01

Column Selection Guide

YMC Columns ---------- 8-9
USP ---------- 10
Column selection guide (Biochromatography) ---------- 11
Features of separation modes ---------- 12-17
Column selection guide
(Low molecular weight organic compounds) ---------- 18
Application ---------- 19-21
Reversed-phase column selection guide ---------- 22
Comparison of hydrophobicity and hydrogen-bonding
capacity of various C18 columns ---------- 22

YMC Columns

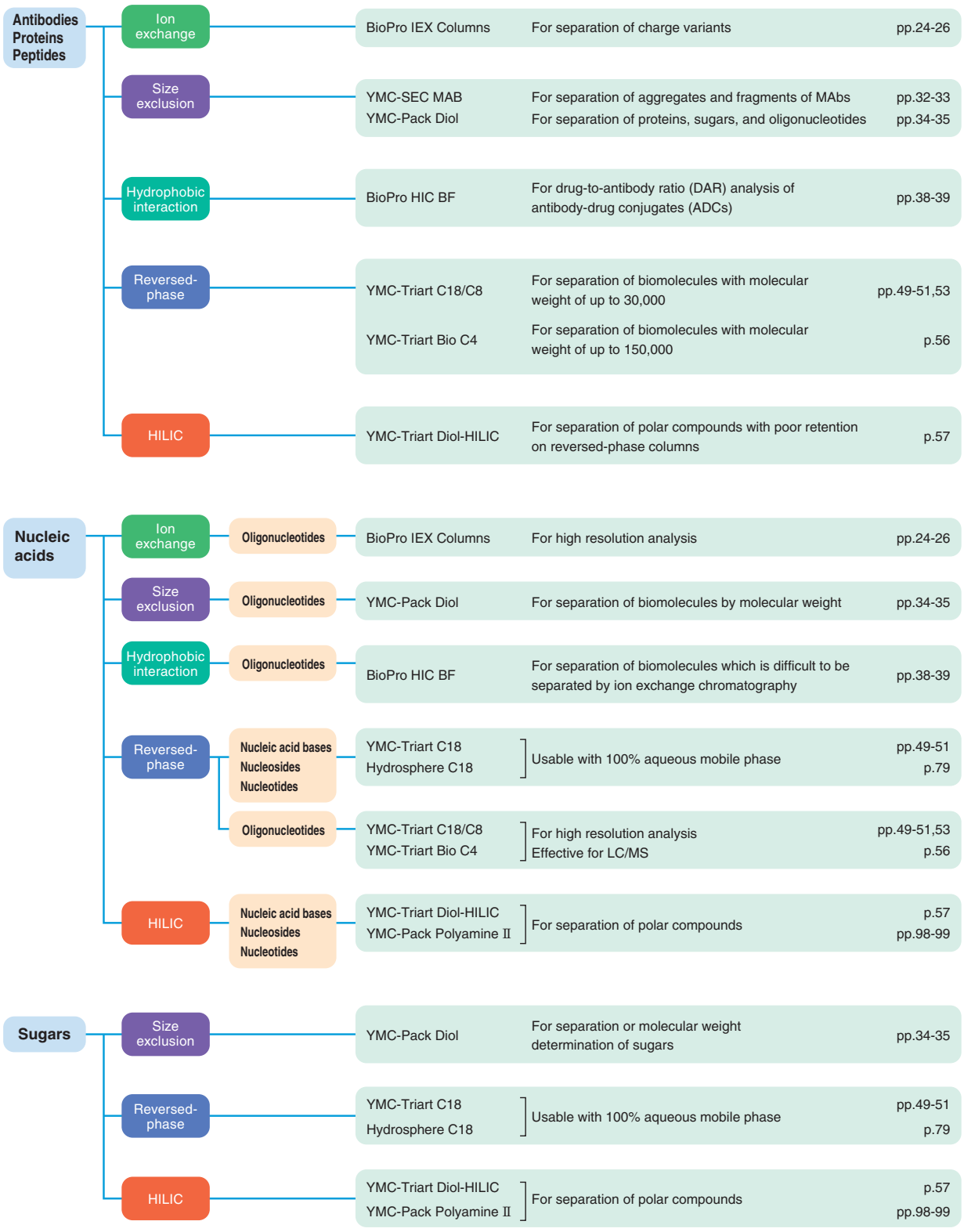
	Product name		USP Class No.	Pore size (Å)	Particle size (µm)	C%	Endcapping	Usable pH range	Page	
									Analytical column	Preparative column
Reversed-phase	YMC-Triart	C18	L1	120	1.9, 3, 5	20	Yes	1-12	49-51	110-114
		C18 ExRS	L1	80	1.9, 3, 5	25			52	
		C8	L7	120	1.9, 3, 5	17			53	
		Phenyl	L11	120	1.9, 3, 5	17		1-10	54	
		PFP	L43	120	1.9, 3, 5	15	No	1-8	55	
		Bio C4	L26	300	1.9, 3, 5	–	Yes	1-10	56	
	Meteoric Core	C18	L1	80	2.7	7	Yes	1.5-10	72-74	–
		C18 BIO	L1	160	2.7	5				
		C8	L7	80	2.7	5		1.5-9		
	Pro series	*Pro* C18	L1	120	2, 3, 5	16	Yes	2-8	78	110-116
		Hydrosphere C18	L1	120	2, 3, 5	12			79	
		Pro C18 RS	L1	80	3, 5	22		1-10	80	
		Pro C8	L7	120	3, 5	10		2-7.5	81	
		Pro C4	L26	120	3, 5	7			81	115-116
	YMC-Pack	ODS-A	L1	120	3, 5	17	Yes	2-7.5	82	110-116
				200	5	12				115-116
				300	3, 5	7				
		ODS-AM	L1	120	3, 5	17			82	115-116
		ODS-AQ	L1	120	3, 5	14			83	110-116
				200	5	10				115-116
		ODS-AL	L1	120	5	17	No		83	115-116
		C_8	L7	120	3, 5	10	Yes		84	115-116
				200	5	7				
				300	5	4				
		C_4	L26	120	3, 5	7			84	115-116
				200	5	5				
				300	5	3				
		TMS	L13	120	3, 5	4			85	115-116
		Ph	L11	120	3, 5	9			85	115-116
		CN	L10	120	3, 5	7			86	115-116
				300	5	3				
		PROTEIN-RP	L26	200	5	4	–	1.5-7.5	86	115-116
	YMCbasic		L7	200	3, 5	7	Yes	2-7.5	87	–
	YMC Carotenoid		L62	–	3, 5	–	–	2-7.5	87	115
	J'sphere ODS	ODS-H80	L1	80	4	22	Yes	1-9	88	115-116
		ODS-M80	L1	80	4	14		2-7.5		
		ODS-L80	L1	80	4	9				
	YMC-Pack PolymerC18		L1	–	6, 10	–	–	2-13	88	–

	Product name		USP Class No.	Pore size (Å)	Particle size (μm)	C%	Endcapping	Usable pH range	Page	
									Analytical column	Preparative column
Normal-phase	YMC-Triart Diol-HILIC		L20	120	1.9, 3, 5	12	–	2-10	57	–
	YMC-Pack	SIL	L3	120	3, 5	–	–	2-7.5	96	115-116
		SIL-06		60	5					
		Diol-NP	L20	60	5				96	115
				120	5					
		CN	L10	120	5				97	115-116
		PVA-Sil	L24	120	5			2-9.5	97	–
		Polyamine Ⅱ	L111	120	5			2-7.5	98-99	115-116
		NH_2	L8	120	5				100	115-116
		PA-G	–	120	5			4-7.5	100	–
Ion exchange	BioPro IEX	QF	–	non-porous	3, 5	–	–	2-12	24-26	–
		SF		non-porous	3, 5					
		QA		porous	5					
		SP		porous	5					
SEC GFC	YMC-SEC MAB		L20 L59	250	3	–	–	5-7.5	32-33	–
	YMC-Pack	Diol-60	L20 L33 L59	60	3, 5				34-35	36
		Diol-120		120						
		Diol-200		200	2, 3, 5					
		Diol-300		300						
SEC GPC	YMC-GPC		–	50	10	–	–	–	–	117-118
				100	10					
				500	10					
				1000	10					
				MIX	10					
HIC	BioPro HIC BF		–	non-porous	4	–	–	2-12	38-39	–
Chiral separation	CHIRAL ART	Amylose-SA	L99	–	3, 5	–	–	2-9	62-66	62-66
		Cellulose-SB	–	–	3, 5					
		Cellulose-SC	L119	–	3, 5					
		Cellulose-SJ	–	–	3, 5					
		Amylose-C Neo	L51	–	3, 5			–		
		Cellulose-C	L40	–	3, 5					
	YMC CHIRAL	NEA (R), (S)	–	300	5	–	–	2-6.5	67	–
		α -CD BR		120	5			3.5-6.5	67	–
		β -CD BR		120	5					
		γ -CD BR		120	5					

