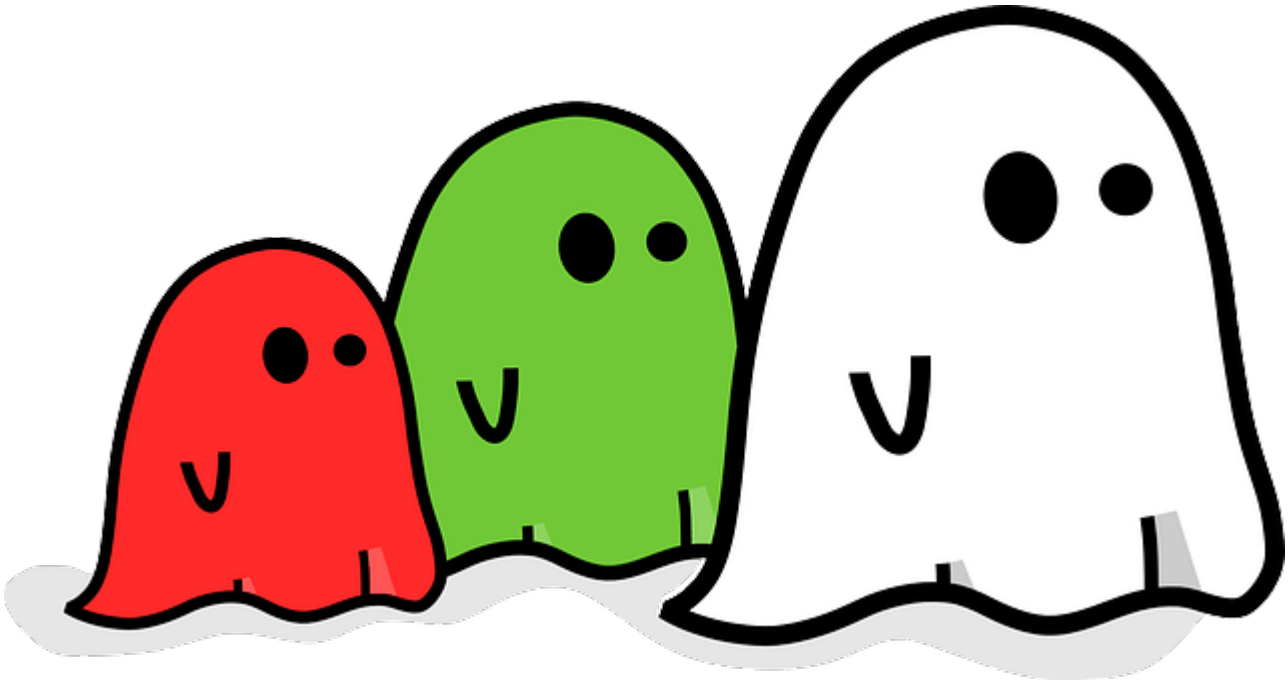
It’s almost Halloween! Let’s talk ghost peaks.

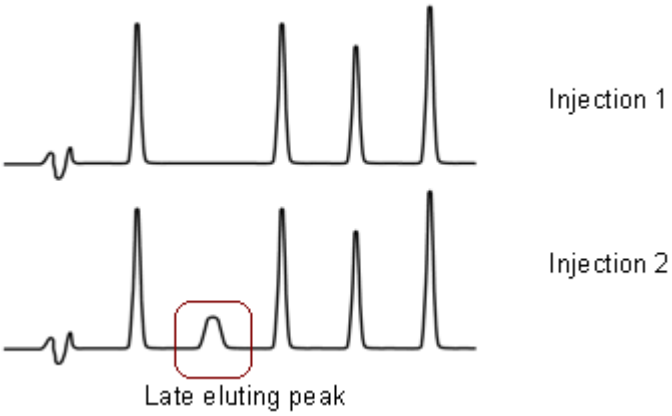


Peaks in the chromatogram not attributed to the sample injected are commonly referred to as **ghost peaks**.

