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Acetonitrile Shortage - Is It Time to Put Your HILIC Columns Away?

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The cost of HPLC grade acetonitrile recently skyrocketed from $10/L to over $70/L, with shortages being an issue too. These events are pressing many companies to revise approaches to method development and testing with the emphasis of reducing acetonitrile consumption.

HILIC and mixed-mode chromatography are well known techniques for retention of polar compounds. HILIC is considered one of a few variations of normal phase chromatography. A typical mobile phase for HILIC chromatography includes acetonitrile with a small water content.

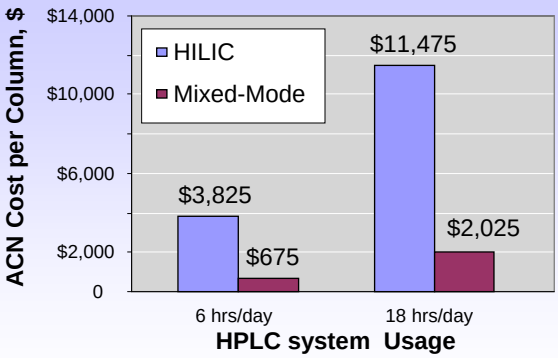

Fig. 1 ACN consumption in HILIC and mixed-mode chromatography

In HILIC, the analyte is distributed between the water-rich stationary layer and the mobile phase with low water contents. Mixed-mode is a variation of reversed-phase chromatography. In mixed-mode chromatography, compounds are retained by reverse-phase and ion-exchange mechanisms. Ion-exchange mechanism can add strong interaction for ionizable analytes and add synergy effect for reverse phase mechanism. This approach allows to readily switch from HILIC chromatography with high consumption of ACN (80-90%), to the very low concentrations of ACN (0-20%) used in mixed-mode chromatography. Based on the average content of ACN in HILIC and mixed-mode chromatography, companies can reduce consumption of ACN by 3x-5x without switching to narrow bore columns. The cost impact can be in thousand of dollars per each column/instrument (Fig. 1). The purpose of this study was to find low ACN content alternative HPLC methods for retention of polar analytes in comparison to HILIC methods

All methods were compared with mixed-mode approach requiring low ACN content. Comparison was done on mixture of amino acids and small basic drugs. Mixed-mode methods show better selectivity and much lower consumption of ACN (Fig. 2 and 3)

