

How to select the ideal column for your separation YMC-Triart selectivities for small molecules

The organic-inorganic hybrid silica particles of the YMC-Triart series make them highly robust (U)HPLC columns. They show high mechanical stability with challenging chromatographic conditions such as high pH values or temperature no longer being a limitation to the day-to-day work.

The YMC-Triart series provides 8 different stationary phases and 3 particle sizes for (U)HPLC to offer maximum flexibility in method development. All have in common excellent reproducibility due to the tightly controlled particle formation technology.

Broad selection of different stationary phases for wide selectivity range

Three phases with a C18 modification are available: YMC-Triart C18, YMC-Triart C18 ExRS and YMC-Triart Bio C18. The balanced hydrophobicity and silanol activity of YMC-Triart C18 make it a truly versatile stationary phase. In contrast, YMC-Triart C18 ExRS provides a high carbon load of 25%. The ultra-high carbon load, together with a pore size of 8 nm, makes it an ideal choice for extremely non-polar or closely related substances. While YMC-Triart C18 has a standard pore size of 12 nm, YMC-Triart Bio C18 is especially designed for the analysis of biomolecules due to its larger pore size of 30 nm. Compared to YMC-Triart C18, YMC-Triart C8 provides a less hydrophobic stationary phase offering reduced retention times, but a comparable selectivity.

Another alkyl modified stationary phase is YMC-Triart Bio C4 with a pore size of 30 nm designed for peptide and protein or even antibody separations. As orthogonal selectivities, aromatic phases such as YMC-Triart Phenyl or YMC-Triart PFP (pentafluorophenyl) are available. Besides hydrophobic interactions π - π - and dipole-dipole interactions are also involved creating a different selectivity compared to C18 modified phases. Since YMC-Triart PFP is not endcapped, hydrogen bonding also plays an important role. The YMC-Triart series is completed by a stationary phase for the separation of polar and hydrophilic compounds which are not retained by reversed phase (RP) chromatography: YMC-Triart Diol-HILIC. It has dihydroxypropyl groups exhibiting low nonspecific adsorption.

