

Analytical and preparative chromatography columns and materials for pharmaceutical applications

○Polymeric partition chromatography columns and materials MCI GEL™ CHP series

Separation mechanism of CHP series

High performance liquid chromatography relies on one of the following physical phenomena for efficient separation of solutes: partition, adsorption, size exclusion, or ion exchange. Of these, partition chromatography is the most commonly used method, and it separates solutes based on their difference in partitioning between a stationary phase and a mobile phase. This technique has currently become the mainstay in industry for the separation of organic compounds such as pharmaceuticals, agricultural chemicals, and other intermediates. Practically, partition chromatography can be performed in two different modes depending on the relative polarities of the stationary and mobile phases. In the normal phase (NP) mode, the mobile phase is less polar than the stationary phase while the situation is reversed in the reverse phase (RP) mode, where the mobile phase is significantly more polar than the stationary phase.

MCI GEL™ specializes in polymer-based packing materials. The use of polymer-based columns has become more widespread thanks to the many advantages of the polymer matrix like excellent selectivity, the absence of specific adsorption which is found commonly with silica-based packing, operability in a wide pH range and good chemical stability due to the inert nature of polymeric materials. The MCI GEL™ partition chromatography columns are based on a polystyrene and polymethacrylate porous polymer. As RP columns, they are applied to the separation of a wide variety of organic compounds, both in the isocratic and gradient elution mode. The compounds include peptides, insulin, small molecule APIs, nutraceutical compounds, water-soluble vitamins and nucleotides. As NP columns, they are used in the separation of various carotenoids, fat-soluble vitamins, steroids, and food additives. These columns tolerate various organic solvents like hexane, heptane, methylene chloride, and alcohols.

As NP columns, they are used in the separation of various carotenoids, fat-soluble vitamins, steroids, and food additives. Various organic solvents like Hexane Heptane, methylene chloride and alcohols can be used.

The MCI GEL™ packing materials are based on the same chemistries offered in the Diaion™ and Sepabeads™ synthetic adsorbent resins. These polymer chemistries, like Diaion™ HP series and Sepabeads™ SP series, are widely used and documented in the biopharmaceutical industry for fermentation extraction, the food industry and in industrial chromatographic separations. The MCI GEL™ packing materials are available as packed columns for analytical applications, and as bulk packing materials for analytical, preparative and production chromatography applications.

●Description of MCI GEL™ columns and materials

MCI GEL™ CHP20/C04

Matrix type

Particle size

C=Column
P=Material

CHP column series

Analytical and preparative chromatography columns and materials for pharmaceutical applications

MCI GEL™ CHP series are suitable for RP and NP chromatography. There are four kinds of columns of various hydrophobicities; porous polystyrene, modified porous polystyrene, polymethacrylate, and modified porous polymethacrylate. This range of packing materials offers tremendous scope for a proper selection of columns based on the properties of the target compounds.

Polystyrene packing: MCI GEL™ CHP20/C04, CHP20/C10

Modified polystyrene packing: MCI GEL™ CHP07/C04, CHP07/C10, CHK40/C04

Polymethacrylate packing: MCI GEL™ CMG20/C10

Modified polymethacrylate packing: MCI GEL™ CHPOD/C04, CHK45/C05

The hydrophobicities of the columns are in the following orders:

MCI GEL™ CHP07/C04, C10 > CHP20/C04, C10 > CHPOD/C04 ≥ ODS columns ≥ CMG20/C04, C10

Polymer columns for HPLC, with their superior chemical resistance, can be used with various mobile phases of broad pH range, acidic through alkaline. They have the following advantages due to their high hydrophobicities:

- 1) In reverse phase chromatographic methods to separate acidic or alkaline compounds, eluents that can suppress the ionic properties of such compounds are generally used. Polymer columns can be applied in these cases where ODS columns would be unsuitable.
- 2) Some extremely hydrophilic compounds, e.g., oligosaccharides, can be separated using strongly hydrophobic CHP07/C04 or CHP07/C10 columns.
- 3) Polymer columns can be washed with acidic and/or basic solutions in case of contamination.

Polymethacrylate columns, CMG20/C04 and CMG20/C10, can be applied both for reverse phase and normal phase chromatography.

Modified polystyrene packing, CHK40/C04, is a mixed-mode type material; both hydrophobic and hydrophilic interactions occur between the packing material surface and the analytes. This material is useful for compounds that are difficult to separate using existing ODS or other polymer-based columns. This column is also used in the normal phase mode and shows a unique separation profile.

All polymeric columns exhibit superior stability and yield in comparison to ODS columns, which may have free silanol groups even when end-capping agents have been used.

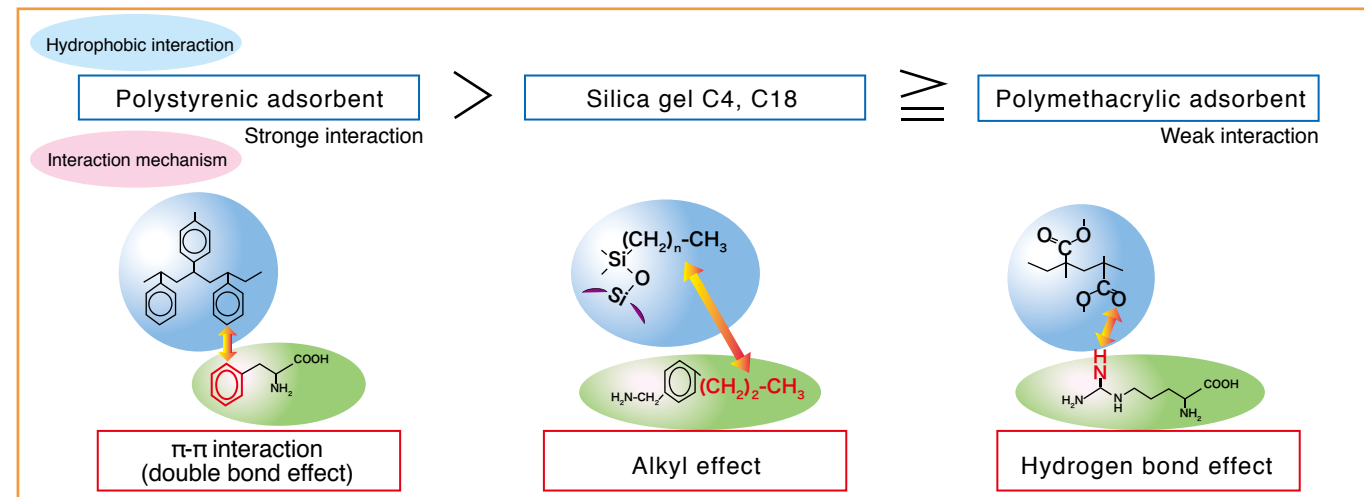
Column list

●CHP column series

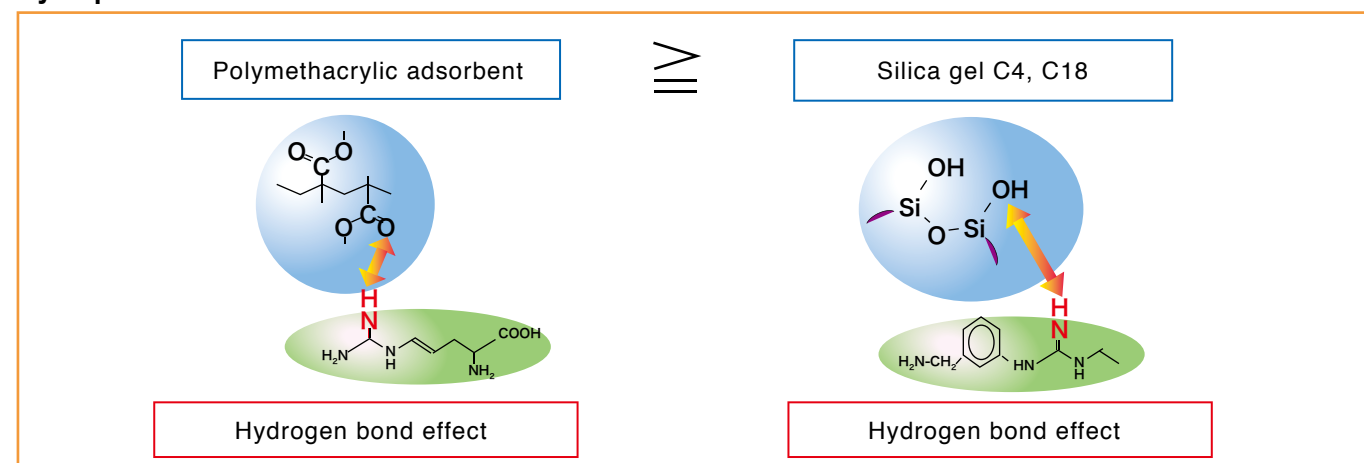
Matrix Type	Functional group	Product name	Particle size [μm]	Column size [mm I.D.×mm]	pH range	USP
Styrene Divinylbenzene	None	CHP20/C04	4	4.6×150 20×150	Full range	L21
		CHP20/C10	10	4.6×150 4.6×250 10×250 20×150 20×250		
	Br	CHP07/C04	4	4.6×150 20×200		
		CHP07/C10	10	4.6×150 4.6×250 10×150 20×150 20×250		
	Cation exchange group		CHK40/C04	4	4.6×150	
Methacrylates	None	CMG20/C04	4	4.6×150 20×150	2~12	
		CMG20/C10	10	4.6×150 4.6×250 10×250 20×150 20×250		
	C18	CHPOD/C04	4	4.6×150 20×200		
	Weak cation exchange group	CHK45/C05	5	4.6×150		

