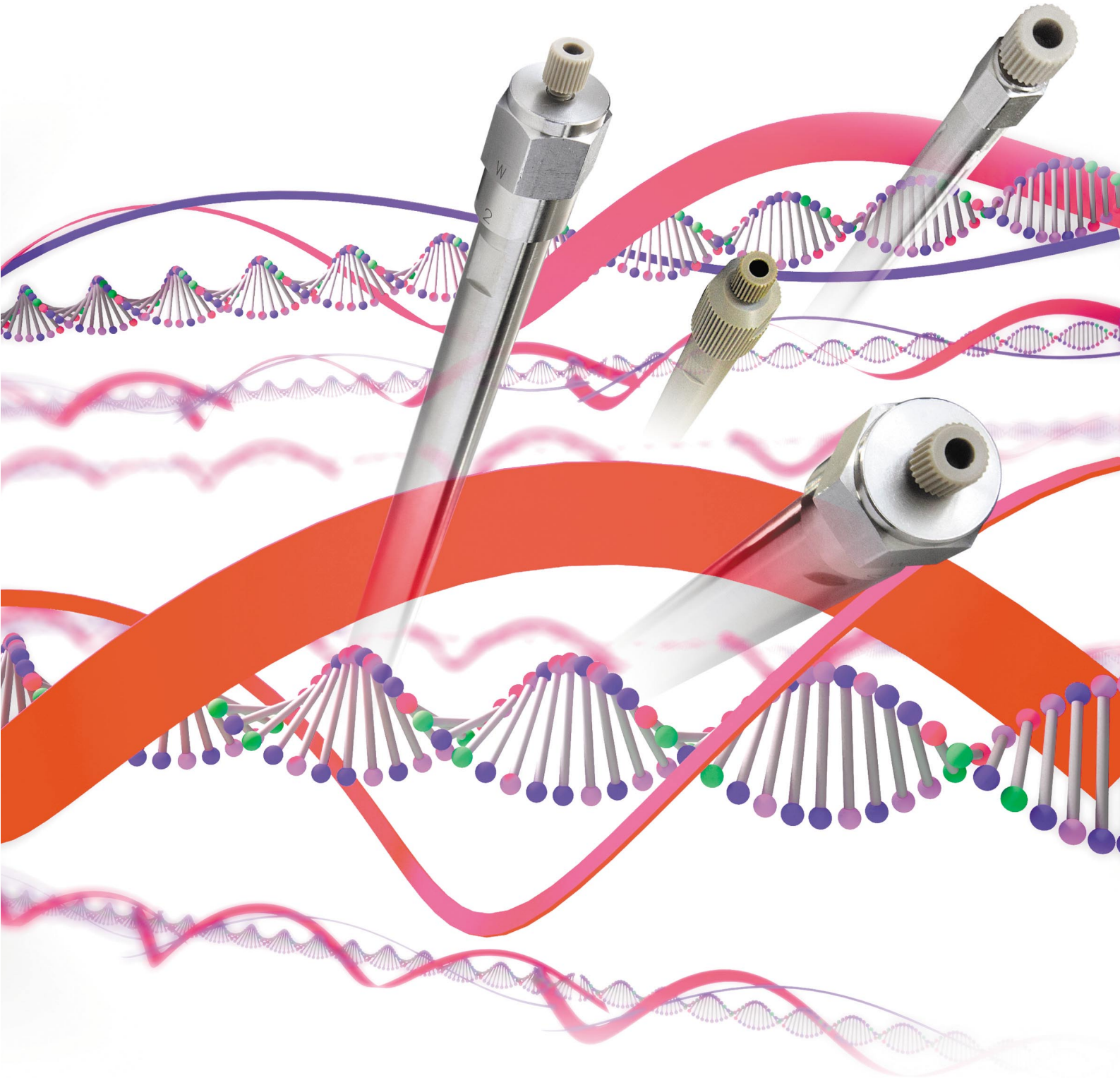
NOMURA CHEMICAL

Targeting low molecular to medium molecular compounds

For Amino Acids, Peptides, and Nucleic acids

HSR C18 Peptide

Develosil®



For Amino Acids, Peptides, and Nucleic acids

HSR C18 Peptide

Develosil®

The Develosil HSR C18 Peptide adopts the HSR series's refined silica gel base material and is Develosil 's first exclusive column, in which the functional group density is controlled so that amino acids, peptides, and nucleic acids can be analyzed efficiently.

