5 MCI GEL™ 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.

CHP column

MCI GEL™ CHP series are sui
hydrophobicities; porous polys
polymethacrylate. This range o
based on the properties of the t

Polystyrene packing: M
Modified polystyrene pa
Polymethacrylate packin
Modified polymethacryl

The hydrophobicities of the colu

MCI GEL™ CHP07/C04, C10 >

Polymer columns for HPLC, wi
broad pH range, acidic through a

1) In reverse phase chromatograp
ionic properties of such compo
columns would be unsuitable.
2) Some extremely hydrophilic
CHP07/C04 or CHP07/C10 col
3) Polymer columns can be wash

Polymethacrylate columns, CMC
chromatography.

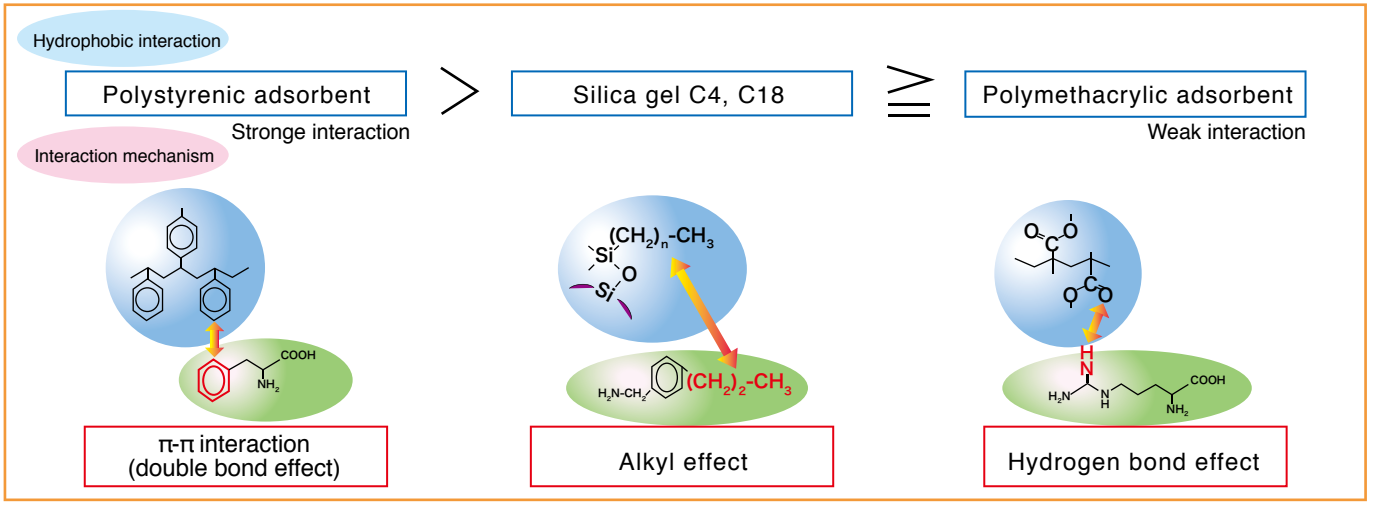
Modified polystyrene packing,
interactions occur between the pa
are difficult to separate using exi
phase mode and shows a unique s

