Tips for Optimization of Peptide Separation

Separation Method Optimization of “Difficult-to-Separate” Peptides

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(S140922AE)

Factors to Be Considered on Method Optimization of Peptides and Proteins

Column

Combination of functional group and the pore diameter

→ Choose optimal combination by molecular weight and hydrophobicity of peptides and proteins.

Generally, a column with large pore size and low surface hydrophobicity is are suitable for a large molecules.

Mobile phase

Gradient elution with 0.1% TFA aq /acetonitrile system as first choice.

→ Change 1) concentration of TFA, 2) acid species and/or pH if a sample is mixture of compounds with various ionic characteristics.

→ Adjust the gradient conditions

2-propanol might be effective for separation of large proteins

Temperature

Effective for changing separation selectivity or improving peak shape. However, usable temperature range is limited by column durability.

(strongly acidic conditions + heating will accelerate the elimination of functional groups =short retention time and/or increase of unfavorable secondary interaction between the packing material and sample)

High durability column, Triart, can offer wider usable temperature range.
Temperature can be used as a tool for method optimization

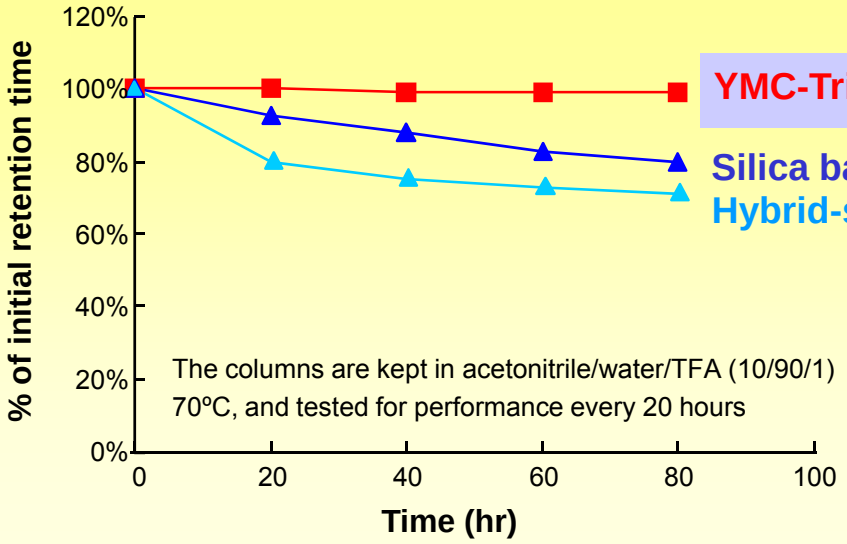
High Performance and Excellent Durability
YMC-Triart C18

Specifications

Base	Organic / inorganic hybrid silica
Bonding	Trifunctional
Functional group	C18
Particle size	1.9 µm, 3 µm, 5 µm
Pore size	12 nm
Carbon content*	20%
Endcapping	Yes
pH range	1~12
Usable temperature range (upper limit)	70℃ for pH 1-7 50℃ for pH 7-12

*Containing 8% carbon for hybrid silica base material

Excellent durability



Excellent durability even at low-pH or high-temperature conditions

Test condition		
	Column	: 5 µm, 50 X 2.0 mmI.D.
	Eluent	: acetonitrile/water (60/40)
	Flow rate	: 0.2 mL/min
	Temperature	: 37ºC
	Sample	: butyl benzoate

Optimization of Antimicrobial Peptides Separation

Structure

Compounds

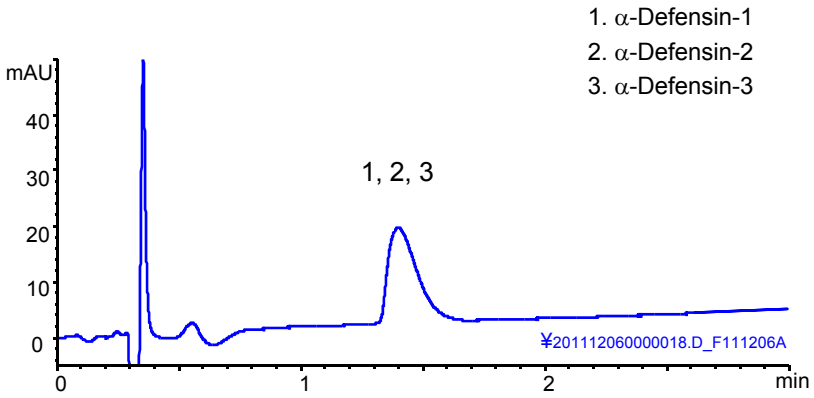
- **α-Defensin-1 : A CYCRIPACIAGERRYGTCIYQGRLWAFCC**
  MW 3,442
- **α-Defensin-2 : CYCRIPACIAGERRYGTCIYQGRLWAFCC**
  MW 3,371
- **α-Defensin-3 : D CYCRIPACIAGERRYGTCIYQGRLWAFCC**
  MW 3,486

