back pressure of the system was measured throughout the entire gradient at all three flow rates without the column. The highest back pressure of the system was approximately 16 bar with 1.4 ml/min and 15 % acetonitrile **(Fig.1** d). The profile of the back pressure curve was similar to those with the column, showing a plateau until 25 % acetonitrile, followed by a constant pressure drop **(Fig.1** d).

For a better overview and comparison of the different measured back pressures, the maximum back pressure of each tested condition is shown **(Fig.2)**. Using the results from the measurements without the column, the back pressure of each column at different flow rates can be calculated. The highest pressure-drop was observed when changing from 5 µm particles to 10 µm particles **(Fig.2)**.

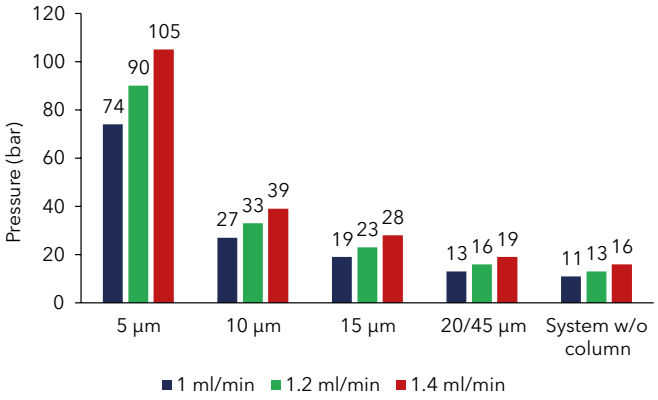

Fig. 2 Maximum back pressure of columns with different particle sizes and various flow rates, acetonitrile/water gradient. maximum pressure peak at 31% acetonitrile; C18 150 x 4.6 mm;

