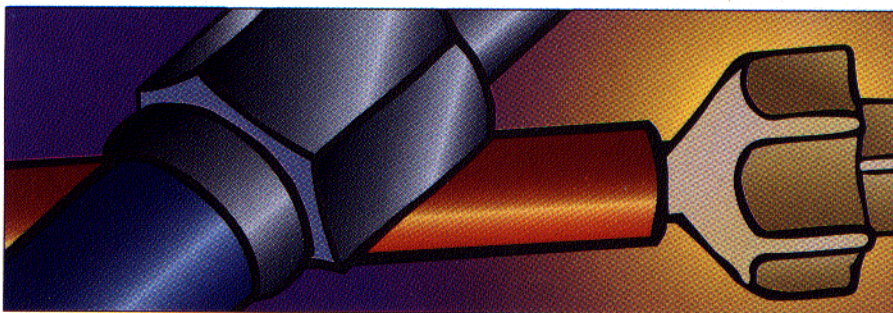


LC Troubleshooting



Ghost Peaks and Aerated Sample Solvent

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Ghost peaks are one of liquid chromatography's most aggravating problems.

In liquid chromatography (LC) runs, analysts occasionally see spurious peaks of unknown origin. These peaks collectively are called ghost peaks. Ghost peaks can be a major concern in application areas such as purity confirmation of pharmaceutical products in which every component in a product must be accounted for. Ghost peaks can arise from various causes, such as dissolved air in the sample solvent. This month's "LC Troubleshooting" column focuses on ghost peaks in the context of reversed-phase LC with UV detection.

PEAKS CAUSED BY DISSOLVED AIR

Analysts commonly observe baseline disturbances at the column dead volume when the sample solvent is different from the mobile phase. Workers usually minimize this problem by carefully matching the injection solvent with the mobile phase. However, ghost peaks can occur even when the sample solvent and mobile phase are matched. One cause of retained ghost peaks is a difference in the amount of dissolved air in the sample solvent compared with the mobile phase. In the case of UV-absorbance detection, dissolved oxygen is the primary concern.

Figure 1 shows ghost peaks of varying size that occurred when samples containing different amounts of dissolved air were injected into

a mobile phase of identical composition. The mobile phase comprised an 85:15 methanol-water mixture, degassed with an in-line membrane degasser. When no sample degassing was used, the sample contained the maximum amount of dissolved air. The center trace of Figure 1a shows a peak of nearly 10 mAU at 210 nm for a 10- μ L injection. Purging this sample with helium displaces the dissolved air and almost eliminates the air peak (bottom trace in Figure 1a). After sparging the sample with oxygen, the sample contains approximately five times the oxygen concentration as the air-saturated sample, and the chromatogram contains a peak roughly five times as large as the first one (top trace in Figure 1a). Notice that the column dead time for Figure 1 is approximately 1.5 min, so the oxygen peak is retained well beyond where unretained materials would be eluted.

It is interesting to contrast the results of Figure 1a, in which the mobile phase was degassed, with the runs of Figure 1b, in which no mobile-phase degassing was used. For this case, the air-saturated sample injected into an air-saturated mobile phase did not generate a peak (center trace in Figure 1b) because there was no difference between the sample solvent and the mobile phase. Oxygen-saturated sample solvent produced a large peak (top trace). After the sample was degassed with helium, the sample solvent contained *less* oxygen than the mobile phase, so a negative peak was produced (bottom trace). When the mobile phase has a higher background absorbance than the sample, a negative peak occurs.

Therefore, the runs of Figure 1 indicated that differences in the amount of dissolved air (oxygen) in the sample solvent and mobile phase can produce a variety of ghost peak appearances.

WAVELENGTH EFFECTS

The detection wavelength can have a pronounced influence on the appearance of ghost peaks due to dissolved air. Figure 2 compares the absorbance spectra of aerated and degassed methanol. Degassed methanol exhibits a lower absorbance; the difference depends on the wavelength. At 210 nm, the two solvents differ by more than 300 mAU, whereas only a 10 mAU difference exists at 254 nm. From these data analysts can calculate the peak height of the ghost peak when injecting aerated methanol in a mobile phase of degassed methanol. For example, at a flow rate of 1 mL/min and an injection volume of 10 μ L, a Gaussian peak with a baseline width of 0.4 min should generate a ghost peak with a height of approximately 15 mAU at 210 nm. The same sample would generate a peak of nearly 0.5 mAU at 254 nm. Thus, ghost peaks arising from dissolved oxygen in the sample should be larger at lower wavelengths.

