

手性分析条件的建立和优化

手性分析中的注意事项

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Daicel手性色谱柱一览表

正相

●直链淀粉衍生物

CHIRALPAK® **AD / AD-H**

CHIRALPAK® **AS / AS-H**

●纤维素衍生物

CHIRALCEL® **OD / OD-H**

CHIRALCEL® **OJ / OJ -H**

CHIRALCEL® OA, OB, OC

CHIRALCEL® OF, OG, OK

CHIRALCEL® CA-1

反相

CHIRALPAK® AD -RH

CHIRALPAK® AS -RH

CHIRALCEL® OD-RH

CHIRALCEL® OJ -RH

共价键合型—新产品

CHIRALPAK® IA

CHIRALPAK® IB

其它

CROWNPAK® **CR (+), (-)**

CHIRALPAK® OT(+), OP (+)

CHIRALPAK® WH, MA (+)

CHIRALPAK® QD-AX

CHIRALPAK® QN-AX

第二代手性色谱柱

— H 系列



CHIRALPAK[®] AD-H

CHIRALCEL[®] OD-H

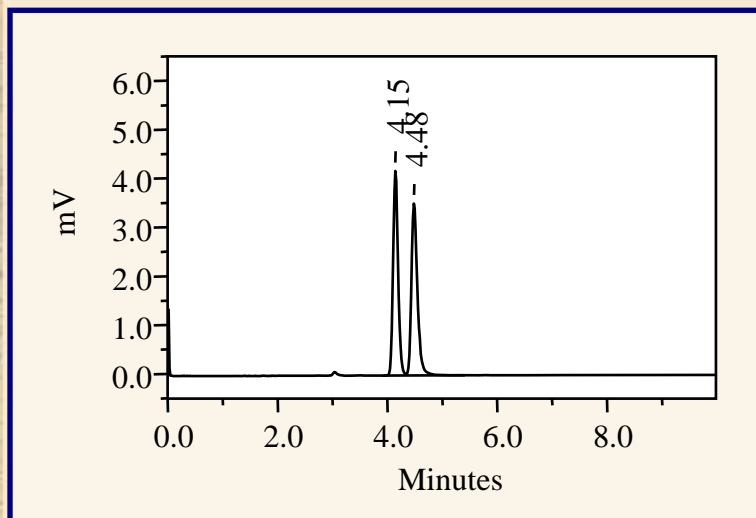
CHIRALPAK[®] AS-H

CHIRALCEL[®] OJ-H

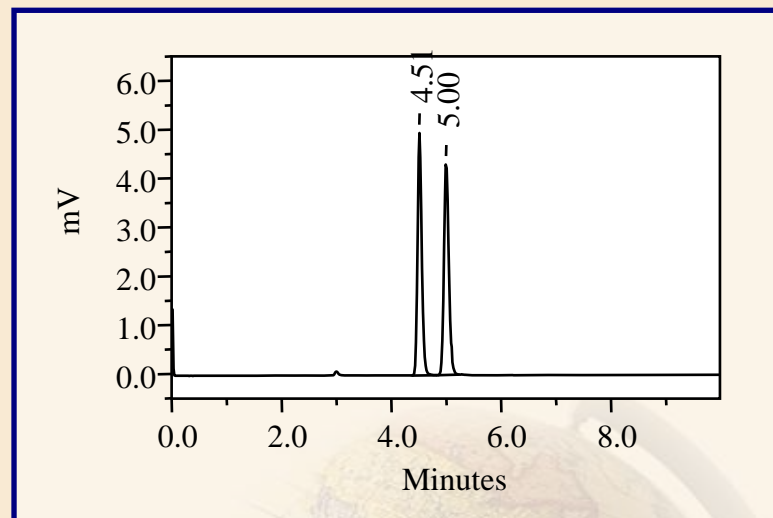
<特性>

- 1) 5 微米硅胶用于获得高的理论塔板数
- 2) 适用于痕量分析
- 3) 采用15cm长的手性柱可节省分析时间

AS vs. AS-H (中性组分)



AS



AS-H

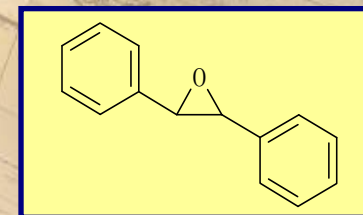
- 柱尺寸：0.46cm I.D. x 25cm L
- 样品：*trans*-Stilbene oxide
- 条件

流动相：正己烷 / 异丙醇 = 90 / 10

流量：1.0 ml/min.

温度：25 ° C

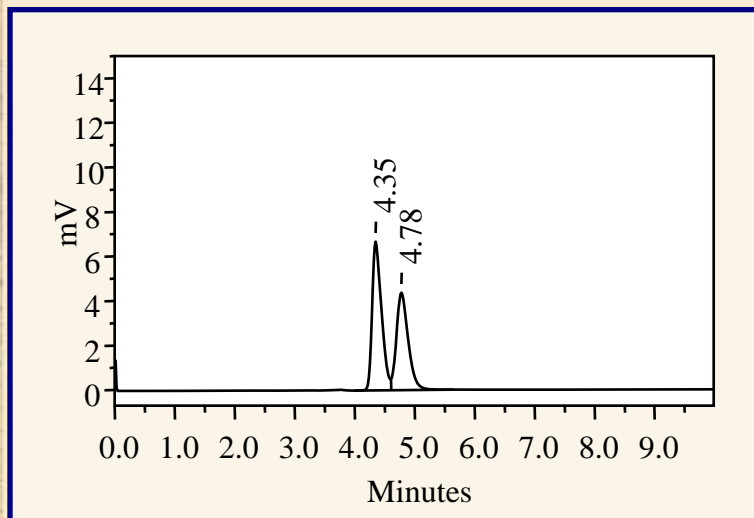
检测波长：UV 254nm



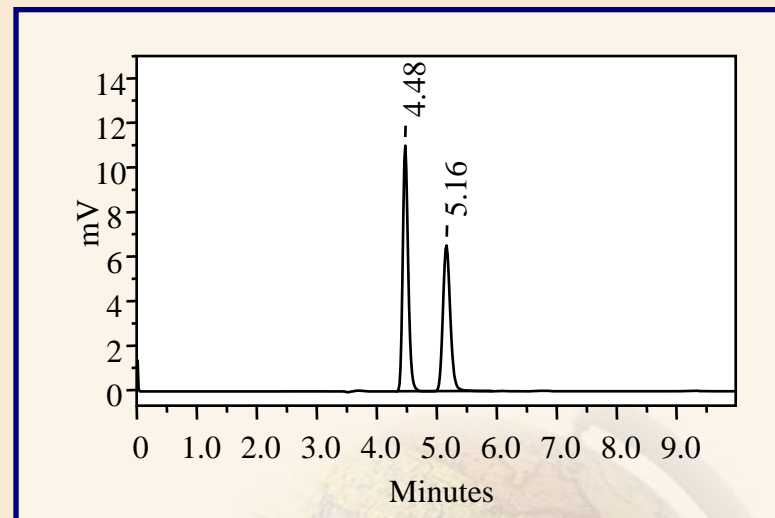
	α	N1
AS	1.30	9800
AS-H	1.32	16100

1.6 times

AS vs. AS-H (碱性组分)



AS



AS-H

- 柱尺寸： 0.46cmI.D. x 25cmL
- 样品： 1-[5-Chloro-2-(methylamino)phenyl]-
1,2,3,4-tetrahydroisoquinoline

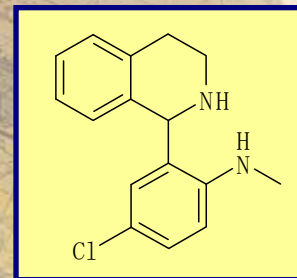
• 条件

流动相： 正己烷 / 异丙醇 / 二乙胺 = 90 / 10 / 0.1

流量： 1.0 ml/min.

温度： 25 ° C

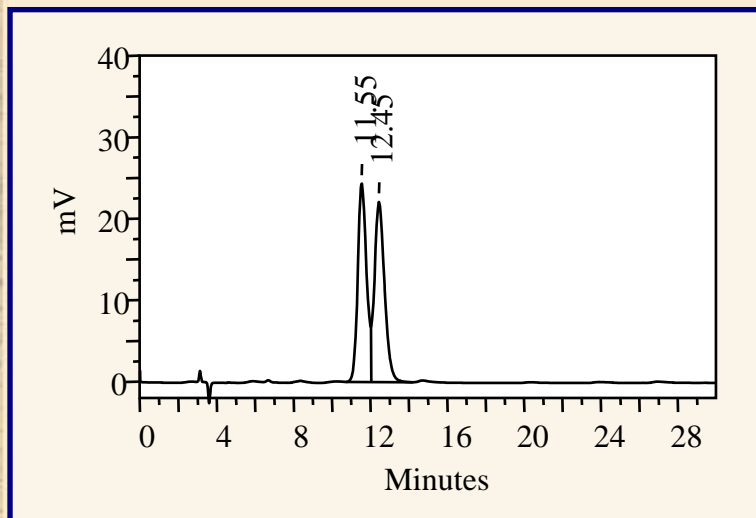
检测波长： UV 254nm



	α	N1
AS	1.33	3600
AS-H	1.46	12200

3.4 times

AS vs. AS-H (酸性组分)



AS

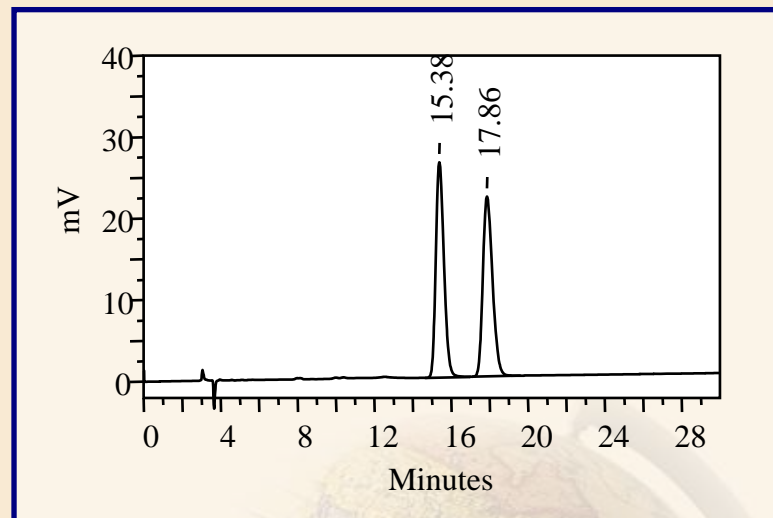
- 柱尺寸： 0.46cmI.D. x 25cmL
- 样品： 2-(4-Hydroxyphenoxy)丙酸
- 条件

流动相： 正己烷 / 异丙醇 / 三氟乙酸 = 90 / 10 / 0.1

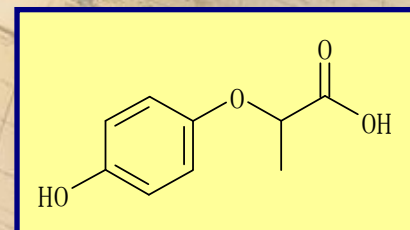
流量： 1.0 ml/min.

温度： 25 ° C

检测波长： UV 254nm



AS-H

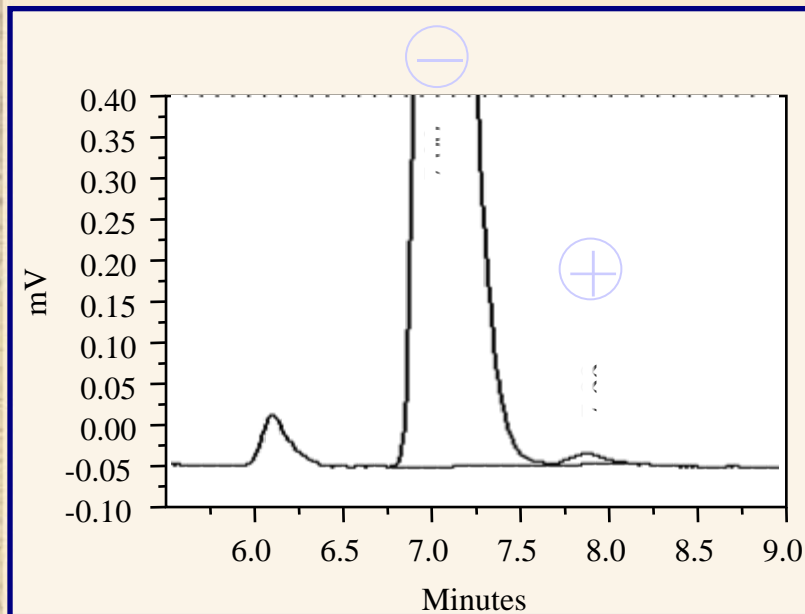


	α	N1
AS	1.11	3000
AS-H	1.20	6200

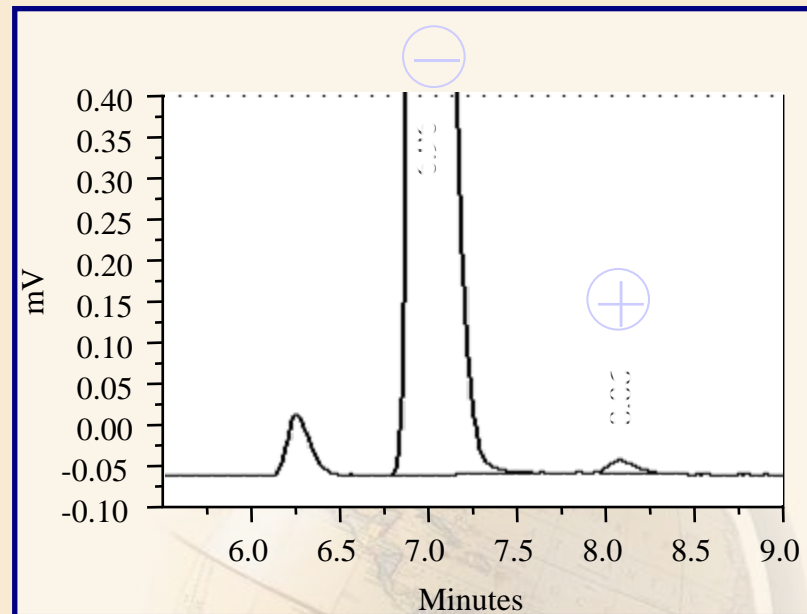
2.1 times

OJ vs.OJ-H (痕量分析)

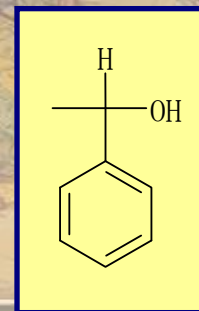
OJ



OJ-H



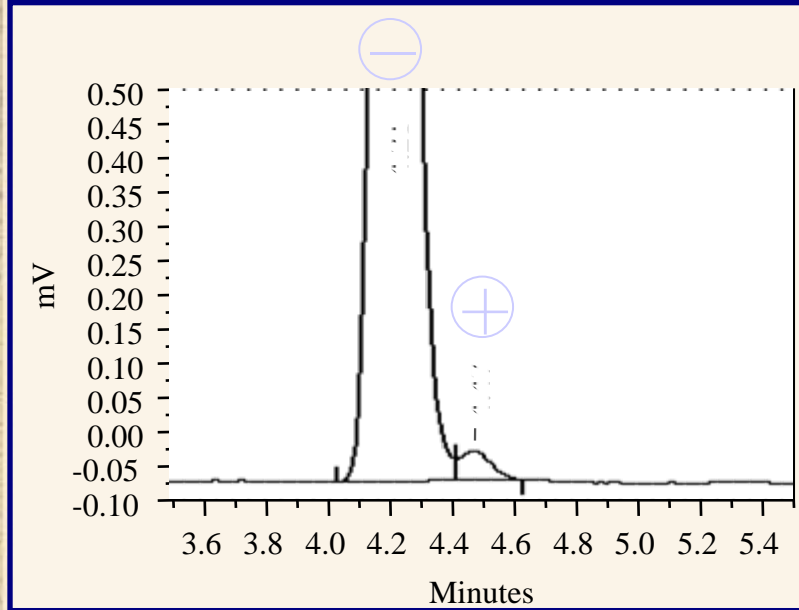
- 柱尺寸： 0.46cmI.D. x 25cmL
- 样品： 1-Phenyl ethanol
- 条件
 - 流动相： 正己烷 / 异丙醇 = 90 / 10
 - 温度： 25 ° C
 - 流量： 1.0 ml/min.
 - 检测波长： UV 254nm



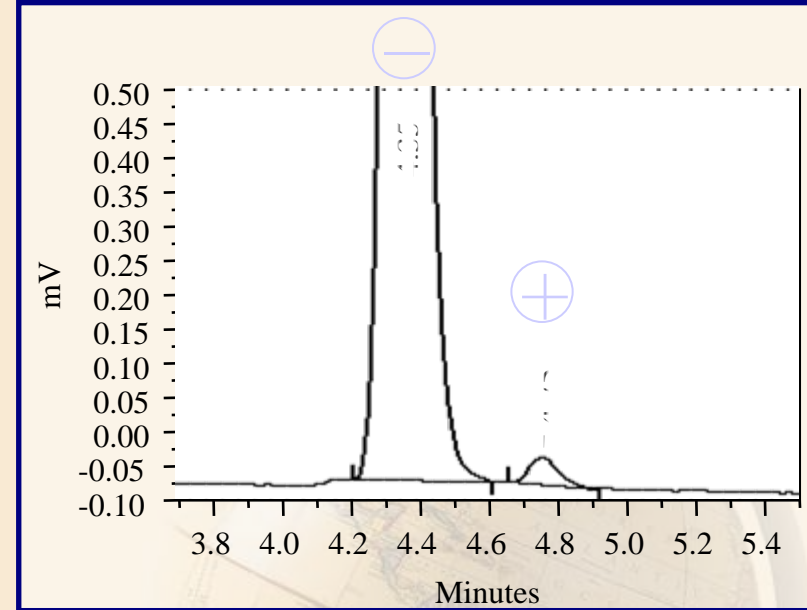
痕量分析 0.5% 手性化合物

AD vs. AD-H (痕量分析)

AD



AD-H



• 柱尺寸： 0.46cmI.D. × 25cmL

• 样品： 2-Phenyl丙酸

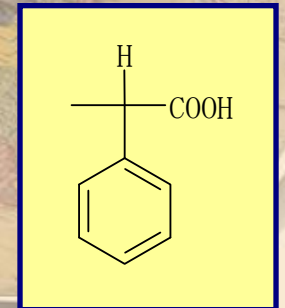
• 条件

流动相： 正己烷 / 异丙醇 / TFA = 80 / 20 / 0.1

温度： 25 ° C

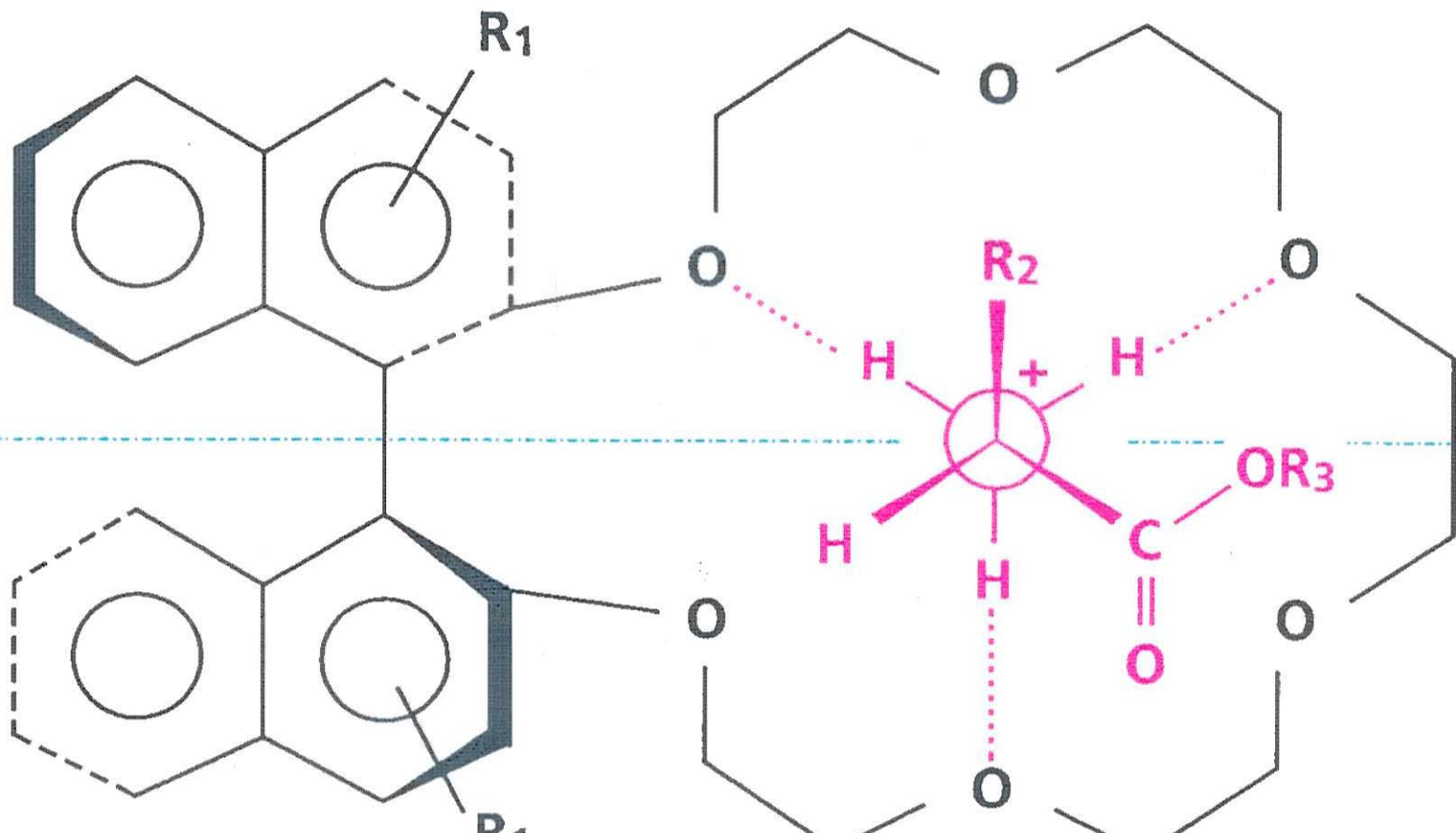
流量： 1.0 ml/min.

