Reducing Extra-Column Effects in HPLC for Minibore and Microbore Applications.

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Introduction

Advances in Drug Discovery, Combinatorial Chemistry and the advent of bench top Mass Spectrometry has placed new demands on HPLC systems. High throughput HPLC and reduced sample volumes have made the short narrow bore column the new "standard analytical."

The effects of dead volume on band broadening and the resulting chromatography have been well studied and documented for many years[1]. When using minibore columns, the effects of dead volume becomes increasingly important as the impact on band broadening is much greater when compared to analytical columns.

Quite often, the column is to blame when less than expected plate count or poor peak shape is encountered. In many cases it is the system itself and this may be due to several factors. To optimize the use of minibore columns, it is essential to reduce the dead volume throughout the flow path. This poster will focus on the optimization of the flow path which has the greatest impact on chromatographic performance.

Results

When moving from a standard analytical (250 x 4.6mm) to a minibore column it is imperative that the HPLC system be re-plumbed. If this is overlooked the chromatography will be less than desirable.

By selecting the correct tubing and fittings, one can expect to obtain similar and in many cases better peak shape and plate count compared to the standard analytical columns of the past.

Improper column connections are typically associated with leaky fittings. HPLC column end fittings vary by manufacturer and improper fit results in poor chromatography.

The use of a universal column connector eliminates the variability of end fitting geometries from one column manufacturer to another. The universal connector also eliminates the variability amongst from one technician to another.

Experimental Conditions

The data to support this poster was produced by packing columns with Kromasil 3.5µ C18 (Courtesy Eka Chemicals. Bohus, Sweden). Plate count and symmetry data was reported for the last eluting peak: Naphthalene.

Mobile Phase:	60:40 acetonitrile:water
Flow Rate:	4.6 mm ID – 1.0 ml/min
	2.1 mm ID – 0.2 ml/min
Detection:	VWD @254nm
	semi-micro flow cell
Sample:	Uracil, acetophenone, methyl
	benzoate, toluene, naphthalene
Injection:	4.6 mm ID – 10µl
	2.1 mm ID – 2µl
Temperature:	25 C
Instrument:	Agilent 1100, Rheodyne 8125

To establish a baseline, analytical columns were tested, and satisfactory results were obtained.

FIGURE 1. Standard analytical and High throughput columns.

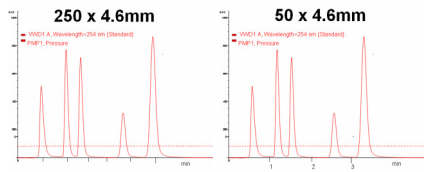
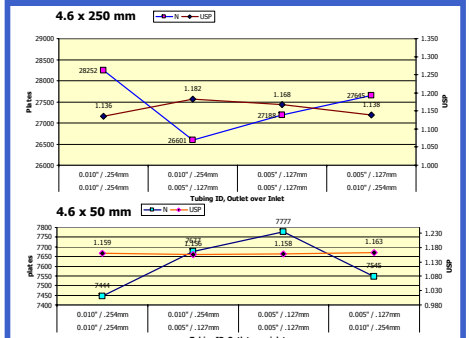


FIGURE 1. The impact of the tubing diameter on analytical columns.



Outlet: Tubing between the Column and detector

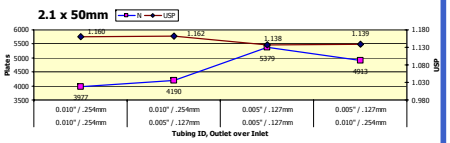
Inlet: Tubing between the Column and Injector

Summary Data	.010" / .254mm	.005" / .127mm	% Change
50 x 4.6mm Plates	7444	7777	5.6%
50 x 4.6mm USP	1.159	1.158	-0.1%
50 x 4.6mm Bar	71	78	9.9%
250 x 4.6mm Plates	28252	27188	-3.8%
250 x 4.6mm USP	1.136	1.168	2.8%
250 x 4.6mm Bar	213	222	4.2%

The impact of changing the tubing diameter on the 250mm column is almost negligible.

