

# Cost-Effective Chiral Separation by Preparative HPLC

Akio Ichikawa\*, Katsunori Taniguchi,  
and Naohiro Kuriyama

**YMC CO., LTD.**

*Kyoto, Japan*





# Introduction

Access to optically pure compounds is getting more and more important in the pharmaceutical and other fields. Optically pure compounds can be produced by various methods including asymmetric syntheses, differential recrystallization of diastereomeric conjugates, enzymatic reactions and HPLC separation. In particular, chiral separation by HPLC has rapidly progressed in recent years and is getting highlighted also from the view point of industrial-scale manufacturing.

The greatest advantage of chiral separation by HPLC is possible simultaneous separation of two enantiomers (R-isomer and S-isomer) at high purity and high recovery rate. Various optically active packing materials for HPLC have been developed and marketed so far. Such packing materials are, however, so expensive that the scope of practical application has been limited. Practical applications to industrial-scale manufacturing are still not common.

The YMC has been engaged in this issue in the course of HPLC-related business. We have been trying to modify the manufacturing processes for the chiral packing materials to reduce the cost so that the chiral separation by HPLC can be applied to industrial-scale production. As a result, we succeeded in developing new packing materials, which can be supplied at remarkably lower prices than the conventional ones.

In this presentation, we are going to refer to the characteristics of the new packing materials and the cost-effective chiral separation by HPLC using those.





## Points to Consider for Cost Effective Preparative HPLC

Needless to say, the purity and recovery of product purified by HPLC greatly depend upon the preparative conditions, which are therefore suggested to be greatly influential on the purification cost. The following points are considered to be essential for reduction of the purification cost.

1. Cost of packing materials  
〔 The major factor for the high cost 〕
2. Optimal particle size and column size.  
〔 To be selected in the light of operating column pressure, loadable amount, resolution performance, etc. 〕
3. Scale-suiting separation system.  
〔 Single column : Batch production  
SMB : Continuous production 〕





## YMC CHIRAL PREP CD Packing Materials

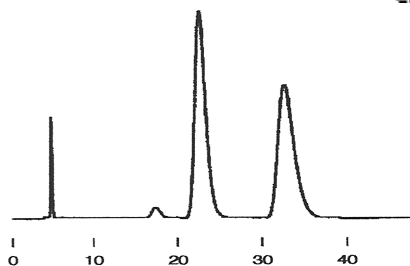
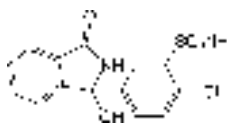
	YMC CHIRAL PREP CD ST	YMC CHIRAL PREP CD PM
Silica	ultra pure	ultra pure
Particle Size	10 $\mu$ m, 20 $\mu$ m, 50 $\mu$ m	10 $\mu$ m, 20 $\mu$ m, 50 $\mu$ m
Pore Size	12nm (120 Å)	12nm (120 Å)
Bonded Phase	$\beta$ -Cyclodextrin without modification of hydroxyl groups	$\beta$ -Cyclodextrin phenyl-modified
Type	Chemically bonded silica gel	Chemically bonded silica gel





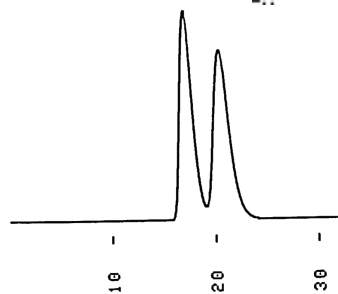
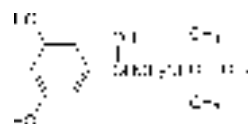
# YMC CHIRAL PREP CD ST Reversed Phase Applications

## Chlorthalidone



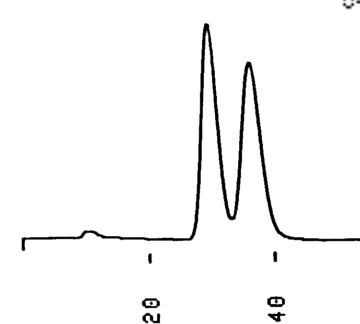
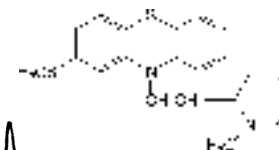
Column : 250 X 4.6 mmI.D. (10 $\mu$ m)  
Eluent : 20mM NH<sub>4</sub>Ac+0.5%AcOH(pH4.1)  
/ MeOH (90/10)  
Flow rate : 1.0 mL/min  
Temperature : ambient  
Detection : UV at 254 nm  
Injection : 5 $\mu$ m (1mg/mL)

