A novel organic/inorganic hybrid C18 stationary phase for efficient method development over a wide pH range

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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 recently developed a new type of hybrid C18 stationary phase, YMC-Triart C18. Triart C18 is based on novel multi-layered organic/inorganic hybrid particles which are produced with a combination of our existing technologies for silica manufacturing and flow microreactor. We also have optimized the surface modification technology for C18 bonding and end-capping.

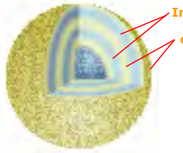
In this poster, we will evaluate the pH stability and chromatographic performance of Triart C18 comparing existing conventional column, and show some application data utilizing characteristics of this material.

The technologies for particles and surface modification

YMC-Triart C18 has been developed using three core technologies: the layered hybrid particle, the precise microreactor granulation, and the multi-stage end-capping.

1 Multi-layered hybrid material

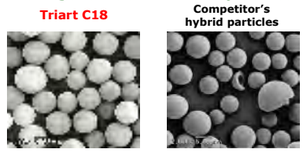
Image structure for hybrid-silica particle



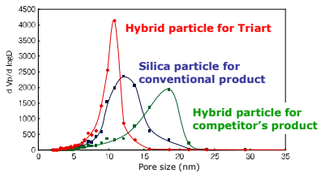
YMC-Triart C18 is based on a multi-layered hybrid particle consisting of two types of layers: a silica based layer (inorganic) and a hybrid polymer based layer (organic). This layered structure contributes to produce physical and chemical durability, and excellent chromatographic performance.

2 New granulation process by microreactor technology

Homogeneous and uniform particles



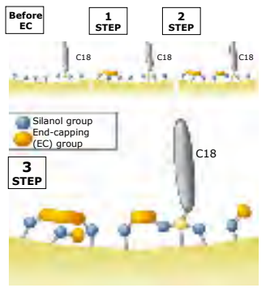
Narrow pore size distribution



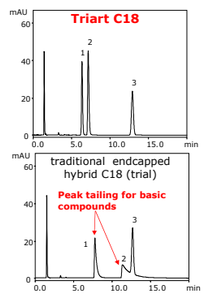
Utilizing of microreactor technology enables the manufacturing of mono-dispersed spherical particles with extremely smooth surface. Furthermore, the pore size distribution is remarkably narrow compared to existing products. This particle uniformity results in low operating pressure and highly reproducible surface modification.

3 Surface modification technology

Reaction image for "multi-stage end-capping"



Evaluation of silanol activity



In addition to proprietary C18 bonding, a new "multi-stage/multi-compound end-capping process" has been developed to overcomes the weaknesses of traditional single-step end-capping and reduce residual silanol groups ultimately. It results in excellent peak shapes, enhanced stability and lot-to-lot reproducibility.

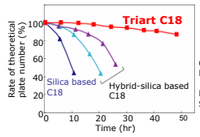
1. Chlorpheniramine (basic compound)
2. Dextromethorphan (basic compound)
3. Propyl paraben (internal standard; I.S.)

Column : 5 μm, 150 X 3.0 mm i. d.
Eluent : 20 mM KH_2PO_4-K_2HPO_4 (pH 6.9)/acetonitrile(65/35)
Flow rate : 0.425 ml/min
Temperature : 40℃
Detectotion : UV at 235 nm

Comparison of chemical durability

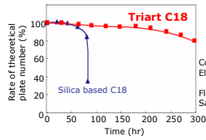
Durability in high pH

Phosphate Buffer, pH 11.5, 40℃



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
Sample : benzylalcohol

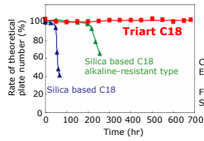
Triethylamine, pH 11.5, 40℃



Column : 5 μm, 150 X 4.6 mm i. d.
Eluent : 50 mM triethylamine (pH 11.5) /methanol (90/10)
Flow rate : 1.0 ml/min
Sample : benzylalcohol