*CHP20/C04, CHP20/C10: USP classification is L21

Retentiveness in reverse phase mode



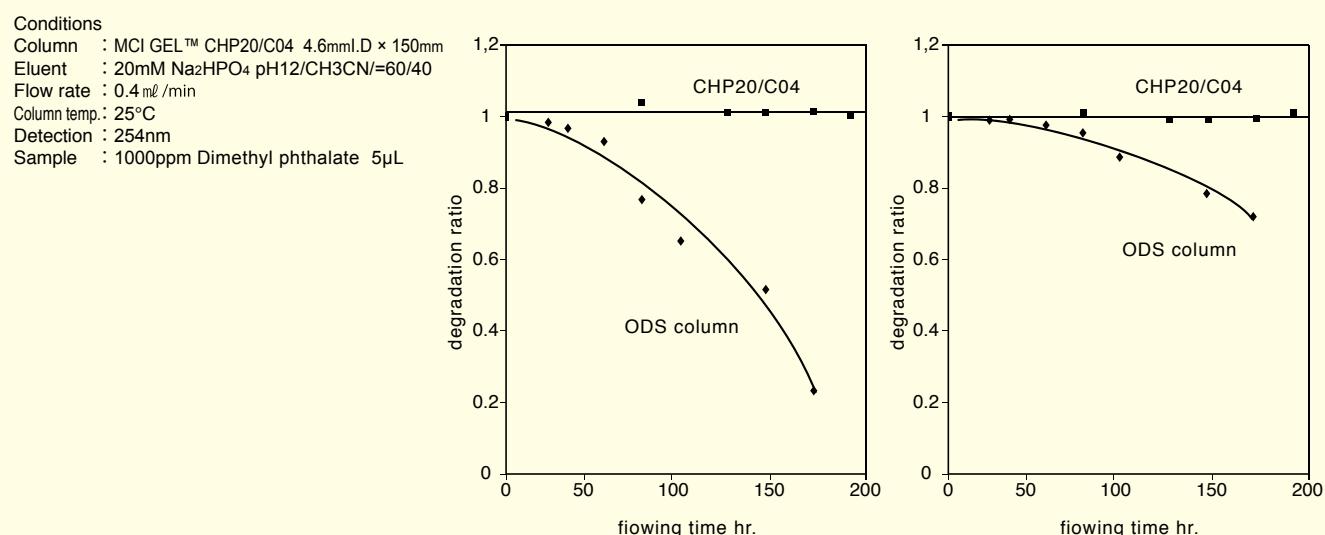
Hydrophobic interaction Interaction mechanism



Durability of polymeric column

The polymeric RP columns are chemically stable. Specifically, the columns have resistance to an alkaline eluent. The following graphs demonstrate stability of the polymeric columns. After feeding a solution of pH 12 into the MCI GEL™ CHP20/C04, there is no change of column performance.

Fig. 5-1 Column durability at pH12 comparison between CHP20/C04 and an ODS column



Application data of CHP series

Fig. 5-2 Separation of catecholamines

Conditions
 Column : MCI GEL™ CHP20/C04
 4.6mm I.D. × 150mm
 Eluent : 50mM Na-phosphate pH2.0,
 1.5% Hexanesulfonic acid /
 CH₃CN=80/20
 Flow rate : 0.25 mL/min
 Column temp.: ambient
 Detection : 280nm
 Sample : 1. Epinephrine
 2. Dopamine
 3. 5-Hydroxy tryptophan
 4. Serotonin
 5. Tryptophan

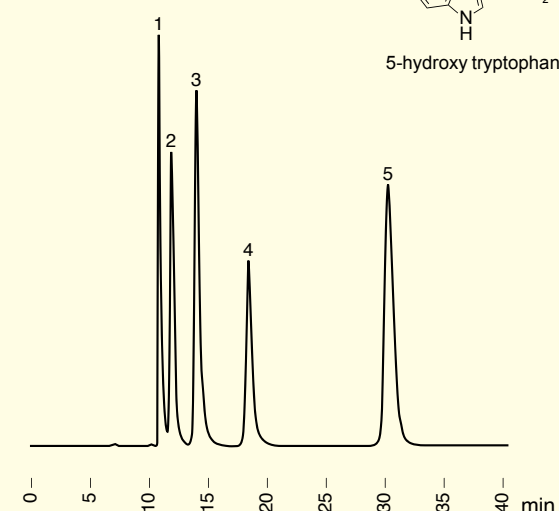
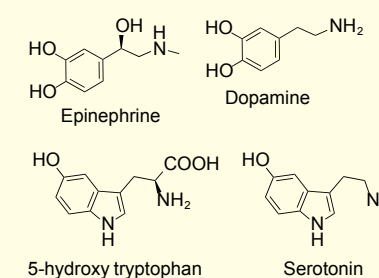


Fig. 5-3 Separation of phthalic acid esters

Conditions
 Column : MCI GEL™ CHP20/C04
 4.6mm I.D. × 150mm
 Eluent : H₂O/CH₃CN=50/50
 Flow rate : 0.75 mL/min
 Column temp.: 60°C
 Detection : 254nm
 Sample : 1. Dimethyl phthalate
 2. Diethyl phthalate
 3. Dipropyl phthalate

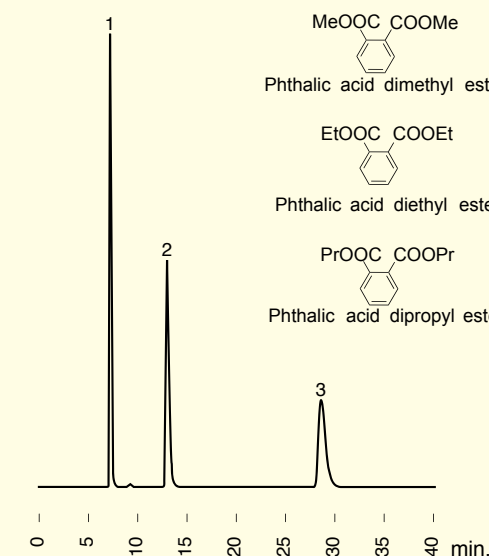
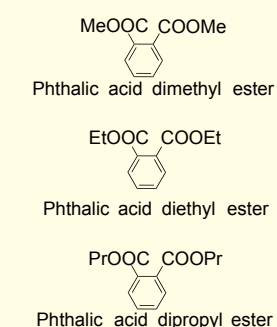


Fig. 5-4 Purine alkaloids

Conditions
 Column : MCI GEL™ CHP20/C04
 4.6mm I.D. × 150mm
 Eluent : H₂O/CH₃CN=10/90
 Flow rate : 0.4 mL/min
 Column temp.: 25°C
 Detection : 275nm
 Sample : 1. Theophylline
 2. Theobromine
 3. Caffeine

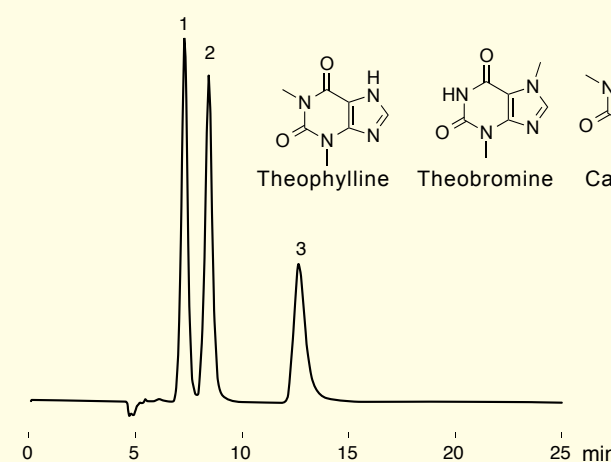
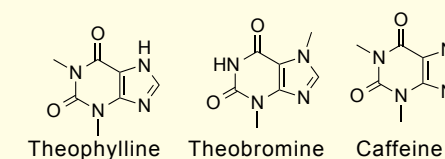
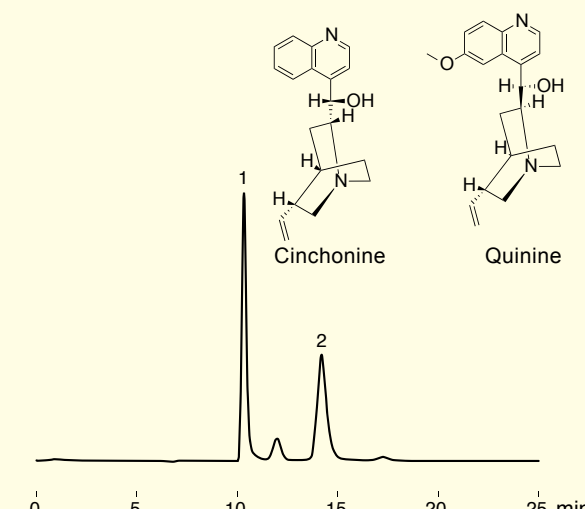
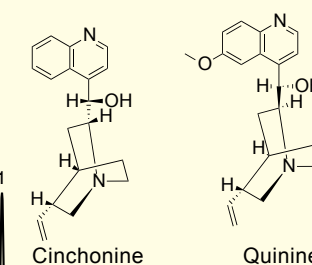


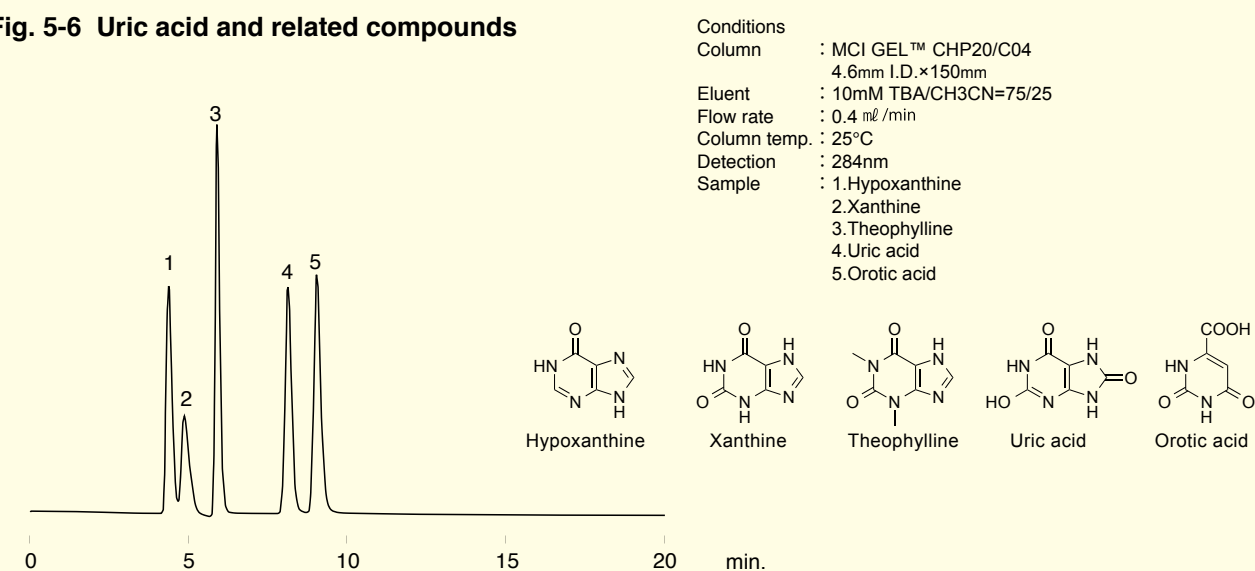
Fig. 5-5 Cinchona alkaloids

Conditions
 Column : MCI GEL™ CHP20/C04
 4.6mm I.D. × 150mm
 Eluent : 0.1M NaH₂PO₄ pH2.0
 CH₃CN=88/12
 Flow rate : 0.3 mL/min
 Column temp.: 25°C
 Detection : 275nm
 Sample : 1. Cinchonine
 2. Quinine