All polymeric columns exhibit s
silanol groups even when end-cap

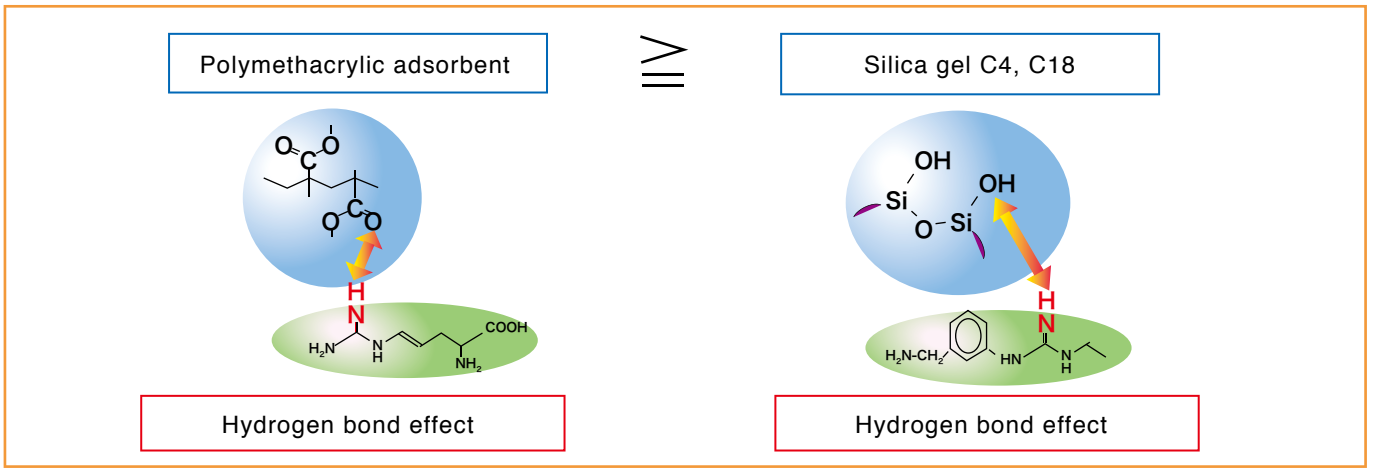
●CHP column series

Matrix Type	Functi
Styrene Divinylbenzene	N

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

Conditions
Column : MCI GEL™ CHP20/C04 4.6mmI.D × 150mm

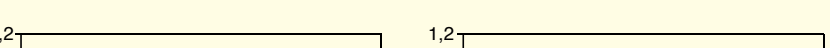


Fig. 5-2 Separation of catecl

Conditions
Column : MCI GEL™ CHP20/C04 4.6mm I.D.×150mm
Eluent : 50mM Na-phosphate pH2.
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

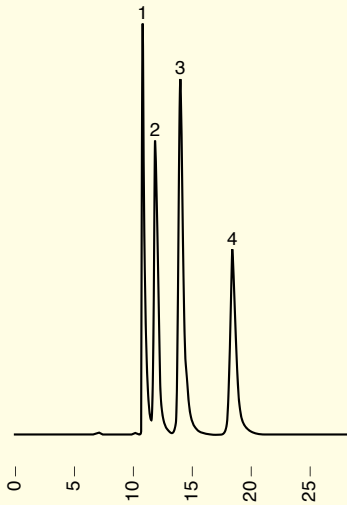
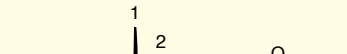