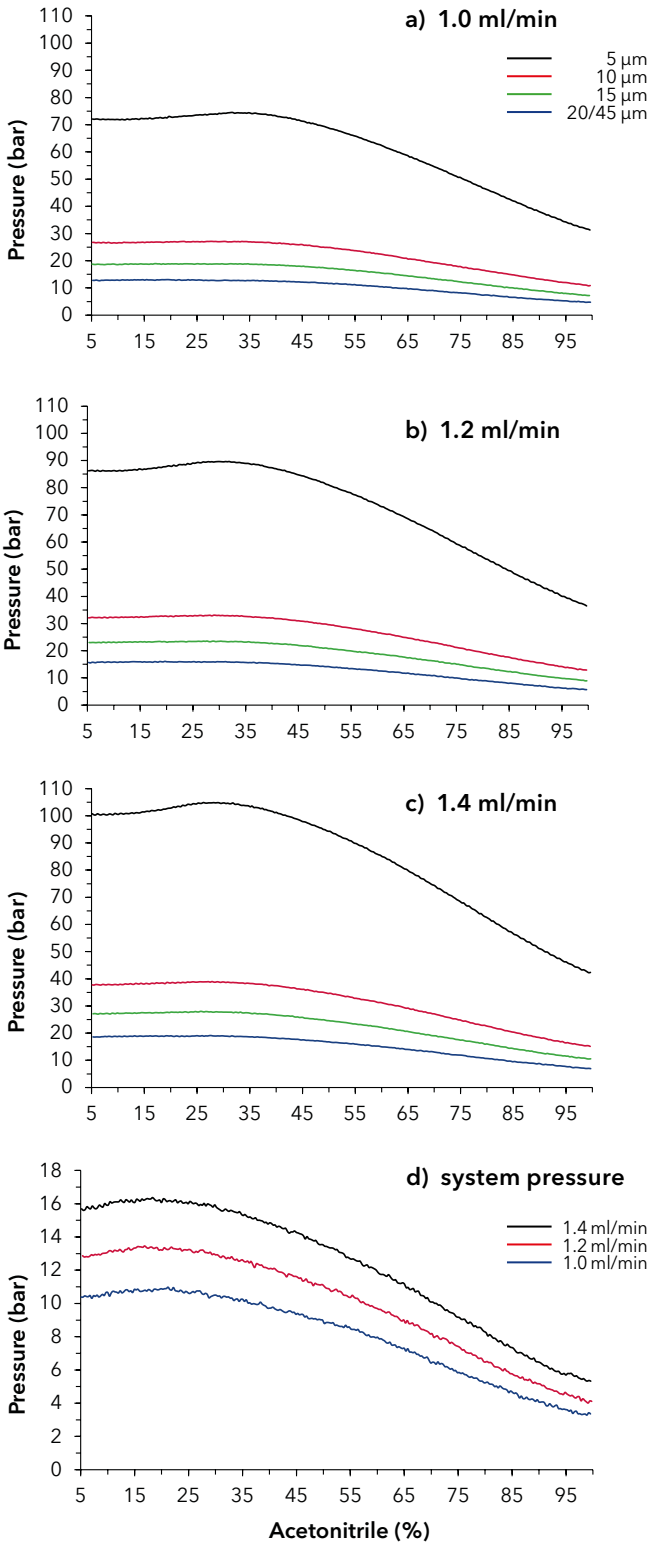

Fig. 1 Pressure traces of columns with different particle sizes at various flow rates, acetonitrile/water gradient; C18 150 x 4.6 mm

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Back pressure profiles in ethanol/water gradients

The back pressure curves for water/ethanol gradients were different when compared to those for acetonitrile. The curves showed a slight increase from 5% to 15% ethanol, followed by a steady increase up to approximately 55% - 60% ethanol and then a drop to a slightly higher value than the starting pressure (Fig.3 a-c).

The back pressure of the system without the column was measured. The maximum back pressure of approximately 40 bar was measured at 1.4 ml/min with 55 % ethanol (Fig.3 d).

For comparison, Fig.4. shows the maximum back pressure of the different columns at the three flow rates as well as the system pressure without columns.

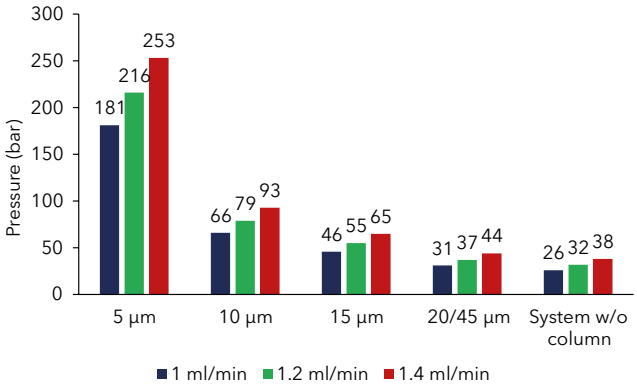

Fig. 4 Maximum back pressure of columns with different particle sizes and various flow rates ethanol/water gradient. maximum pressure peak at 61% ethanol, w/o at 55%; C18 150 x 4.6 mm;

