Reduced Back Pressure on HPLC Columns for High-Throughput Chemical Analysis.

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Introduction

High throughput chemical analysis has placed greater demands on HPLC column design as operating pressures have increased up to five times what has been traditionally accepted. The recent introduction of sub 2µ particles have increased operating pressures in excess of 10,000 PSI and have raised the "bar" for durable HPLC column hardware.

In the past many columns would reach the end of their lifetime due to loss of resolution and peak shape. In the high throughput environment, many HPLC columns require replacement upon reaching the maximum operating pressure of the HPLC system. In these instances a column hardware design which exhibits lower operating backpressure is a significant advantage. It can be assumed that a column with lower initial backpressure will outlast a column of higher starting pressure.

This poster will focus on recent advances in HPLC column design which reduce backpressure up to 20% compared to conventional columns.

Results

Accel-X™ patent pending narrow bore column hardware has been engineered to reduce backpressure without sacrificing peak shape and plate count. There are two primary differences with Accel-X hardware. There is a reduction in dead volume in the end fittings and a different frit composition which has the greatest impact on pressure.

By reducing the frit thickness (Figures 5 and 6) we achieved improvements in peak shape and plate count. Further reduction in dead volume was gained by re-configuring the end fitting (Figures 8 and 9) and as a result improvements in peak shape and plate count were observed.

The use of an ultra-fine frit combined with the reconfigured end fitting produced a dramatic improvement in overall column performance. As shown in Figures 11 - 13, plate count and peak symmetry were improved and a significant reduction in backpressure was achieved.

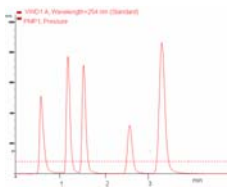
Experimental Conditions

The data to support this poster was produced by packing 50 x 2.1mm columns with Kromasil 3.5µ C18 (Courtesy Eka Chemicals. Bohus, Sweden). Each experiment was repeated five times to ensure reproducibility. The reported plate count, symmetry and backpressure are an average of the five experiments. Plate count and symmetry data was reported for the last eluting peak: Naphthalene.

Mobile Phase:	60:40 acetonitrile:water
Flow Rate:	0.2 ml/min
Detection:	VWD @ 254nm semi-micro flow cell
Sample:	Uracil, acetophenone, methyl benzoate, toluene, naphthalene
Injection:	2µl
Temperature:	25 C
Instrument:	Agilent 1100, Rheodyne 8125

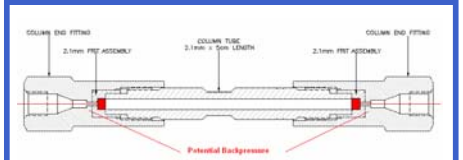
To establish a baseline, five columns were packed and tested. The data in Figure 1 demonstrates that these are acceptable columns for use under isocratic and ballistic gradient conditions.

FIGURE 1. Isocratic separation 50 x 2.1mm, conventional column hardware



Conventional Hardware	Plates/m	Symmetry	Bar
Standard End Fitting & Frit	93,100	1.173	48

FIGURE 2. Conventional column hardware



Suspecting that backpressure was present in the frits, it was thought that reducing the frit thickness would reduce the pressure drop. Figure 3 demonstrates the impact of reducing frit thickness from 1/16" to 1/32".

FIGURE 3. Effect of frit thickness

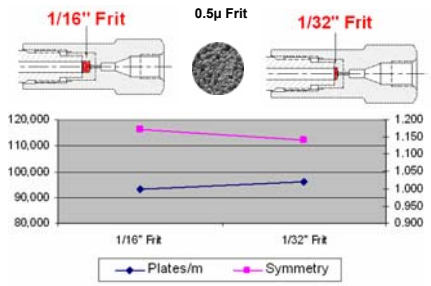


FIGURE 4. 1/16" vs. 1/32"Frit	Plates/m	Symmetry	Bar
1/16" Frit Standard End Fitting	93,100	1.173	48
1/32" Frit Standard End Fitting	96,089	1.142	48
Δ, Thinner Frit	**3.2%**	**-2.6%**	**0.0%**

The reduced frit thickness improved column performance but does not affect backpressure.

