

● 赛默飞液相色谱柱产品选择与推荐

胡学桥 18516519922

Xueqiao.hu@thermofisher.com

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色谱柱的正确认识与选择

**2**

色谱柱的针对性选择

**3**

色谱柱常见问题与维护

# Thermo Scientific 色谱柱系列概览

## LC Columns

Accucore

Acclaim

Hypersil GOLD

Synchronis

Hypercarb

Legacy Hypersil

## Bio Columns

MABPac

ProPac

DNAPac

GlycanPac

ProSwift

DNASwift

## IC Columns

IonPac AS

IonPac CS

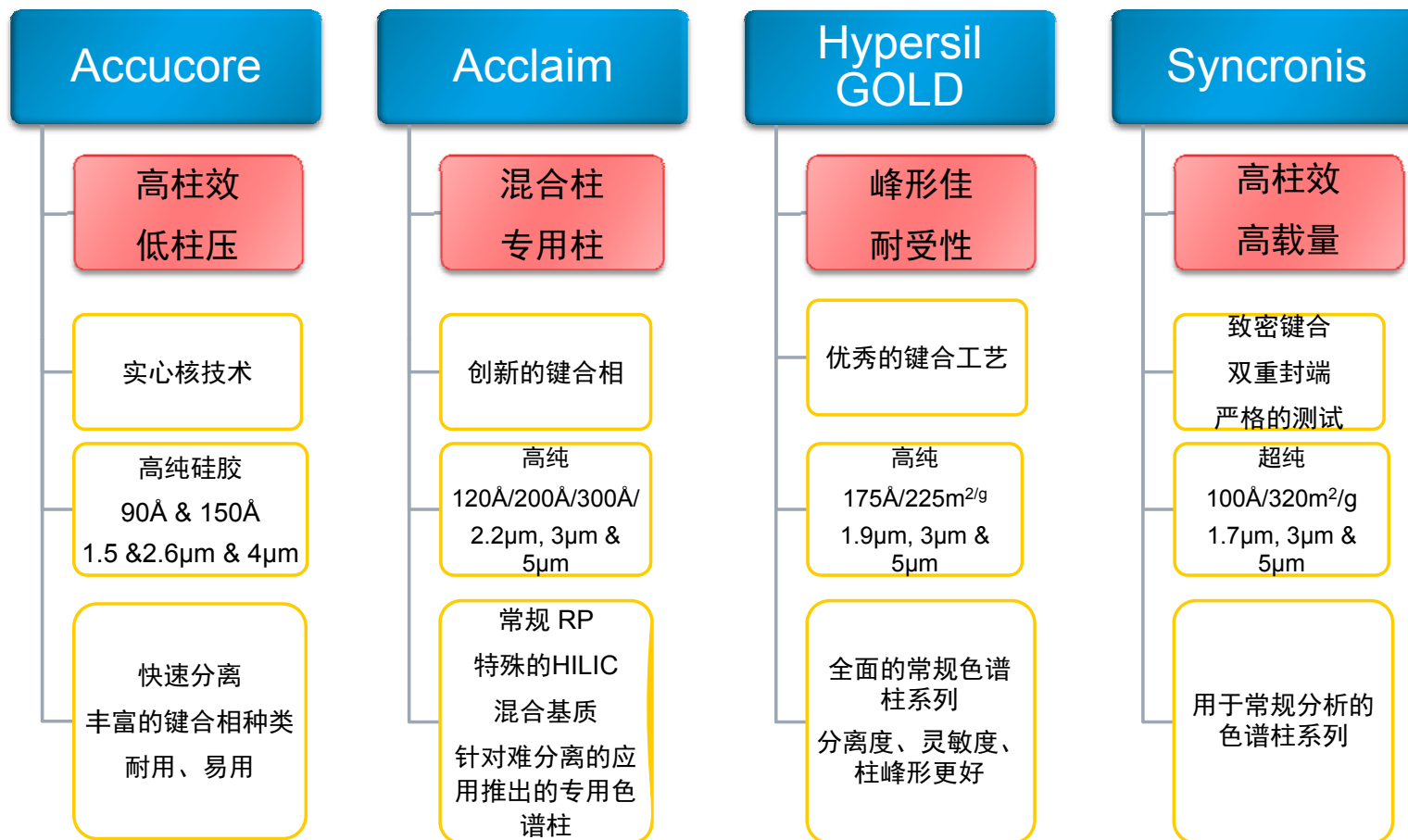
CarboPac

AminoPac

IonSwift

# 旗舰液相色谱柱系列

—听从分析工作者的心声







## 填料的正确认识与选择

硅胶+键合相

——选择合适的键合相是建立方法的首要任务

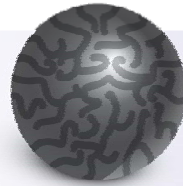
Large, irregular particles



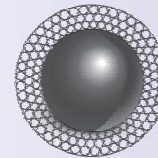
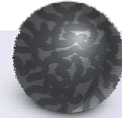
5µm particles