USP

USP Class No.	USP Description	Bonded phase	YMC product	Page
L1	Octadecyl silane chemically bonded to porous or nonporous silica or ceramic microparticles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	C18	YMC-Triart C18	49-51
			YMC-Triart C18 ExRS	52
			Meteoric Core C18	72-74
			Meteoric Core C18 BIO	
			YMC-UltraHT *Pro* C18	78
			YMC-Pack *Pro* C18	
			YMC-UltraHT Hydrosphere C18	79
			Hydrosphere C18	
			YMC-Pack *Pro* C18 RS	80
			YMC-Pack ODS-A	82
			YMC-Pack ODS-AM	82
			YMC-Pack ODS-AQ	83
			YMC-Pack ODS-AL	83
			J'sphere ODS-H80	88
			J'sphere ODS-M80	
			J'sphere ODS-L80	
L3	Porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	Silica	YMC-Pack SIL	96
			YMC-Pack SIL-06	
L7	Octylsilane chemically bonded to totally porous or superficially porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod.	C8	YMC-Triart C8	53
			Meteoric Core C8	72-74
			YMC-Pack *Pro* C8	81
			YMC-Pack C_8	84
			YMCbasic	87
L8	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 1.5 to 10 µm in diameter.	NH_2	YMC-Pack NH_2	100
L10	Nitrile groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter.	CN	YMC-Pack CN	86
L11	Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter.	Phenyl	YMC-Triart Phenyl	54
			YMC-Pack Ph	85
L13	Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter.	C1	YMC-Pack TMS	85
L20	Dihydroxypropane groups chemically bonded to porous silica or hybrid particles, 1.5 to 10 µm in diameter.	Diol	YMC-Triart Diol-HILIC	57
			YMC-Pack Diol-NP	96
			YMC-SEC MAB	32-33
			YMC-Pack Diol-60	34-35
			YMC-Pack Diol-120	
			YMC-Pack Diol-200	
			YMC-Pack Diol-300	
L24	Polyvinylalcohol chemically bonded to porous silica particle, 5 µm in diameter.	Polyvinylalcohol	YMC-Pack PVA-Sil	97
L26	Butyl silane chemically bonded to totally porous silica particles, 1.5 to 10 µm in diameter.	C4	YMC-Triart Bio C4	56
			YMC-Pack *Pro* C4	81
			YMC-Pack C_4	84
			YMC-Pack PROTEIN-RP	86
L27	Porous silica particles, 30 to 50 µm in diameter.	Silica	YMC-Pack SIL-HG	127,132
L33	Packing having the capacity to separate dextrans by molecular size over a range of 4,000 to 500,000 Da. It is spherical, silica-based, and processed to provide pH stability.	Diol	YMC-Pack Diol-60	34-35
			YMC-Pack Diol-120	
			YMC-Pack Diol-200	
			YMC-Pack Diol-300	
L40	Cellulose tris-3,5-dimethylphenylcarbamate coated porous silica particles, 3 to 20 µm in diameter.	Cellulose tris-3,5-dimethylphenylcarbamate	CHIRAL ART Cellulose-C	62-66
L43	Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 1.5 to 10 µm in diameter.	PFP	YMC-Triart PFP	55
L51	Amylose tris-3,5-dimethylphenylcarbamate-coated, porous, spherical, silica partilces, 3 to 10 µm in diameter.	Amylose tris-3,5-dimethylphenylcarbamate	CHIRAL ART Amylose-C Neo	62-66
L59	Packing for the size-exclusion separation of proteins (separation by molecular weight) over the range of 5 to 7,000 kDa. The packing is a spherical 1.5 to 10 µm, silica or hybrid packing with a hydrophilic coating.	Diol	YMC-SEC MAB	32-33
			YMC-Pack Diol-60	34-35
			YMC-Pack Diol-120	
			YMC-Pack Diol-200	
			YMC-Pack Diol-300	
L62	C30 silane bonded phase on a fully porous spherical silica, 3 to 15 µm in diameter.	C30	YMC Carotenoid	87
L99	Amylose tris-(3,5-dimethylphenylcarbamate), immobilized on porous, spherical, silica particles, 3 to 5 µm in diameter.	Amylose tris-3,5-dimethylphenylcarbamate	CHIRAL ART Amylose-SA	62-66
L111	Polyamine chemically bonded to porous spherical silica particles, 5 µm in diameter.	Polyamine	YMC-Pack Polyamine Ⅱ	98-99
L119	Cellulose tris-(3,5-dichlorophenylcarbamate), immobilized on porous, spherical, silica particles, 3 to 5 µm in diameter.	Cellulose tris-3,5-dichlorophenylcarbamate	CHIRAL ART Cellulose-SC	62-66

Column selection guide (Biochromatography)

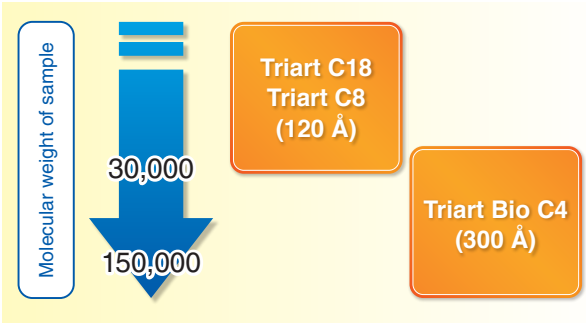


Features of separation modes

	Ion exchange BioPro IEX Columns	Size exclusion YMC-SEC MAB YMC-Pack Diol	Hydrophobic interaction BioPro HIC BF	Reversed-phase YMC-Triart etc.
Principle of separation	Electric charge	Molecular weight	Hydrophobicity	Hydrophobicity
Molecular weight range	Up to several millions	Up to about 1,000,000	Up to several millions	Up to about 150,000
Resolution	+++	++	+++	+++
Speed	++ - +++	+	+++	+++
Loading	+++	++	+++	++
Sample stability	+++	+++	+++	+ - ++
Typical applications	Charge variants analysis	Separation of aggregates Separation of fragments	Drug-to-antibody ratio analysis of antibody-drug conjugates	Peptide mapping LC/MS Structural analysis

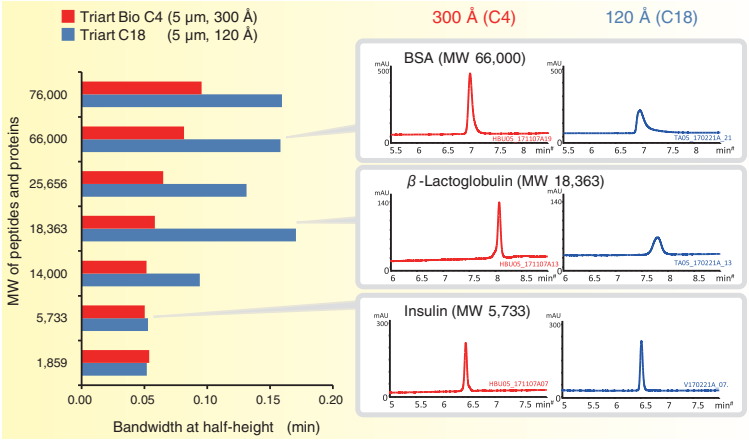
Reversed-phase separation of biomolecules

How to select reversed-phase columns



Columns are selected based on molecular weights (MW) of the target substances to separate proteins and peptides. Triart C18/C8 with pore size 120 Å provide the good separation of substances with MW of up to 30,000 under high temperature. For separation of substances with larger molecules, wide pore columns are effective. Triart Bio C4 with pore size 300 Å can perform separation of substances with MW of up to 150,000 under high temperature.
An elevated temperature can improve efficiency and peak shape by lowering the viscosity of the mobile phase and improving mass transfer. The appropriate MW range for a given pore size of Triart can be expanded compared to using the same pore size at lower temperature.