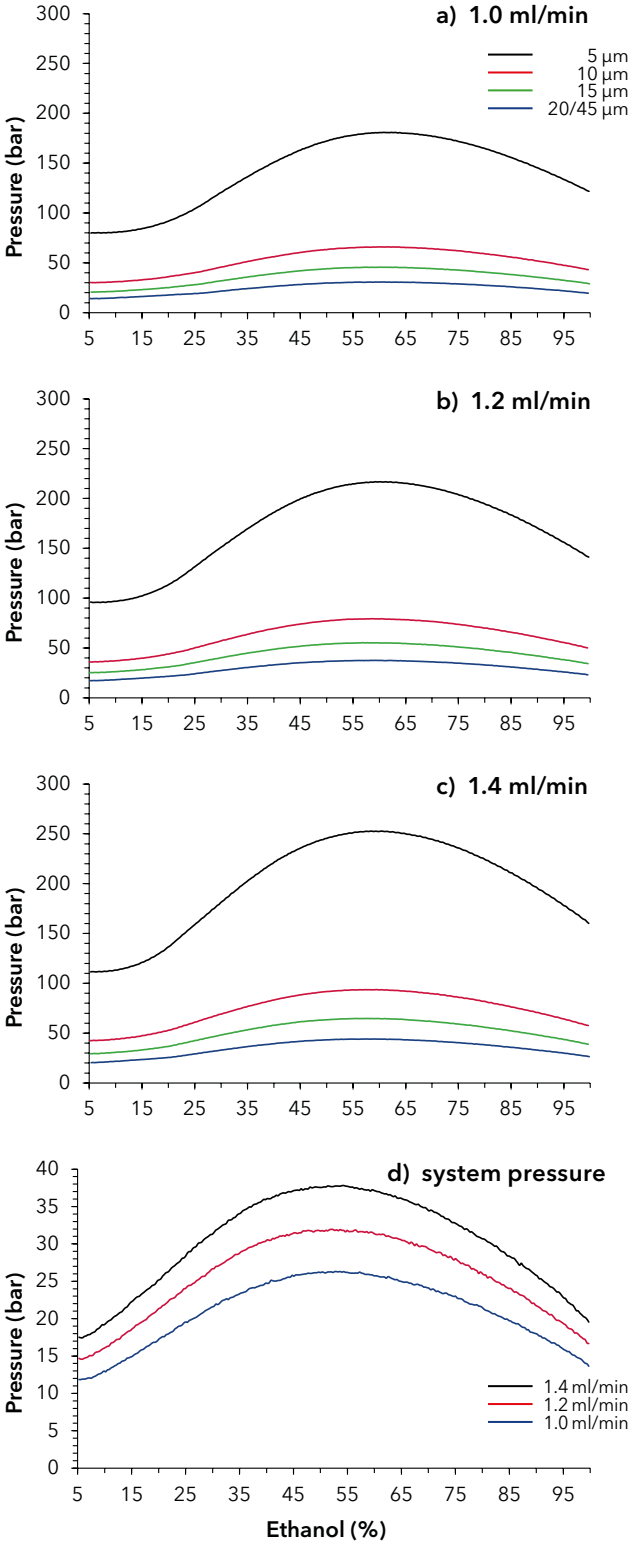

Fig. 3 Pressure traces of columns with different particle sizes at various flow rates with ethanol/water gradien; C18 150 x 4.6 mm

Back pressure profiles in methanol/water gradients
The water/methanol back pressure curves showed similar behavior to the ethanol measurements **(Fig.5** a-c). The back pressure curves increased slightly from 5% to 15% methanol and then continuously to a maximum at approximately 55% methanol. The pressure curves then dropped below the starting pressure at 100% methanol **(Fig.5** a-c). This drop is different from that seen in the ethanol back pressure curves. Also, the generate pressures are at all tested conditions lower then those generated with ethanol/water gradient. Measurements of the system back pressure without the column showed a similar curve with a maximum pressure at approximately 50% ethanol **(Fig.5** d).

Figure 6 shows a comparison of the maximum back pressures of all the tested conditions.

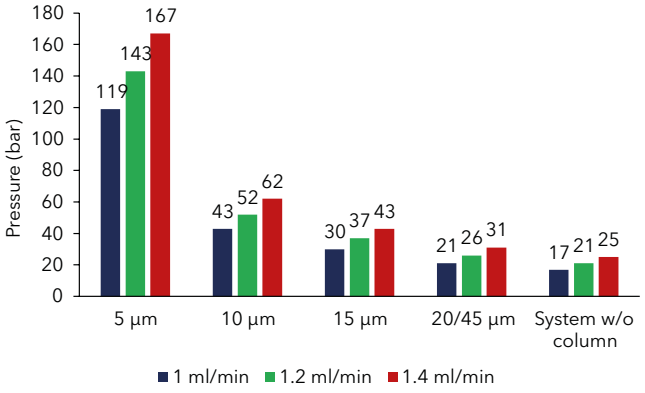

Fig. 6 Maximum back pressure of columns with different particle sizes and various flow rates, methanol/water gradient. maximum pressure peak at 53% methanol, w/o 46%; C18 150 x 4.6 mm;

