LC Troubleshooting Series
Ghost Peaks

Introduction

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Ghost peaks can come from a number of areas:

- Elution of a analytes retained from a previous injection
- Mobile phase contamination
- Sample Preparation
- System contamination
- Column contamination

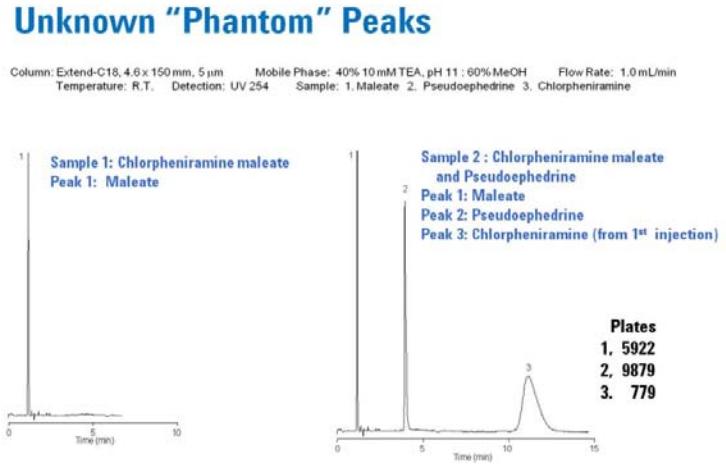
Elution of Analytes Retained from Previous Injection

Analytes retained from a previous injection that show up on a new run are referred to as "wraparound peaks". They can be a cause of confusion for chromatographers, especially in isocratic runs.

A Clue for Wraparound Peaks: They tend to be wider than the other close peaks in the run.

Figure 1 is an example of chlorpheniramine, an antihistamine. The chromatographer running this analysis initially thought that she had co-elution of chlorpheniramine and maleate, which is not likely. The chromatographer did another injection adding pseudophedrine, and obtained the results on the right.

Figure 1. Chlorpheniramine injection exhibiting wraparound peaks.



In **Figure 1** (pg. 1):

- The first peak is maleate in the ionized form.
- The second peak is the pseudoephedrine.
- After leaving the instrument running for a while, a third peak, chlorpheniramine, is also observed. It was from the previous injection and the operator did not wait long enough for the compound to elute.

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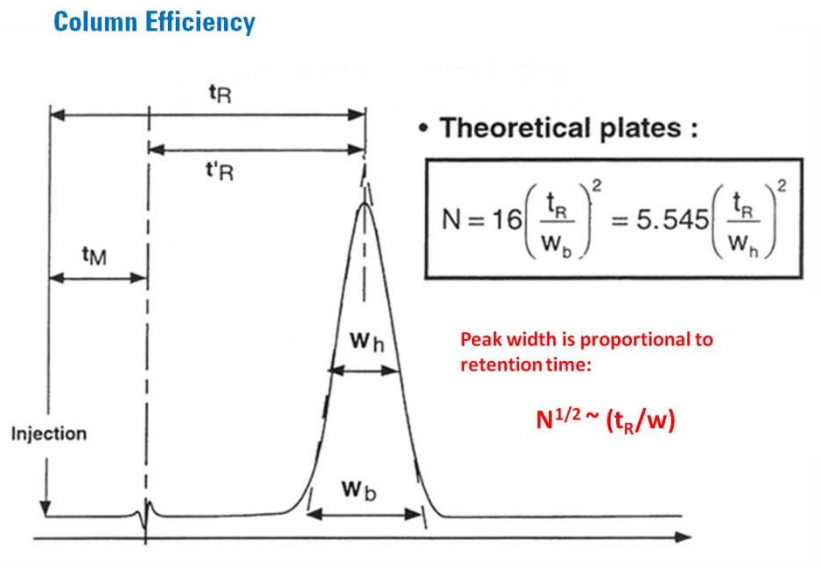
Use a fast gradient– such as 10% ACN to 90% ACN in 10 or 15 minutes-- to get a feel for the number of components in a sample and their relative retention. Even with isocratic separations, it's a good practice to start with strong mobile phase, such as 75% MeOH, and/or higher flow rate to get the components to come off the column in a more reasonable amount of time.