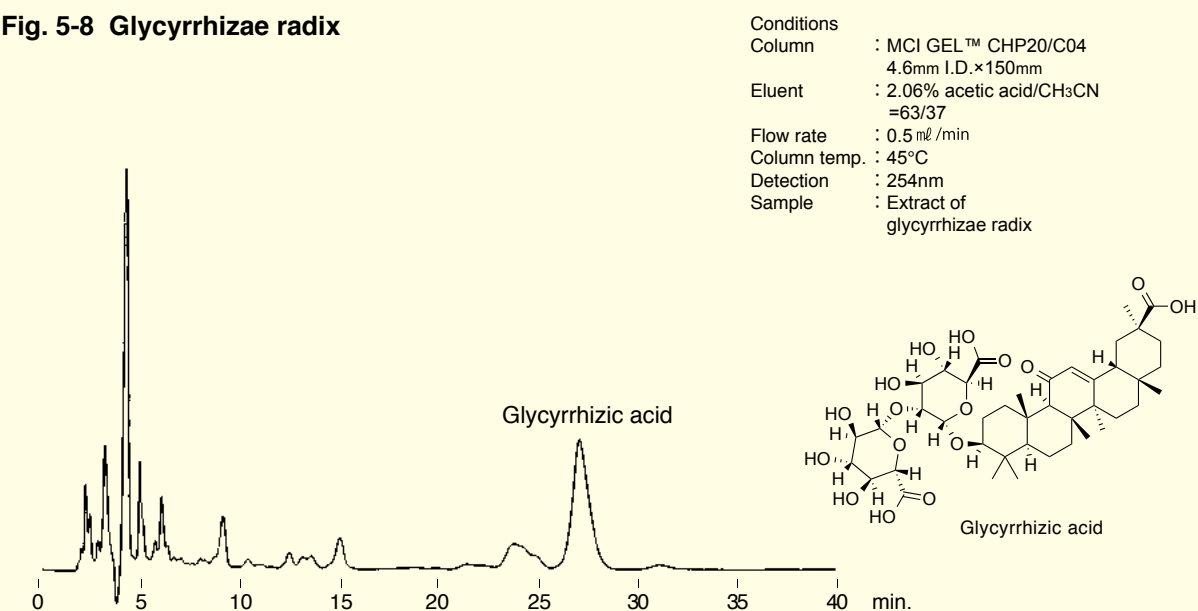
Application data of CHP series

Fig. 5-6 Uric acid and related compounds



Application data of CHP series

Fig. 5-8 Glycyrrhizae radix



Comparison with an ODS column

Fig. 5-7 Bile acids

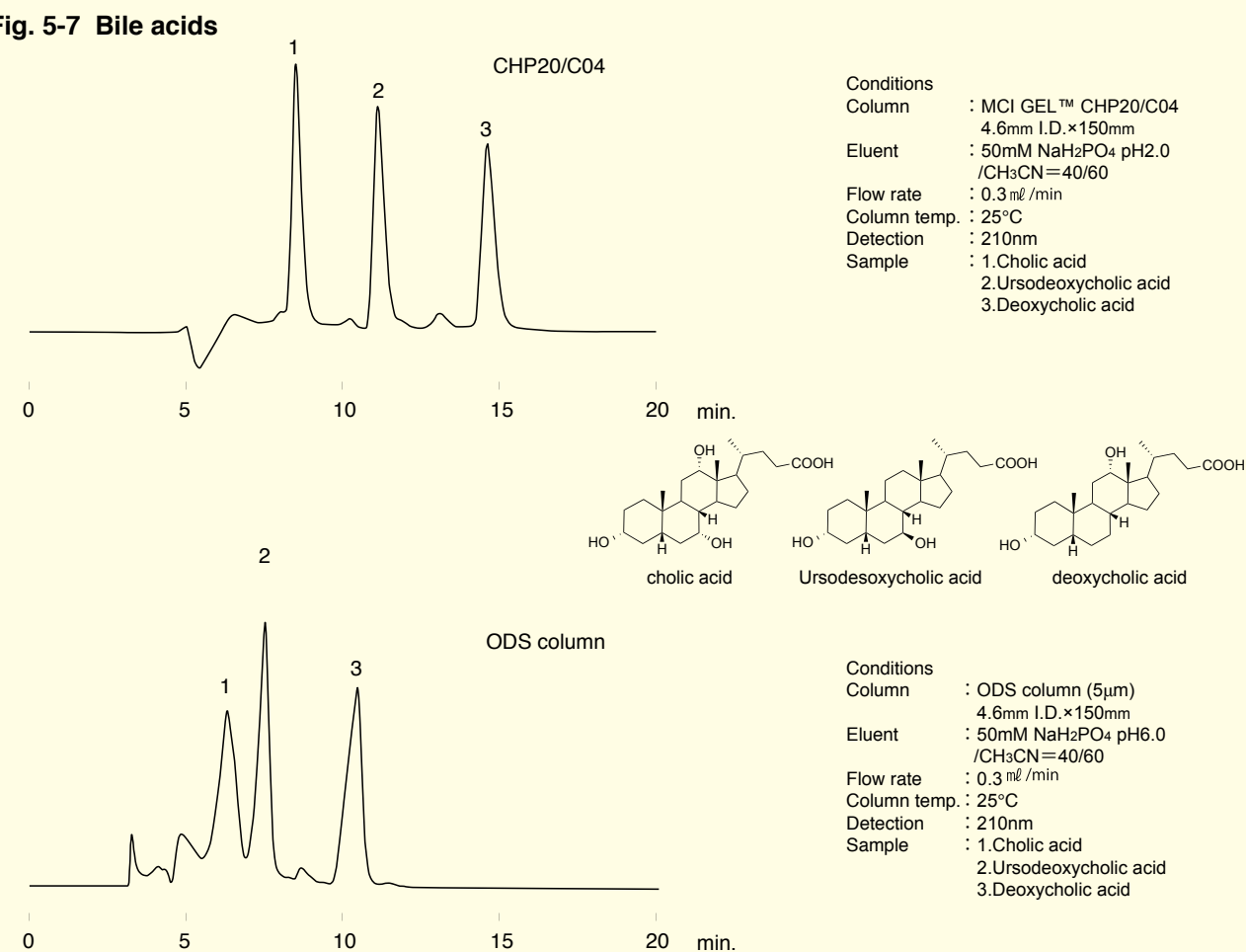
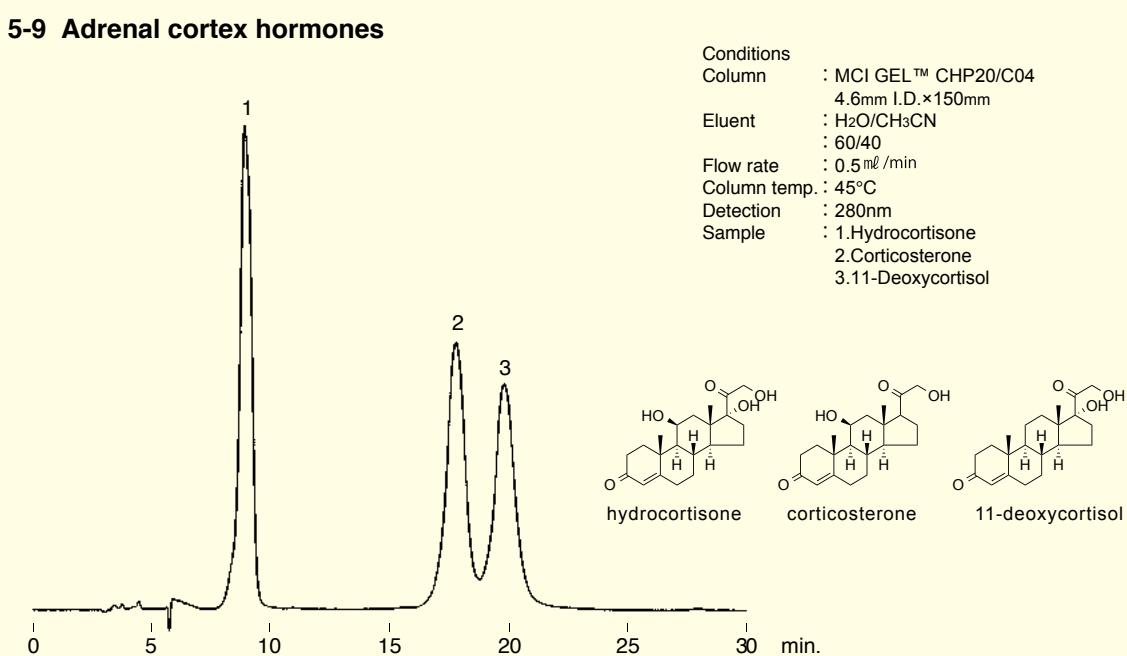
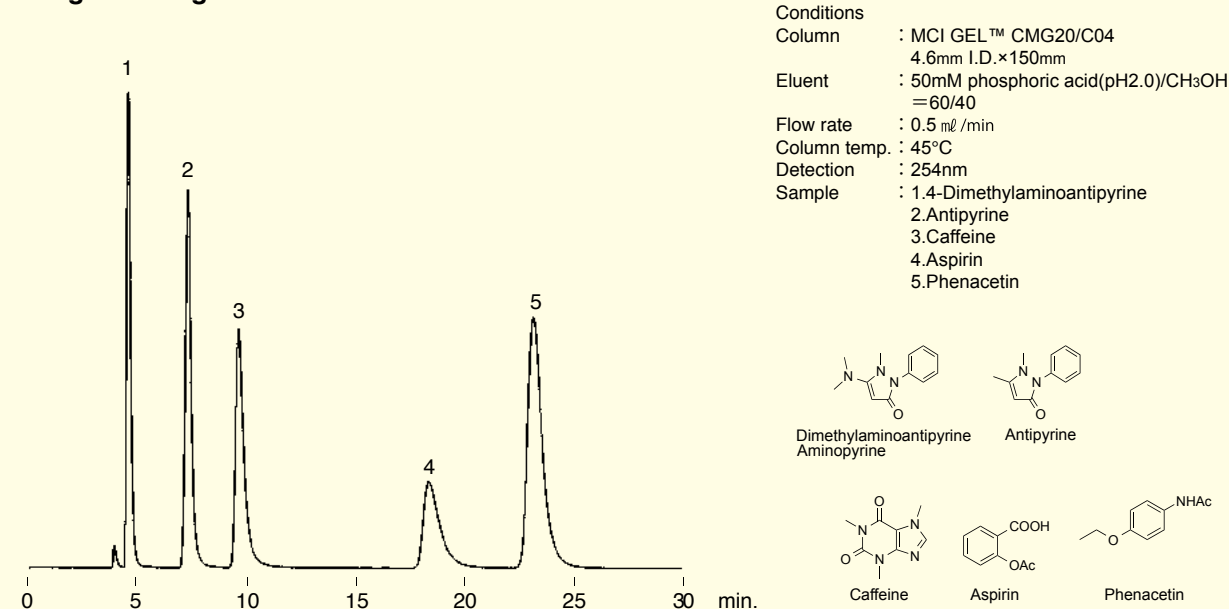