## Terbutaline



Column : 250 X 4.6 mmI.D. (10 $\mu$ m)  
Eluent : 20mM KH<sub>2</sub>PO<sub>4</sub> (pH4.6)  
/ CH<sub>3</sub>CN (99/1)  
Flow rate : 1.0 mL/min  
Temperature : ambient  
Detection : UV at 220 nm  
Injection : 5 $\mu$ m (1mg/mL)

## Thioridazine



Column : 250 X 4.6 mmI.D. (10 $\mu$ m)  
Eluent : 20mM KH<sub>2</sub>PO<sub>4</sub> (pH4.6)  
/ CH<sub>3</sub>CN (75/25)  
Flow rate : 1.4 mL/min  
Temperature : ambient  
Detection : UV at 254 nm  
Injection : 5 $\mu$ m (1mg/mL)

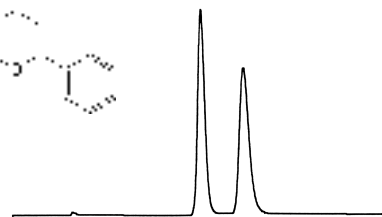
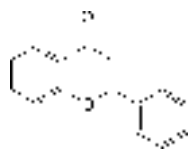




# YMC CHIRAL PREP CD PM

## Normal Phase Applications

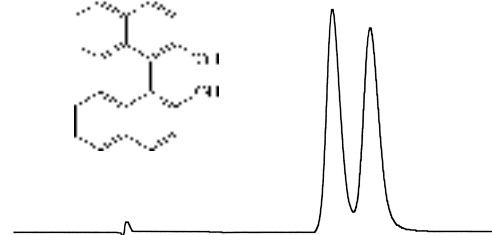
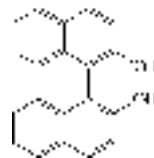
### Flavanone



0 10 20 30

Column : 250 X 4.6 mmI.D. (10 $\mu$ m)  
Eluent : Hexane / EtOH (95/5)  
Flow rate : 0.5 mL/min  
Temperature : ambient  
Detection : UV at 254 nm  
Injection : 5 $\mu$ m (1mg/mL)

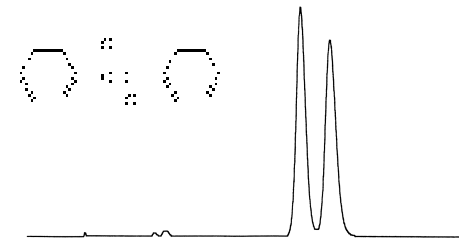
### 1,1'-Bi-2-Naphthol



0 5 10 15 20

Column : 250 X 4.6 mmI.D. (10 $\mu$ m)  
Eluent : Hexane / CH<sub>2</sub>Cl<sub>2</sub> / EtOH (70/30/2)  
Flow rate : 0.5 mL/min  
Temperature : ambient  
Detection : UV at 254 nm  
Injection : 5 $\mu$ m (1mg/mL)

### Benzoin



0 10 20 30 40

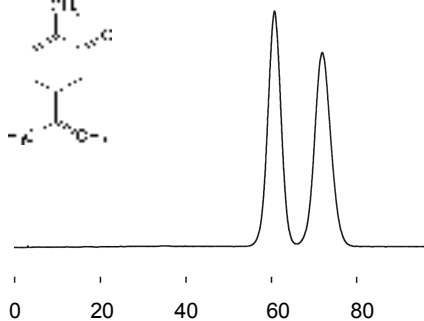
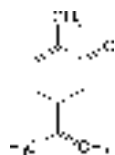
Column : 250 X 4.6 mmI.D. (10 $\mu$ m)  
Eluent : Hexane / MeOH/ IPA (93/5/2)  
Flow rate : 0.5 mL/min  
Temperature : ambient  
Detection : UV at 254 nm  
Injection : 5 $\mu$ m (1mg/mL)



