Rapid Resolution HT Columns–Maintain High Resolution with Agilent In-Line (Pre-Column) Filters

Application

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Introduction

To reduce premature column failure and the associated downtime, chromatographers use guard columns or pre-column filters to protect their HPLC analytical columns from the potential damage caused by harmful particulate materials in the flow stream. However, these protection devices can sometimes compromise a separation by adding extra column volume that significantly reduces the resolving power of very efficient, short, low-volume analytical columns. Recent data show that Agilent low volume in-line filters (p/n 01090-68702) are very effective in retaining the resolving power of both the 4.6 × 30 mm and 2.1 × 30-mm high-efficiency Rapid Resolution high throughput (HT) columns (1.8 µm) when compared to another commercially available alternative. Thus, the Agilent in-line filter can effectively be used to protect any analytical column from damage, thereby lengthening the analytical column's useful life.

Instrumentation

All work was performed using an Agilent 1100 LC (binary pump) optimized to minimize band broadening by using 0.12-mm id tubing from the autosmpler to the detector and a 1.7-µL flow cell in the diode array detector (DAD). A capillary kit (p/n 5065-9947) is available to convert a standard 1100 LC for use with Rapid Resolution HT columns. The 1100 LC was also equipped with a solvent degasser, autosampler, and temperature controlled column compartment.

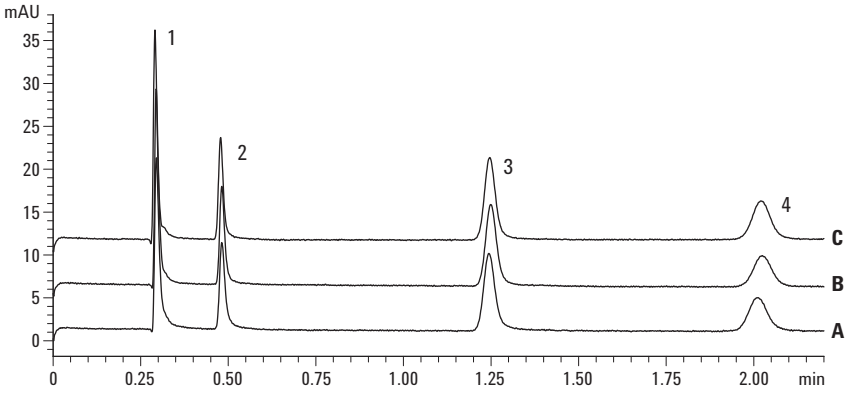
Results

Figure 1 shows resulting chromatograms and data for high-throughput analyses of a standard QC mixture on a ZORBAX Rapid Resolution HT 4.6 × 30 mm, 1.8-µm, analytical column without a pre-column filter (A), with an Agilent in-line filter (B), and with a competitor's direct-connect pre-column filter (C). In each case, six sample runs were conducted to provide adequate data to calculate average plates (N), relative standard deviation (RSD), and to determine the effect of using either pre-column filter. In a similar way, Table 1 provides additional comparative data using a smaller diameter, low-volume column commonly used in liquid chromatography/mass spectrometry (LC/MS) electrospray applications–the ZORBAX Rapid Resolution HT, 2.1 × 30 mm, 1.8-µm analytical column. Agilent in-line filters maintain the resolving power of the narrow-bore Rapid Resolution HT columns.

Highlights

- Agilent low volume in-line (pre-column) filters provide high performance protection for analytical columns including the ZORBAX Rapid Resolution HT, 1.8-µm, analytical columns.
- When protecting ZORBAX Rapid Resolution HT columns, Agilent in-line filters retain high resolution with minimum change in peak retention time and minimal plate reduction (<10% loss). When using another popular commercially available direct-connect pre-column filter under identical conditions, larger losses in efficiency are observed (up to 24% loss).

The results presented in Figure 1 and Table 1 show that the high-resolving power of the short, high-efficiency Rapid Resolution HT columns are maintained when an Agilent in-line filter is employed. While protecting these high-performance LC columns from premature failure, the Agilent in-line filter does not significantly affect analyte efficiency of early eluting peaks (10% or less reduction in N) or change the analyte peak retention time. In contrast, a popular alternative direct-connect pre-column filter reduces analyte efficiency by up to 24%. In each case, as expected, the early eluting peaks are more sensitive to extra column volume introduced with use of the pre-column filters than later eluting peaks.



Mobile phase

60% Methanol, 40% Water

Peak identification

Peak	Compound
1	Uracil
2	Phenol
3	4-Chloro-Nitrobenzene
4	Toluene

Conditions

Columns	**Rapid Resolution HT, Eclipse XDB-C18** 4.6 × 30 mm, 1.8-µm particles p/n 923975-902
Sample	QC test mix dissolved in mobile phase
Temperature	Ambient
Injection volume	1 µL
Flow rate	1.0 mL/min
Detection	UV (254 nm)

(A) No pre-column filter

Peak	Average plates (N)	% RSD
1	3437	1.4
2	5792	1.2
3	7633	1.4
4	6928	1.6

(B) With Agilent in-line filter

Agilent p/n 01090-68702 (Replacement 2-µm frits (10/pk) p/n 280959-904)

Peak	Average plates (N)	% RSD	Plate reduction with pre-filter (%)
1	3082	2.1	–10.3
2	5355	1.1	–7.5
3	7388	1.0	–3.2
4	6873	1.3	–0.8

(C) With direct connect pre-column filter

Peak	Average plates (N)	% RSD	Plate reduction with pre-filter (%)
1	2597	3.2	–24.5
2	4668	3.4	–19.4
3	6982	0.4	–8.5
4	6668	2.1	–3.8

Figure 1. Agilent in-line filters maintain the resolving power of the short, high-efficiency Rapid Resolution HT analytical columns.

Table 1. Plate Reduction Comparison for Two Filter Types

Peak	Peak identification	Retention time no filter (min.)	Plate reduction Agilent in-line filter (%)	Plate reduction direct connect pre-column filter (%)
1	Uracil	0.21	–7.8	–21.8
2	Phenol	0.32	–8.1	–24.3
3	4-Chloro-Nitrobenzene	0.78	–4.5	–16.9
4	Toluene	1.23	–1.0	–9.5

ZORBAX Rapid Resolution HT, 2.1 × 30 mm, 1.8-µm analytical column, Agilent part number 923700-902

Conditions same as in Figure 1 except flow rate is 0.4 mL/min.

Agilent in-line filters maintain the resolving power of the narrow-bore Rapid Resolution HT columns.

Conclusions

- Peak efficiency is consistent with or without the Agilent in-line filter.
- Peak retention is essentially the same with or without the Agilent in-line filter.
- Plate (N) reduction with Agilent in-line filter is less than with the competitor's direct connect pre-column filter, which shows 2 to 10 times larger losses in efficiency.

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