Typically, various conditions need to be examined to analyze biological samples, but these repeated changes do not have a positive impact on the column.

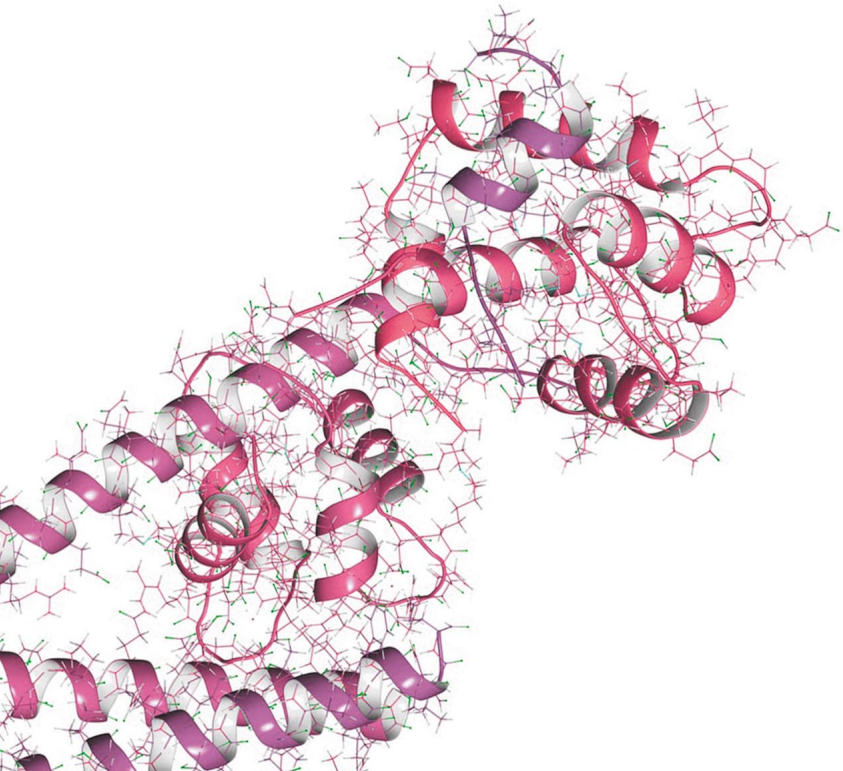
The HSR C18 Peptide's newly constructed bonding technology greatly contributes to durability and smoother method development.

Selection of optimal silica base material

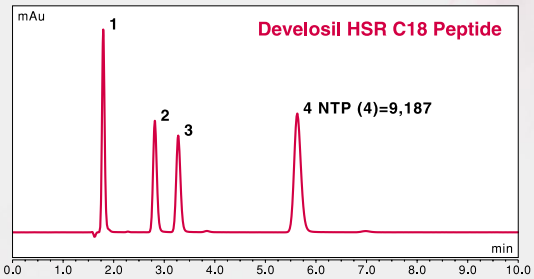
For the Develosil HSR C18 Peptide, the optimal performing substrate is selected considering the molecular weight and the structure expansion in low to medium molecular compounds.