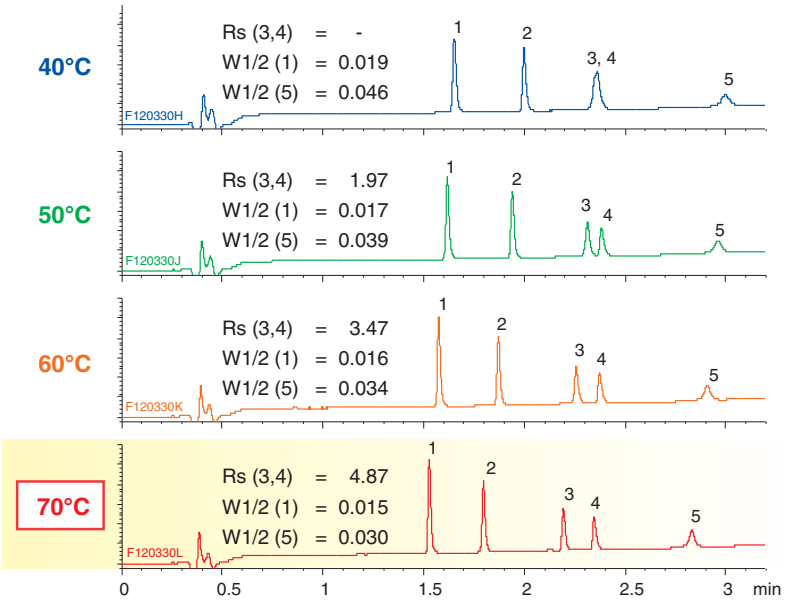
Influence on separation of peptides and proteins by bonded phase and pore sizes



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)
Sample	: γ -Endorphin, Insulin, Lysozyme, β -Lactoglobulin, α -Chymotoripsinogen A, BSA, Conalbumin

The left figure shows separation of peptides and proteins with MW from 1,859 to 76,000 with Triart Bio C4 (5 µm, 300 Å) and Triart C18 (5 µm, 120 Å). With a C18 (120 Å) column, peak broadening is observed for proteins with MW of 10,000 or more. On the other hand, a wide pore (300 Å) C4 column provides good peak shapes even for proteins with high MW proteins. Thus, in general, a wide pore C4 column such as Triart Bio C4 is suitable for separation of proteins with MW of 10,000 or higher.

Effect of column temperature on separation



1. Oxytocin (MW 1,007)
2. Leu-Enkephalin (MW 556)
3. β -Endorphin (MW 3,465)
4. Insulin (MW 5,733)
5. β -Lactoglobulin A (MW 18,400)

Column	: YMC-Triart C18 (1.9 µm, 120 Å) 50 X 2.0 mmI.D.
Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 10-80%B (0-5 min)
Flow rate	: 0.4 mL/min
Detection	: UV at 220 nm

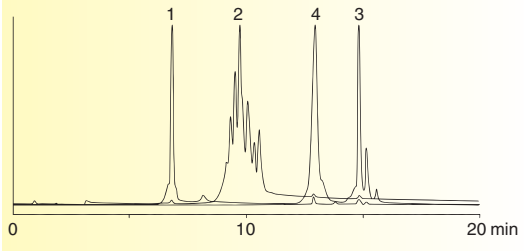
Increasing column temperature to 70°C provides selectivity change, sharper peaks, and therefore improved resolution especially for larger molecules.

Separation of Antibodies/Proteins/Peptides

Charge variants analysis of monoclonal antibodies

Ion exchange **BioPro IEX SF 5 µm, 100 X 4.6 mmI.D.**

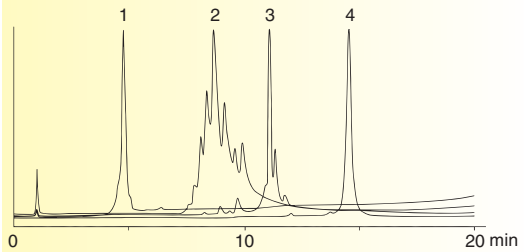
Salt gradient mode



1. Natalizumab (Humanized IgG4, pI=7.3)
2. Cetuximab (Chimeric IgG1, pI=7.9)
3. Adalimumab (Human IgG1, pI=8.4)
4. Denosumab (Human IgG2, pI=8.8)

Eluent	: A) 10 mM MES-NaOH (pH 5.7) B) 10 mM MES-NaOH (pH 5.7) containing 1 M NaCl 0-20%B (0-20 min)
Flow rate	: 0.6 mL/min

pH gradient mode



Eluent	: A) CX-1 pH Gradient Buffer A* (pH 5.6) B) CX-1 pH Gradient Buffer B* (pH 10.2) 0-100%B (0-20 min)
Flow rate	: 0.6 mL/min

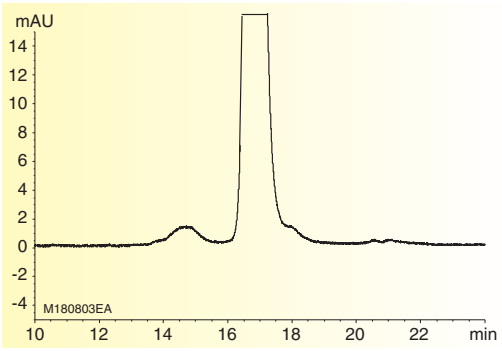
*Purchased from Thermo Fisher Scientific Inc.

J. Pharm. Biomed. Anal., 2015, 111, 169-176.

Monoclonal antibodies were separated by ion exchange chromatography in salt gradient mode and in pH gradient mode. Sharp peaks were obtained in both modes with BioPro IEX columns since they have extremely low nonspecific adsorption. This shows that BioPro IEX columns are effective for separation of charge variants and isoforms.

Aggregates analysis of antibody-drug conjugate (ADC)

Size exclusion **YMC-SEC MAB 3 µm, 300 X 4.6 mmI.D.**

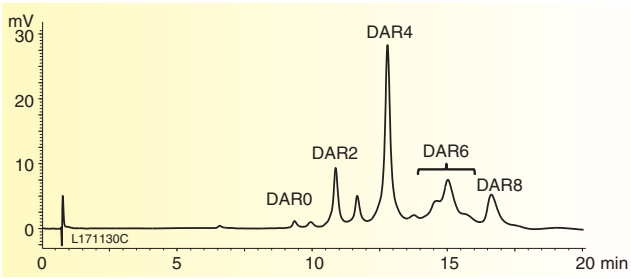


Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl/2-propanol (85/15)
Flow rate	: 0.165 mL/min
Temperature	: 25°C
Detection	: UV at 280 nm
Injection	: 4 µL
Sample	: Cysteine-conjugated ADC mimic (2.5 mg/mL)

Size exclusion chromatography is useful for separation of substances with different molecular weights such as MAbs, their dimers and aggregates or ADCs, their dimers and aggregates.

Drug-to-antibody ratio (DAR) analysis of ADC

Hydrophobic interaction **BioPro HIC BF 4 µm, 100 X 4.6 mmI.D.**

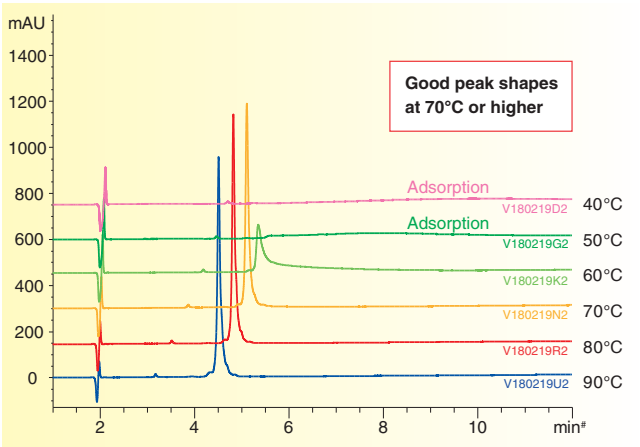


Eluent	: A) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0) containing 1.5 M $(NH_4)_2SO_4$/2-propanol (95/5) B) 50 mM NaH_2PO_4-Na_2HPO_4 (pH 7.0)/2-propanol (80/20) 0%B (0-1 min), 0-100%B (1-15 min), 100%B (15-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 25°C
Detection	: UV at 280 nm
Injection	: 5 µL
Sample	: Cysteine-conjugated ADC mimic (5 mg/mL)

Hydrophobic interaction chromatography column BioPro HIC BF provides superior separation for ADCs and is effective for DAR determination.

