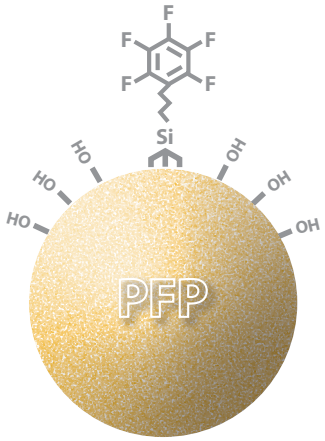
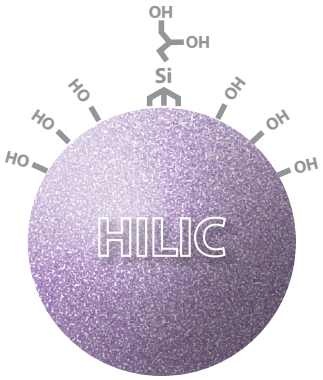
Improving the retention behaviour of hydrophilic compounds on YMC-Triart PFP and YMC-Triart Diol-HILIC

Stationary phase modification of Pentafluorophenyl (PFP) or Diol (1,2-Dihydroxypropyl) are a great tool to resolve hydrophilic compounds. They are delivering enhanced H-bonding, dipole-dipole and ionic interactions and thus have a beneficial influence on the separation of polar compounds. Due to the nature of retention mechanisms, the eluent's pH and the organic solvent used greatly influence the resolution.



YMC-Triart PFP



YMC-Triart Diol-HILIC

Effect of pH

In order to evaluate pH influence on retention behaviour three model analytes are chosen:

1. Basic compound = serotonin HCl
2. Acidic compound = 5-hydroxyindoleacetic acid
3. Zwitterionic compounds = glycine and tyrosine

The retention factor was determined at three different pH values (2.2, 3.6 and 6.5) with a mobile phase containing 90% acetonitrile.

pH	Analyte	k' YMC-Triart PFP	k' YMC-Triart Diol-HILIC
2.2	Basic Compound	+++[1]	+++[1]
	Acidic Compound	–[4]	–[4]
	Zwitterionic Compound	+++[1]	+++[1]
3.6	Basic Compound	+[3]	+[3]
	Acidic Compound	–[4]	+[3]
	Zwitterionic Compound	+[3]	++[2]
6.5	Basic Compound	+[3]	+[3]
	Acidic Compound	–[4]	+[3]
	Zwitterionic Compound	+[3]	++[2]

[1] Retention factor very high
[2] Retention factor high
[3] Retention factor low
[4] Retention factor too low

Findings

1. High retention factor for basic and zwitterionic compounds at acidic pH. Within 30 minutes, no peaks could be observed for 5-HT and glycine on the YMC-Triart PFP column, so it is assumed that the retention factor is very high. Positively charged analytes interact strongly with negatively charged free silanols. Even though a formate buffer is used, the pKa of formic acid increases up to around 8 in 90 % acetonitrile which contributes to the ionisation of residuals silanols[1].
2. Lower retention factor at elevated pH for basic and zwitterionic compounds: Suppression of electrostatic interaction due to NH_4^+ cation, originated from ammonium formate in the mobile phase.
3. Retention of 5-hydroxyindoleacetic acid was only achievable using the YMC-Triart Diol-HILIC column. The retention factor improved with increasing pH due to increasing ionisation of the compound.

This indicates that the retention of basic or zwitterionic compounds on the PFP phase is mainly based on ionic interaction using highly organic conditions.