# YMC CHIRAL PREP CD PM

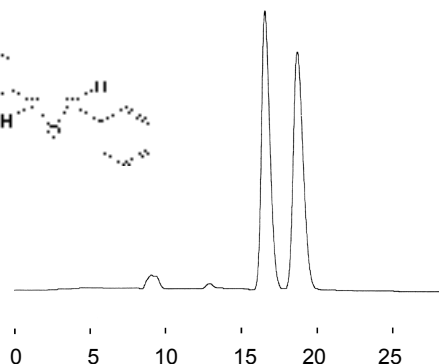
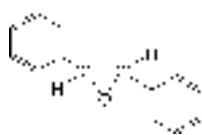
## Reversed Phase Applications

### Carvone



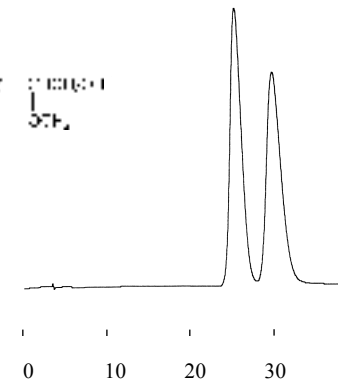
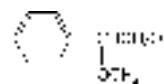
Column : 250 X 4.6 mmI.D. (10 $\mu$ m)  
Eluent : MeOH / H<sub>2</sub>O (40/60)  
Flow rate : 1.0 mL/min  
Temperature : ambient  
Detection : UV at 254 nm  
Injection : 5 $\mu$ m (1mg/mL)

### trans-Stilbene Oxide



Column : 250 X 4.6 mmI.D. (10 $\mu$ m)  
Eluent : MeOH / H<sub>2</sub>O (80/20)  
Flow rate : 0.5 mL/min  
Temperature : ambient  
Detection : UV at 220 nm  
Injection : 5 $\mu$ m (1mg/mL)

### 2-Methoxy-2-Phenylethanol



Column : 250 X 4.6 mmI.D. (10 $\mu$ m)  
Eluent : EtOH / H<sub>2</sub>O (20/80)  
Flow rate : 1.0 mL/min  
Temperature : ambient  
Detection : UV at 220 nm  
Injection : 5 $\mu$ m (1mg/mL)



# Listing of Applications

Compound	YMC CHIRAL PREP CD ST	YMC CHIRAL PREP CD PM	Competitor A	Competitor B	Competitor C	Competitor D	Competitor E	Competitor F
Chlormezanone	○	○				○	○	
Chlorpheniramine	○			○	○			
Chlorthalidone	○				○	○		
Hexobarbital	○				○	○		
Homatropine	○		○		○			○
Terbutaline	○							
Thioridazine	○							
Tolperisone	○	○		○				
Trimipramine	○							
Benzoin	○	○	○	○	○			
1,1'-Bi-2-Naphthol		○		○		○		
4-Chloroamphetamine		○						
Carvone		○						
Flavanone		○	○				○	
Isoproterenol		○						
Metoprolol		○	○		○			○
2-Methoxy-2-phenylethanol		○						
1-(1-Naphthyl)ethylamine		○					○	
Norphenylephrine		○						○
Propranolol		○	○		○			
t-Stilbene oxide		○	○	○				

○ resolved

**Most of the compounds separable with the competitor's packing materials can be separated with YMC CHIRAL PREP CD ST/PM, indicating the applicability of the latter to a wide variety of chiral substances.**





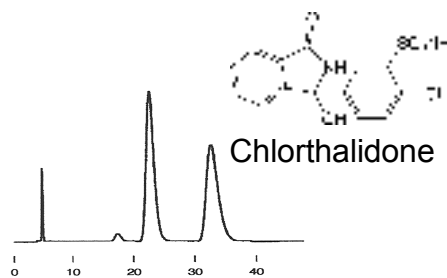


# From Analytical to Preparative

Column: **YMC CHIRAL PREP CD ST**  
(10 $\mu$ m)

• Large-scale separation immediately feasible, if separable with an analytical column. **Excellent scalability.**