检测波长： UV 254nm



痕量分析 0.5% 手性化合物

CROWNPAK CR(+) 冠醚性手性色谱柱



- * Chiral Crown Ether coated on silica
- * For amino acids (primary amino group near the chiral center)
- * Dipeptides

CROWNPAK CR(+) の拆分实例

Resolution of Amino acids on CROWNPAK CR(+)

Amino acids	K_D'	α	R_s	pH	Flow rate (ml/min.)	Temp.	Amino acids	K_D'	α	R_s	pH	Flow rate (ml/min.)	Temp.
Alanine	0.38	1.86	3.17	1.5	0.4	25	Cysteine	0.44	1.67	3.31	1.5	0.4	25
Valine	1.09	1.51	3.47	1.5	0.4	0	Tyrosine	2.88	1.28	2.47	2.0	0.8	25
Norvaline	0.69	1.69	2.74	2.0	0.8	25	Asparagine	0.53	1.69	3.15	1.5	0.4	0
Leucine	1.44	1.67	3.73	2.0	0.8	25	Glutamine	0.25	2.13	3.11	2.0	0.4	25
Norleucine	1.76	1.66	3.38	2.0	0.8	25	Asparadic acid	0.61	2.01	4.07	2.0	0.4	0
Isoleucine	1.76	1.58	4.29	2.0	0.4	0	Gultamic acid	0.33	2.81	5.32	2.0	0.4	25
tert-leucine	2.06	1.10	0.70	1.0	0.4	0	Ornithine	0.65	1.49	2.82	1.5	0.4	25
Phenylalanine	3.88	1.27	2.80	2.0	0.8	25	Lysine	1.18	1.26	2.20	1.5	0.4	25
DOPA	2.02	1.32	2.62	2.0	0.8	25	Arginine	0.65	2.21	5.18	1.5	0.8	25
Methionine	1.05	2.00	5.87	2.0	0.8	25	Citruline	0.43	2.18	3.97	1.5	0.4	25
Ethionine	2.43	1.93	6.03	2.0	0.8	25	Proline	0.73	1.00	---	1.5	0.4	0
Phenylglycine	1.06	2.35	7.14	2.0	1.0	40	Histidine	0.90	1.82	528	1.5	0.4	0
Serine	0.48	1.75	3.04	1.5	0.4	0	Tryptophan	18.45	1.19	2.22	2.	1.2	25
Threonine	0.39	2.58	4.20	2.0	0.4	0							

第三代手性色谱柱

CHIRALPAK® IA, IB



CHIRALPAK® IA与IB研发背景

涂敷型

正相：正己烷，异丙醇，乙醇

反相：水，乙腈，甲醇，乙醇，异丙醇

键合型

CHIRALPAK® IA, CHIRALPAK® IB

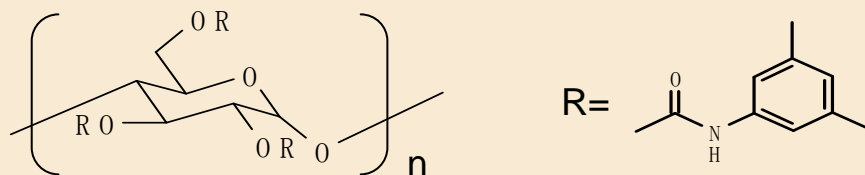
- 在广泛的流动相条件下具有很强的手性分离能力
- 色谱试验过程中，用于溶解样品的溶剂没有任何限制
- 在各种溶剂中柱性能异常稳定

CHIRALPAK® IA, IB 的基本特征

CHIRALPAK® IA

载体：硅胶

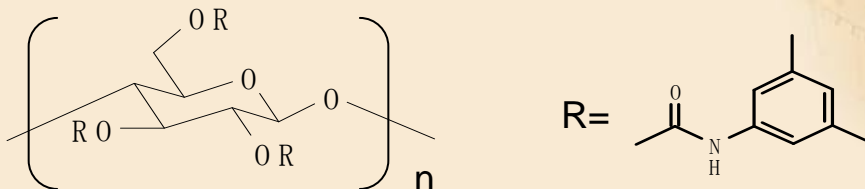
手性选择剂：直链淀粉-三(3,5-二甲基苯基氨基甲酸酯)



CHIRALPAK® IB

载体：硅胶

手性选择剂：纤维素-三(3,5-二甲基苯基氨基甲酸酯)



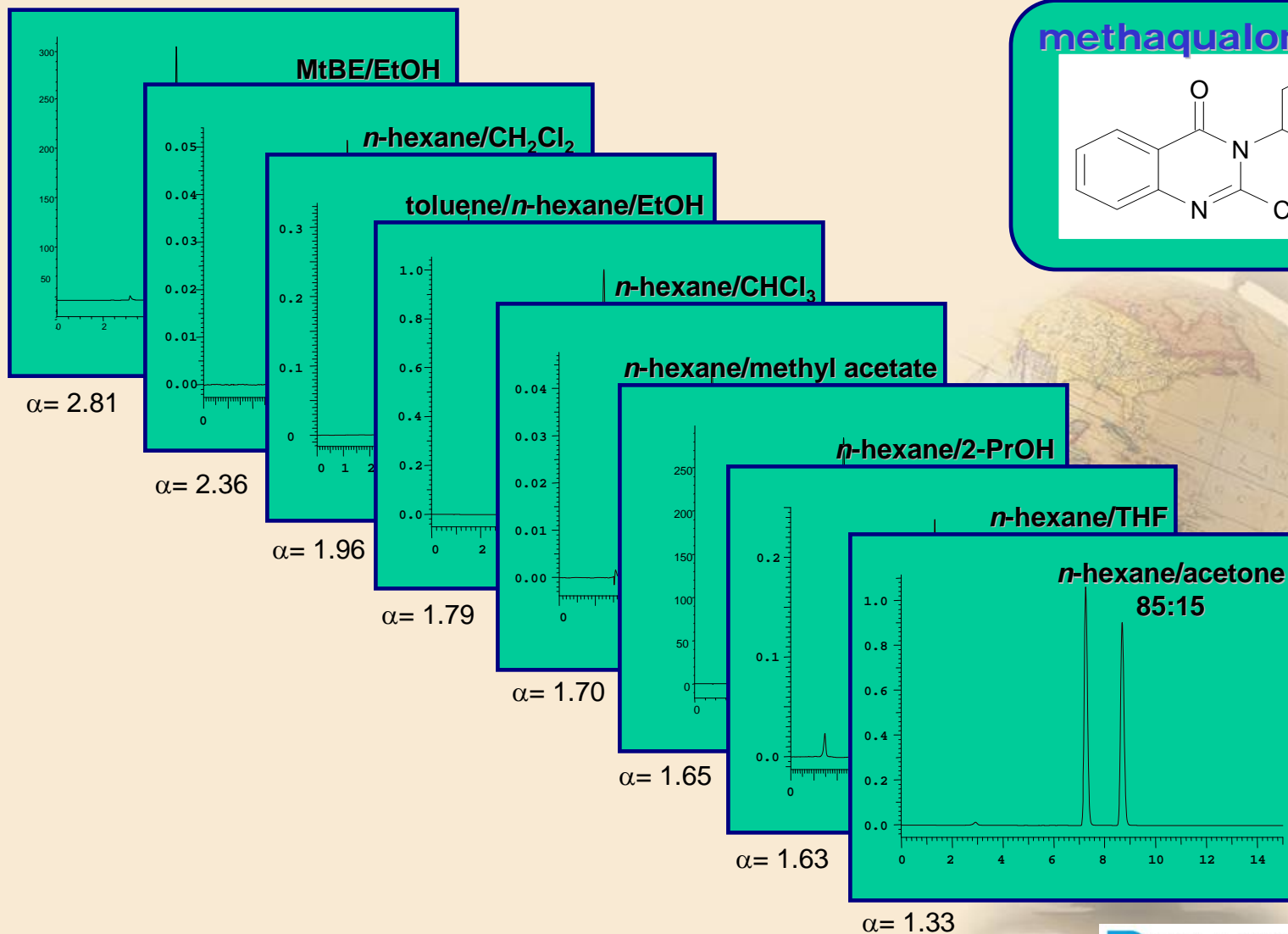
规格：150 或 250 X 4.6 mmID, 250 X 10 或 20 mmID

柱温：0 to 40 °C

柱压：10 MPa

CHIRALPAK® IA柱应用实例

不同流动相体系下手性分离



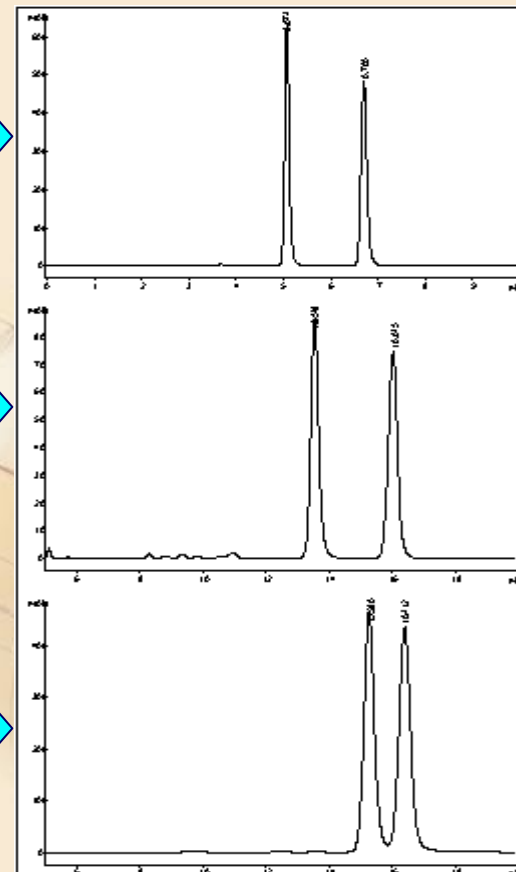
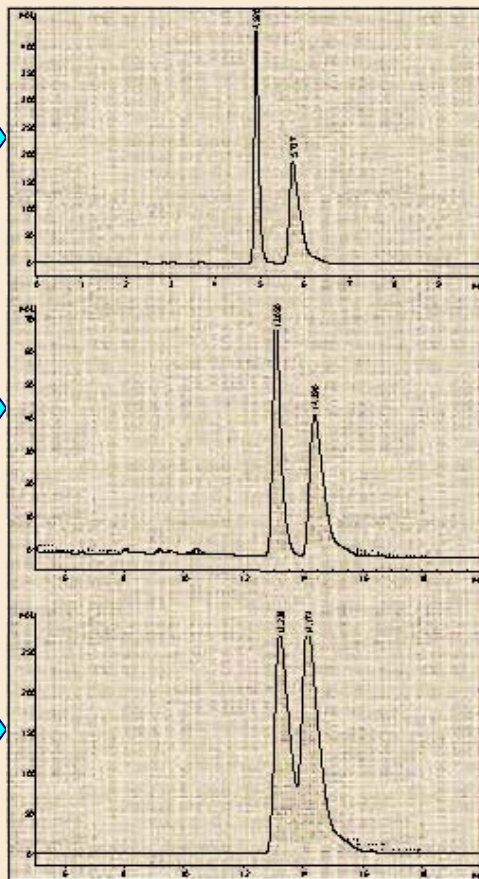
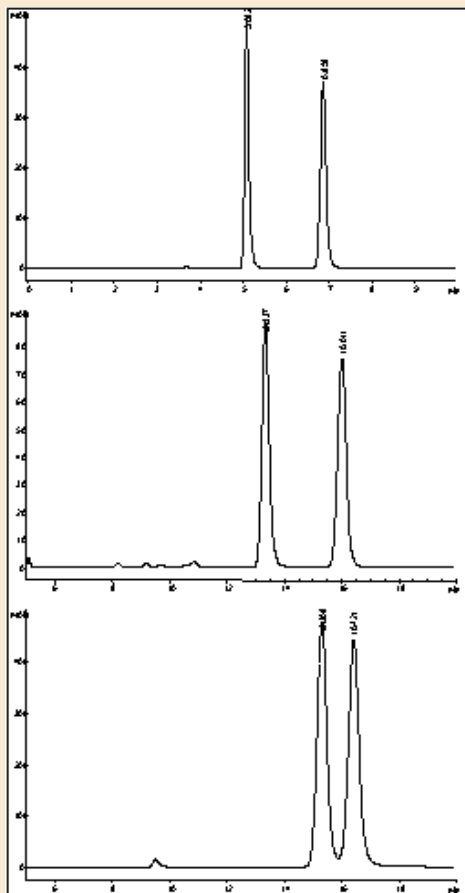
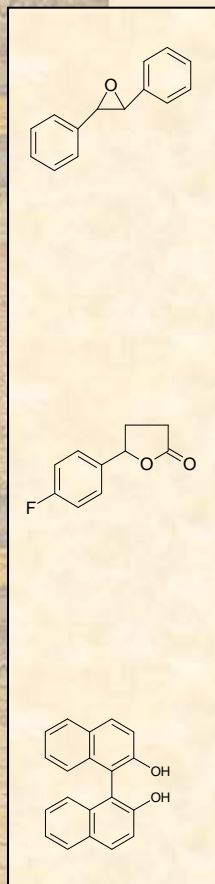
IA,IB柱的修复再生

样品

新色谱柱

柱性能受损
(如受强吸附杂质的影响)

修复再生后
(可使用各种强极性溶剂)

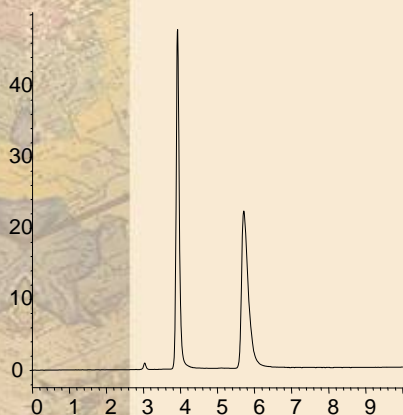
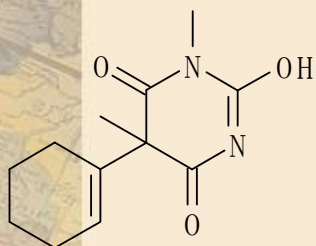


流动相:
n-hexane/EtOH 90/10
1 ml/min, 25° C

CHIRALPAK® IA 分离实例

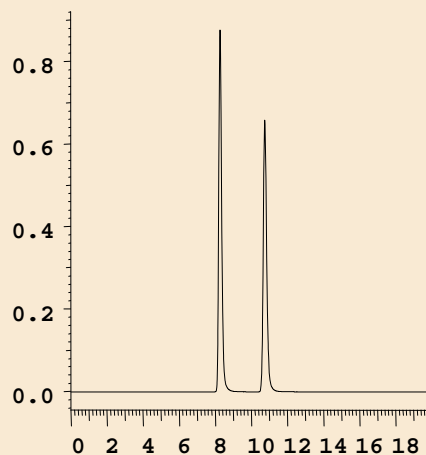
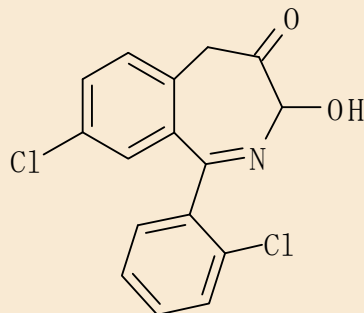
---中性样品---

Hexobarbital



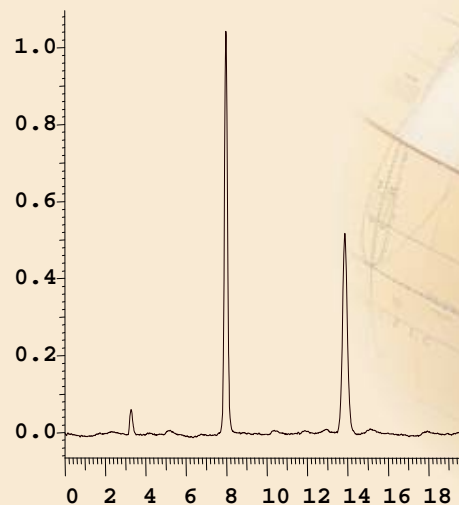
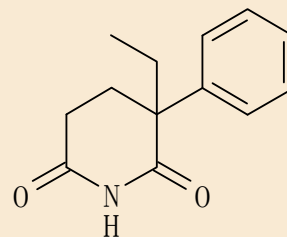
甲基叔丁基醚
=100
 $k_1' = 0.31, \alpha = 2.94$

Lorazepam



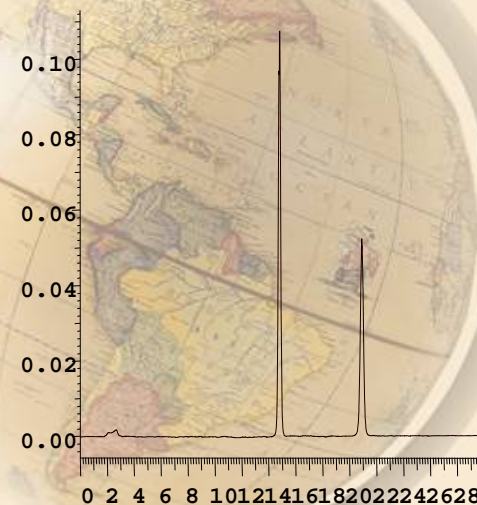
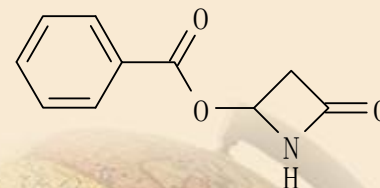
正己烷/丙酮
=60/40
 $k_1' = 1.75, \alpha = 1.47$

Glutethimide



正己烷/1,4-二氧环乙烷
=70/30
 $k_1' = 1.65, \alpha = 2.19$

4-Benzoyloxy-2-azetidinone

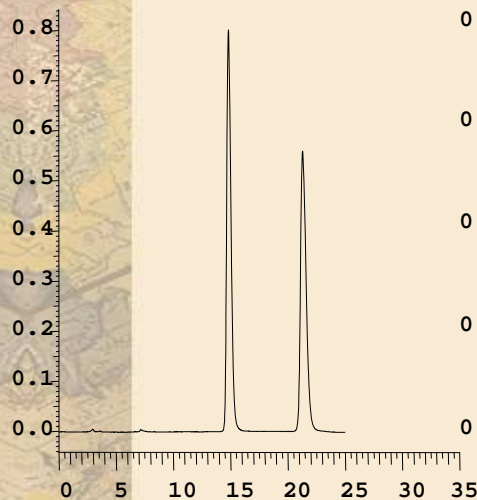
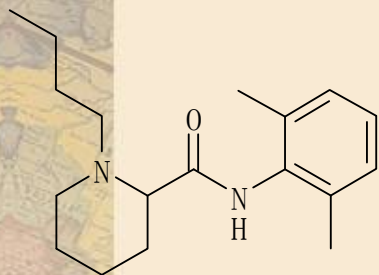


正己烷/二氯甲烷
=25/75
 $k_1' = 3.93, \alpha = 1.52$

CHIRALPAK® IA 分离实例

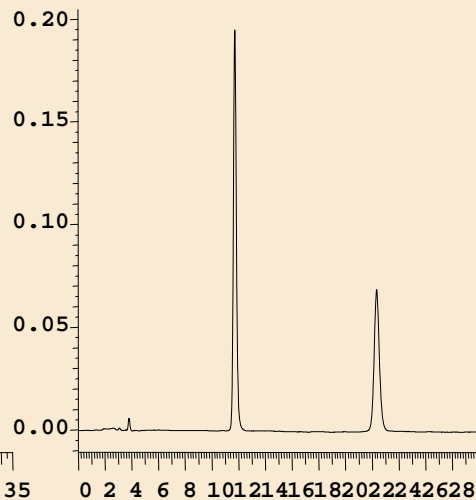
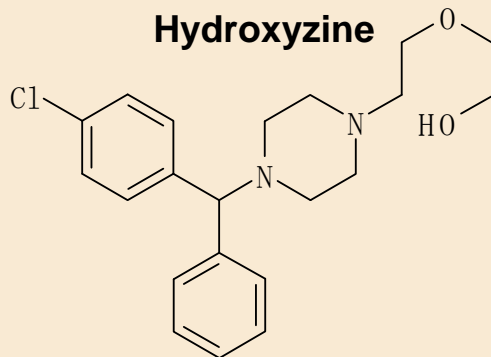
---碱性样品---

Bupivacaine



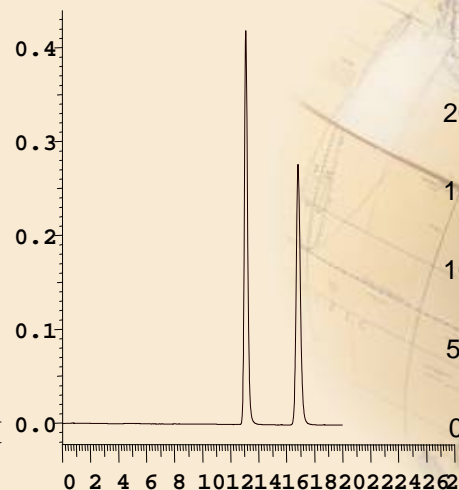
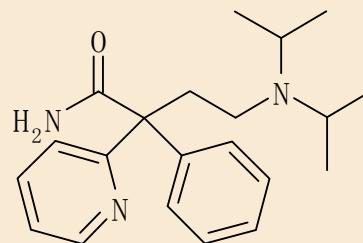
Hex/THF/DEA
=90/10/0.1
 $k_1' = 3.94$, $\alpha = 1.55$