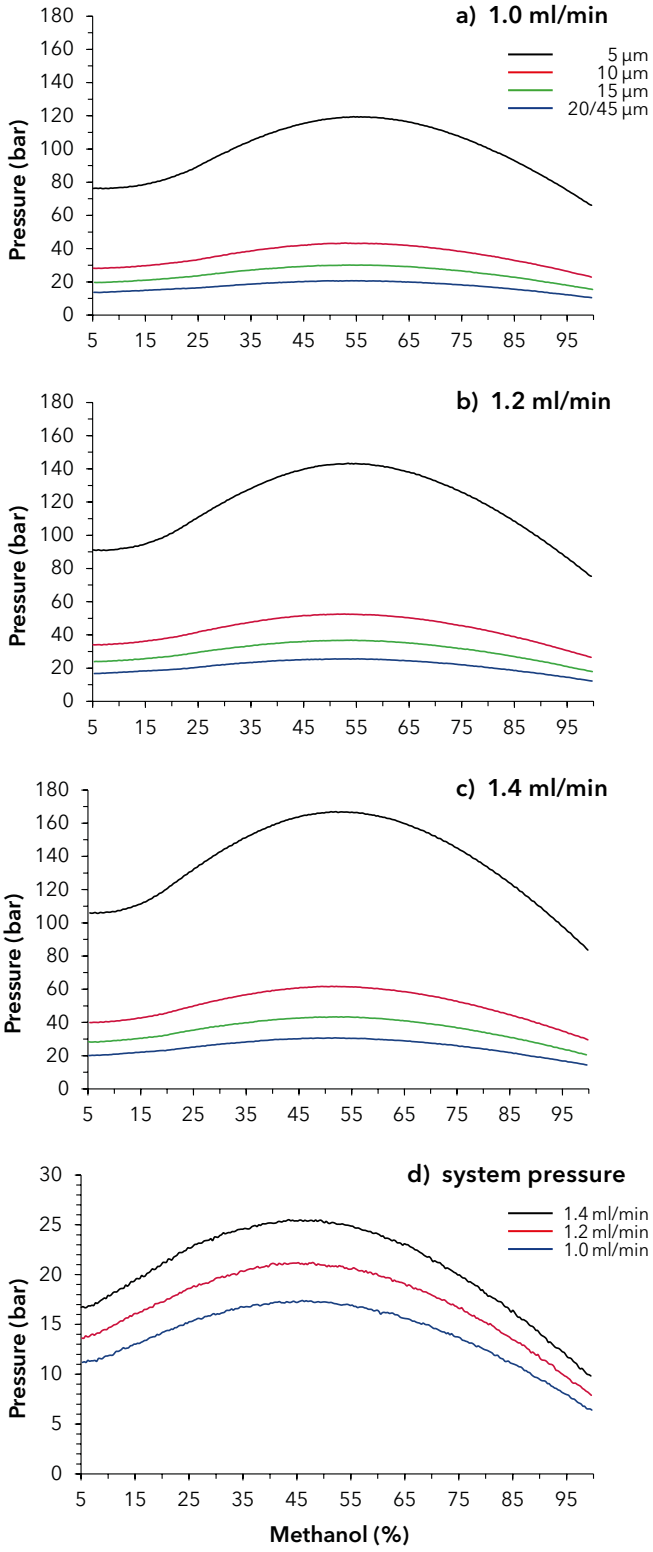

Fig. 5 Pressure traces of columns with different particle sizes at various flow rates, methanol/water gradient; C18 150 x 4.6 mm

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Determining back pressure in preparative scale

During a linear scale up from analytical to preparative scale the back pressure should remain unaffected if only the inner diameter of the column is increased. Here, a scale-up from a C18 150 x 4.6 mm 10 µm column to a C18 150 x 50 mm 10 µm column was performed. For the scale-up the flow rate of 1.2 ml/min was chosen at the analytical condition resulting in a flow rate of 141.78 ml/min for the 50 mm ID column. The gradients of the three previously tested solvents were measured, as well as the system pressure without a column.

The back pressure curve with acetonitrile showed a similar behavior as it was seen with the analytical columns **(Fig.7** a). The pressure remains stable until approximately 35% acetonitrile and then drops slightly. Similar back pressure curves to those observed in analytical scale were seen for ethanol and methanol **(Fig.7** b and c).

The maximum back pressure of the column with the three different solvents without the system pressure was compared, showing the highest back pressure at 50 bar with 60% ethanol and the lowest at 24 bar with 34% acetonitrile **(Fig.8)**.