Fig. 5-9 Adrenal cortex hormones



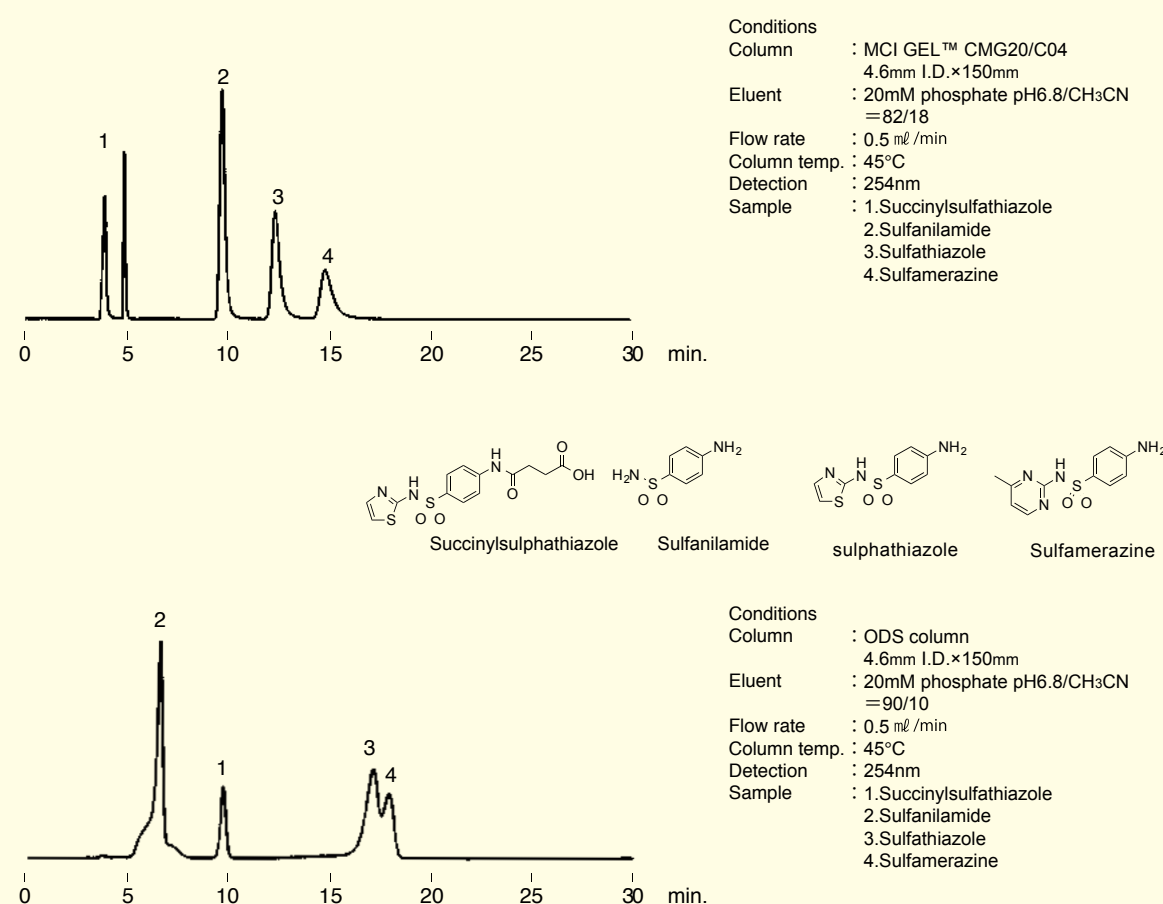
Application data of CHP series

Fig. 5-10 Ingredients of medicine



Comparison with an ODS column

Fig. 5-11 Sulfa drugs



Application data of CHP series

Fig. 5-12 Peptides

Conditions
Column : MCI GEL™ CMG20/C04
4.6mm I.D.×150mm
Eluent : 0.1%TFA/CH₃CN
=70/30
Flow rate : 0.5 mL/min
Column temp. : 25°C
Detection : 220nm
Sample : 1.Gly-Tyr
2.Met Enkephalin
3.Leu Enkephalin
4.Angiotensin II

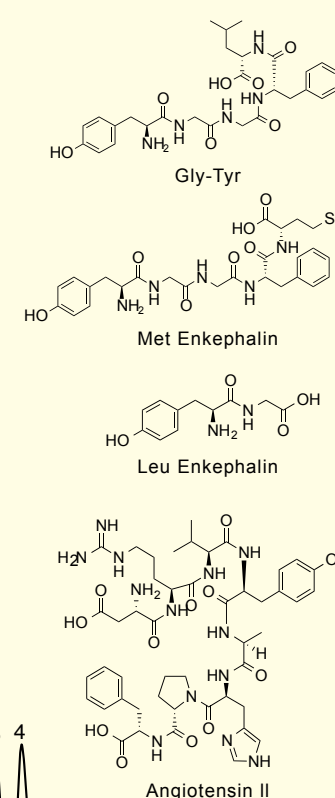


Fig. 5-13 Proteins

Conditions
Column : MCI GEL™ CMG20/C04
4.6mm I.D.×150mm
Eluent : A;0.05%TFA/CH₃CN
=80/20
B;0.05%TFA/CH₃CN
=20/80
A→B 30min.linear
Flow rate : 0.5 mL/min
Column temp. : 25°C
Detection : 280nm
Sample : 1.Ribonuclease A
2.Cytochrome c
3.α-Chymotrypsinogen A

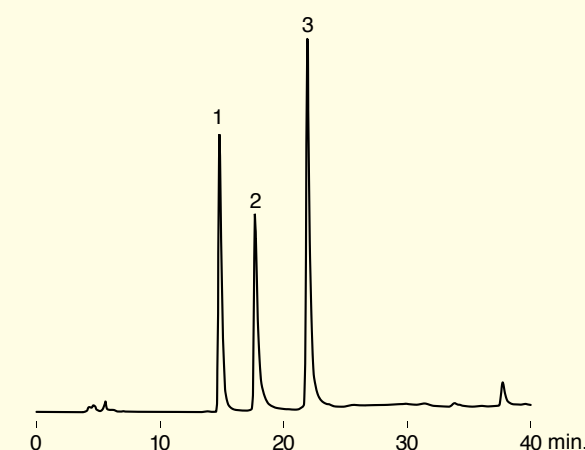


Fig. 5-14 Procainamide, Procaine

Conditions
Column : MCI GEL™ CMG20/C04
4.6mm I.D.×150mm
Eluent : 20mM phosphate pH7.2/CH₃CN
=65/35
Flow rate : 0.5 mL/min
Column temp. : 45°C
Detection : 254nm
Sample : 1.Procainamide
2.Procaine

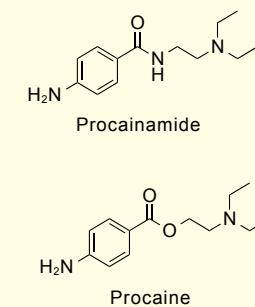
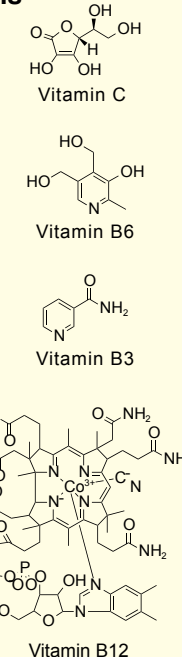


Fig. 5-15 Water-soluble vitamins

Conditions
Column : MCI GEL™ CMG20/C04
4.6mm I.D.×150mm
Eluent : 8mM Na₂HPO₄ pH7.0/CH₃CN
=85/15
Flow rate : 0.5 mL/min
Column temp. : 25°C
Detection : 254nm
Sample : 1.Vitamin C
2.Vitamin B6
3.Vitamin B3
4.Vitamin B12



Application data of CHP series

Fig. 5-16 Pravastatin sodium

Conditions
Column : MCI GEL™ CHP20/C10
(10μm 250 ×4.6mm I.D.) and
ODS (10μm 250 ×4.6mm I.D.)
Eluent : A :0.1% Formic acid;
B :0.1% Formic acid in AcCN;
Gradient : 45%B-95%B over 29min.
Flow rate : 1.00 mL/min
Column temp.: 25°C
Detection : UV238nm
Sample : Pravastatin sodium, Mevastatin and Simvastatin, 1mg/ml each;
Injection : 5μl

