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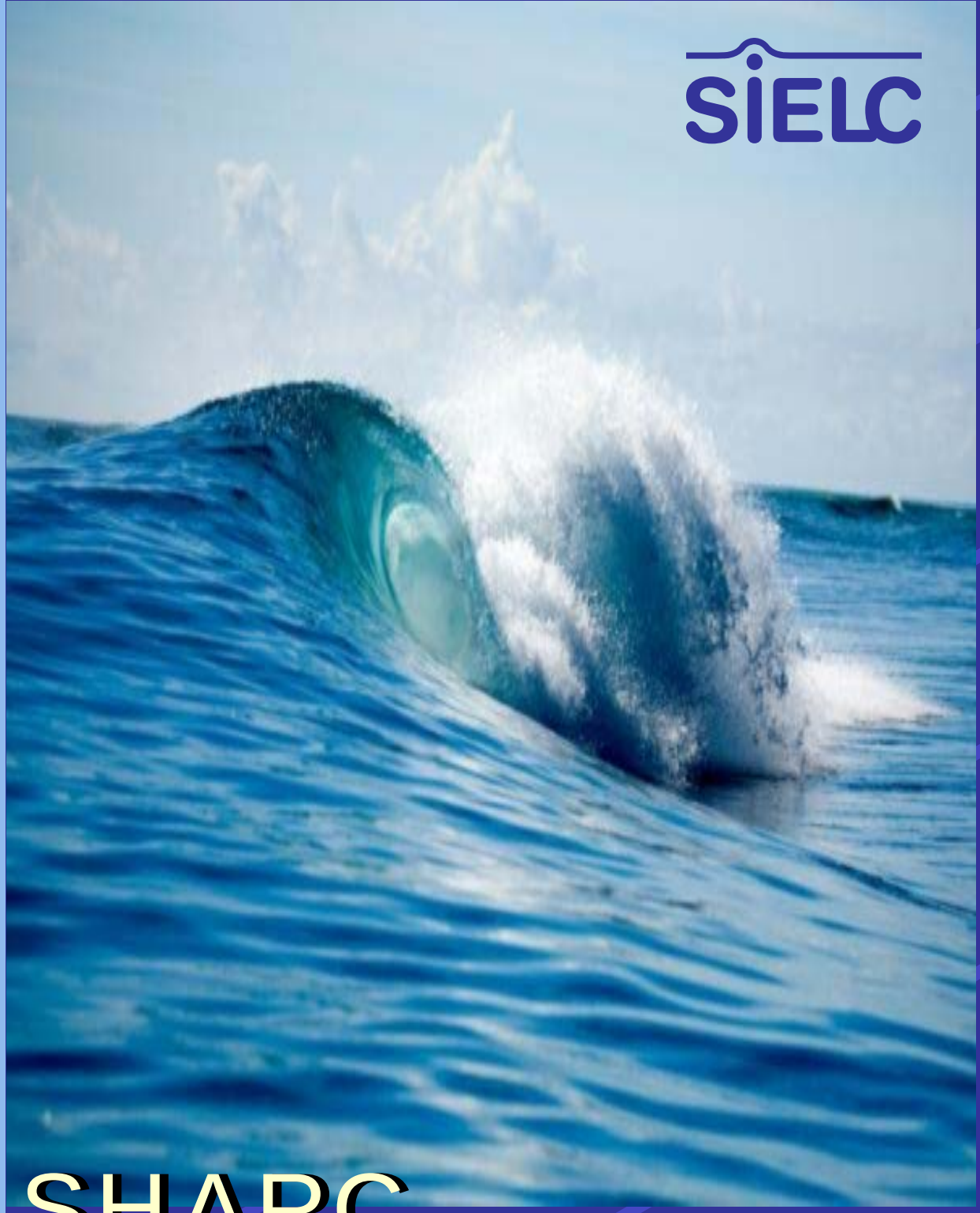


SHARC Separation Technology

LC Column with Hydrogen Bonding Based Separation

SHARC™ HPLC Columns

Specific **H**ydrogen-bond **A**dsorption **R**esolution **C**hromatography.

SHARC™ HPLC columns are the latest, innovative column from SIELC Technologies, the inventors of Primesep®.

