Development of novel reversed-phase packing material for improved separation of protein biopharmaceuticals including intact antibodies

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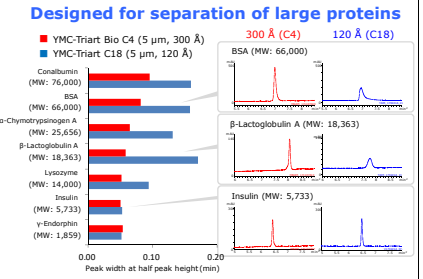
Introduction

On the development and quality control of biopharmaceuticals (proteins, monoclonal antibodies, antibody drug conjugate, etc.), high-performance liquid chromatography (HPLC) is an important tool for analysis and characterization of their structural heterogeneity. We have developed a novel C4 bonded reversed-phase (U)HPLC column named YMC-Triart Bio C4, which is based on organic/inorganic hybrid silica particles with pore diameter of 300 Å, designed for biopharmaceuticals separation. Optimized pore size with narrow pore distribution and advanced surface modification that suppresses interaction between an analyte and residual silanol group improve resolution, peak shape, sensitivity and reproducibility on analyses of biomolecules such as intact and subunits of monoclonal antibodies. In this poster, we will show some examples of effective method development for biopharmaceuticals such as intact monoclonal antibodies and their fragments with this new hybrid C4 column.

Specifications

Bonded Phase	YMC-Triart Bio C4
	Si−C_4H_9
USP class	L26
Base material	inorganic/organic hybrid silica
Particle size (µm)	5, 3, 1.9
Pore size (Å)	300
Bonding	trifunctional
End-capping	Yes
Usable pH range	1-10
Temperature limit (Recommendation)	90°C for pH 1-7 50°C for pH 7-10

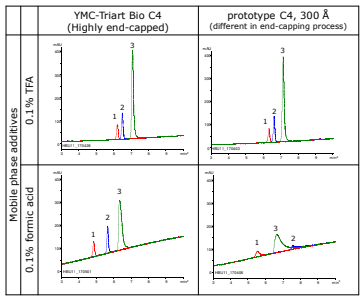
Designed for separation of large proteins



Column size : 150 X 3.0 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
10-95%B (0-15 min)
Flow rate : 0.4 mL/min
Temperature : 40°C
Detection : UV at 220 nm
Injection : 4 µL (0.1 ~ 0.5 mg/mL)

- The optimized pore size with narrow distribution of Triart Bio C4 improves peak shape and intensity of proteins with high molecular weight.

Effect of surface modification on peak shape of proteins and peptides



- The chromatograms of protein analysis are compared between Triart Bio C4 column and its prototype which is different in end-capping process. Although the results obtained with 0.1% TFA (upper figures) are comparable, the results obtained with 0.1% formic acid (lower figures) are significantly different.
- The optimized end-capping process of Triart Bio C4 suppresses interaction between an analyte and residual silanol group and improves peak shape of proteins with 0.1% formic acid mobile phase.

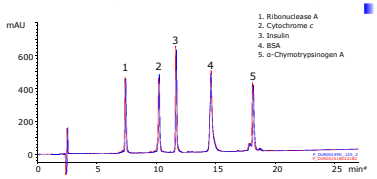
1. Cytochrome c
2. Insulin
3. BSA

Column size : 150 X 3.0 mmI.D.
Eluent : A) water/TFA or formic acid (100/0.1)
B) acetonitrile/TFA or formic acid (100/0.1)
10-95%B (0-15 min)
Flow rate : 0.4 mL/min
Temperature : 40°C
Detection : UV at 220 nm
Injection : 4 µL (0.1~ 0.5 mg/mL)

High chemical durability

Acidic condition (containing 0.1% TFA at 70℃)

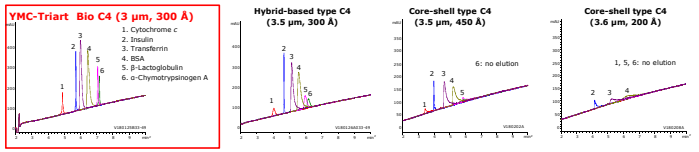
Red : Initial
Blue : After 125 hr (3,000 column volumes, 150 injections)



- No change in retention time and peak shape is observed on Triart Bio C4 under chemically harsh condition such as the strongly acidic and high temperature.