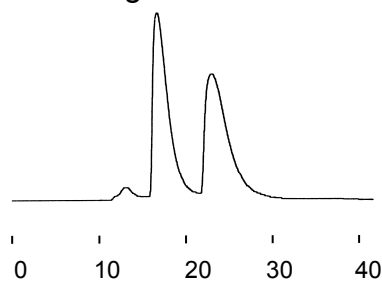
Analytical



Chlorthalidone

Loading Study

Load: 5mg

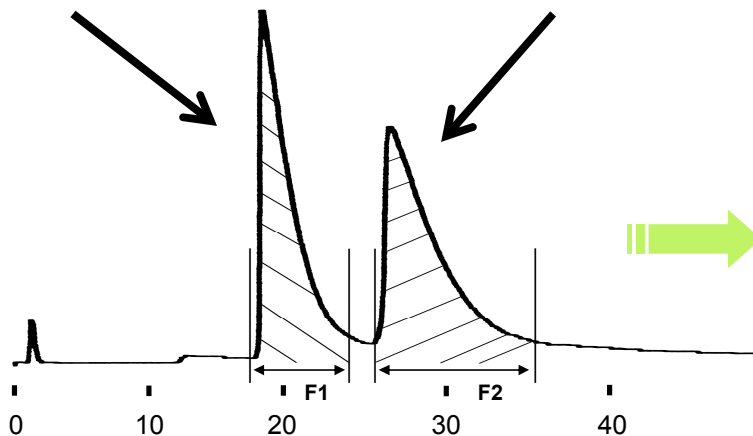


Column size : 250 x 4.6 mm I.D.

Semi-preparative  
Load: 100 mg

Optical Purity: >99 %  
Recovery: 94.8 %  
Collection: 47.4 mg

Optical Purity: >99 %  
Recovery: 97.9 %  
Collection: 49.0 mg



Column size : 250 x 20 mm I.D.

Tentative Preparative  
Load: >360 g

$$\left[ 100 \text{ mg} \times \left( \frac{600 \text{ mm I.D.}}{20 \text{ mm I.D.}} \right)^2 \times \left( \frac{1000 \text{ mm}}{250 \text{ mm}} \right) \right]$$

F1  
Collection 171 g / cyc.

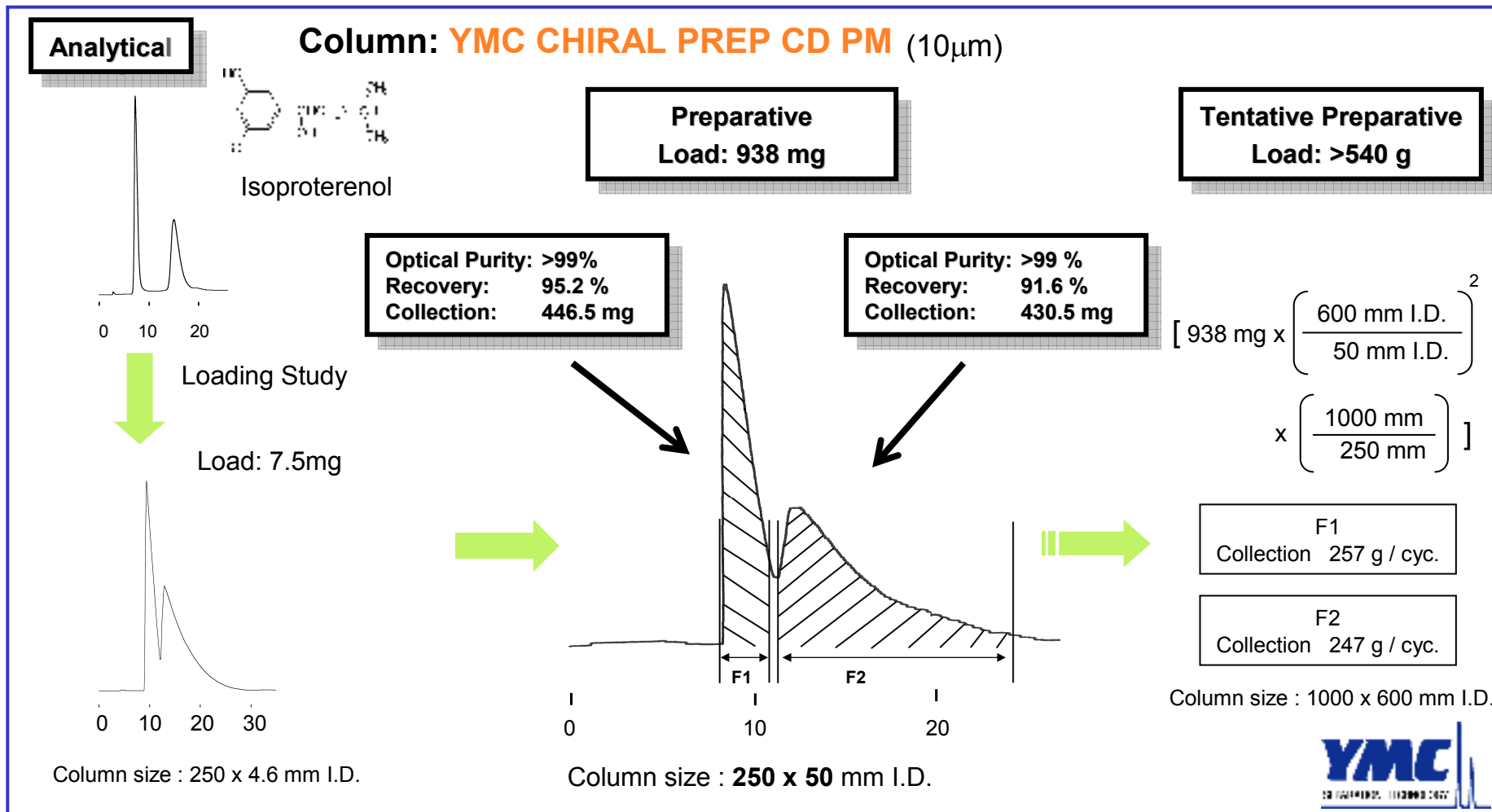
F2  
Collection 177 g / cyc.