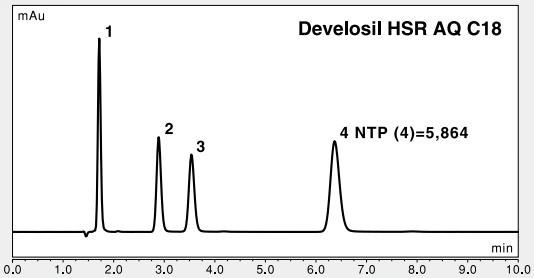
Spec of Develosil HSR C18 Peptide

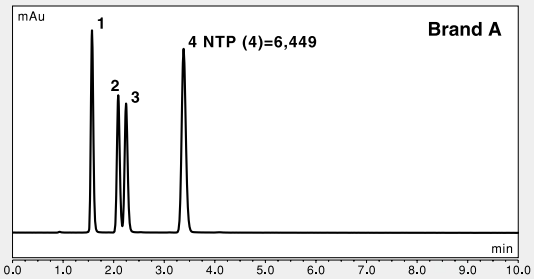
	HSR C18 Peptide
Functional	ODS
Surface area	300m²/g
Pore diameter	140 Å
Carbon	17%
End-cap	○
pH range	pH1 - 10
Temperature range	5 - 80℃
Particle size	2.5, 3, 5㎛

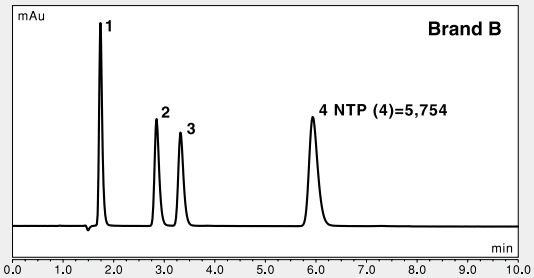


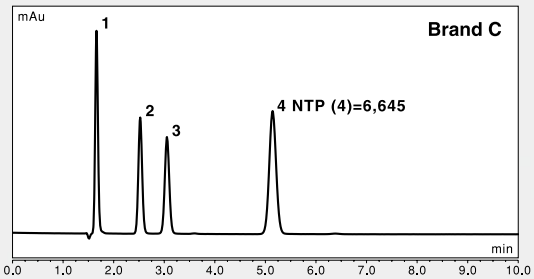
Performance comparison using dNTP











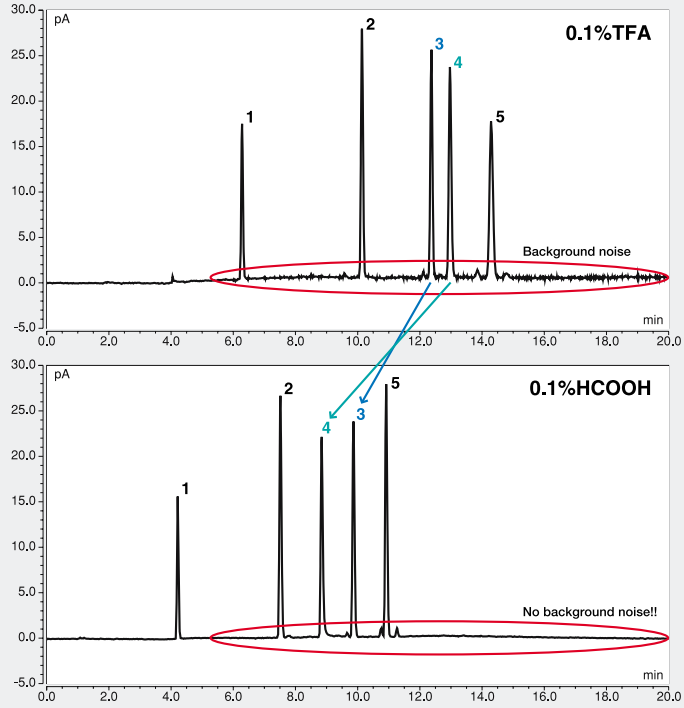
Analytical Conditions:

Column	: **C18 Column, 5um (4.6x150mm)**
Mobile phase	: 25mM Phosphate buffer, pH7.0
Flow rate	: 1.0mL/min
Temperature	: 40℃
Detection	: UV254nm
Sample	: 1.dCTP 2.dTTP 3.dGTP 4.dATP (each 0.13mM)
Injection volume	: 2.0uL

Improvement of mobile phase selectivity

There are many cases where formic acid is used for peptide analysis instead of TFA to reduce background noise. HSR C18 peptide can also accommodate these conditions.