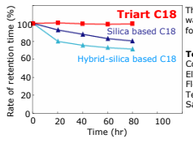
Durability in high temperature

pH 6.9, 70℃



Column : 5 μm, 50 X 2.0 mm i. d.
Eluent : 20 mM KH_2PO_4-K_2HPO_4 (pH 6.9) /acetonitrile (90/10)
Flow rate : 0.2 ml/min
Sample : phenol

pH 1, 70℃

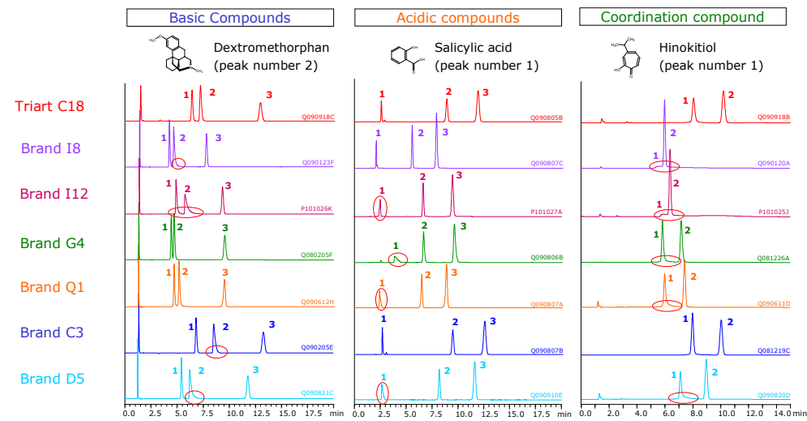


The columns are kept in acetonitrile/water/TFA (10/90/1) at 70℃, and tested for performance every 20 hours.

Test condition
Column : 5 μm, 50 X 2.0 mm i. d.
Eluent : acetonitrile/water (60/40)
Flow rate : 0.2 ml/min
Temperature : 37℃
Sample : butyl benzoate

Newly developed hybrid particles and surface modification of Triart C18 provide excellent durability in difficult conditions such as acidic or alkaline mobile phases, and at a high temperature. This advantage allows to utilize a wide pH range to optimize separation of complex mixture or increase loading amount of a compound which is poorly soluble at a certain pH.

Comparison of chromatographic performance for ionic compounds

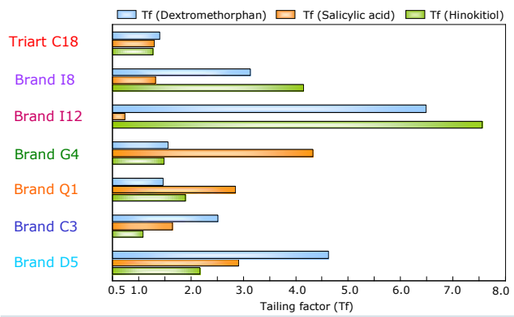


Column : 5 μm, 150 X 4.6 mm i. d.
Flow rate : 1.0 ml/min
Temperature : 40℃

Eluent : 20 mM KH_2PO_4-K_2HPO_4 (pH 6.9) /acetonitrile (65/35)
Sample : 1. Chlorpheniramine
2. Dextromethorphan
3. Propyl paraben (I.S.)

Eluent : 10 mM CH_3COOH-CH_3COONH_4 (pH4.2) /acetonitrile (75/25)
Sample : 1. Salicylic acid
2. Methyl paraben (I.S.)
3. Cinnamic acid

Eluent : acetonitrile/0.1% H_3PO_4 (40/60)
Sample : 1. Hinokitiol
2. Methyl benzoate (I.S.)

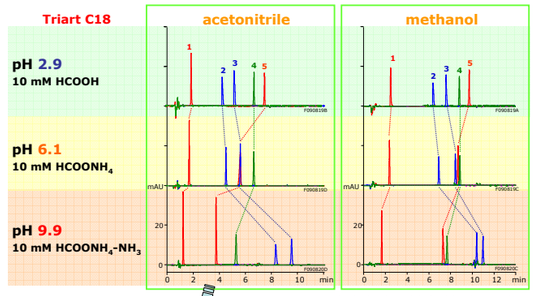


The peak tailing or fronting of ionic compounds are often caused by adsorption to residual silanol groups and/or surface impurities resulting from base materials or manufacturing process.

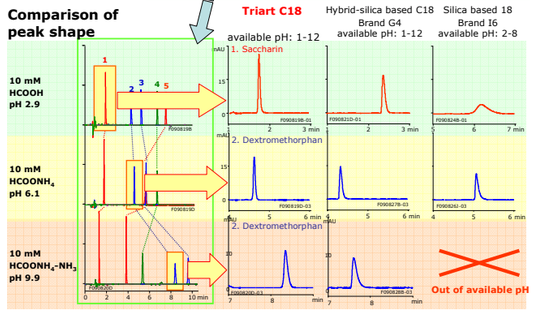
As shown in the upper chromatograms and the left graph, the chromatographic performance of commercially available C18 columns is compared in difficult separation of basic (Dextromethorphan), acidic (Salicylic acid), and coordination compound (Hinokitiol). Triart C18 provides symmetrical peaks for all types of compounds, unlike other C18 columns.

