Benefits of Hydrogen in Liquid Chromatography. Introducing SHARC1 HPLC Columns.

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The use of hydrogen bonding interactions as a primary retention mechanism in chromatography is usually associated with gas chromatography. However, we have recently developed stationary phases which benefit from the presence of hydrogen in the molecule and offer the possibility for hydrogen-bonding chromatography in the liquid phase.

Hydrogen-bonding chromatography has been neglected in favor of reversed-phase, HILIC, ion-exchange, normal phase and other chromatographic modes. Stationary phases rarely achieve retention and separation of analytes based purely on a single mechanism and SIELC Technologies have developed many commercial stationary phases benefiting from the ability to exploit multiple interactions in a controlled way. Hydrogen bonding, is omnipresent in each of the techniques listed above, especially in normal phase chromatography.
The contribution of hydrogen bonding can be significant. SHARC-1 (**S**pecific **H**ydrogen-bond **A**dsorption **R**esolution **C**hromatography) is the first column intentionally designed to achieve separation based entirely on the analytes ability to act as a hydrogen atom donor or acceptor.

Unique Mobile Phase, but Common Solvents.

SHARC1 column operating conditions are unique. Solvents used for SHARC separations are acetonitrile (MeCN) as the weak solvent and methanol (MeOH) as the strong solvent. Pure MeCN has a very insignificant degree of hydrogen bonding with the SHARC stationary phase while MeOH interacts strongly, reducing retention of analytes capable of hydrogen interactions. By altering the ratio of MeCN/MeOH, the optimum retention profile can be obtained for many types of molecules with high selectivity, good peak shape, efficiency, and speed.

SHARC1 Methods and Applications

Hydrogen-bonding interaction offers unique selectivity based on number of “interaction points” available for hydrogen bonding. Not every hydrogen bond participates in intermolecular hydrogen interactions with the stationary phase. Therefore the proximity of “interaction points” to each other within a molecule also needs to be considered since molecules can form intramolecular hydrogen-bonds, which compete with intermolecular interactions between analyte and stationary phase. Intramolecular interactions reduce retention time in hydrogen-bonding mode and such structural factors provide unique selectivity among structural isomers, homologs, degradation products and precursors.

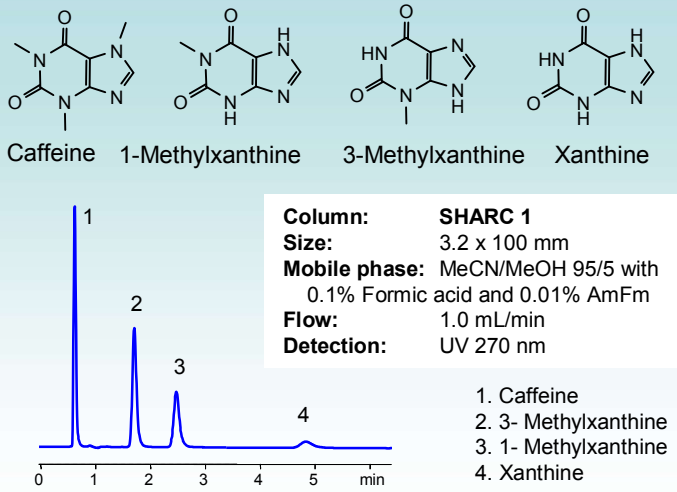

Fig. 2. The separation of caffeine, 3-methylxanthine, xanthine, and 1-methylxanthine.