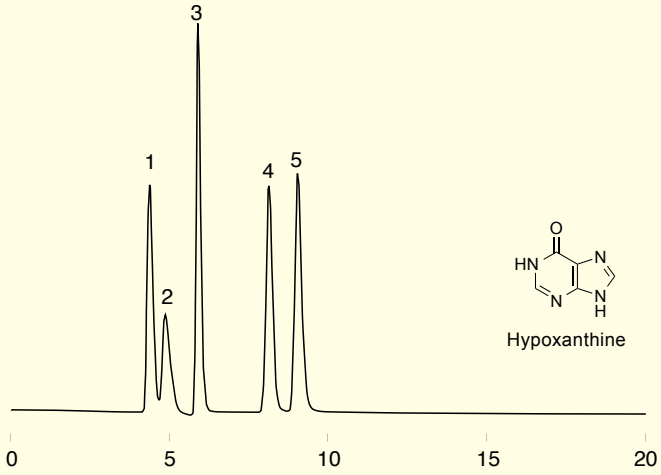
Fig. 5-4 Purine alkaloids

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

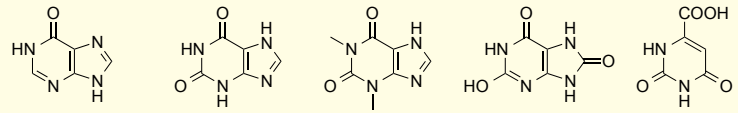


Application data of CHP series

Fig. 5-6 Uric acid and related compounds

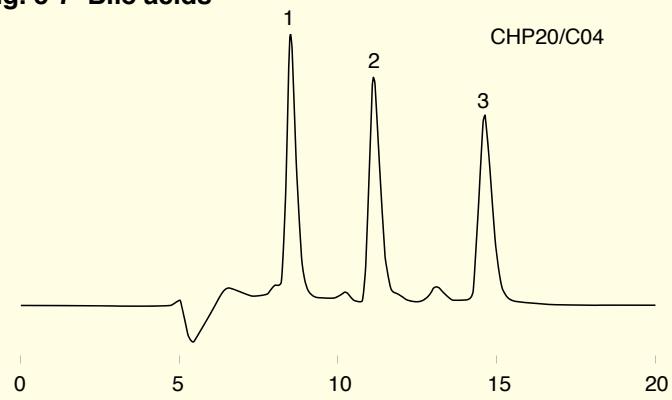


Conditions
Column : MCI GEL™ CHP20/C04
4.6mm I.D.×150mm
Eluent : 10mM TBA/CH3CN=75/25
Flow rate : 0.4 mℓ/min
Column temp. : 25°C
Detection : 284nm
Sample : 1.Hypoxanthine
2.Xanthine
3.Theophylline
4.Uric acid
5.Orotic acid



Comparison with an ODS column

Fig. 5-7 Bile acids



Conditions
Column : MCI GEL™ CHP20/C04
4.6mm I.D.×150mm
Eluent : 50mM NaH_2PO_4 pH2.0
/CH_3CN=40/60
Flow rate : 0.3 mℓ/min
Column temp. : 25°C
Detection : 210nm
Sample : 1.Cholic acid
2.Ursodeoxycholic acid
3.Deoxycholic acid

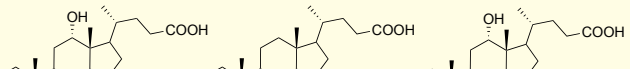


Fig. 5-8 Glycyrrhizae ra

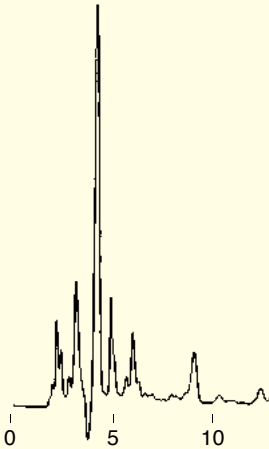
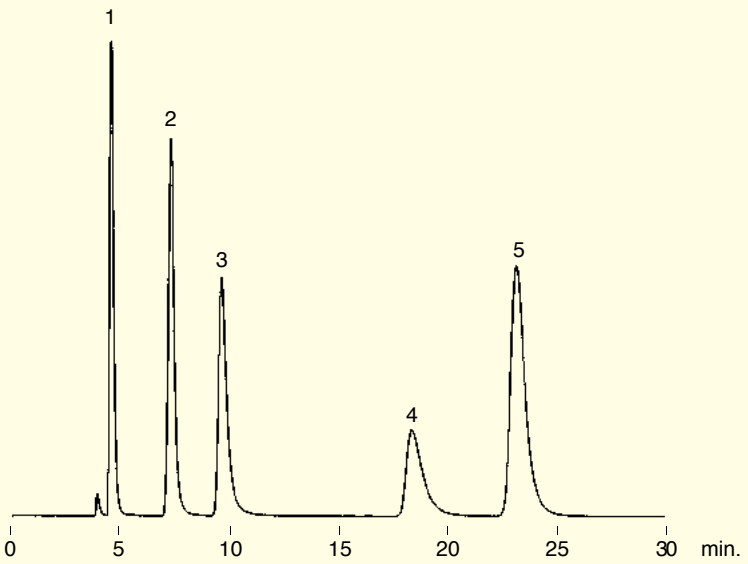