[Retention behavior of peptides in different mobile phases]



Analytical Conditions:

Column	: **Develosil HSR C18 Peptide, 5um (4.6x150mm)**
Mobile phase	: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA A) Water + 0.1%HCOOH B) Acetonitrile + 0.1%HCOOH

Gradient :

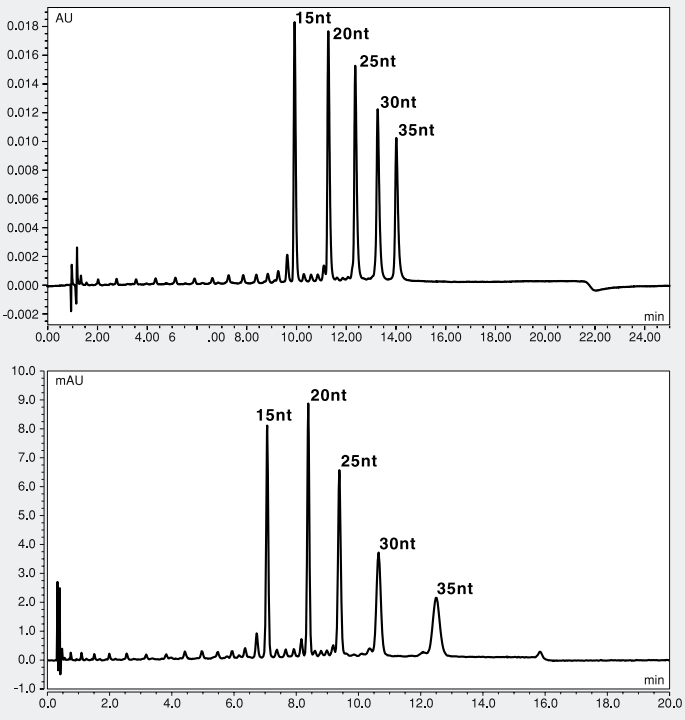
Time(min)	%B	Curve
0.0	0	5
10.0	25	5
20.0	25	5
20.1	0	5

Flow rate	: 1.0mL/min
Temperature	: 50℃
Detection	: CAD
Injection volume	: 10uL
Sample	: 1. Gly-Tyr (0.05mg/mL) 2. Val-Tyr-Val (0.05mg/mL) 3. Met-Enk (0.05mg/mL) 4. Angiotensin II (0.05mg/mL) 5. Leu-Enk (0.05mg/mL)
System	: Thermo Fisher SCIENTIFIC Ultimate 3000
Mixer volume	: 150uL

Support analysis from HPLC to UHPLC

Develosil HSR C18 Peptide can analyze peptides and nucleic acids in both HPLC and UHPLC conditions.

[Analysis of oligo deoxy thymidine by HPLC and UHPLC]



Analytical Conditions:

Column	: **Develosil HSR C18 Peptide, 3um (4.6x75mm)** **Develosil HSR C18 Peptide, 2.5um (2.0x50mm)**
Mobile phase	: A) 0.1M TEAA, pH7.0 B) 0.1M TEAA, pH7.0/Acetonitrile=40/60

Gradient (HPLC) :

Time(min)	Flow rate (mL/min)	%B	Curve
0.0	1.0	15	6
8.0	1.0	20	6
20.0	1.0	25	6
20.1	1.0	15	6
25.0		End	

(UHPLC) :