Monoclonal antibody

Reversed-phase **YMC-Triart Bio C4 5 µm, 150 X 3.0 mmI.D.**

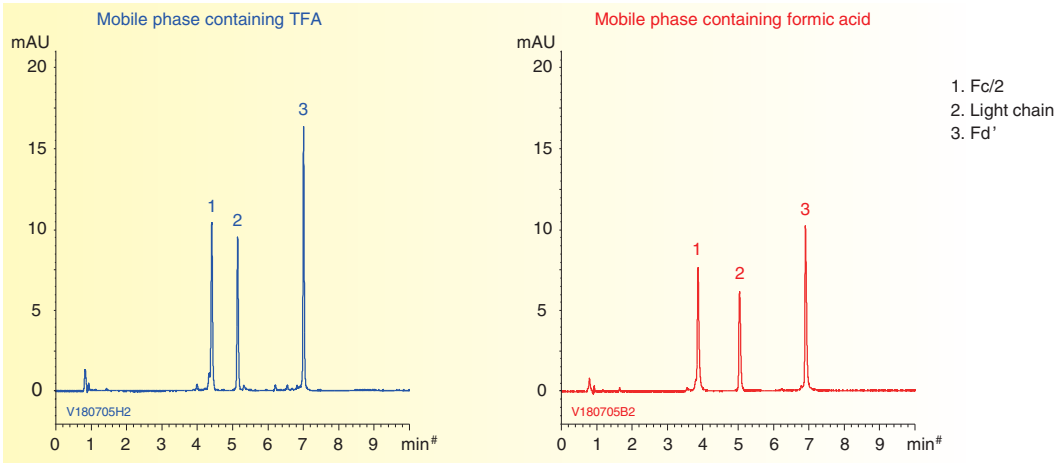


Eluent	: A) water/TFA (100/0.1) B) acetonitrile/TFA (100/0.1) 30-60%B (0-15 min), 90%B (15-30 min)
Flow rate	: 0.4 mL/min
Detection	: UV at 220 nm
Injection	: 4 µL
Sample	: Humanized monoclonal IgG1

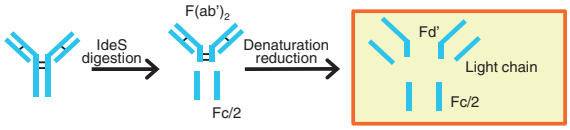
Intact monoclonal antibody was analyzed at temperatures between 40°C and 90°C using Triart Bio C4. Good peak shapes were acquired at 70°C and higher while there was no elution at 50°C and lower. Highly durable Triart Bio C4 can perform stable analysis even at 90°C and is suitable for the reversed-phase analysis of antibodies at high temperatures.

Antibody fragments

Reversed-phase **YMC-Triart Bio C4 1.9 µm, 150 X 2.1 mmI.D.**



【Sample】

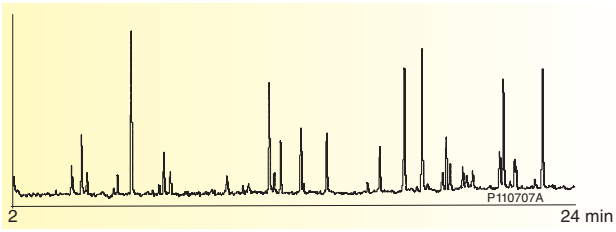


Eluent	: A) water/TFA (100/0.1)
<TFA>	B) acetonitrile/TFA (100/0.1)
	25-50%B (0-10 min), 90%B (10-12.5 min)
Eluent	: A) water/formic acid (100/0.1)
<Formic acid>	B) acetonitrile/formic acid (100/0.1)
	20-45%B (0-10 min), 90%B (10-12.5 min)
Flow rate	: 0.4 mL/min
Temperature	: 80℃
Detection	: UV at 280 nm
Injection	: 4 µL (0.25 mg/mL)

Above are the chromatograms of monoclonal antibody fragments analysis with mobile phase containing either TFA or formic acid. Triart Bio C4 achieves excellent peak shape even when using mobile phase containing formic acid. Therefore it is ideal for the high sensitive LC/MS analysis.

Peptide mapping

Reversed-phase **YMC-Triart C18 1.9 µm, 200 X 2.0 mmI.D. (100 X 2.0 mmI.D. two coupled)**

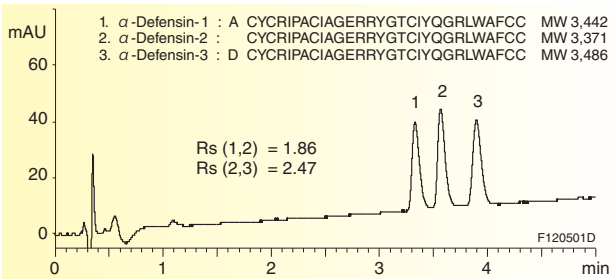


Eluent	: A) water/TFA (100/0.1)
	B) acetonitrile/TFA (100/0.08)
	5-40%B (0-30 min)
Flow rate	: 0.4 mL/min
Temperature	: 70°C
Detection	: UV at 220 nm
Injection	: 20 µL
Sample	: Triptic digest of Bovine Hemoglobin

The outstanding efficiency obtained by a coupling of two 100 mm length of Triart 1.9 µm columns allows the precise separation in peptide mapping.

Analysis of antimicrobial peptides

Reversed-phase **YMC-Triart C18 1.9 µm, 50 X 2.0 mmI.D.**



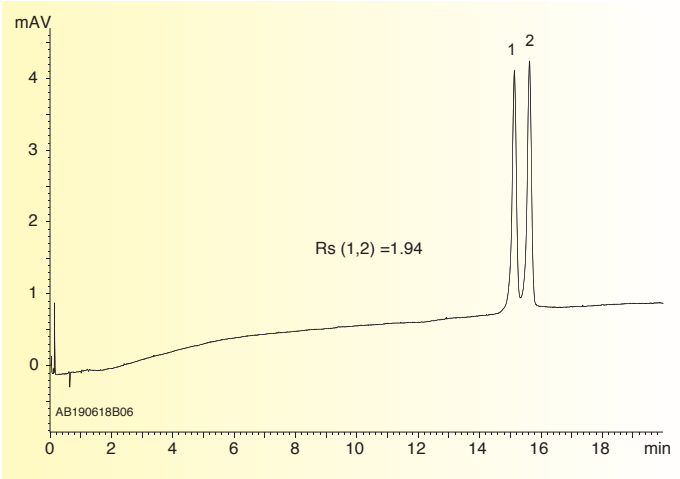
Eluent	: A) water/formic acid (100/0.1)
	B) acetonitrile/2-propanol/formic acid (50/50/0.08)
	10-25%B (0-10 min)
Flow rate	: 0.4 mL/min
Temperature	: 70°C
Detection	: UV at 220 nm

High temperature condition improves separation of peptides and proteins on reversed-phase chromatography. Since Triart columns are highly durable at high temperatures, they are effective for analysis of peptides and proteins under such harsh conditions.

Separation of Nucleic Acids

Phosphorothioate oligonucleotides

Reversed-phase **YMC-Triart C8 [Metal free column] 1.9 µm, 100 X 2.1 mmI.D.**



1. 5'-U^C^A^U^C^A^C^A^C^U^G^A^A^U^A^C^C^A^A^U-3'
   (RNA 20 mer All PS)
2. 5'-G^U^C^A^U^C^A^C^A^C^U^G^A^A^U^A^C^C^A^A^U-3'
   (RNA 21 mer All PS)

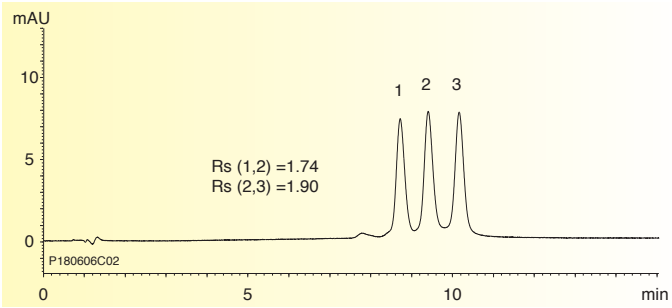
^=Phosphorothioated

Eluent	: A) 15 mM triethylamine-400 mM HFIP*
	B) methanol
	10-20%B (0-20 min)
Flow rate	: 0.42 mL/min
Temperature	: 70°C
Detection	: UV at 260 nm
Injection	: 1 µL (each 1.25 nmol/mL)

*1,1,1,3,3,3-hexafluoro-2-propanol

Triart C8 [Metal free column] provides superior resolution of full-length (n) and n-1 failure seaquence oligonucleotides.

