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

Ernest J. Sobkow[1*], Noriko Shoji[2], Chie Yokoyama[2], Chiaki Iwata[2], Takashi Sato[2], and Takatomo Takai[2]

[1]YMC America, Inc., [2]YMC CO., LTD.

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, use of inorganic/organic hybrid particle based C18 phases is increasing due to their high efficiency and good chemical stability/long lifetime, and, so far, a number of hybrid particle based C18 phases have been marketed by various vendors. However, most of those phases have been developed based on a strategy of making a "Standard C18" that has moderate hydrophobicity and hydrophilicity. This sometimes impeded the flexible method development.

In order to enable a wide variety of method development using C18 phase, we developed a new high-coverage and fully-endcapped C18 phase based on robust hybrid particles. This phase offers complementing selectivity to standard C18 phases, rendering it efficacious in separating hydrophobic drug substance from structurally similar impurities. Also, its chemical durability, especially under neutral/alkaline conditions, enables the rapid optimization of mobile phase conditions without limitation of the usable pH range.

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

Specifications of RP columns used in this poster

Bonded Phase	Triart C18 ExRS	Triart C18	Triart C8	Triart Phenyl	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℃	

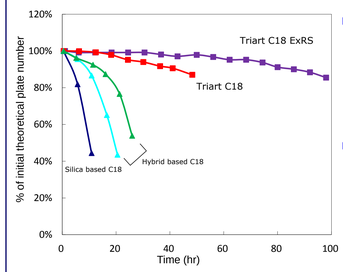
*Containing 8% for hybrid silica base material.

- Specifications of Triart RP columns are compared in above table. Triart C18 ExRS is based on hybrid particles with smaller pore size and higher surface area than other Triart RP columns. The higher bonding density of C18 is achieved by the combination of this newly-designed particles and advanced surface modification. It offers significantly different retention behavior and selectivity to various compounds.

Comparison of Hydrophobicity and Hydrogen-bonding capacity among various alkyl bonded phases

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

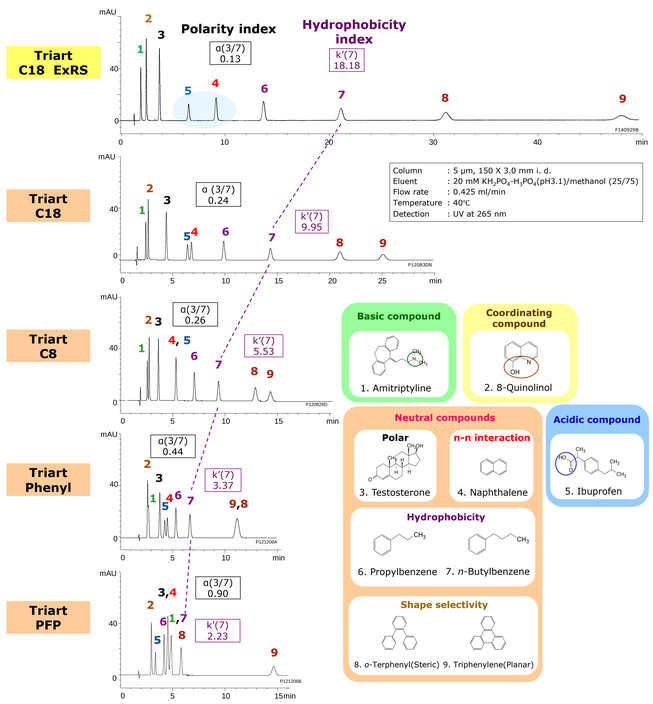
Comparison of alkaline durability



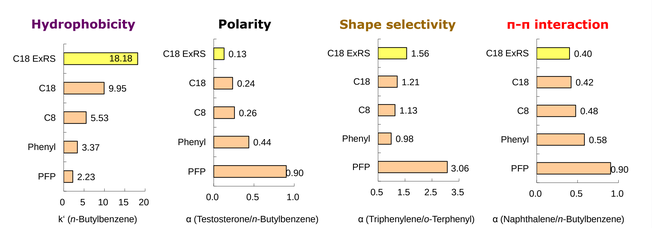
- The lifetime of Triart C18 ExRS under strongly alkaline conditions is more than 10 times greater than conventional silica based C18 column. This excellent chemical durability is achieved by combined technologies of novel hybrid particles, reproducible surface modification with high-density trifunctional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

Column	: 5 µm, 150 X 4.6 mm i. d.
Eluent	: 50 mM K_2HPO_4-K_2PO_4 (pH 11.5)/methanol (90/10)
Flow rate	: 1.0 ml/min
Temperature	: 40℃
Sample	: benzyl alcohol

Comparison of selectivity and characteristics among Triart RP columns



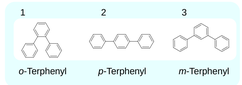
Column	: 5 µm, 150 X 3.0 mm i. d.
Eluent	: 20 mM KH_2PO_4-H_3PO_4(pH3.1)/methanol (25/75)
Flow rate	: 0.425 ml/min
Temperature	: 40℃
Detection	: UV at 265 nm



- The retention behavior of Triart RP columns is compared in the separation of nine compounds illustrated in above. The compounds are selected as indicators of several types of chromatographic properties, such as hydrophobicity, polar and π-π interactions, shape selectivity, retention and peak shapes for ionic compounds. These chromatograms show each bonded phase has different capacity and selectivity.
- Triart C18 ExRS has higher hydrophobicity and lower polarity than other bonded phases. Naphthalene and Ibuprofen (peak 4 and 5), which are not separated by other Triart RP columns under the tested condition, can be completely separated on Triart C18 ExRS. And also Triart C18 ExRS shows improved shape selectivity than other phases except PFP.
- The big difference in retention behavior between Triart C18 ExRS and Triart C18 indicate that Triart C18 ExRS would be useful as a alternative selectivity for standard C18 column in method development.

