Liquid Chromatography Problem Solving and Troubleshooting

Question:

Recently, I encountered a situation that puzzles me. I put a fresh HPLC column into my system, and I noticed a slight green tinge in my waste reservoir. Luckily, I had a clean and empty reservoir because I had previously used my system in normal phase with dichloromethane as the solvent; therefore, I had cleaned my system before I switched over to the reversed-phase solvent and column. I originally speculated that my column was the cause of this contamination, but once the eluent became clear, my chromatography seemed alright. Do you have any thoughts on this?

Answer:

In many situations, what is thought to be a column problem is actually a solvent problem. It is assumed by users that the phrase "HPLC grade" will always provide the adequate level of quality and reproducibility that is required for good chromatography. Unfortunately, sometimes this may not be the case. This is not because the suppliers of solvent do not have adequate quality control; it is because the demands of chromatographic separations are varied. While there is a high level of control in making HPLC-grade solvents, the impurity detection levels that determine the specifications may be above what is required for your needs, there may be unique impurities, or the aging of the solvent may change the performance.

With regard to the situation you mentioned, I have observed a similar phenomenon with another chlorinated solvent. After using chloroform for a normal-phase separation, the HPLC was switched to a reversed-phase system and the methanol-water mobile phase that eluted from the system had a slight green color. The column was removed and the HPLC was placed in chloroform; when I switching over to methanol-water, the green color reappeared, so the source of the problem was not the column. The hypothesis we developed was that chloride in the chloroform was attacking the stainless steel and forming iron chloride. In chloroform, the metal complex was not soluble, but on introduction of a solvent that dissolved the complex, it washed off and out of the system. The metal attack was probably initiated by the presence of small amounts of phosgene, which is created by oxidation or degradation of the chloroform. Changing to another bottle of chloroform and repeating this experiment did not result in a green color. This was additional support that the chloroform was the source of the green color.

A recent article (1) noted peak shape problems with varying retention times with the use of HPLC-grade dichloromethane on a silica gel column. The investigator suspected the solvent and extracted the dichloromethane with water whereupon the aqueous phase became strongly acidic. The investigator postulated this solvent contained hydrochloric acid, which likely was formed by the hydrolysis of phosgene present in the dichloromethane as a contaminant. Another bottle of solvent from a different lot immediately cleared up the problem.

Also, the investigator purified the "bad" dichloromethane by treatment with Florisil. While this report did not mention a green color, it does support the hypothesis that the source of your problem was a "bad" batch of dichloromethane. If you still have the solvent available, go through the same experiment I mentioned and see if the green color is present and then repeat the experiment with another bottle of solvent from a different batch.

A chromatographer should always purchase the best purity possible at the best price. Further, it is important for a chromatographer to understand what the term *HPLC-grade* means in terms of the level of merit. Solvent purity should never be taken for granted. If a problem exists, the operator should troubleshoot the situation, and if the solvent appears to be the problem, the chromatographer should contact the manufacturer and determine what can be done.

References

1. J.J. Kirkland. Experiences with HPLC-grade solvents. *J. Chromatogr.* **715:** 199-200 (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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