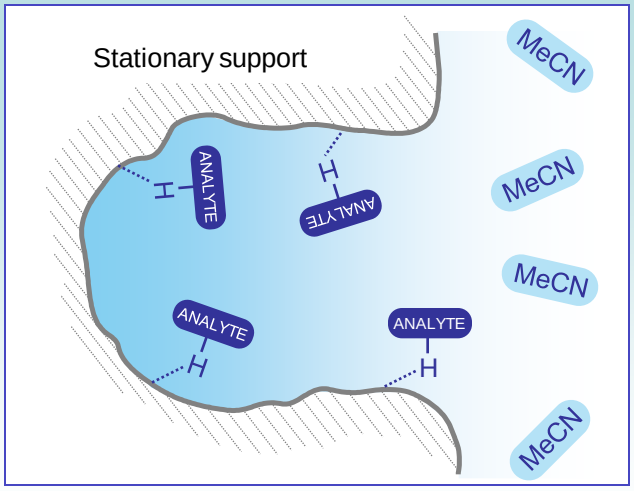
SHARC™ columns are the first commercially available in which the primary separation mode is hydrogen bonding and use the hydrogen bonding interaction of hydrogen atoms within the stationary phase with analyte molecules containing electronegative atoms such as oxygen, nitrogen, fluorine. This is typically a weak interaction, especially when the separation is performed in aqueous solution. However, used in conjunction with purely organic eluent systems, these hydrogen bonding interactions can be exploited to achieve alternative selectivity than that achieved with reversed phase, normal phase or other mixed mode separations.

Liquid chromatography separation modes have evolved as tools for the separation of different molecules based on their physico-chemical properties. The most common interactions in which can be exploited to achieve a separation include:

- Hydrophobic separation based on the degree of hydrophobicity of the molecule
- Ion-exchange separation based on the number, nature, and distribution of charges in the molecule.
- Normal phase separation based on hydrophilic properties of the molecules including molecule dipoles value and dipoles position
- Size exclusion separation (SEC) based on molecule size and shape

Stationary phases rarely undergo retention and separation of analytes based purely on a single mechanism. Hydrogen bonding, for example, is omnipresent in each of the techniques listed above and in some cases, especially in normal phase chromatography, the contribution of hydrogen bonding can be significant. SHARC-1 is the first column specifically designed to achieve separation based entirely on the analytes ability to act as a hydrogen atom donor or acceptor.

Technology



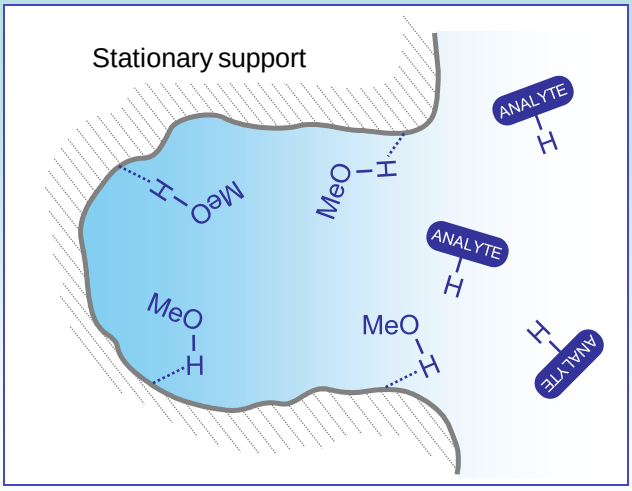


Fig. 1 SHARC surface interacts with hydrogen bonding analytes in acetonitrile (weak solvent) (a); methanol (strong solvent) competes with analytes for stationary phase surface sites (b)

SHARC-1 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 (fig. 1). 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.

O–H ····· :N	(29 kJ/mol or 6.9 kcal/mol)
O–H ····· :O	(21 kJ/mol or 5.0 kcal/mol)
N–H ····· :N	(13 kJ/mol or 3.1 kcal/mol)
N–H ····· :O	(8 kJ/mol or 1.9 kcal/mol)

Fig. 2 Hydrogen bond energy (J. Phys. Chem. A **111**/12, 2253)

Hydrogen bond energies are typically in range 30 kJ/mol or less (fig. 2) and depending upon the nature of the functional groups and their number and orientation in the molecule. This energy difference and orientation of active groups is the basis for the selectivity mechanism of the stationary phase.

Analytes can be retained on the stationary phase by more than one hydrogen bond and act as a donor or acceptor of the stationary phase hydrogen atom. Retention, therefore, strongly depends on the chemical nature of the stationary phase surface. The presence of a polarized hydrogen atom is not always enough to gain retention by hydrogen bonding mechanisms. An analyte may form intramolecular as opposed to intermolecular hydrogen bonds which preclude the stationary phase interaction (fig. 4).
The SHARC 1 column is a hydrogen atom acceptor type stationary phase showing increased retention toward molecules with higher numbers of polar X-H bonds such as alcohols, amines, acids, amides, phenols etc.

