Improvement in productivity of the method development for the drug analyses by using a novel hybrid reversed phase column

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Introduction

Silica based reversed phase columns have been widely used for analytical and preparative chromatographic field. The silica based packing materials have low stability under alkaline conditions, and have a limited usable pH range. Recently, there has been much attention given to hybrid materials that have two aspects of inorganic and organic character to improve the chemical stability.

We have developed a new type of hybrid reversed phase, YMC-Triart C18 and YMC-Triart C8. YMC-Triart columns are based on multi-layered organic/inorganic hybrid particles with 5 μm, 3 μm and a novel 1.9 μm diameter which are produced with a combination of our existing technologies for silica manufacturing and flow microreactor. We also have applied the optimized technology of surface modification for hybrid silica of YMC-Triart columns to all particle sizes for improving durability, scalability, selectivity and peak shapes for various types of compounds.

In this poster, we will evaluate the pH stability and chromatographic performance of YMC-Triart columns comparing commercially available columns, and show some application data utilizing characteristics of this material.

Features & benefits of YMC-Triart columns

- Three core technologies for particles and surface modification
  1. A multi-layered organic/inorganic hybrid particle
  2. A precise granulation with microreactor technology
  3. A proprietary C18/C8 bonding and a multi-stage, multi-compound end-capping

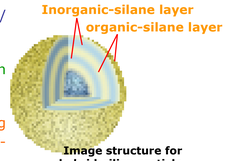


Image structure for hybrid-silica particle

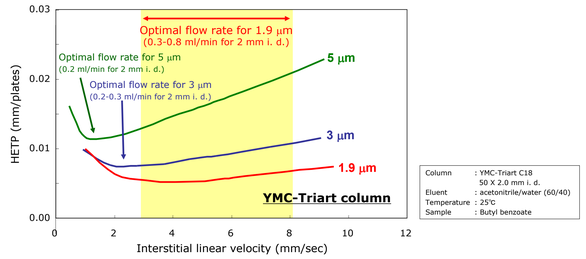
- Symmetrical peak shapes and reproducible retention for all types of compounds under a variety of mobile phase conditions
- Improved speed and resolution in UHPLC analysis on 1.9 μm columns with operating pressure up to 100 MPa (14,500 psi)
- Superior column-to-column and lot-to-lot reproducibility provided by YMC's rigorous manufacturing control system
- Outstanding chemical and physical durability over a wide pH range at a high temperature

Specifications of YMC-Triart columns

Base material	Multi-layered organic/inorganic hybrid
Stationary phase	Polymerically bonded C18 group (USP L1) and C8 group (USP L7)
Particle size	1.9 μm, 3 μm, 5 μm
Pore size	120 Å
Carbon loading	C18: Approx. 20%, C8: Approx. 17%
End-capping	Yes ("multi-stage end-capping" technology)
pH range	1-12
Temperature limit (Recommendation)	70℃ for pH 1-7 50℃ for pH 7-12

Characterization and evaluation of YMC-Triart columns

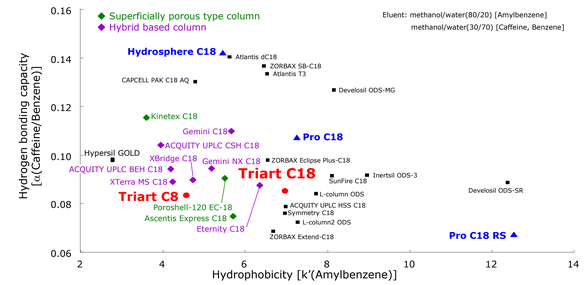
Comparison of column efficiency among different particle sizes