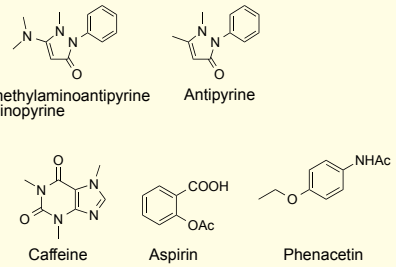
Fig. 5-9 Adrenal cortex

Application data of CHP series

Fig. 5-10 Ingredients of medicine

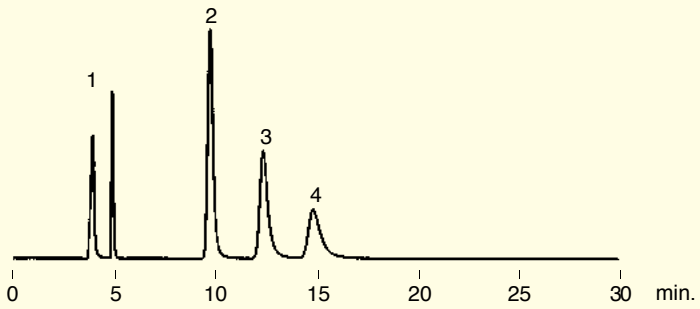


Conditions
Column : MCI GEL™ CMG20/C04
4.6mm I.D.×150mm
Eluent : 50mM phosphoric acid(pH2.0)/CH_3OH
=60/40
Flow rate : 0.5 mℓ/min
Column temp. : 45°C
Detection : 254nm
Sample : 1.4-Dimethylaminoantipyrine
2.Antipyrine
3.Caffeine
4.Aspirin
5.Phenacetin



Comparison with an ODS column

Fig. 5-11 Sulfa drugs



Conditions
Column : MCI GEL™ CMG20/C04
4.6mm I.D.×150mm
Eluent : 20mM phosphate pH6.8/CH_3CN
=82/18
Flow rate : 0.5 mℓ/min
Column temp. : 45°C
Detection : 254nm
Sample : 1.Succinylsulfathiazole
2.Sulfanilamide
3.Sulfathiazole
4.Sulfamerazine

Fig. 5-12 Peptides

Conditions
Column : MCI GEL™ CMG20/C04
4.6mm I.D.×150mm
Eluent : 0.1%TFA/CH_3CN
=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

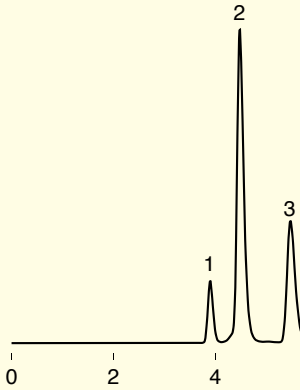


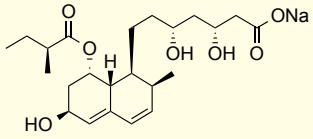
Fig. 5-14 Procainamide,

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

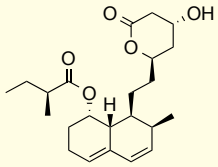
Application data of CHP series

Fig. 5-16 Pravastatin sodium

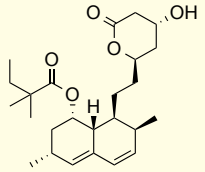
Conditions
Column : MCI GEL™ CHP20/C10 (10μm 250 ×4.6mm I.D.) and ODS (10μm 250 ×4.6mm I.D.)
Eluent : A :0.1% Formic acid; B :0.1% Formic acid in AcCN;
Gradient : 45%B-95%B over 29min.
Flow rate : 1.00 mℓ/min
Column temp. : 25℃
Detection : UV238nm
Sample : Pravastatin sodium, Mevastatin and Simvastatin,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