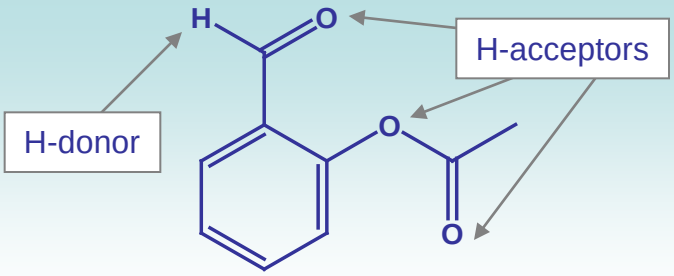


Fig. 3 Some molecules can act as both hydrogen atom donors and acceptors

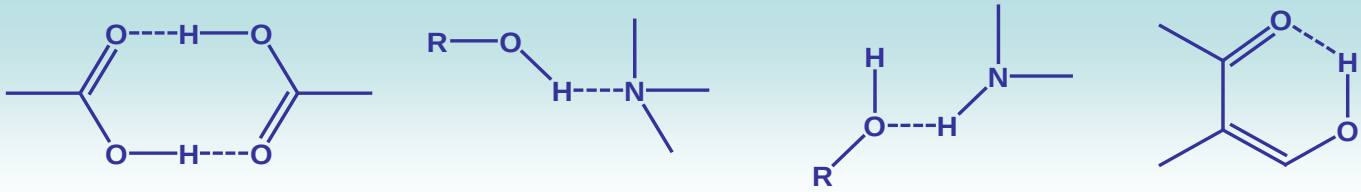


Fig. 4 Typical examples of intermolecular and intramolecular hydrogen bond formation with hydrogen containing functional groups

Advantages

SPEED

MeCN/MeOH mixtures have 2-3 times lower viscosity than water/MeOH or water/MeCN mixtures (fig. 5). 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 shorter columns with smaller particle size. Increases in analysis speed of up to 5 times are routinely achieved using this combination (fig. 10). Use of UPLC (RRLC, UHPLC) equipment allows a further increase in analysis speed of 5-6 times.

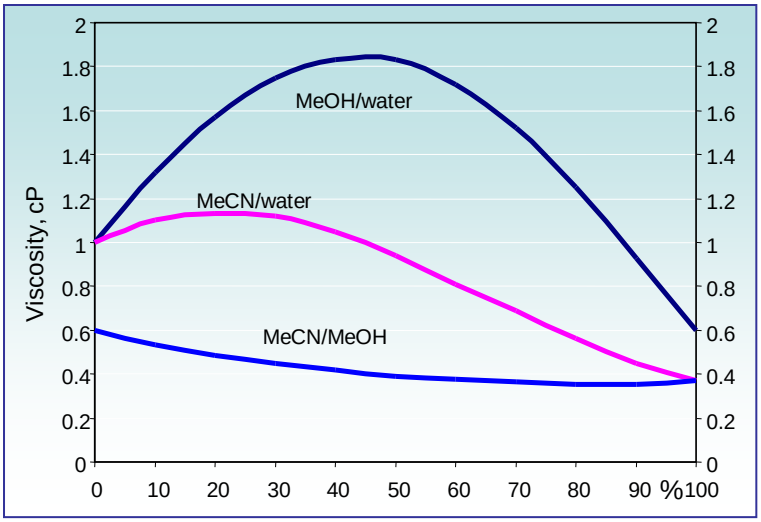


Fig. 5 Viscosity profile of mixtures MeOH/water, MeCN/water, and MeCN/MeOH

SOLUBILITY

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, di-ols, 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.

PREPARATIVE SEPARATION

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. Additional, the benefit of the low viscosity eluent systems allows preparative separations with higher throughput. 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.

WIDE RANGE OF COMPOUNDS

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.

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.

Hydrogen-bonding interaction offers unique selectivity based on number of “interaction points” available for hydrogen bonding. One of the useful characteristics to determine retention patterns in hydrogen-bonding mode is the molecular polar surface area (PSA). This calculated parameter is usually used for prediction of drug transport properties, but it has been successfully applied to hydrogen-bonding interactions. Polar surface area is defined as a sum of surfaces of polar atoms (usually oxygens, nitrogens and heteroatom-attached hydrogens) in a molecule. Since the polar atoms can participate in hydrogen-bonding interactions, an estimation of the elution order can often be made based on PSA. While PSA is a good indicator of retention, it must be noted that it does not account for the accessibility of hydrogen-interaction sites. Not every polar surface 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 an intramolecular hydrogen-bonding, which competes with intermolecular interactions between analyte and stationary phase.

Intramolecular interactions reduce retention time in hydrogen-bonding mode. Such structural factors provide unique selectivity among structural isomers, homologs, degradation products and precursors.

Since the SHARC 1 is a mixed-mode column, pKa is another useful parameter in method development for these columns. SHARC columns operate in a non-aqueous mobile phase, but some charge interactions will occur between the stationary phase and ionizable molecules and contributes to the retention profile

Hydrogen-bonding interactions were successfully used for the separation of drugs containing hydroxyl and amino groups (fig. 6). The strength of interaction depends on the number of interaction points and/or PSA as described above. Pyrilamine has four “interaction points”, however two of these points are not easily accessible.