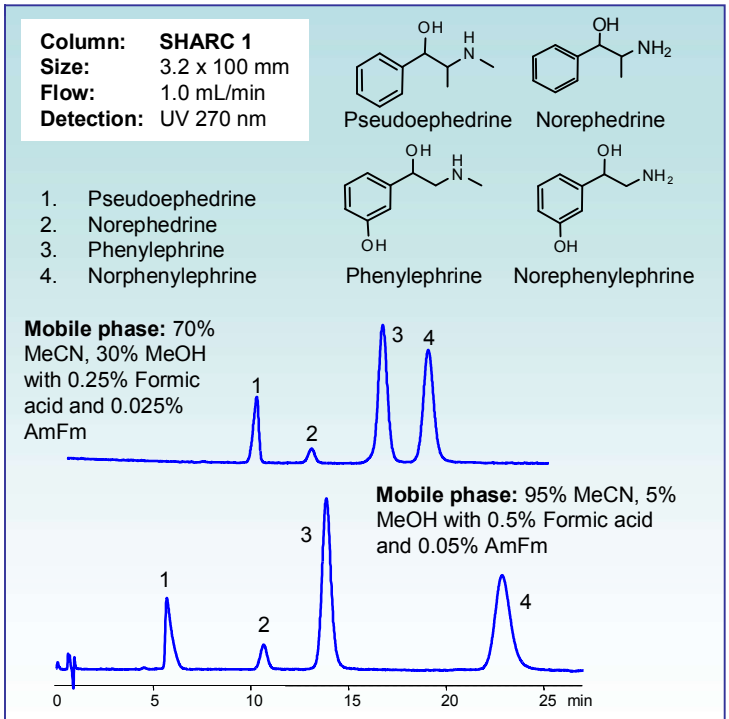

Fig. 1. The effect of the mobile phase composition on the separation of pseudoephedrine, norephedrine, phenylephrine, and norphenylephrine.

Xanthines are good candidates for separation by hydrogen bonding chromatography with SHARC1 (fig. 2). In caffeine, three of the nitrogens have substituted methyls, which makes these groups unavailable for hydrogen bonding interaction with the stationary phase , whereas 3-methyl-, and 1-methylxanthine only have one substituent and thus are retained longerand Xanthine, which has no substitution on the nitrogen, is retained the longest.

In another example, a mixture of 4 neurotransmitters (fig. 1) were separated based on their ability to form hydrogen bonds with the SHARC 1 stationary phase. Pseudoephedrine and norephedrine have 2 interaction points, phenylephrine and norphenylephrine have three. The presence of N-methyl substitution reduces hydrogen-bonding interaction of nitrogen functional groups and reduces retention. This approach can be used for the separation of unsubstituted and N-substituted amines. Accessibility of hydrogen-donors or hydrogen-acceptors play a critical role in how compounds are retained and eluted from SHARC column. Longer retention is achieved with more accessible interactions and offers a wider range of mobile phase conditions to optimize a separation.

