Utilising Alternative Selectivity Provided by Novel High-Coverage C18 Phase Based on Robust Hybrid Particles for UHPLC/HPLC Method Development

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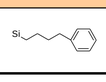
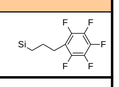
Introduction

More than 90% of UHPLC (Ultra High-Performance Liquid Chromatography) and HPLC analyses in reversed phase (RP) mode have been considered to be feasible by using C18 phases. Recently, the use of inorganic/organic hybrid particle based C18 phases has increased due to their high efficiency and good chemical stability/long lifetime. In addition the number of hybrid particle based C18 phases from various vendors has increased. However, most of these phases have been developed based on a strategy of making a "standard C18" which has moderate hydrophobicity and hydrophilicity. This is sometimes an impediment to flexible method development.

In order to broaden the scope of method development using C18 phases, YMC has developed a new high-coverage and fully-endcapped C18 phase based on robust hybrid particles. This phase offers complementary selectivity to standard C18 phases, making it especially suitable for separating hydrophobic drug substance from structurally similar impurities. In addition, its chemical stability, especially under neutral/alkaline conditions, enables the rapid optimisation of mobile phase conditions without limitation of the usable pH range.

In this poster, we introduce the unique characteristics of this new high-coverage C18 phase "YMC-Triart C18 ExRS", comparing it to our other hybrid based RP columns with C18, C8, Phenyl, and PFP chemistries. We also show an example of seamless method transfer between UHPLC and HPLC using by using this high-coverage C18 phase.

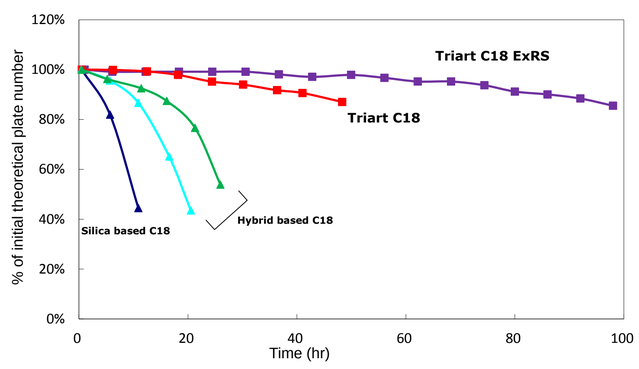
Specifications of YMC RP columns used in this poster

Bonded Phase	YMC-Triart C18 ExRS	YMC-Triart C18	YMC-Triart C8	YMC-Triart Phenyl	YMC-Triart PFP
	Si−$C_{18}H_{37}$		Si−C_8H_{17}		
Base material	inorganic/organic hybrid silica				
Particle size (µm)	5, 3, 1.9				
Pore size (Å)	80	120			
Specific surface area (m^2/g)	430	360			
Carbon content* (%)	25	20	17	17	15
Bonding	trifunctional				
End-capping	Yes ("multi-stage end-capping" technology)				No
Usable pH range	1-12			1-10	1-8
Temperature limit (Recommendation)	70℃ for pH 1-7/ 50℃ for pH 7-12			50℃	

*Containing 8% for hybrid silica base material.

- Specifications of YMC-Triart RP columns are compared in the above table. YMC-Triart C18 ExRS is based on hybrid particles with a smaller pore size and higher surface area than the other YMC-Triart RP columns. The higher bonding density of C18 is achieved by the combination of these newly-designed particles and advanced surface modification. We demonstrate its significantly different retention behaviour and selectivity towards a range of different compounds.