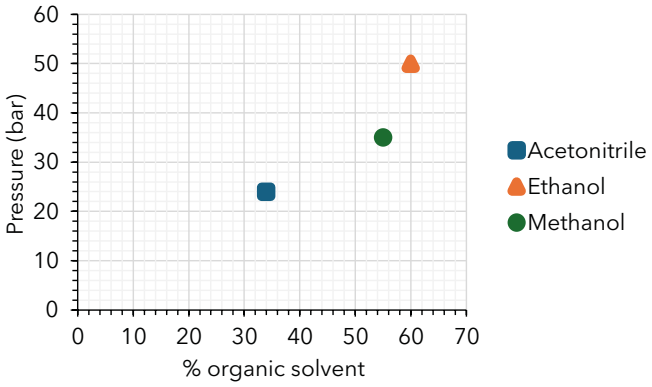

Fig. 8 Maximum pressure at organic solvent content of preparative C18 10 µm 150 x 50 mm column, 142 ml/min

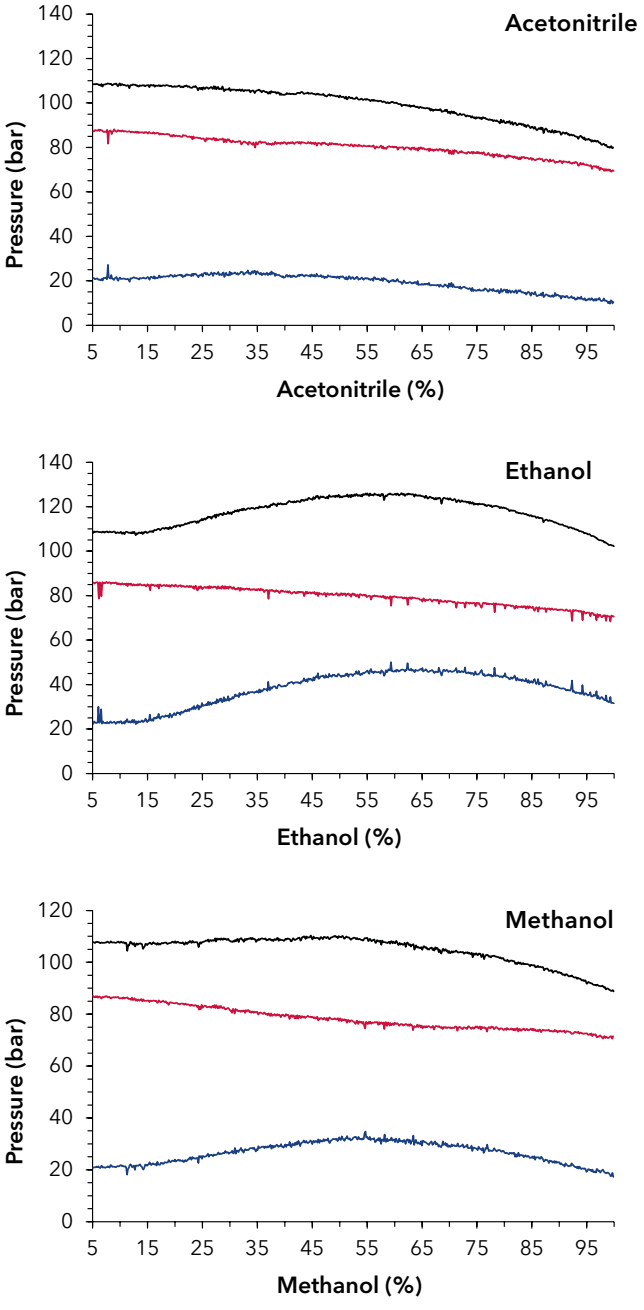

Fig. 7 Pressure traces of preparative C18 10 µm 150x50 mm column at 142.0 ml/min; **black** – total pressure, **red** – system pressure, **blue** – column pressure

Comparison of back pressure for analytical – preparative scale

The back pressure from the analytical C18 10 µm 150 x 4.6 mm and preparative C18 10 µm 150 x 50 mm columns were compared to determine if the back pressure remained the same during a linear upscale, as expected. The flow rate at preparative scale was 142 ml/min, corresponding to 1.2 ml/min at analytical scale for a linear scale up.