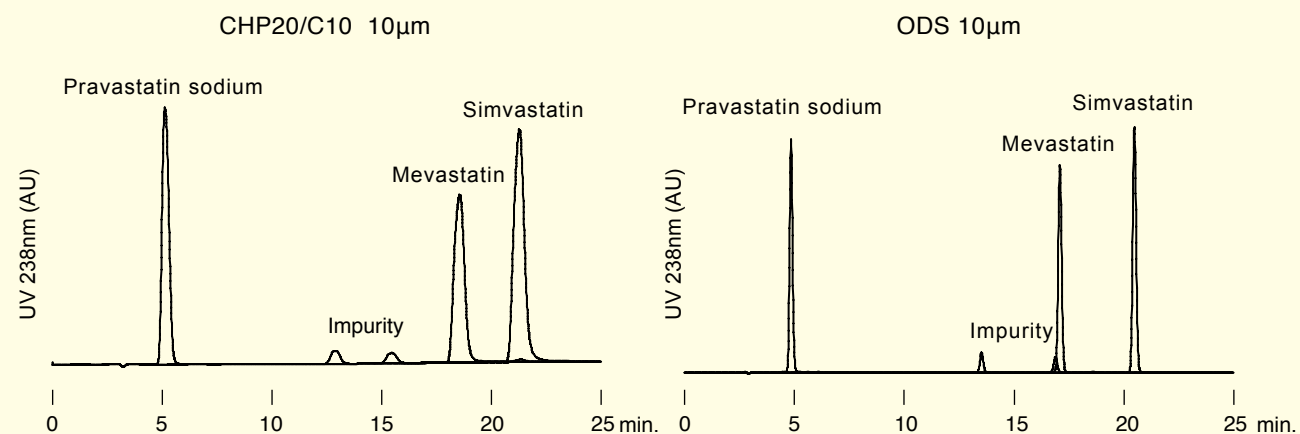
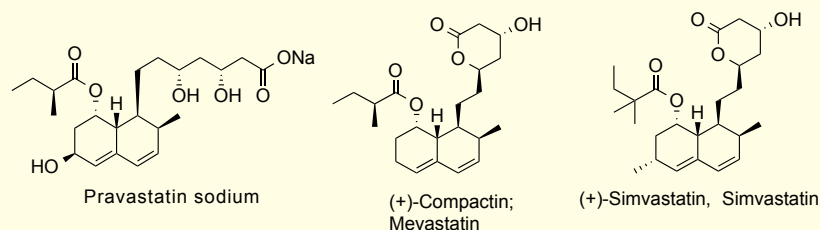
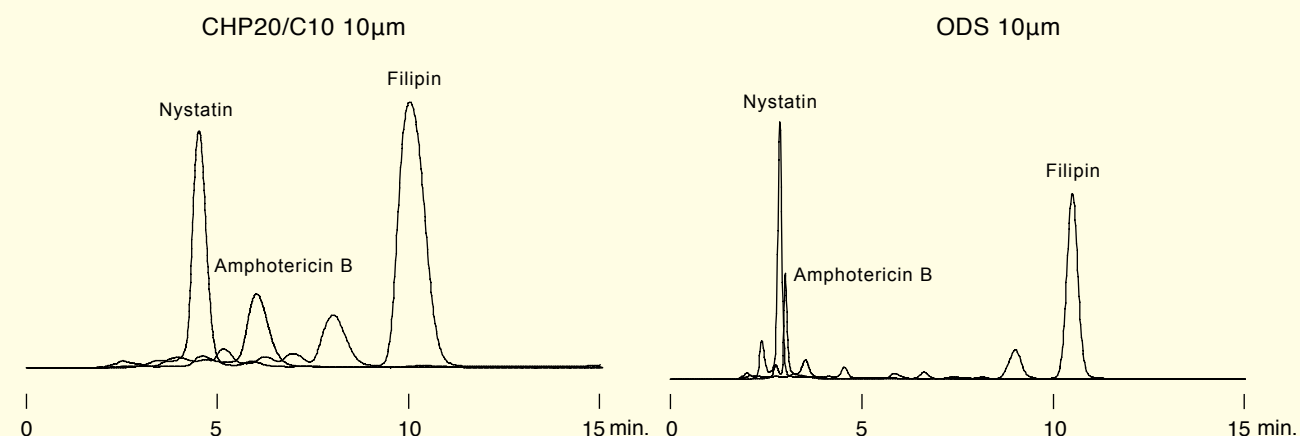
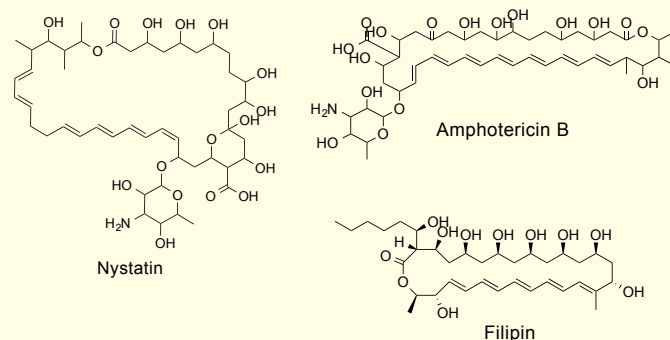


Fig. 5-17 Polyene antibiotics

Conditions
Column : MCI GEL™ CHP20/C10 (10μm 250 ×4.6mm I.D.) and
ODS (10μm 250 ×4.6mm I.D.)
Eluent : A :0.1% Formic acid;
B :0.1% Formic acid in AcCN; A/B=60/40;
Flow rate : 1.00 mL/min
Column temp.: 25°C
Detection : UV305nm for Nystatin, VIS405nm for
Amphotericin B and UV340nm for Filipin;
Sample : Pravastatin sodium, Mevastatin and
Simvastatin, 1mg/ml each;
Injection : 10μl



Application data of CHP series

Fig. 5-18 Proteins

Conditions
Column : MCI GEL™ CMG20/C10
4.6mm I.D.×250mm
Eluent : A :0.05% TFA/CH₃CN=80/20
B :0.05% TFA/CH₃CN=30/70
A → B 45min linear gradient
Flow rate : 0.5 mL/min
Column temp.: 25°C
Detection : 280nm
Sample : 1. Ribonuclease A
2. Cytochrome C
3. Transferrin
4. α-Chymotrypsinogen A
5. β-Lactoglobulin

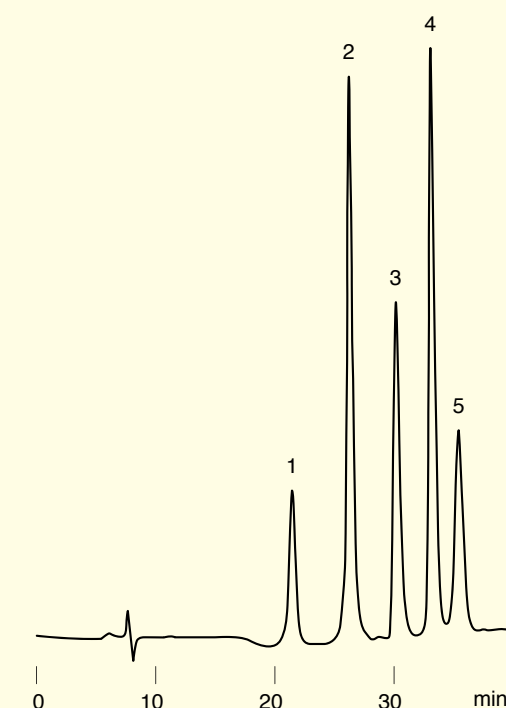
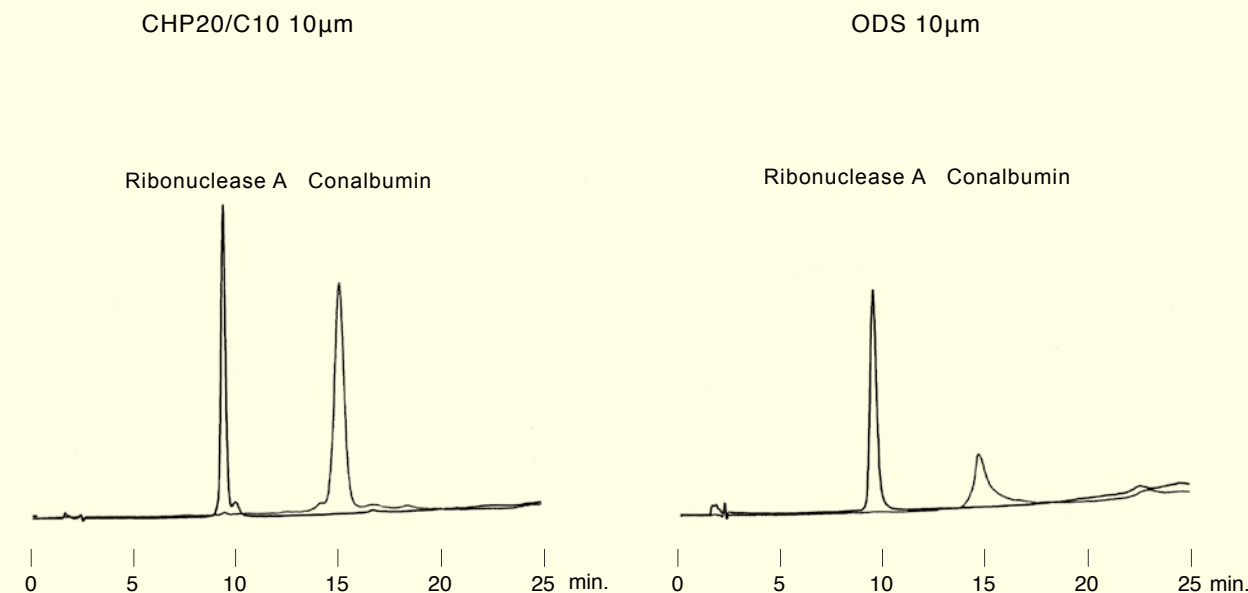


Fig. 5-19 Proteins



Conditions
Column : 150 ×4.6mm I.D.
Eluent : A :0.1% TFA;
B :0.1% TFA in AcCN
Flow rate : 1.00 mL/min
Column temp.: 20%B-60%B over 20min;
Detection : UV280nm;
Sample : Ribonuclease A and Conalbumin 2mg/ml;
Injection : 10μl

Application data of CHP series

Fig. 5-20 Insulin

Conditions
 Column : MCI GEL™ CHP20/C10
 MCI GEL™ CMG20/C10
 ODS 10μm
 4.6mm I.D.×150mm
 Eluent : A) 0.1%TFA, H₂O
 B) 0.1%TFA, CH₃OH
 Gradient : 20%B→60%B over 20min.
 Flow rate : 1.0 ml/min
 Column temp.: 40°C
 Detection : 280nm
 Sample : Insulin Glargine and human recombinant, 1mg/ml each
 Injection : 10μl

