How to improve the analysis of hydrophilic compounds

Hydrophilic compounds can naturally form hydrogen bonds. Therefore, they dissolve well in water or other polar solvents. This characteristic, combined with the non-polar nature of the stationary phases in reversed phase liquid chromatography (RP-LC), poses a major challenge for their analysis using RP-LC. However, strategies exist to successfully achieve the separation of hydrophilic compounds with RP-LC. An effective alternative to RP-LC is hydrophilic interaction liquid chromatography (HILIC).
This technical note outlines strategies to enhance the analysis of hydrophilic compounds using both RP-LC and HILIC.

Topic overview

- Qualification of hydrophilicity by log*P*
- RP-LC
- IP-RP
- HILIC

1. Qualification of hydrophilicity by log*P*

The partition coefficient (*P* = [organic]/[aqueous]) or distribution coefficient is the ratio of a compound's concentration in an aqueous and a lipophilic phase. Typically, water and 1-octanol are used to determine hydrophilicity. The value is most often expressed as the log*P* value.

$$\log P = \log_{10}(P) = \log_{10}\left(\frac{[\text{organic}]}{[\text{aqueous}]}\right) \quad [1]$$

A negative log*P* value indicates that a compound has a higher affinity for the aqueous phase, making it more hydrophilic. Molecules with a log*P* value of 0 are distributed equally between both phases. At a log*P* value > 0, the compound shows a stronger preference for the lipophilic phase, making it more hydrophobic. A log*P* value of 1 suggests a molecule's distribution ratio of 10:1 in favour of the organic phase over the aqueous phase. Therefore, the log*P* value can be used to estimate a compound's hydrophilicity, thereby guiding the choice of the most suitable separation mode. Common examples for hydrophilic substances include nucleic acids, sugars such as glucose, amino acids such as glycine and water-soluble vitamins.

2. Analysis of hydrophilic compounds by RP-LC

In RP-LC, hydrophobic or less polar compounds are retained because of their affinity for the hydrophobic stationary phase. Conversely, interactions with hydrophilic compounds are less pronounced, which may result in lower selectivity among different analytes. Very polar compounds might even elute with the mobile phase. Nevertheless, specific phase modifications and mobile phases can be employed to enhance elution.

2.1. The stationary phase

In RP-LC, the separation of hydrophilic compounds often requires highly aqueous mobile phase conditions to achieve sufficient retention on a C18 stationary phase. When using a standard C18 column, the retention times can decrease over time, leading to irreproducible results.
One explanation could be a so-called dewetting of the stationary phase, in which the aqueous eluents are extruded from the particle pores due to their hydrophobic surface. If the pores are no longer completely wetted, approximately 99.9% of the specific surface area becomes inaccessible [2,3], accounting for the observed loss of retention and increased tailing (Figure 1). To mitigate this issue, using a stationary phase (AQ column) that remains stable under 100% aqueous conditions is beneficial.

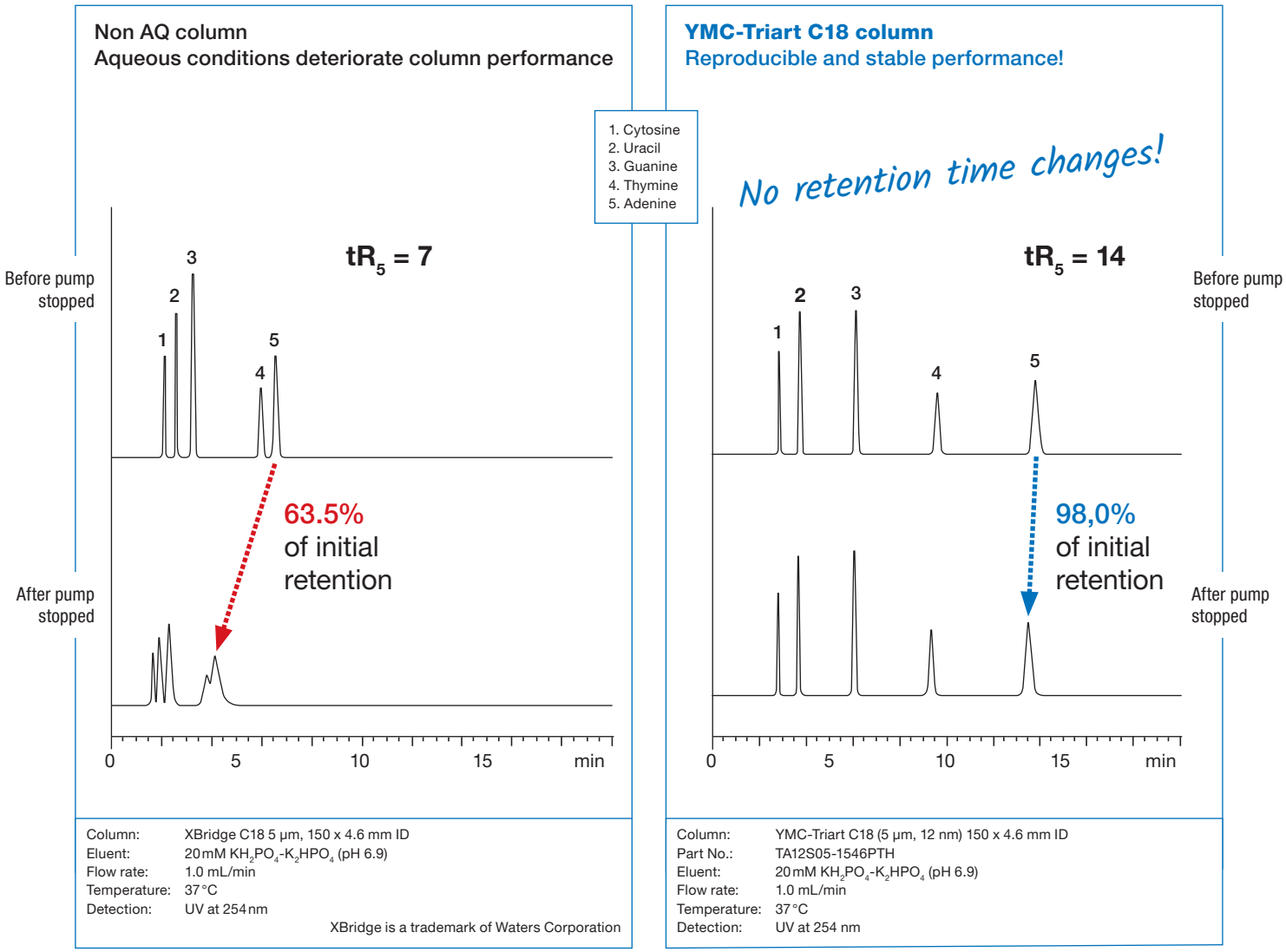
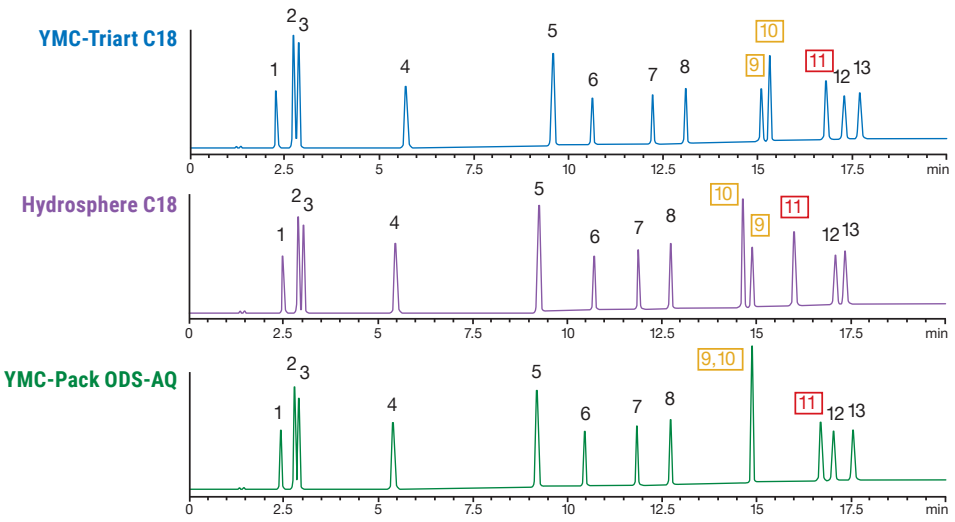