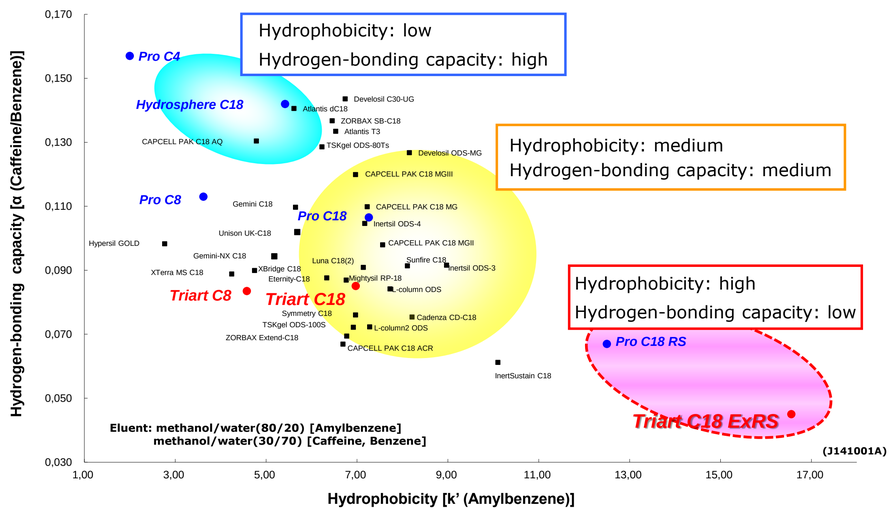
Comparison of alkaline stability



Column	: 5 µm, 150 X 4.6 mm i. d.
Eluent	: 50 mM K_2HPO_4-K_3PO_4 (pH 11.5)/methanol (90/10)
Flow rate	: 1.0 ml/min
Temperature	: 40℃
Sample	: benzyl alcohol

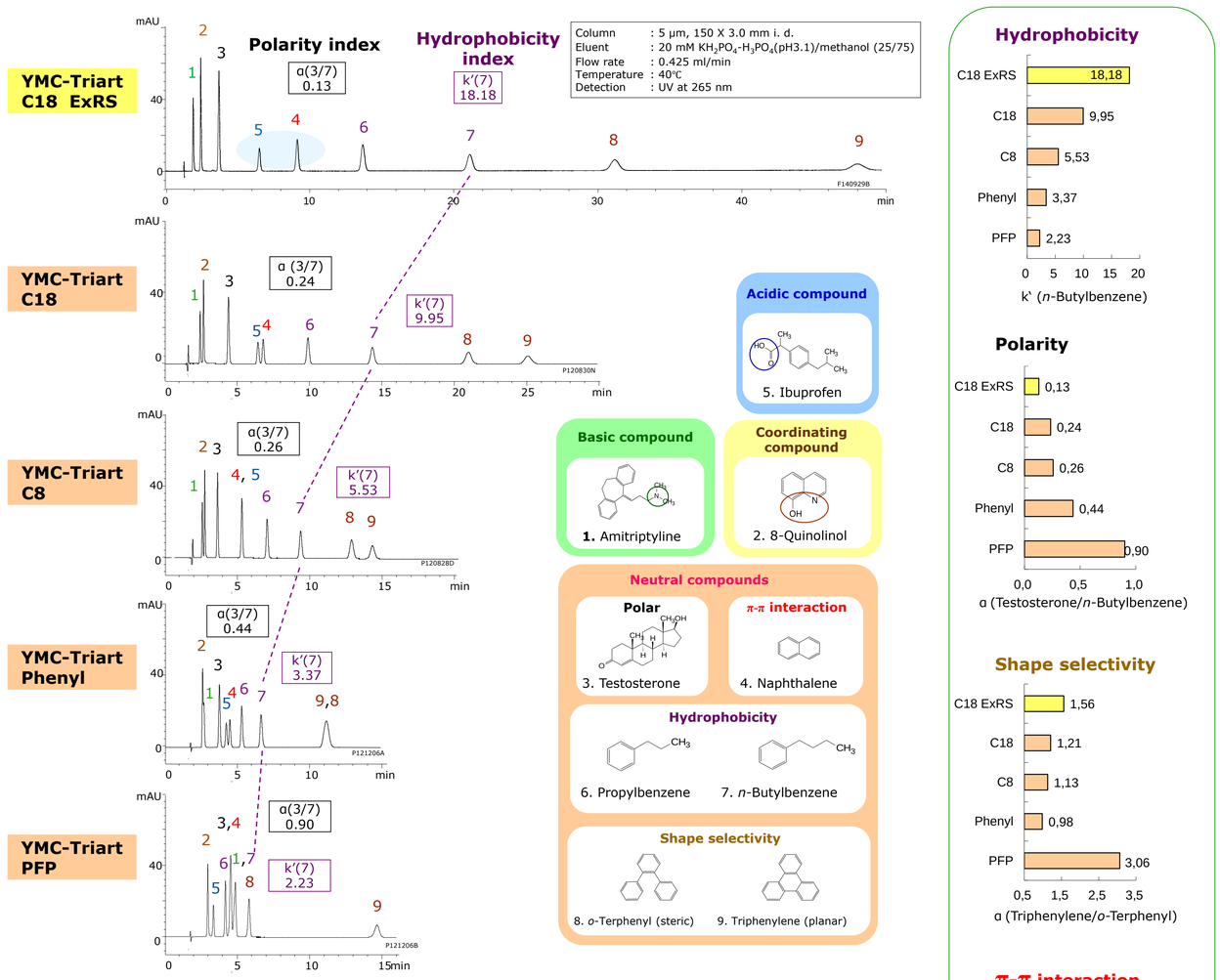
- The lifetime of YMC-Triart C18 ExRS under strongly alkaline conditions is more than 10 times greater than conventional silica-based C18 column. This excellent chemical stability is achieved by the combined technologies of novel hybrid particles, reproducible surface modification with high-density trifunctional C18 groups, and multi-stage endcapping.
- This stability enables rapid optimisation of mobile phase conditions without the limitation of the normal usable pH range.