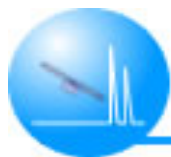
Column size : 1000 x 600 mm I.D.



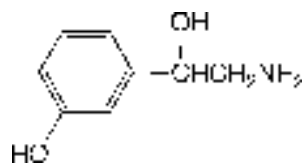


# From Analytical to Preparative

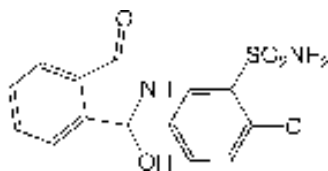




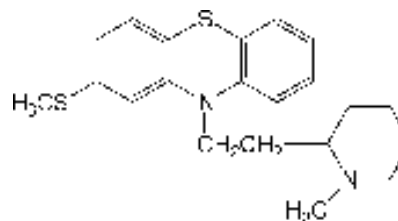
## Purification of Optical Isomer by Using YMC CHIRAL PREP CD ST/PM



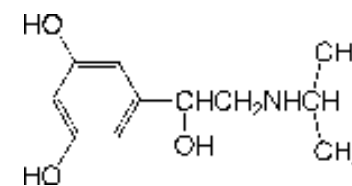
1. Norphenylephrine



2. Chlorthalidone



3. Thioridazine



4. Isoproterenol

Compound	Column*	Column Size	Loading	Optical purity (F1)	Collection (F1)	Recovery (F1)
1	PM	<b>250 x 50</b> mml.D.	625mg	>99%	261.9mg	83.8%
2	ST	250 x 20 mml.D.	100mg	>99%	47.4mg	94.8%
3	ST	250 x 20 mml.D.	100mg	>99%	38.5mg	77.0%
4	PM	250 x 20 mml.D.	200mg	>99%	96.5mg	96.5%
4	PM	<b>250 x 50</b> mml.D.	938mg	>99%	446.5mg	95.2%