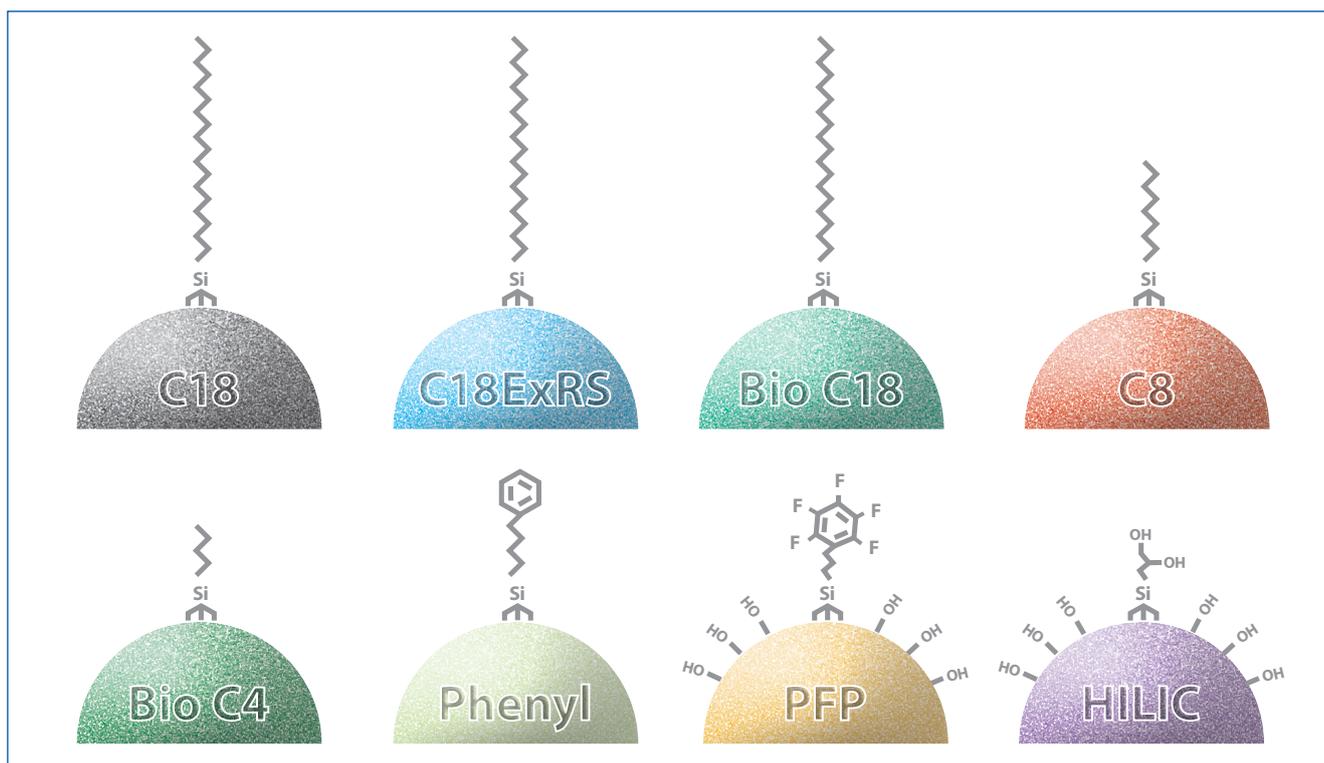


Figure 1: 8 different modifications within the YMC-Triart series.

Table 1: Specifications of YMC-Triart.

	C18	C18 ExRS	Bio C18	C8	Bio C4	Phenyl	PFP	Diol-HILIC
Base	organic/inorganic silica							
Particle size	1.9, 3 and 5 µm							
Pore size	12 nm	8 nm	30nm	12nm	30nm	12nm	12nm	12nm
Carbon content	20%	25%	—	17%	—	17%	15%	—
Endcapping	multi-stage	multi-stage	multi-stage	multi-stage	multi-stage	multi-stage	none	none
pH range	1 ~ 12	1 ~ 12	1 ~ 12	1 ~ 12	1 ~ 10	1 ~ 10	1 ~ 8	2 ~ 10
Temperature range	pH<7: 90°C pH>7: 50°C	50°C	50°C	50°C				
100% aqueous eluents	✓	✗	✓	✗	✓	✓	✓	✓

Selectivity comparison based on Tanaka

How do these characteristics look like in comparison? In Figure 2 the five classical RP modifications are compared in a Tanaka diagram according to primary and secondary interactions. Retention time for nonpolar substances, hydrophobic and steric selectivity are related to primary interactions, while tailing tendency, ionic sensitivity at neutral pH and silanol contribution at acidic pH are related to secondary interactions.

The differences between the stationary phases now become clear according to the different peak heights: e.g. due to the high hydrophobicity of YMC-Triart C18 ExRS the retention time for nonpolar substances is much greater compared to the other phases. For YMC-Triart PFP the secondary interactions have to be pointed out. They are much higher since this stationary phase is not endcapped and therefore the free silanols are able to interact with the analytes.

