Gas Chromatography Problem Solving and Troubleshooting

Question:

I occasionally see some extra peaks in my chromatograms that I suspect are from septum bleed. How do I determine whether septum bleed is the source of the extra peaks?

Answer:

Extra peaks in a chromatogram can originate from several sources. Because septum bleed is suspected as the cause of the problem, the first step is to confirm or eliminate septum bleed as the cause of the extra peaks. If septum bleed is the cause, the extra peaks correspond to compounds released by septa upon heating in an injector. Sometimes these compounds may appear more like humps or blobs in the baseline. The appearance and severity of septum bleed often depends on the brand of septa and the time and oven temperature maintained between analyses.

The other possible sources of extra peaks or baseline problems need to be eliminated before the blame can be placed solely on septum bleed. The best method to determine if the septa is responsible is to remove the septa from the GC. Because this is rarely practical, another method can be used. Wrap a new septum with a single layer of aluminum foil. Make sure the entire septum is covered, but use only enough to cover the septum with one layer of foil. Place the side with the seam toward the septum nut and the side without the seam (i.e., the continuous piece of foil) toward the injector body. Do not overtighten the septum nut or the foil will tear. This is especially true for septum nuts with a knife edge intended to cut into the septum. The idea is to obtain a leak-free seal at the septum but not to expose the septum to the carrier gas flow. Any compounds released by the septum are trapped within the foil wrapping and cannot enter the column. After installing the wrapped septum, heat the GC oven to the highest temperature used and

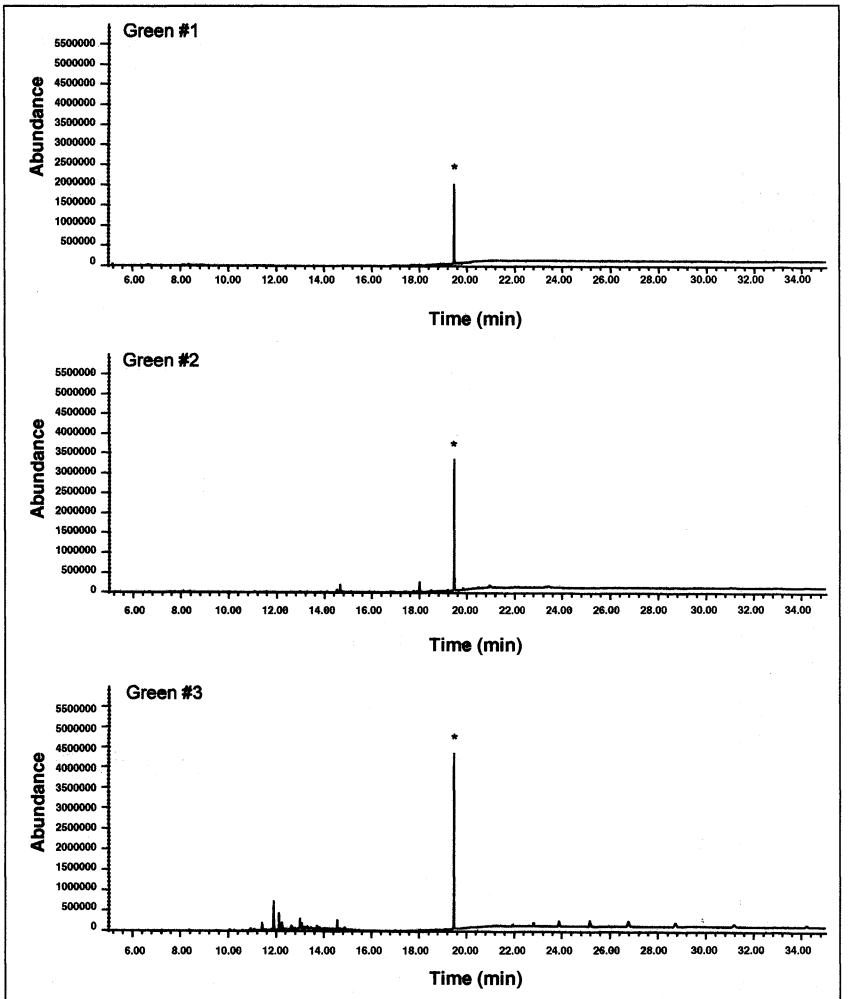

Figure 1. Methylene chloride extracts of green septa. Conditions: DB-1 column (30 m x 0.25-mm i.d., 0.25-µm film thickness), split injector (1:30 split ratio, 250°C), FID (300°C), helium carrier gas (32 cm/s), 40°C column temperature for 1 min followed by an increase of 15°C/min to 40–325°C, then 325°C for 15 min. Asterisks denote sample contaminants not originating from the septa. All chromatograms were plotted using the same scale.