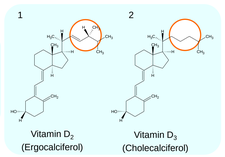
Separation of hydrophobic compounds with highly similar structures

Structural isomers

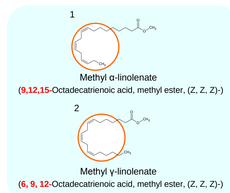


Column	: 5 µm, 150 X 3.0 mm i. d.
Eluent	: Written in each chromatograms
Flow rate	: 0.425 ml/min
Temperature	: 30℃
Detection	: UV at 254 nm

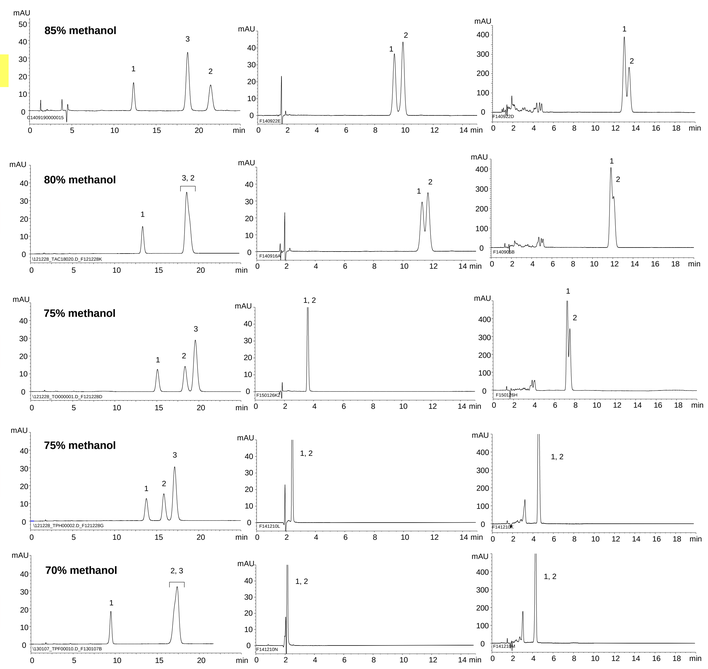
Structurally similar substances



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



Comparison table of resolution in each condition

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

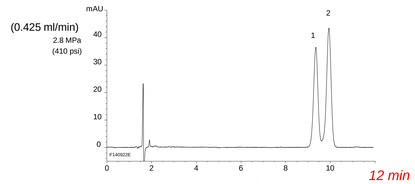
	Terphenyl (peak 2 and 3)	Vitamin D	Methyl linolenate
Triart C18 ExRS	++	++	+
Triart C18	-	+	-
Triart C8	++	-	+
Triart Phenyl	++	-	-
Triart PFP	-	-	-

- The above chromatograms show the comparison of separation of structural isomers and structurally similar substances with high hydrophobicity. For all of these compounds, Triart C18 ExRS shows superior resolution than other Triart RP columns.
- In Terphenyl isomers' separation, baseline resolution is achieved on Triart C18 ExRS, Triart C8, and Triart Phenyl. The difference of selectivity is shown from difference in elution order of p- and m-Terphenyl (peak 2 and 3).
- The separation of hydrophobic structural analogs like Vitamin D_2 and D_3 or Methyl α- and γ-linolenate is also improved on Triart C18 ExRS.
- The higher C18 bonding density of Triart C18 ExRS can contribute to recognize a small difference in structure. This feature would be preferable also for separation of a hydrophobic pharmaceutical compound and its structurally similar impurities.

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

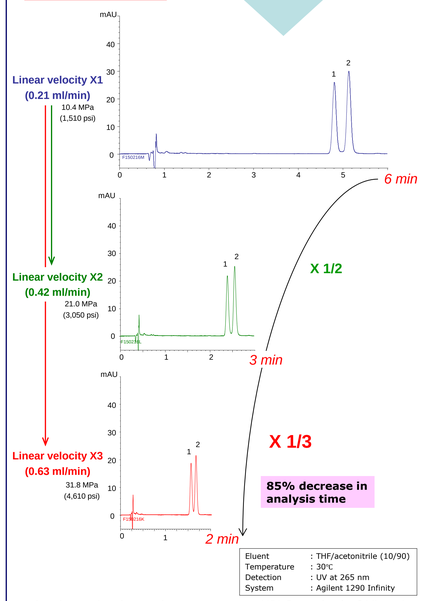
HPLC method
Triart C18 ExRS
150 X 3.0 mm i. d. ***5 µm***
L/dp=30

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



Method transfer maintaining equivalent L/dp

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



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

- As shown in above chromatograms, HPLC method of Vitamin D_2 and D_3 separation using 5 µm Triart C18 ExRS column is transferred to UHPLC method using 1.9 µm column with 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 to 15% compared to HPLC method without losing resolution by increasing linear velocity up to triple of the initial flow rate.
- The identical chromatographic performance and selectivity of Triart C18 ExRS across different particle sizes enables an easy method transfer between HPLC and UHPLC.

Conclusions

- The improved resolution of structural isomers and hydrophobic structural analogs is achieved on Triart C18 ExRS column, which is novel stationary phase bonded with high-density of trifunctional C18 groups on inorganic/organic hybrid silica particles. Furthermore, the excellent separation of Vitamin D2 and Vitamin D3 is maintained in method transfer between HPLC and UHPLC.
- These results indicate that the alternative selectivity and its reproducibility in different particle sizes of Triart C18 ExRS enable an efficient and rapid method optimization especially in separation of structural similar compounds which is hardly achieved on other RP columns.