Figure 2. Theoretical plates and column efficiency.



Theoretical plates are proportional to the square of the ratio of retention time and peak width (see **Figure 2**). If calculated theoretical plates for the peak are unreasonably low it could be indicative of a "wrap-around" peak**. The peak width is proportional to the retention time,** so unusually broad peaks in the midst of much narrower peaks are likely wraparound peaks.

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Mobile Phase Contamination

Using high purity HPLC Grade solvents is very important for good chromatographic results.

Symptoms of mobile phase contamination:

- Even after extensive flushing, the "contamination" does not decrease in size.
- The peak (or peaks) shows up only when a gradient is run.
- The peak (or peaks) shows up even when the gradient is run without making an injection and they do not decrease in size with subsequent injections.

There are several causes of contamination from the mobile phase:

- The contamination can be in the mobile additives. Run the gradient without any additives.
- The contamination may be in the water. Sometimes people use "purified" water, but not HPLC water. Try HPLC grade water, either from an in-house purification system or a bottled HPLC grade water (see **Figure 3**).
- The organic solvent may also be contaminated. Use fresh HPLC grade solvent, possibly even from another manufacturer. If you are using acetonitrile, try replacing it with methanol or vice-versa (see **Figure 4**).

Figure 3. Comparison of two different water sources.

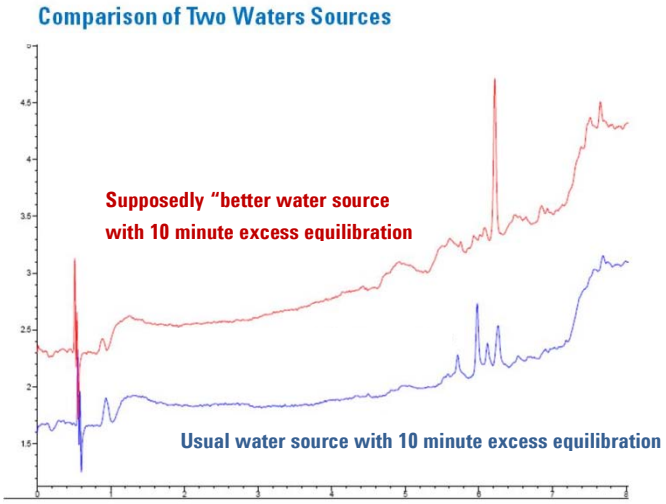

Figure 3 shows the difference on baseline from different sources of water.

Figure 4. Comparison of Acetonitrile Samples.

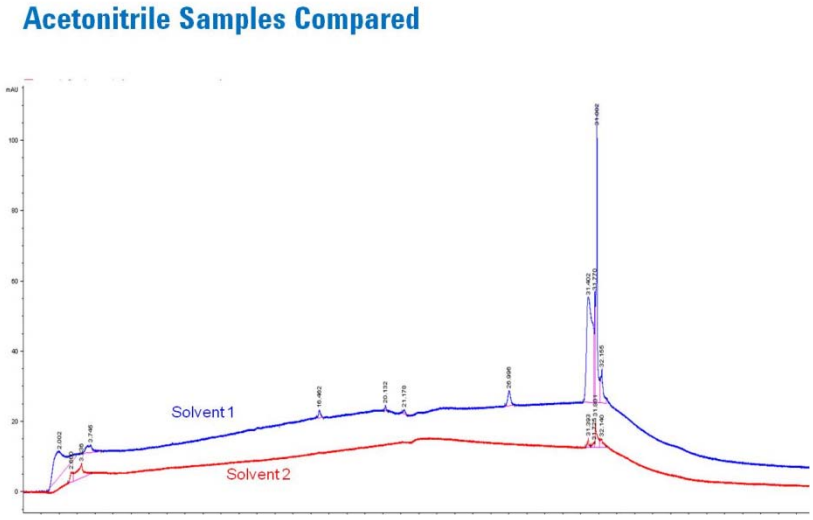

Figure 4 shows the background response from different sources of acetonitrile. Note in the lower chromatogram the absence of small peaks present in the upper trace.