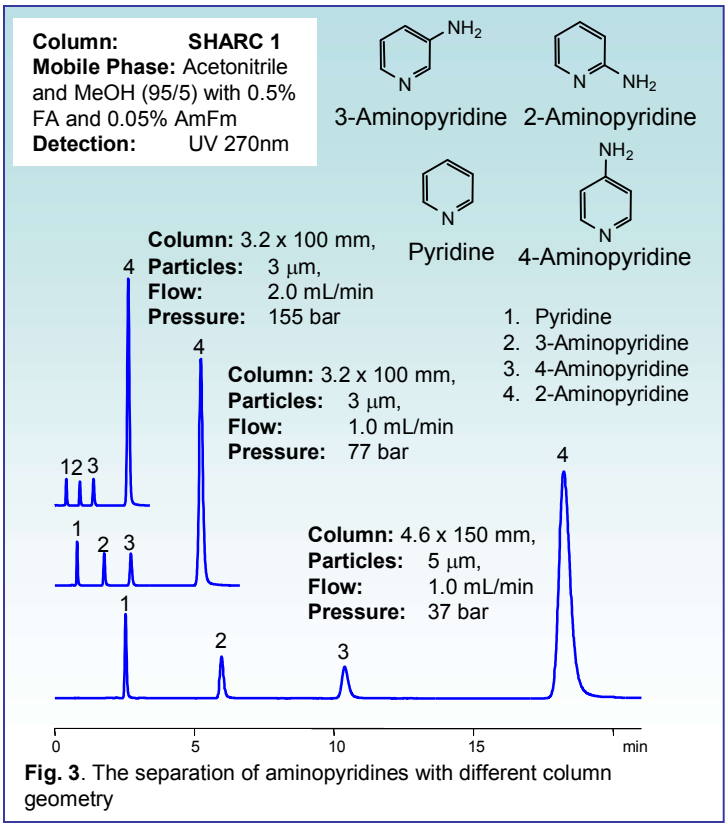

Fig. 3. The separation of aminopyridines with different column geometry

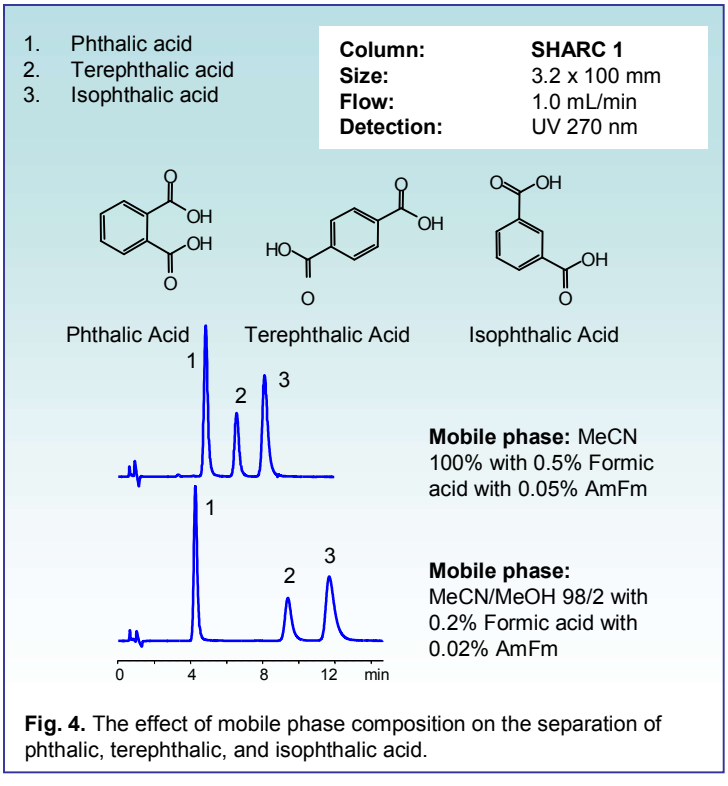

Fig. 4. The effect of mobile phase composition on the separation of phthalic, terephthalic, and isophthalic acid.

Isomers of aminopyridine were separated by hydrogen-bonding chromatography (fig. 3). The difference in retention time was attributed to accessibility of hydrogen-bonding sites.

The separation of phthalic acid isomers (fig. 4) is an example which demonstrates the effect of intramolecular bonding (phthalic acid). It is less retentive compared to the other isomers (terephthalic and isophthalic acids), which do not form internal hydrogen bonds.

Hydrogen-bonding proved to be a good approach to retain and separate simple organic acids (fig. 5). The mobile phase for the separation of acids typically contains acetonitrile/methanol and ammonia or triethylamine as an additive. The mobile phase is fully MS-compatible and the presence of ammonia allows high sensitivity in LC/MS.

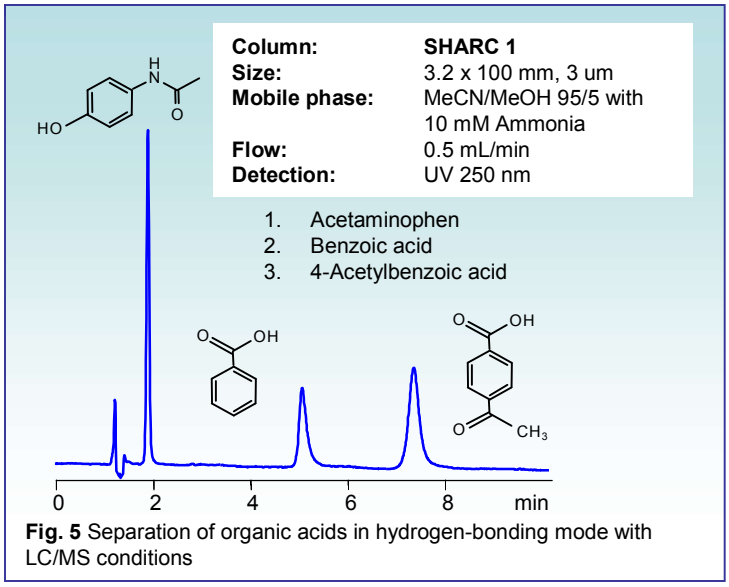

Fig. 5 Separation of organic acids in hydrogen-bonding mode with LC/MS conditions

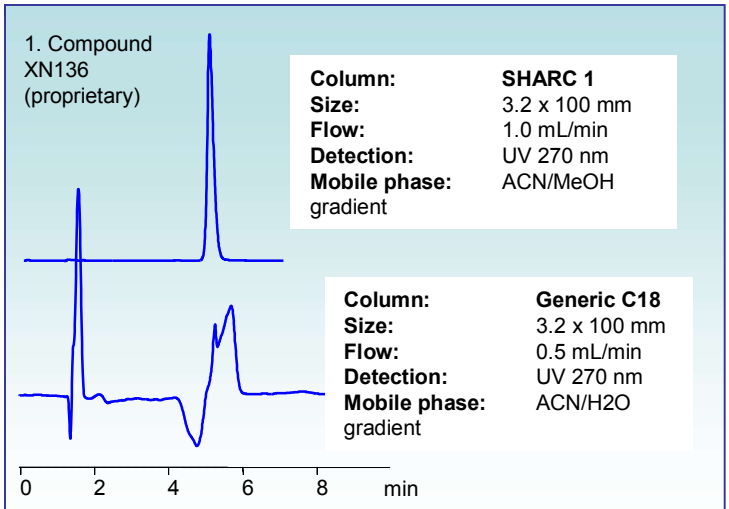

Fig. 6. The effect of the column and mobile phase composition on analysis of water-sensitive compound

The separation of phthalic acid isomers (fig. 5) is another example of the separation of compounds with identical numbers of interaction sites. This example also demonstrates that when intramolecular bonding exists (phthalic acid) compounds are less retentive compared to the other isomers (terephthalic and isophthalic acids).