Significant changes in plate count, peak shape and backpressure are observed when tubing diameter changes are made on the shorter 50mm column.

FIGURE 2. The impact of the tubing diameter on minibore columns.



Summary Data	.010" / .254mm	.005" / .127mm	% Change
50 x 2.1mm Plates	3977	5379	35.3%
50 x 2.1mm USP	1.16	1.138	1.9%
50 x 2.1mm Bar	81	80	-1.2%

The impact of changing the tubing diameter on the 2.1 x 50mm column is dramatic.

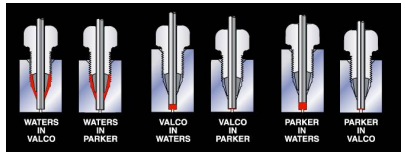
A significant improvement in peak shape and plate count is observed with virtually no change in backpressure.

The connections made to the HPLC columns used thus far were of the fixed ferrule variety. Another common connector is a polymeric fitting which does not have a fixed ferrule.

FIGURE 3. Common connectors in HPLC

To ensure a connection with no dead volume, the connector must match the end fitting geometry. Connections in Figure 4 show that dead volume can be created when the female and male fittings do not match.

FIGURE 4. End fitting geometry varies by column manufacturer



In order to account for the variability in end fitting geometry one has to either use dedicated connectors for each manufacturers' column or use polymeric fittings. A third option is a universal connector which incorporates a spring loaded design that ensures a zero dead volume connection regardless of end fitting geometry. Figure 5 outlines the mechanical aspects of this universal connector.

FIGURE 5. A spring loaded universal connector compensates for variability in end fitting geometry

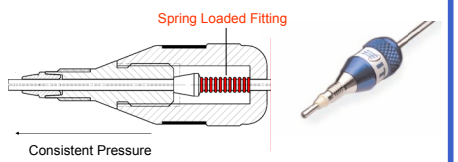
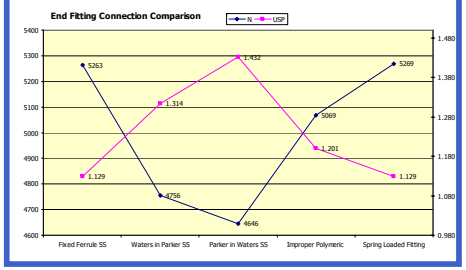


Figure 6 shows the data obtained using improper and proper connections. The tubing diameter at the inlet and outlet was 0.005" / 0.127mm.

FIGURE 6. The impact of the improper connections on minibore columns.



Change vs. Fixed Ferrule

Summary Data	USP	N	Bar	USP	N
Fixed Ferrule SS	1.129	5263	81	-	-
Waters in Parker SS	1.314	4756	83	16.4%	-9.6%
Parker in Waters SS	1.432	4646	83	26.8%	-11.7%
Improper Polymeric	1.201	5069	84	6.4%	-3.7%
Spring Loaded Fitting	1.129	5269	84	0.0%	0.1%

Compared to the changes in tubing diameter, similar changes in performance are observed when improper connections are made on the 50 x 2.1mm column. The use of a spring loaded connector offered similar performance to fixed ferrule connectors.

Conclusion

In this poster we have demonstrated the importance of proper tubing selection. It can be seen that the difference in tubing ID is magnified at the smaller column geometries, in particular for 2.1mm ID columns. In each of the tests, the performance of the column improved when the 0.005 ID tubing was used on the instrument.

Proper connections are also critical as these can lead to the introduction of dead volume which ultimately has a negative impact on peak shape and plate count.

Universal connectors address the variability in end fitting geometry and offer the added benefit of being finger-tight. The consistent pressure from connection to connection, end-user to end-user also contribute to a more reproducible connection.

References

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

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