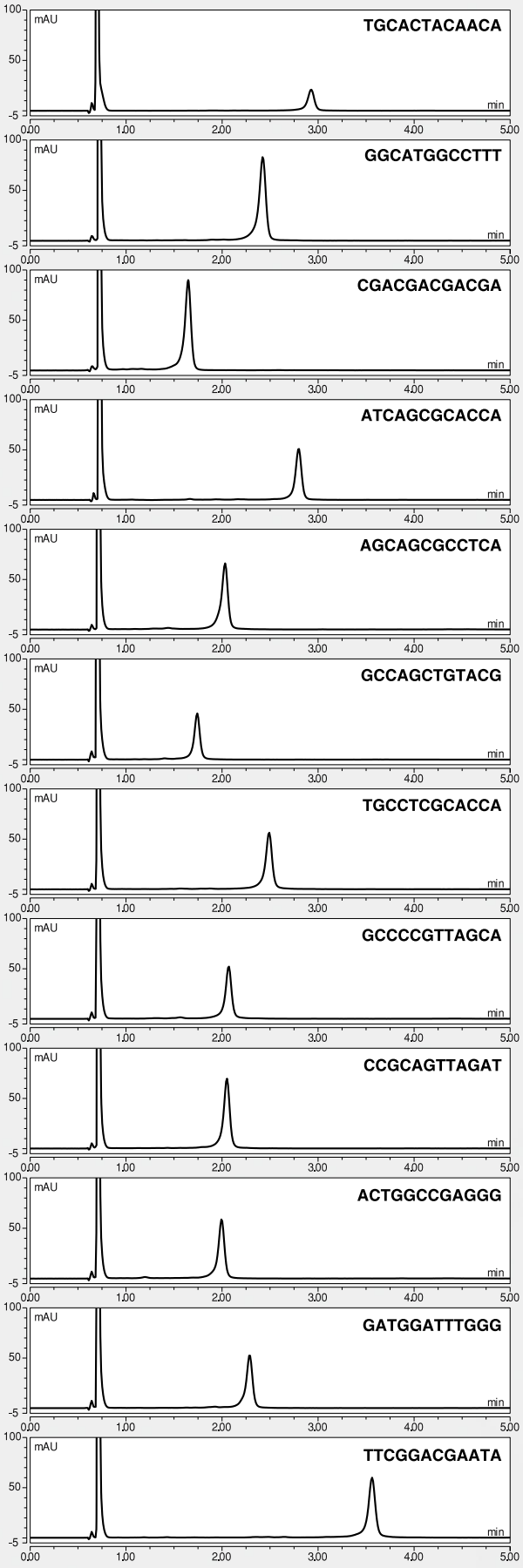
Time(min)	Flow rate (mL/min)	%B	Curve
0.0	0.4	15	5
8.0	0.4	20	5
15.0	0.4	20	5
15.1	0.4	15	5
20.0		End	

Temperature	: 50℃
Detection	: UV260nm
Sample	: Oligo Deoxy Thymidine (15, 20, 25, 30, 35nt)
Injection volume	: (HPLC) 10.0uL (UHPLC) 2.0uL
System	: (HPLC) Waters alliance (UHPLC) Thermo Fisher SCIENTIFIC Vanquish Horizon
Mixer volume	: (UHPLC) 10uL

The Silica gel of all Develosil series is produced in-house. The manufactured silica gel is strict inspected and controlled. Therefore, there is almost no deviation in physical properties such as surface area and pore volume due to the difference in particle size. This has an important meaning when transferring methods from HPLC to UHPLC.