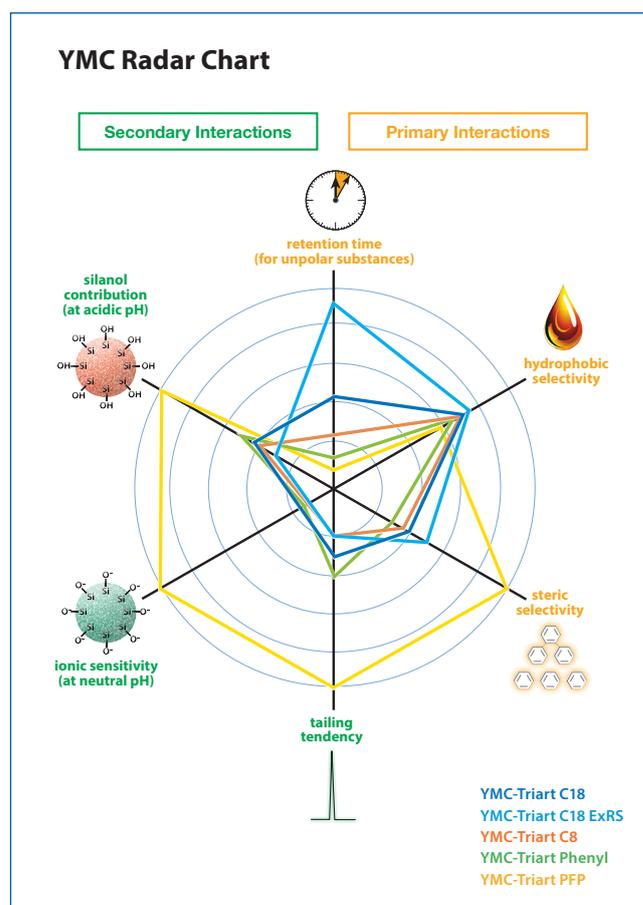


Figure 2: Tanaka diagrams of YMC-Triart C18, YMC-Triart C18 ExRS, YMC-Triart C8, YMC-Triart Phenyl and YMC-Triart PFP.

Differences in selectivity shown by aliphatic and aromatic YMC-Triart phases

The separation of selected compounds with very different properties was performed under the same chromatographic conditions using four different YMC-Triart RP columns. It is shown that the selectivity of YMC-Triart C8 is similar to YMC-Triart C18 but shorter retention times are achieved. This is also represented by the hydrophobicity index which is lower for YMC-Triart C8. This means, if the resolution between two compounds is sufficient on a C18 column, it could be possible to reduce the runtime by choosing a C8 modification.

In contrast, YMC-Triart Phenyl and YMC-Triart PFP show different selectivities since the elution order has changed. Besides the hydrophobic interactions, π - π - and polar interactions contribute to the retention creating a different elution profile compared to YMC-Triart C18. In addition the remarkable steric selectivity of YMC-Triart PFP is shown since the shape recognition ability index for peaks 8 and 9 is more than doubled compared to YMC-Triart C18.