Hydroxyzine



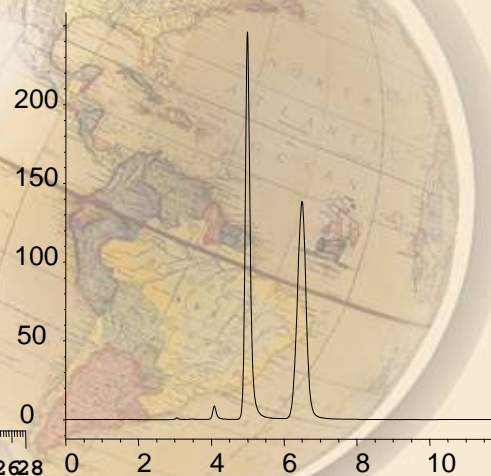
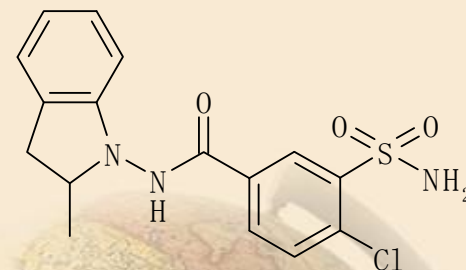
Hex/EtOAc/DEA
=40/60/0.1
 $k_1' = 2.90$, $\alpha = 2.22$

Disopyramide



Hex/Acetone/DEA
=80/20/0.1
 $k_1' = 3.36$, $\alpha = 1.37$

Indapamide

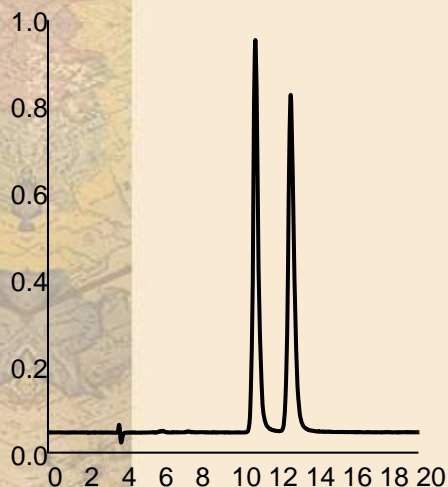
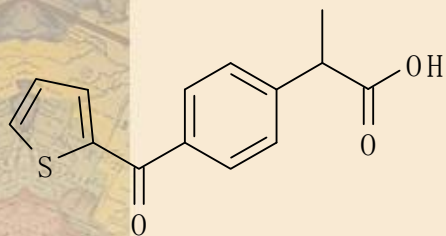


MTBE/EtOH/DEA
=80/20/0.1
 $k_1' = 0.67$, $\alpha = 1.75$

CHIRALPAK® IA 分离实例

---酸性样品---

Suprofen

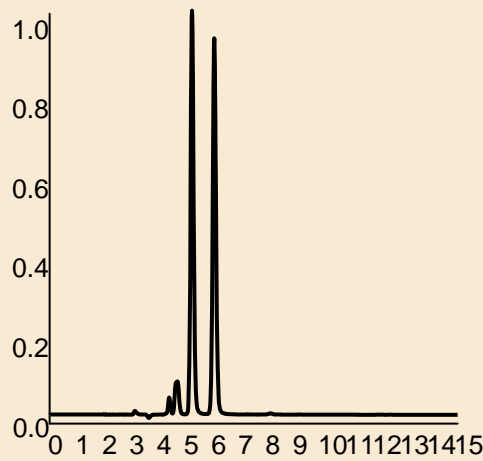
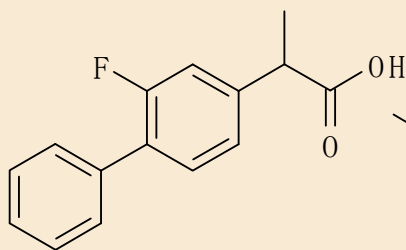


Hex/EtOAc/TFA

=70/30/0.1

$k_1' = 2.83, \alpha = 1.18$

Flurbiprofen

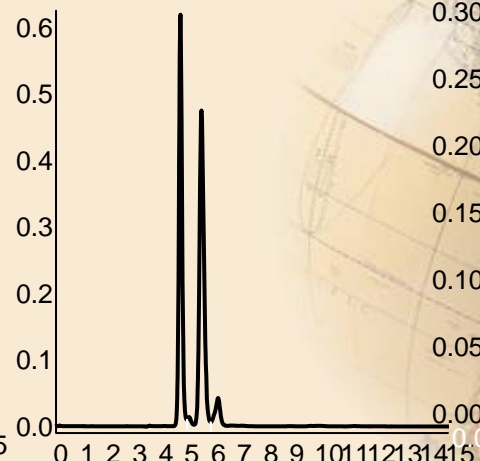
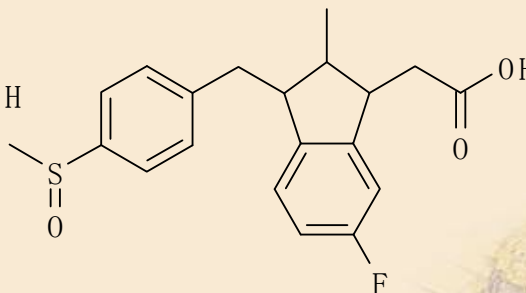


Hex/EtOH/TFA

=85/15/0.1

$k_1' = 0.74, \alpha = 1.37$

Sulindac

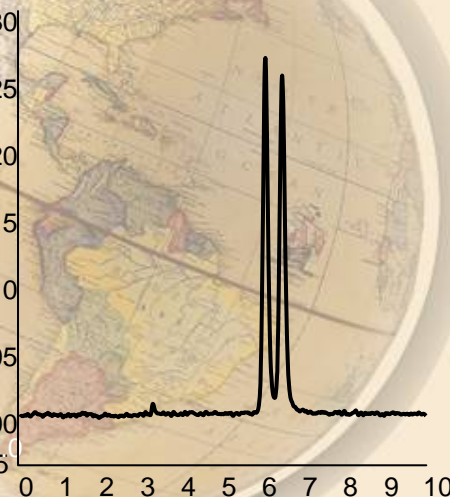
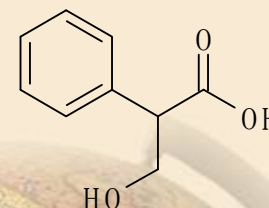


MeOH/EtOH/TFA

=50/50/0.1

$k_1' = 0.59, \alpha = 1.45$

Tropic acid



Hex/THF/TFA

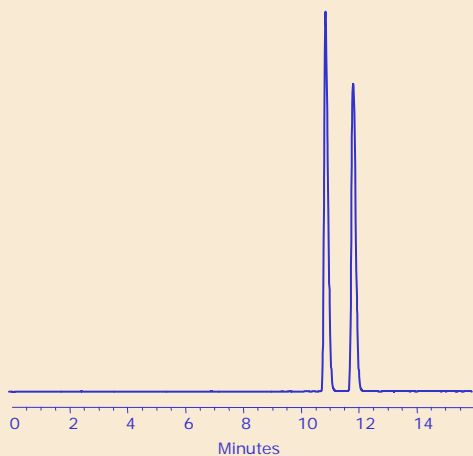
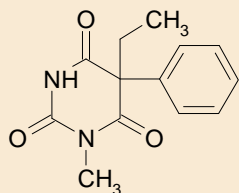
=65/35/0.1

$k_1' = 1.01, \alpha = 1.14$

CHIRALPAK® IB 分离实例

---中性样品---

Mephobarbital

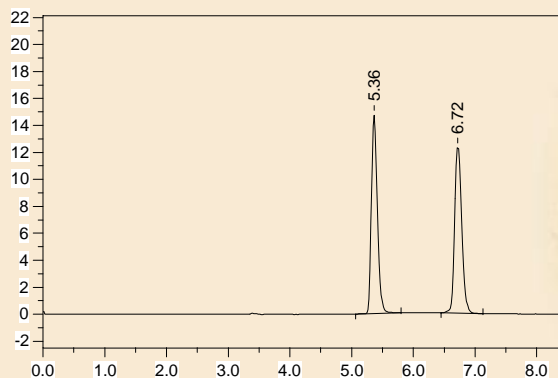
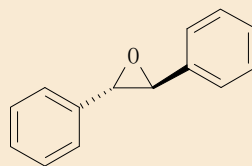


正己烷/三氯甲烷

=50/50

$\alpha=1.12$

trans-Stilbene oxide

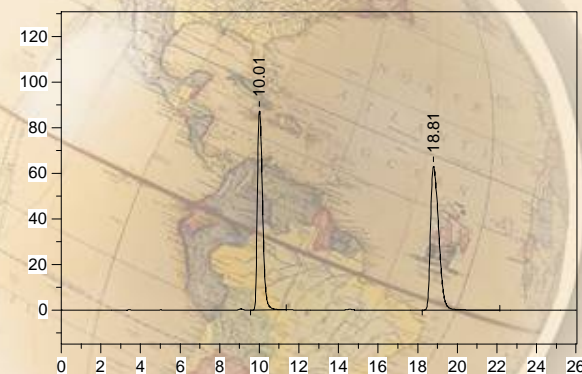
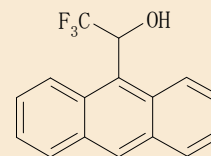


正己烷/二氯甲烷

=80/20

$k_1'=0.69, \alpha=1.62$

2,2,2-Trifluoro-1-(9-anthryl)ethanol



正己烷/四氢呋喃

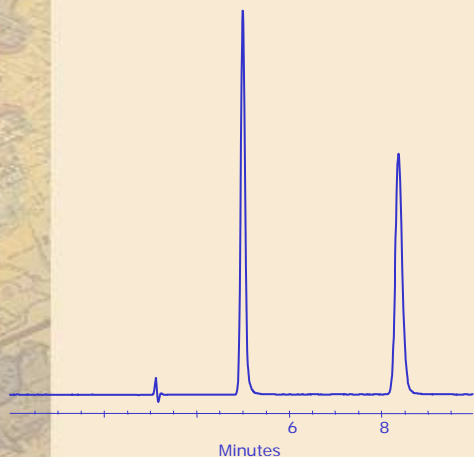
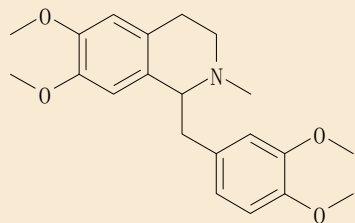
=90/10

$k_1'=2.28, \alpha=2.26$

CHIRALPAK® IB 分离实例

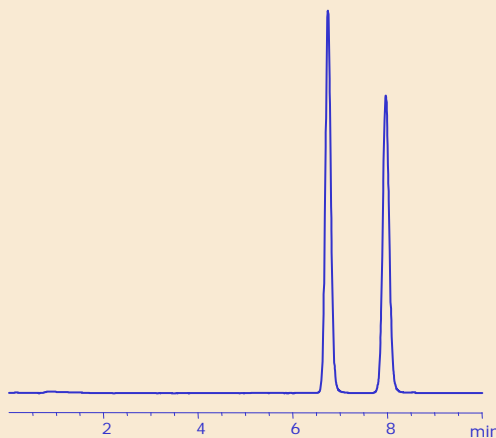
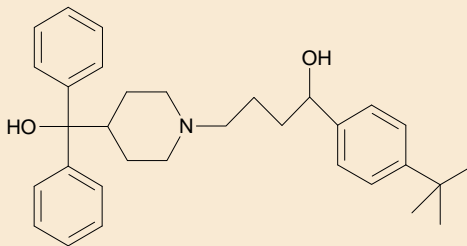
---碱性样品---

Laudanosine



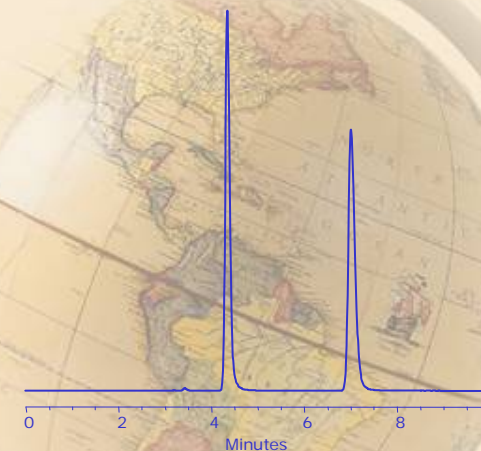
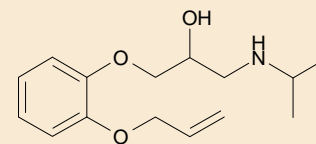
正己烷
/THF/EDA
=70/30/0.1
 $k_1' = 0.67, \alpha = 2.68$

Terfenadine



正己烷
/CHCl₃/EtNA
=65/35/0.1
 $k_1' = 1.20, \alpha = 1.33$

Oxprenolol

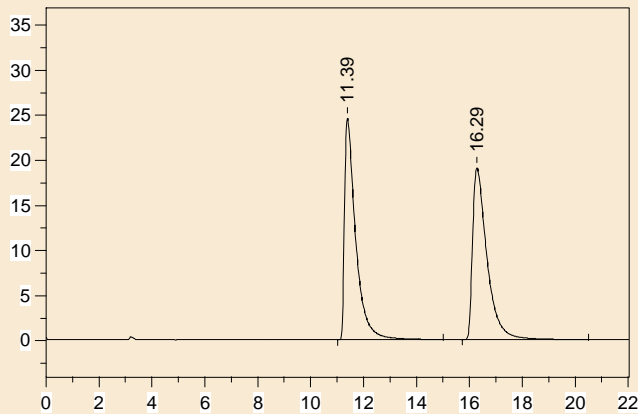
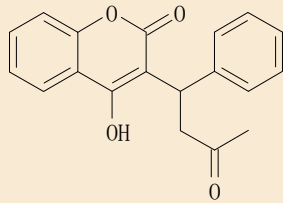


MTBE/EtOH/EDA
=95/5/0.1
 $k_1' = 0.45, \alpha = 2.98$

CHIRALPAK® IB 分离实例

---酸性样品---

Warfarin

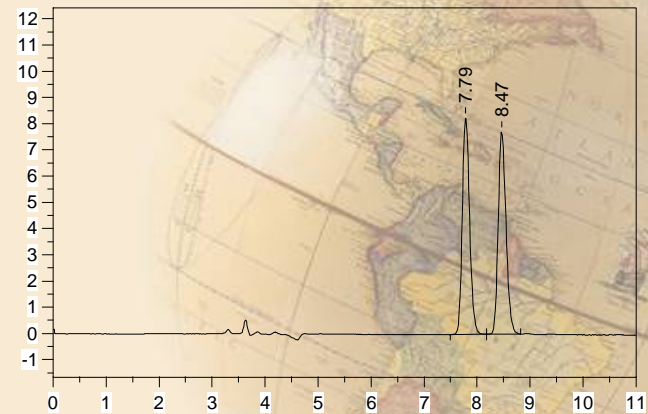
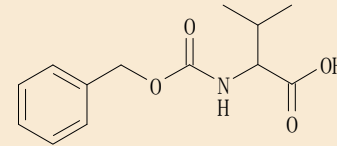


正己烷/乙酸乙酯/TFA

=70/30/0.1

$k_1' = 2.69$, $\alpha = 1.59$

N-Benzyloxycarbonyl-valine



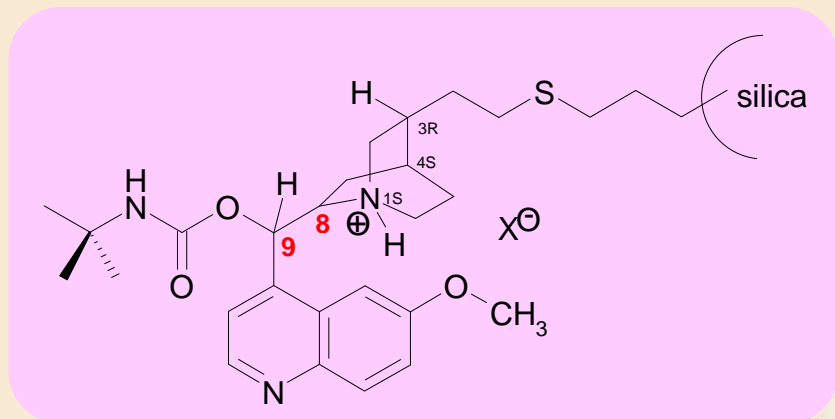
正己烷/ CHCl_3 /甲醇/TFA

=70/30/1/0.1

$k_1' = 1.52$, $\alpha = 1.14$

离子交换型新手型色谱柱

维也纳大学 W.Lindner 教授研究开发的离子交换型手性色谱柱



不对称炭原子临近的胺正离子和酸性官能基(X^-)所形成的不对称环境是手性化合物分离的基本作用力

手性识别剂

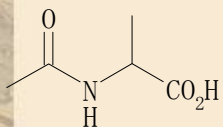
Quinine衍生物

CHIRALPAK® QN-AX:(8S, 9R)

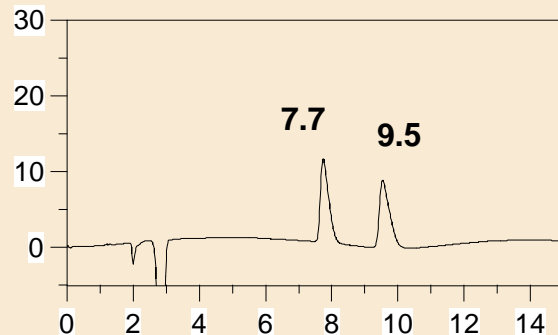
Quinidine衍生物

CHIRALPAK® QD-AX:(8R, 9S)

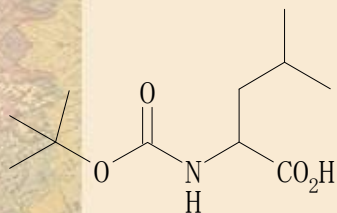
N-保护氨基酸的分析实例



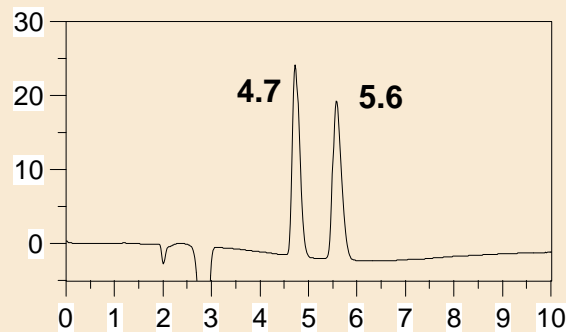
N-Ac-Ala



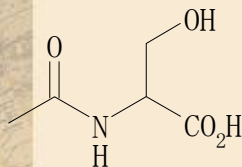
CHIRALPAK® QD-AX
MeOH/AcOH=100/1(v/v)



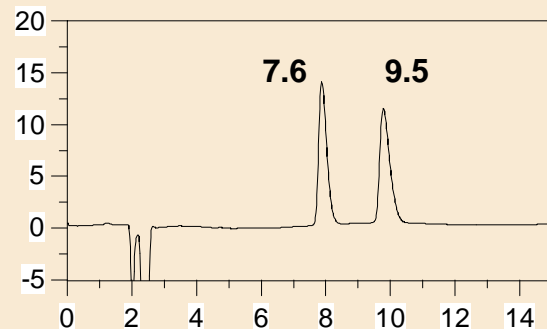
N-Boc-Leu



CHIRALPAK® QN-AX
MeOH/AcOH=100/1(v/v)



N-Ac-Ser



CHIRALPAK® QD-AX
MeOH/AcOH=97/3(v/v)

内容提纲

1. 手性HPLC分析条件的建立和优化

1-1 正相色谱

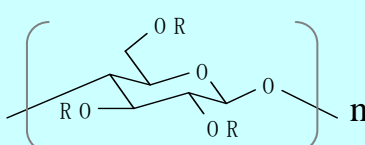
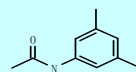
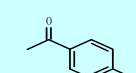
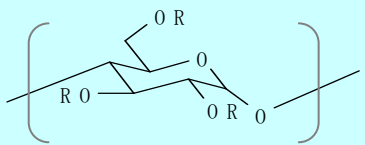
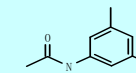
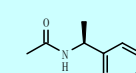
2-2 反相色谱

2. 手性色谱柱的自动筛选

3. 手性分析中的注意事项

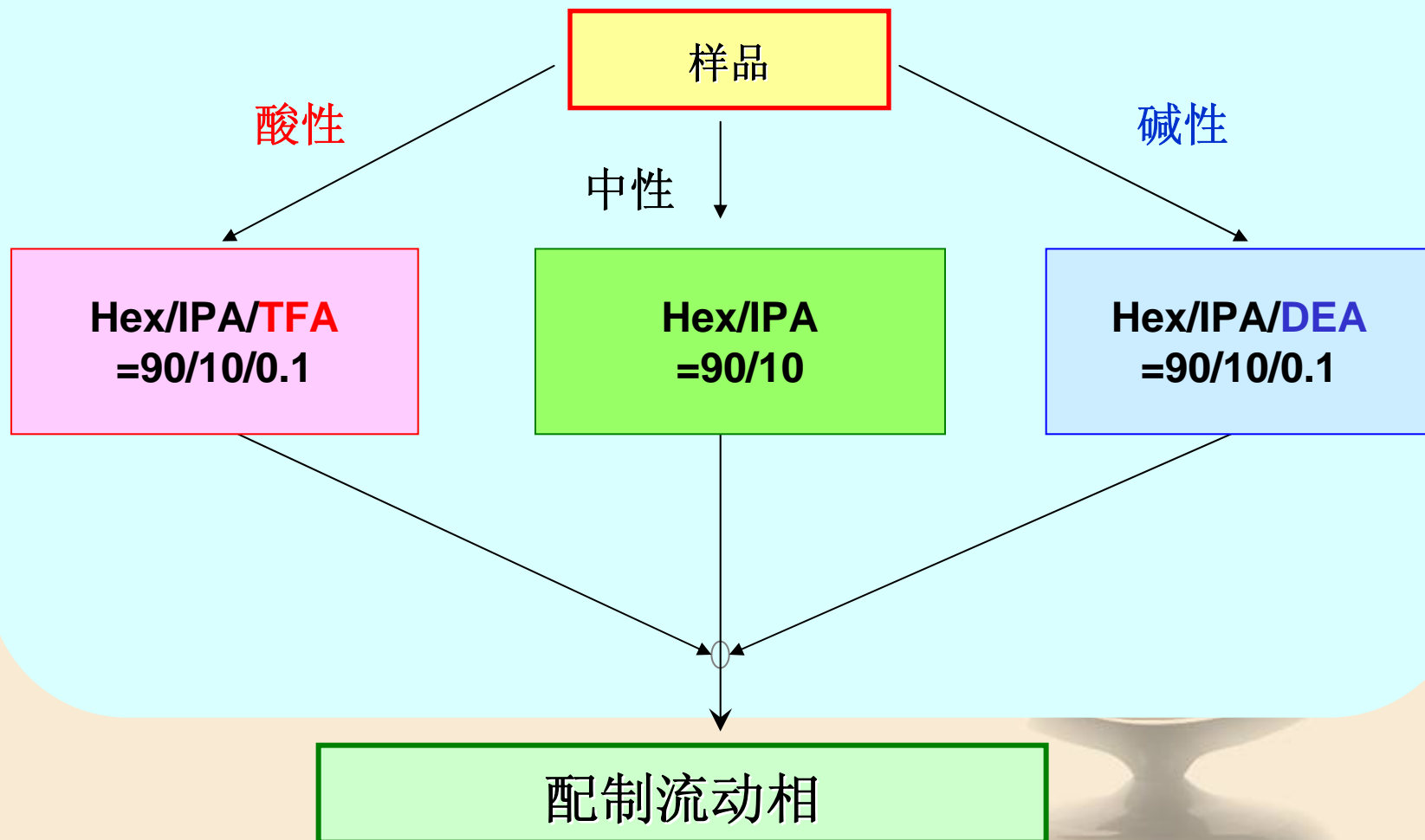


4种主要的正相柱和反相柱

	正相		反相
	普通	H 系列	
CHIRALCEL® 系列 	R =  OD	OD-H	OD-RH
R =  OJ	OJ-H	OJ-RH	
CHIRALPAK® 系列 	R =  AD	AD-H	AD-RH
R =  AS	AS-H	AS-RH	
柱长 (I.D. : 0.46cm)	25cm	25cm	N.A.
	N.A.	15cm	15cm