Comparison of hydrophobicity and hydrogen-bonding capacity among various alkyl bonded phases



- YMC-Triart C18 ExRS has significantly higher hydrophobicity and lower hydrogen bonding capacity than many commercial C18 phases. This unique selectivity is provided by high coverage of C18 chains bonded to hybrid particles with large surface area. This complements the standard YMC-Triart C18 and other RP columns especially for the separation of hydrophobic compounds with similar structures.

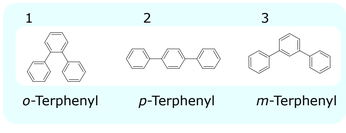
Comparison of selectivity and characteristics of YMC-Triart RP columns



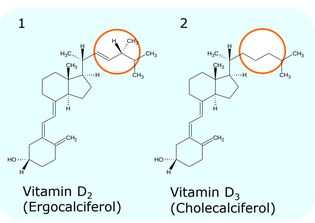
- The retention behaviour of YMC-Triart RP columns is compared for the separation of nine compounds illustrated as shown above. The compounds were selected as indicators of several types of chromatographic properties, including hydrophobicity, polar and π-π interactions, stereoselectivity, retention and peak shapes for ionic compounds. These chromatograms show the different capacity and selectivity for each bonded phase.
- YMC-Triart C18 ExRS has higher hydrophobicity and lower polarity than other bonded phases. Naphthalene and Ibuprofen (peaks 4 and 5), which are not separated by other Triart RP columns under the test conditions, can be completely separated using YMC-Triart C18 ExRS. YMC-Triart C18 ExRS also shows improved shape selectivity than any other phase except YMC-Triart PFP.
- The big difference in retention behaviour between YMC-Triart C18 ExRS and YMC-Triart C18 indicates that YMC-Triart C18 ExRS would be useful as an alternative selectivity to standard C18 columns in phase screening for method development.

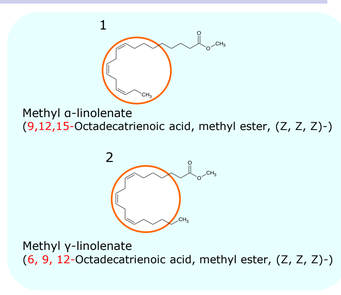
Separation of hydrophobic compounds with highly similar structures