Effect of mobile phase conditions on selectivity and peak shapes

Effect of pH and organic solvent on selectivity



Comparison of peak shape



Acidic compounds

1. Saccharin pka=2.2
5. Ibuprofen pka=4.4

Basic compounds

2. Dextromethorphan pka=8.3
3. Amitriptyline pka=9.4

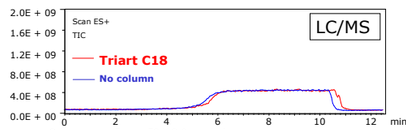
Neutral compound

4. *n*-Butylparaben pka=8.3

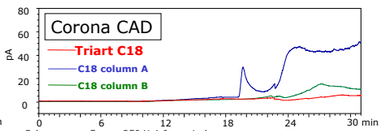
Column : 5 μm, 50 X 2.0 mm i. d.
Eluent : A) 10 mM ammonium formate buffer
B) organic solvent
5-90%B (0-10 min), 90%B (10-15 min)
Flow rate : 0.2 ml/min
Temperature : 25℃
Detection : UV at 230 nm

In reversed-phase HPLC, pH and organic solvent are the most important factor to control retention and selectivity as shown in upper chromatograms. On Triart C18, the peak shapes are symmetrical independent of a mobile phase condition, even for acidic or basic compounds that often exhibit peak tailing with conventional C18 columns. This enables the efficient method development based on selectivity and resolution.

Column bleeding test with high-sensitivity detector



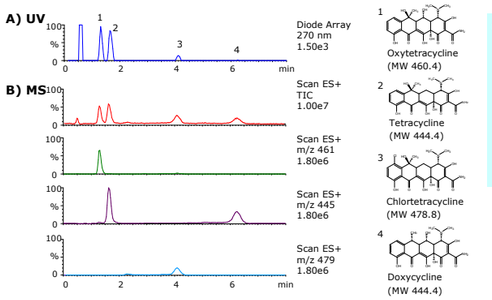
Column : 5 μm, 50 X 2.0 mm i. d.
Eluent : A) water/HCOOH (100/0.1) B) acetonitrile/HCOOH (100/0.1) 5%B (0-1 min), 5-100%B (1-5 min), 100%B (5-6 min)
Flow rate : 0.4 ml/min
Temperature : 40 ℃



Column : 5 μm, 250 X 4.6 mm i. d.
Eluent : A) water/HCOOH (100/0.1) B) acetonitrile/HCOOH (100/0.1) 5%B (0-5 min), 5-100%B (5-20 min), 100%B (20-30 min)
Flow rate : 1.0 ml/min
Temperature : 40 ℃

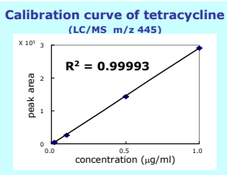
Bleeding of stationary phase can often cause unexpected peak and background noise on a high-sensitivity detector. Triart C18 shows remarkably reduced background signal in our evaluation using ESI-MS (TIC) and Corona charged aerosol detector with the typical mobile phase condition.

Application – High sensitivity LC/MS analysis of tetracycline antibiotics -



1 Oxytetracycline (MW 460.4)
2 Tetracycline (MW 444.4)
3 Chlortetracycline (MW 478.8)
4 Doxycycline (MW 444.4)

Calibration curve of tetracycline (LC/MS m/z 445)



Column : Triart C18 5 μm, 50 X 2.0 mm i. d.
Eluent : acetonitrile/water/formic acid (15/85/0.1)
Flow rate : 0.4 ml/min
Temperature : 40℃
Detection : A) UV at 270 nm B) ESI positive-mode
Injection : 10 μl (0.02-1 μg/ml)

Conclusions

- The combination of three core technologies - the multi-layered hybrid particle, the precise microreactor granulation, and the multi-stage end-capping - produces a material with outstanding chemical and physical durability, and also provides excellent peak shape for any kind of compounds under a variety of mobile phase conditions.
- This enhanced durability and chromatographic performance offers the maximum flexibility in separation conditions over a wide pH range.