Figure 1: Effect of dewetting at 100% aqueous mobile phase when using a non AQ column in comparison to an AQ column at the same conditions.

Today, there are various phase modifications that allow 100% aqueous conditions. One approach involves reducing the overall hydrophobicity of the stationary phase by decreasing the density of the C18 chains. Another strategy is to replace remaining silanol groups for hydrophilic units by end-capping. The YMC-Pack ODS-AQ was among the first columns on the market to feature hydrophilic end-capping [3]. Subsequently, second-generation columns such as the Hydrosphere C18 column, which incorporate high-purity silica and hydrophilic end-capping, were developed. End-capping can significantly alter a column's selectivity. Residual silanol groups are naturally acidic and therefore ionic at basic pH. This leads to secondary interactions with ionised basic analytes resulting in peak tailing. The hydrophilic end-capping holds the water within the stationary phase's pores via dipole interactions and hydrogen bonds [4]. This enables the separation of hydrophilic compounds under 100% aqueous conditions while maintaining optimal peak shape. Stationary phases based on organic-inorganic hybrid silica particles with improved end-capping processes such as YMC-Triart C18 show improved efficiency and peak shape for ionised compounds. Differences in selectivity between these AQ columns are shown in Figure 2.



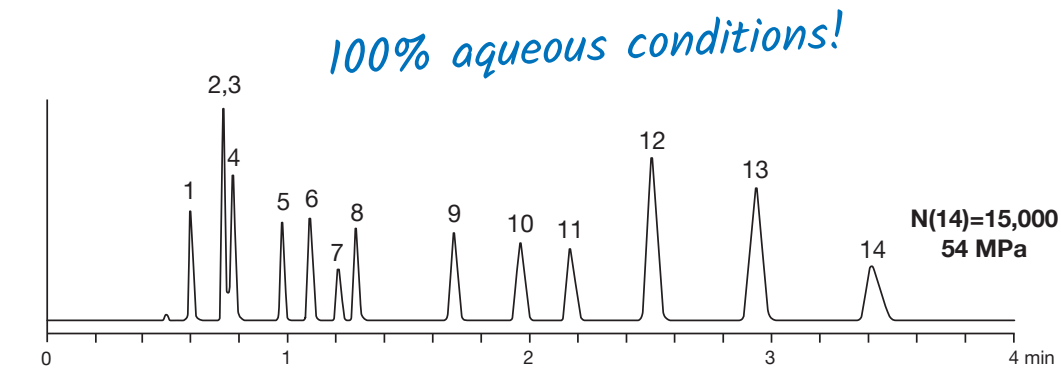
1. L-Ascorbic acid 2-glucoside
2. L-Ascorbic acid (Vitamin C)
3. D-Isoascorbic acid
4. Nicotinic acid (Vitamin B_3)
5. Nicotinamide
6. D-(+)-Pantothenic acid calcium salt (Vitamin B_5)
7. Pyridoxal hydrochloride (Vitamin B_6)
8. Pyridoxine hydrochloride (Vitamin B_6)
9. Folic acid (Vitamin B_9)
10. Thiamine hydrochloride (Vitamin B_1)
11. Cyanocobalamin (Vitamin B_{12})
12. D-Biotin (Vitamin B_7)
13. Riboflavin (Vitamin B_2)