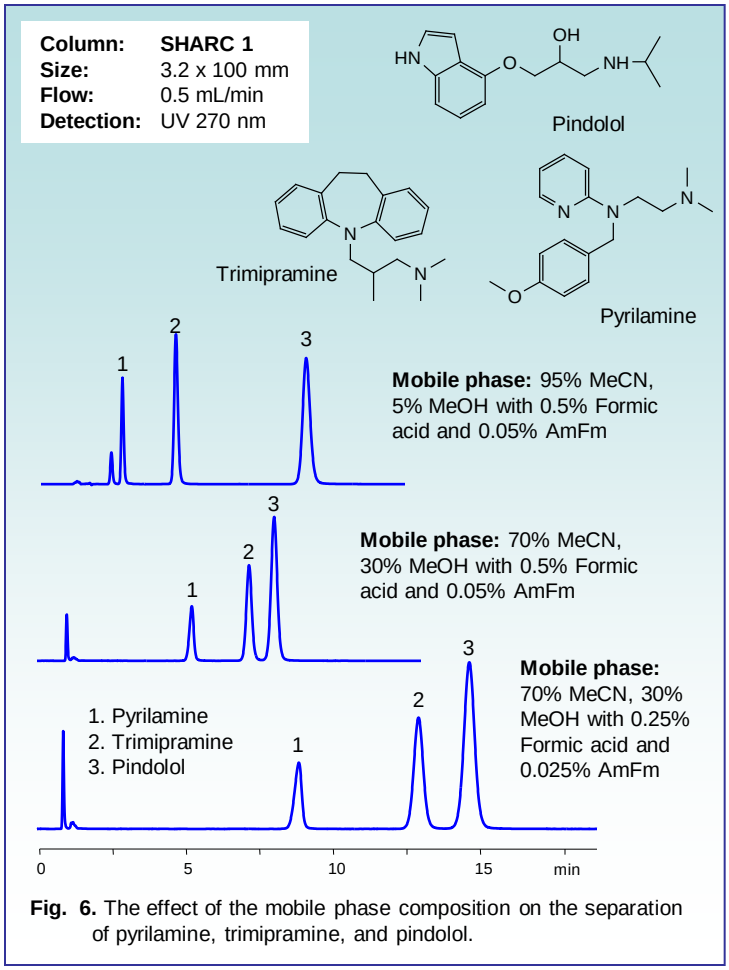


Fig. 6. The effect of the mobile phase composition on the separation of pyrilamine, trimipramine, and pindolol.

Thus, the compound has the lowest PSA (6.5). Pindolol has a much higher PSA value (57.3) with 4 interaction points which are more accessible, this causes pindolol to retain longer than trimipramine and pyrilamine (PSA value 42.9). Resolution and retention of compounds can be adjusted by variations in mobile phase.

In another example, a mixture of four neurotransmitters (fig. 7) were separated based on their ability to form hydrogen bonds with the SHARC 1 stationary phase. Elution order corresponds to number and strength of interaction points as well as PSA numbers for pseudoephedrine (32.3), norephedrine (46.3), phenylephrine (52.5) and norphenylephrine (66.5). 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.

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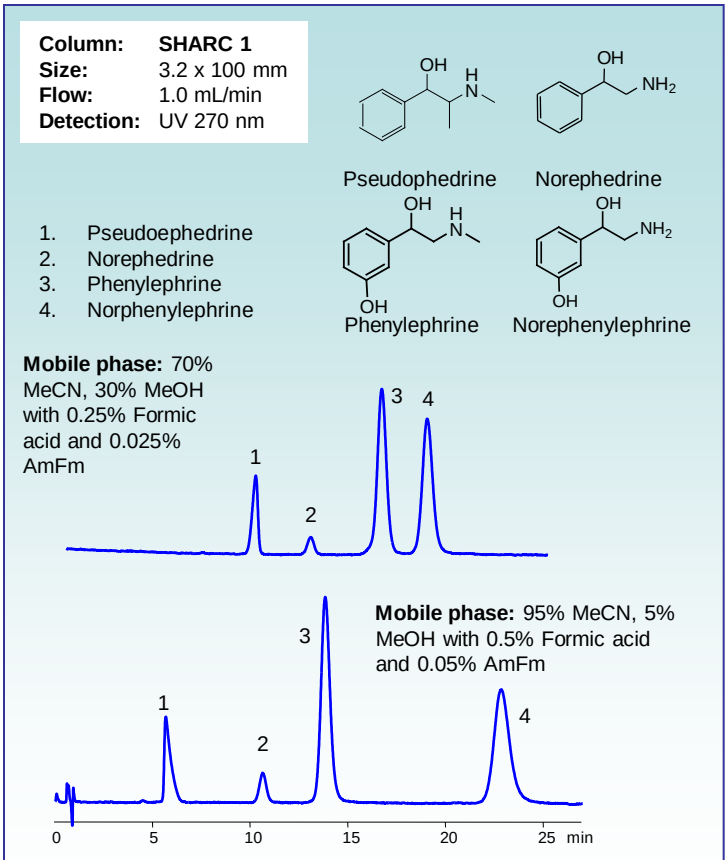


Fig. 7. The effect of the mobile phase composition on the separation of pseudoephedrine, norephedrine, phenylephrine, and norphenylephrine.

