

Gas Chromatography Problem Solving and Troubleshooting

Question:

When analyzing derivatized samples, my capillary columns seem to degrade much faster than normal. Are there methods to prevent or minimize the amount of column damage?

Answer:

Compared with underivatized samples, more frequent column maintenance or replacement is required for the analysis of derivatized samples prepared by using typical reagents and methods. The column is often not permanently damaged but only contaminated with sample residues. In many cases, solvent rinsing restores column performance (1). Sometimes, removal of only 10–50 cm of the front of the column restores performance. The amount and speed of performance degradation is dependent on the type of derivatives, reagents, and the preparation method. In general, derivatized samples should not be injected into polyethylene-glycol-based columns (Carbowax, FFAP, OV-351, etc.) unless all of the excess derivatization reagent is removed from the sample or permanently neutralized. Any derivatization reagent that reacts with hydroxyl groups may react with polyethylene-glycol-based columns and cause irreversible damage to the stationary phase.

Trimethylsilyl (TMS) and acyl derivatives are among the most common ones used for GC analyses. The acyl derivatives usually cause more problems than the TMS derivatives. The imidazole (trifluoroacetylimidazole [TFAI], pentafluoropropionylimidazole [PFPI], and heptafluorobutyrylimidazole [HFBI]) and anhydride (trifluoroacetic acid anhydride [TFAA], pentafluoropropionic acid anhydride [PFPA], and heptafluorobutyric acid anhydride) forms of the acyl derivitization reagents are the most commonly used. The imidazoles are very reactive and generate a relatively inert byproduct; however, their cost is 2–3 times greater than the corresponding anhydride. The anhydrides generate perfluoroacids (trifluoroacetic acid, pentafluoropropanoic acid, or heptafluorobutyric acid) as the main reaction byproducts. These acids can rapidly damage the stationary phase in the column; thus they need to be removed or neutralized before injecting the sample.

Various derivatization methods have been devised to deal with the perfluoroacid formation problem. One of the best methods uses a water-immiscible derivatization solvent such as toluene or toluene–acetonitrile (95:5); these are the most commonly used solvents. After heating and subsequent cooling of the sample, aqueous 5% sodium bicarbonate is added to the sample. Two layers form; the toluene is the upper layer. The sample is mixed with a vortex mixer until the top layer is clear; additional mixing is needed if any cloudiness is observed. If the two layers are not well defined after mixing (complex or dirty samples may cause this to occur), the sample can be centrifuged at 1000–1500 rpm for a few minutes to speed up the formation of two distinct layers. A portion of the upper layer is removed for dilution or injection. The sodium bicarbonate neutralizes the acid and also performs some sample cleanup.

Another neutralization technique is to add a base to the derivatization solvent. The base reacts with the acid byproducts and rapidly drives the reaction toward completion. Triethylamine is most commonly used for this purpose. If an amine is added to the sample and an aqueous wash step is used (like the bicarbonate wash previously described), it is recommended that a buffer below pH 6 is used to avoid potential hydrolysis of the derivatives. In another method, anhydride (usually along with an equal volume of solvent) is added directly to the dry sample residue. After heating, the sample is evaporated to dryness. Although this removes most of the excess reagent and reaction byproducts, the evaporation step is a potential source of compound loss and method irreproducibility.

TMS derivatives usually are less problematic than acyl derivatives. BSTFA, MSTFA, and BSA are the most common TMS reagents. These reagents are not harmful to capillary columns with polysiloxane (i.e., silicone) stationary phases. Sometimes 1–10% of trimethylchlorosilane (TMCS) is added to improve the derivatization reaction. TMCS generates a small amount of HCl as a reaction byproduct. If the column is maintained at low temperatures (below 50–60°C) for more than 5 min during the GC run, the HCl may cause a small amount of stationary phase damage. Usually the damage is localized to the front of the column. Removing 0.5–1 m from the front of the column often restores column performance. If a very large number of TMS samples are injected and analyzed and a flame-ionization detector (FID) is used, some reagents (most notably BSA) may produce silicone deposits in the FID. Sensitivity changes or difficulty in lighting the FID flame are the typical symptoms of this problem. Cleaning the FID collector and jet is required to remove the deposits.

In general, the chromatogram of a derivatized sample has a larger solvent front and more peaks than that of the corresponding underivatized sample chromatogram. Also, derivatized samples contaminate GC systems at a much faster rate. It is easy to confuse derivatization artifacts with actual damage to the capillary column. In most cases, simple cleaning of the injector or column, or trimming the front of the column is all that is needed to restore column performance after analyzing derivatized samples.

Reference

1. D. Rood. Troubleshooting. *J. Chromatogr. Sci.* **33**: 596 (1995).

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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