Pravastatin sodium



(+)-Compactin; Mevastatin



(+)-Simvastatin, Simvastatin

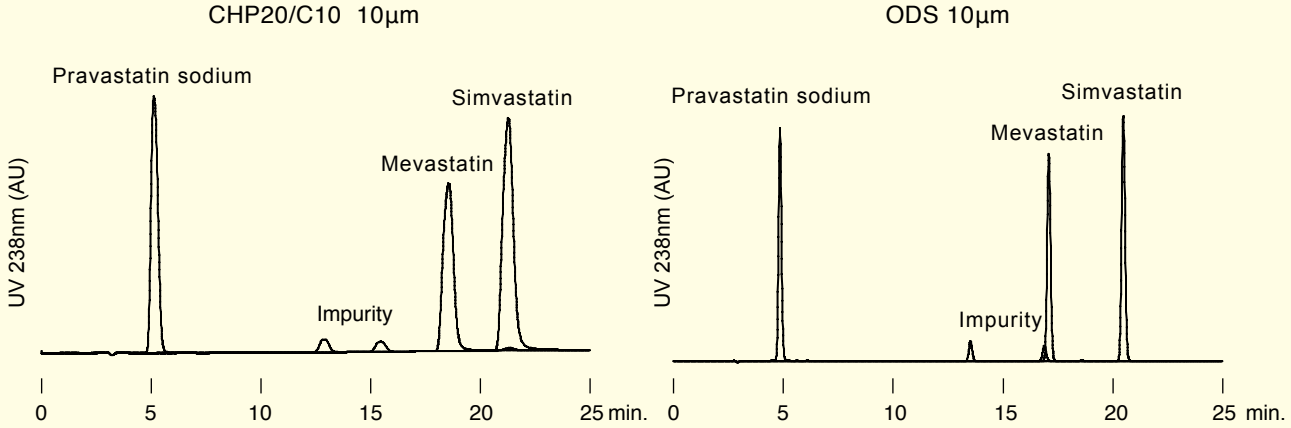
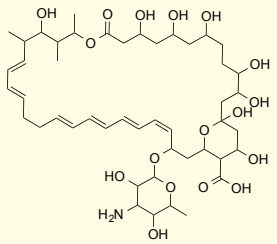
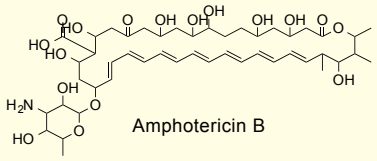


Fig. 5-17 Polyene antibiotics

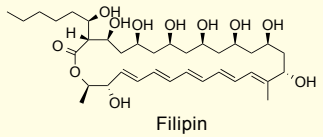
Conditions
Column : MCI GEL™ CHP20/C10 (10μm 250 ×4.6mm I.D.) and ODS (10μm 250 ×4.6mm I.D.)
Eluent : A :0.1% Formic acid; B :0.1% Formic acid in AcCN; A/B=60/40;
Flow rate : 1.00mℓ/min
Column temp. : 25℃
Detection : UV305nm for Nystatin, VIS405nm for Amphotericin B and UV340nm for Filipin;
Sample :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



Nystatin



Amphotericin B



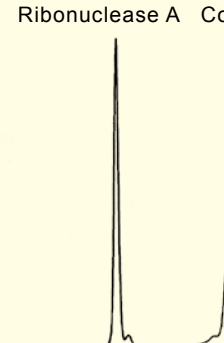
Filipin

A

Fig. 5-18 Proteins

Conditions
Column : MCI GEL™ CMG20/
4.6mm I.D.×250mm
Eluent : A 0.05% TFA/CH3CI
B 0.05% TFA/CH3CI
A → B 45min linear g
Flow rate : 0.5 mℓ/min
Column temp. : 25°C
Detection : 280nm
Sample : 1. Ribonuclease A
2. Cytochrome C
3. Transferrin
4. α-Chymotrypshino
5. β-Lactoglobulin