The purpose of *Chromatography Problem Solving and Troubleshooting* is to have selected experts answer chromatographic questions in any of the various separation fields (GC, GC–MS, HPLC, TLC, SFC, HPTLC, open column, etc.). If you have questions or problems that you would like answered, please forward these to the *Journal* editorial office with all pertinent details: instrument operating conditions, temperatures, pressures, columns, support materials, liquid phases, carrier gas, mobile phases, detectors, example chromatograms, etc. In addition, if you would like to share your expertise or experience in the form of a particular question accompanied by the answer, please forward to JCS Associate Editor, *Chromatography Problem Solving and Troubleshooting,* P.O. Box 48312, Niles, IL 60714. All questions/answers are reviewed to ensure completeness. The *Journal* reserves the right not to publish submitted questions/answers.

Dean Rood
Associate Editor

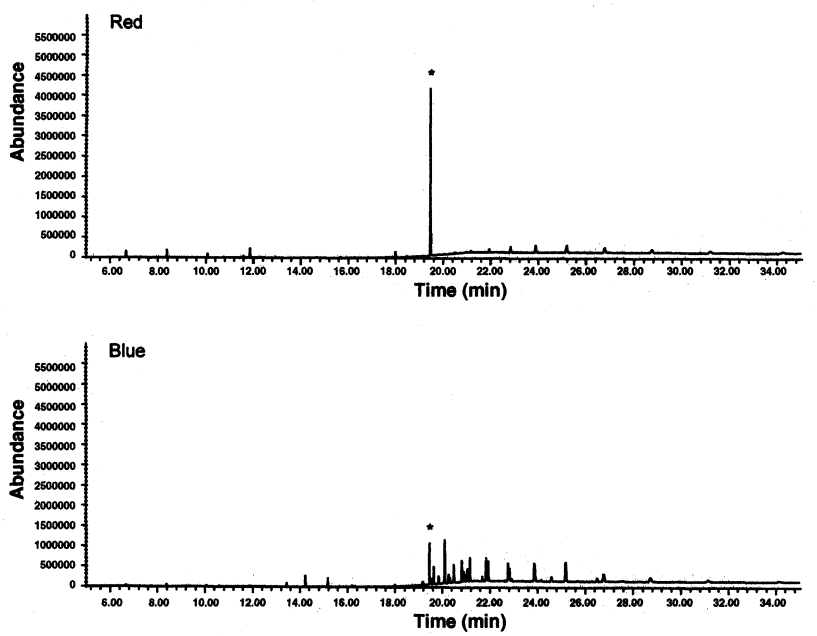

Figure 2. Methylene chloride extracts of red and blue septa. Conditions are listed in Figure 1.

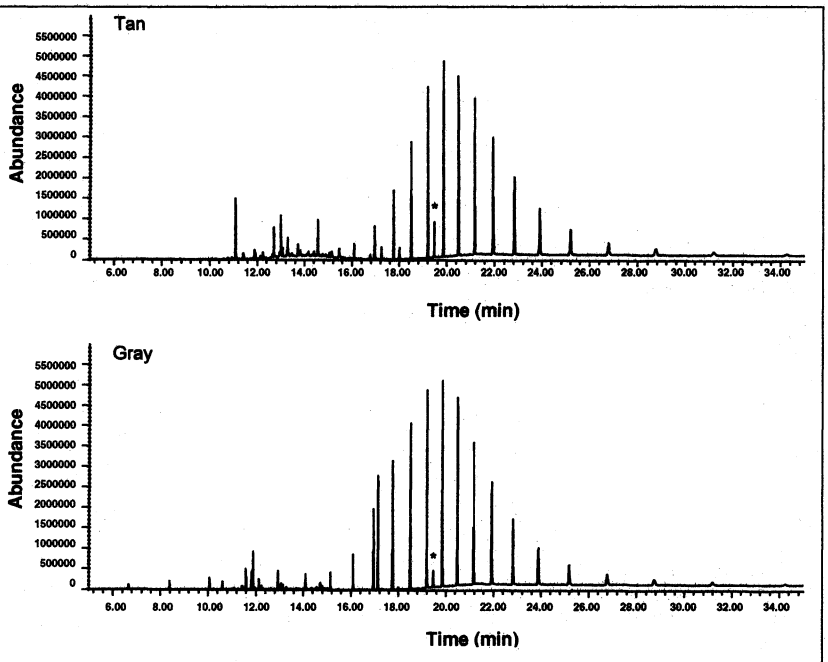

Figure 3. Methylene chloride extracts of tan and gray septa. Conditions are listed in Figure 1.