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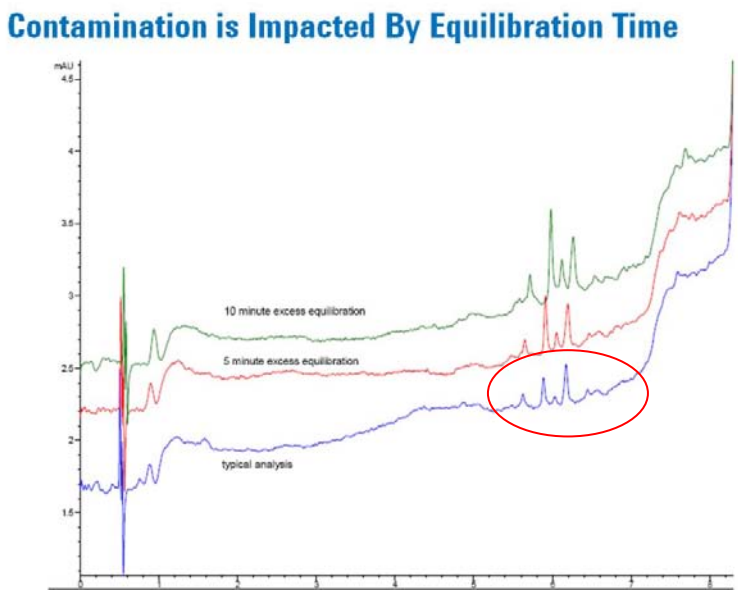
Determining if Ghost Peaks are a Result of Mobile Phase Contamination

A method to determine if ghost peaks are a result of mobile phase contamination:

- Vary the gradient and watch your baseline during re-equilibration.
- The more equilibration time you have, the more time your contaminants have to build up on the column.

Figure 5. Contamination is impacted by equilibration time.



In **Figure 5,** each of the 3 blank chromatograms represents a different equilibration time. Observe the peaks showing up in the 5.5- to 6.2 minute timeframe—they could interfere with peaks of interest. The **blue** chromatogram represents the original gradient with equilibration time of 10 minutes already established. One can see the evidence of 6 or so peaks with their respective peak heights (areas) in the 5.5-6.2 minute timeframe.

To determine if the contaminants were originating from the mobile phase:

- We ran an extra 5 minutes of equilibration for a total of 15 min (see the red chromatogram).
  Note: The impurity peaks slightly increased in their peak height (area**).**
- Next, we ran an extra 10 minutes of equilibration.
- As can be seen in the green chromatogram, the peak heights (areas) have increased even further.

This series of experiments shows that the impurities originated from some trace impurities in the mobile phase building up with an increase in equilibration time. From these results, one cannot tell in which of the two mobile phase solvents the impurities were present, so each may have to be further investigated.

Contamination of Mobile Phase Additives

Be aware of contamination of mobile phase additives.
Note: The UV absorbance of TFA increases as the ACN level is increases. This gives a baseline rise.

There are two ways to address this:

- Use TFA in the aqueous mobile phase solvent and TFA at a lower concentration in the organic mobile phase solvent (e.g. 0.1% TFA in water / 0.086% in ACN) These percentages balance out the gradient drift and provide a more stable baseline.
- You can also use a longer wavelength – e.g. 235 nm, 254 nm, 280 nm, where TFA has poorer absorbance.

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Sample Preparation

Sample preparation is very helpful for reducing column wear and minimizing contamination. Use sample cleanup such as solid-phase extraction (SPE), liquid-liquid extraction or protein precipitation before injection.

Filtering your sample helps prevent blocking of capillaries.

Dissolved contaminants are different than particulate contaminants. SPE and liquid-liquid extraction help to remove dissolved contaminants.

Be wary of sources of contamination from sample preparation:

- Impurities in the sample solvent.
- Impurities in the components added during sample prep, such as buffers, internal standard, etc.
- Degradation of the sample or impurities in the autosampler vial on standing.