Ion exchange **BioPro IEX QF 5 µm, 100 X 4.6 mmI.D.**



Single-stranded DNA (15 mer)

1. 5'-T^T^T^T^T^T^T^T^T^T^T^T^TTT-3'
   (DNA 15 mer 12PS, 2PO)
2. 5'-T^T^T^T^T^T^T^T^T^T^T^T^TT-3'
   (DNA 15 mer 13PS, 1PO)
3. 5'-T^T^T^T^T^T^T^T^T^T^T^T^T^T-3'
   (DNA 15 mer All PS)

^=Phosphorothioated

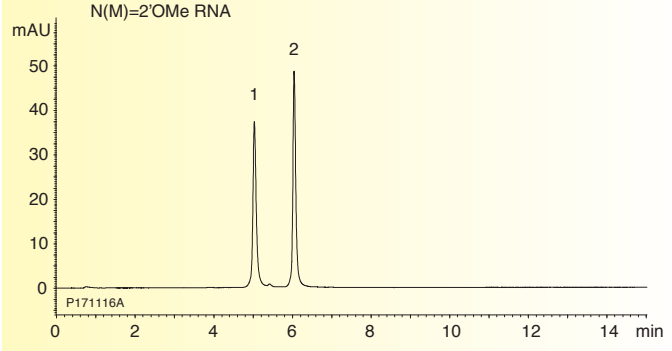
Eluent	: A) 10 mM NaOH
	B) 10 mM NaOH containing 1.0 M $NaClO_4$
	40-70%B (0-15 min)
Flow rate	: 1.0 mL/min
Temperature	: 25°C
Detection	: UV at 260 nm
Injection	: 6 µL (each 3.3 nmol/mL)

[All PS], [13PS, 1PO] and [12PS, 2PO] of DNA 15 mer were clearly separated by BioPro IEX column.

2'-*O*-methyl oligonucleotides

Ion exchange **BioPro IEX QF 5 µm, 100 X 4.6 mmI.D.**

1. 5'-U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)A(M)A(M)U(M)-3' (20 mer)
2. 5'-G(M)U(M)C(M)A(M)U(M)C(M)A(M)C(M)A(M)C(M)U(M)G(M)A(M)A(M)U(M)A(M)C(M)C(M)A(M)A(M)U(M)-3' (21 mer)
   N(M)=2'OMe RNA



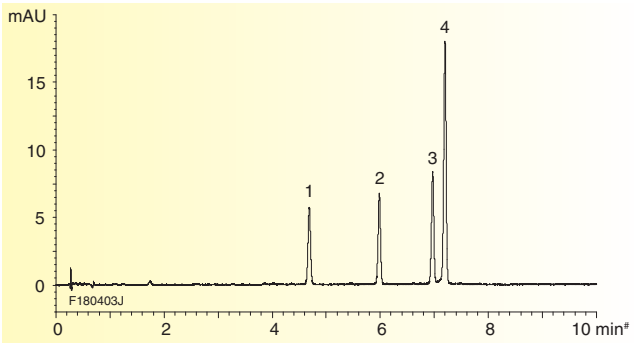
Eluent	: A) 10 mM NaOH
	B) 10 mM NaOH containing 1.0 M $NaClO_4$
	25-55%B (0-15 min)
Flow rate	: 1.0 mL/min
Temperature	: 25°C
Detection	: UV at 260 nm
Injection	: 4 µL (5 nmol/mL)

100 mm length column of BioPro IEX QF, which has high separation ability, is suitable for separation of oligonucleotides by one nucleotide difference.

Oligonucleotides

Reversed-phase

YMC-Triart C18 1.9 µm, 50 X 2.1 mmI.D.



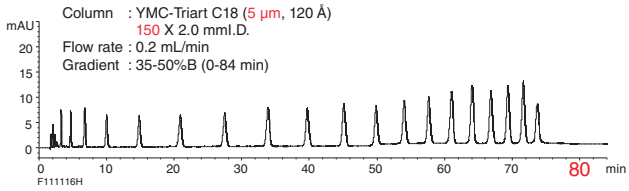
1. 5'-CAC UGA AUA CCA AU-3' (14 mer)
2. 5'-UCA CAC UGA AUA CCA AU-3' (17 mer)
3. 5'-UCA UCA CAC UGA AUA CCA AU-3' (20 mer)
4. 5'-GUC AUC ACA CUG AAU ACC AAU-3' (21 mer)

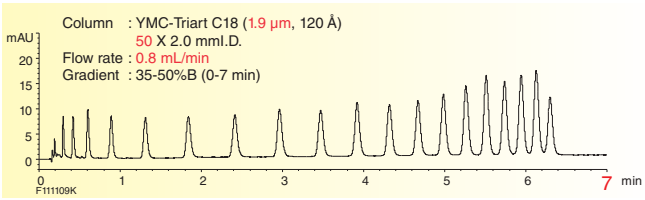
Eluent	: A) 8 mM triethylamine-200 mM HFIP B) methanol 10-20%B (0-10 min)
Flow rate	: 0.42 mL/min
Temperature	: 65°C
Detection	: UV at 260 nm
Injection	: 1 µL (2-4 nmol/mL)

Triart C18 is suitable for separation of hydrophilic compounds such as oligonucleotides.

Reversed-phase

YMC-Triart C18





Oligonucleotides $d(T)_{2\text{-}20}$

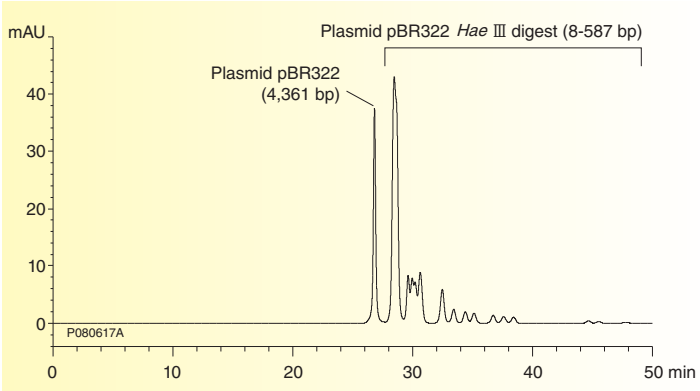
Eluent	: A) 10 mM di-*n*-butylamine-acetic acid (pH 6.0) B) methanol
Temperature	: 35°C
Detection	: UV at 269 nm
Injection	: 1 µL (5 nmol/mL)

In the separation of oligonucleotides, 19 peaks are completely resolved within 7 minutes using a Triart C18 1.9 µm UHPLC column. The separation is achieved within one tenth of the analysis time of the conventional HPLC method.