Structural isomers



Structurally similar substances

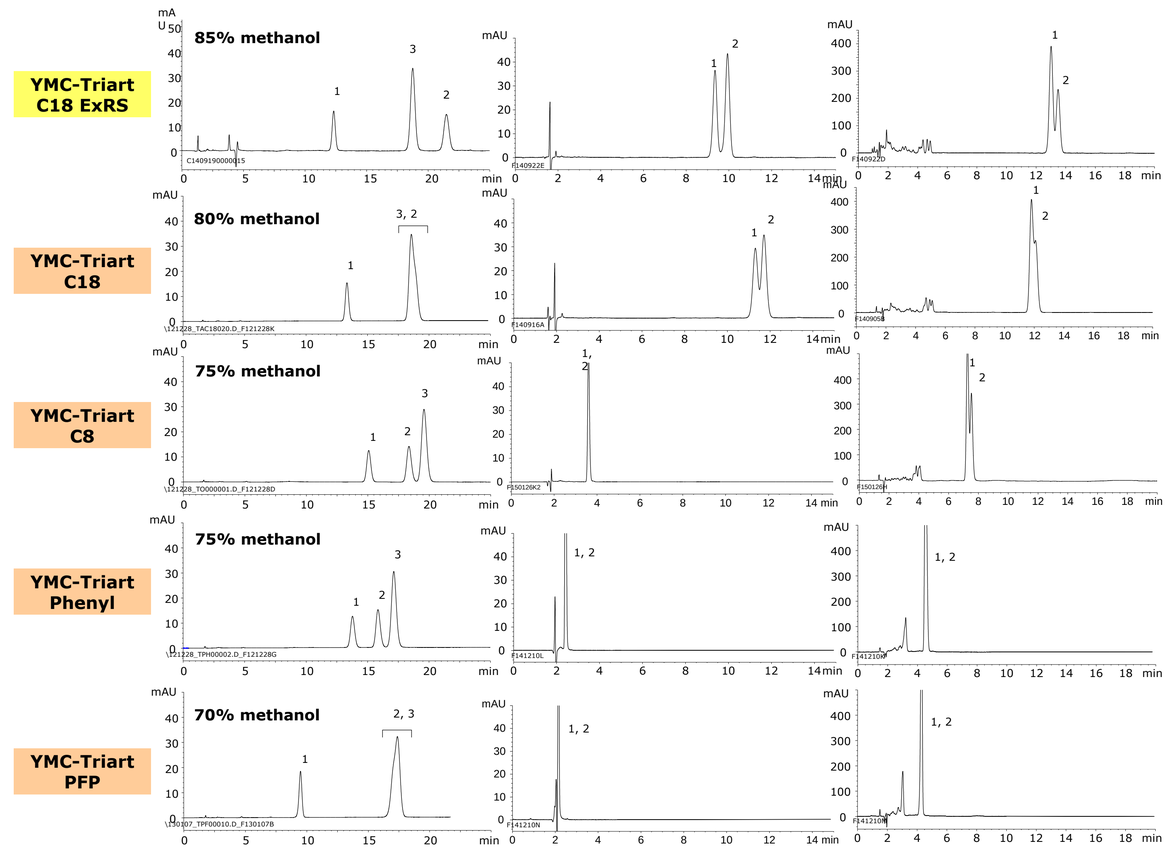




Column	: 5 µm, 150 X 3.0 mm i. d.
Eluent	: Written in each chromatograms
Flow rate	: 0.425 ml/min
Temperature	: 30℃
Detection	: UV at 254 nm

Column	: 5 µm, 150 X 3.0 mm i. d.
Eluent	: THF/acetonitrile (10/90)
Flow rate	: 0.425 ml/min
Temperature	: 30℃
Detection	: UV at 265 nm

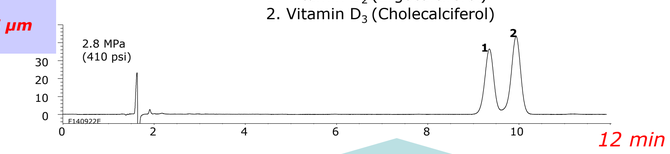
Column	: 5 µm, 150 X 3.0 mm i. d.
Eluent	: THF/acetonitrile/water (35/35/30)
Flow rate	: 0.425 ml/min
Temperature	: 35℃
Detection	: UV at 210 nm