Application Data

[Analysis of DNA oligomer (12-mer) base sequence difference]



Analytical Conditions:

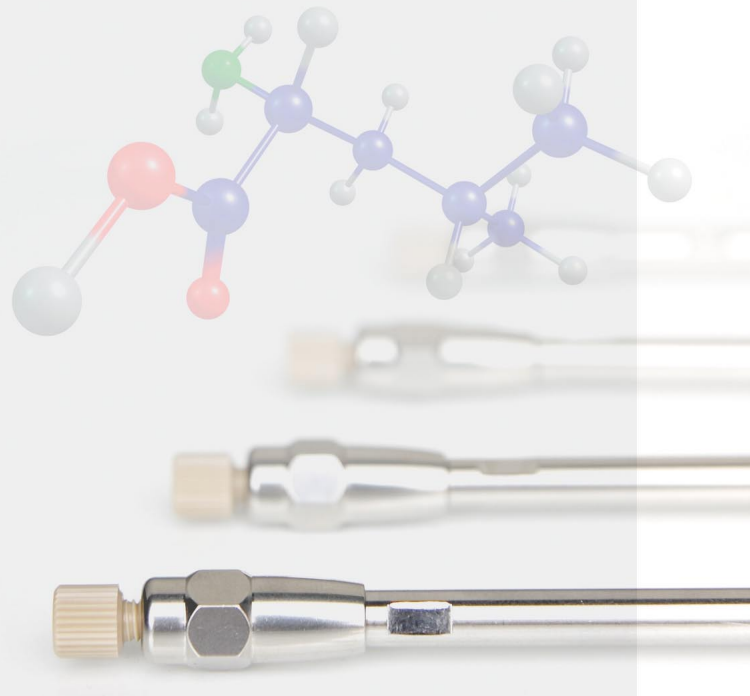
Column	: **Develosil HSR C18 Peptide, 2.5um (2.0x100mm)**				
Mobile phase	: A) 0.1M TEAA, pH7.0				
	B) 0.1M TEAA, pH7.0/Acetonitrile=40/60				
Gradient	:	Time(min)	Flow rate (mL/min)	%B	Curve
		0.0	0.4	15	5
		8.0	0.4	20	5
		8.1	0.4	25	5
		10.0		End	
Temperature	: 50℃				
Detection	: UV260nm				
Sample	: DNA Oligomer (12-mer)				
Injection volume	: 2.0uL				
System	: Thermo Fisher SCIENTIFIC Vanquish Horizon				
Mixer volume	: 10uL				

■Perform analysis with HPLC

Analytical Conditions:

Column	: **Develosil HSR C18 Peptide, 5um (4.6x150mm)**				
Mobile phase	: A) 0.1M TEAA, pH7.0				
	B) 0.1M TEAA, pH7.0/Acetonitrile=40/60				
Gradient	:	Time(min)	Flow rate (mL/min)	%B	Curve
		0.0	1.0	15	6
		8.0	1.0	20	6
		8.1	1.0	25	6
		10.0		End	
Temperature	: 50℃				
Detection	: UV260nm				
Sample	: DNA Oligomer (12-mer)				
Injection volume	: 10uL				
System	: Waters alliance				

There are examples in the above analysis conditions.
For details, please visit our website:
www.develosil.net
for application data.



Application Data

[Digested Protein Analysis]

■Protein digestion procedure

Weigh 1.0 to 2.0 mg each of insulin, BSA, monoclonal antibody and thyroglobulin, add 1.0 ml of Smart Digest buffer※1 and stir thoroughly.