Xanthines are good candidates for separation by hydrogen bonding with SHARC1 (fig. 8). They are capable of forming multiple hydrogen bonding interactions. The theory of PSA and accessibility of groups explains the retention time and order of elution very well. Caffeine (PSA-61.8), 3-methylxanthine (PSA-83,6), 1-methylxanthine (PSA-83.6) and xanthine (PSA-94.4) eluted according to the PSA number. In caffeine, three of the nitrogens have substituted methyls, which makes these groups unavailable for hydrogen interaction. 3-methyl-, and 1-methylxanthine only have one substitution and thus are retained longer. Xanthine which has no substitution on the nitrogen is retained the longest.

Nucleobases, like xanthines, have a lot of interaction points due to the presence of multiple nitrogens and oxygens. Nucleobases in most cases elute according the number of interaction points and PSA value (fig. 9). Thymidine (3 interaction points, PS value of 104), uridine (4 interaction points, PS value of 124), adenosine (4 interaction points, PS value of 139), guanosine (5 interaction points, PS value of 159), and cytidine (4 interaction points, PS value of 139) were separated based on hydrogen-bonding properties.

Isomers of aminopyridine were separated by hydrogen-bonding. All the aminopyridines have the same PSA value (fig. 11). Therefore, the difference in retention time was attributed to accessibility of hydrogen-bonding sites and basicity of the analytes. The order of elution for aminopyridines can be changed by variation of the mobile phase.