Fig. 5-19 Proteins

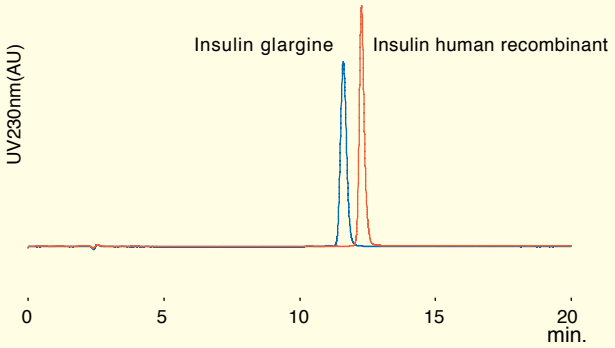


Application data of CHP series

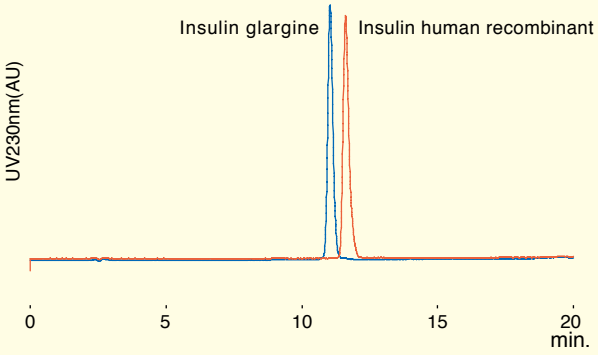
Fig. 5-20 Insulin

Conditions
Column : MCI GEL™ CHP20/C10
MCI GEL™ CMG20/C10
ODS 10μm
4.6mm I.D.×150mm
Eluent : A) 0.1%TFA, H_2O
B) 0.1%TFA, CH_3OH
Gradient : 20%B→60%B over 20min.
Flow rate : 1.0㎖/min
Column temp. : 40°C
Detection : 280nm
Sample : Insulin Glargine and human recombinant , 1mg/ml each
Injection : 10μl

CHP20/C10 10μm



CMG20/C10 10μm



ODS 10μm

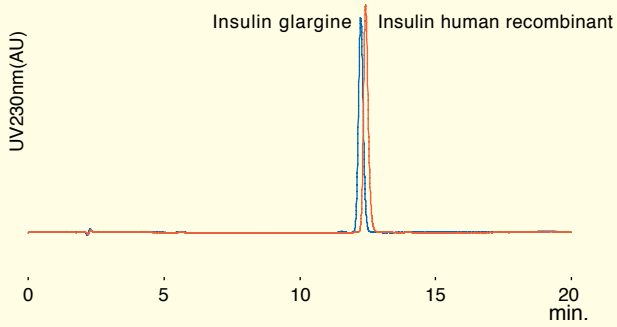


Fig. 5-21 Ghrelin

Conditions
Column : MCI GEL™ CMG20/C10
ODS 10μm
4.6mm I.D.×150mm
Eluent : A) 0.1%TFA, H_2O
B) 0.1%TFA, AcCN
Gradient : 10%B→60%B over 25min.
Flow rate : 1.0 ㎖/min
Column temp. : 40°C
Detection : 230nm
Sample : Ghrelin rat and Ghrelin human ,0.1mmol/l each
Injection : 10μl

CMG20/C10 10μm

ODS 10μm

Fig. 5-22 Leuprorelin

Conditions
Column : MCI GEL™ CHP20/C10
MCI GEL™ CMG20/C10
ODS 10μm
4.6mm I.D.×150mm
Eluent : A) 0.1%TFA, H_2O
B) 0.1%TFA, AcCN
Gradient : 20%B→60%B over 20min.
Flow rate : 1.0 ㎖/min
Column temp. : 40°C
Detection : 280nm
Sample : Leuprorelin,LHRH human, LHRH salr
Injection : 10μl