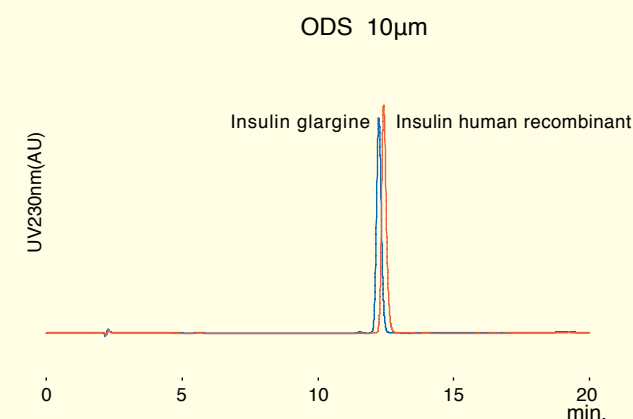
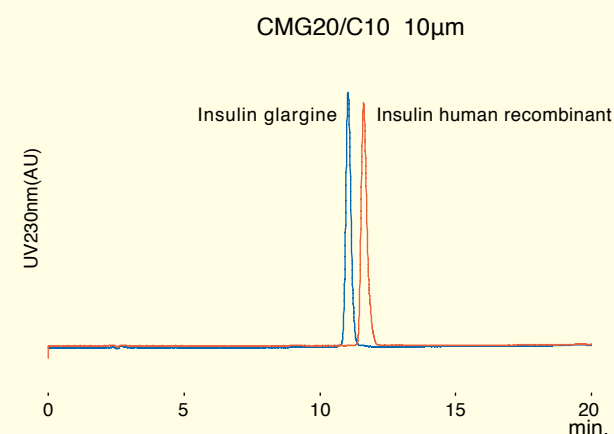
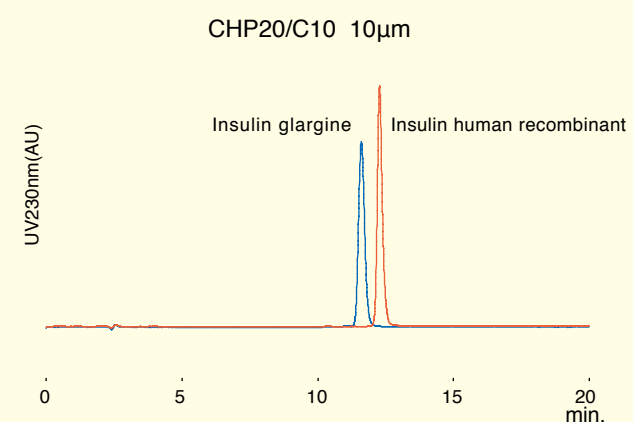
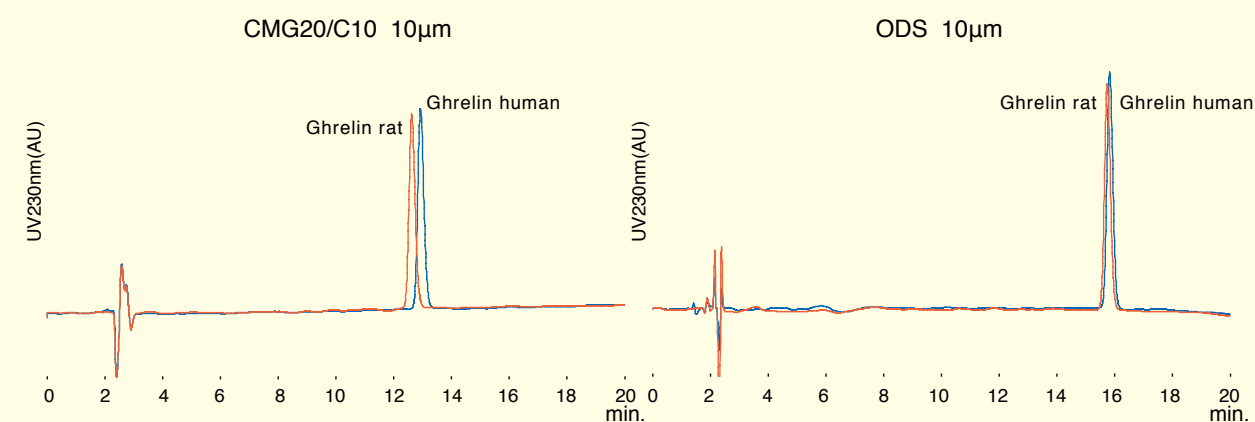


Fig. 5-21 Ghrelin

Conditions
 Column : MCI GEL™ CMG20/C10
 ODS 10μm
 4.6mm I.D.×150mm
 Eluent : A) 0.1%TFA, H₂O
 B) 0.1%TFA, AcCN
 Gradient : 10%B→60%B over 25min.
 Flow rate : 1.0 ml/min
 Column temp.: 40°C
 Detection : 230nm
 Sample : Ghrelin rat and Ghrelin human, 0.1mmol/l each
 Injection : 10μl



Application data of CHP series

Fig. 5-22 Leuporelin

Conditions
 Column : MCI GEL™ CHP20/C10
 MCI GEL™ CMG20/C10
 ODS 10μm
 4.6mm I.D.×150mm
 Eluent : A) 0.1%TFA, H₂O
 B) 0.1%TFA, AcCN
 Gradient : 20%B→60%B over 20min.
 Flow rate : 1.0 ml/min
 Column temp.: 40°C
 Detection : 280nm
 Sample : Leuporelin, LHRH human, LHRH salmon and Buserelin, 1mg/ml each
 Injection : 10μl

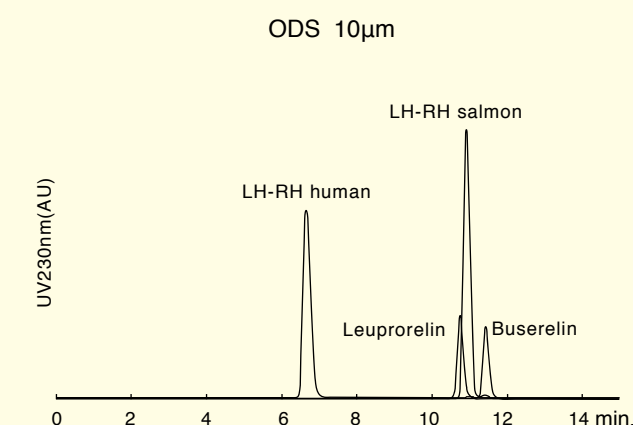
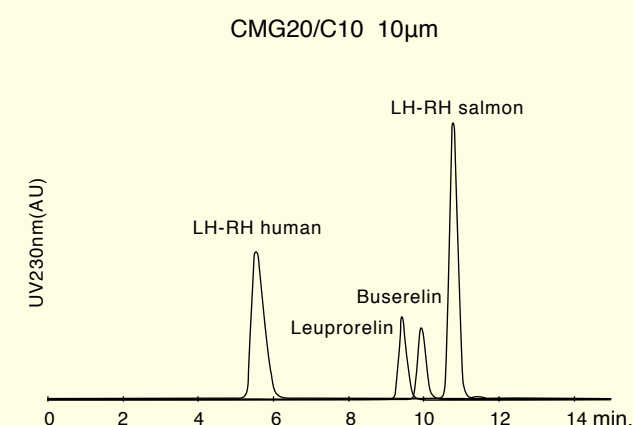
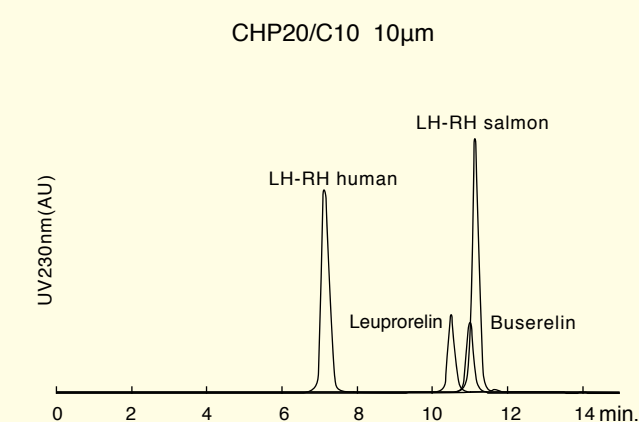
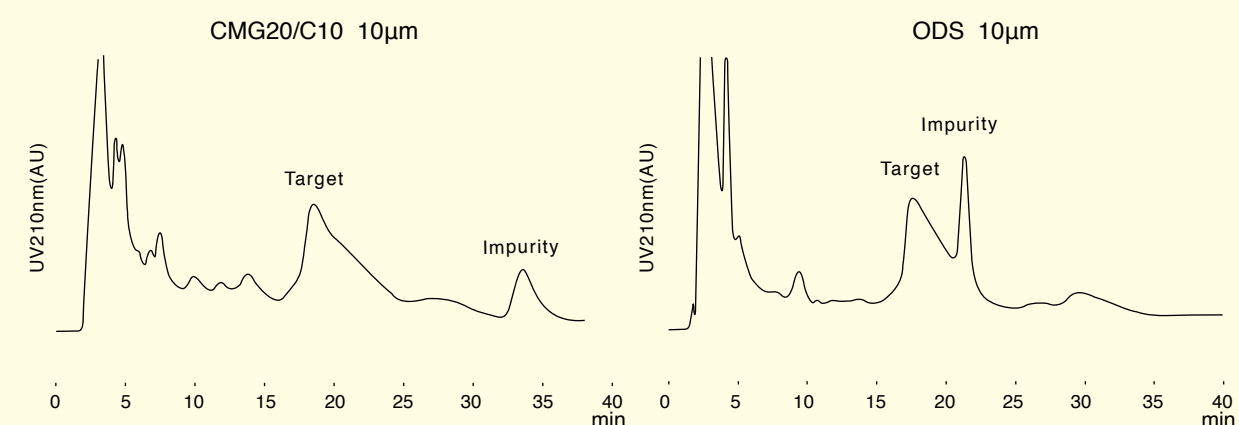


Fig. 5-23 Sifuvirtide

Conditions
 Column : MCI GEL™ CMG20/C10
 ODS 10μm
 4.6mm I.D.×150mm
 Eluent : 0.1%TFA./CH₃CN=68/32
 Flow rate : 1.0ml/min
 Column temp.: 40°C
 Detection : 210nm
 Sample : Sifuvirtide crude(purity 35.5%) 2.1mg/ml
 Injection : 0.4ml