Direct comparison of the maximum pressure of both columns excluding the system pressure, using all three solvents, showed that the preparative column generated a slightly higher back pressure than the analytical column **(Fig.9)**. The difference for all three solvents was approximately 4 bar.

This difference in pressure between the analytical and preparative columns could be due to the packing procedure. Also, the preparative column had 1/16" connections and not 1/8" at the inlet and outlet which might generate a higher back pressure at 146 ml/min due to the resulting small bore size of the 1/16" inlet. Overall, the trend was similar with ethanol showing the highest back pressure and acetonitrile the lowest.

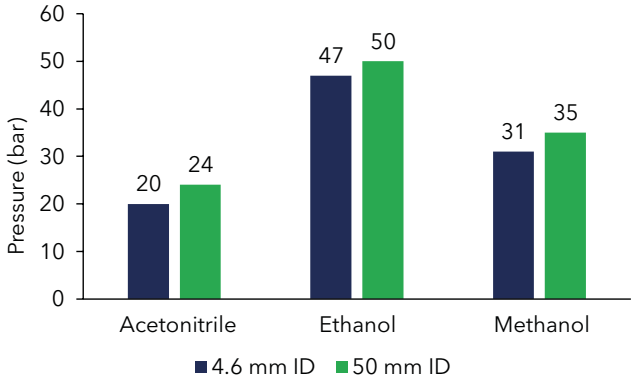

Fig. 9 Comparison maximum pressure analytical and preparative column after linear up-scale

CONCLUSION

The back pressures of four C18 150 x 4.6 mm columns, which differed only in particle size were measured and compared at different flow rates and solvent compositions. As expected, the 5 µm column showed the highest and the 20/45 µm column the lowest back pressure at all tested conditions.

The pressure curves of ethanol and methanol gradients were similar, with a maximum pressure at approximately 50% solvent. The pressure curve of the acetonitrile gradient differed from the other two, showing the highest pressure at a lower organic solvent content of approximately 35%, after which the pressure dropped. Ethanol showed the highest pressure, followed by methanol and then acetonitrile.

Determining the column back pressure at an analytical scale is an important tool during preparative method development. Ideally, if a linear scale up from the analytical to the preparative scale is conducted, the column back pressure should stay the same. This helps to predict if the method can be scaled up to the desired size in terms of back pressure.

User Tips:

- What is the finale scale you want to operate later?
- What are the pressure limitations of the preparative system?
- Be aware of the solvent back pressure
- Consider particle size not only for best separation
- Consider the flow rate

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MATERIAL AND METHODS

Tab. 1 Analytical system configuration

Instrument	Description	Article No.
Pump	AZURA® P 6.1LHPG, 10 ml, sst	APH35EA
Autosampler	AZURA AS 6.1L	AAA50AA
Detector UV	AZURA DAD 2.1L	ADC01
Flow cell	Pressure proof 10 mm, 10 µl, 300 bar	AMC38
Thermostat	CT2.1	ATC00
Columns	Eurospher II 100-5 C18, 150 x 4.6 mm Eurospher II 100-10 C18, 150 x 4.6 mm Eurospher II 100-15 C18, 150 x 4.6 mm Eurospher II 100-20/45 C18, 150 x 4.6 mm	15EE181E2J 15EE181E2N 15EE181E2Q 15EE181E2X
Software	ClarityChrom 10.1 ClarityChrom 10.1 PDA extension	A1670 A1676

Tab. 2 Preparative system configuration

Instrument	Description	Article No.
Pump	AZURA P2.1L 250 ml sst AZURA LPG ternary module	APE20LA AZZ00AB
Assistant module	ASM 2.2L, basic unit	AYASM
Detector UV	AZUAR UVD 2.1S, left position ASM 2.2L	ADA03XA
Flow cell	Preparative UV flow cell, 1/16", 200 bar, 250 ml/min	A4069
Valve drive	AZURA Valve unifier VU 4.1, right position ASM 2.2L	AWA04
Injector	2-position valve, 6 port, 1/16", sst	AVD26AE
Capillaries	1/16" sst ID 1 mm	A0134
Column	Eurospher II C18 150x50 mm 10 µm, 1/16" connection	
Software	PurityChrom 6 full license	A2681