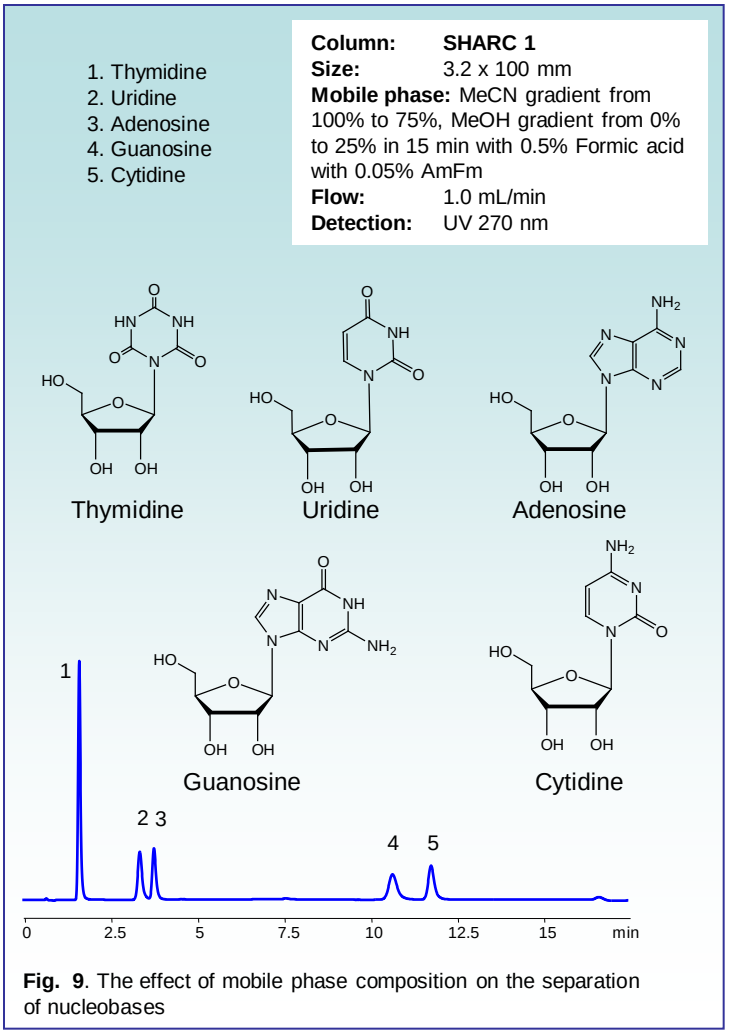


Fig. 9. The effect of mobile phase composition on the separation of nucleobases

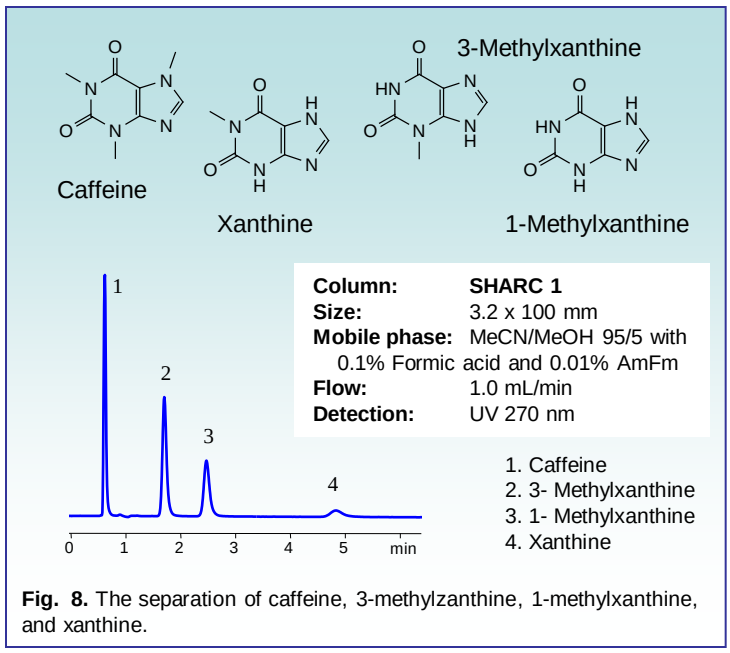


Fig. 8. The separation of caffeine, 3-methylzanthine, 1-methylxanthine, and xanthine.

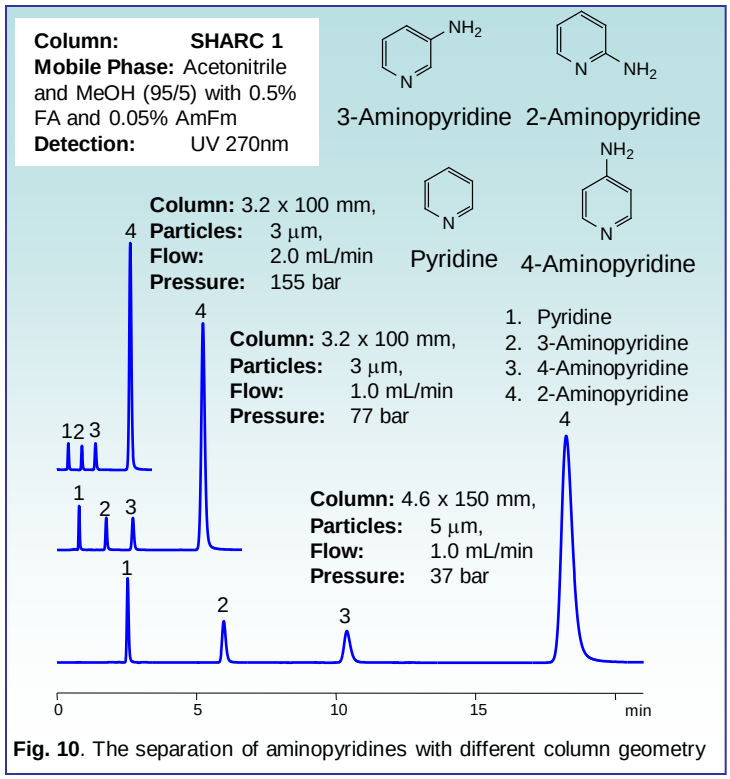


Fig. 10. The separation of aminopyridines with different column geometry

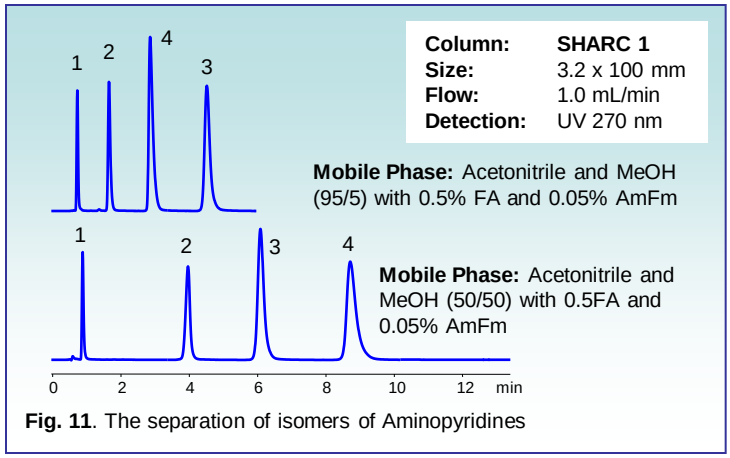


Fig. 11. The separation of isomers of Aminopyridines

The separation of phthalic acid isomers is another example of the separation of compounds with identical numbers of interaction sites and PSA value (74.6) (fig. 12). This example also demonstrates that when intramolecular bonding exists (phthalic acid) compounds are less retentive compared to the other isomers (terephthalic and isophthalic acids).

Mixtures of basic and acidic compounds containing hydroxy and amino groups can be separated in one analysis utilizing hydrogen-bonding interaction between the stationary phase and the hydrogen-bonding groups of analytes (fig. 13). The method was used for the separation of three neurotransmitters

However, the retention time of dihydroxybenzoic acids is harder to predict based on PSA number alone, because all four compounds have the an identical PSA value (fig. 14). If compounds have an identical PSA index, accessibility and internal hydrogen bonding can be considered in explaining elution order. Acids, which can undergo internal hydrogen bonding (2,6-dihydroxybenzoic acid) retain less than the acids where hydrogen interactions are less restricted, such as 3,5- and 2,5- dihydroxybenzoic acids.