Plasmid pBR322 *Hae* Ⅲ restriction fragments

Size exclusion

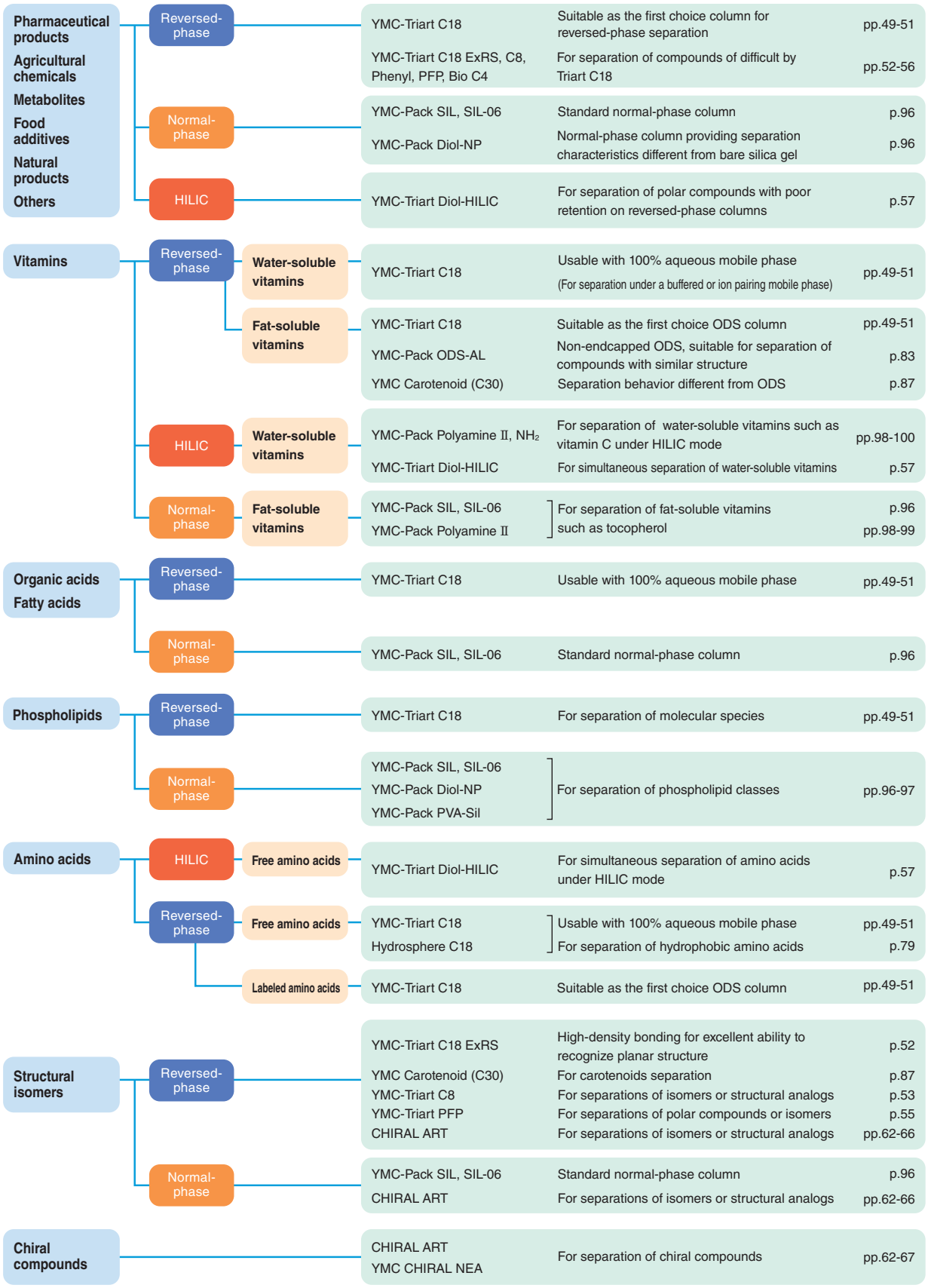
YMC-Pack Diol-300 + Diol-200 5 µm, 500 X 8.0 mmI.D. X 2



Eluent	: 0.1 M KH_2PO_4-K_2HPO_4 (pH 7.0) containing 0.2 M NaCl
Flow rate	: 0.7 mL/min
Temperature	: ambient (25°C)
Detection	: UV at 260 nm
Injection	: 10 µL

In size exclusion mode, separation is expected to be improved by using coupled columns with different pore sizes.

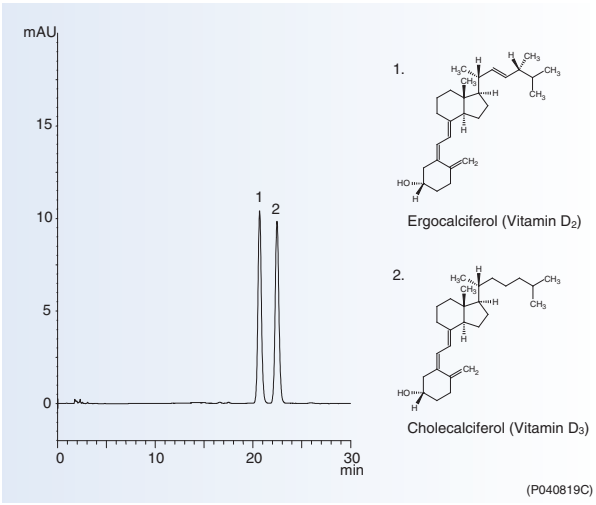
Column selection guide (Low molecular weight organic compounds)



Application

Reversed-phase Vitamin D

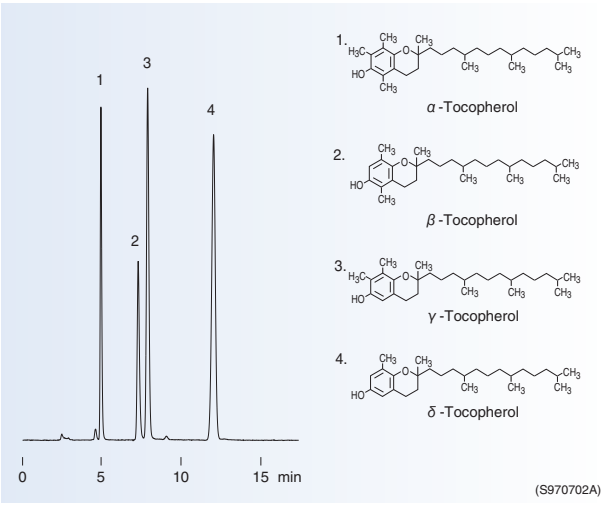
Separation of structurally similar compounds



Column	: YMC-Pack ODS-AL (5 µm, 120 Å) 150 X 4.6 mmI.D.
Eluent	: acetonitrile/water (95/5)
Flow rate	: 1.0 mL/min
Temperature	: 40°C
Detection	: UV at 265 nm
Injection	: 10 µL (0.01 mg/mL)

Normal-phase Vitamin E (Tocopherols)

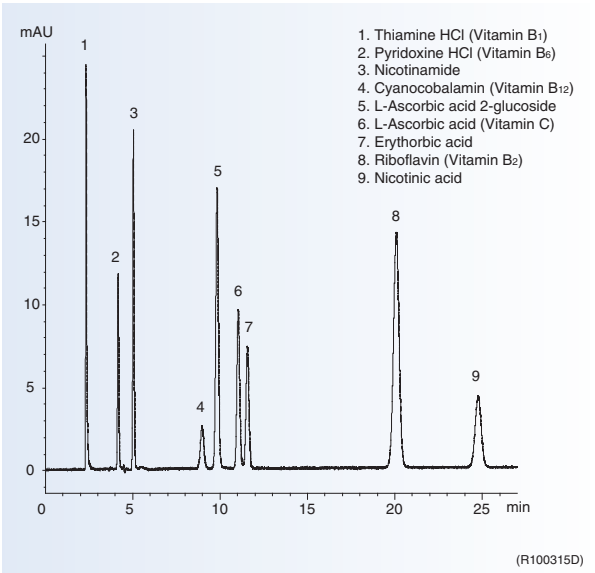
Separation of tocopherol homologues



Column	: YMC-Pack SIL (5 µm, 120 Å) 250 X 4.6 mmI.D.
Eluent	: *n*-hexane/2-propanol/acetic acid (1000/6/5)
Flow rate	: 1.4 mL/min
Temperature	: 35°C
Detection	: FLS at Ex 298 nm, Em 325 nm
Injection	: 20 µL (5-20 µg/mL)

Reversed-phase Water-soluble vitamins

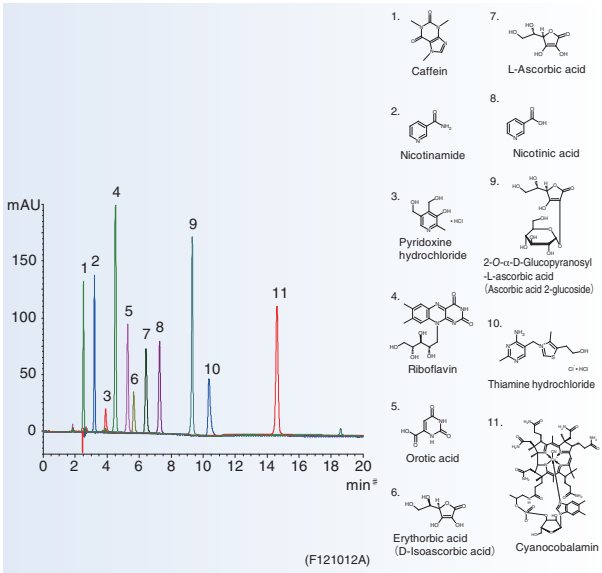
Simultaneous separation of water-soluble vitamins under ion pairing mobile phase



Column	: YMC-Triart C18 (5 µm, 120 Å) 250 X 4.6 mmI.D.
Eluent	: phosphate buffer*/acetonitrile (90/10) **Dissolve 1.4 g KH_2PO_4 in 800 mL water → add 26 mL 10% TBA·OH → adjust pH 5.2 by 20% H_3PO_4 → add water to make 1000 mL*
Flow rate	: 0.8 mL/min
Temperature	: 40°C
Detection	: UV at 260 nm
Injection	: 10 µL (5 µg/mL)