*** Difference in amino acid residue on N-terminal**

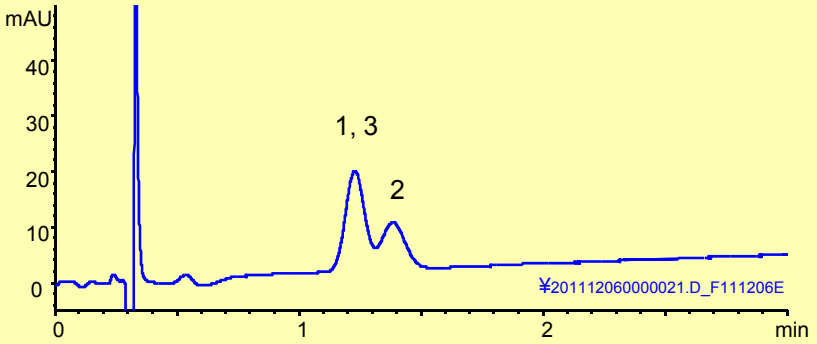
Optimization of Antimicrobial Peptides Separation

Effect of Column Temperature

40℃



70℃



Column	: **YMC-Triart C18** (1.9 µm, 12 nm),
	: 50 X 2.0 mmI.D.
Eluent	: A) water/TFA (100/0.1)
	B) **acetonitrile**/TFA (100/0.1)
	25-45%B (0-5 min)
Flow rate	: 0.4 mL/min
Detection	: 220 nm

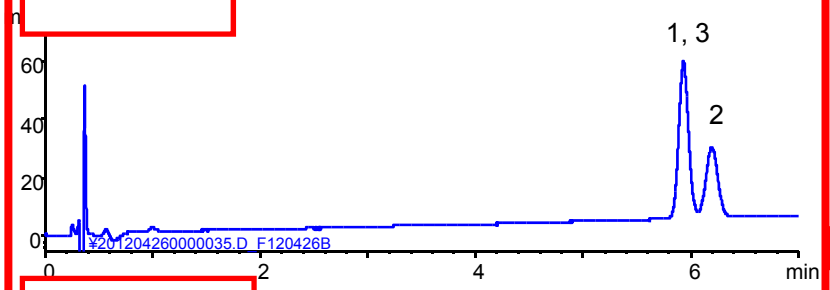
At 70℃, resolution of peak1, 3 and peak2 are improved.

Optimize other conditions

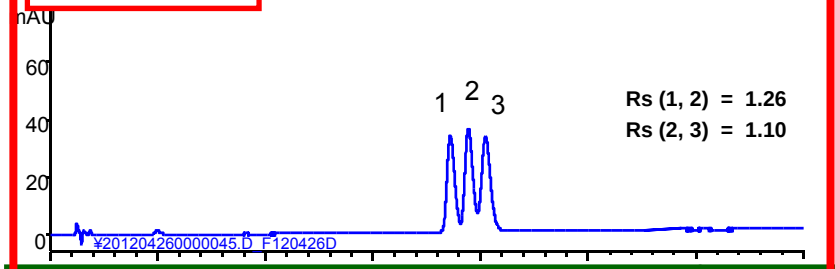
Optimization of Antimicrobial Peptides Separation

Effect of acid type, acid concentration and gradient conditions (at 70℃)

0.1% TFA



0.01% TFA



Column	: **YMC-Triart C18** (1.9 µm, 12 nm), 50 X 2.0 mmI.D.
Eluent	: **A) acid-containing aqueous solution**
	B) acid-containing acetonitrile solution
	(0.1 % HCOOH in B solution is 0.08 %)
Flow rate	: 0.4 mL/min
Detection	: 220 nm
Temperature	: **70℃**

