VYDAC®

Application Note #9805

Are ghost peaks haunting your chromatograms?

Ghost peaks in gradient chromatogram blank runs, with no sample, are the most frequent problem described by callers to Vydac's technical staff. What causes them? There are several possible answers. Systematic, logical investigation will pinpoint the cause.

RI Effects

Rapid changes in solvent composition can create false peaks due to refractive index (RI) sensitivity with some detectors. This is similar to the baseline blip that results from sample injection. If there is a rapid composition change in the gradient, try making it more gradual. This should broaden an RI ghost into mere baseline drift. All other ghosts result from contamination.

The Column

The easiest source to rule out is the column itself. Because it is a finite source, column contamination will decrease with time if the column is washed with solvents stronger than needed to elute the ghost peak(s).

Perform several blank gradient runs in quick succession. If possible, incorporate extended washes with strong eluant. If ghost-peak sizes remain the same from run to run, the source is not the column. If their size decreases, the source may be protein aggregates or other difficult-to-dissolve contaminants in the column. Ion exchange columns with purely aqueous buffers are suspect for bacterial contamination. Larger peaks may appear again after an idle period. In this case, clean the column as recommended in the maintenance guide.

Solvent A

If ghost peaks remained of fairly constant size during successive gradients, the source is probably a mobile phase component. Contaminants in the weak solvent ("A") can accumulate on the column during equilibration and the early part of a gradient. Try varying equilibration time before successive runs. Figure 1 shows an example. If the size of the peaks changes in direct relation to equilibration time, the probable source is solvent A.

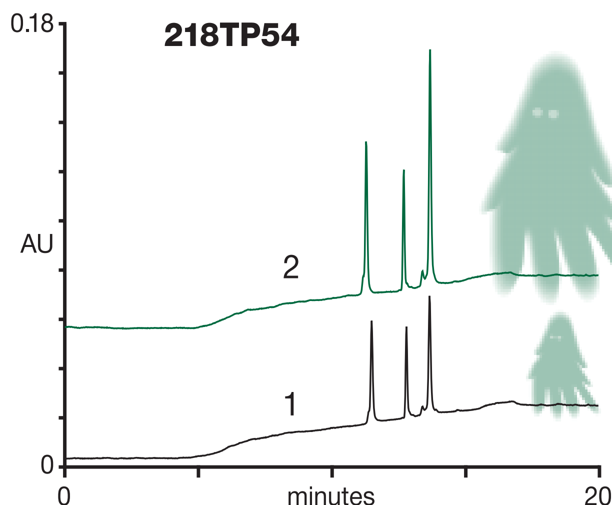

Figure 1. Effect of varying equilibration times with contaminated Solvent A. Column: 218TP54, 5µm, 300Å, C18 reversed phase, 4.6mm ID x 250mm L. Sample: none. Conditions: 214 nm. 1.5 mL/min. A=10% ACN and 0.1% TFA (v/v) in water, spiked with 25ng/mL insulin, RNase, and lysozyme. B=90% ACN and 0.1% TFA (v/v). Gradient from 100% A to 100% B in 20 min. Equilibration: (1) 10 min. (2) 30 min.

Solvent B

Contaminants from the strong solvent ("B") can also accumulate on the column during early portions of a gradient. In this case, ghost-peak size should be independent of equilibration time. Try equilibrating the column with a weak mixture of B in A for a time before pure A. This should increase the size of the peaks if B is the source, as shown in Figure 2.

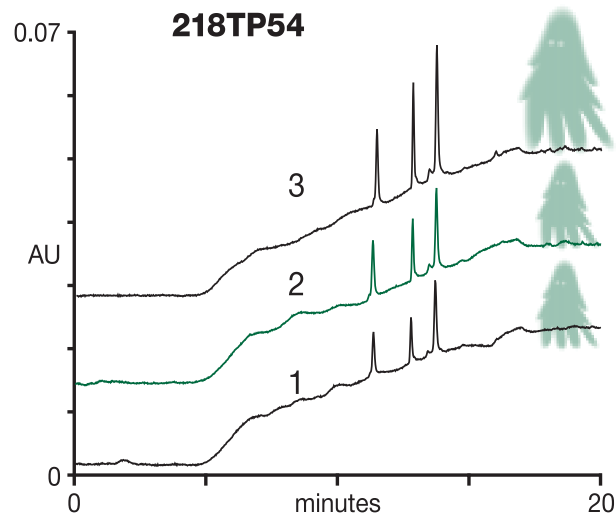

Figure 2. Effect of varying equilibration times with contaminated Solvent B. Column and Conditions: Same as Figure 9, except A was clean and B was spiked at 50ng/mL. Equilibration: (1) 10 min. (2) 30 min. (3) 30 min with 10%B, then 10 min with A.

The Nest Group • 45 Valley Road • Southborough, MA 01772.1306 • USA
Toll-Free: 800.347.6378 • Tel: 508.481.6223 • Fax: 508.485.5736 • EMail: info@nestgrp.com

The Autosampler

Are you using an autosampler? Contamination in blank or wash solution from an autosampler can also cause ghosts. If the peak-size tests have not isolated the problem, try bypassing the sampler to see if this cures it.

In Most Cases

Our experience with customers who encounter ghost peaks is that in almost every case the source is mobile phase, usually solvent A. Invariably, the solvent-pickup filter is also contaminated. A labyrinthine haven for bacterial growth, it should be replaced frequently, along with the solvent.

Reversed-phase protein and peptide separations where solvent A is water with TFA but no acetonitrile are especially problematic. We suggest including at least a 5% ACN concentration in solvent A for several reasons:

(1) Reversed-phase protein chromatography works better with a little ACN.

(2) There's less outgassing during the run if solvent and water are premixed.

(3) Better reproducibility, especially with single-pump gradient systems.

(4) ACN inhibits microbial growth.

To order contact The Nest Group, 800.347.6378 your local Vydac distributor.

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