Liquid Chromatography Problem Solving and Troubleshooting

Question:

I am aware that amines are commonly encountered as impurities in methanol and acetonitrile, and that this type of impurity can significantly influence my reversed-phase chromatography. Are there other impurities that I should be aware of?

Answer:

Solvent impurities can play havoc with chromatography, and their presence makes troubleshooting difficult for several reasons. First, because they are trace impurities, they are often below the testing abilities of the analytical method that was used to measure the solvent specifications; therefore, only the "analysis" of the presence of an impurity affects chromatography. This means that a trace impurity will only be noticed when it causes a problem with the chromatography; its impact is not immediate but will occur sometime after it is introduced. Additionally, the impurity may only affect certain analytes in a mixture, which further complicates troubleshooting. A solvent may be perfectly good for one analysis, but that same "good" solvent may be quite inappropriate for a second analysis. Keeping appropriate records of when mobile phases were made and which solvent lot was used is very important for identifying solvent-induced problems.

Certainly, as you point out, the most worrisome type of impurity commonly encountered in solvents used for reversed-phase chromatography is an amine. Because the amine can absorb onto the stationary phase and is positively charged, it will influence the retention time and peak shapes of basic analytes. The difference between chromatographic behavior when using methanol containing an amine impurity and the behavior using impurity-free methanol is quite dramatic (1). I might point out that if you were analyzing for a neutral molecule, this trace impurity would not be a problem.

Specifically in response to your question, what you are analyzing will determine whether or not an impurity will be a problem. For example, another impurity which has been identified in methanol is formaldehyde (2). In this specific example (2), the reported level of active material, the diuretic bendroflumethiazide, was lower than expected and a drug impurity was present. These observations were difficult to accept as correct in light of prior testing of the drug and drug product. There was no change in the chromatography of the peak, nor was there a retention time shift. After checking the HPLC, the column, etc., a solvent impurity, formaldehyde, was hypothesized to have caused the loss of peak area due to a reaction with the drug, forming a side product ("the drug impurity") that eluted at a later time, resulting in the low assay. The solvent lot was assayed and was found to contain 94 ppm formaldehyde. When "good" methanol was used for the HPLC assay, the result was a higher level of the diuretic.

Re-creation of the interaction between formaldehyde and bendroflumethiazide was tested by dissolving equal amounts of the drug in volumes of "good" methanol and "good" methanol with 40 ppm formaldehyde. The solutions were mixed and stored overnight. Only the formaldehyde-spiked drug solution showed a lower peak height and a small second peak.

In addition to the two impurities mentioned, there is no doubt that other impurities in solvents are potential problems. However, if the observations are never reported in the literature, we never learn of them. Other times, only a hypothesis of the contaminant is developed and the cuprit (trace impurity) is never completely proven to exist (3). I am sure we have all heard verbal reports of problems which are resolved by using another source or new batch of solvent. Only with publications of these situations will we become more aware of the problems and the solutions to solvent-induced problems.

If you have an example of a solvent-induced problem which you would like to share with other chromatographers, send it to me care of this journal.

References

1. B.A. Bidlingmeyer. Liquid chromatography problem solving and troubleshooting. *J. Chromatogr. Sci.* **30:** 287 (1992).
2. H. Edelstein and P. Olkolvotch. A forum for chromatographers. *J. Chromatogr. Sci.* **21:** 288 (1983).
3. B.A. Bidlingmeyer. Liquid chromatography problem solving and troubleshooting. *J. Chromatogr. Sci.* **34:** 351 (1996).

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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