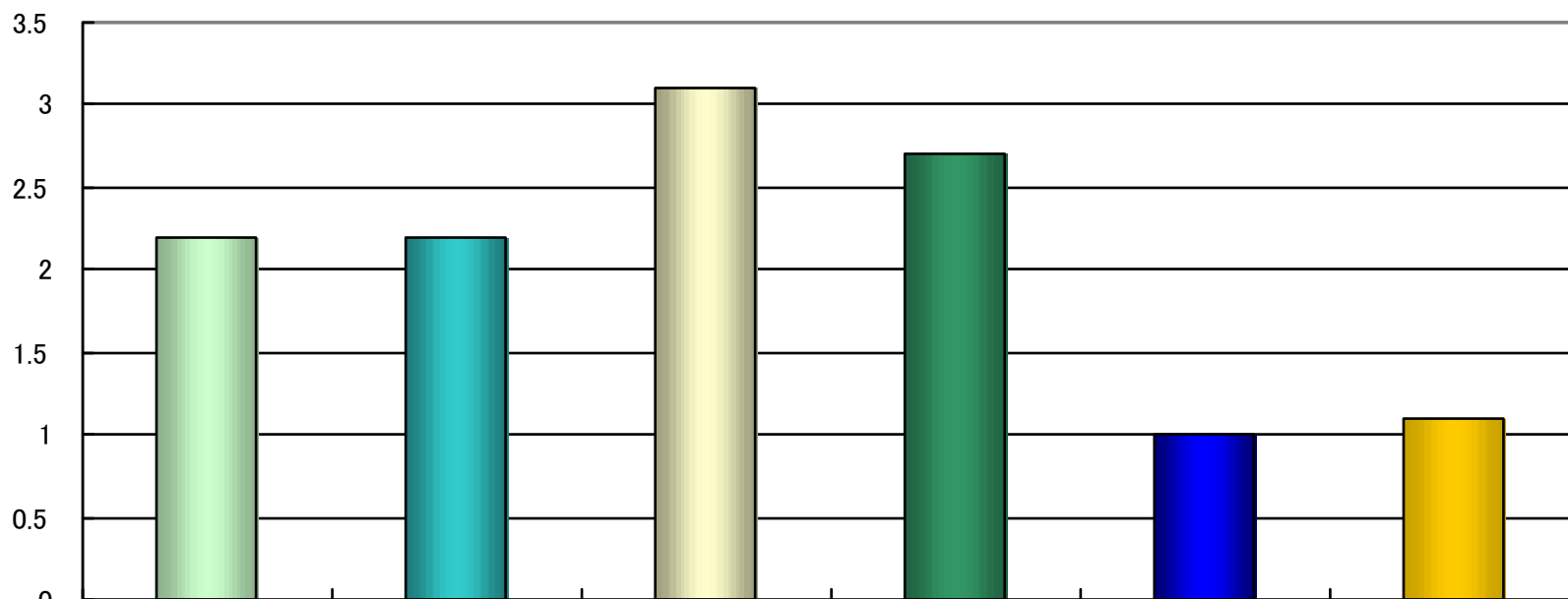
\* (10 $\mu$ m, 12nm)





## Market Prices of Various Chiral Packing Materials

Market Price  
(Index)



Competitor A

Competitor B

Competitor C

Competitor D

YMC CHIRAL  
PREP CD ST

YMC CHIRAL  
PREP CD PM

(In-house data)