We will be discussing three common problems that can manifest as ghost peaks:

- Late eluting peaks from the previous injection
- Injector carryover
- Solvent impurities in the mobile phase

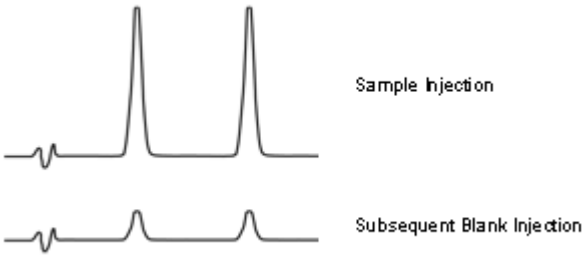
Late eluting peaks from the previous injection



In this example, a strongly retained compound has eluted from a previous injection—an event most commonly occurring in isocratic runs. The indicted peak, relative to the closely eluting expected peaks, is typically broad.

Extending the run-time to allow the compound to elute before the next injection, or adding a step gradient, are typical solutions that won't otherwise affect the chromatography.

Injector carryover

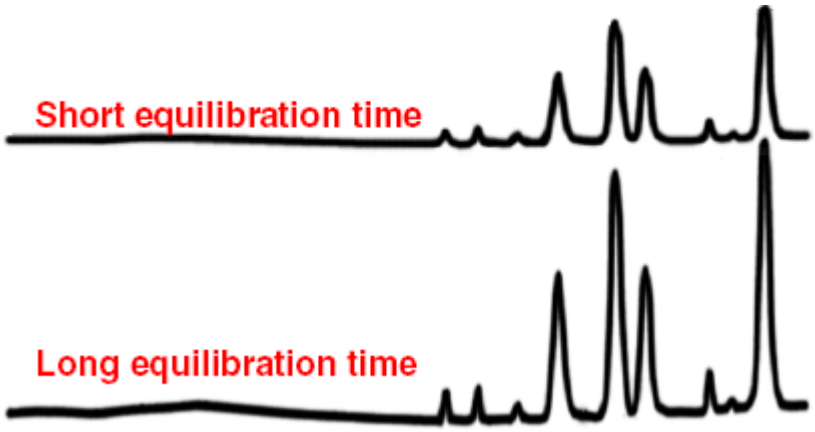


The peaks in the blank injection can be attributed to injector carryover as they have the same retention times and chromatographic characteristics as the known peaks in the sample, but are at a lower level.

The cause can be from a faulty rotor seal on the injector valve, needle wash contamination,

or an inappropriate sample or needle wash solvent. Performing injector maintenance or needle wash/diluent method development are the best solutions. In some cases, a simple solution can be to inject multiple blanks after sample injections to fully remove the carryover before the next quantitative injections.

Solvent impurities in the mobile phase



Peaks eluting during a gradient run with nothing being injected are typically attributed to impurities in the mobile phase. This can be confirmed by equilibrating in the initial mobile phase A:B ratio for a longer period of time prior to running the gradient profile. If the subsequent peaks have the same profile but increase in size, it suggests a greater amount are accumulating on the column due to the longer equilibration time.

Sources of these components can be:

1. **Impurities in the solvents**; be sure to use HPLC-grade or better solvents—sometimes different solvent vendors are worth testing, as well.
2. **Contamination from the glassware**; residual washing detergents are somewhat common concerns.
3. **Water purification contaminants**; it may be time to change the filter, or if recently

changed, it could have been installed improperly.

4. **Buffer impurities or degradents**; organic acids (particularly trifluoroacetic acid) can degrade over time and/or if exposed to light, and it is good practice to prepare such buffers fresh daily. Ion-pair reagents are also a common source of buffer impurities.

If these peaks are consistent in retention time, consistently present, and do not co-elute with any components of interest, it may not be a problem and can be subtracted from the chromatogram.

If there's something strange in your chromatogram, don't call the Ghostbusters…

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