Column:	3 µm, 150 x 3.0 mm ID
Part Nos.:	TA12S03-1503PTH HS12S03-1503WT AQ12S03-1503WT
Eluent:	A) 20 mM KH_2PO_4-H_3PO_4 (pH 2.8) containing 5 mM $CH_3(CH_2)_5SO_3Na$ B) 20 mM KH_2PO_4-H_3PO_4 (pH 2.8)/acetonitrile (80/20) containing 5 mM $CH_3(CH_2)_5SO_3Na$
Gradient:	0%B (0–3 min), 0–75%B (3–13 min), 75%B (13–20 min)
Flow rate:	0.425 mL/min
Temperature:	40 °C
Detection:	210 nm
Injection:	2 µL (0.01~0.1 mg/mL)

Figure 2: Differences in selectivity of AQ columns due to the varying balance of hydrophobicity and hydrogen bonding capacity between the stationary phases.

2.2. The mobile phase

For the analysis of hydrophilic compounds by RP-LC, a high proportion of aqueous eluent is required. Therefore, the proportion of organic modifier should be reduced. Polar organic solvents such as methanol can further improve the hydrophilicity of the mobile phase. In combination with a suitable stationary phase, the analysis can be carried out below 100% aqueous conditions (Figure 3). For entirely aqueous methods, adjustments are limited to pH and buffer composition. Yet, these methods have limited LC-MS compatibility due to the eluents' low volatility.



1. Oxalic acid
2. Tartaric acid
3. Glycolic acid
4. Formic acid
5. L-Malic acid
6. Malonic acid
7. Lactic acid
8. Acetic acid
9. Maleic acid
10. Citric acid
11. Succinic acid
12. Fumaric acid
13. Acrylic acid
14. Propionic acid

Column:	YMC-Triart C18 (1.9 µm, 12 nm) 100 x 3.0 mm ID
Part Nos.:	TA12SP9-1003PT
Eluent:	20 mM H_3PO_4
Flow rate:	0.85 mL/min
Temperature:	37 °C
Detection:	UV at 220 nm

Figure 3: Separation of hydrophilic analytes with RP-LC using 100% aqueous conditions.

In RP mode, the pH of the mobile phase significantly influences retention and selectivity. Altering the pH of the eluent affects the ionisation state of ionisable compounds. Naturally, the ionised form is more hydrophilic, resulting in lower retention. Acidic analytes are neutral at low pH, while basic analytes are neutral at high pH. Therefore, acidic conditions (pH<2.5) are recommended for hydrophilic analytes using a silica-based stationary phase. At very low pH, acidic analytes are neutral and therefore retained more strongly (Figure 4). This enables the separation of acidic analytes which otherwise might not be adequately retained (Figure 3). Additionally, the remaining silanol groups of the stationary phase are protonated. This prevents secondary interactions of otherwise charged silanol groups with basic ionised analytes, which lead to peak tailing [3].