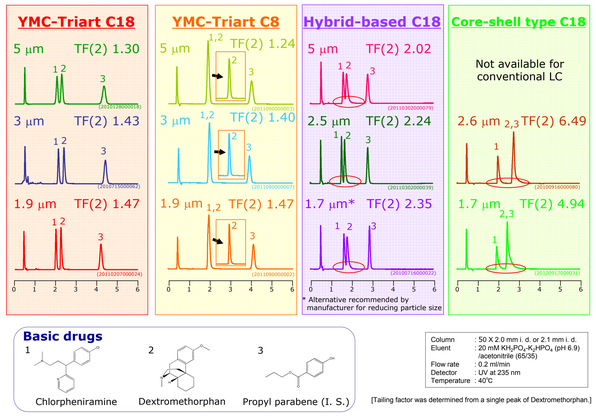
- 1.9 μm particle exhibits higher efficiency and maintains efficiency over a wide range of flow rate compared to 5 μm and 3 μm particles.
- 1.9 μm YMC-Triart columns enable ultra high throughput analysis by using shorter length column and increasing flow rate.

Comparison of selectivity and scalability among various C18/C8 columns

Selectivity chart -Hydrophobicity and Hydrogen bonding capacity-



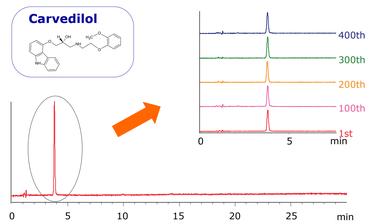
Comparison of scalability across different particle sizes

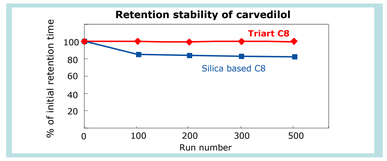


- YMC-Triart C18 has moderate hydrophobicity and hydrogen bonding capacity as a standard C18 phase and its selectivity is consistent across all of the particle sizes.
- YMC-Triart C8 has a different selectivity compared to YMC-Triart C18.
- YMC-Triart columns show the identical selectivity and the excellent peak shapes of basic (ionic) compounds across all of the particle sizes including 1.9 μm. It allows predictable scale up from UHPLC to conventional HPLC and even to semi-preparative LC, and vice versa.
- In contrast, commercially available C18 columns often show some differences in selectivity, retention, and peak shape across the different particle sizes.

Chemical stability under acidic and alkaline conditions

Analysis of carvedilol at pH 2 and 55℃

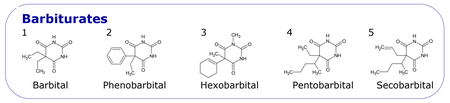


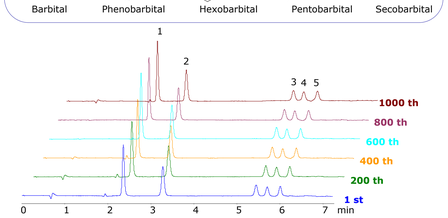


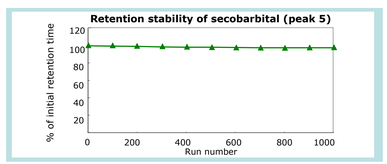
Column : YMC-Triart C8 (5 μm, 150 X 2.0 mm i. d.)
Eluent : phosphate buffer (pH 2.0)*/acetonitrile (65/35)
*Dissolve 2.72 g of KH_2PO_4 in 900 ml water, adjust pH 2.0 with H_3PO_4, and add water to make 1000 ml
Flow rate : 0.28 ml/min (adjust the flow rate so that the retention time of carvedilol is about 4 min)
Temperature : 55℃
Detection : UV at 240 nm

- No change in retention time of carvedilol was observed even at low pH and elevated temperature.

Analysis of barbiturates at pH 9.5







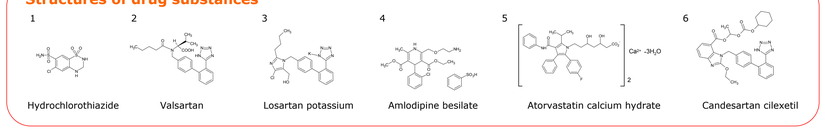
Column : YMC-TriartC18 (5 μm, 50 X 2.0 mm i. d.)
Eluent : A) 20 mM $HCOONH_4$-NH_3 (pH 9.5) B) methanol 0-90%B (0-7 min)
Flow rate : 0.2 ml/min
Detection : UV at 240 nm
Temperature : 25℃

- No change in retention time of barbiturates was observed even after 1,000 runs at elevated pH.
- High chemical durability of YMC-Triart columns achieved by applying hybrid particles and novel surface modification allows to utilize a wide pH range for better method development.