Method transfer of vitamin D_2 and D_3 separation between HPLC and UHPLC

HPLC method
Triart C18 ExRS
150 X 3.0 mm i. d. ***5 µm***
L/dp=30
(0.425 ml/min)

1. Vitamin D_2 (Ergocalciferol)
2. Vitamin D_3 (Cholecalciferol)

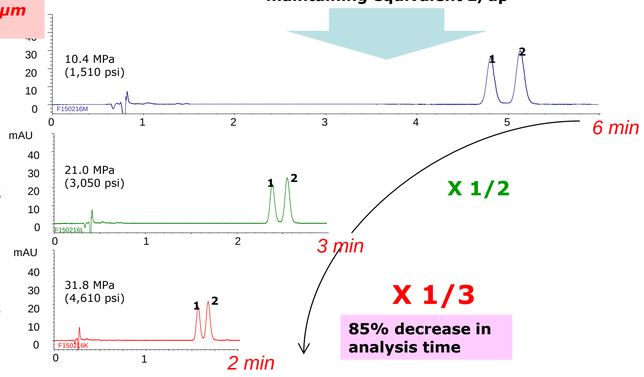


Eluent	: THF/acetonitrile (10/90)
Temperature	: 30℃
Detection	: UV at 265 nm
System	: Agilent 1290 Infinity

Method transfer maintaining equivalent L/dp

UHPLC method
Triart C18 ExRS
75 X 2.1 mm i. d. ***1.9 µm***
L/dp=39

Linear velocity X1 (0.21 ml/min)
Linear velocity X2 (0.42 ml/min)
Linear velocity X3 (0.63 ml/min)



- As shown in the above chromatograms, the HPLC method for vitamin D2 and D3 separation using 5 µm YMC-Triart C18 ExRS column can be transferred to a UHPLC method using a 1.9 µm column with a similar L/dp (ratio of column length to particle diameter).
- The efficiency of 1.9 µm packed column is maintained at higher flow rate, and the analysis time is reduced by 85% compared to the HPLC method without loss of resolution by increasing the linear velocity by factor of 3.
- The identical chromatographic performance and selectivity of YMC-Triart C18 ExRS across different particle sizes enables an easy method transfer between HPLC and UHPLC.

Comparison table of resolution in each condition

	Terphenyl (peak 2 and 3)	Vitamin D	Methyl linolenate
YMC-Triart C18 ExRS	++	++	+
YMC-Triart C18	-	+	-
YMC-Triart C8	++	-	+
YMC-Triart Phenyl	++	-	-
YMC-Triart PFP	-	-	-

++:baseline resolution, +: partial resolution, -: no resolution

- The above chromatograms show the comparison of separations of structural isomers and structurally similar substances with high hydrophobicity. For all of these compounds, YMC-Triart C18 ExRS shows superior resolution than any other YMC-Triart RP columns.
- Baseline resolution is achieved for Terphenyl isomers separation on YMC-Triart C18 ExRS, YMC-Triart C8, and YMC-Triart Phenyl. The difference in selectivity is demonstrated by the difference in elution order of p- and m-Terphenyl (peaks 2 and 3).
- The separation of hydrophobic structural analogues such as vitamin D2 and D3 or methyl α- and γ-linolenate is also improved by use of YMC-Triart C18 ExRS.
- The higher C18 bonding density of YMC-Triart C18 ExRS contributes to the recognition of small structural differences which makes this phase ideal for the separation of a hydrophobic pharmaceutical compound and their structurally similar impurities.

Conclusions

- The improved resolution of structural isomers and hydrophobic structural analogues is achieved using YMC-Triart C18 ExRS columns. This is a novel stationary phase with high-density trifunctional C18 groups onto inorganic/organic hybrid silica particles. Furthermore, the excellent separation of vitamin D2 and D3 demonstrates the facile method transfer between HPLC and UHPLC.
- These results indicate that the alternative selectivity and the reproducibility across different particle sizes for YMC-Triart C18 ExRS enable efficient and rapid method optimisation especially for the separation of structural similar compounds which are difficult to achieve with other RP columns.