Utilizing of a novel organic/inorganic hybrid reversed phase column for efficient method development over a wide pH range

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Introduction

Method development of high-performance liquid chromatography (HPLC) methods requires optimization of several conditions, such as bonded-phase, column efficiency, solvent type, pH and temperature. Especially pH is a most important parameter to control retention, selectivity and sensitivity of ionic compounds in reversed phase HPLC. Although silica based reversed phase columns have been widely used for analytical and preparative separation, they have low stability under alkaline conditions and a limited usable pH range.

Recently, we have developed a new type of organic/inorganic hybrid reversed phase column, YMC-Triart C18 and YMC-Triart C8, to improve the chemical stability at expanded pH range and temperature. The novel technologies of manufacturing particles and surface modification provide outstanding chemical stability and excellent peak shape for many types of compounds under a variety of mobile phase conditions.

In this poster, we will show characteristics of this new hybrid column, and some example cases of efficient method development in separation of pharmaceutical compounds and natural products.

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

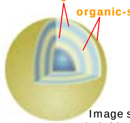


Image structure for hybrid-silica particle

- Outstanding chemical and physical durability over a wide pH range at a high temperature
- 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

Specification 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	Approx. 20%
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

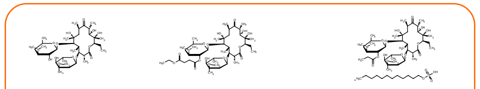
Conclusions

- Highly sensitive, selective and reproducible HPLC methods can be developed with a novel hybrid C18 column using pH and temperature as key tools for optimization.
- YMC-Triart columns offer significant advantages for simple and rapid method development of a variety of compounds.

The advantages of pH and temperature as tools for optimizing resolution and increasing sensitivity

Method optimization of erythromycin and its derivatives by pH and temperature

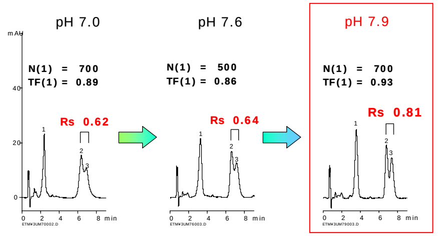
Structures of erythromycins



1. Erythromycin (EA) 2. Erythromycin ethylsuccinate (EES) 3. Erythromycin estolate (EE)

- Erythromycin and its derivatives are macrolide antibiotics which have a broad antimicrobial spectrum.
- Erythromycins are shown to be easily degraded under acidic (< pH 6.5) or strongly alkaline condition. This instability limits the choice of mobile phase condition.

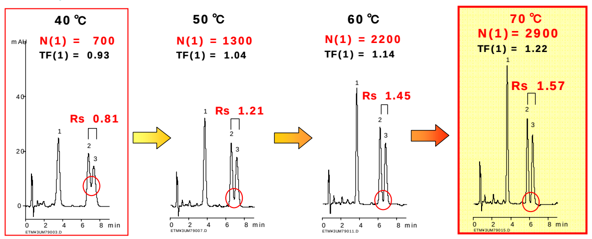
Effect of pH change on separation



- The pH is optimized within the range in which the compounds are stable.
- The peak shapes and the resolutions are slightly improved by raising the pH.

Column	YMC-Triart C18 (3 µm, 120 Å) 50 X 2.0 mm i.d.
Eluent	20 mM phosphate buffer/acetonitrile/methanol (40/45/15)
Flow rate	0.2 ml/min
Temperature	40℃
Detection	UV at 210 nm
Injection	1 µl (2.0 mg/ml)

Effect of temperature change on separation

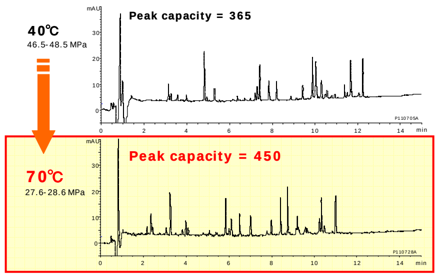


- The column temperature is increased at pH 7.9, and the higher temperature provides sharper peaks.
- The baseline separation of EES and EE (peak 2 and 3) is achieved at 70℃.
- The impact of temperature is stronger than that of pH for separation of erythromycins.

Column	YMC-Triart C18 (3 µm, 120 Å) 50 X 2.0 mm i.d.
Eluent	20 mM KH_2PO_4-K_2HPO_4 (pH 7.9)/acetonitrile/methanol (40/45/15)
Flow rate	0.2 ml/min
Detection	UV at 210 nm
Injection	1 µl (2.0 mg/ml)

Peptide mapping

Comparison of separation between low temperature (40℃) and high temperature (70℃)

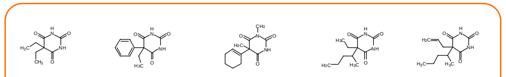


- 23% more peaks can be resolved by increasing the column temperature to 70℃ in peptide mapping.
- The higher column temperature often exhibits sharper peak on an analysis of high-molecular-weight compounds, such as peptides or proteins.