THE INFLUENCE OF SOLVENT TYPE

Figure 3 shows UV spectra for several common LC solvents saturated with air. In each case, the spectrum shown represents the absorbance spectrum after correcting for the absorbance of the corresponding degassed solvent. The additional absorbance caused by air is much greater for some solvents than for others. This factor should translate directly to the expected size of air-caused ghost peaks in these solvents. The intensity of the ghost peak, however, does not seem to be correlated with the degree of air saturation. For example, hexane allows greater dissolved oxygen uptake than methanol, but the absorbance change is smaller. Therefore, the absorbance is not only related to the concentration of oxygen but also to the interaction of the oxygen with the solvent.

PEAK RETENTION

Analysts may assume that an artifact peak caused by dissolved air would appear early in the chromatogram. However, the retention of the ghost peak shows reversed-phase retention in the present example. Figure 4 shows that retention increases as the amount of methanol in the mobile phase is reduced. In each case shown in Figure 4 the sample solvent was the same as the mobile phase. Similar behavior occurs when a phosphate buffer is used instead of water. Because ghost peaks from dissolved air will change retention with mobile-

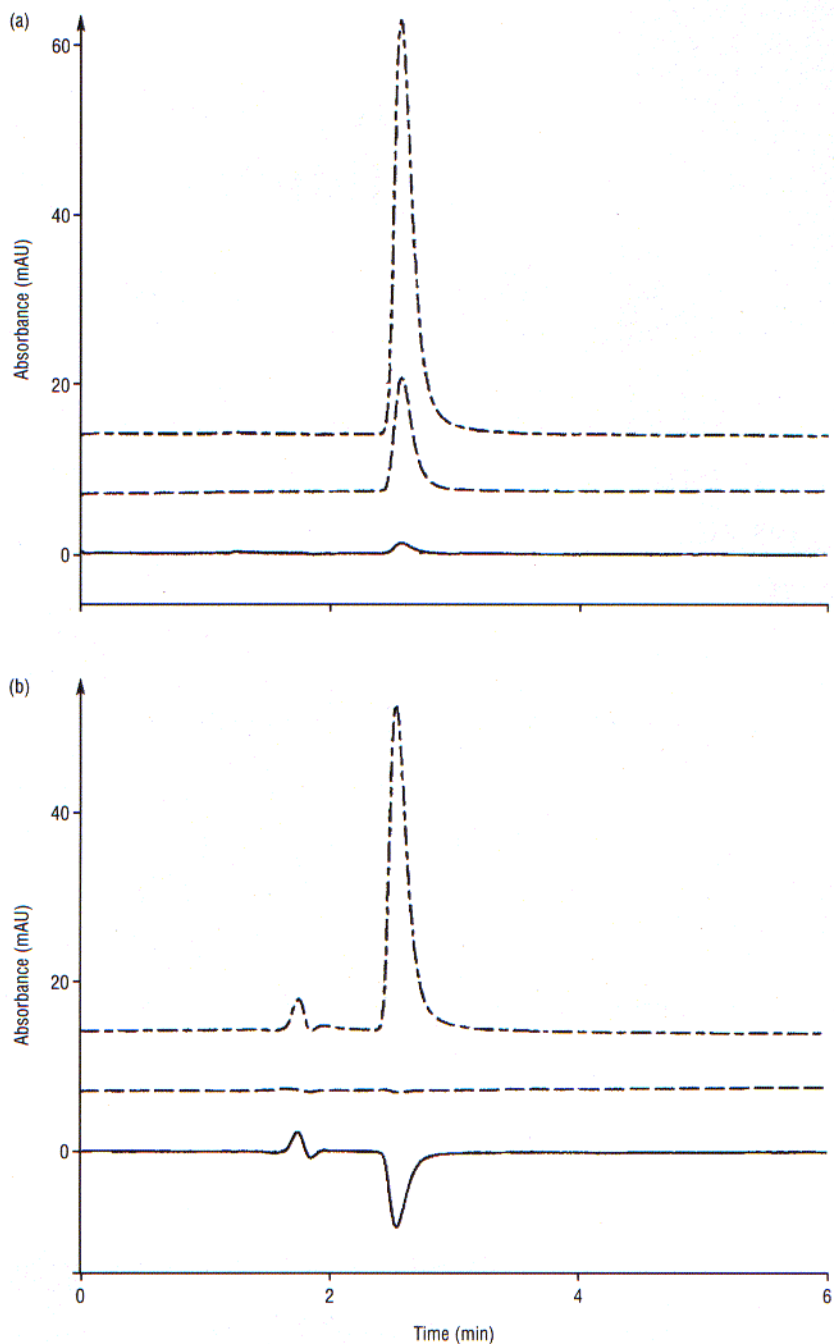


FIGURE 1: Peaks observed when a sample of mobile phase is injected into (a) degassed and (b) nondegassed mobile phase. The center, bottom, and top traces show results from 10- μ L mobile-phase samples saturated with air, purged with helium, and purged with oxygen, respectively. Column: 15 cm \times 4.6 mm STR-ODS II (Shimadzu Corp., Kyoto, Japan); mobile phase: 85:15 methanol-water; flow rate: 1 mL/min; temperature: 40 $^{\circ}$ C; detection: UV absorbance at 210 nm.

phase changes, the identification of the source of these peaks can be confusing.

IDENTIFICATION OF ARTIFACT PEAKS

When degassed mobile phase is used and ghost peaks are observed, the following tests will help to determine if the artifactual peak