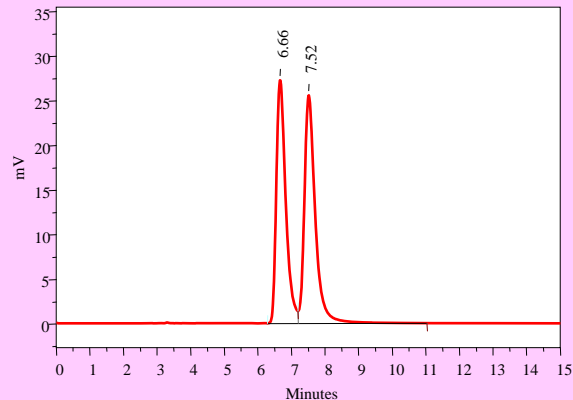
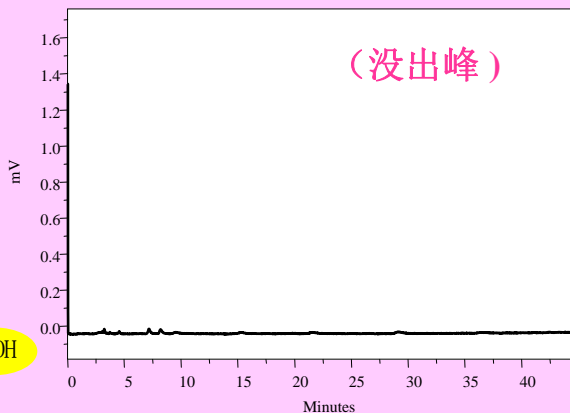
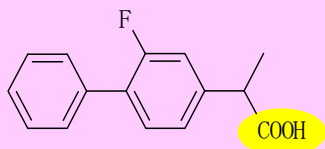
分析条件优化流程图 (1)

1. 流动相中添加物的必要性



添加物在分离中的作用

酸性
样品

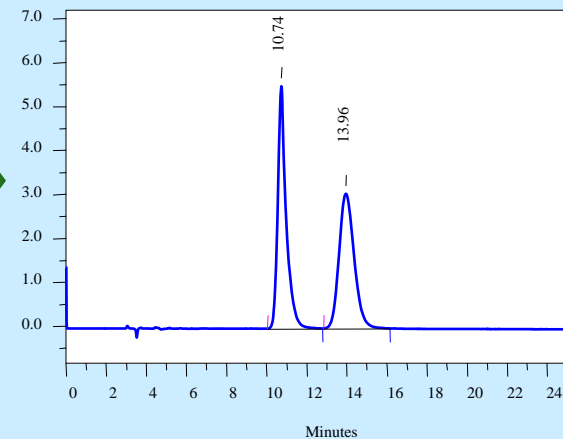
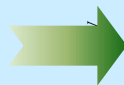
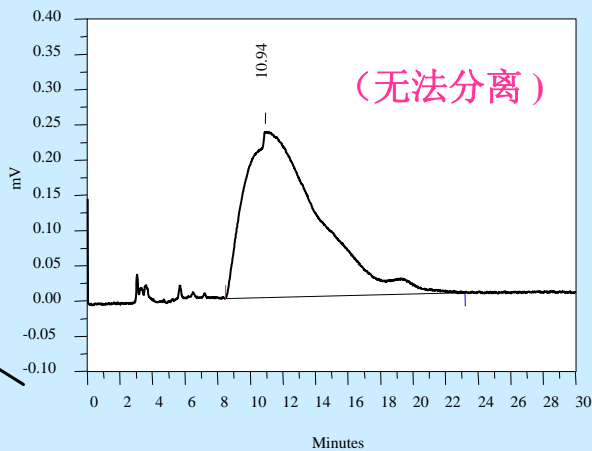
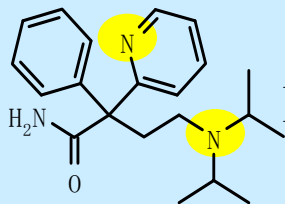


流量: 1.0mi/min. 温度: 40℃、检测波长: U V (254 n m)

流动相 Hex/IPA=90/10

Hex/IPA/**AcOH**=90/10/**0.1**

碱性
样品



流量: 1.0mi/min. 温度: 25℃、检测波长: U V (254 n m)

流动相 Hex/IPA=90/10

Hex/IPA/**DEA**=90/10/**0.1**

添加物的种类和用量

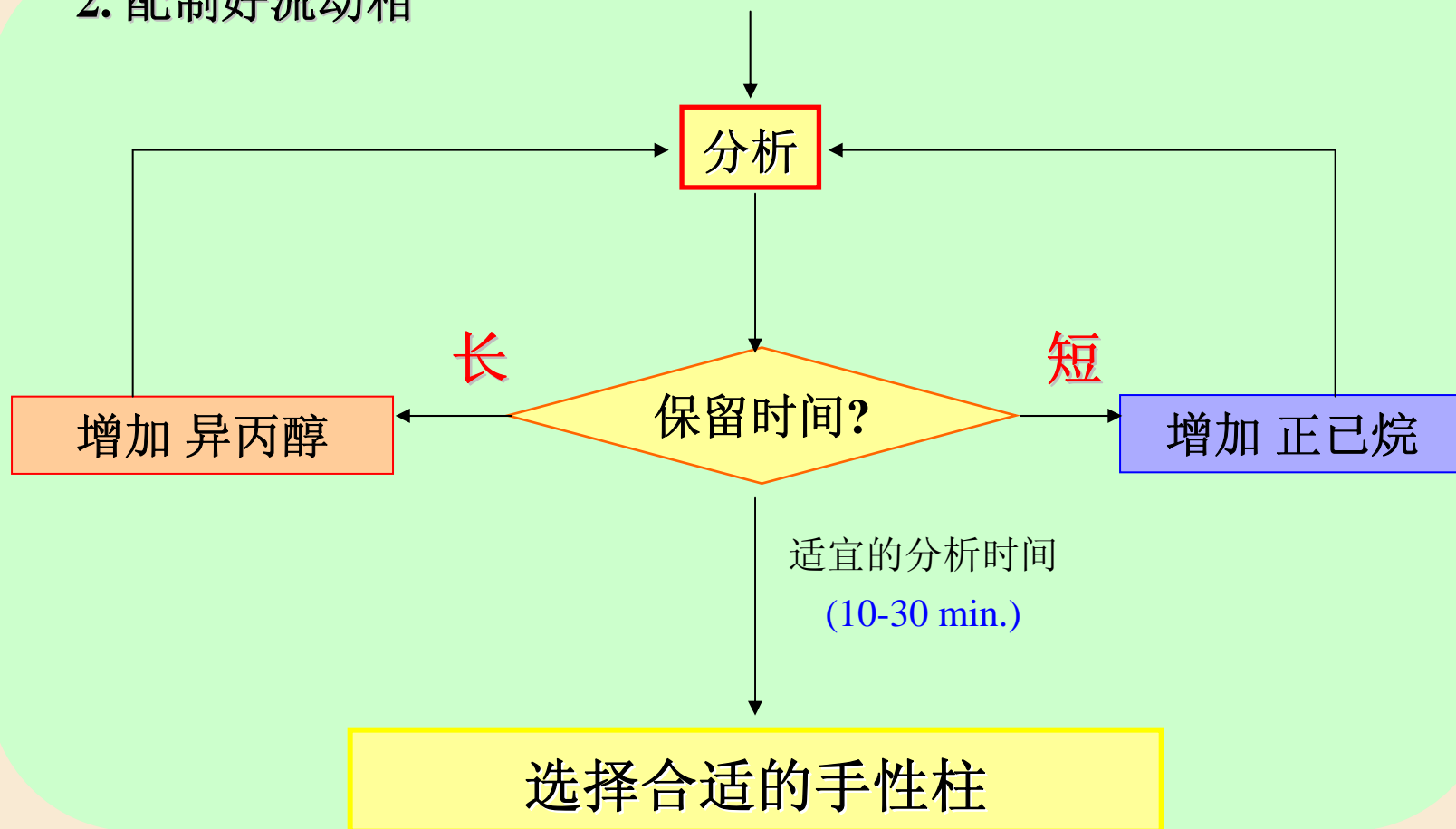
样品酸碱性	添加物种类	添加量
中性	不需要	
碱性	二乙胺 (DEA) (n-丁胺*1) (乙醇胺*2)	$\leq 0.5\%$ 通常0.1%
酸性	三氟乙酸 (TFA) 醋酸	$\leq 0.5\%$ 通常0.1%

*1 主要用于1级胺

*2 主要用于1级氨基醇

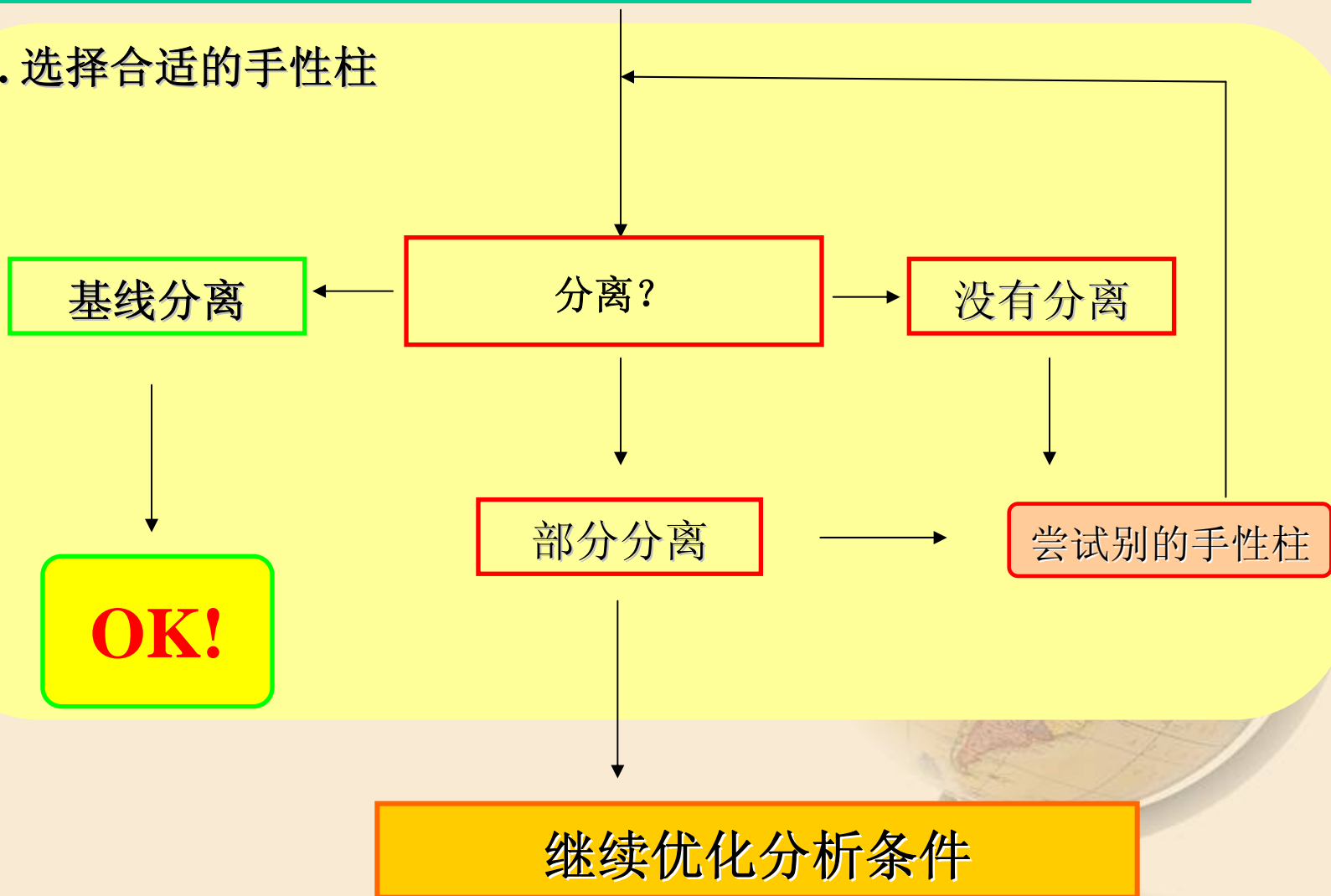
分析条件优化流程图 (2)

2. 配制好流动相



分析条件优化流程图 (3)

3. 选择合适的手性柱



筛选结果

色谱条件

流动相:

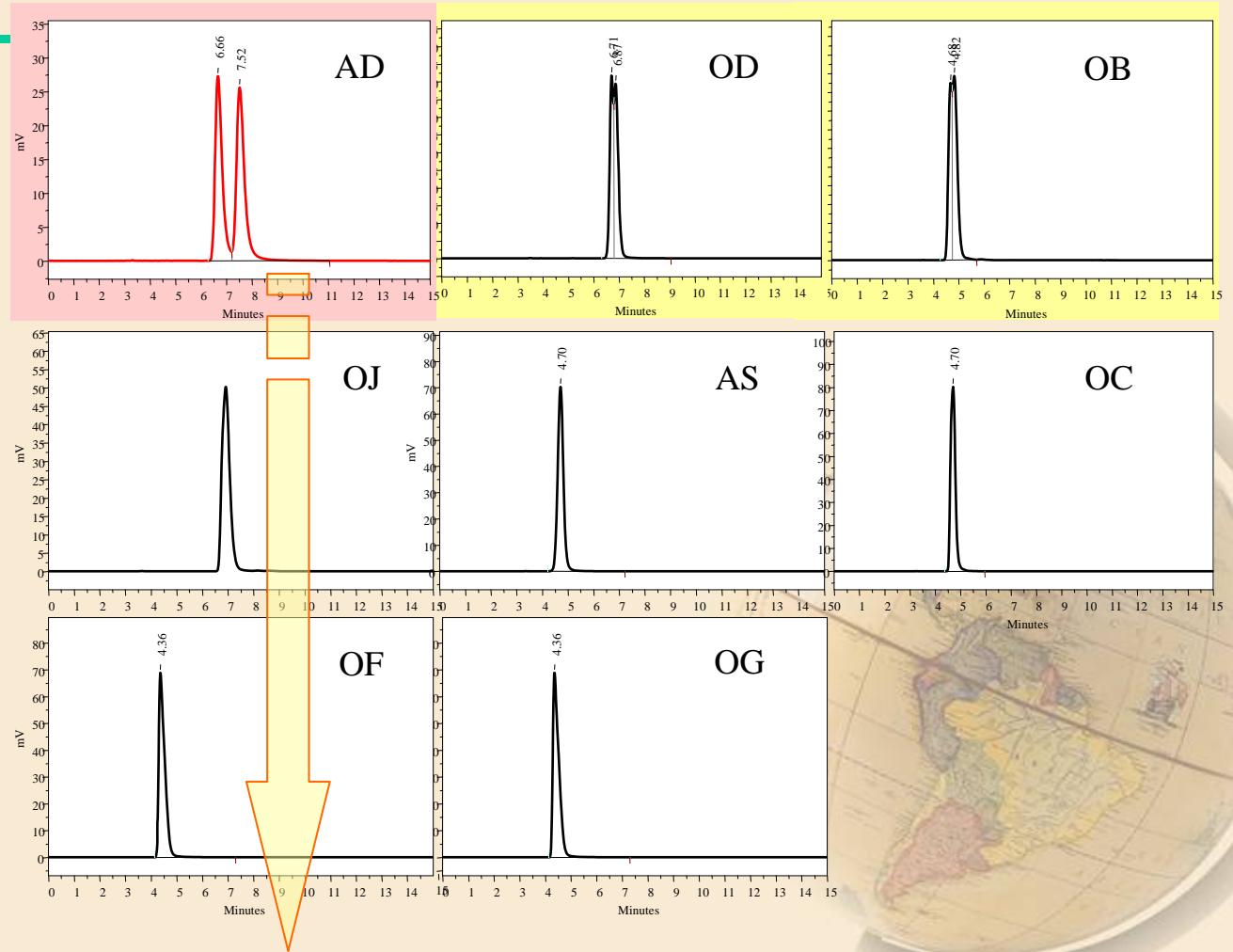
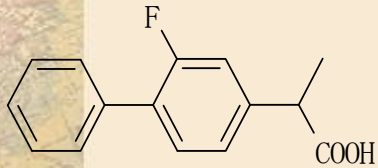
Hex./IPA/ AcOH =
90/10/0.1

流量 : 1.0ml/min.

温度 : 40°C

检测波长 : UV254nm

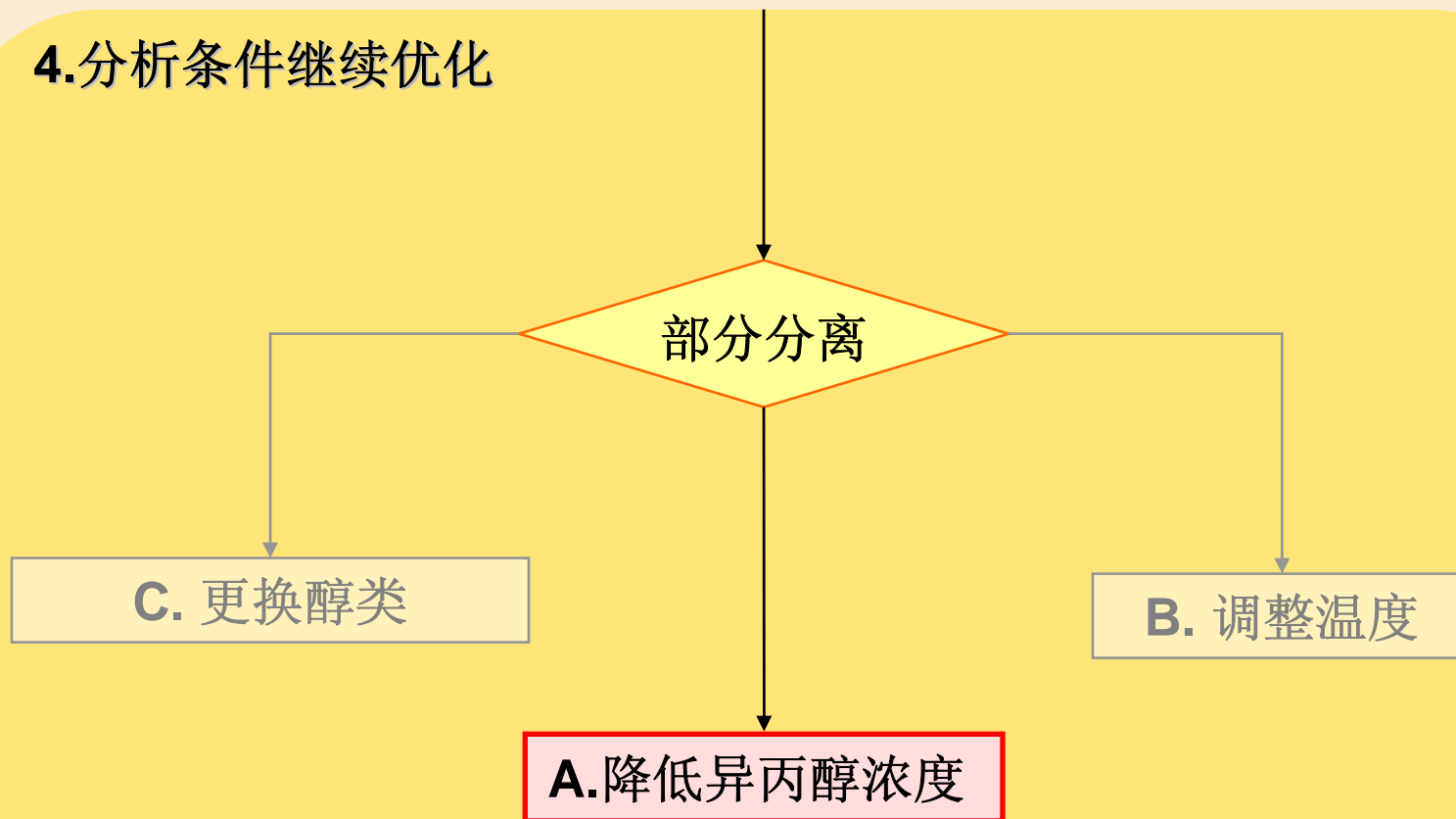
样品 :



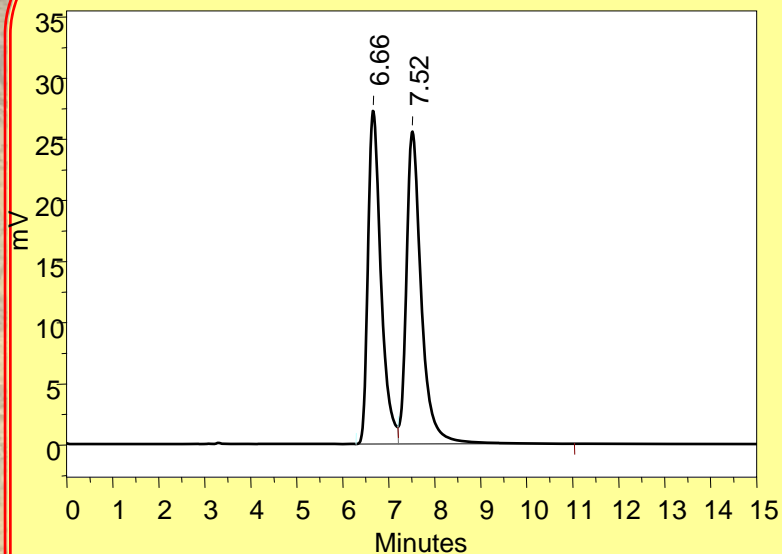
先择效果最好的手性柱, 然后继续优化分析条件

分析条件优化流程图 (4-A)

4. 分析条件继续优化

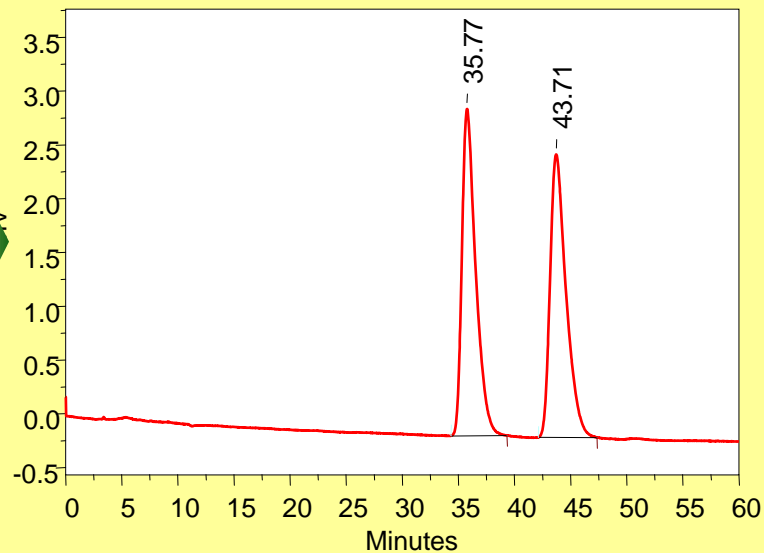


醇浓度的影响



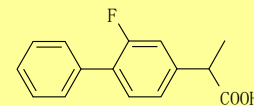
Hex./IPA/ AcOH = **90/10/0.1**

Column: AD, Temp.: 40°C, Flow rate: 1.0ml/min.