Various order of elution depending on the conditions can be obtained for molecules containing very different numbers of hydrogen-bonding sites. A good example is nucleosides and nucleobases (fig. 15).

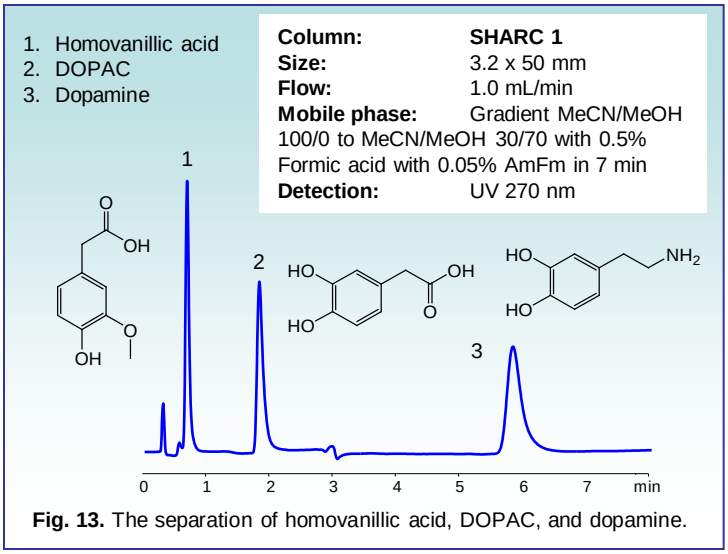


Fig. 13. The separation of homovanillic acid, DOPAC, and dopamine.

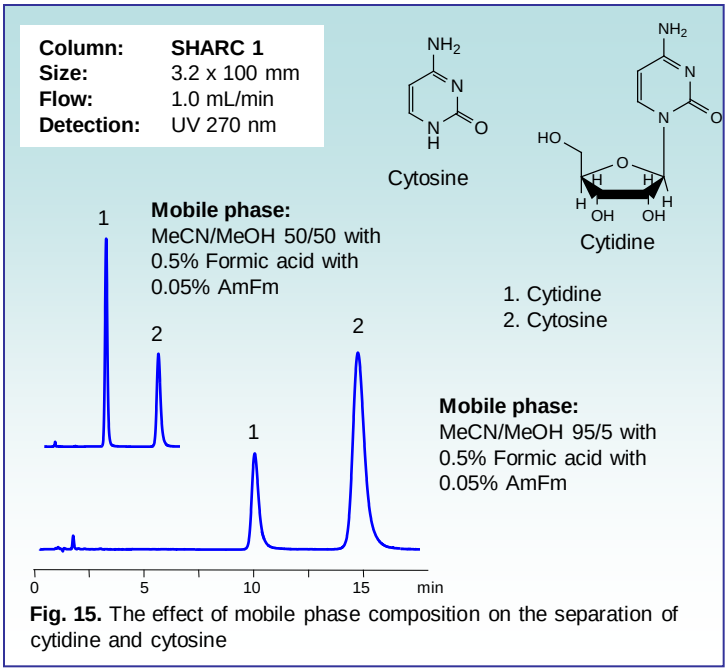


Fig. 15. The effect of mobile phase composition on the separation of cytidine and cytosine

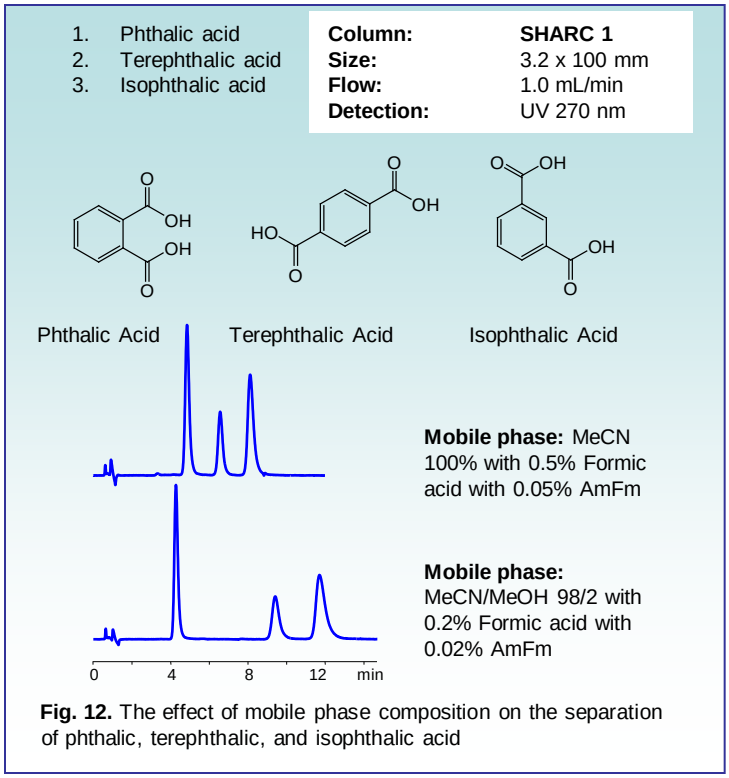


Fig. 12. The effect of mobile phase composition on the separation of phthalic, terephthalic, and isophthalic acid