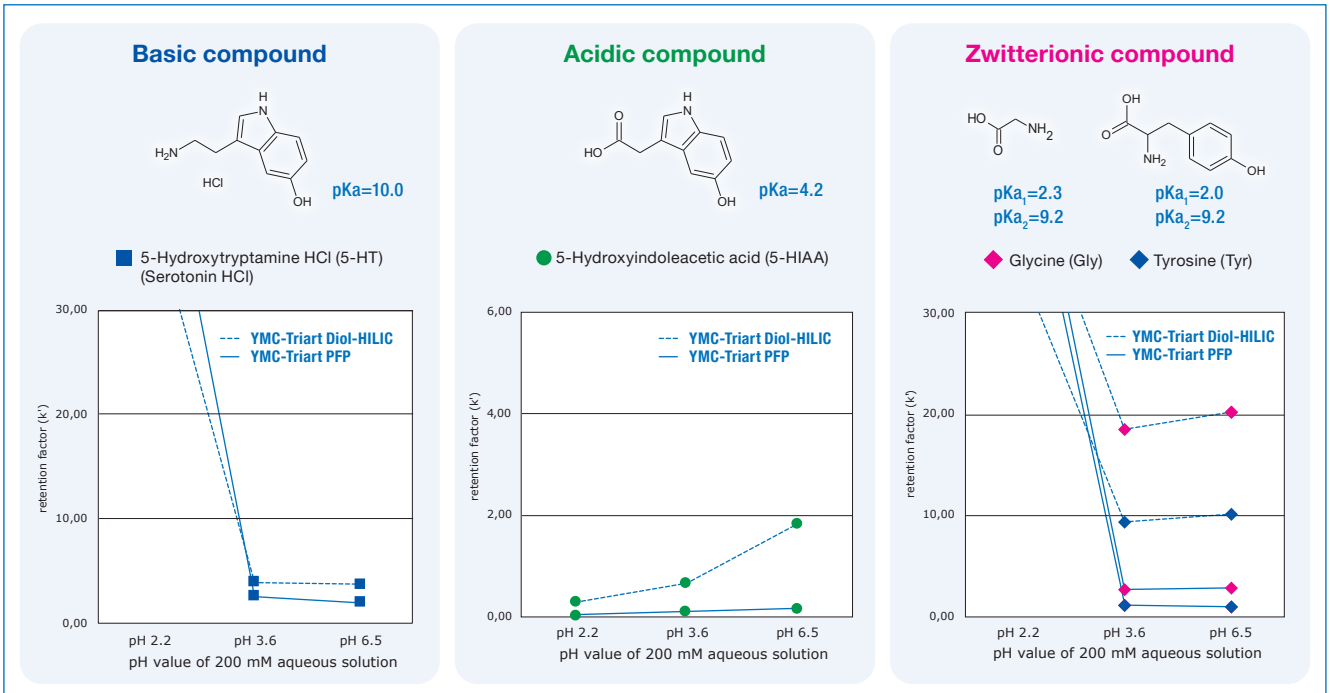


Figure 1: Effect of different pH values (2.2, 3.6 and 6.5) on retention factor using YMC-Triart PFP or YMC-Triart Diol-HILIC columns for analysis of basic, acidic and zwitterionic compounds

Columns:	YMC-Triart PFP (5 µm, 12 nm) 50 x 4.6 mm ID
	YMC-Triart Diol-HILIC (5 µm, 12 nm) 50 x 4.6 mm ID
Part nos.:	TPF12S05-0546PTH
	TDH12S05-0546PTH
Eluent:	acetonitrile/water (90/10) containing 10 mM formate buffer
Flow rate:	1.0 mL/min
Temperature:	40 °C
Detection:	UV at 280 nm

Effect of organic solvent

The influence of the organic solvent on the retention behaviour on serotonin HCl and tyrosine was examined as basic and zwitterionic compound respectively. The acidic analyte was not included due to the low retention. Acetonitrile (solid line) was compared to methanol (dashed line).

By using YMC-Triart PFP the retention factor increased for both mobile phases containing organic solvent of less than 20% and more than 60%. This retention behaviour represents a mixture of RP- as well as HILIC-mode. A simple change in organic solvent content leads to more or less retention.

The YMC-Triart Diol-HILIC column showed the typical HILIC retention behaviour. By increasing the organic solvent content to more than around 70% the retention also increases.