**YMC CHIRAL PREP CD ST/PM have achieved remarkably lower prices compared with the competitor's products.**





## Guideline for Choosing Optimal Particle Size and Column Size

High ← Resolution performance → Low  
← Column pressure →

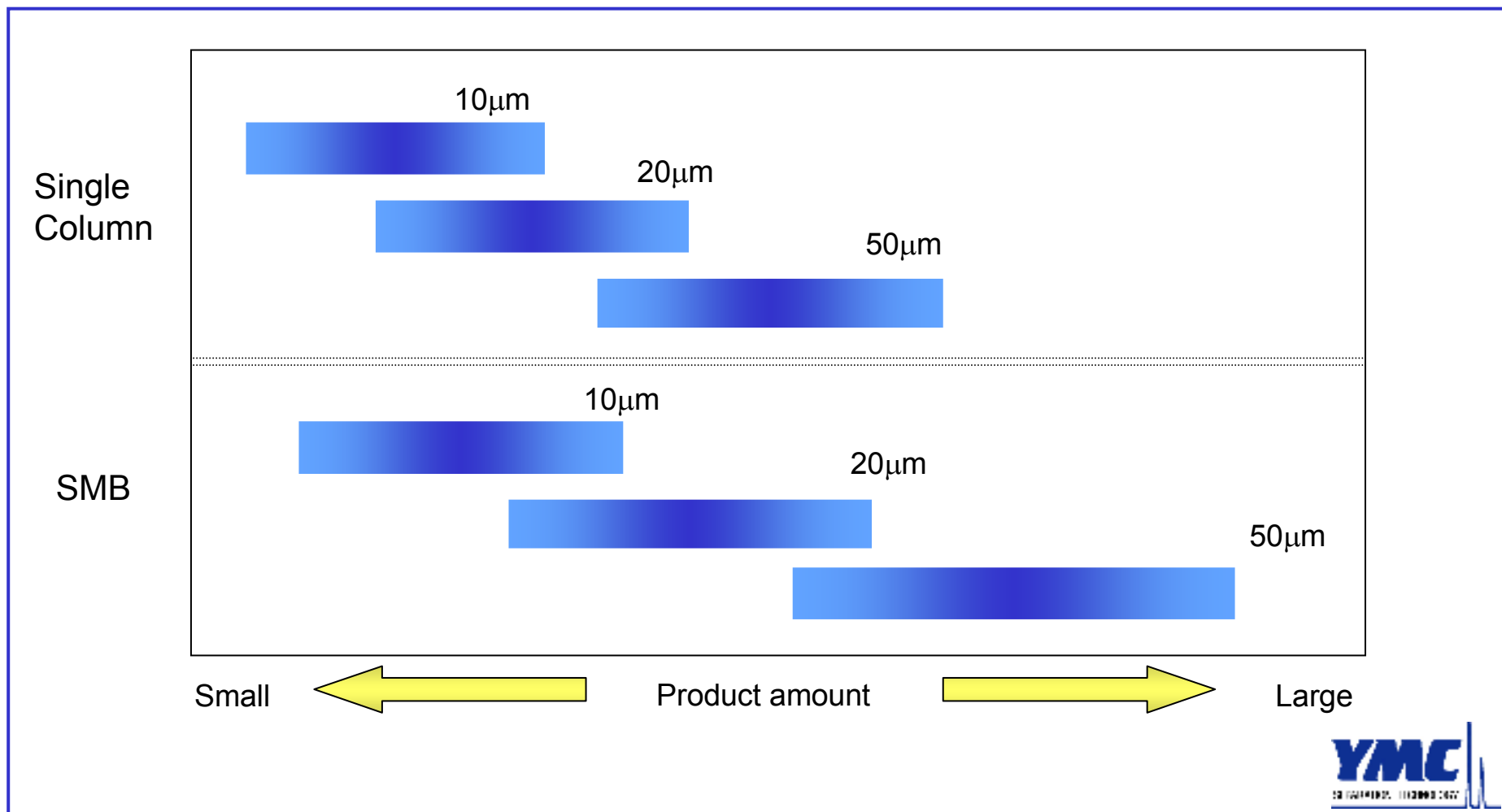
● Optimal

Internal diameter (mm)	Particle size (μm)			Loading amount	Purification capacity /day
	10	20	50		
20	●	○	○	0.1g	1g
50	●	○	○	1g	10g
100	○	●	○	10g	100g
200	○	●	●	10g	100g
400	○	○	●	100g	1000g





## Guideline for Choosing Optimal Particle Size by Scale and by System





## Preparative HPLC Systems : Single Column vs. SMB

Key point for cost-effective HPLC Separation = Selection of scale-suiting system

Single Column : Easy to set operating conditions, easy to scale up, easy to switch to other product.

But, requires a large amount of solvent per amount of product.

(DAC Column) : Possible self-packing, easy handling

➡ Suitable to short-term production, small-scale production, batch production.



SMB : High productivity per amount of packing material, requires a smaller amount of solvent per amount of product.

➡ Suitable to continuous production, large-scale production

**You can achieve cost-effective HPLC production with less expensive YMC CHIRAL PREP CD ST/PM in either case.**





# Single Column-based Separation of Optical Isomer : Comparison of Purification Cost with the Case Using Competitor's Packing Material

Sample : Propranolol

**YMC CHIRAL PREP CD PM**

Competitor A

Column	250 x 50 mm I.D.	250 x 50 mm I.D.
Particle size	10 $\mu$ m	10 $\mu$ m
Purification capacity	3.0g/day	3.6g/day
Target amount	20g	20g
Necessary number of days	7days	6days
Purity	>99.0%	>99.0%
Recovery	95.0%	89.6%
<b>Purification cost ratio</b>	<b>0.66</b>	<b>1.0</b>

With YMC CHIRAL PREP CD, you can reduce the purification cost, compared with conventional packing materials.







## Example Chiral Separation by SMB

Sample : Synephrine

**YMC CHIRAL PREP CD PM**

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Column number	250 x 30 mm I.D. x 8 *
Particle size	10 $\mu$ m
Purification Capacity	30.6g/day *
Total amount of packing material	1413cm <sup>3</sup>
Purity	95%
Recovery	80%

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\* Experimentally applied to SMB system for simulation.





## Conclusions

- Newly marketed two kinds of cyclodextrin-based chiral packing materials
- Widely applicable chiral packing materials usable under both normal and reversed phase conditions.
- Remarkably low prices and excellent separation performance
- Contributable to cost reduction in any type of preparative HPLC system
- Lined-up particle sizes (10 $\mu$ m,20 $\mu$ m,50 $\mu$ m) enabling application to the scale-suited separation system

