Gas Chromatography Problem Solving and Troubleshooting

Question:

I have been experiencing some inconsistent peak sizes when using BSTFA to derivatize some drug standards and samples. The dry sample residue is dissolved in 50 µL chloroform and an equal volume of BSTFA. After heating at 45°C for 15 min, 2 µL is injected using an autosampler. A GC–MS system with a splitless injector is used. The peak sizes for one set of equal concentration samples are consistent, whereas the peaks for another set of identical samples are substantially smaller and vary by 25–50%. GC–MS system evaluation samples do not exhibit the same problem. What is causing the inconsistent peak sizes for select samples?

Answer:

There are several possible causes for this type of problem. The use of a system evaluation or checkout sample greatly simplifies the situation. It is very unlikely that the GC–MS is at fault because the evaluation samples are satisfactory even when the derivatized samples are exhibiting the problem. Derivatized samples rarely suffer from adsorption problems, thus column activity or contamination is also an unlikely cause. The satisfactory performance of the evaluation sample strongly indicates that the problem is related to the samples.

One area to investigate is whether the various samples are being exposed to different conditions or environments. Before they are injected, are the problem samples stored differently (temperature, time, light conditions, etc.) than the satisfactory samples? If they are, usually there will be some correlation between the sample storage conditions and the appearance of the problem. Are different types or brands of vials, caps, pipettes, solvents, reagents, or related items being used? Are different people handling the samples during any part of the sample workup? Are there any other possible variables involved with the handling of the samples? All of these areas need to be investigated because a seemingly small detail in one of these areas can have a significant influence on the GC–MS results.

Derivatization reagents are susceptible to interferences that may inhibit their complete reaction with the sample. The most common source is the presence of hydroxyl- or amine-containing solvents such as water, methanol, isopropanol, or diethylamine. The derivatization reagents preferentially react with these light solvents rather than with higher molecular weight compounds. The less reactive sample compounds often do not react or only partially react with the derivatization reagents in the presence of these types of solvents. This type of problem would account for the variable peak sizes of some of the samples. Traces of water- or alcohol-based solvents in the final sample due to incomplete evaporation are one possible source of interference.

It is sometimes recommended to use a water-immiscible solvent as the derivatization solvent. This helps to minimize the amount of trace water present in the solvent. Solvents such as acetone may have a significant amount of residual water and therefore can be difficult to keep dry for long periods of time. Chloroform is being used in the method described above. One potential problem with using chloroform as a derivatization solvent is the presence of preservatives. Amylene (2-methyl-2-butene) and ethanol are the most common preservatives used with chloroform; ethanol concentrations of 1% are often used. Amylene does not cause a problem, but ethanol interferes with most derivatization reactions. Check the chloroform being used to determine whether two different brands or grades are being used. Using ethanol-preserved chloroform for the problem samples may account for the reduced and variable peak sizes. The satisfactory samples may have been prepared with an amylene-preserved chloroform.

The presence of ethanol may affect the solubility of some dry sample residues in chloroform. A greater amount of polar compounds in the residue may dissolve in the ethanol-preserved chloroform, thus changing the concentration of the final sample. This may affect extraction efficiencies and result in variable quantitative results. If chloroform is used in a chromatography column cleanup technique, elution of the analytes may differ between the ethanol- and amylene-preserved chloroform.

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.

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Associate Editor