Liquid Chromatography Problem Solving and Troubleshooting

Question:

I have a method using the ion pair (IP) reagent octane sulfonic acid in an acetonitrile–water (pH 3) mobile phase. I would like to clean the column of this mobile phase and use another mobile phase on this column, but my colleagues tell me that once you use a column with an IP reagent, the column cannot be used in any other system. Is this true? How should I clean the column?

Answer:

I too have heard that once you use a column with an IP reagent, the column must by dedicated to this system, because the reason for this is that the IP reagent cannot be removed from the stationary phase. I consider this an opinion rather than an absolute. Certainly, if you can afford to dedicate a column to a particular mobile phase, this is always the best option. However, if you need to use one column with several different mobile phases, cleaning is indeed possible. Also, remember that if you use an aggressive mobile phase that attacks the stationary phase or underlying silica, the column will change. IP reagents are used at either pH 1–3 or pH 7–8; these pH values are considered aggressive to some stationary phases.

Before I discuss how to clean the column, it is important to understand a little about how the IP reagent works in the retention mechanism. HPLC separations that use an IP reagent obtain retention of otherwise nonretained compounds because the IP reagent is adsorbed onto the stationary phase, which enables the stationary phase to attract and hold the oppositely charged analyte ions. A good discussion can be found elsewhere (1). To accomplish the goal of cleaning the column and removing the IP reagent, one needs to solublize the reagent off of the column while not precipitating the reagent and buffer salts in the system. Thus, the cleaning is a stepwise process, rather than a matter of just increasing the solvent strength of the mobile phase. Also, removing an IP reagent will depend upon whether it is a sulfonic acid type, as yours is, or an amine-containing IP reagent. Each has a slightly different strategy.

Specifically for your IP system that contains a sulfonic acid, I have found that washing first with the same mobile phase without the IP reagent is a good idea. By keeping the mobile phase organic–water ratio constant, there is no risk of precipitation of the buffer. After flushing with the mobile phase without IP reagent, prepare a solution of the mobile phase without buffer and wash the system. In your case, this mobile phase would be acetonitrile–water. Because many IP reagents are more soluble in methanol, my recommendation would be to prepare a methanol–water (50:50, v/v) mobile phase and do another wash of the column with this solution. Each wash should be 20 column volumes. Generally, this should be sufficient to have flushed out the IP reagent. If you are using a longer-chained IP reagent, then it may be necessary to go to a very strong solvent like tetrahydrofuran–water (80:20). This procedure should remove all IP reagent from the column.

But what if you have removed all of the IP reagent from the column, and the column does not work as it did before in the non-IP mobile phase? The major risk in using a column after cleaning off the sulfonic acid IP reagent is that when you used the column at pH 3 (or lower pH values), there was some loss of stationary phase due to the acid cleavage of the bonded phase or the endcapping reagent. This can be a slow process or a fast process, or not even occur, depending upon the type of stationary phase that you purchased. Unfortunately, most endcapped columns will lose the endcapping reagent quite quickly in acid mobile phase (2). So, if you find that the column changes before and after being used with an IP sulfonic acid reagent, it is probably the column that has changed and not due to the IP reagent not being removed. I believe that this is the origin of the perception: "Once a column has been exposed to a mobile phase containing an IP reagent, you can't wash off an IP reagent." It may not be IP reagent that is remaining, but the column that has changed in usage.

Therefore, as I mentioned above, to attain the best results and maximize the useful column life of a bonded-phase column, you should dedicate a column to IP use. At the minimum, you should dedicate a column for use with only IP sulfonate reagents. Changing from an IP system to a non-IP system may exhibit changes, but this is probably because the stationary phase has changed, not because the IP reagent was not removed.

References

1. B.A. Bidlingmeyer. Separation of ionic compounds by reversed-phase liquid chromatography: an update of ion-pairing techniques. *J. Chromatogr. Sci.* **18:** 525–39 (1980).
2. J.J. Kirkland and J.W. Henderson. Reversed-phase HPLC selectivity and retention characteristics of conformationally different bonded alkyl stationary phases. *J. Chromatogr. Sci.* **32:** 473–80 (1994).

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