Application data of CHP series

Fig. 5-24 ssRNA Ladder Marker

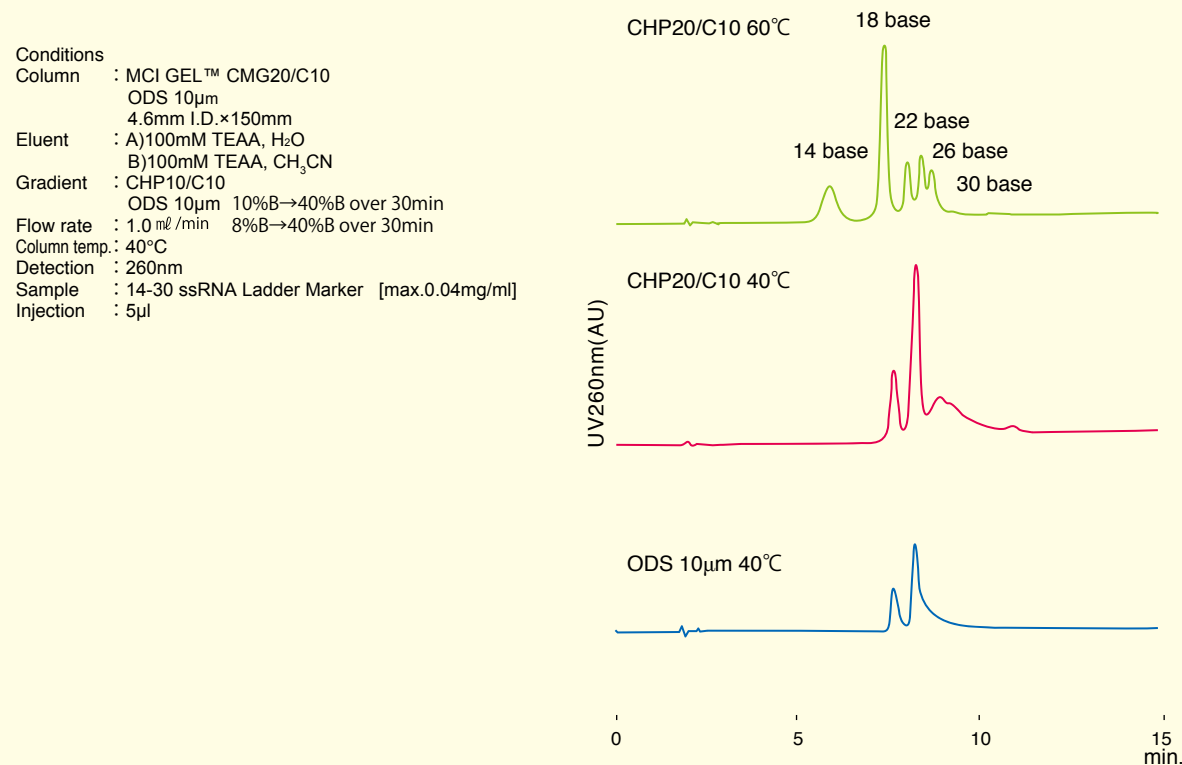
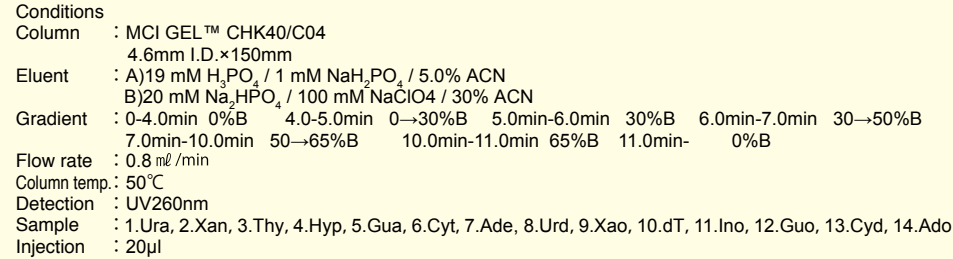


Fig. 5-25 Nucleotide



(Data provided by Professor Yokoyama of Yokohama National University)

Application data of CHP series

Fig. 5-26 Linalool

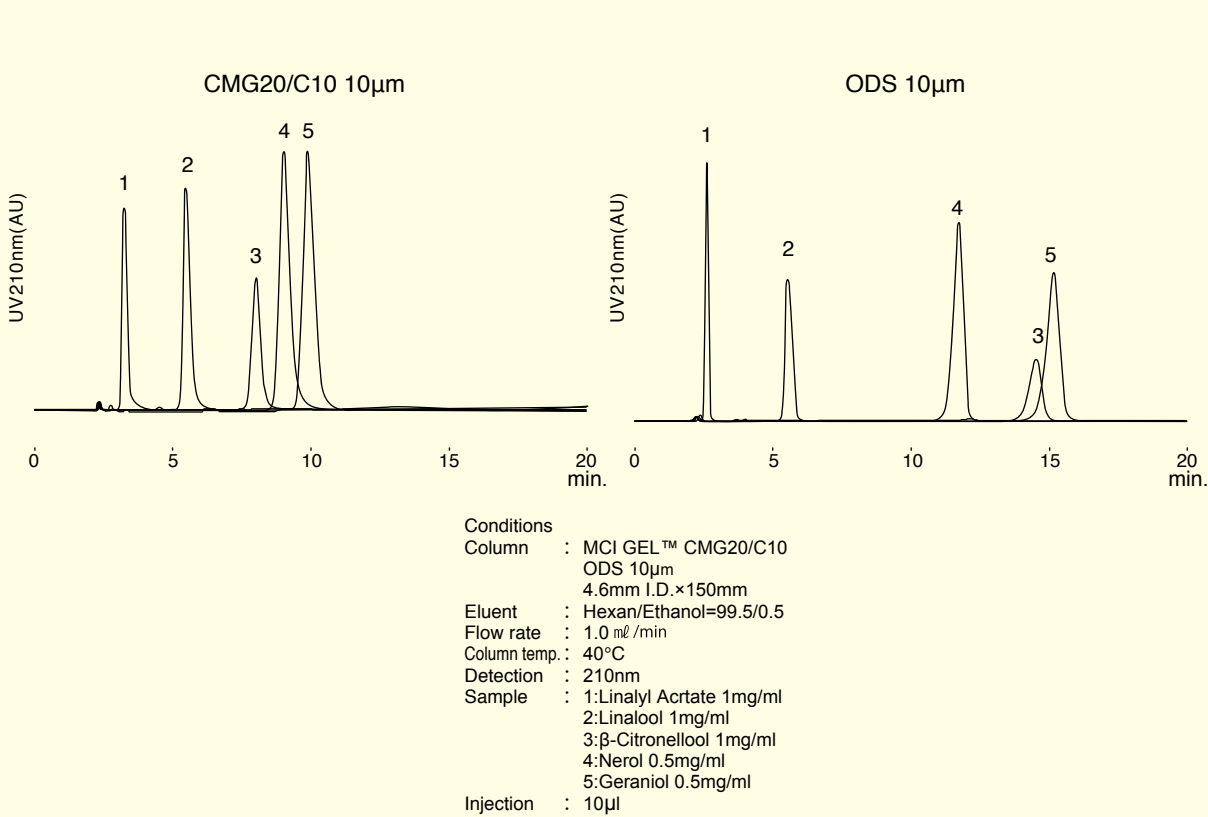
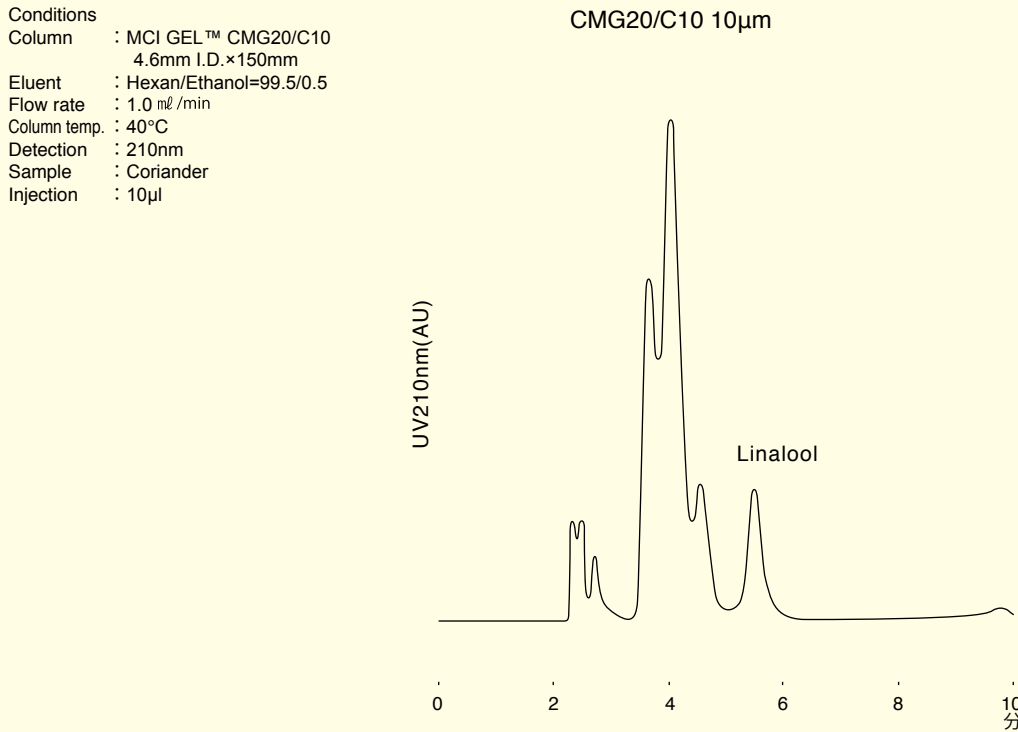