results from dissolved air in the sample solvent:

- The sample solvent is the same as the mobile phase and the ghost peak is observed with almost the same retention time and size in each run.

Increasing or decreasing the amount of dissolved air in the sample should change

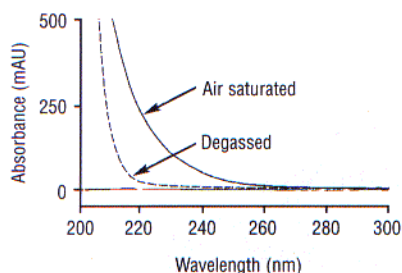


FIGURE 2: UV spectra of methanol saturated with air and methanol degassed by helium sparging.

the size of the ghost peak. Stir or shake the sample to increase the amount of air and the peak height should increase. Degas the sample with helium sparging or vacuum to reduce the dissolved air in the sample, and the peak should be smaller.

- Aerated mobile phase is injected as a sample and the retention time of the observed peak coincides with that of the ghost peak.

Degassing the sample (mobile phase) before injection by purging with helium or vacuum degassing should reduce the peak height.

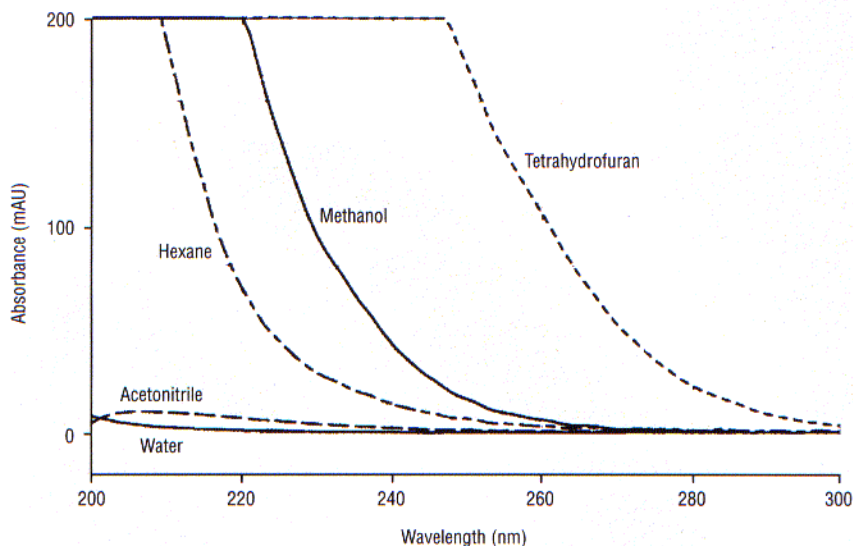


FIGURE 3: Spectra of common HPLC-grade solvents saturated with air. Each plot represents a difference spectrum between air-saturated solvent and degassed solvent.

- Use of nondegassed mobile phase results in smaller ghost peaks.

When the aerated mobile phase is injected as sample, the observed peak always is

smaller than in the case above, because the difference in composition between the mobile phase and sample solvent is smaller.

COUNTERMEASURES

Although it is difficult to eliminate ghost peaks caused by dissolved air completely, the following measures will help to minimize this problem. In the cases below, assume that the mobile phase is methanol-water.

- Substitute HPLC-grade acetonitrile for the methanol in the mobile phase.

The plots of Figure 3 show that ghost peaks caused by dissolved air in acetonitrile should be much smaller than those in methanol or tetrahydrofuran. A change in mobile-phase solvent will require some re-optimization for most methods.

- Reduce the ratio of methanol in the mobile phase.