Hex./IPA/ AcOH = **100/1/0.1**

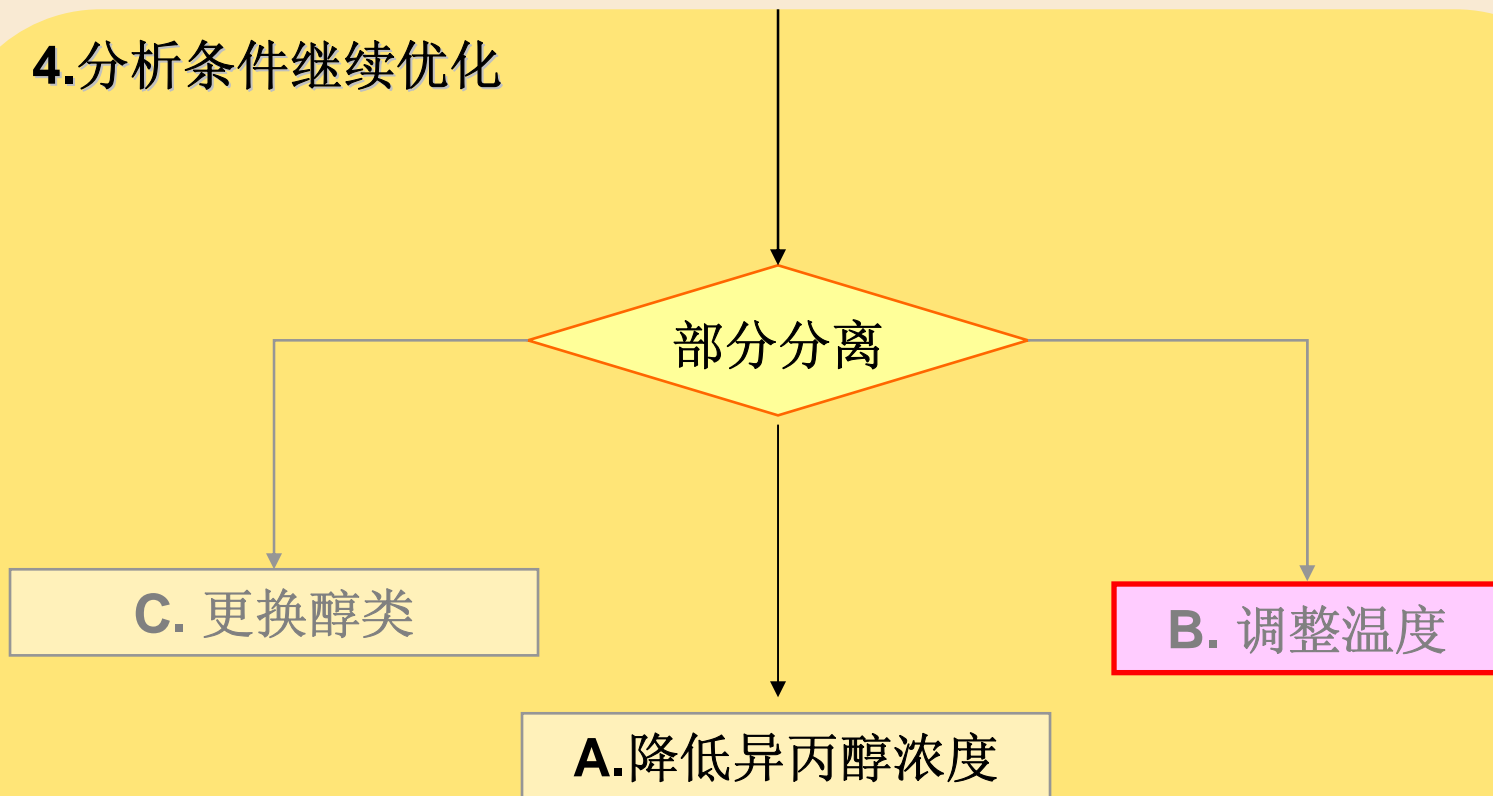
Sample: Flurbiprofen



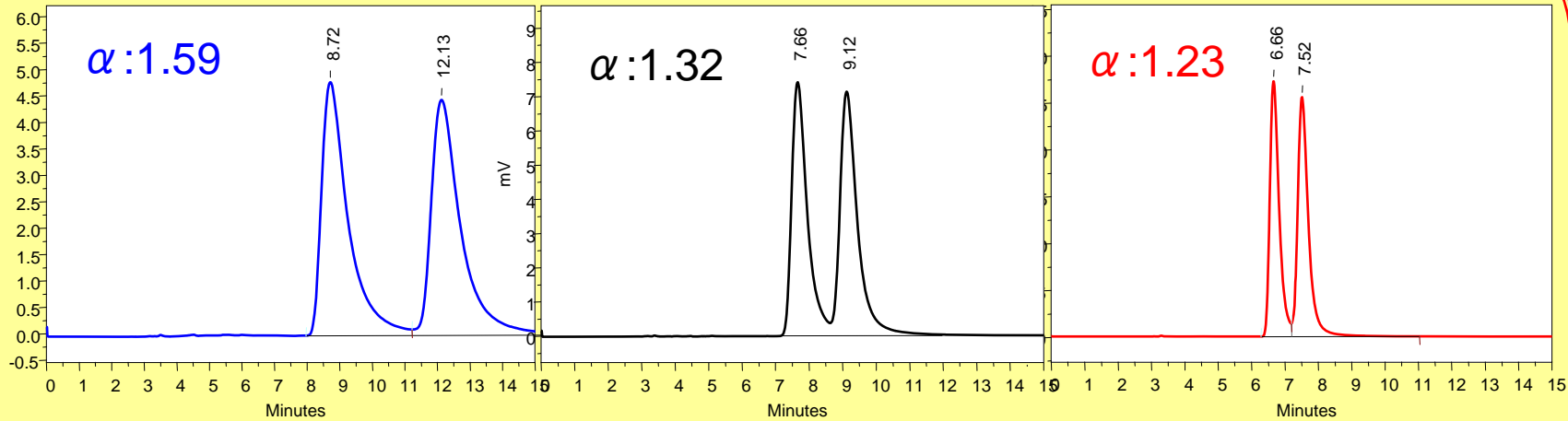
在多数情况下，降低醇的浓度可延长保持时间，改善分离度

分析条件优化流程图 (4-B)

4.分析条件继续优化



温度的影响



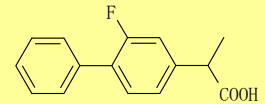
Temp. : **5°C**

25°C

40°C

Column : CHIRALPAK AD

Mobile phase : Hex./IPA/ AcOH =90/10/0.1, Flow rate : 1.0ml/min.

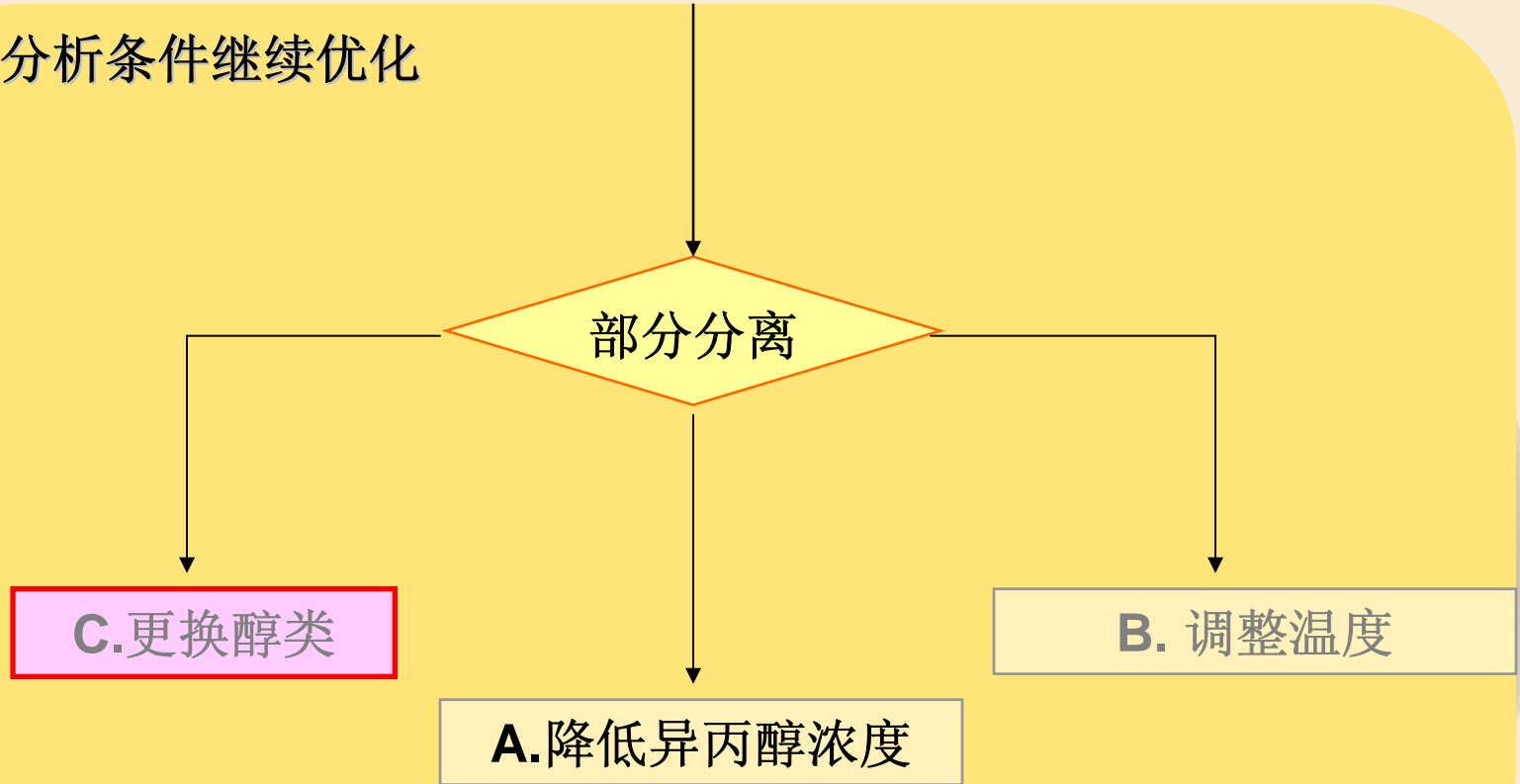


降低柱温可增大 α 值，但会使峰形变宽

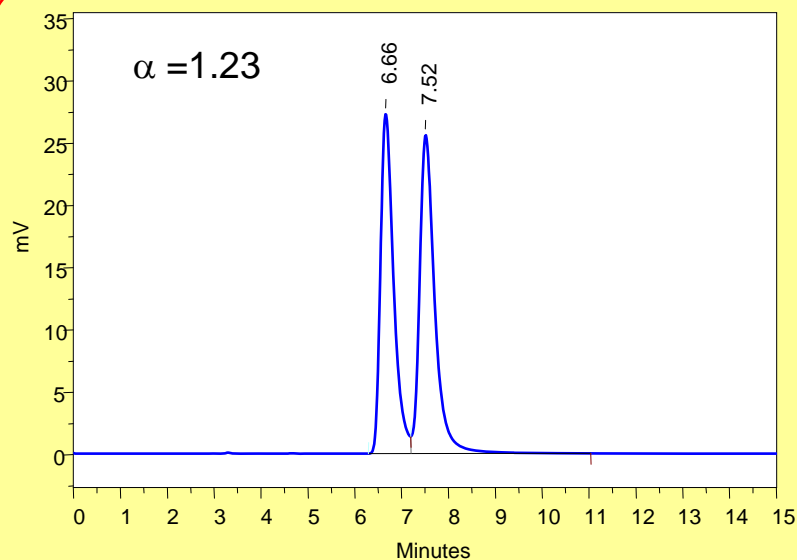
升高柱温会减少 α 值，但会使峰形尖峰

分析条件优化流程图 (4-C)

4.分析条件继续优化

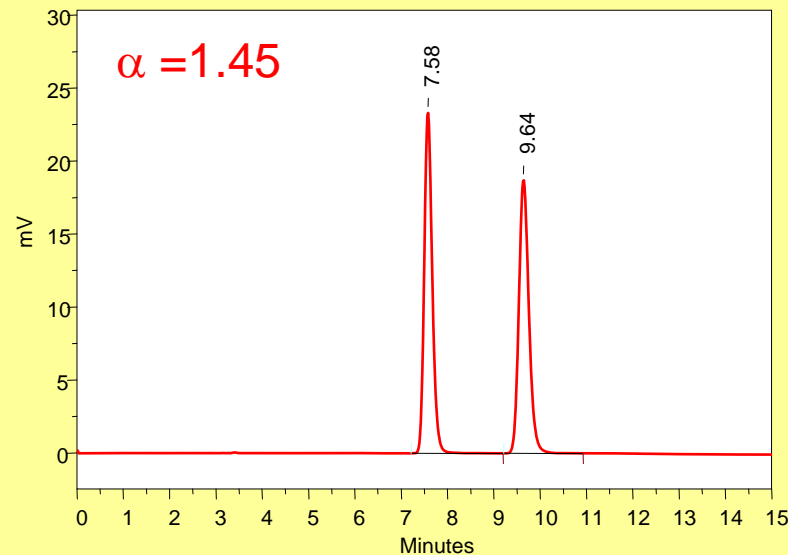


醇种类的影响



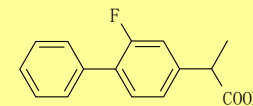
Hex./IPA/AcOH=90/10/0.1

Column: CHIRALPAK AD
Temp.: 40°C, Flow rate: 1.0ml/min.



Hex./EtOH/AcOH=95/5/0.1

Sample: Flurbiprofen



IPA和EtOH有时会使峰形和 α 值发生明显变化

正相柱分析条件优化的总结

1. 根据样品性质判断是否需要往流动相中加添加剂
2. 调整正己烷/醇的比例，从而选择适当的保留时间
3. 其它优化方法
 - a. 流动相的醇浓度
 - 降低醇的浓度能改善分离度，但会延长分析时间
 - b. 色谱柱温度
 - 降低柱温能增加 α 值，但会使峰形变宽
 - c. 流动相中醇的种类
 - 改换醇的种类(IPA \leftrightarrow EtOH)，有时会使选择性发生一些变化

内容提纲

1. 手性色谱柱分析条件的建立和优化

2-1 正相色谱

2-2 反相色谱

2. 手性色谱柱的自动筛选

3. 手性分析中的有关注意事项



RH系列（反相用）的规格

	流动相	耐压 (MPa)	温度范围 (°C)	pH范围
OD-RH	水溶液/有机溶剂*1 =90/10~0/100	10	5~40	2~7
OJ-RH				
AS-RH			5~40*2	2~9*2,3
AD-RH				

*1 有机溶液:醇（甲醇, 乙醇, 异丙醇）或乙腈

*2 pH>7使用时, 温度在5~25°C

*3 pH超过8时, 使用硼酸缓冲液

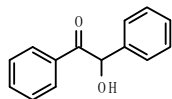
根据样品性质选择流动相

样品性质	OD-RH、OJ-RH	AD-RH、AS-RH
中性	水/乙腈	
酸性	磷酸水溶液(pH2)/乙腈 0.1M 磷酸缓冲液(pH2)/乙腈	
碱性	0.1M KPF_6 水溶液/乙腈 0.1M $NaPF_6$ 水溶液/乙腈	20mM 硼酸缓冲液(pH9)/乙腈 20mM 磷酸缓冲液(pH8)/乙腈

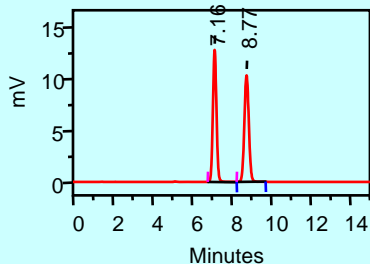
反相流动相的pH值对分离度的影响(AS-RH)

中性化合物

Benzoin

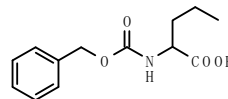


水溶液/CH₃CN=60/40
流速: 1.0ml/min.

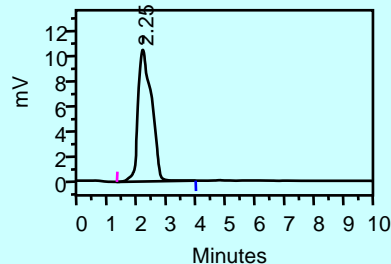


酸性化合物

N-CBZ-Norvaline

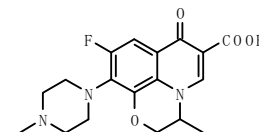


水溶液/CH₃CN=60/40
流速: 1.0ml/min.

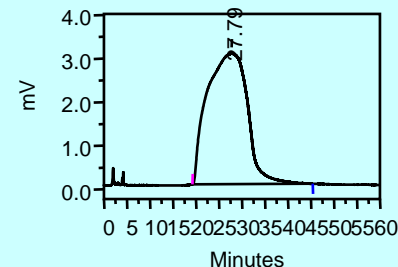


碱性化合物

Ofloxacin ethyl ester



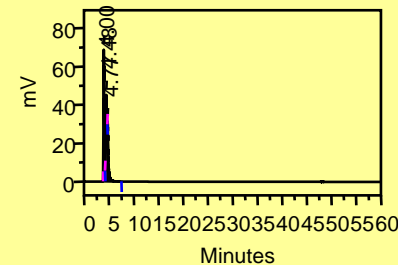
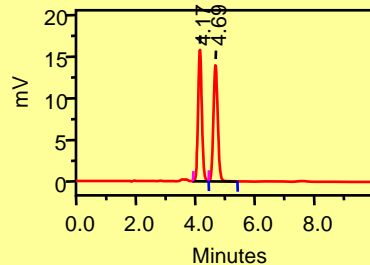
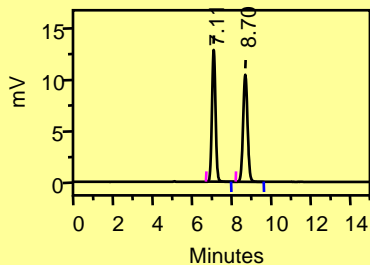
水溶液/CH₃CN=80/20
流速: 0.5ml/min.