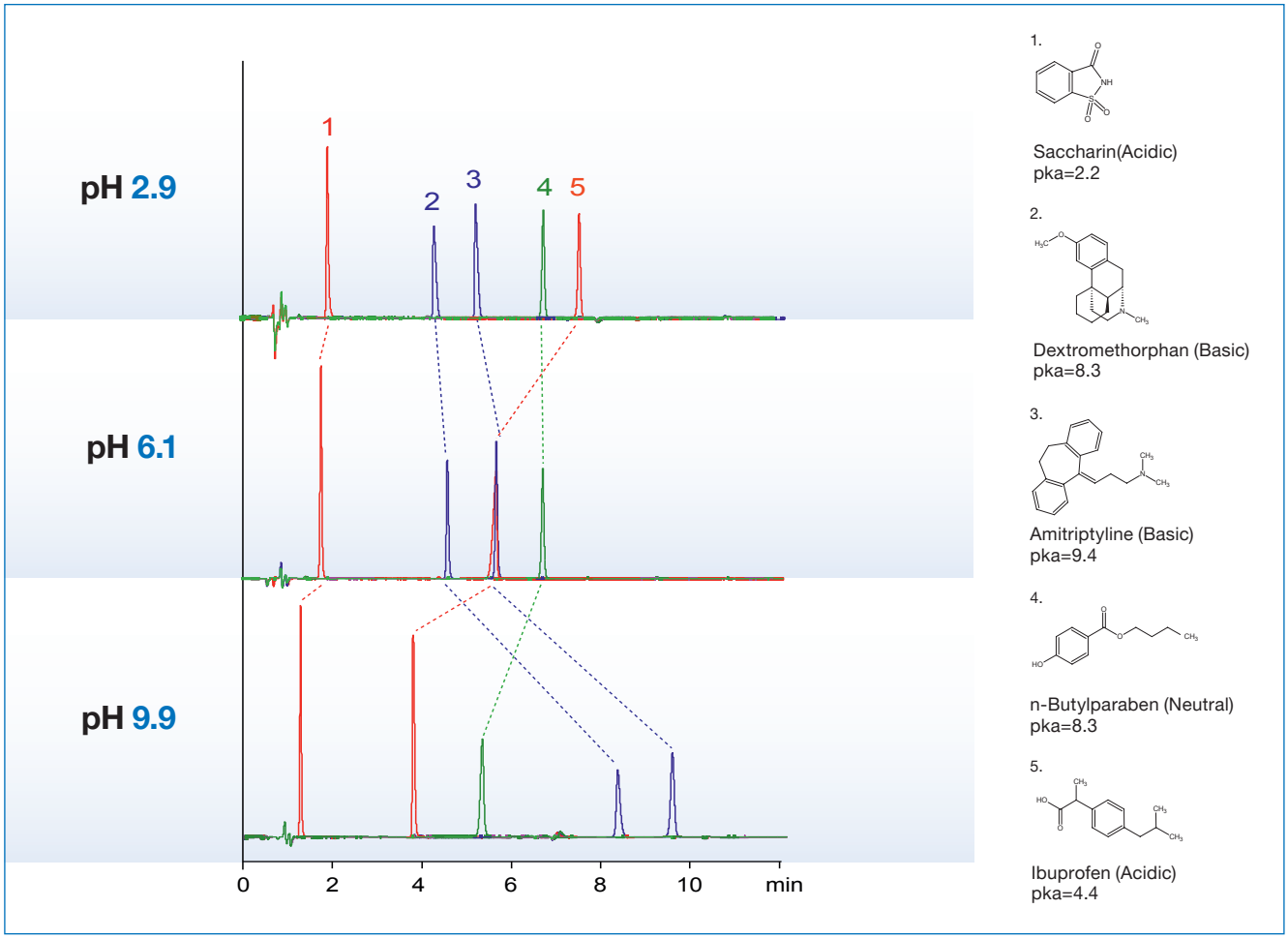


Figure 4: Influence of pH on the retention of analytes with different pKa values.

2.3. The use of ion paring reagents - Ion pair reversed phase chromatography

If hydrophilic analytes are not retained with 100% aqueous eluents, the use of ion pair reagents is an option. In ion-pair reversed phase chromatography (IP-RP), oppositely charged ion-pair reagents that have an ionic end and a nonpolar tail are added to the mobile phase. The separation mechanism in IP-RP is still not fully understood, but some theoretical models exist. According Bidlingmeyer's ion pair model, the ion pair reagents form a pair with the charged analyte so that they have no net electric charge (electroneutral) [3]. This increases the hydrophobicity of the analyte, which enables retention on the stationary phase. The ion exchange model states that the nonpolar tail of the ion pair reagent adsorbs on the stationary phase. This leads to an ion exchange between the charged analyte and the free ionic end of the ion pair reagent [3]. The correct choice and concentration of the ion pair reagent are crucial for the success of the analysis [5].

For pairing with anions, ammonium or tetraalkylammonium ions such as tetrabutylammonium hydroxide (TBAH) or tetrabutylammonium phosphate (TBAP) are used [6,7]. Alkylamines such as triethylamine (TEA) and dibutylamine (DBAA) are more commonly used for oligonucleotide analysis [8]. For IP-RP of cations, alkyl sulphates or alkyl sulphonates are employed as ion pair reagents [3,5]. The hydrophobicity of the ion pair reagents is determined by their chain length; the longer the chain length, the stronger the retention due to its hydrophobic properties [5]. The appropriate ion pair reagent and concentration must be determined experimentally for each analyte. The typical working concentration varies between 0.5 and 20 mM. If the concentration of the ion pair reagent is too low, the analyte will not interact sufficiently with the stationary phase. Conversely, if the concentration is too high, the analyte may be retained too strongly in the stationary phase [3,5].

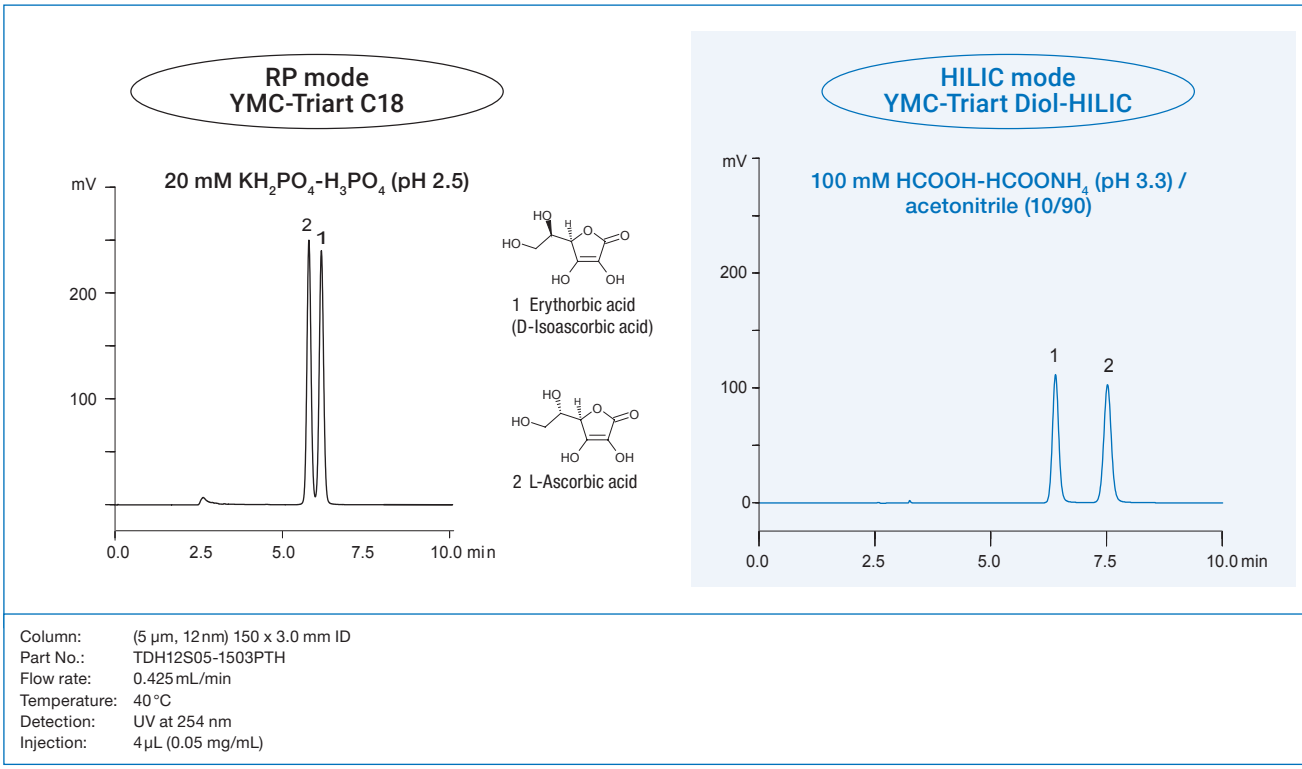
Several other factors influence retention in IP-RP. Organic modifiers affect the polarity of the mobile phase and hence the solubility of the ion pair reagent [5]. The optimal amount of organic modifier largely depends on the chosen ion pairing reagent. As the hydrophobicity of the ion pair reagent increases, a greater amount of organic modifier is needed to decrease the polarity of the mobile phase. Common organic modifiers include acetonitrile or methanol [5]. Other modifiers may be used, for instance, if sample solubility is an issue. The success of IP-RP is heavily dependent on the pH of the mobile phase [5]. The pH must be adjusted to keep the analyte and ion pair reagent ionised. Therefore, the pH value should be set two units above the pKa value of acidic compounds and two units below the pKa value of basic compounds.