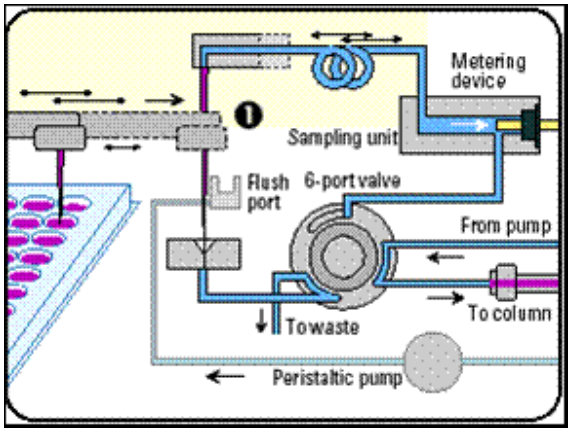
Good tips to prevent contamination from sample preparation:

- Inject the sample solvent alone to ensure that there is nothing in this solvent that contributes to spurious or ghost peaks
- Make blank injections during the course of multiple runs to demonstrate that there are no ghost peaks present due to carry-over.

System Contamination

The autosampler is a common source of system contamination, particularly for high sensitivity LC/MS and LC/MS/MS. You can verify if the autosampler is the source by removing it from the flowpath and making a blank run to see if your ghost peaks disappear.

Figure 6. Diagram of a modern autosampler.



Quick Tip: Try disconnecting the column from the instrument and replacing it with a narrow piece of narrow bore capillary tubing, such as 0.12 mm ID, and make a blank run. If your ghost peak still appears, then you know you likely have a system contamination issue. You can work backwards through key system components – such as the column inlet or the autosampler.

One short-cut to use is to set no vial location in the ChemStation Sequence Table—this will result in the autosampler being skipped.

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Column Contamination – Column Clean-Up/Column Flush

Column contamination is rarely the cause of repeatable ghost peaks. Column particulate contamination will more likely lead to increased pressure. If you think you have contamination in your column, **check your column documentation to see if it can be backflushed.**

To backflush a column:

- Take the column, remove it from the system, and connect the outlet to the inlet tubing from the pump.
- Do not connect your column to the detector when you are backflushing. Instead, run the inlet end into a beaker to capture the solvent or water.
- Do not run a high flow rate. Start out slow and build to a higher flow rate as needed to dislodge the particulates.

You should flush an amount that is sufficient to clean the column. We measure this in "column volumes", which can be determined using a formula. Generally, we recommend 10 column volumes for a starting point.

Tips for cleaning columns:

- Flush with stronger solvents than your mobile phase.
- Make sure the detector is taken out of the flow path.
- Do not add your organic solvent directly to the buffer, as this may cause the buffer salts to precipitate out and lead to more backpressure.

For Reversed Phase:

Use at least 10 column volumes of each solvent for analytical columns.

1. Start with your mobile phase without buffer salts (water/organic)
2. 100% Organic (MeOH or ACN)
3. Check pressure to see if this has returned it to normal; if not, then
4. Discard column or consider more drastic conditions: 75% Acetonitrile / 25% Isopropanol
5. 100% Isopropanol
6. 100% Methylene chloride, solvent wash for very nonpolar compounds.
7. Hexane

Summary

Ghost peaks may come from a number of sources.

1. If you suspect wraparound peaks, increase your flow rate, increase your mobile phase strength, or run a gradient.
2. Always use HPLC grade solvents.
3. Use sample preparation, but test your sample preparation solvents, and make blank injections during the course of multiple runs to demonstrate that there are no ghost peaks present due to carry-over.
4. If you think you have system contamination, start by bypassing the autosampler and running a blank gradient to see if you can isolate the source.
5. If you suspect your column, you can try backflushing it, if your column is able to be backflushed.

As always, if you have tried to troubleshoot your issue without success, you can always contact Agilent technical support for assistance. Visit us on the web at www.agilent.com/chem/contactus to find your technical support contact.

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