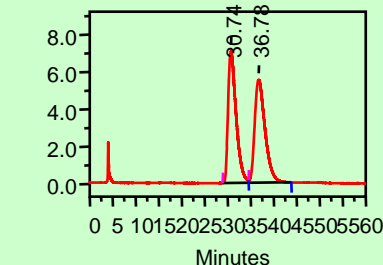
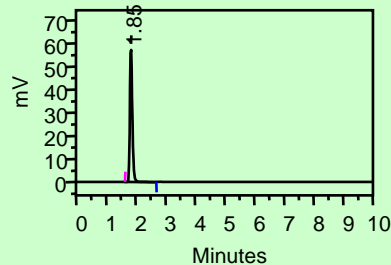
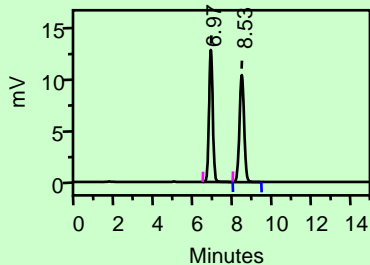
水溶液

水

磷酸水溶液
(pH2)

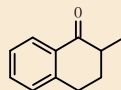


硼酸缓冲液
(pH9)

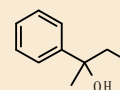


有机溶剂种类对分离的影响

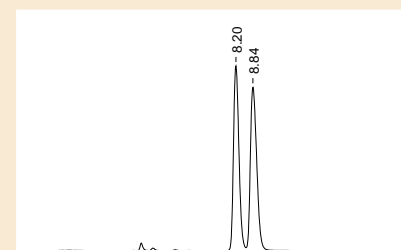
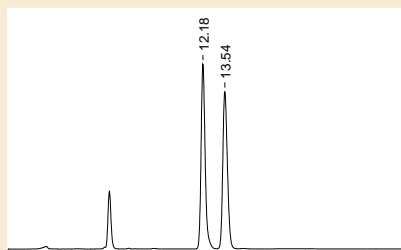
2-Methyl-1-tetralone



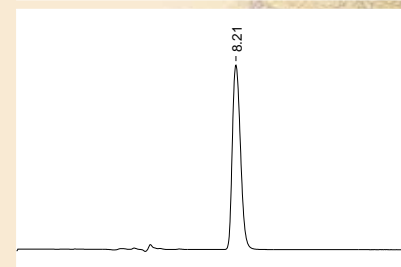
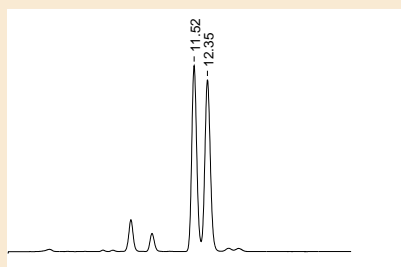
2-Phenyl-2-butanol



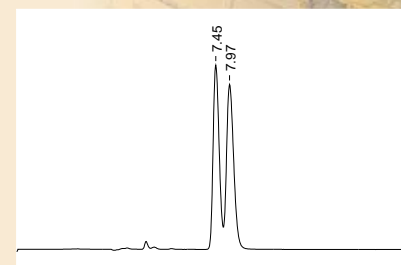
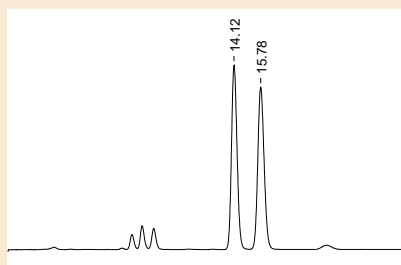
水/乙腈
=50/50



水/乙醇
=35/65



水/甲醇
=25/75



手性柱: CHIRALPAK AD-RH、流速: 0.5ml/min、温度: 25°C

反相柱分析条件优化的总结

1. 根据样品性质选择流动相

※中性，酸性样品对4种反相柱的流动相是一致的。

但是，碱性样品对OD-RH、OJ-RH的流动相和AD-RH、AS-RH的流动相是不同的。

2. 探索适当的保留时间和流动相组成

3. 其它优化条件

a. 水溶液和有机溶液比例

➤ 多数情况下降低有机溶液的浓度能改善分离，但会延长分析时间。

b. 色谱柱温度

➤ 降低柱温能增加 α 值，但会使峰形变宽。

c. 有机溶剂的种类

➤ 有机溶剂的种类不同，其洗脱强度也不同。有时其分离效果也发生一些变化



内容提纲

1. 手性色谱柱分析条件的建立和优化

2-1 正相色谱

2-2 反相色谱

2. 手性色谱柱的自动筛选

3. 手性分析中的注意事项



手性色谱柱组合筛选

ダイセルHシリーズ

キラルカラム® スクリーニングセット

CHIRALPAK® AD-H : 0.46cm I.D. × 15cmL
CHIRALPAK® AS-H : 0.46cm I.D. × 15cmL
CHIRALCEL® OD-H : 0.46cm I.D. × 15cmL
CHIRALCEL® OJ-H : 0.46cm I.D. × 15cmL

ダイセル化学工業株式会社
Chiralpak® Chiral Pharmaceutical Separators

手性色谱柱组合

CHIRALPAK® AD-H

CHIRALPAK® AS-H

CHIRALCEL® OD-H

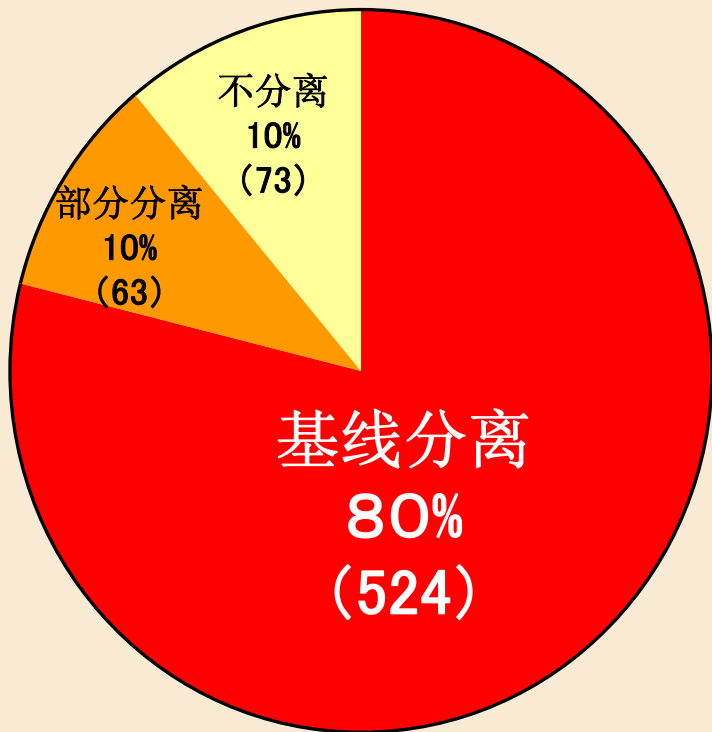
CHIRALCEL® OJ-H

手性色谱柱尺寸:

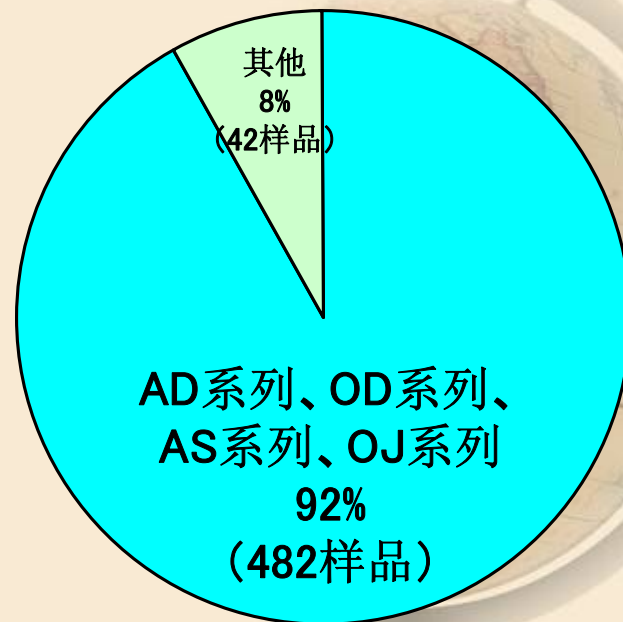
0.46cm I.D. × 15cmL

为何选用AD-H,AS-H,OD-H,OJ-H这4种柱子？

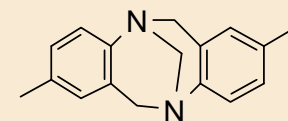
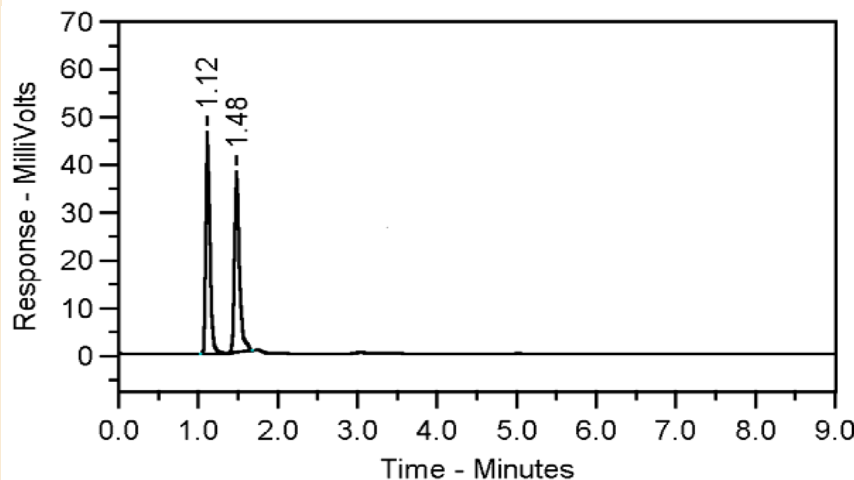
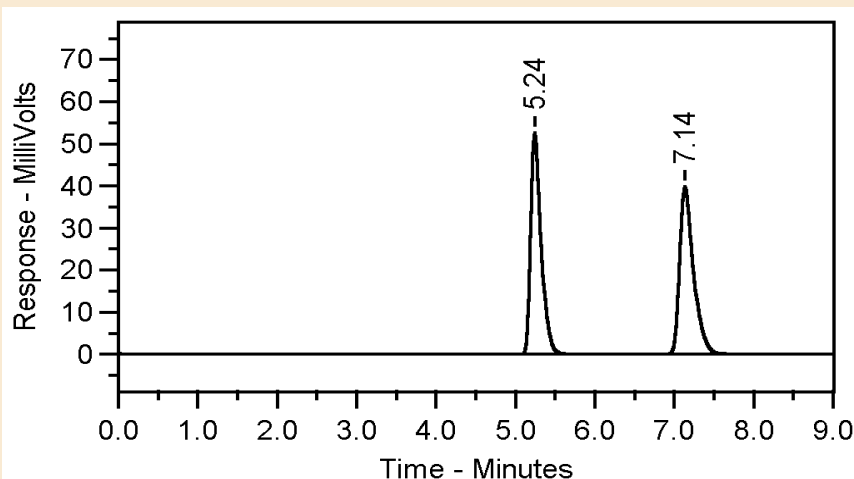
2000~2004年 客户样品分析 660个样品的分析结果



达到基线分离的手性柱比例：



为何选用15cm长度手性色谱柱呢？

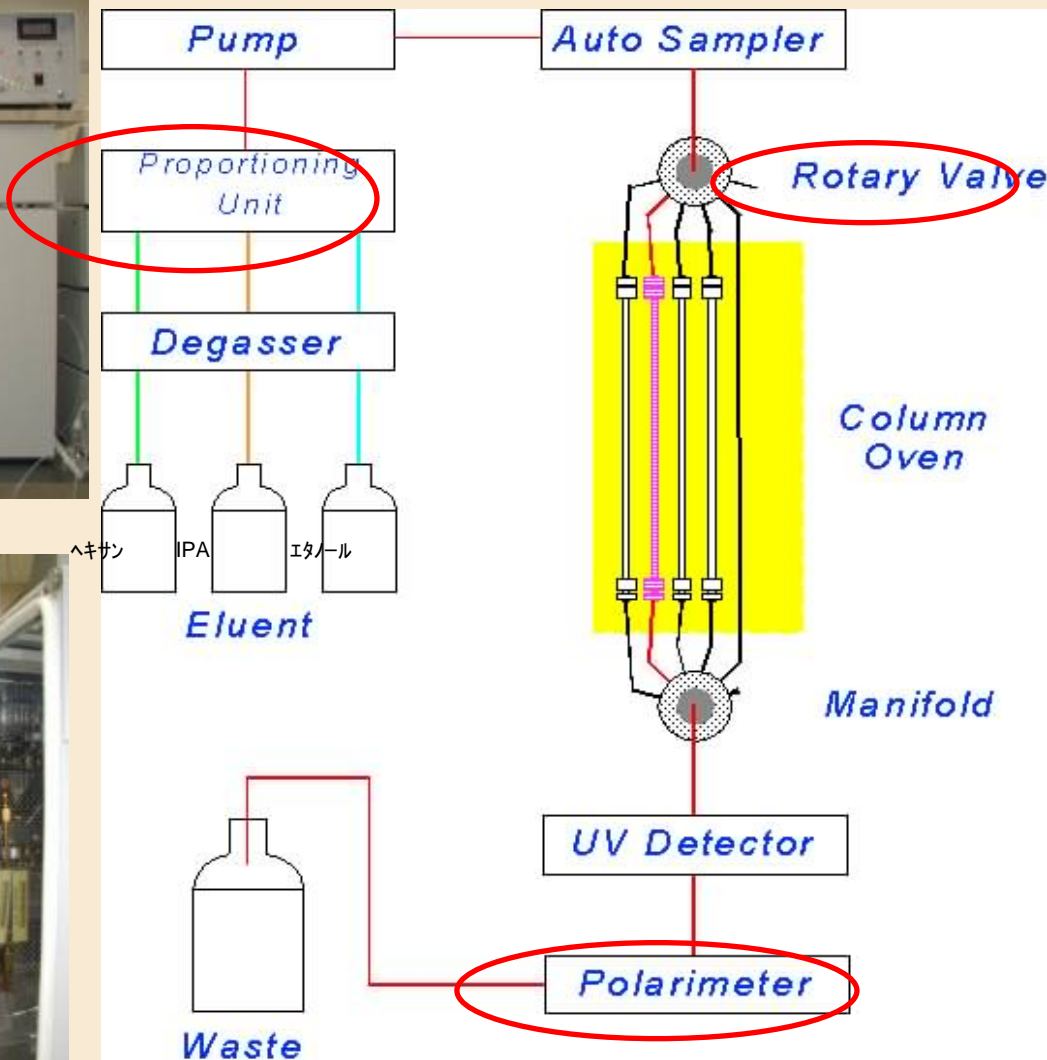


标准条件

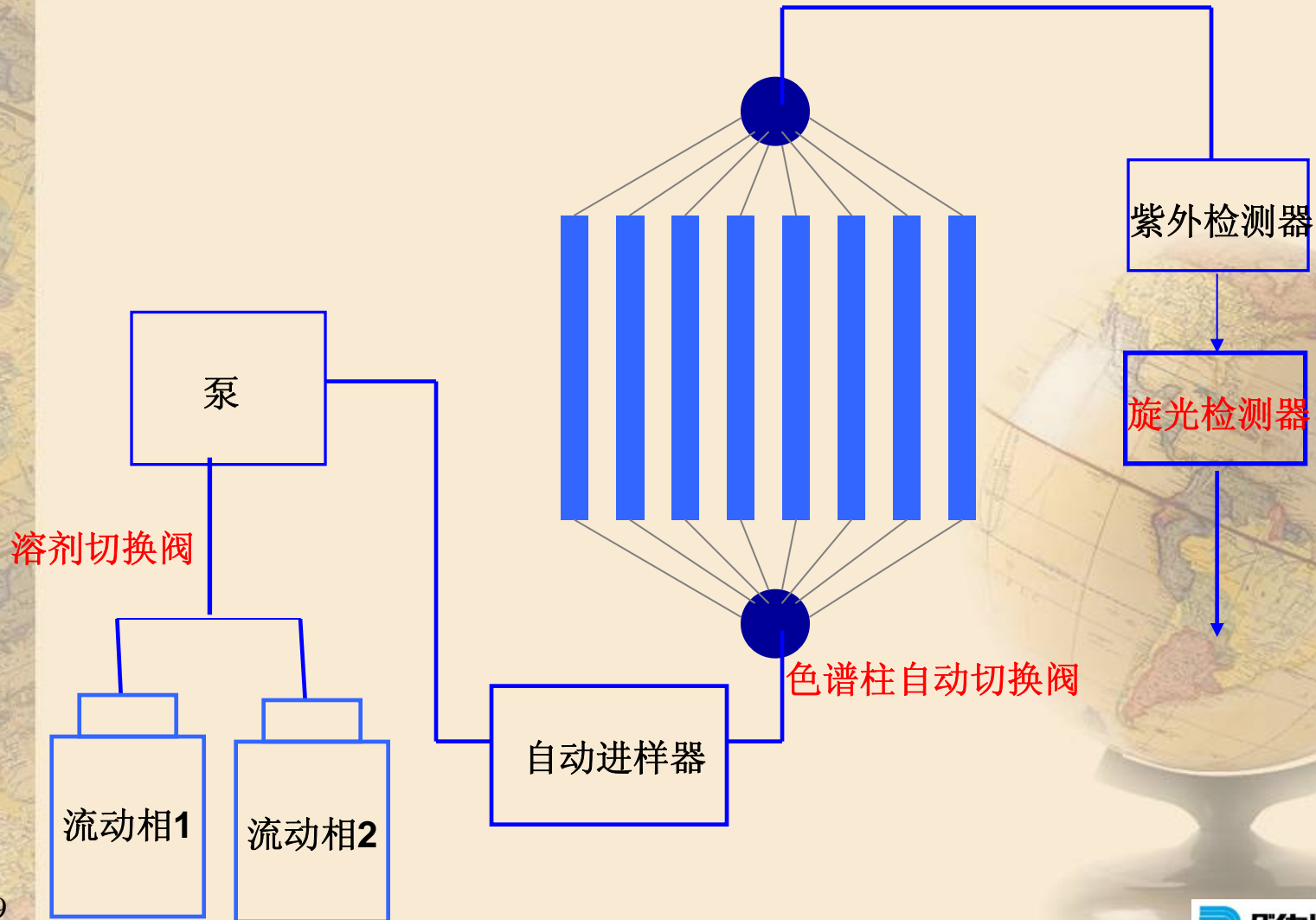
柱长 (15/25) × 流速 (1/3) =

分析时间短缩为 (1/5)

组合筛选设备

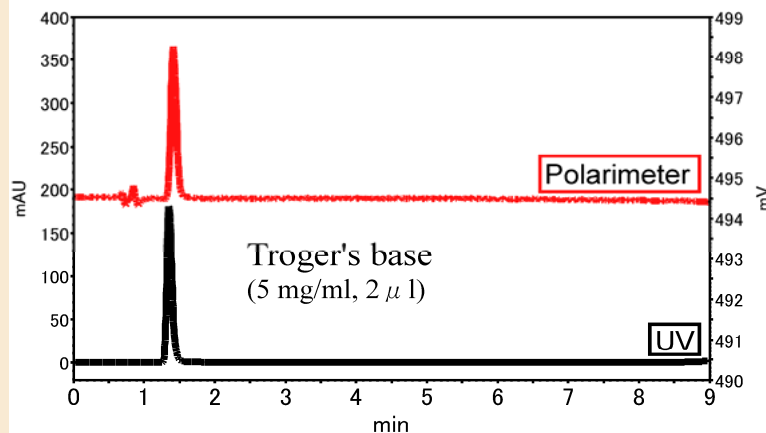
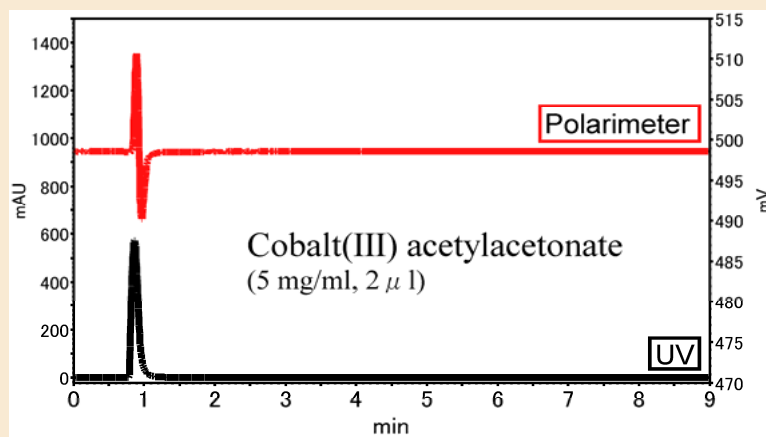


组合筛选设备示意图



串联旋光检测器的好处 1

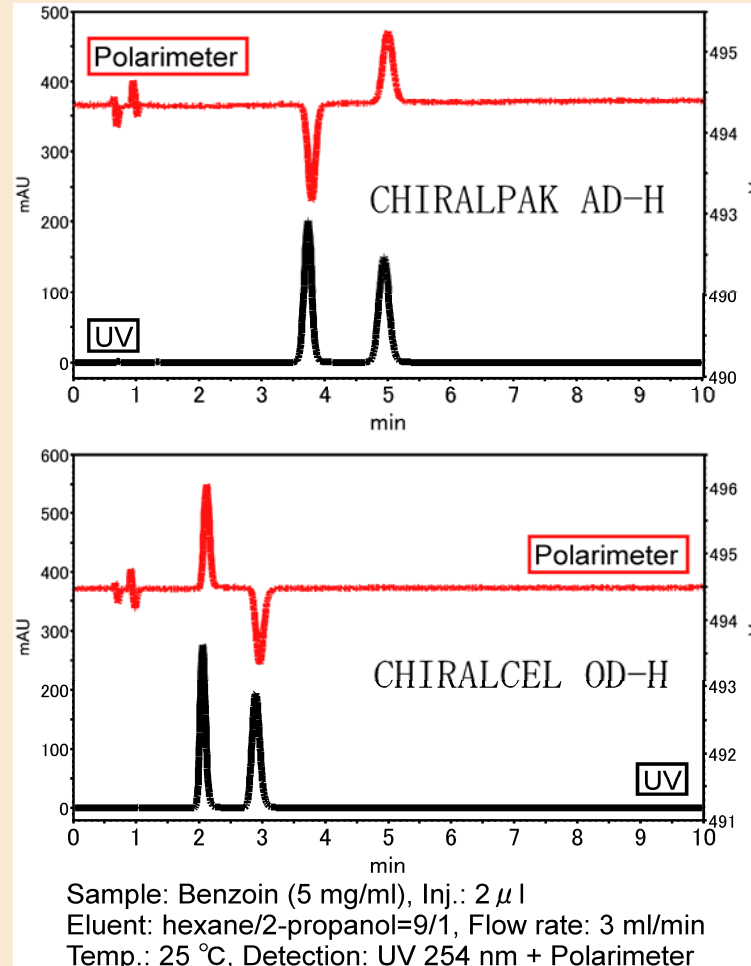
1. 用UV检测器只出现“1峰”的时候
→应用旋光检测器可确认其分离状况



Column: CHIRALCEL OJ-H
Eluent: hexane/2-propanol=9/1, Flow rate: 3 ml/min
Temp.: 25 °C, Detection: UV 254 nm + Polarimeter