maintain this temperature for 15–30 min. This removes any materials that may have previously condensed in the column. After this heating period, maintain the GC oven at 40–50°C for about 60 min. Then run the same temperature program as used while experiencing the problem and record the full chromatogram. If the extra peaks are no longer present, the septum is probably not responsible for the extra peaks. After the test, install an unwrapped septum to verify that the problem returns. If the extra peaks are still present with a wrapped septum, another source is probably responsible. Contaminated carrier gas, gas lines, injector, and regulators and expired gas impurity traps are some of the other more common sources of extra peaks or baseline disturbances.

The amount of septum bleed is very dependent on the quality of the septum. In general, the more expensive septa exhibit lower bleed and residual contaminants. An unofficial standard concerning the color of septa seems to have evolved over the years. Green septa often exhibit the lowest peak bleed and are often marketed as the highest quality septa from the various suppliers. The red (rust or brick red color) and blue septa exhibit moderate bleed levels, whereas tan and gray septa exhibit the worst bleed levels. This is not a universal or exact classification system; however, septa bleed levels seem to follow this color pattern.

A crude test of potential septa bleed levels is to solvent-extract the septa and analyze the extracts by GC. The presence and size of any peaks can be used as an indicator of residual contaminants in the septa. This technique does not exactly duplicate septum bleed conditions, but comparing different septa provides a relative measure of their bleed levels. A variety of septa were examined using this method. Each septa was cut into four pieces and weighed, and a volume five times the septa weight (e.g., 1.2 mL solvent added to 0.24 g of septa) was added to a vial containing the pieces. The vial was shaken for 10 min, then 2 µL of the solvent was injected. Methylene chloride extracts are shown in the following figures.* There were few differences between the three green septa (Figure 1). One showed a few more contaminants (green #3). Each septa contained the same main contaminant, whose mass spectrum indicated a large hydrocarbon or hydrocarbon-based compound. This compound was not detected in a blank solvent sample, thus it did not originate from the GC–MS system, vial, solvent, or syringe. Further investigation showed that the

* The same brands of septa were also extracted using hexane and methanol. The same contaminants were observed; however, the amounts of the contaminants were slightly less.

compound originated from the blade used to cut the septa into pieces and not from the septa. This is a classic example of an apparent contaminant peak originating outside of the GC system. Whenever a contamination problem ocurs, it is imperative to eliminate all other possible outside sources of the contaminant or faulty conclusions may be reached.

The solvent extracts of blue and red septa had higher levels of contaminants than the green septa (Figure 2). Most of the contaminant peaks correspond to hydrocarbons and silicones. The blue septa extract showed a few more contaminants and also contained a few phthalates. Figure 3 shows the extracts of the tan and gray septa. It is obvious that these septa contain very high levels of contaminants. The evenly-spaced distribution of peaks corresponds to a homologous series of silicones. The mass spectra of these compounds are very similar to the spectra of stationary phase bleed. Sometimes these types of contaminants are incorrectly attributed to column bleed and not to septa bleed (or another contamination source) (1). The peaks in earlier regions of these chromatograms are hydrocarbons and phthalates. The costs of the gray and tan septa were significantly less than those of the other septa. Again, there seems to be a correlation between septa cost and bleed levels.

Plastic boxes or bags were used to store the blue, gray, and tan septa. It is not a coincidence that these septa contained the highest levels of hydrocarbons and phthalates. The other septa were packaged in metal cans or glass vials. To maintain the highest septa cleanliness, clean tweeters should be used to handle the septa. Although this is cumbersome, it is the best way to minimize septa contamination. In most cases, handling septa with one's hands is satisfactory, provided the hands are clean. Lotions, soaps, food residues, finger oils, and other miscellaneous substances are all possible contaminants that can be transferred to a clean septa from the hands. Hands should be thoroughly cleaned and rinsed before touching septa to avoid the possibility of contamination.

One method to reduce septa residues and contaminants is to preheat the septa prior to use. Placing the septa in a clean beaker and heating for several hours at or near the injector temperature is sufficient to remove most contaminants. Using a GC oven to heat the septa is convenient and allows the septa to be consistently maintained at an elevated temperature. Be careful not to exceed the temperature limit of the septa. Septa can also be soaked in solvent for 1 h and dried prior to use. Although this technique works, it has the tendency to reduce septa life (i.e., number of injections before replacement).

References

1. D. Rood. Gas chromatography problem solving & troubleshooting. *J. Chromatogr. Sci.* **35:** 136 (1997).