CMG20/C10 10

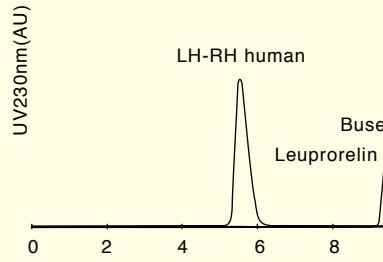


Fig. 5-23 Sifuvirtide

Application data of CHP series

Fig. 5-24 ssRNA Ladder Marker

Conditions
Column : MCI GEL™ CMG20/C10
ODS 10μm
4.6mm I.D.×150mm
Eluent : A)100mM TEAA, H_2O
B)100mM TEAA, CH_3CN
Gradient : CHP10/C10
ODS 10μm 10%B→40%B over 30min
Flow rate : 1.0 mℓ/min 8%B→40%B over 30min
Column temp. : 40°C
Detection : 260nm
Sample : 14-30 ssRNA Ladder Marker [max.0.04mg/ml]
Injection : 5μl

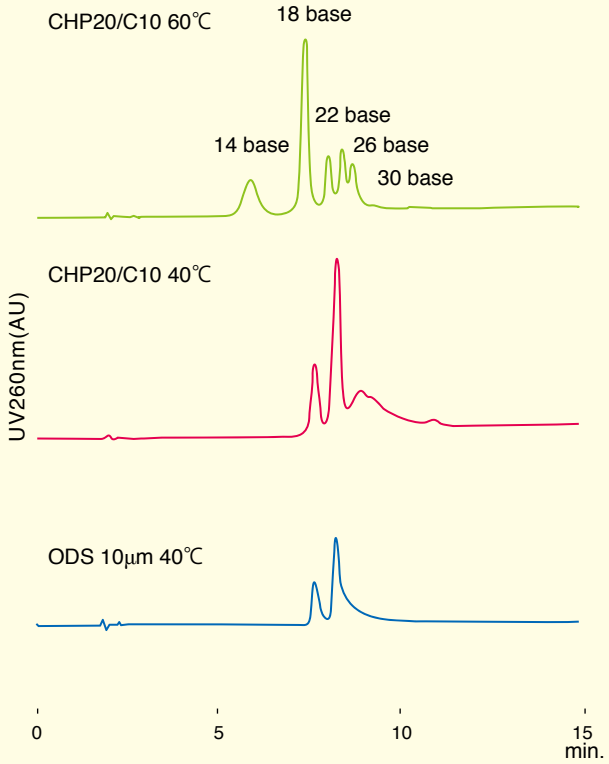


Fig. 5-25 Nucleotide

Conditions
Column : MCI GEL™ CHK40/C04
4.6mm I.D.×150mm
Eluent : A)19 mM H_3PO_4 / 1 mM NaH_2PO_4 / 5.0% ACN
B)20 mM Na_2HPO_4 / 100 mM NaClO4 / 30% ACN
Gradient : 0-4.0min 0%B 4.0-5.0min 0→30%B 5.0min-6.0min 30%B 6.0min-7.0min 30→50%B
7.0min-10.0min 50→65%B 10.0min-11.0min 65%B 11.0min- 0%B
Flow rate : 0.8 mℓ/min
Column temp. : 50℃
Detection : UV260nm
Sample : 1.Ura, 2.Xan, 3.Thy, 4.Hyp, 5.Gua, 6.Cyt, 7.Ade, 8.Urd, 9.Xao, 10.dT, 11.Ino, 12.Guo, 13.Cyd, 14.Ado
Injection : 20μl