UV and fluorescence detection are most commonly used in IP-RP. Many ion pairing reagents exhibit UV absorption. Therefore, ion pairing reagents that show absorption in the selected wavelength range should be avoided to prevent interference with detection. Special caution is required when LC is coupled with MS. Generally, ion pairing agents are not well-suited for LC-MS because they can suppress ion formation, reduce sensitivity, and have a tendency to adhere to the instrument's components. Consequently, volatile ion pairing agents are recommended for use in LC-MS. Formic acid (FA) is suitable for the analysis of cations, while TEA in combination with 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) is often used for anion analysis in combination with MS [3,8].

IP-RP significantly enhances the retention of hydrophilic compounds by RP-LC and can improve their detection limit. At the same time, peak shape and resolution are improved. However, there are several drawbacks to IP-RP that must be considered. Ion pairing agents tend to adhere to the stationary phase, making them difficult or even impossible to remove. Their adherence can irreversibly alter the characteristics of the stationary phase and can shorten the lifetime of a column. Consequently, columns used for IP-RP should be designated exclusively for that use and not repurposed for other types of chromatographic analyses. Additionally, the use of ion-pairing agents leads to prolonged equilibration times, with equilibration often being carried out overnight.

3. Analysis of hydrophilic compounds by HILIC

When hydrophilic analytes are unretained or poorly retained on RP-LC columns, using hydrophilic interaction liquid chromatography (HILIC) can be a suitable alternative (Figure 5).



Column:	(5 µm, 12 nm) 150 x 3.0 mm ID
Part No.:	TDH12S05-1503PTH
Flow rate:	0.425 mL/min
Temperature:	40 °C
Detection:	UV at 254 nm
Injection:	4 µL (0.05 mg/mL)

Figure 5: Improved retention and resolution of the hydrophilic L-ascorbic acid and its stereoisomer by HILIC in contrast to RP-LC.

With HILIC, hydrophilic stationary phases with reversed phase eluents, primarily acetonitrile and water are used. The aqueous part of the mobile phase forms a hydrophilic layer on the hydrophilic stationary phase. Thereby, the analytes partition between the aqueous and the organic layer of the mobile phase (Figure 6). The more hydrophilic the analyte, the greater is the preference for the aqueous layer in the stationary phase. As a result, the hydrophilic analytes are stronger retained. However, it is assumed that HILIC relies not only on partitioning but rather on a combination of mechanisms such as hydrogen bonding, dipole-dipole interactions or ion exchange depending on the analyte, mobile phase composition and the stationary phase selected.

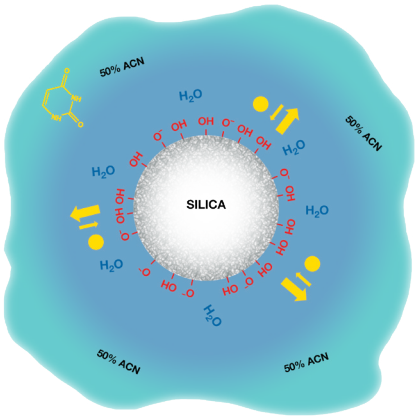


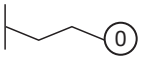
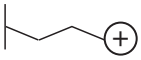
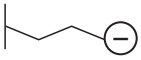
Figure 6: Dispersion of analytes between mobile phase and the surface water layer on the stationary phase.

3.1. The stationary phase

The selectivity of the stationary phase varies with its modification and the predominant interaction mechanism. In HILIC, various types of interactions occur, including hydrophobic interactions, ion exchange interactions, and adsorption processes, which encompass hydrogen bonds, electrostatic interactions, and dipole-dipole interactions. The choice of stationary phase is largely dependent on the nature of the analytes. Here it is important to know the log*P* values of the analytes, or whether the respective analytes are negatively or positively charged. Larger differences in hydrophilicity favour the use of a stationary phase based primarily on partitioning and polar interactions. Where analytes differ in their ionisation (pK_a value), stationary phases which depend on ion exchange mechanisms should be chosen. However, it is important to note that some properties of the analytes such as solubility and ionisation are influenced by the mobile phase. A set of columns with varying selectivities can be helpful in finding the optimal separation mechanism.

Most HILIC stationary phases are silica-based, modified with polar units. Based on their modification, HILIC columns can be categorised into neutral, charged and zwitterionic columns. Table 1 provides an overview of the different phases, divided into the groups mentioned above.