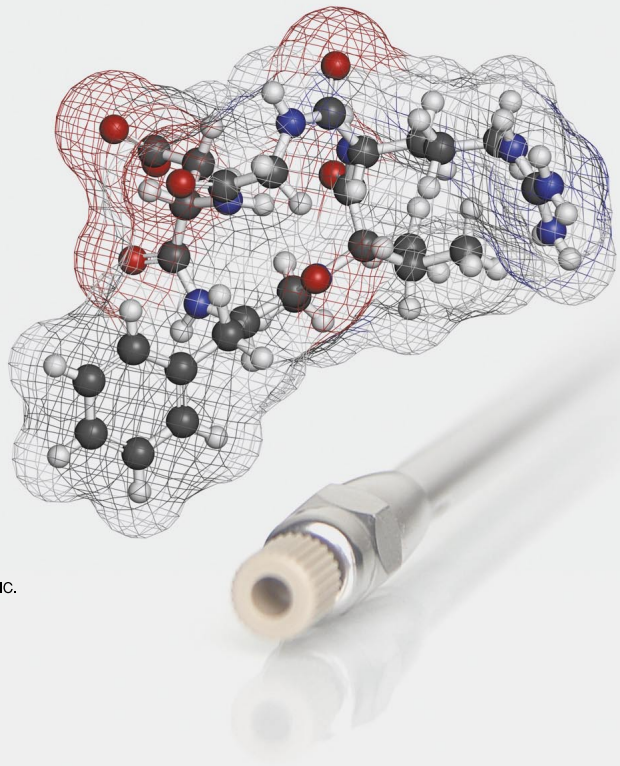
▼

Place 200 μL of the above sample in a SMART Digest Tube※1, and mixed at a temperature of 70℃-1,400 rpm for 2 hours at Thermomixer C※2 for a predetermined time.

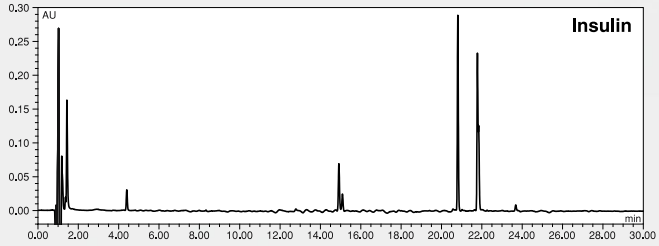
▼

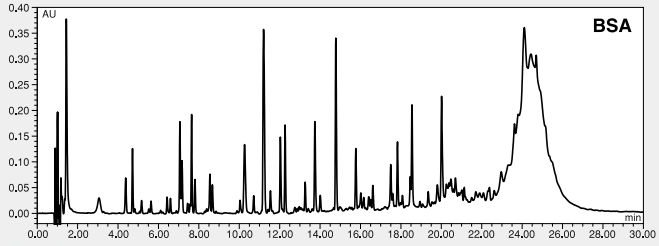
Centrifuge the prepared, mixed samples for injection.

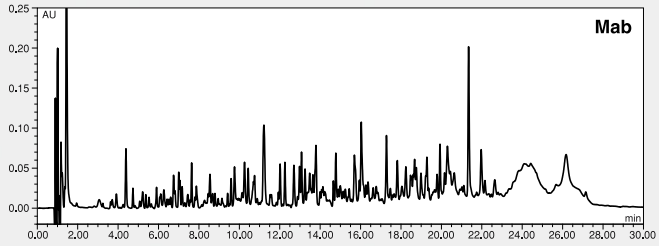
※1 The "Smart Digest Kit" used in this research is a registered product of Thermo Fisher SCIENTIFIC.
※2 The "Thermomixer C" used in this research is a registered product of Eppendorf.

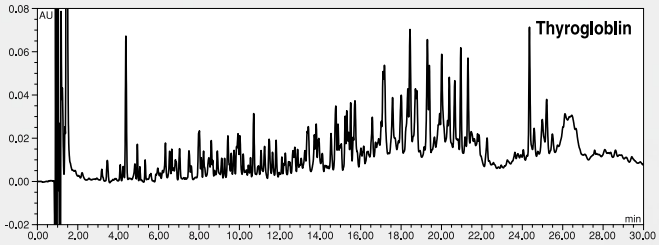


■Digested Protein analysis (HPLC Method)