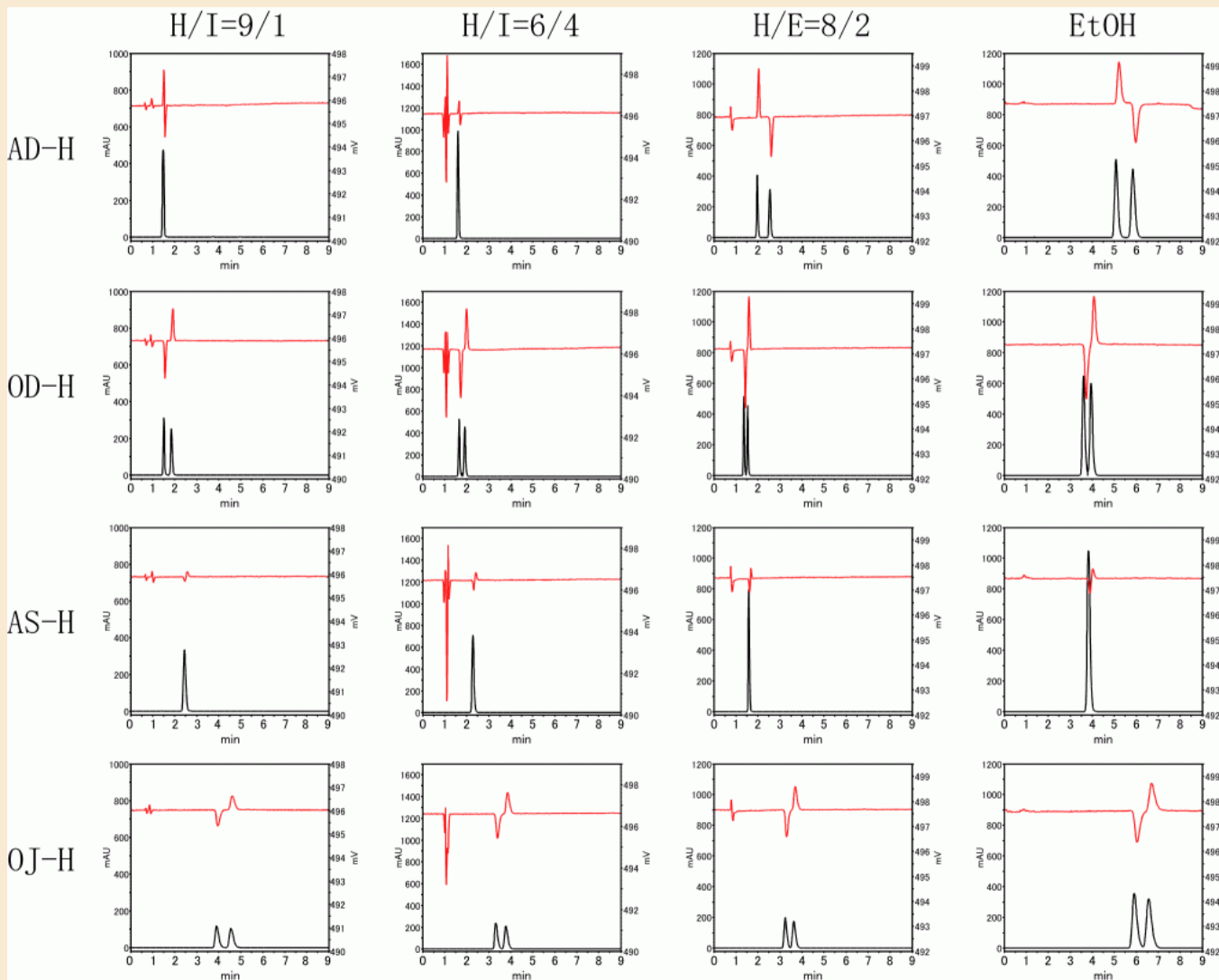
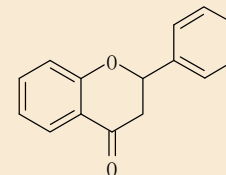
串联旋光检测器的好处 2

2. 可确认洗脱顺序

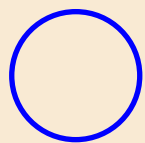


筛选结果示例

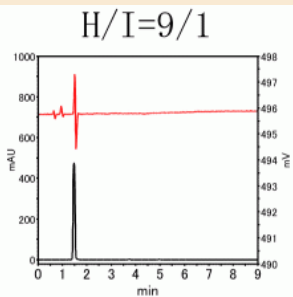
样品:



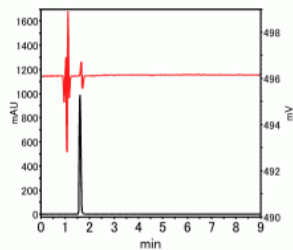
信息1: 判别具有分离可能性的色谱柱



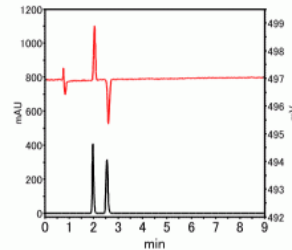
AD-H



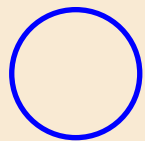
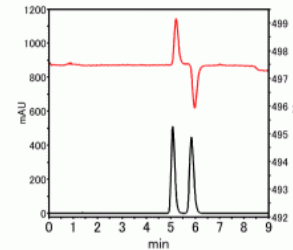
H/I=6/4



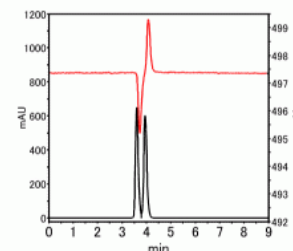
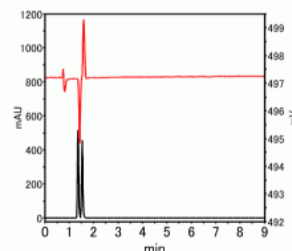
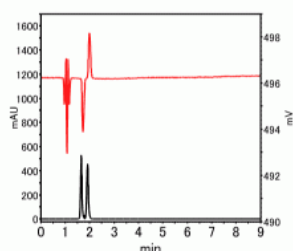
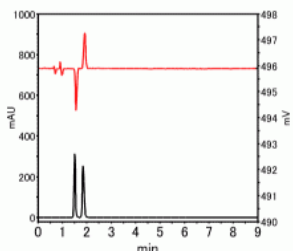
H/E=8/2



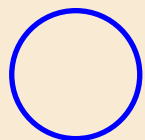
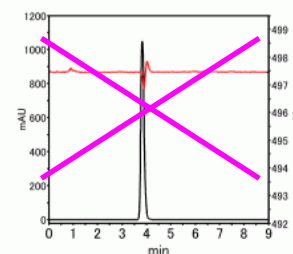
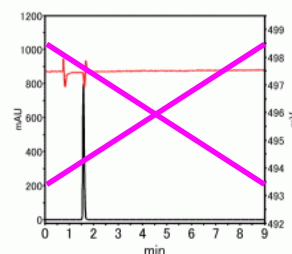
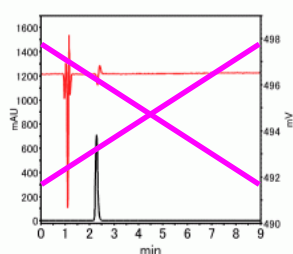
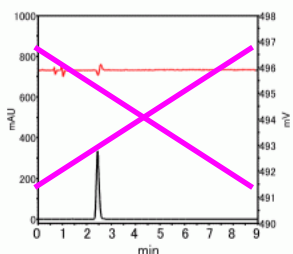
EtOH



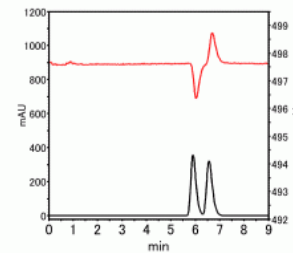
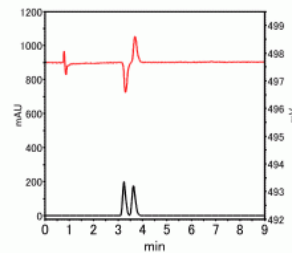
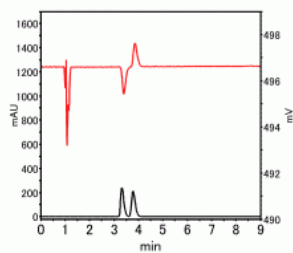
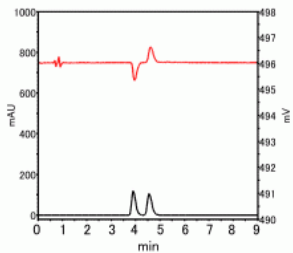
OD-H



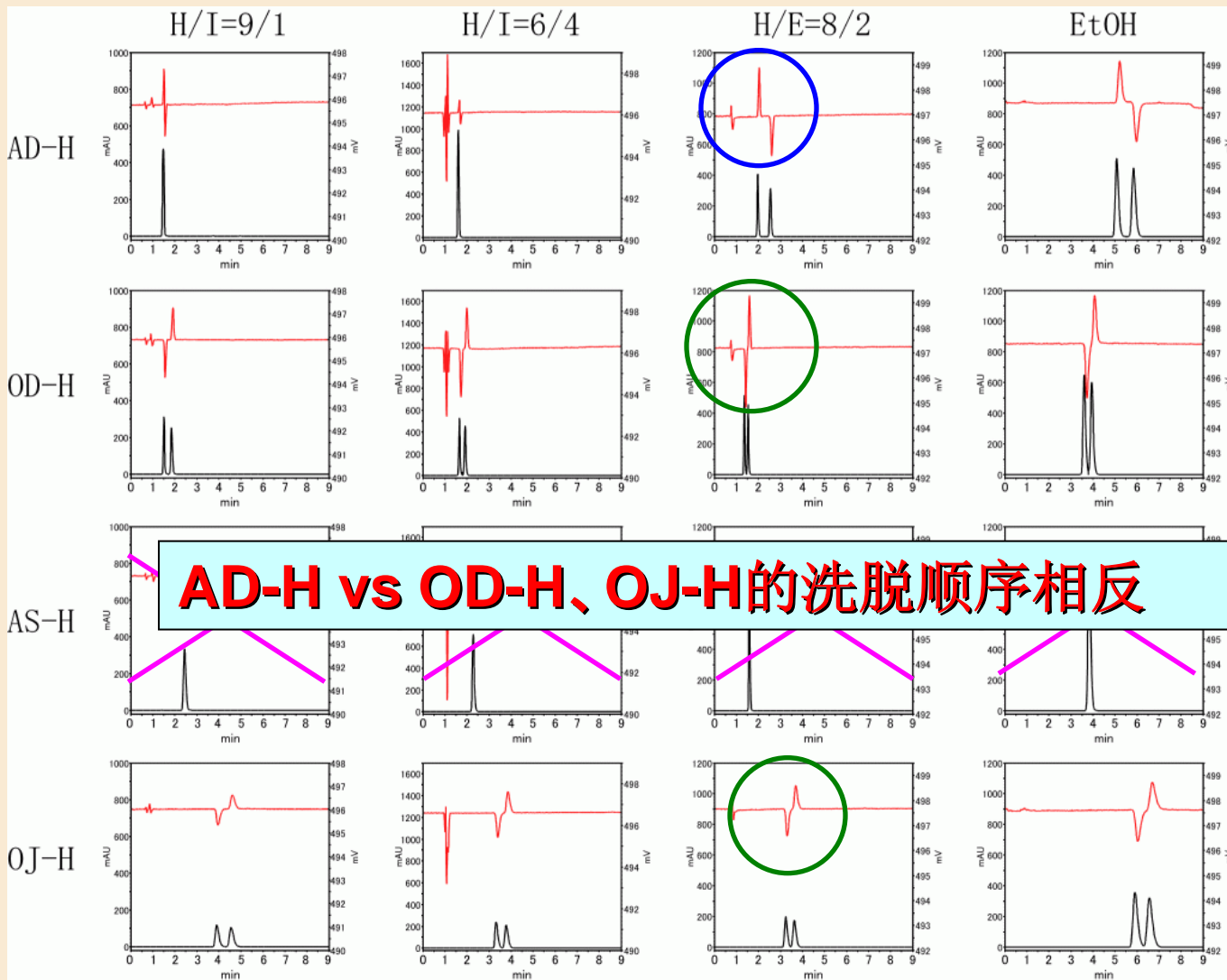
AS-H



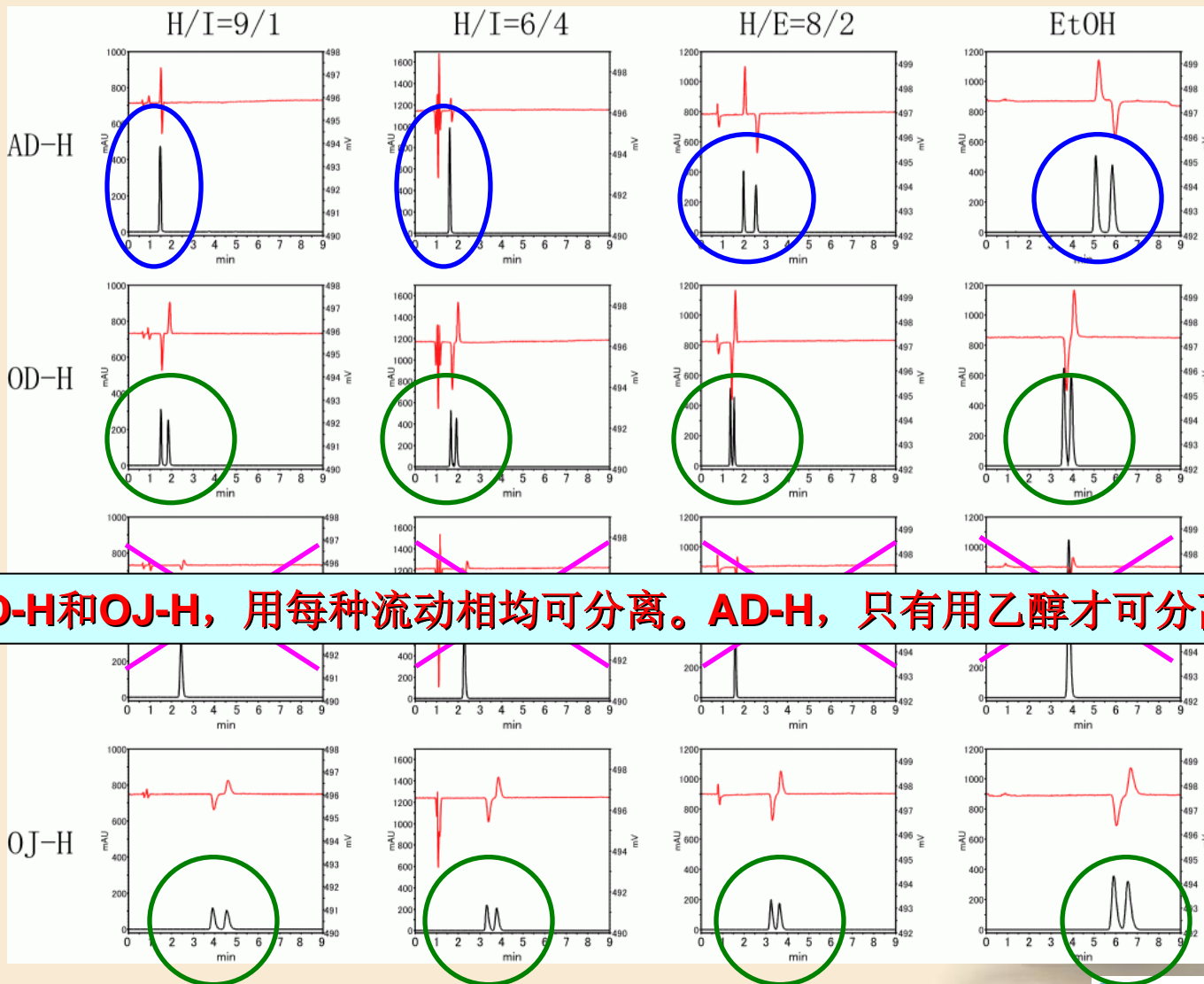
OJ-H



信息2: 色谱柱和洗脱顺序

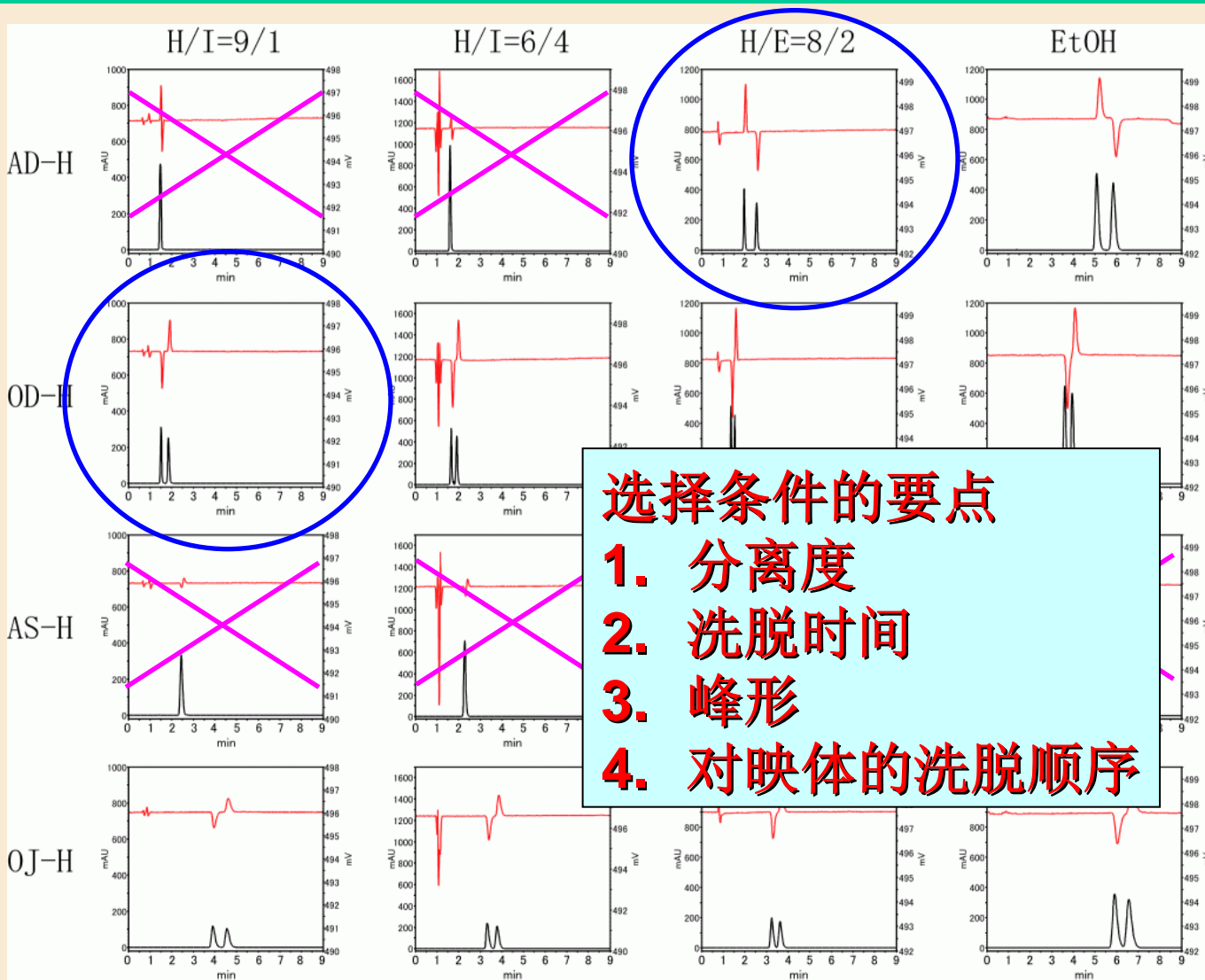


信息3: 醇的种类对分离的影响



OD-H和OJ-H，用每种流动相均可分离。AD-H，只有用乙醇才可分离。

分析条件的选择



若筛选没得到理想的分析条件时

1. 选择最接近基线分离的条件
2. 调整流动相中醇的比例
3. 调整流速
4. 调整温度
5. 变更为同种类长度为**25cm**的色谱柱。
6. 尝试其他的色谱柱



应用组合筛选设备探索分析条件总结

1. 使用具有广普分离能力的的**4**种手性色谱柱
2. 使用适合高流速的**15cm**的手性色谱柱
3. 组合应用**2**种醇，考察其分离情况
4. 和旋光检测器并用，可一次性获得更多的判断信息



内容提纲

1. 手性HPLC分析条件的建立和优化

2-1 正相色谱

2-2 反相色谱

2. 手性色谱柱的自动筛选

3. 手性分析中的注意事项



常见使用故障=溶剂错误 1

- **Daicel**大部分手性柱是涂敷型产品，在市场上具有独特性。
- 若导入错误的溶剂(**CH₂Cl₂**、乙酸乙酯、甲苯等)，柱子会马上被破坏。
- 当流动相或样品溶液导入错误的溶剂时，柱压剧增，分离突然变差。
- 一旦错误地使用了溶剂，重新恢复柱子性能的可能性很低。
- 若您遇到此类分析故障，请对照出厂报告重新确认柱子的性能或与大赛璐联系

