Liquid Chromatography Problem Solving and Troubleshooting

Question:

When should I use a cyano column?

Answer:

Cyano columns are usually prepared by bonding a cyanopropyl silane to the silica. This results in a column that has some polar functionality due to the CN group and some nonpolar functionality due to the propyl group. Thus, the column has intermediate polarity and is suitable for use in both the normal-phase and the reversed-phase modes. In the reversed-phase mode, the column is less retentive than a C8 column and somewhat similar in polarity to a C3 column for the retention of neutral compounds. This makes the column ideal for situations where there is too much retention using a C8 column. Additionally, the column may be useful when there is a need to pull polar compounds away from nonpolar compounds. An example is shown in the Figure. The neutral compounds are slightly retained and elute early in the chromatogram while the polar, basic compounds (propranolol [P] and amitriptyline [A]) are held on the column longer and elute at longer retention times well resolved from the other components in the mixture.

In the normal-phase mode, the cyano column is a less active polar phase than silica, which gives the user more flexibility in separation development. When silica is used in the normal-phase mode, the chromatographer must prepare mobile phases carefully to preserve the "activity" of the column. Specifically, the water in the mobile phase will adsorb to the surface silanols and influence the activity of the silanols. As the amount of absorbed water changes, the retention of the compounds on the silica column will also change. Because cyano is a bonded phase, it is more tolerant of trace amounts of water in the mobile phase; thus, the activity of the surface does not change significantly with variations in the water content. Since the water content is less critical, moderate amounts of polar organic solvents can be used as mobile phase modifiers to obtain different retention characteristics. This makes method development faster. Also, as a result of the bonded phase, gradient separations can be run without fear of "poisoning" the column with water or other polar additives. Furthermore, gradient re-equilibration in the normal-phase mode is quicker than that required when a silica phase is used. In short, the cyano column has much of the retentive character of the silica column, but it is more rugged and relatively insensitive to the water content of the nonpolar mobile phase.

Since the cyano column can be used in either mode, a wide range of mobile phases could be used. Switching between normal-phase and reversed-phase solvents requires proper washing with a solvent that is compatible with both a nonpolar solvent like hexane and a polar solvent like methanol. This requires careful operation and, if not done properly, can affect chromatographic performance. The resulting column problems can even include the collapse of the column bed structure. So, to facilitate usage and enhance the lifetime of the cyano column, many manufacturers supply the columns in two different types: one prepared and shipped in a solvent that is appropriate for use in the normal-phase mode and one that is suitable for use in the reversed-phase mode.

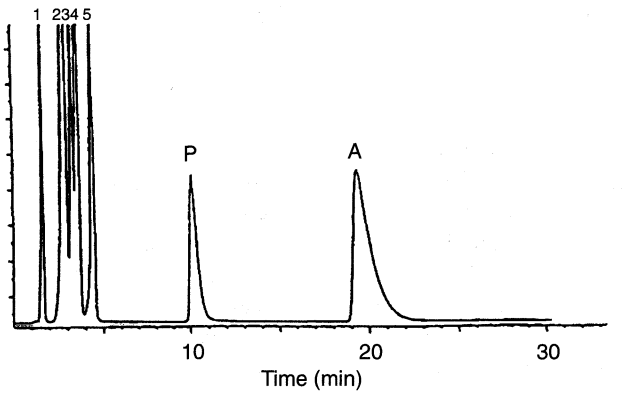

Figure. Separation of polar, basic compounds in a mixture with a cyano bonded phase column. Conditions: mobile phase, 20mM potassium phosphate (pH 7.0) and methanol (35:65); flow rate, 1 mL/min; detection, 254 nm. Compounds: 1, uracil; 2, butylparaben; 3, naphthalene; 4, dipropyl phthalate; 5, acenaphthene; P, propranolol; and A, amitriptyline.

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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