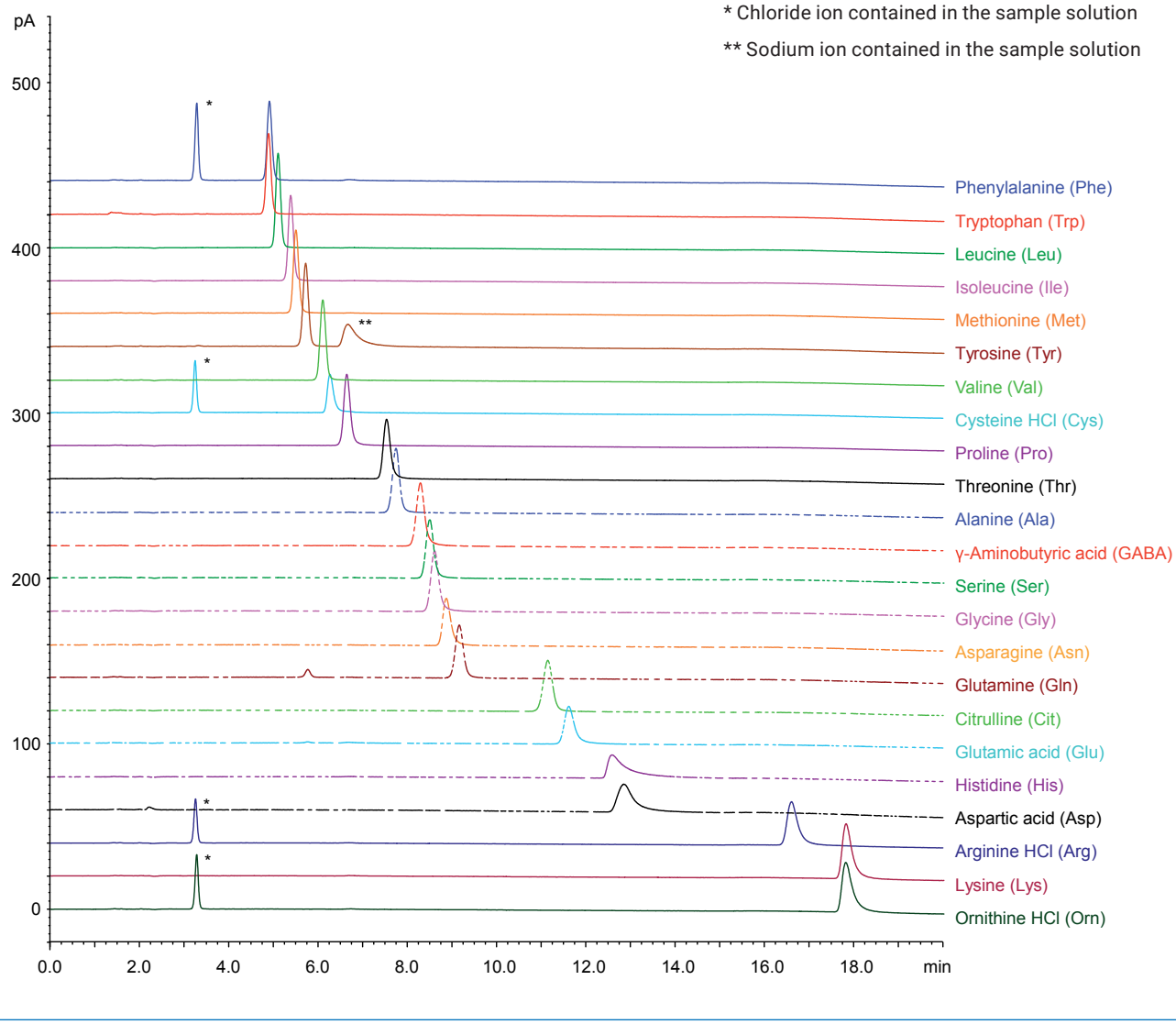
Table 1: HILIC stationary phases arranged by their chemical properties.

Chemical properties	Electrostatic interactions	Phases	YMC Column examples
neutral	No	 diol, cyano, PFP, amide	YMC-Triart Diol-HILIC, YMC-Triart PFP
charged	strong	 amino, polyamine, imidazole	YMC-Pack NH2, YMC-Pack Polyamine II
		 plain silica, polyaspartic acid	YMC-Pack SIL
zwitterionic	weak	 peptides, sulfobetaine	–

Many HILIC stationary phases consist of classic pure silica. Retention on pure silica is mainly attributed to distribution, adsorption and ion exchange [9]. This can be greatly influenced by the properties of the analyte and the composition of the mobile phase. At high pH values, the silanol groups on the stationary phase tend to ionise, facilitating cation exchange. This is particularly relevant for the separation of basic analytes that carry a positive charge, as they are strongly retained by stationary silica phases [10]. Furthermore, the acidity of pure silica is notably affected by its metal content. Early generations of silica phases often contained metal impurities, giving them a relatively acidic surface, which could lead to strong retention, asymmetric peaks, and complexation issues.

As a result, using pure silica stationary phases with very low metal concentrations, such as YMC-Pack SIL, is advisable to mitigate these problems [10]. In addition to pure silica, there are various stationary phases with polar functional groups that provide additional selectivity [9,10].

Stationary phases with neutral ligands rely primarily on hydrophilic interactions and thereby retain various analytes (Figure 7). An example are the diol stationary phases such as YMC-Triart Diol-HILIC, which are usually modified with 2,3-dihydroxypropyl ligands and are uncharged. Its strong hydrogen bonding properties and low secondary interactions influence the separation of ionised compounds. This often improves peak symmetry and sensitivity. Another example of neutral stationary phases are phases modified with a carbonyl or amide moiety [9,10].