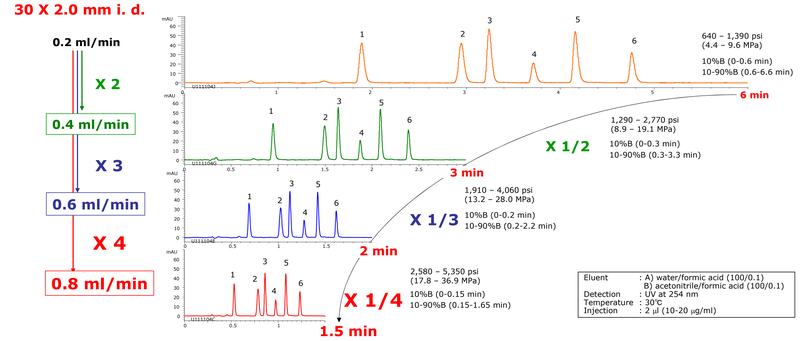
Application

Development of Ultra Fast separation method for drug substances

Structures of drug substances



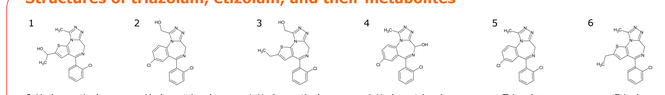
YMC-Triart C8 1.9 μm 30 X 2.0 mm i. d.



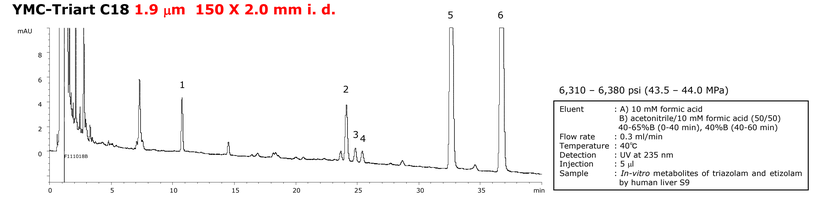
- 1.9 μm YMC-Triart C8 provides a ultra fast separation of six drug substances which are different in polarity and hydrophobicity within 1.5 minutes by using the short column and increasing flow rate.
- 1.9 μm YMC-Triart is useful for drug discovery research where a high throughput analysis is required.

Simultaneous separation of benzodiazepines and their metabolites

Structures of triazolam, etizolam, and their metabolites



YMC-Triart C18 1.9 μm 150 X 2.0 mm i. d.



- Triazolam and etizolam are sleep-inducing drugs, and recently focused as abused substances of addicts. The identification of these two drugs and their hydroxylated metabolites is usually very difficult because of the similarity in structure, molecular weight and mass spectra, even by using MS or MS/MS detection. Therefore the distinct separation on an analytical column is required.
- The baseline separation of triazolam, etizolam, and their metabolites formed *in vitro* by human liver S9 can be achieved by 1.9 μm and 150 mm length of YMC-Triart C18 column which has an extremely high efficiency. The longer length (or coupled) columns packed with 1.9 μm particle are useful for the screening, identification, and quantification of drugs and their metabolites in a complex biological matrix.

Conclusions

- The enhanced durability and chromatographic performance of YMC-Triart columns offer the maximum flexibility in separation conditions across an expanded pH range.
- 1.9 μm YMC-Triart columns with excellent chromatographic performance and 100 MPa of maximum operating pressure enable ultra-fast and reliable analysis.
- Identical chromatographic performance and selectivity of Triart columns across different particle sizes provides mutual method transfer among UHPLC, HPLC and even semi-preparative LC.