Column	YMC-Triart C18 (1.9 µm, 120 Å) 100 X 2.0 mm i.d.
Eluent	A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.08) 5-40%B (0-15 min)
Flow rate	0.4 ml/min
Detection	UV at 220 nm
Injection	10 µl
Sample	Tryptic digest of Bovine Hemoglobin
System	Agilent 1290

Method optimization of barbiturates in human serum by pH and detection wavelength

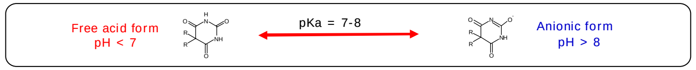
Structures of barbiturates



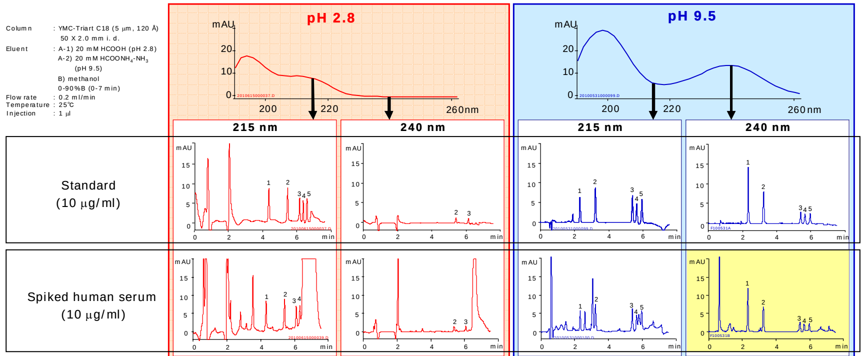
1. Barbital 2. Phenobarbital 3. Hexobarbital 4. Pentobarbital 5. Secobarbital

- Barbiturates have been widely used as psychotropic substances, and some of them are designated to regulation by the Convention on Psychotropic Substances.
- The structures and UV spectra of barbiturates vary depending on pH. The anionic form at alkaline pH (pH > 8) has a maximum absorption at 240 nm.

Comparison of separation and sensitivity between low pH and high pH



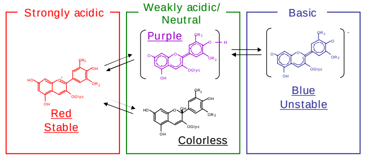
Column	YMC-Triart C18 (5 µm, 120 Å) 50 X 2.0 mm i.d.
Eluent	A-1) 20 mM HCOOH (pH 2.8) A-2) 20 mM $HCOONH_4$-NH_3 (pH 9.5) B) methanol 0-90%B (0-7 min)
Flow rate	0.2 ml/min
Temperature	25℃
Injection	1 µl



- UV detection with low wavelength is required for high-sensitive analysis at pH 2.8
- The strong UV absorption at low wavelength of the impurities in serum interfere quantitative determination of barbiturates.
- The highly sensitive and selective detection of barbiturates in serum is achieved by setting pH to 9.5 and wavelength to 240 nm.

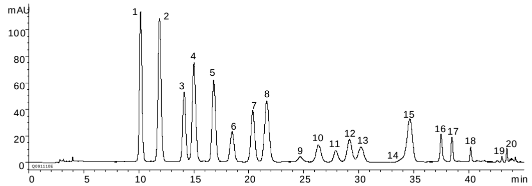
Analysis of anthocyanins under strongly acidic eluent condition (pH 1.5)

The change of structures and colors of anthocyanins by pH



- Anthocyanin is a kind of flavonoid pigment found in a various fruits and vegetables.
- The chemical structures and the colors of anthocyanins vary depending on pH. The strongly acidic condition is required for reproducible and highly sensitive analysis.

Analysis of bilberry extract with Triart C18



1. Delphinidin-3-O-galactoside
2. Delphinidin-3-O-glucoside
3. Cyanidin-3-O-galactoside
4. Delphinidin-3-O-arabinoside
5. Cyanidin-3-O-glucoside
6. Petunidin-3-O-galactoside
7. Cyanidin-3-O-arabinoside
8. Petunidin-3-O-glucoside
9. Peonidin-3-O-galactoside
10. Petunidin-3-O-arabinoside
11. Delphinidin
12. Peonidin-3-O-glucoside
13. Malvidin-3-O-galactoside
14. Peonidin-3-O-arabinoside
15. Malvidin-3-O-glucoside
16. Malvidin-3-O-arabinoside
17. Cyanidin
18. Petunidin
19. Peonidin
20. Malvidin

Column	YMC-Triart C18 (5 µm, 120 Å) 250 X 4.6 mm i.d.
Eluent	A) water/formic acid (90/10) B) acetonitrile/methanol/water/formic acid (22.5/22.5/40/10) 20-28%B (0-30 min), 28-70%B (30-40 min), 100%B (40-45 min)
Flow rate	1.0 ml/min
Temperature	25℃
Detection	UV/VIS at 535 nm
Injection	10 µl
Sample	Commercial bilberry extract

- Triart C18 which has high durability under the strongly acidic condition is suitable for the quantitative analysis and quality control of anthocyanins in bilberry extracts and foods.