HILIC Water-soluble vitamins

Simultaneous separation of water-soluble vitamins under HILIC mode

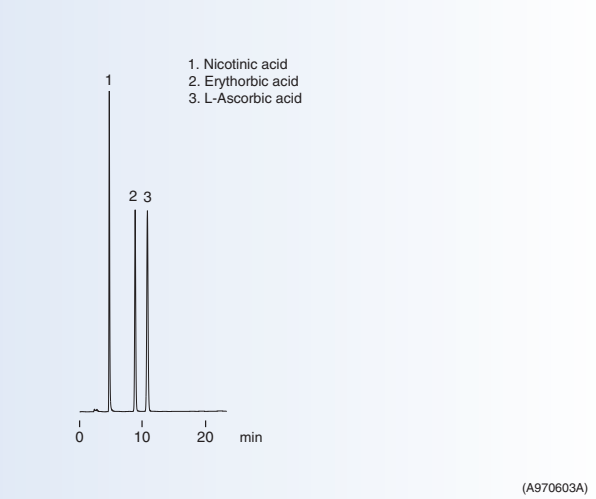


Column	: YMC-Triart Diol-HILIC (5 µm, 120 Å) 150 X 3.0 mmI.D.
Eluent	: A) acetonitrile/200 mM HCOOH-$HCOONH_4$ (pH 3.6)/water (90/5/5) B) acetonitrile/200 mM HCOOH-$HCOONH_4$ (pH 3.6)/water (50/5/45) 0-75%B (0-20 min)
Flow rate	: 0.425 mL/min
Temperature	: 40°C
Detection	: UV at 254 nm
Injection	: 4 µL (50 µg/mL)

Application

HILIC Vitamin C (Ascorbic acid)

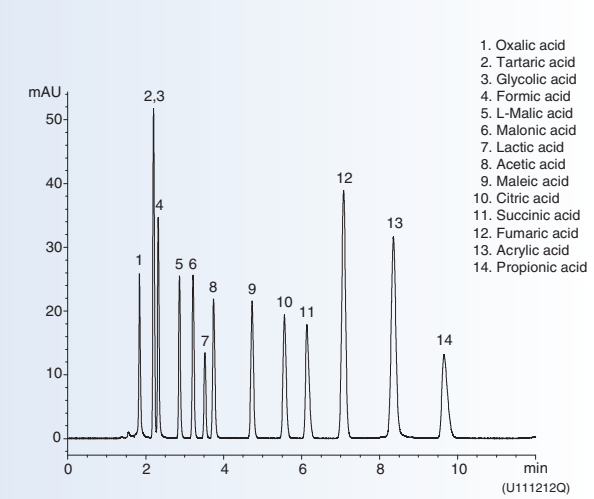
Separation of ascorbic acid under HILIC mode



Column	: YMC-Pack Polyamine Ⅱ 250 X 4.6 mmI.D.
Eluent	: acetonitrile/50 mM $NH_4H_2PO_4$ (70/30)
Flow rate	: 1.0 mL/min
Temperature	: 30°C
Detection	: UV at 250 nm
Injection	: 10 µL (0.05-0.1 mg/mL)

Reversed-phase Organic acids

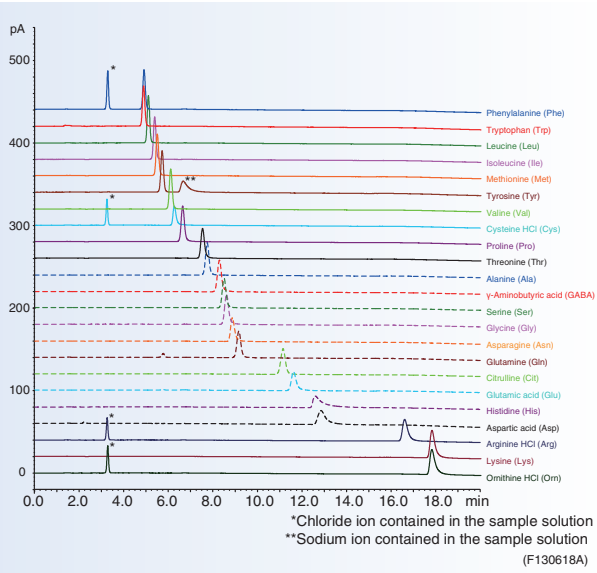
Simultaneous separation of organic acids under 100% aqueous mobile phase



Column	: YMC-Triart C18 (3 µm, 120 Å) 150 X 3.0 mmI.D.
Eluent	: 20 mM phosphoric acid
Flow rate	: 0.425 mL/min
Temperature	: 37°C
Detection	: UV at 220 nm
Injection	: 2 µL (0.005-1.5 mg/mL)

HILIC Amino acids

Simultaneous separation of amino acids under HILIC mode

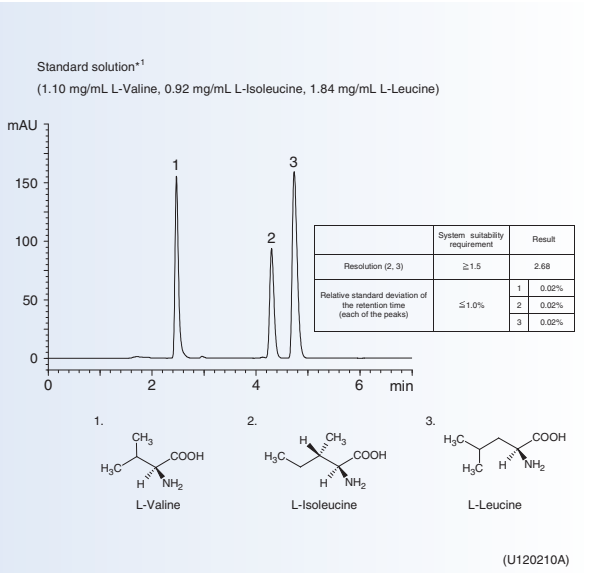


Column	: YMC-Triart Diol-HILIC (5 µm, 120 Å) 150 X 4.6 mmI.D.
Eluent	: A) 100 mM HCOOH-$HCOONH_4$ (pH 3.6) B) acetonitrile 83-80%B (0-12 min), 80-68%B (12-20 min)
Flow rate	: 1.0 mL/min
Temperature	: 40°C
Detection	: Corona® CAD® (Charged Aerosol Detector)
Injection	: 10 µL (0.1 mg/mL)

Corona and CAD are trademarks of Thermo Fisher Scientific.

Reversed-phase Amino acids

Separation of hydrophobic amino acids under highly aqueous mobile phase



Column	: YMC-Triart C18 (3 µm, 120 Å) 150 X 4.6 mmI.D.
Eluent	: phosphate buffer (pH 2.8)*2/acetonitrile (97/3)
Flow rate	: 0.9 mL/min *(adjust the flow rate so that the retention time of L-Valine is about 2.5 min)*
Temperature	: 40°C
Detection	: UV at 210 nm
Injection	: 20 µL
(The Japanese Pharmacopoeia 16th; Identification)	

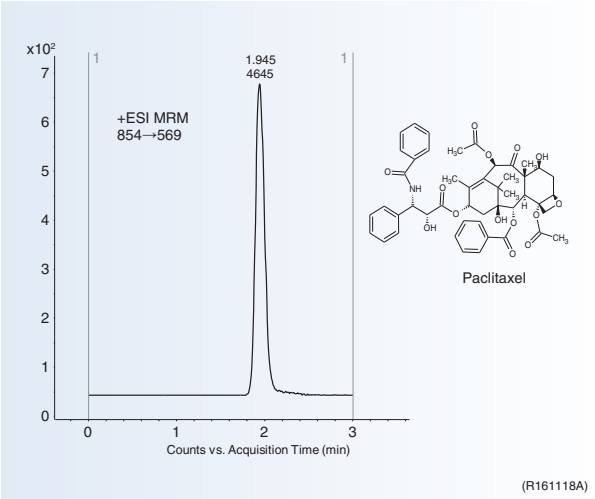
*2 *Dissolve 31.2 g of $NaH_2PO_4 \cdot 2H_2O$ in 1000 mL of water and adjust pH 2.8 with H_3PO_4*

*1 Standard solution was prepared from L-Valine, L-Isoleucine and L-Leucine supplied as a reagent for laboratory use.

