RESTEK HPLC

Applications note

Ultra C4 HPLC Column Provides High Stability At Low pH

The use of a low pH (< 2.0) mobile phase can cause acid hydrolysis of the HPLC column stationary phase, resulting in reduced retention and poor reproducibility. During hydrolysis the phase is stripped from the silica backbone. Generally, acid hydrolysis of alkyl bonded phases increases with a decrease in the chain length of the stationary phase ligand. Typically, stationary phase ligands as short as C4 will be much less stable than C18 ligands. To overcome the problem, some HPLC column manufacturers offer C4 phases made with relatively high ligand density, which are more stable than lower density C4 phases. However, these high ligand density phases still exhibit gradual loss of retention when exposed to low pH mobile phases.

The research chemists at Restek have designed the new Ultra C4 phase to further increase stability by using both high ligand density and a unique bonding chemistry to reduce acid hydrolysis. The improved stability of the Ultra C4 phase was confirmed using a controlled study comparing the Ultra C4 to another leading C4 phase. This leading C4 phase contains a relatively high ligand density, making it more stable than other C4 columns (Table 1). The results of the study are shown in Figure 1.

Experimental Conditions

Both columns were simultaneously exposed to repeated acetonitrile/water mobile phase gradients in the presence of 0.1% (v/v) trifluoroacetic acid (TFA) and a temperature of 50°C. The pH of 0.1% TFA in water is 1.9. As the mobile phase pH is decreased towards 2, all bonded phase silicas become less stable because the siloxane (Si-O-Si) linkage to the stationary phase is susceptible to acid hydrolysis, resulting in loss of stationary phase[1]. Elevated column temperature accelerates acid hydrolysis and the resulting loss of the stationary phase.

The hydrophobic retention of each column was measured before exposure to hydrolysis conditions, and periodically during the acid hydrolysis experiment. The capacity factor (k') of phenylheptane was used to measure hydrophobic retention, and was calculated using uracil as the void marker (t_o). After equilibration in a mobile phase of H_2O:ACN (25:75, v/v) at a flow rate of 1.25mL/min, each column was injected with 5µL of a mixture of uracil (0.1mg/mL) and phenylheptane (10mg/mL). UV detection was performed at 254nm.

In addition to the Ultra C4 and competitor C4 columns that were exposed to hydrolytic conditions, an additional Ultra C4 column was used as a control. The control column was periodically analyzed for hydrophobic retention along with the test columns. While the test columns were exposed to the TFA gradients, the control column was only exposed to

Figure 1

Ultra C4 shows greater retention and stability due to its unique bonding chemistry

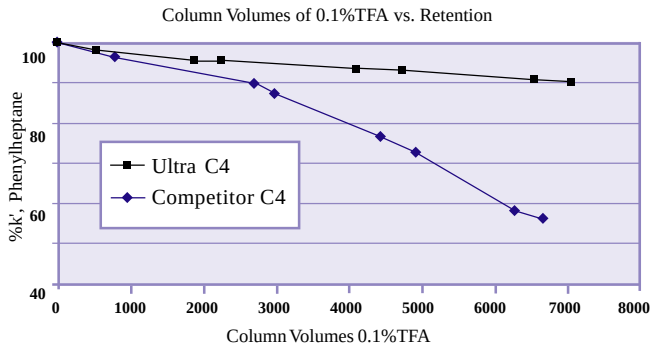

Table 1

C4 Columns Evaluated

Column	Dimensions	Particle Size	Pore Size	Carbon %
Competitor C4	50x4.6mm	5µm	100Å	8
Ultra C4	50x4.6mm	5µm	100Å	9

Table 2

Acid Hydrolysis Conditions

Mobile phase A: 0.1% TFA in deionized water
Mobile phase B: 0.1% TFA in acetonitrile
Temperature: 50°C

Gradient:	Time (min.)	%A	%B
	0	100	0
	20	0	100
	40	0	100
	40.1	100	0
	60	100	0

H_2O:ACN (25:75). The hydrophobic retention of the control column varied less than 2% throughout this experiment.

The acid hydrolysis conditions of the experiment (Table 2) were modeled after those of Kirkland, Glajch, and Farlee.[2] Both test columns were exposed simultaneously to repeated cycles of the mobile phase gradient listed in Table 2**.** A flow rate of 2.0mL/min was split between the two test columns using a tee. The eluent from each column was collected and monitored. A restrictor was added to the outlet of one of the columns to make the flow rate as similar as possible. The total flow through each column at the conclusion of the experiment was comparable—3784mL for the Ultra C4

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column versus 3671mL for the competitor C4 column. Mobile phase volumes were converted to column volumes using the initial elution volumes for uracil (0.553 mL for competitor C4, 0.538mL for Ultra C4). Following the 50th gradient cycle, the columns were exposed to 8.5 hours of continuous flow of 100% mobile phase A. The test columns were exposed to a total of 54 gradient cycles.

Results

Figure 1 shows the percent change in capacity factor (k') of phenylheptane plotted against the column volumes of the TFA mobile phase. After approximately 3000 column volumes of TFA, the competitor C4 column showed twice the loss in retention as compared to the Ultra C4 column. Even after 7000 column volumes, the Ultra C4 column lost less than 10% of its original retention, while the competitor C4 column showed a 44% loss in retention.

Conclusions

Restek's Ultra C4 column combines high ligand density and a unique bonding chemistry to attain excellent resistance to acid hydrolysis. This resistance to stationary phase loss ensures highly reproducible and consistent retention even when using a very acidic mobile phase.

References

1. J.J. Kirkland, J.W. Henderson, J.J. Destefano, M.A. Van Straten, H.A. Claessens, J. Chromatogr., A, 762 (1-2), 97-112 (1997).
2. J.J. Kirkland, J.L. Glajch, and R.D. Farlee, *Anal. Chem*. **61**, 2-11 (1989).

References not available from Restek.

Ultra C4, 3µm Columns

Particle Size: 3µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9102331	9102332	9102333	9102335
50mm length	9102351	9102352	9102353	9102355
100mm length	9102311	9102312	9102313	9102315

Ultra C4, 3µm Columns with Trident™ Inlet

Particle Size: 3µm		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	—	9102332-700	9102333-700	9102335-700
50mm length	—	9102352-700	9102353-700	9102355-700
100mm length	—	9102312-700	9102313-700	9102315-700

Ultra C4, 5µm Columns

Particle Size: 5µm	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	9102531	9102532	9102533	9102535
50mm length	9102551	9102552	9102553	9102555
100mm length	9102511	9102512	9102513	9102515
150mm length	9102561	9102562	9102563	9102565
200mm length	9102521	9102522	9102523	9102525
250mm length	9102571	9102572	9102573	9102575

Ultra C4, 5µm Columns with Trident™ Inlet

Particle Size: 5µm		2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm length	—	9102532-700	9102533-700	9102535-700
50mm length	—	9102552-700	9102553-700	9102555-700
100mm length	—	9102512-700	9102513-700	9102515-700
150mm length	—	9102562-700	9102563-700	9102565-700
200mm length	—	9102522-700	9102523-700	9102525-700
250mm length	—	9102572-700	9102573-700	9102575-700