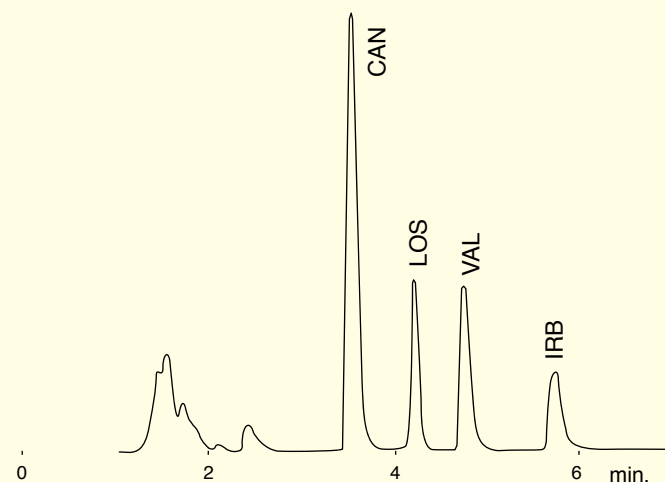
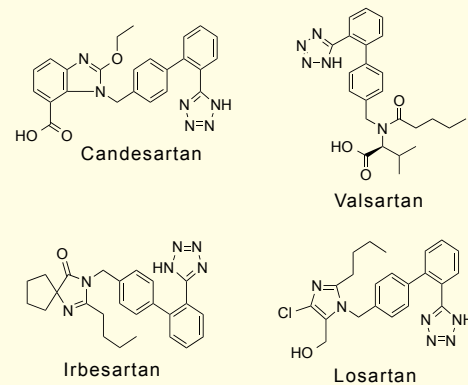
Fig. 5-27 Coriander



Application data of CHP series

Fig. 5-28 Application data of CHK40/C04: Separation of Sartans

Conditions
 Column : MCI GEL™ CHK40/C04
 4.6mm I.D.×150mm
 Eluent : A) 10 mM NaH₂PO₄ / 4 + 0.2 mM Na₂HPO₄ / 4 (25%ACN)
 B) 10 mM NaH₂PO₄ / 4 + 1.0 mM Na₂HPO₄ / 4 (40%ACN)
 Gradient : 0.5min 0%B 0.5-2.0min 50%B
 2.0min- 90%B
 Flow rate : 1.0 mL/min
 Column temp. : 50°C
 Detection : UV
 Sample : Candesartan(CAN), Losartan(LOS),
 Valsartan(VAL), Irbesartan(IRB)
 Injection : 20μL



(Data provided by Professor Yokoyama of Yokohama National University)

(Polyphenon 60)

Fig. 5-29 Modified Styrene Divinylbenzene CHP07/C04

Conditions
 Column : MCI GEL™ CHP07/C04
 4.6mm I.D.×150mm
 Eluent : CH₃OH/10mM-Acetic acid=60/40
 Flow rate : 0.46 mL/min
 Column temp. : 60°C
 Detection : 280nm
 Sample : Polyphenon 60(10mg/mL) each 10μL

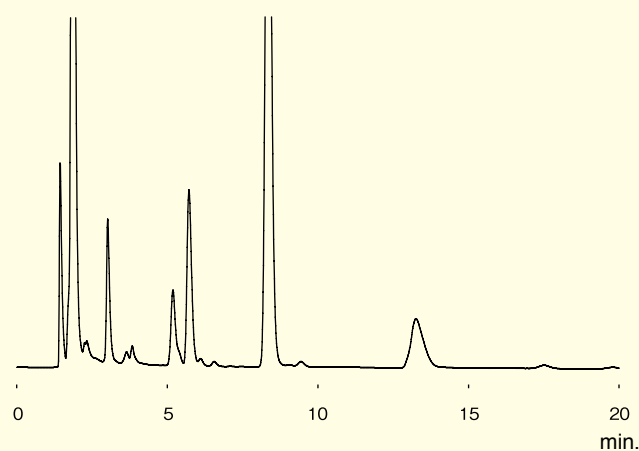
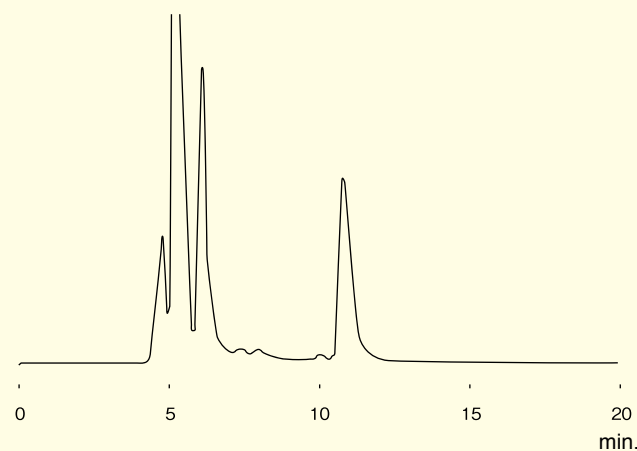


Fig. 5-30 Styrene Divinylbenzene CHP20/C04

Conditions
 Column : MCI GEL™ CHP20/C04
 4.6mm I.D.×150mm
 Eluent : CH₃OH/10mM-Acetic acid=60/40
 Flow rate : 0.46 mL/min
 Column temp. : 60°C
 Detection : 280nm
 Sample : Polyphenon 60(10mg/mL) each 10μL



Application data of CHP series

(TritonX-100)

Fig. 5-31 C18-alkylated aliphatics CHPOD/C04

Conditions
 Column : MCI GEL™ CHPOD/C04
 4.6mm I.D.×150mm
 Eluent : 50vol%CH₃CN
 Flow rate : 0.50 mL/min
 Column temp. : 40°C
 Detection : 254nm
 Sample : Triton X-100
 (Polyoxyethylene octyl phenyl ether)
 1% each 10μL

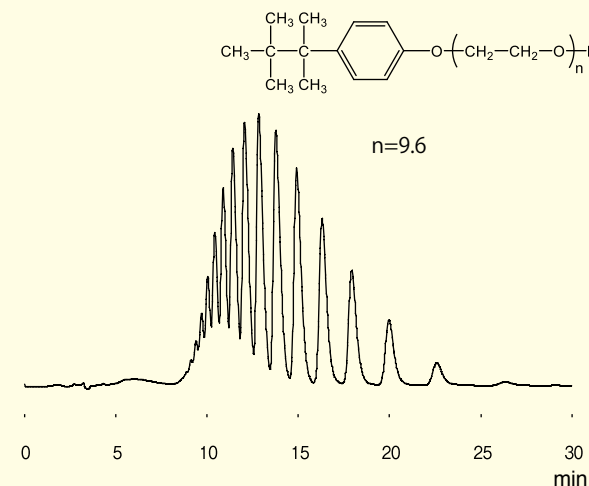


Fig. 5-32 ODS-1HU (ODS)

Conditions
 Column : MCI GEL™ ODS-1HU
 4.6mm I.D.×250mm
 Eluent : 50vol%CH₃CN
 Flow rate : 1.00 mL/min
 Column temp. : 40°C
 Detection : 254nm
 Sample : Triton X-100
 (Polyoxyethylene octyl phenyl ether)
 1% each 10μL

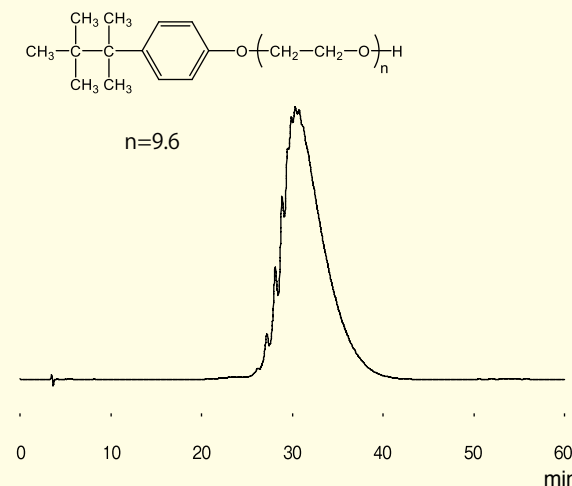
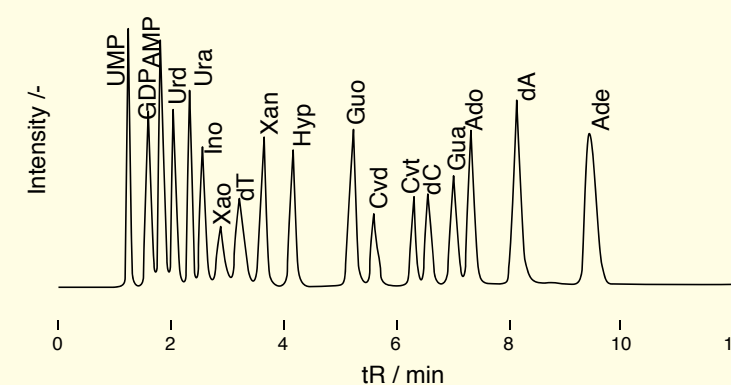
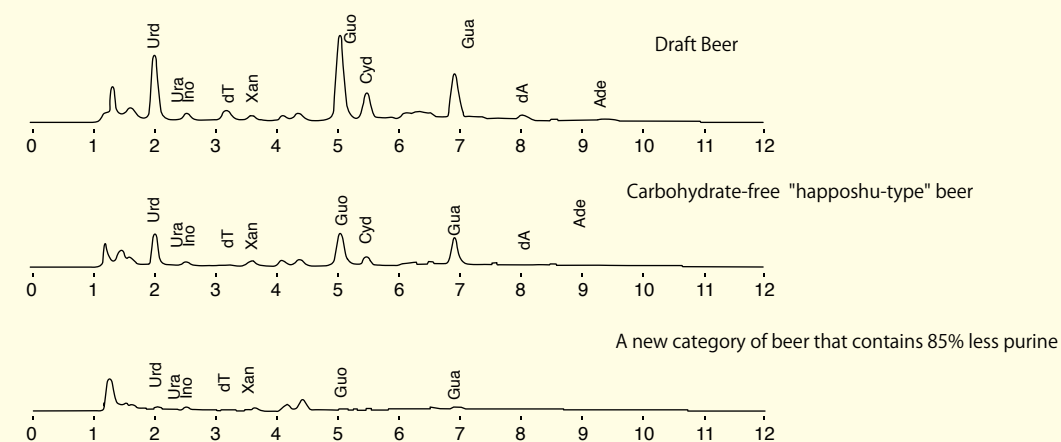


Fig. 5-33 Application data of Nucleic base/Nucleoside and Beer

Conditions
 Column : MCI GEL™ CHK45/C05
 4.6mm I.D.×150mm
 Eluent : A) 8 mM H₃PO₄
 B) 10 mM H₃PO₄ / 30% ACN
 Gradient : 0-0.7min 0%B 0.7-3.0min 0-40%B 3.0-3.2min 40%B
 3.2-3.5min 40-80%B 3.5-8.0min 80%B 8.0min- 0%B
 Flow rate : 1.3mL/min
 Column temp. : 45°C
 Detection : UV(260nm)
 Injection : 20μL



Analysis of various category beer



(Data provided by Professor Yokoyama of Yokohama National University)