1. α-Defensin-1
2. α-Defensin-2
3. α-Defensin-3

Acid Type / Acid Concentration

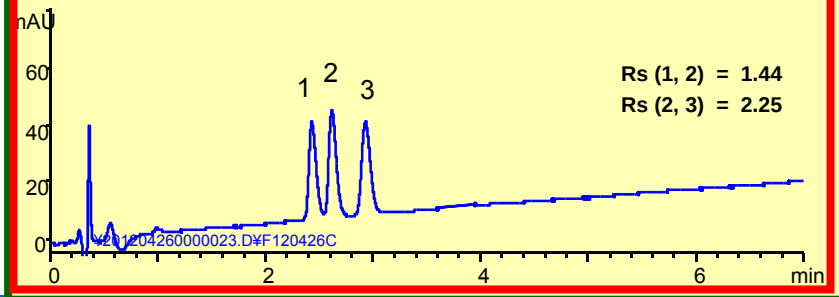
Change of acid type and acid concentration improves the resolution. On this case, use of formic acid instead of TFA is effective.

Gradient slope

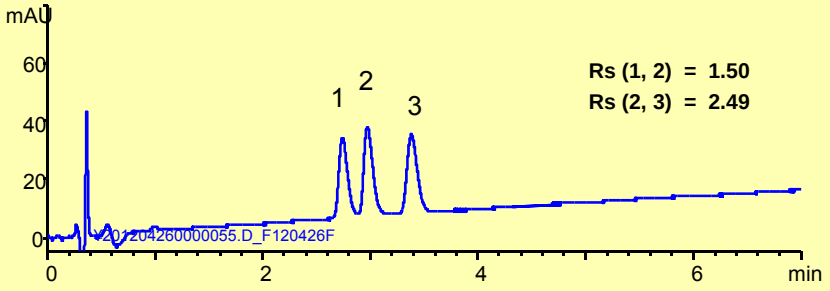
Resolution is improved by a gentle gradient slope.

0.1% HCOOH

15-35 %B (0-10 min)
slope : 2.0%B/min



15-30%B (0-10 min)
slope : 1.5%B/min

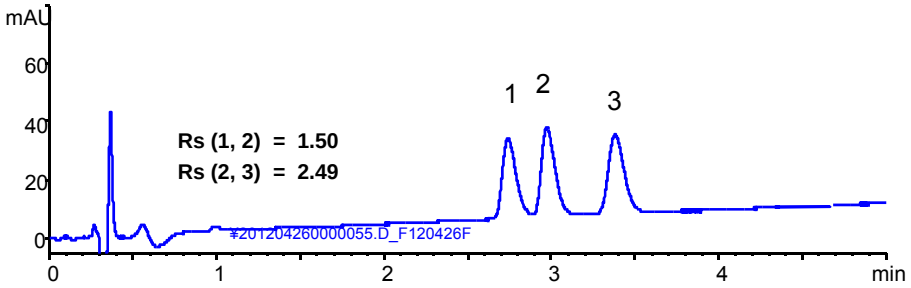


Optimization of Antimicrobial Peptides Separation

Addition of 2-propanol in a mobile phase

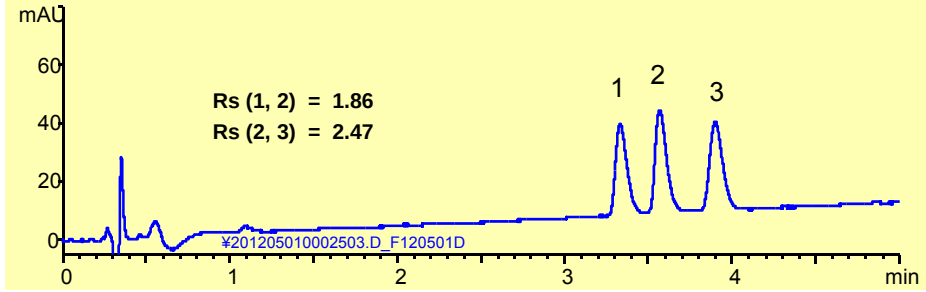
acetonitrile

15-30%B (0-10 min)
slope : 1.5%B/min



2-propanol/acetonitrile (50/50)

10-25%B (0-10 min)
slope : 1.5%B/min



1. α-Defensin-1
2. α-Defensin-2
3. α-Defensin-3

Column	: YMC-Triart C18 (1.9 μm, 12 nm) 50 X 2.0 mmI.D.
Eluent	: A) 0.1% formic acid in water B) 0.08% formic acid in **organic solvent**
Flow rate	: 0.4 mL/min
Detection	: 220 nm
Temperature	: 70℃

2-propanol is added in the mobile phase, and gradient slope is optimized.