常见使用故障=溶剂错误 2

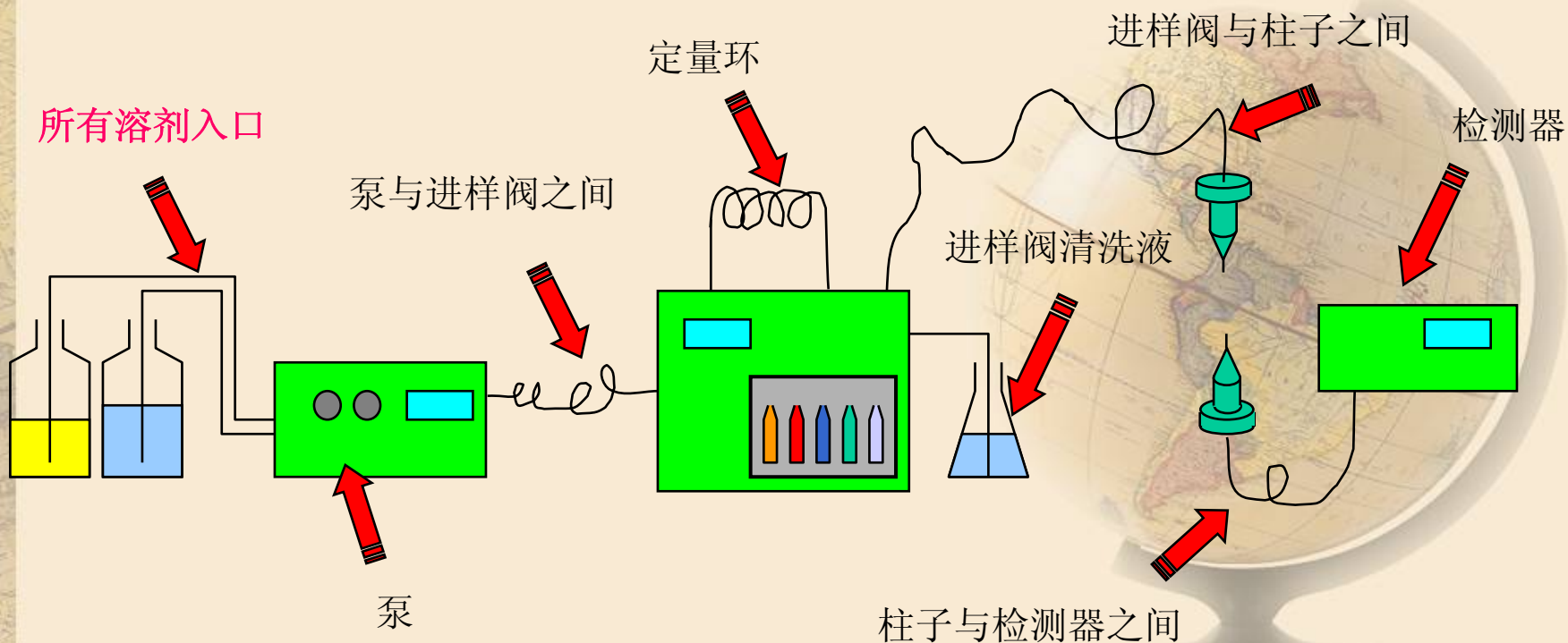
- **Daicel**正相手性柱的流动相和样品溶液不能使用水溶液
- 若不小心导入水，柱子不会彻底受损。
- 只需要使用色谱级无水乙醇低流速将水置换出来。



使用前彻底清洗管路

将手性柱接入液相色谱仪之前

1. 用异丙醇将HPLC所有管路彻底清洗。
2. 用流动相将HPLC所有管路彻底清洗。



压力

后果:

- 长期超压会引起柱头塌陷，反压上升，柱效下降。

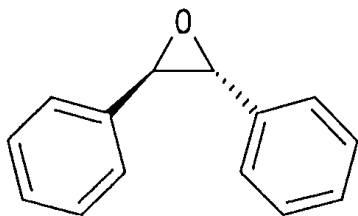
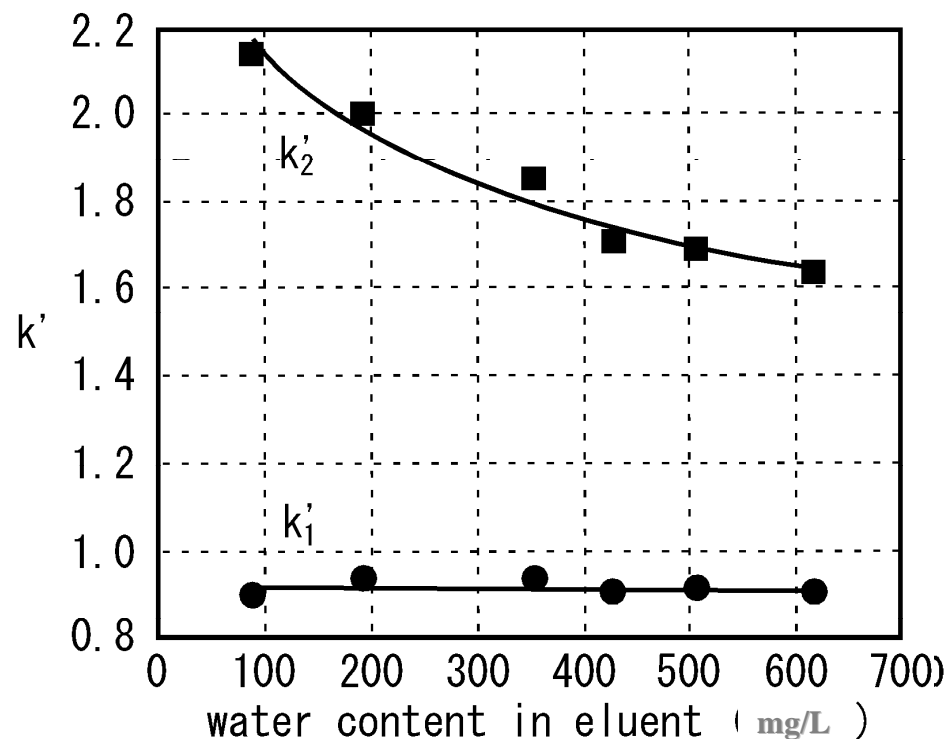
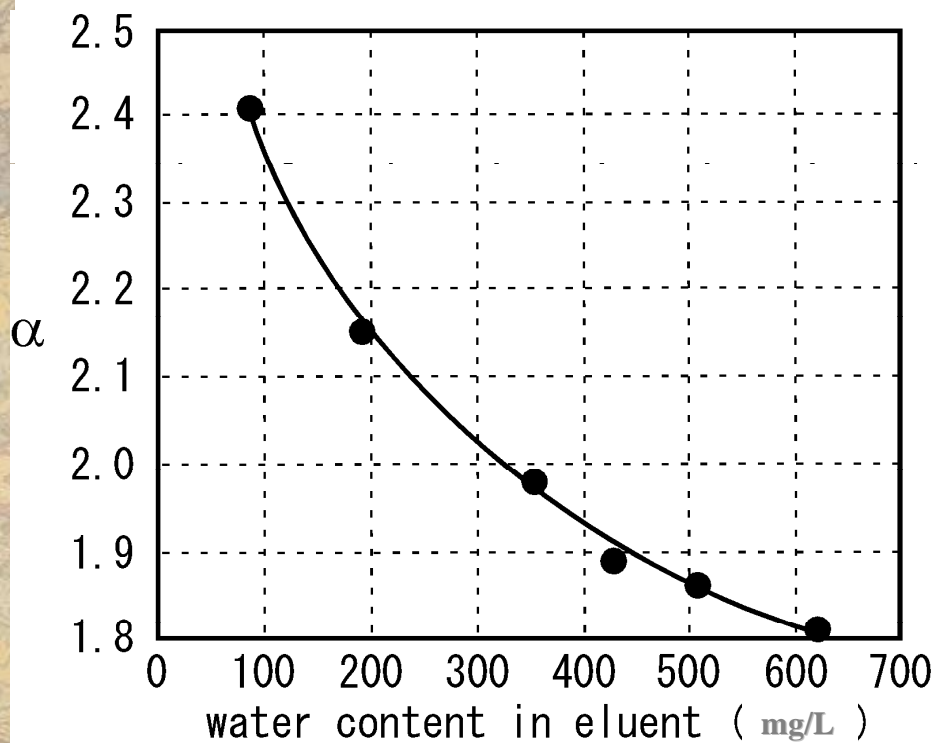
原因:

- 流动相或样品溶液过滤不彻底，固体颗粒堵塞管路或柱头，会引起柱压过高。
- 样品中成分在柱头析出或强烈吸附，会引起柱压升高。
- 流速过快，会引起柱压过高

解决方法:

- 每种柱子有特定的承压范围，请仔细参照相应的《色谱柱说明书》。
- 彻底过滤流动相及样品溶液。
- 样品预处理
- 更换流动相时，或者刚把柱子刚接上仪器的时候，逐步增加流速

流动相中水含量的影响



Conditions;

Column: CHIRALCEL OD-H

Flow rate: 1.0 ml/min

Sample conc.: 1.0 mg/ml

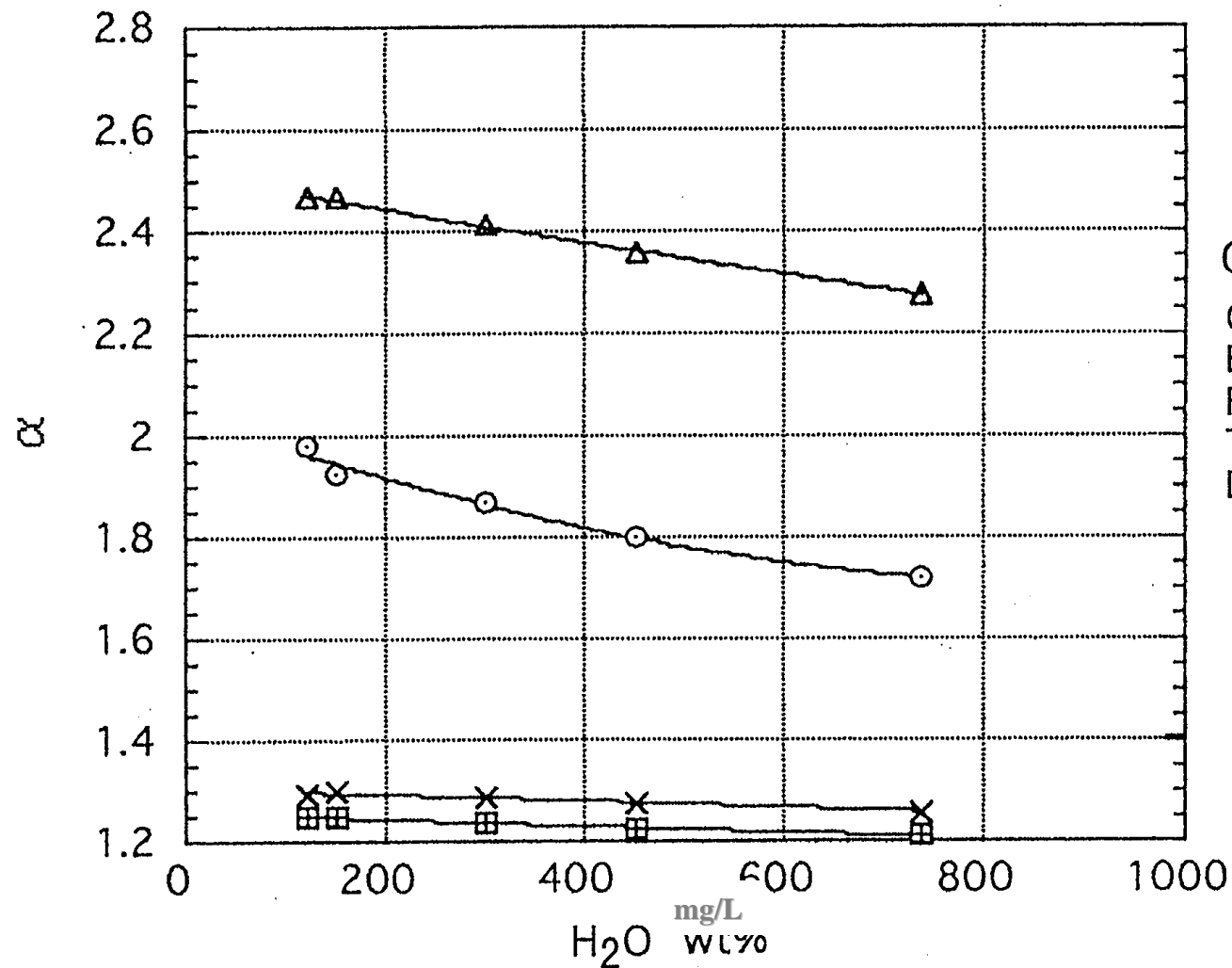
Detect.: UV 254 nm

Eluent: Hexane / IPA = 9 / 1

Temp.: 25 °C

Inj. volume: 5 μ l

流动相中水含量的影响



Conditions;

Column: CHIRALPAK AS

Eluent: Hexane/IPA = 9/1

Flow Rate: 1.0 ml/min

Temp.: 25 °C

Detect.: UV 254 nm

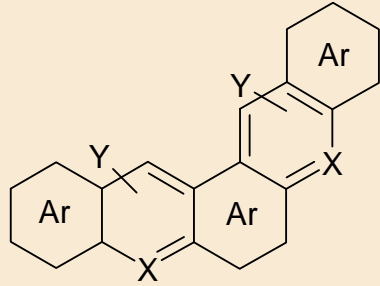
○ 2,2,2-Trifluoro-1-(9-anthryl)ethanol

× trans-Stilben oxide

△ troger's base

□ 2-Phenyl-cyclohexanone

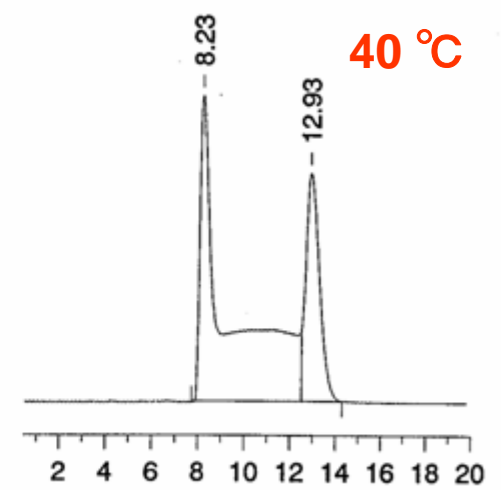
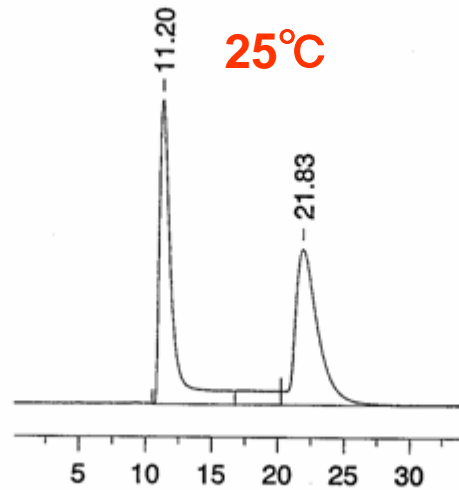
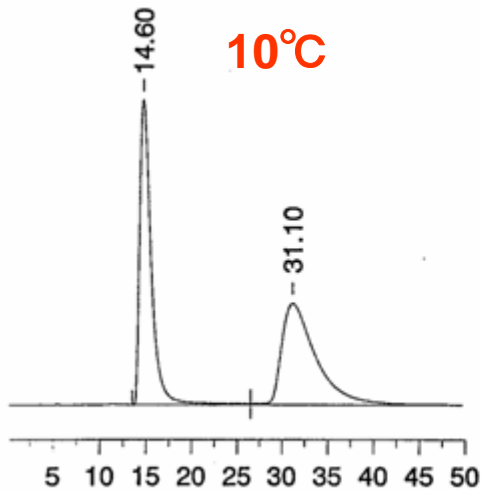
峰变形 (消旋化)



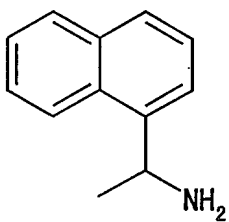
CHIRALCEL OJ

正己烷/乙醇=90/10

1.0 ml/min, 275 nm



添加物残留效应



Conditions;

Column: CHIRALCEL OJ

Flow rate: 1.0 ml/min

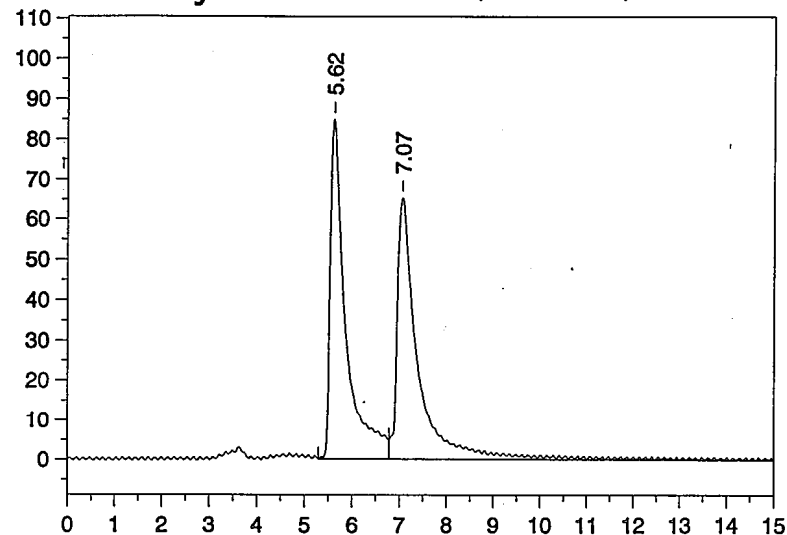
Temp.: 25 °C

Detect.: UV 254 nm

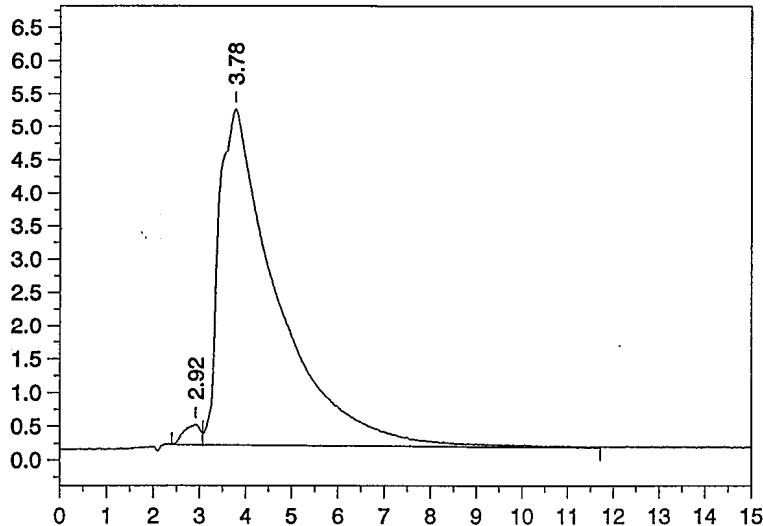
Sample conc.: 1.0 mg/ml

Inj. volume: 5 μ l

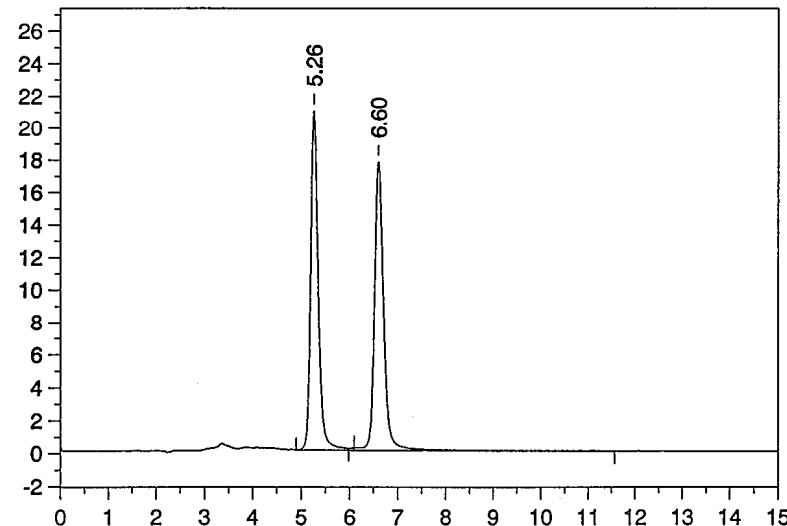
New Column,
analyzed with Hex/IPA=9/1



After flushing with Hex/IPA=9/1+0.1% TFA,
analyzed with Hex/IPA=9/1



After flushing with Hex/IPA=9/1+0.1% TFA,
analyzed with Hex/IPA=9/1+0.1% DEA



如何避免“残留效应”

- 残留物在普通流动相中可长时间地稳定保留
- ➡ 用醇洗涤可除去大部分的碱性残留物
- ➡ 用酸洗涤也可除去碱性残留物
- ➡ 用碱洗涤可除去酸性残留物
- ➡ 或者分开使用酸性条件手性柱和碱性条件手性柱



大赛璐（中国）手性技术服务中心



位于上海有机化学研究所内

1. 开发手性分析方法
2. 为用户制备手性化合物
3. 大赛璐手性柱售前售后服务



大赛璐（中国）手性技术服务中心

1. 开发手性分析方法

如果您想买一根手性柱来分析某个样品，但是吃不准买哪个型号



免费筛选，优化



最佳手性柱，最佳分析条件



用户放心购买合适的手性柱



大赛璐（中国）手性技术服务中心

2. 为用户制备手性化合物

如果您想得到光学纯的手性化合物，手头没有手性制备柱



免费评估，可行性实验，成本核算



用户请大赛璐制备手性化合物或者购买制备柱



大赛璐（中国）手性技术服务中心

2.大赛璐手性柱售前售后服务

如果您想购买或者购买了大赛璐的手性柱



免费技术咨询，疑问解答



用户放心使用大赛璐手性柱



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北京崇文区左安门内大街8号伟图大厦301室 PC: 100061

Tel : 010-67136152/67100708 Fax : 010-67114016/67113925

http: //www.jinouya.cn

E-mail: china.hplc@163.com