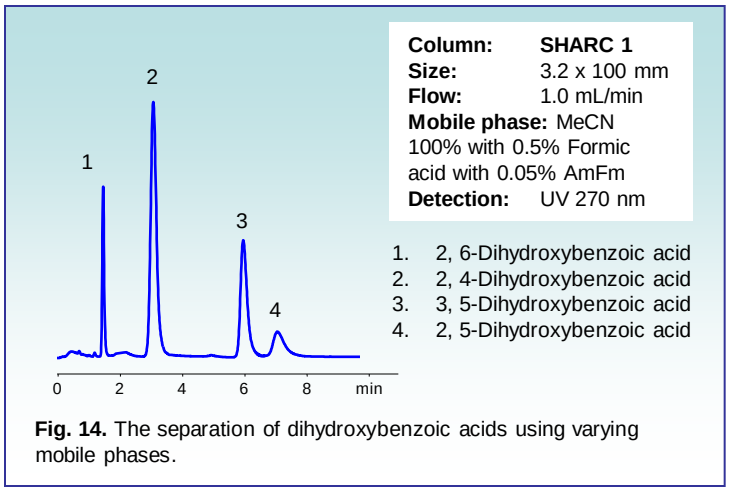


Fig. 14. The separation of dihydroxybenzoic acids using varying mobile phases.

Hydrogen bonding separation can be demonstrated in Triton X-100 example where degree of retention is correlated to the number of oxygen atoms in the molecule (fig. 16).

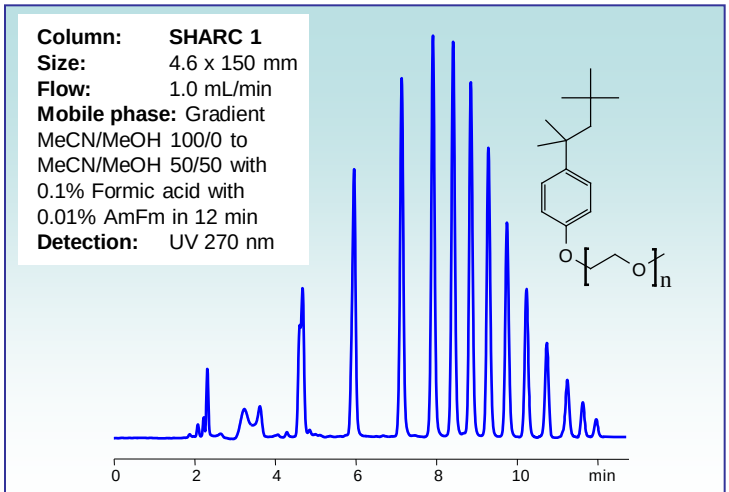
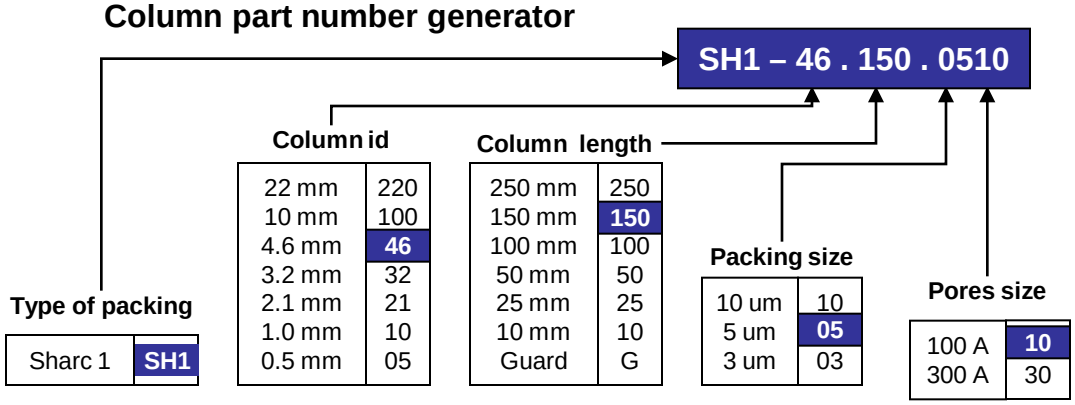


Fig. 16. The separation of components of Triton X 100 surfactant

Column part number generator



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