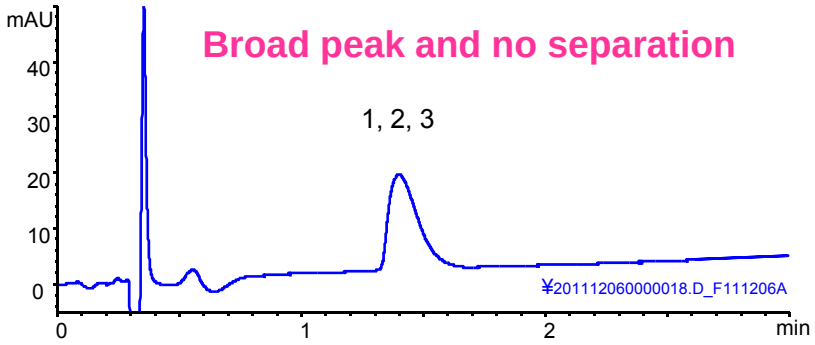
Resolution between peak 1 and 2 is improved while maintaining the similar analysis time.

Separation optimization example of antimicrobial peptide

Conclusion

YMC-Triart C18 (1.9 μm, 12 nm), 50 X 2.0 mmI.D.

Initial result



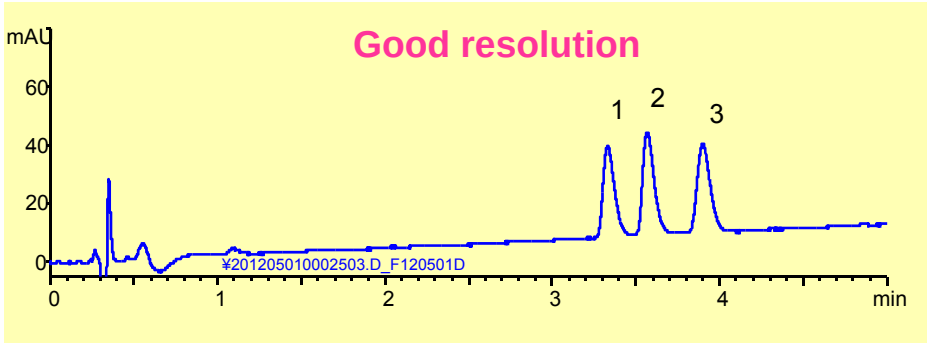
1. α-Defensin-1
2. α-Defensin-2
3. α-Defensin-3

Eluent	: A) water/TFA (100/0.1)
	B) acetonitrile/TFA (100/0.1)
	25-45%B (0-5 min)
Flow rate	: 0.4 mL/min
Temperature	: 40℃
Detection	: 220 nm

Separation condition optimization

- Changing column temperature
- Changing type of acid and organic solvent
- Changing gradient slope

Optimized condition



Eluent	: A) water/formic acid (100/0.1)
	B) 2-propanol/acetonitrile/formic acid (50/50/0.08)
	10-25%B (0-10 min)
Flow rate	: 0.4 mL/min
Temperature	: 70℃
Detection	: 220 nm

Tips for method optimization of peptides and proteins for reversed phase HPLC analysis

Concentration and type of acid

Selectivity change provided by other acid type and/or different concentration is expected

It is effective when there is a large difference in ionic characteristics of compounds.

Concentration and type of organic solvent

It is effective for improvement of resolution/peak shape to use an organic solvent with stronger elution ability for large molecular weight proteins and proteins with high hydrophobicity.

Column temperature

Changing the column temperature will provide selectivity change as well as peak shape improvement.

In particular, a good separation of proteins whose molecular weight is 10,000 or larger could be obtained under high temperature condition.

YMC-Triart C18, which offers excellent durability even under an elevated temperature condition, is effective for the separation of peptides and proteins.