Due to the improved performance observed in the previous experiment, further reduction of dead volume was pursued. The original columns with 1/16" frits were used for the following set of experiments. The end fittings were replaced with a modified version with minimal dead volume **(MDV).** The **MDV** end fittings incorporate a reduced through hole orifice in the portion of the end fitting between the bottom of the port and top of the frit.

FIGURE 5. Effect of end fitting orifice diameter

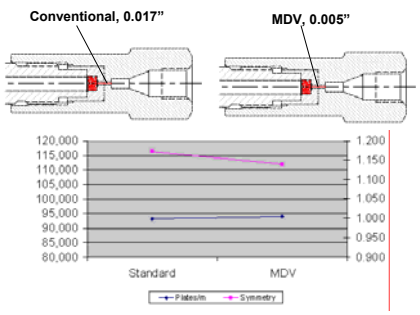


FIGURE 6. Standard vs. MDV End Fittings	Plates/m	Symmetry	Bar
Standard End Fitting with 1/16" Frit	93,100	1.173	48
MDV End Fitting with 1/16" Frit	94,057	1.139	48
Δ, MDV End Fittings	**1.0%**	**-2.9%**	**0.0%**

A reduction in dead volume in the end fittings improved column performance but does not affect backpressure.

At last – Reduced Backpressure

The results in the previous experiments provide sound evidence that reduced dead volume in the end fitting and frit, improve the performance of the HPLC column. Our goal to reduce backpressure remained elusive. The next step in our research culminated in a holistic approach to HPLC column design. A thorough investigation of flow dynamics in the end fitting and frit were conducted to provide optimum HPLC column performance. In the end, reduced backpressure was finally achieved.

The following set of data demonstrates the effectiveness of Accel-X HPLC column hardware. The combination of a minimal dead volume flow path and an integrated frit provides the ultimate in HPLC column performance.

The original columns with 1/16" frits were used for this set of experiments. The end fittings were replaced with Accel-X MDV fittings and frits.

FIGURE 7. Overlaid Chromatograms: Standard vs. Accel-X MDV

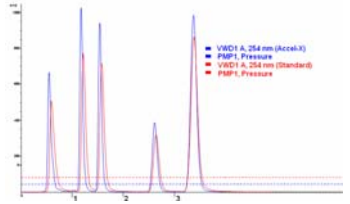


FIGURE 8. Accel-X MDV Column Design

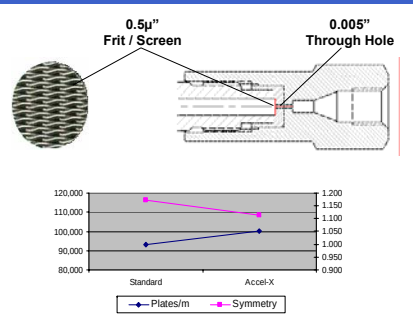


FIGURE 13. Standard vs. Accel-X MDV	Plates/m	Symmetry	Bar
Standard Column Hardware	93,100	1.173	48
Accel-X Column Hardware	100,176	1.113	38
Δ, Accel-X	**7.6%**	**-5.4%**	**21.0%**

The impact of the Accel-X MDV fitting and frit provides increased plate count, reduced band broadening and lower backpressure.

Conclusion

It has been demonstrated that the use of a new HPLC column design can be utilized to improve chromatographic performance as follows:

- **Reduced backpressure**
- **Reduced band broadening**
- **Increased plate count**

These performance enhancements offer many distinct advantages for the analyst. Reduced backpressure allows for use of higher flow rates with longer column lifetimes. Reduced band broadening leads to improvements in peak height, resolution and accuracy of quantitation. This can be especially appealing when sensitivity of the analysis is critical. We believe that this integrated hardware design is the ultimate configuration for narrow bore HPLC columns possessing all of the strengths and none of the weaknesses of previous generations.

References

(1) Neue, Uwe D. HPLC Columns: Theory, Technology, and Practice, Wiley-VCH, Inc.: New York, 1997: pp 352-359

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