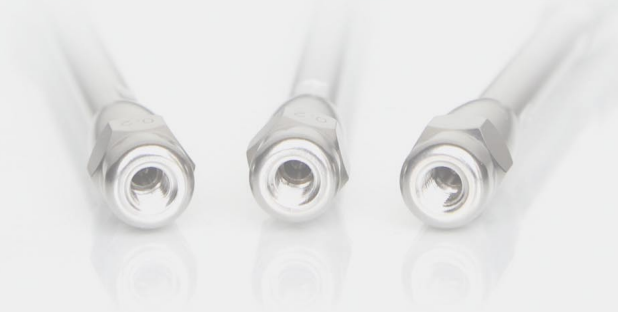
Analytical Conditions:

Column	: **Develosil HSR C18 Peptide, 3um (4.6x75mm)**			
Mobile phase	: A) Water + 0.1%TFA			
	B) Acetonitrile + 0.1%TFA			
Gradient	: Time(min)	Flow rate (mL/min)	%B	Curve
	0.0	1.0	0	6
	30.0	1.0	50	6
	40.0	1.0	50	6
	40.1	1.0	0	6
	50.0		End	
Temperature	: 50℃			
Ditection	: UV210nm			
Sample	: (1) Digest of Insulin			
	(2) Digest of BSA			
	(3) Digest of Mab			
	(4) Digest of Thyrogloblin			
Injection volume	: (1) 10uL			
	(2)-(4) 20uL			
System	: Waters alliance			

The above analysis example is an application using a HPLC system.
Save solvent and sample as well as get high sensitivity with a 2.5 um UHPLC column (inner diameter 2.0 mm) and a UHPLC system.
For details, please visit our website:

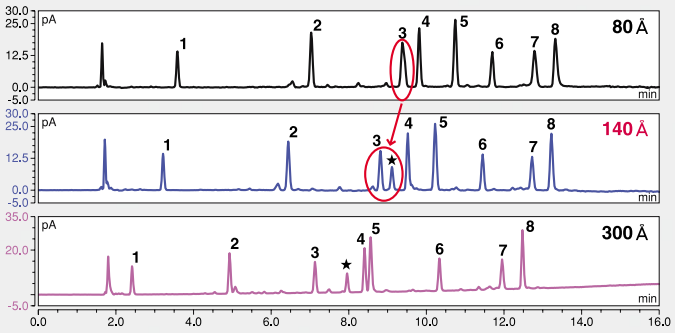
www.develosil.net

for application data.



Application Data

[Comparison of peptide separation by difference in pore size]



Analytical Conditions:

Column	: **Develosil HSR AQ C18, 5um (4.6x150mm)**
	Develosil HSR C18 Peptide, 5um (4.6x150mm)
	Other Campany 300 Å C18, 5um (4.6x150mm)
Mobile phase	: A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA

Gradient :

Time(min)	Flow rate (mL/min)	%B	Curve
0.0	1.0	0	6
30.0	1.0	50	6
30.1	1.0	0	6
40.0		End	

Temperature	: 50℃
Detection	: CAD
Sample	: 1. Gly-Tyr (M.W.=238.24)
	2. Val-Tyr-Val (M.W.=379.5)
	3. Met-Enkephalin (M.W.=573.7)
	4. Angiotensin II (M.W.=1046.2)
	5. Leu-Enkephalin (M.W.=555.6)
	6. γ-Endorphin (M.W.=1859.1)
	7. β-Endorphin (M.W.=3465.0)
	8. Insulin (M.W.=5807.6)
Injection volume	: 20uL
System	: Thermo Fisher SCIENTIFIC Ultimate 3000
Mixer volume	: 150uL

Small to medium molecular peptides and nucleic acids can be analyzed with Develosil HSR C18 peptide.
If the target are these compounds, 300 Å is not necessary.
This means lower cost is achieved.
Also, since the mobile phase is composed of TFA, formic acid, and TEA for this analysis, precipitation hardly occurs.
Columns that can be used under such conditions can explore many examination conditions and analyze many specimens.

【CONTACT】

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