Column : YMC-Triart Bio C4 (5 µm, 300 Å)
150 X 3.0 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
20-60%B (0-27 min), 90%B (27-35 min), 20%B (35-50 min)
Flow rate : 0.4 mL/min
Temperature : 70°C
Detection : UV at 220 nm
Injection : 10 µL (0.25 ~ 0.50 mg/mL)

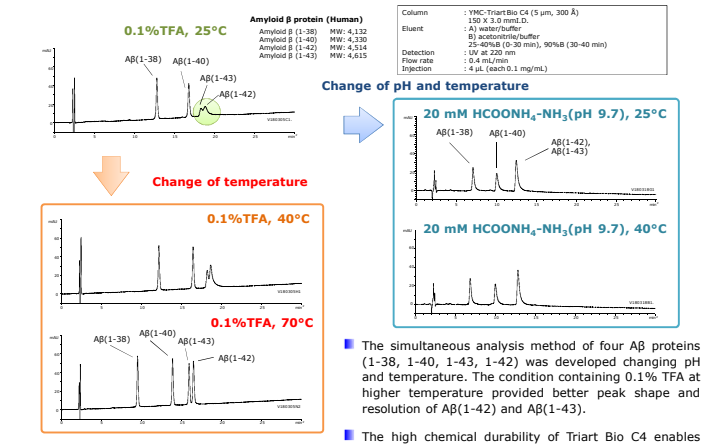
Comparison of protein separation with 0.1% formic acid mobile phase



Eluent : A) water/formic acid (100/0.1)
B) acetonitrile/formic acid (100/0.1)
10-95%B (0-15 min)
Temperature : 40°C
Detection : UV at 220 nm

- Triart Bio C4 shows better peak shape and recovery compared to commercially available C4 columns designed for bioseparation, with a mobile phase containing 0.1% formic acid, which is commonly used for LC/MS(/MS) analysis.

Method development of simultaneous analysis of Amyloid β (Aβ) proteins

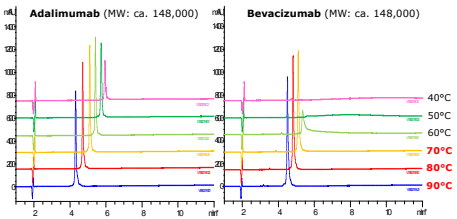


Column : YMC-Triart Bio C4 (5 µm, 300 Å)
150 X 3.0 mmI.D.
Eluent : A) water/buffer
B) acetonitrile/buffer
25-40%B (0-30 min), 90%B (30-40 min)
Detection : UV at 220 nm
Flow rate : 0.4 mL/min
Injection : 4 µL (each 0.1 mg/mL)

- The simultaneous analysis method of four Aβ proteins (1-38, 1-40, 1-43, 1-42) was developed changing pH and temperature. The condition containing 0.1% TFA at higher temperature provided better peak shape and resolution of Aβ(1-42) and Aβ(1-43).
- The high chemical durability of Triart Bio C4 enables rapid method optimization for complex mixture of peptides and proteins utilizing the wide pH and temperature range.

Analysis of intact monoclonal antibody (mAb)

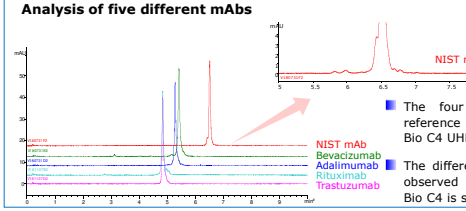
Effect of increasing temperature



Column : YMC-Triart Bio C4 (3 µm, 300 Å),
150 X 3.0 mmI.D.
Eluent : A) water/TFA (100/0.1).
B) acetonitrile/TFA (100/0.1)
30-60%B (0-15 min), 90%B (15-30min)
Detection : UV at 220 nm
Flow rate : 0.4 mL/min
Injection : 4 µL (0.5 mg/mL)

- The chromatograms of monoclonal antibody analysis with different column temperature from 40°C to 90°C are compared.
- Increasing column temperature improves peak shape and sensitivity of monoclonal antibody.

Analysis of five different mAbs

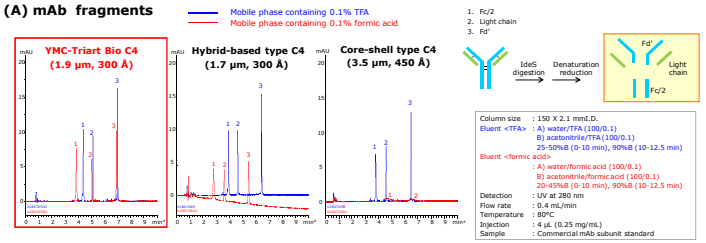


Column : YMC-Triart Bio C4 (1.9 µm, 300 Å)
50 X 2.1 mmI.D.
Eluent : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
25-45%B (0-10 min), 90%B (10-12.5 min)
Detection : UV at 280 nm
Flow rate : 0.4 mL/min
Temperature : 80°C
Injection : 2 µL (0.5 mg/mL)

- The four therapeutic mAbs and NIST mAb reference material are analyzed on 1.9 µm Triart Bio C4 UHPLC column at 80°C.
- The difference in retention and peak profiles are observed among these mAbs and it shows Triart Bio C4 is suitable for mAb characterization.