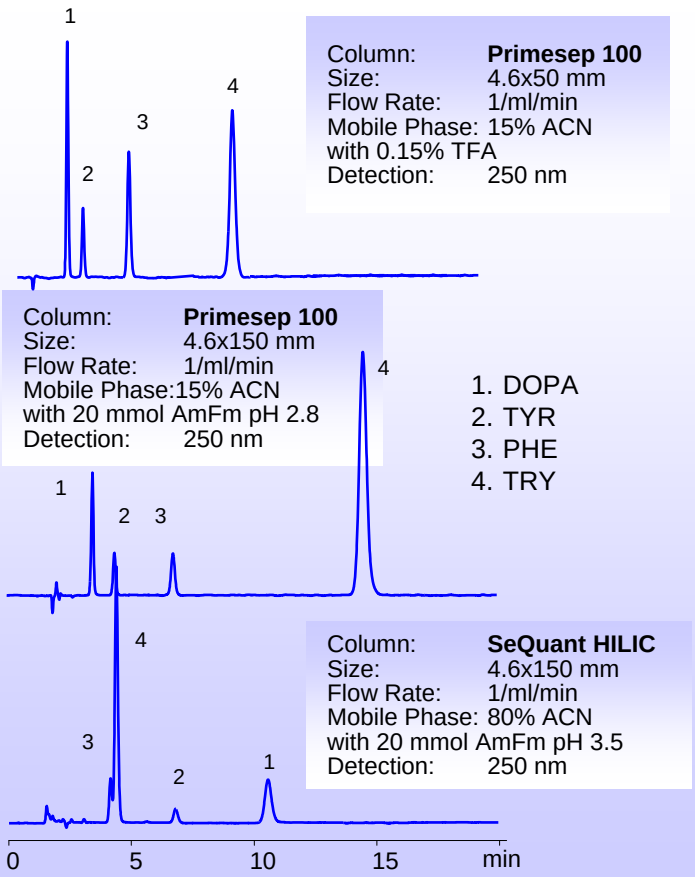

Fig. 2 Retention of Amino Acids in HILIC and mixed-mode chromatography

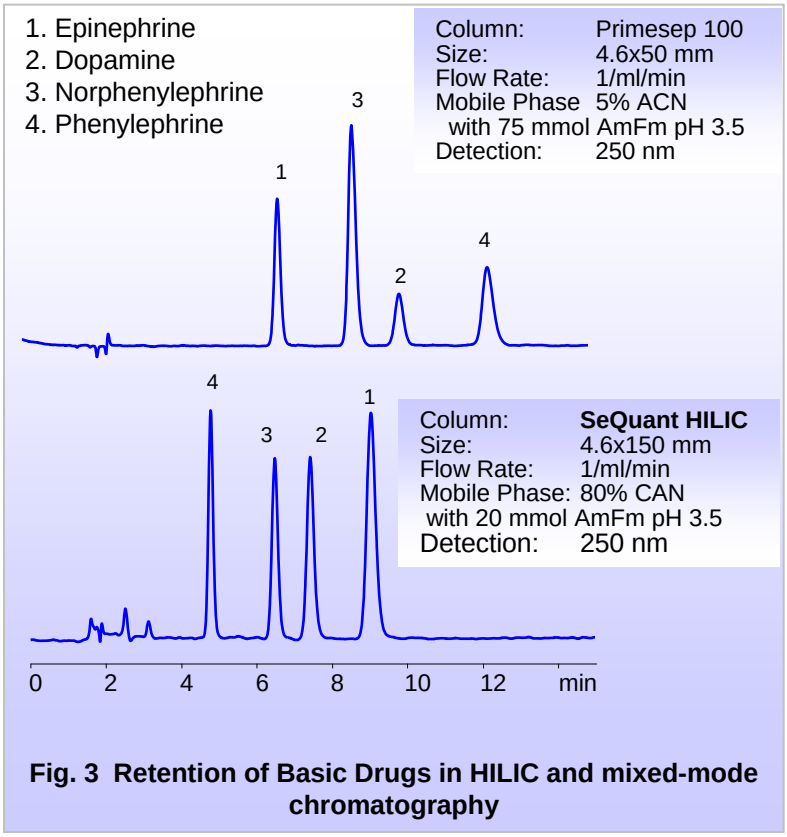

Fig. 3 Retention of Basic Drugs in HILIC and mixed-mode chromatography

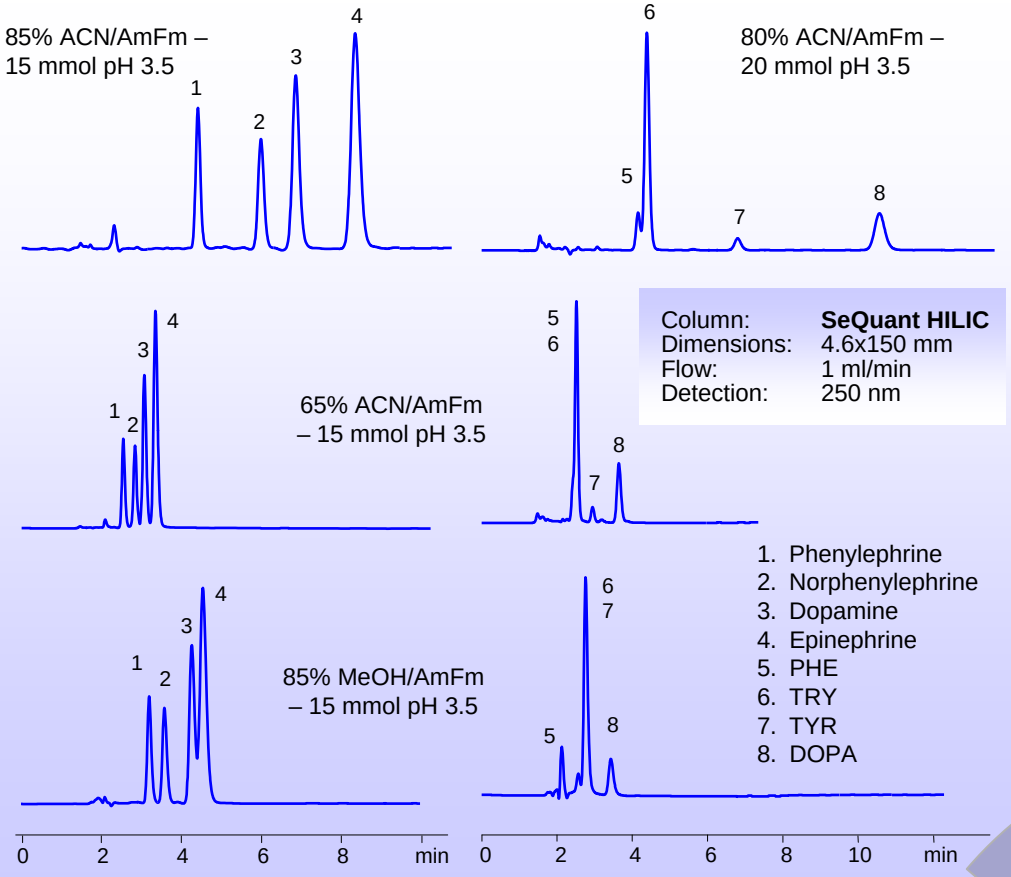

Fig. 4 Effect of ACN/MeOH concentration on retention of amino acids and basic drugs

Addressing acetonitrile consumption sounds like a reasonable effort, even when cost impact on the overall analysis is not great. When methods move to validation and QC, significant savings can be achieved at productions sites. Also reducing amount of ACN can save money on stage of method development, when chemists try different conditions and different HILIC columns.

An example of the analysis of four drugs on Fig. 4 gives up to 90% saving in acetonitrile consumption when converted to a mixed-mode method on Fig. 5.

Some of the general approaches to reduce consumption of acetonitrile in HILIC are:

1. Reduce content of acetonitrile in the mobile phase: Unfortunately for a lot of polar compounds no retention is observed on HILIC columns when acetonitrile content is reduced below 65% (Fig. 4).