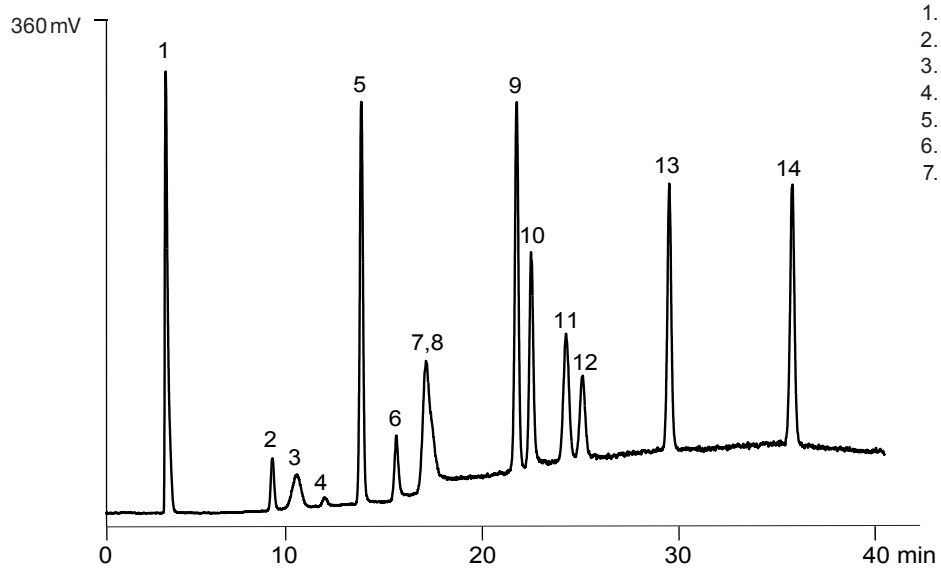


Column:	YMC-Triart Diol-HILIC (5 µm, 12 nm) 150 x 4.6 mm ID
Part No.:	TDH12S05-1546PTH
Eluent:	A) 100 mM $HCOOH\text{-}HCOONH_4$ (pH 3.6)
	B) acetonitrile
Gradient:	83–80%B (0–12 min), 80–68%B (12–20 min)
Flow rate:	1.0 mL/min
Temperature:	40 °C
Detection:	Corona® CAD® (Charged Aerosol Detector)
Injection:	10 µL (0.1 mg/mL)

Corona and CAD are trademarks of Thermo Fisher Scientific.

Figure 7: Analysis of free amino acids in HILIC mode using the YMC-Triart Diol-HILIC column, a stationary phase with neutral diol ligands.

Among the most favoured charged stationary phases are those with amino functionalities. For the separation of acidic compounds, amino phases such as the YMC-Pack NH_2, which is modified with primary amine groups, are commonly used [9]. These phases are highly hydrophilic and have a positive charge. Therefore, they have a high affinity for anionic compounds. This can sometimes lead to irreversible adsorption to the stationary phase [10]. Other amino phases, such as the YMC-Pack Polyamine II, contain secondary and tertiary amine groups that cannot form Schiff bases with aldehydes, greatly improve the separation of carbonyl compounds such as carbohydrates (Figure 8) [9].



Column:	YMC-Pack Polyamine II (5 µm, 12 nm) 250 x 4.6 mm ID
Part No.:	PB12S05-2546WT
Eluent:	A) water
	B) acetonitrile
Gradient:	80–60%B (0–30min, linear)
	60% (35–40min)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Detection:	ELSD
Injection:	5 µL (2–40 mg/mL)

Figure 8: Analysis of saccharides using the YMC-Pack Polyamine II, an amino phase with secondary and tertiary amine groups.

Zwitterionic materials consist of positively and negatively charged groups. Sulfobetaine-bonded phases also strongly adsorb water through hydrogen bonding, so that the water adheres to their surface more easily and determines the distribution mechanism [10].
Since the charged units are in a 1:1 ratio, their net charge is almost 0 and they show only weak electrostatic interactions. This enables the separation of neutral, acidic and basic analytes [9].

Choosing the optimum stationary phase is a challenge. The performance of seemingly similar phases may vary depending on the provider. Moreover, the separation mechanisms of stationary phases which incorporate ligands may be affected by the acidity of the silica base particles [10]. The density of the ligand can also influence the selectivity and the extent to which the remaining silanol groups are shielded. However, the selectivity of the stationary phase is also highly dependent on the mobile phase.

3.2. The mobile Phase

In HILIC, the mobile phase usually consists of an organic-rich mobile phase with 5–40% water or buffer. A minimum of water (2–3%) is required to enable formation of a water layer on the stationary phase surface. Water ratios exceeding 40% can hinder the formation of this layer, significantly reducing the efficiency of hydrophilic partitioning and forcing separation to rely on alternative mechanisms [11]. Since stabilisation of the water layer is time intensive, equilibration times are much longer than in RP-LC. This also has to be considered for column equilibration, where a minimum of 20 CVs is recommended.
Acetonitrile is the most common organic solvent in HILIC. Its aprotic characteristics promote hydrogen bonding between the stationary phase and the analytes. Increasing the concentration of acetonitrile in the mobile phase typically results in a higher retention of analytes [11]. However, alternative organic solvents such as methanol, ethanol, isopropanol, tetrahydrofuran or acetone can be used.
The relative solvent strength of the organic solvents in HILIC mode is as follows:
acetone < acetonitrile < isopropanol < ethanol < methanol < water [10, 11].
Mobile phases containing organic solvents other than acetonitrile often cause poor analyte retention or broad/asymmetrical peaks. This is especially true for methanol, which is rarely used. The reason might be the protic characteristics of methanol similar to water, which leads to strong hydrogen bonding interactions of both solvents [9].

To determine the suitable ratio of organic modifier and aqueous buffer, first analyses can be started with a high ratio of aqueous buffer (e.g. 40%) to achieve elution of all samples. In further experiments, elution strength can be adjusted by increasing the amount of organic solvent, generally acetonitrile until the desired resolution is achieved [9]. Analyses in HILIC are mostly performed isocratically. When applying a gradient, it should start with a high concentration of organic solvent, since water is the strongest solvent in HILIC [10]. Furthermore, a rather flat gradient has to be used.