Hydrogen bonding separation can be demonstrated with Triton X100, where degree of retention is correlated to the amount of oxygen in the molecule. This approach is suitable for other surfactants with different alkyl chain length substituents. The Hydrogen-bonding approach can also be successfully used when analytes are water sensitive. Employing non-aqueous mobile phases keep water-sensitive compounds intact (fig. 6). When developing a method using SHARC conditions, reactivity of analytes with alcohols must be considered.

Additional benefits

Fast Analysis due to Low Back Pressure

MeCN/MeOH mixtures have 2-3 times lower viscosity than water/MeOH or water/MeCN mixtures (fig. 7). As a result, smaller particles for column packing can be used without an increase of working pressure compared to conventional HPLC. Separation UPLC-like conditions can be easily obtained on standard HPLC instruments with a 2-3 times higher eluent linear velocity using columns with smaller particle size. Increases in analysis speed of up to 5 times are routinely achieved using this combination (fig. 3). Use of UPLC (RRLC, UHPLC) equipment allows a further increase in analysis speed of 5-6 times.

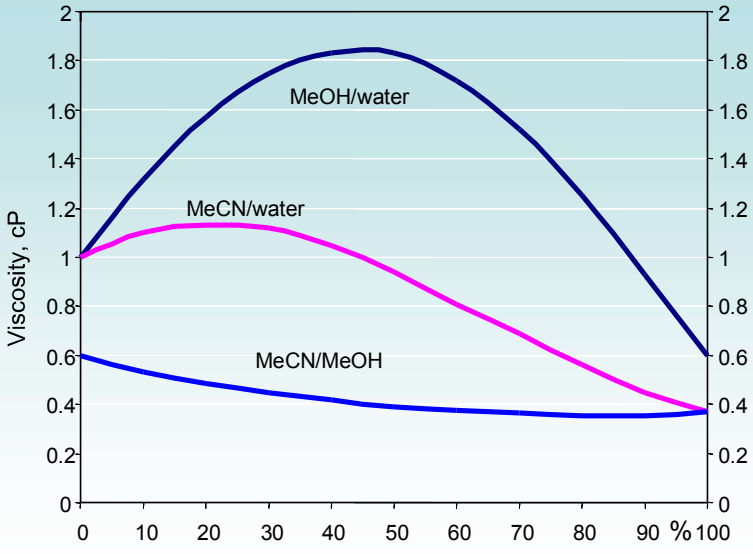

Fig. 7 Viscosity profile of mixtures MeOH/water, MeCN/water, and MeCN/MeOH

Solubility of Analytes

MeOH is one of the most universal solvents for organic compounds. Combinations of MeOH with MeCN will dissolve almost any molecules with very high or very low polarity. Highly hydrophobic molecules such as surfactants, lipids and oil soluble vitamins are easily soluble in this solvent combination. Very polar molecules such as sugars, diols, salts of amino compounds and carboxylic acids also can also be efficiently dissolved in these solvent systems. Other organic solvents can be used as diluents without affecting the separation.

LC/MS and Preparative Chromatography Compatibility

MeCN/MeOH mixtures have a low boiling point and are much easier to evaporate than water. As result, this solvent system is much more convenient for preparative chromatography. Additionally, the benefit of the low viscosity eluent systems allows preparative separations with higher throughput/flow rate. These eluents are MS friendly, which enables mass directed preparative strategies. In most cases isocratic methods can be used due to the high selectivity of the column which permits eluent recycling - minimizing solvent consumption. High organic content of the mobile phase guarantees high sensitivity in MS applications.

Alternative Selectivity

Since hydrogen bond formation is very specific in terms of interaction energy and strongly depends on molecule geometry as well as the number and position of functional groups, the separation of similar molecules such as isomers or oxidation or reduction products can be achieved in SHARC with high selectivity. A wide range of molecules can be analyzed with the SHARC technique. Practically any molecule with functional groups containing oxygen and nitrogen can be retained and separated from similar compounds using this technology.

Contact SIELC for additional information on column performance, method development, ordering, and availability. Phone 847-229-2629; Fax 847-655-6079; Email: mail@sielc.com