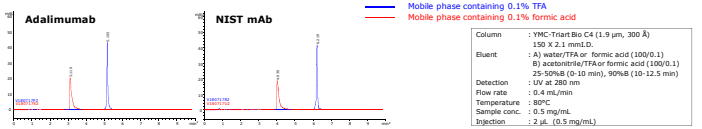
Analysis of monoclonal antibody and related substances with LC/MS compatible mobile phase

(A) mAb fragments



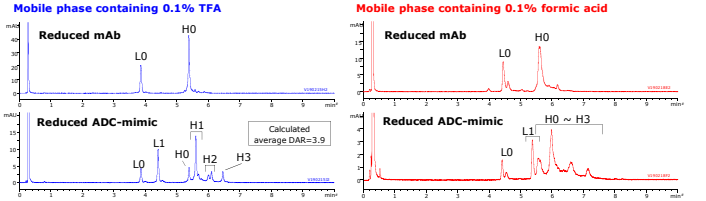
Column size : 150 X 2.1 mmI.D.
Eluent <TFA> : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
25-50%B (0-10 min), 90%B (10-12.5 min)
Eluent <formic acid> : A) water/formic acid (100/0.1)
B) acetonitrile/formic acid (100/0.1)
20-45%B (0-10 min), 90%B (10-12.5 min)
Detection : UV at 280 nm
Flow rate : 0.4 mL/min
Temperature : 80°C
Injection : 4 µL (0.25 mg/mL)
Sample : Commercial mAb subunit standard

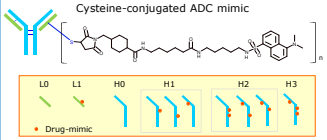
(B) Intact mAbs



Column : YMC-Triart Bio C4 (1.9 µm, 300 Å)
150 X 2.1 mmI.D.
Eluent : A) water/TFA or formic acid (100/0.1)
B) acetonitrile/TFA or formic acid (100/0.1)
25-50%B (0-10 min), 90%B (10-12.5 min)
Detection : UV at 280 nm
Flow rate : 0.4 mL/min
Temperature : 80°C
Sample conc. : 0.5 mg/mL
Injection : 2 µL (0.5 mg/mL)

(C) Antibody-drug conjugate (ADC)





Column : YMC-Triart Bio C4 (1.9 µm, 300 Å)
50 X 2.1 mmI.D.
Eluent <TFA> : A) water/TFA (100/0.1)
B) acetonitrile/TFA (100/0.1)
27-42%B (0-10 min), 90%B (10-12.5 min)
Eluent <formic acid> : A) water/formic acid (100/0.1)
B) acetonitrile/formic acid (100/0.1)
22-37%B (0-10 min), 90%B (10-12.5 min)
Detection : UV at 280 nm
Flow rate : 0.4 mL/min
Temperature : 80°C
Sample : mAb and ADC-mimic were reduced with 10 mM DTT at 30°C, 1 hr
Injection : 2 µL (2.0 mg/mL) for mAb
4 µL (0.725 mg/mL) for ADC-mimic

- The analysis results of (A) mAb fragments, (B) intact mAbs, and (C) reduced mAbs and ADCs are compared between 0.1% TFA added condition and 0.1% formic acid added condition. The excellent resolution and peak shape are obtained with Triart Bio C4 and 0.1% TFA added condition for a variety of mAb and related substances which are different in their molecular size and hydrophobicity.
- Formic acid is a more compatible additive for MS detection than TFA and commonly used in LC/MS analysis of low molecular compounds, however, it usually produces peak broadening and low intensity for proteins as shown in the results with two commercially available C4 columns and (A) mAb fragments. On Triart Bio C4 column, although slightly broader peaks and shorter retention are provided under 0.1% formic acid added condition for larger molecules such as (B) intact mAbs or (C) reduced mAb and ADC, the separation would be applicable for the structural analysis using LC/MS(/MS).

Conclusions

- The combination of newly developed hybrid particles with uniform 300 Å pore diameter and advanced surface modification of YMC-Triart Bio C4 column provide excellent peak shape for a variety of proteins and sufficient chemical durability in wide pH and temperature range. This advantage enables a rapid and efficient method optimization of a complex mixture of peptides and proteins.
- The superior peak shape and intensity even for larger biopharmaceutical proteins such as intact mAbs, mAb fragments and ADCs, are obtained on YMC-Triart Bio C4 with LC/MS compatible mobile phase containing 0.1% formic acid.