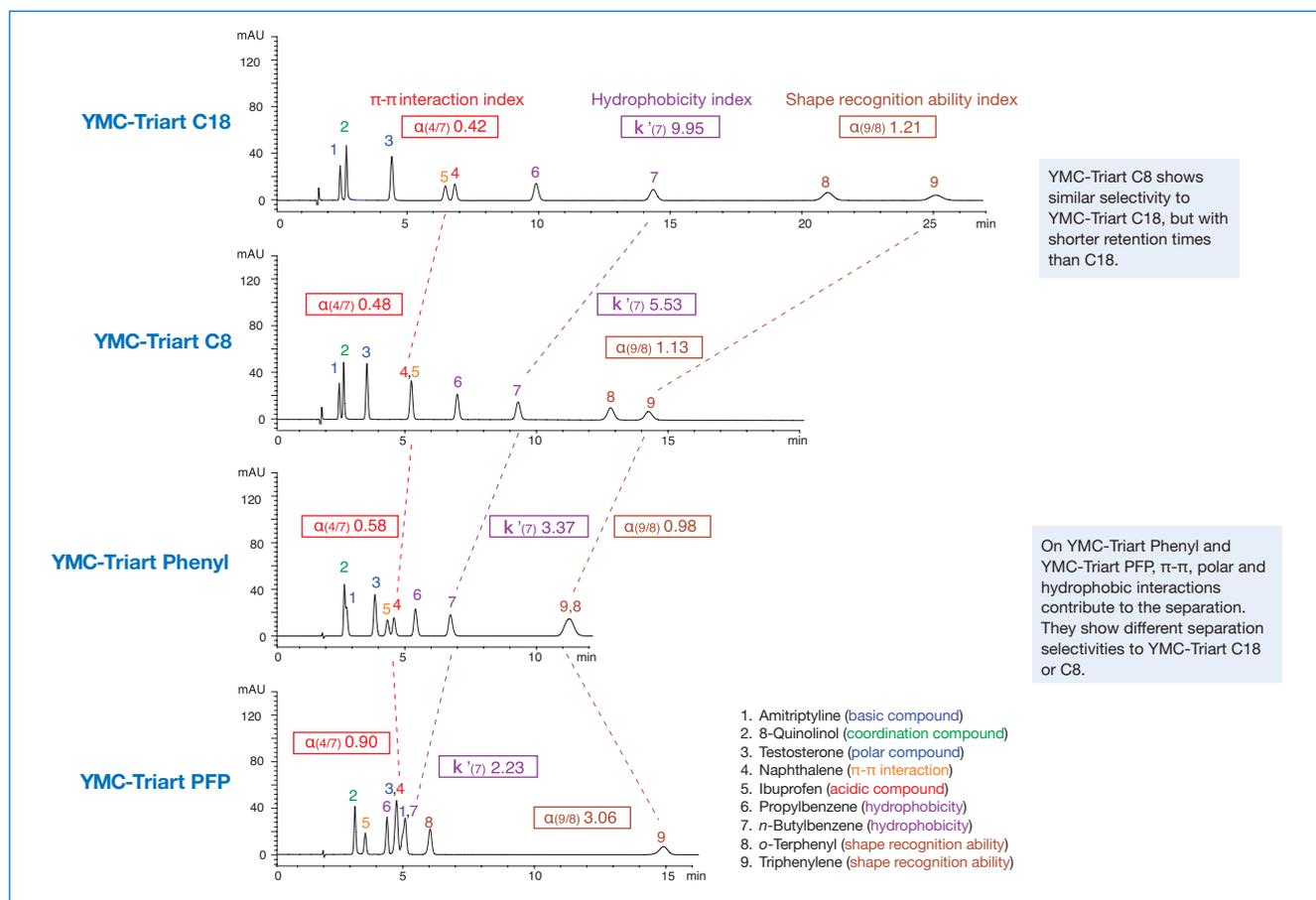


Figure 3: Chromatograms of the separation of compounds with different properties using YMC-Triart C18, YMC-Triart C8, YMC-Triart Phenyl and YMC-Triart PFP.