3.3. Use of additives

Retention of ionisable analytes increases with their degree of ionisation due to the increase in polarity. Acidic compounds show this behaviour in mobile phases with basic pH and basic compounds in mobile phases with acidic pH [12]. A mixture of ionised and non-ionised analytes may cause band broadening, peak asymmetry and peak splitting [9]. It is therefore important to control the mobile phase pH. For this purpose, ionic additives should be used [10]. However, it has to be considered that some salts are poorly soluble in organic solvent-rich mobile phases and if added at low concentrations the buffer capacity might not be sufficient. This may result in pH variations during the analysis. For an acidic pH ammonium acetate or ammonium formate are recommended, whereas carbonate and NH_4OH are suitable salts to achieve a basic pH [12]. To improve the peak shape of basic compounds, trifluoroacetic acid (TFA) can be added to the mobile phase, which suppresses ion exchange interactions [9]. The pH of the buffer in the mobile phase may change when mixed with organic solvent. Therefore, the actual pH of the mobile phase under analytical conditions should be closely monitored.

3.4. The sample diluent

Pure acetonitrile is the first choice as sample solvent in HILIC providing the best peak shapes and resolution. A mixture of water and acetonitrile as solvent will reduce the efficiency, which decreases in proportion to the ratio of water. Therefore, a mixture of acetonitrile with other organic solvents such as isopropanol are preferred if the analyte is not soluble in 100% acetonitrile. In this case, other organic solvents such as isopropanol or ethanol can also be used [11].

3.5. The influence of temperature

Temperature has only a relatively small effect on retention compared to the mobile phase composition. However, an increase in temperature is usually associated with a decrease in retention. This is probably due to reduced cohesive energy and polar interactions between the mobile phase and the stationary phase. Often higher temperatures positively influence the efficiency caused by the reduction in viscosity and higher diffusivity [9].

3.6. Detection methods

Various HPLC detectors such as UV-VIS, fluorometric or evaporative light-scattering detectors (ELSD) are compatible with HILIC. However, one of the biggest advantages of HILIC, is its high compatibility with MS. Due to the high ratio of organic solvent in the mobile phase, which can be easily evaporated during electrospray ionisation (ESI), ion formation is strongly improved. This leads to a massive increase in sensitivity compared to RP-MS [10]. However, when coupling HILIC to MS, the use of non-volatile salts and TFA should be avoided. Instead, volatile buffers such as ammonium formate or acetate have to be applied [9].

4. Summary

Both RP-LC and HILIC offer unique advantages and challenges for analysing hydrophilic analytes. RP-LC enables the simultaneous analysis of hydrophilic and hydrophobic compounds. All LC detectors are compatible. Another advantage is the rapid equilibration time. However, one of the primary limitations of RP-LC is its difficulty in separating highly polar compounds effectively. For these compounds, IP-RP presents a viable alternative. The peak shape and resolution of hydrophilic analytes are significantly improved and the detection limit can be increased. However, MS compatibility is limited.

In contrast, one of the biggest advantages of HILIC is its MS compatibility. The high organic solvent content of the mobile phase not only reduces back pressure but also enhances the sensitivity of evaporation detectors such as ELSD, Charged Aerosol Detector (CAD), or ESI-MS. The viscosity of the eluents also enables extremely fast separations. However, to use the appropriate stationary phase in HILIC, an understanding of the physiochemical properties of an analyte is required. Sometimes multiple stationary phase screening may be necessary to determine the optimal stationary phase. Problems with sample solubility may also occur due to the preferred sample solvent, acetonitrile. Therefore, the separation mode for the analysis of hydrophilic analytes should be selected individually according to the analytical focus and preferences.

References:

[1] Leo, A. et al. (1971) Partition coefficients and their uses. Chem. Rev., 71, 525–616.

[2] Nagae, N. et al. (2002) The retention behavior of reversed phase HPLC columns with 100% aqueous mobile phase, LC GC, North America 20, 964–972.

[3] Kromidas, S. (2014) The HPLC Expert: Possibilities and Limitations of Modern High Performance Liquid Chromatography (1st ed.). Wiley.

[4] Taylor, T. (2018) The LCGC Blog: Do You Really Know Your Stationary-Phase Chemistry?, LC GC International

[5] Gloor, R. & Johnson E. (1977) Practical Aspects of Reverse Phase Ion Chromatography, J. Chromatogr. Sci., 15, 413-423

[6] Wangkarn, S. & Pergantis, A. (2000) High-speed separation of arsenic compounds using narrow-bore high-performance liquid chromatography on-line with inductively coupled plasma mass spectrometry, J. Anal. At. Spectrom., 15, 627-633

[7] Hu et al. (2012) Determination of Trace Vitamin C by Ion-Pair HPLC with UV Detection in Calcium Gluconate and Vitamin C Compound Oral Solution, J. Chromatogr. Sci., 50, 102-107

[8] Liu, A. et al. (2022) Bioanalysis of Oligonucleotide by LC-MS: Effects of Ion Pairing Regents and Recent Advances in Ion-Pairing-Free Analytical Strategies, In. J. Mol. Sci., 23, 15474

[9] Jandera, P. (2011) Stationary and mobile phases in hydrophilic interaction chromatography: a review, Anal. Chim. Acta, 692, 1-25

[10] Buszweski, B. & Noga, S. (2012) Hydrophilic interaction liquid chromatography (HILIC) – a powerful separation technique, Anal. Bioanal. Chem., 402, 231-247

[11] Fadhil, A. et al. (2023) A review of Recent Advances in The Estimation of Pharmaceutical Products Using Hydrophilic Interaction Chromatography (HILIC) Technology, Egypt. J. Chem., 66, 399-417

[12] Subirats X. et al. (2023) Effect of the solvent on the chromatographic selectivity in reversed phase and HILIC, 6, 100063