Liquid Chromatography Problem Solving and Troubleshooting

Question

In a previous article, you discussed the way to clean a reversed-phase column after using a mobile phase containing an alkyl sulfonate Ion-Pair (IP) reagent. My question is how can I clean my column having an alkyl amine IP reagent? Is the same procedure you described for the sulfonate IP reagent applicable to an amine IP reagent?

Answer

Before I discuss how to clean off the column it is important to restate how the IP reagent contributes to the retention mechanism of the analytes. HPLC separations that use an IP reagent do so to obtain retention of oppositely charged compounds to the IP reagent. These compounds because they are charged have insufficient retention in a non-IP system. The IP reagent is adsorbed onto the stationary phase and as a result enables the stationary phase to attract and hold the oppositely charged analyte ions. A good discussion of the mechanism can be found elsewhere (1).

The strategy is the same, but the procedure is different. There are some distinct differences between using an alkylamine IP reagent and an alkyl sulfonate reagent. As I mentioned in the earlier article, it is more appropriate, if you can afford it, to dedicate a column to a particular IP mobile phase. But, if you need to use one column with several different mobile phases, cleaning is indeed possible. However, remember that if you use an aggressive mobile phase that attacks the stationary phase or underlying silica, the column will change. In the case of using an amine as an IP reagent, these reagents are basic and are somewhat corrosive to the analytical column because it tends to dissolve the base-silica particle. Having made a note of this, it may be more difficult to "clean" the column. I will return to this aspect after I discuss the cleaning process.

The goal of cleaning the column and removing the IP reagent needs to be solubilizing the reagent off of the column while not participating the reagent and buffer salts in the system. Thus, the cleaning is a stepwise process rather than just increasing the mobile phase solvent strength. Specifically for your IP system (which contains an amine), the pH usually is on the basic or slightly basic side, and this may complicate matters (I will discuss this point later). The reagent can be removed by first washing the column with the same mobile phase without the IP reagent. By keeping the mobile phase organic water ratio constant, there is no risk of precipitating the buffer in the column or HPLC. After this flush is completed, prepare a solution of the mobile phase water with an acid phosphate buffer at pH 3 and wash the system. This step is done due to the fact that because most amines are protonate at this pH, they will be ionized, and also the silica will favor being in the non-ionic state so that the surface will be in the SiOH form. Because of this, there will be minimum or no electrostatic forces acting to bind the amine to the surface. Another eluent made up of only organic water with no buffer is now prepared. Because many IP reagents are more soluble in methanol, my recommendation would be to prepare a methanol–water (50:50, v/v) eluent, and flush 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 really strong solvent like tetrahydrofuran–water (50:50). This procedure will remove all IP reagent from the column.

This wash should have removed the entire IP reagent from the column. However, what if the column does not work as it did before in the non-IP mobile phase? Often this occurs and the user believes that the column was not "cleaned" of the IP reagent. This, however, is not necessarily true, and in fact, it is possible that a column can be completely cleaned of the IP reagent and the column will not behave in a non-IP system as it did previously. This is because the IP mobile phase supplied an environment that changed the nature of the column's surface.

Specifically, as I mentioned earlier, the major risk in using a column after cleaning off the alkyl amine IP reagent is that because you used the column at a pH greater than 7, there was risk that some change to the stationary phase from the dissolution of the underlying silica could occur (2,3). This can be a slow process or a fast process depending upon the type of stationary phase purchased (3). A densely bonded phase that is well-endcapped will be the best column to minimize the

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dissolution of the underlying silica (2,3). Additionally, a column that contains bonded phase on silica made from a sol-gel process has been reported to have longer life than bonded phases made from silica prepared by using the xerogel process (2,3). Therefore, if you find that the column changes before and after being used with an alkylamine IP reagent, it is probably the column that has changed and not the fact that the IP reagent has not been removed. I believe that this is how the perception "you can't wash off an IP reagent once a column has been exposed to a mobile phase containing an IP reagent" was established. It's not the IP reagent remaining that caused the behavior but the column that changed in usage when the IP reagent was present.

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, especially when using alkylamine IP reagents. At the minimum, one should dedicate a column for use with different IP amine reagents. Changing from one IP reagent to another of the same class should have only minimum variations. However, changing from an IP system to a non-IP system may exhibit changes, but not because the IP reagent has not been removed.

References

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