The more water the mobile phase contains, the smaller the impact of dissolved air in the sample. Analysts may need to use shorter or weaker columns to maintain an acceptable run time with a weaker mobile phase.

- Discontinue on-line degassing of the mobile phase.

This measure will work only with certain LC systems. In general, LC systems using low-pressure mixing will require some form of degassing for reliable operation. Some LC systems using high-pressure mixing may not require degassing. If degassed mobile phase is not used, be sure to use a back-pressure restrictor after the detector cell to minimize bubble problems in the cell.

- Degas the sample.

It may be sufficient to degas the sample for approximately 10 s with helium. Alternatively, vacuum degassing may be effective at

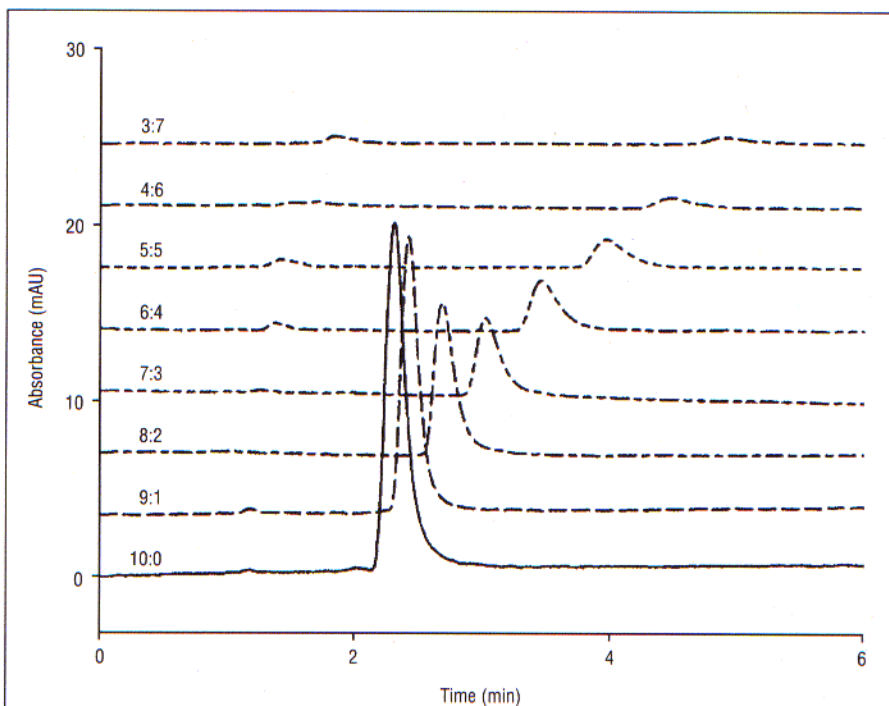


FIGURE 4: Chromatograms showing the effect of the mobile phase's methanol-water ratio on retention of an air-saturated mobile-phase sample. Sample: 10 μ L air-saturated mobile phase. Methanol-water ratios are shown in the figure. Other conditions were the same as in Figure 1.

removing excess air from the sample solvent.

- Change the mobile-phase selectivity.

It may be possible to change the mobile-phase selectivity by adjusting pH, temperature, percent organic, organic type, or column type so the air peak moves to a portion of the chromatogram where it does not interfere with any desired peaks. Although this procedure will not eliminate the peak, it may provide a satisfactory solution for many situations.

OTHER CONSIDERATIONS

The present discussion assumed use of a UV-absorbance detector. Other LC detectors may respond differently to dissolved air. For example, when analysts use refractive index detectors under the same conditions, dissolved air causes a negative peak. The negative peak occurs because the degassed mobile phase has a larger refractive index than the aerated mobile phase. In this case, nitrogen and oxygen contribute to the peak.

CONCLUSION

Most workers understand the need to degas the mobile phase for reliable pump and detector operation, but this discussion points out other potential problems. Ghost peaks can occur when the sample solvent and mobile-phase air content are not matched — or even when they are the same. Ghost peaks vary in size because of different types of organic solvents and their ratios. The magnitude of these

problems depends on the air content, the mobile-phase type, and the mobile-phase water content. Workers can minimize air-related ghost peaks by degassing both the sample and the mobile phase. In reversed-phase chromatography, retention of ghost peaks may be increased by reducing the organic solvent ratio. Understanding these behaviors will help chromatographers in developing HPLC methods.

Editor's Note: The information contained here was reported in a different format in the English language publication LCWorldTalk I(1), 8-11 (1996), published by Shimadzu Corp., Kyoto, Japan.

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