Fig. 5-26 Linalool

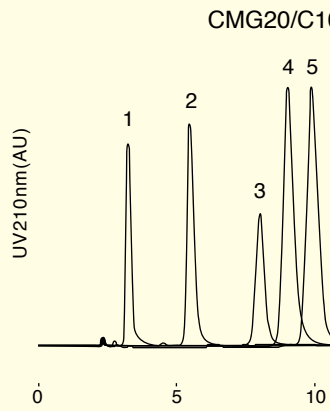


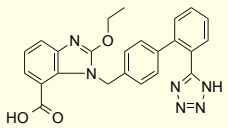
Fig. 5-27 Coriander

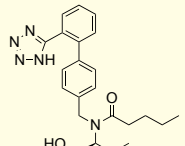
Conditions
Column : MCI GEL™ CMG20
4.6mm I.D.×150mm
Eluent : Hexan/Ethanol=99.
Flow rate : 1.0 mℓ/min
Column temp. : 40°C
Detection : 210nm
Sample : Coriander
Injection : 10μl

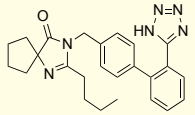
Application data of CHP series

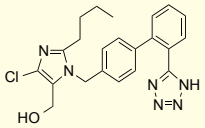
Fig. 5-28 Application data of CHK40/C04：Sepatation of Sartans

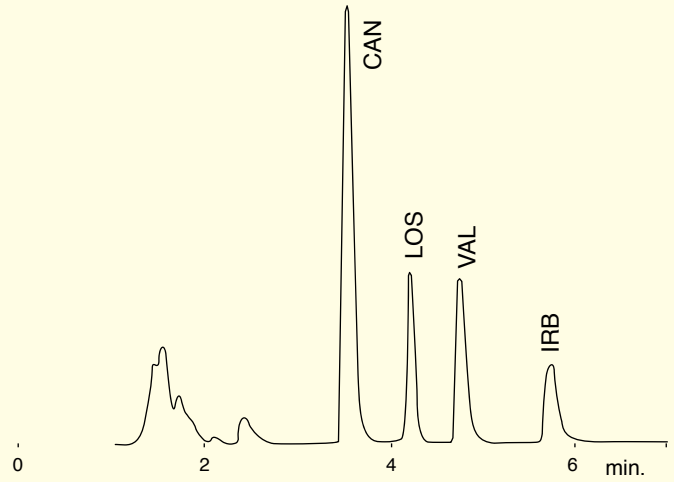
Conditions
Column ：MCI GEL™ CHK40/C04
4.6mm I.D.×150mm
Eluent ：A) 10 mM Na H↓2 PO↓4 +0.2 mM Na↓2 HPO↓4 (25%ACN)
B) 10 mM Na H↓2 PO↓4 +1.0 mM Na↓2 HPO↓4 (40%ACN)
Gradient ：0.5min 0%B 0.5-2.0min 50%B
2.0min- 90%B
Flow rate ：1.0 mℓ /min
Column temp. ：50°C
Detection ：UV
Sample ：Candesartan(CAN),Losartan(LOS),
Valsartan(VAL), Irbesartan(IRB)
Injection ：20µl


Candesartan


Valsartan


Irbesartan


Losartan



(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

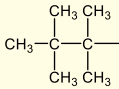
Fig. 5-30 Styrene Divinylbenzene CHP20/C04

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

(TritonX-100)

Fig. 5-31 C18-alkylated alip

Conditions
Column ：MCI GEL™ CHPOD
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 oct
1% each 10µL



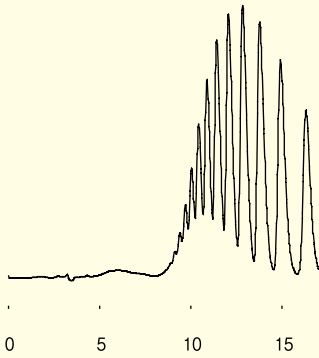


Fig. 5-33 Application data c

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
3.2-3.5min 40→80%B 3.5-8.0min 80%
Flow rate ：1.3mℓ /min
Column temp ：45℃
Detection ：UV(260nm)
Injection ：20µl