Application

Reversed-phase Pharmaceutical products

LC-MS analysis of pharmaceutical product

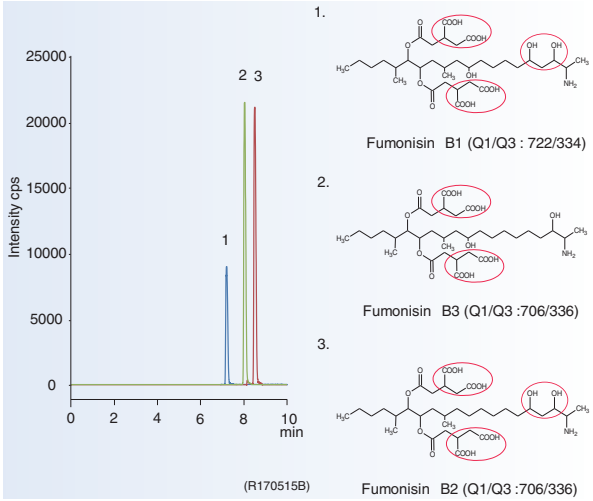


Courtesy of Gyeong Sang National University

Column	: YMC-Triart C18 (3 µm, 120 Å) 50 X 2.0 mmI.D.
Eluent	: acetonitrile/0.1% formic acid (45/55)
Flow rate	: 0.3 mL/min
Temperature	: 30°C
Detection	: ESI positive-mode
Injection	: 2 µL (1 µg/mL)

Reversed-phase Natural products

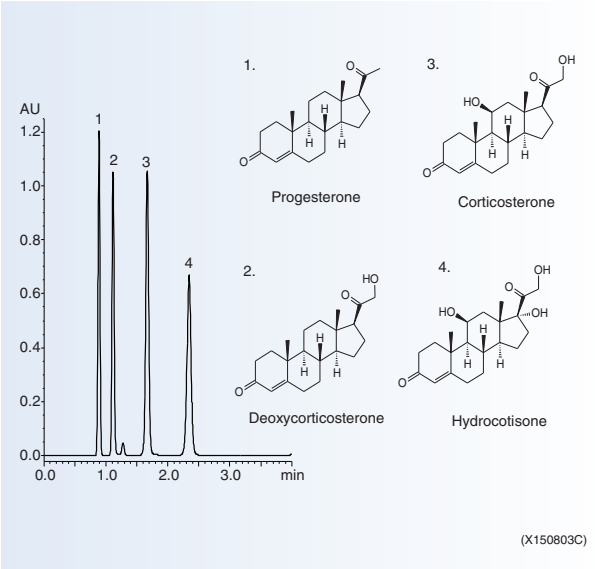
LC-MS/MS analysis of fumonisin mycotoxins using metal free column



Column	: YMC-Triart C18 [Metal free column] (3 µm, 120 Å)150 X 2.1 mmI.D.
Eluent	: A) water/HCOOH (100/0.1) B) acetonitrile 25-50%B (0-5 min), 50%B (5-8 min), 50-90%B (8-10 min)
Flow rate	: 0.2 mL/min
Temperature	: 40°C
Detection	: ESI positive, Scheduled MRM
Injection	: 5 µL (0.1 µg/mL)
Instrument	: LC) Shimadzu Prominence UFLC MS) AB Sciex 3200 QTRAP

SFC Steroids

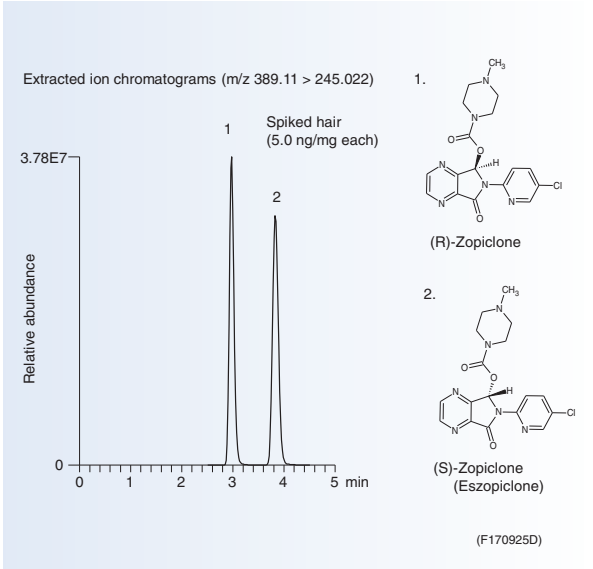
Separation of steroids by SFC



Column	: Alcyon SFC Triart Diol (5 µm, 120 Å) 150 X 4.6 mmI.D.
Eluent	: CO_2/methanol (80/20)
Flow rate	: 3.0 mL/min
Temperature	: 40°C
Detection	: UV at 254 nm
Back pressure	: 13.8 MPa (2000 psi)
Injection	: 5 µL (0.8 mg/mL)

Reversed-phase Chiral compounds

Analysis of zopiclone in human hair by chiral LC/HRMS

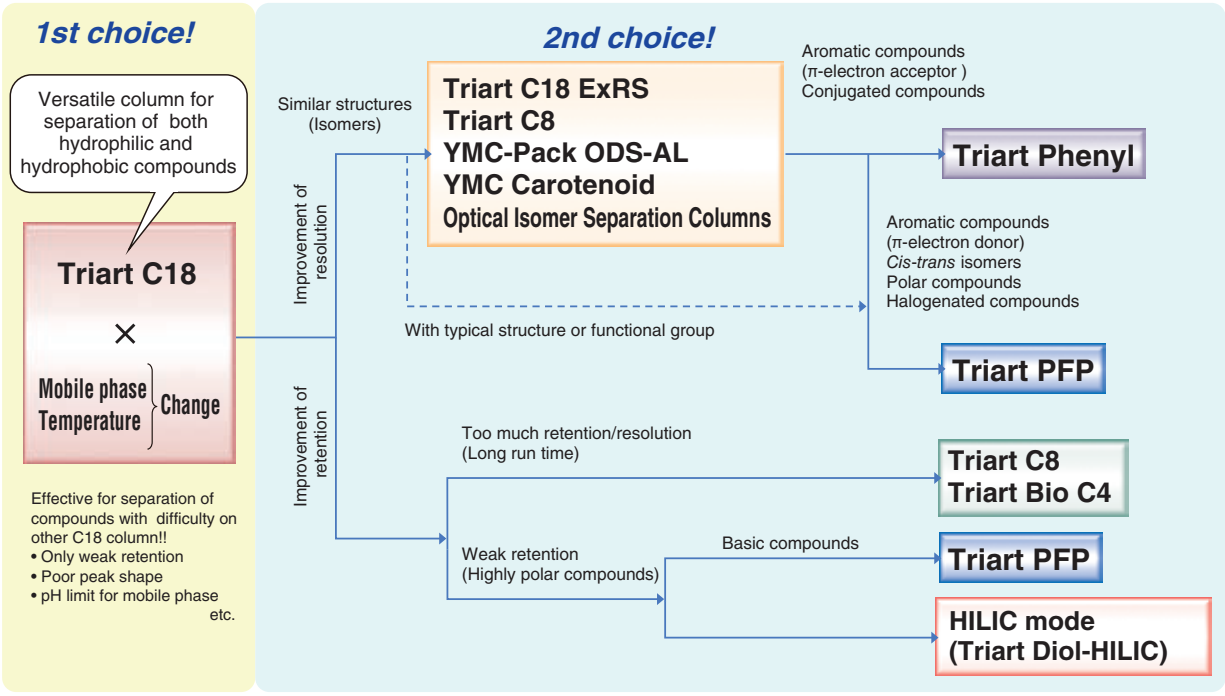


Courtesy of H. Miyaguchi, National Research Institute of Police Science.

Column	: CHIRAL ART Cellulose-SC (3 µm) 150 X 2.0 mmI.D.
Eluent	: 10 mM ammonium bicarbonate (pH 8.0 with aqueous ammonia) /acetonitrile (25/75)
Flow rate	: 0.2 mL/min
Temperature	: 25°C
Detection	: ESI, positive
Injection	: 10 µL (Hair sample extracted by micropulverized extraction)
Instrument	: LC) Ultimate™ 3000 liquid chromatograph (Thermo Fisher Scientific) HRMS) Q Exactive™ mass spectrometer (Thermo Fisher Scientific)

Reference
H. Miyaguchi, K. Kuwayama, Enantioselective determination of (*R*)-zopiclone and (*S*)-zopiclone (eszopiclone) in human hair by micropulverized extraction and chiral liquid chromatography/high resolution mass spectrometry, *J.Chromatogr.* A 1519 (2017) 55-63.

Reversed-phase column selection guide



Comparison of hydrophobicity and hydrogen-bonding capacity of various C18 columns

