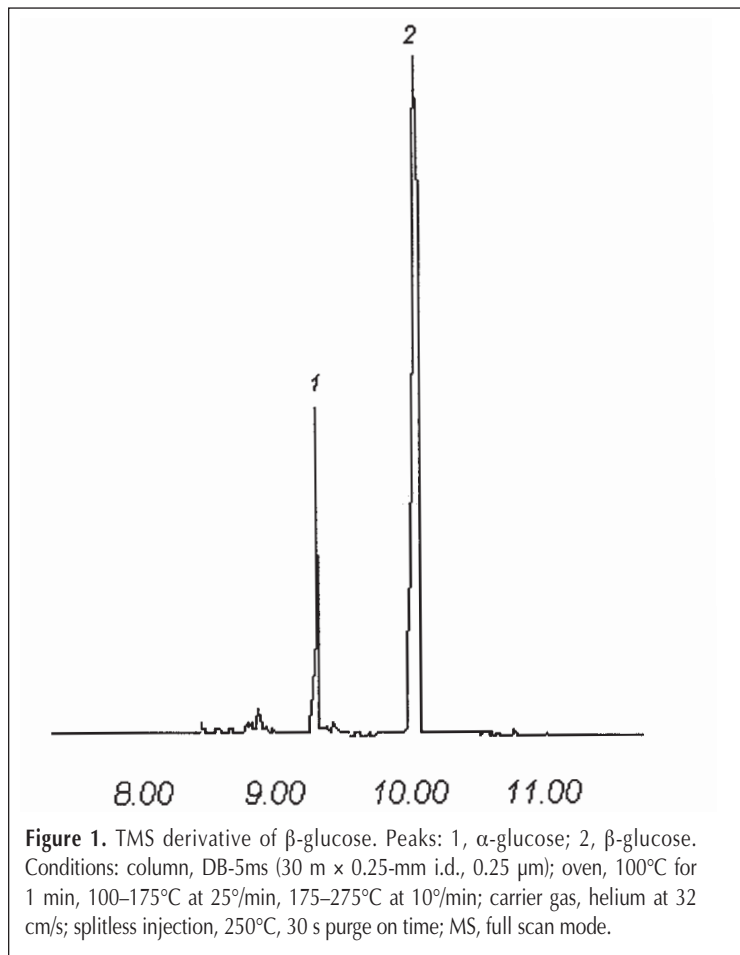# Gas Chromatography Problem Solving and Troubleshooting

Question:

I consistently obtain two or more peaks for many TMS derivatized sugars. This problem occurs with standards and samples. I have tried adjusting the derivatization and GC conditions; however, multiple peaks always appear. How do I fix this problem?

Answer:

Occurrences of multiple peaks for a single compound are usually related to the sample or injector. If the injector is responsible, the injected sample is being introduced into the column at two different times. Injector leaks, broken or misinstalled liners, incorrect column installation, and poor injection technique are the most common injector related problems. If this type of problems exists, split peaks (i.e., partial coelution) are more typical. Sometimes fully resolved peaks are seen, but this usually happens for highly retained compounds. The two peaks obtained for β-glucose (Figure 1) are separated by nearly 1 min, thus it is very unlikely that injector-induced peak splitting is occurring.

It is unlikely that injector contamination is responsible, because the multiple peak problem is fairly consistent. Extraneous peaks caused by injector contamination tend to be erratic in occurrence, size, and retention. Because the sample appears to be the most likely source, there are several steps to find or eliminate potential sources. A solvent blank should be prepared and injected. This involves preparing a sample that does not contain the target analytes. If the extra peak is caused by a sample contaminant, it should appear in the solvent blank. If the peak appears, one or more of the solvents or reagents is the source, or the contaminant is present in anything the sample contacts. Glassware, sample vials and caps, pipettes, or the syringe are only some of the possible contamination sources. If the peak does not appear in the solvent blank, sample contamination can be eliminated as a possible source.

Sample decomposition or degradation in the injector is another possibility. This problem can sometimes be difficult to verify. The easiest method is to change the injector temperature by 75–100°C. Lowering the temperature should reduce the amount of degradation; thus, the degradation product peak should decrease in size while the

Figure 1. TMS derivative of β-glucose. Peaks: 1, α-glucose; 2, β-glucose. Conditions: column, DB-5ms (30 m × 0.25-mm i.d., 0.25 μm); oven, 100°C for 1 min, 100–175°C at 25°/min, 175–275°C at 10°/min; carrier gas, helium at 32 cm/s; splitless injection, 250°C, 30 s purge on time; MS, full scan mode.

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

parent or original compound peak should become larger. Increasing the temperature should have the opposite effect. Although this technique is not definitive, and is sometimes inconclusive, sample degradation in the injector can often be verified or eliminated as a possible cause.

The extra peak occurs for both the standard and sample. This eliminates an impurity in the β-glucose, unless the standard and sample are prepared from the same source of β-glucose. Derivatization involves chemically changing the structure of analytes to improve their chromatographic characteristics. In a few cases, the derivatization process does not proceed as desired. Unfortunately, this occurs for sugars when using typical TMS derivatization methods and reagents. Common sugars contain an anomeric center, and mutarotation occurs during the formation of their TMS derivatives. This results in the formation of alpha and beta forms of the sugar derivative. These forms are easily separated by GC; thus, two peaks are obtained in the chromatogram. In Figure 1, the mass spectra of both peaks are consistent with the TMS derivative of glucose. The other peak in Figure 1 corresponds with the retention time for the TMS derivative of α-glucose. In addition, the TMS derivatization of α-glucose also produced a corresponding β-glucose peak. Some sugars (e.g., galactose) have pyranose and furanose forms; thus, four peaks are obtained upon TMS derivatization.

There are several solutions for this multiple peak problem. One is to sum the areas of the two (or more) peaks for quantitation purposes. Although there is some uncertainty with this technique, it often results in satisfactory results. Another choice is to change or alter the derivatization. If the oxime form of the sugar is formed prior to TMS derivatization, mutarotation does not occur, and a single peak is obtained. This method involves additional steps, and the final sample often needs further cleanup to remove interferences. Finally, a different derivative can be selected. For sugars, derivatization to form alditol acetates is common. Although this eliminates the multiple peak problems, the formation of the same alditol acetate for several different sugars occurs. This means that several sugars will be indistinguishable as their respective alditol acetates.