Acetonitrile provided stronger retention than methanol under high organic mobile phase conditions for both stationary phases. It is assumed that methanol may disturb the formation of the water-enriched layer on the surface of the stationary phases by replacing water molecules.

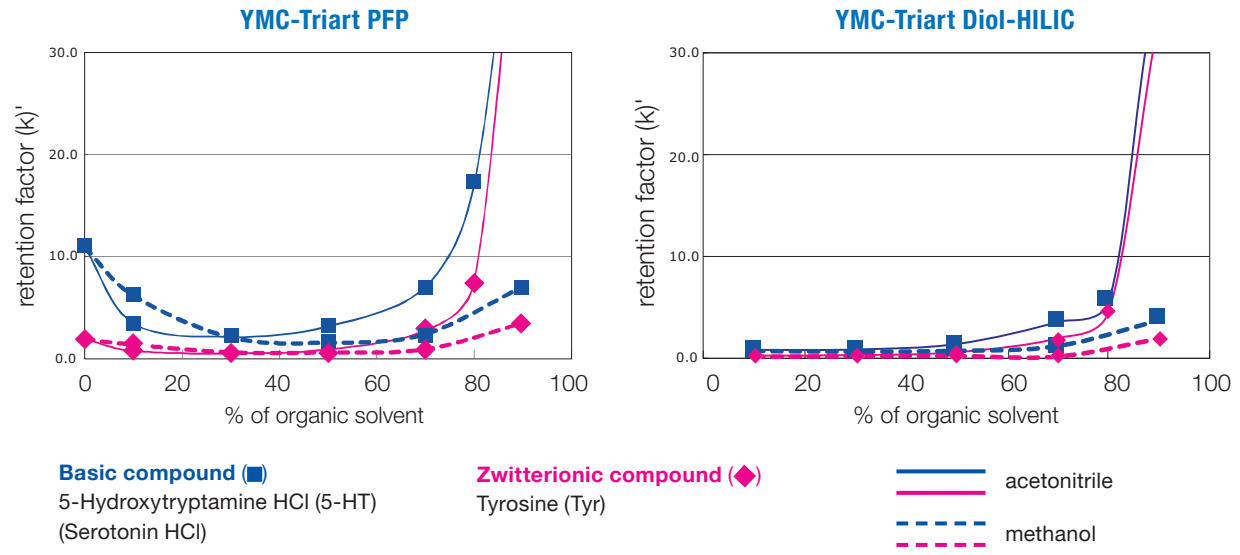


Figure 2: Effect of organic solvent content on the retention factor using YMC-Triart PFP or YMC-Triart Diol-HILIC columns for basic and zwitterionic compound separations

Columns:	YMC-Triart PFP (5 µm, 12 nm) 50 x 4.6 mm ID YMC-Triart Diol-HILIC (5 µm, 12 nm) 50 x 4.6 mm ID	Eluent:	A) water containing 10 mM formic acid B) acetonitrile or methanol containing 10 mM formic acid
Part nos.:	TPF12S05-0546PTH TDH12S05-0546PTH	Flow rate: Temperature: Detection:	1.0 mL/min 40 °C UV at 280 nm

Conclusion

In summary, retention behaviour can easily be influenced by changing pH or the amount of organic solvent. For hydrophilic basic and zwitterionic compounds the retention is increased on YMC-Triart PFP by using low pH or highly organic conditions. Whereas a stationary phase such as YMC-Triart Diol-HILIC can be a good approach for acidic compounds. It seems that interactions on YMC-Triart PFP are mainly ionic based, while on YMC-Triart Diol-HILIC additional interactions are provided.

[1] J.M. Padró, A. Acquaviva, M. Tascon, L.G. Gagliardi, C.B. Castells, Effect of temperature and solvent composition on acid dissociation equilibria, I: Sequenced (s)(s)pK(a) determination of compounds commonly used as buffers in high performance liquid chromatography coupled to mass spectroscopy detection, Analytica chimica acta, 2012, 725, 87-94, 10.1016/j.aca.2012.03.015.