2. Replacement of ACN with alcohols, THF or other solvents: This approach is not practical for HILIC columns (Fig .4), and in some case of methanol drastic change in retention and selectivity occurs. Replacement of ACN with THF or dioxane can damage PEEK tubing and suppress ionization in mass spectroscopy. Other solvents might be not mixable with water (ethyl acetate) or have high UV activity (acetone).

3. Go to the smaller bore columns: Smaller bore columns require optimization of detection cell of detector, solvent lines and connections in order to reduce peak broadening. This approach demands changing, or even replacing, parts of the HPLC system.

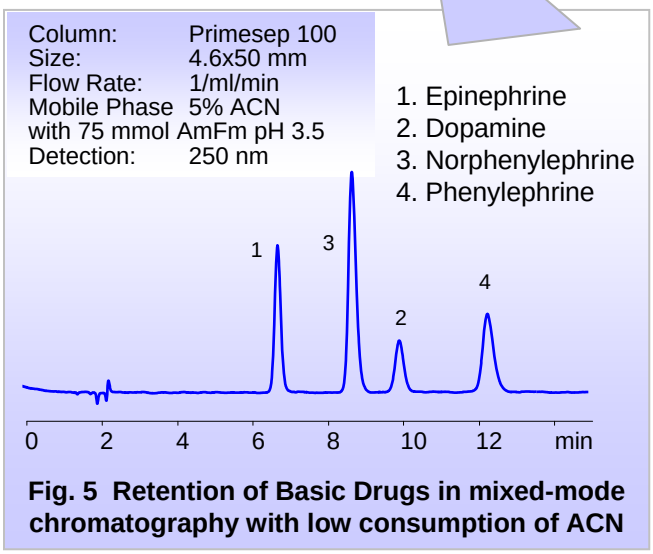

Fig. 5 Retention of Basic Drugs in mixed-mode chromatography with low consumption of ACN

Examples for HILIC (Fig. 4) show that in many cases it is impossible to reduce amount of ACN. If you consider other drawbacks of HILIC chromatography, you might want to reconsider your initial steps when choosing column and approach to method development and switch to mixed-mode columns.