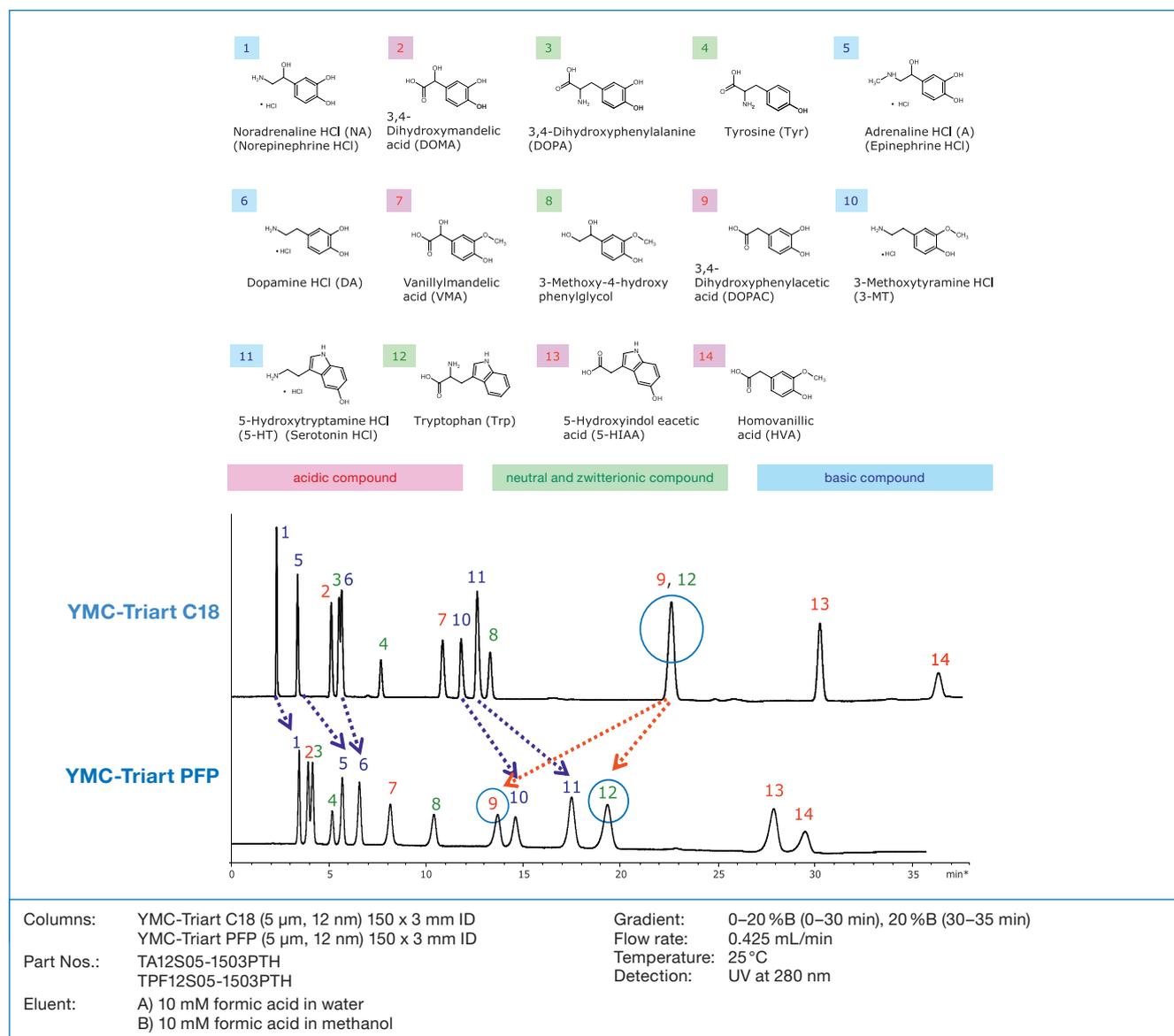
Table 2: Chromatographic conditions of the separation of compounds with different properties.

Columns:	YMC-Triart C18 (5 μ m, 12 nm) 150 x 3 mm ID YMC-Triart C8 (5 μ m, 12 nm) 150 x 3 mm ID YMC-Triart Phenyl (5 μ m, 12 nm) 150 x 3 mm ID YMC-Triart PFP (5 μ m, 12 nm) 150 x 3 mm ID
Part Nos.:	TA12S05-1503PTH TO12S05-1503PTH TPH12S05-1503PTH TPF12S05-1503PTH
Eluent:	20 mM H ₃ PO ₄ -KH ₂ PO ₄ (pH 3.1)/methanol (25/75)
Flow rate:	0.425 mL/min
Temperature:	40 °C
Detection:	UV at 265 nm

Orthogonal separation using YMC-Triart C18 and YMC-Triart PFP

In a second example, basic, acidic and neutral/zwitterionic catecholamines were analysed using a YMC-Triart C18 and a YMC-Triart PFP column. The orthogonal selectivity of YMC-Triart PFP leads to a different elution order. All the acidic compounds show a reduced retention time, whilst the basic compounds elute later when using the YMC-Triart PFP column. This results from the different retention mechanisms occurring using PFP columns.

Since not all silanols are neutral at acidic pH, this explains why the positively charged bases interact with the negatively charged silanols. On the other hand, the partial charge arising from the PFP ring system results in ion-exchange interactions. In contrast, the acidic compounds which are negatively charged are repulsed from the stationary phase and therefore eluting earlier.



Conclusion

Choosing the ideal column for a separation is not always easy. However, by considering the YMC-Triart series you can choose between 8 stationary phases providing different selectivities. Orthogonal selectivities, such as for YMC-Triart C18 and YMC-Triart PFP, are based on differences in primary and secondary interactions.

The Tanaka test visualises these differences. The Tanaka test and the analytes' properties both contribute greatly to the column selection for a separation.