3µm particles

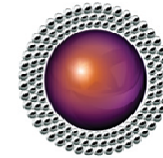


Sub-2µm particles

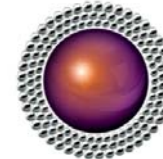


Solid Core

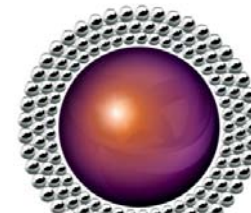
Solid Core Particles



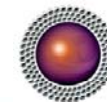
2.6 µm  
80 Å



2.6 µm  
150 Å

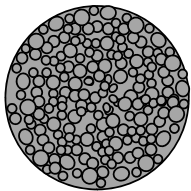


4 µm  
80 Å

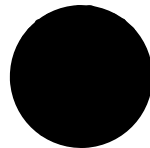


1.5 µm  
80 Å

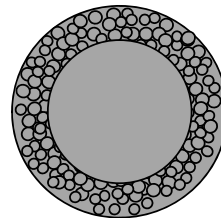
全多孔



无孔



表面多孔

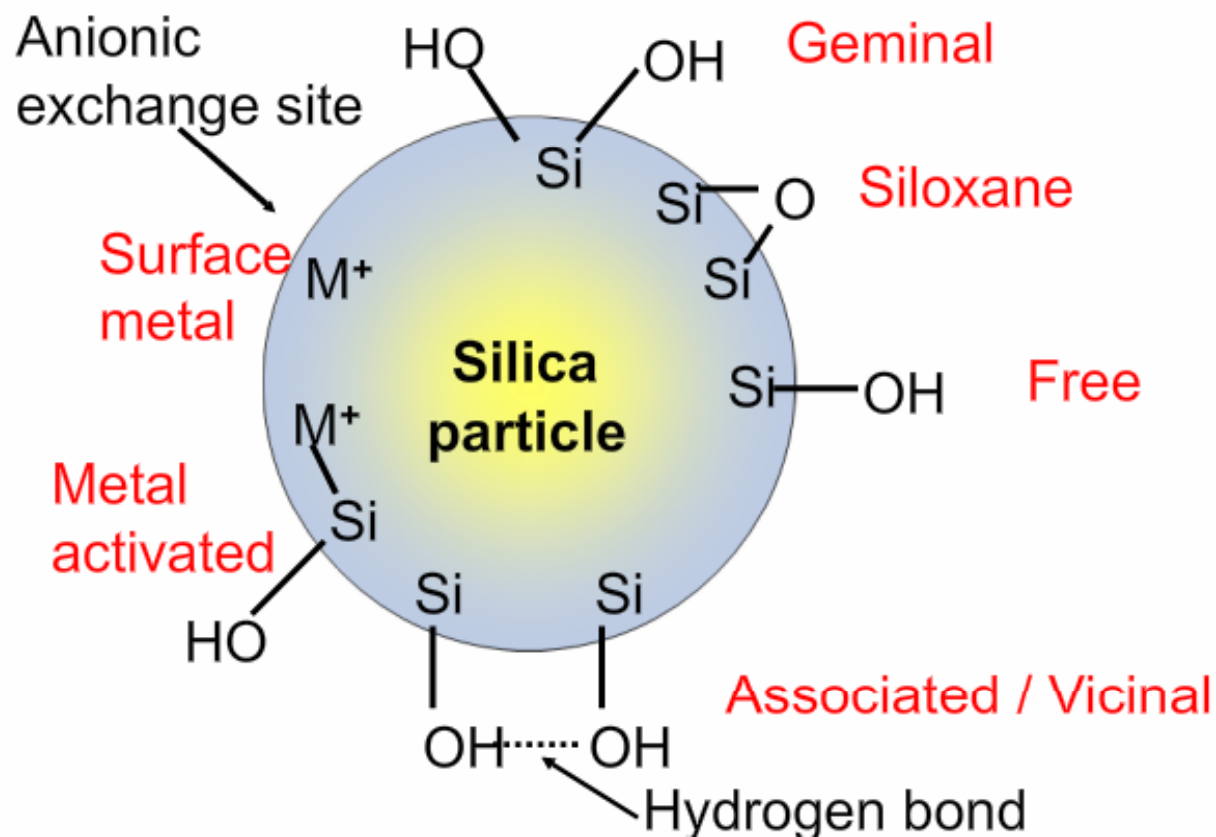


上样量大，通过减小粒径增加柱效，柱压升高

上样量小分离效率高，柱压高

上样量大，分离效率高，柱压较低

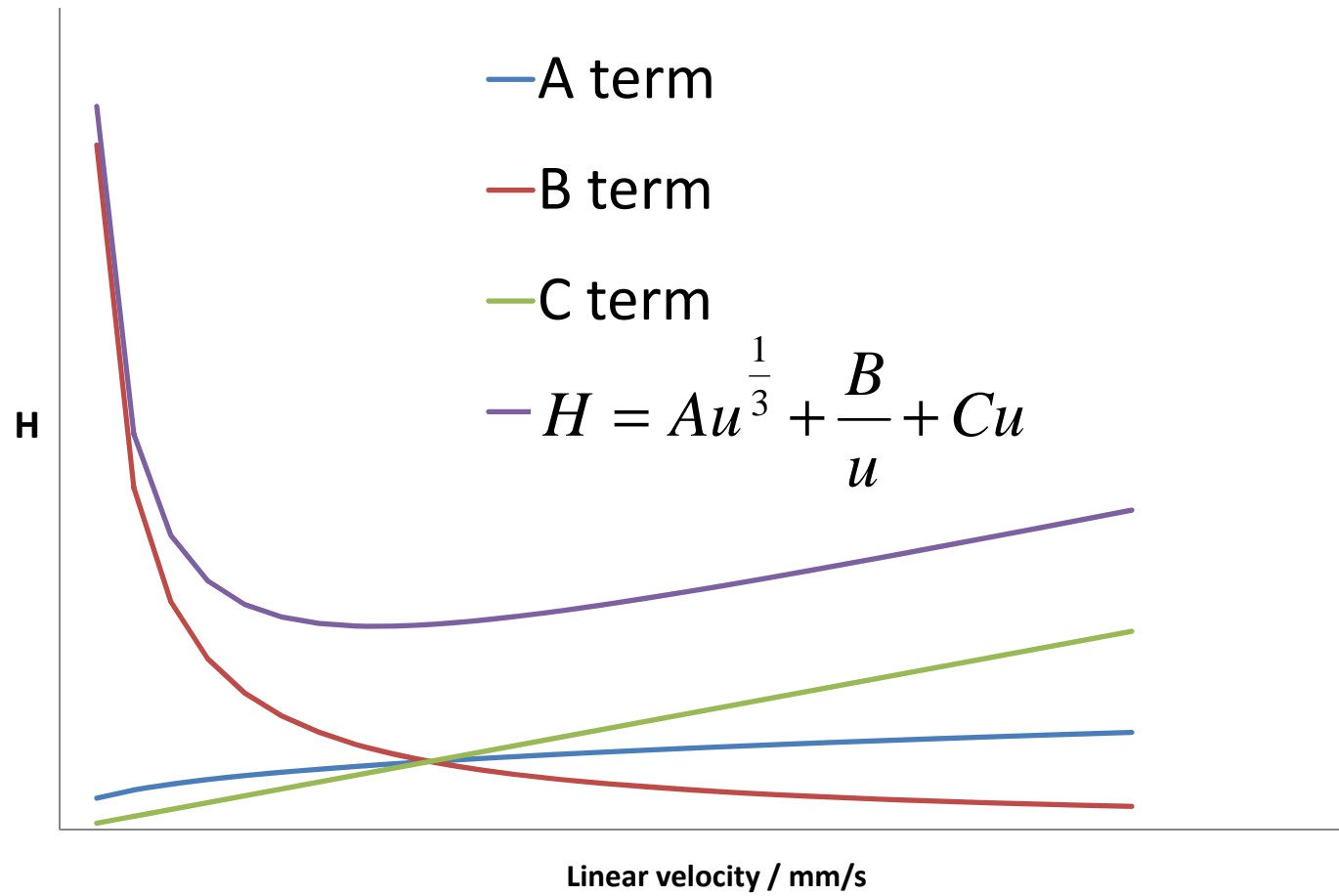
# 物理参数



## 物理参数

	Mg	Fe	K	Na	Ca	Ti	Al
Hypersil	31	240	10	2000	27	57	250
Hypersil BDS 10		88	10	1350	10	28	130
HyPurity	<1	<20	<1	<5	<1	<1	<1
GOLD	<1	<20	<1	<5	<1	<1	<1
Synchronis	<1	<10	-	<2	<1	<5	<2
Accucore	<1	<20	<1	<5	<1	<1	<1
Acclaim	<1	<20	<1	<5	<1	<1	<1

# 理论基础

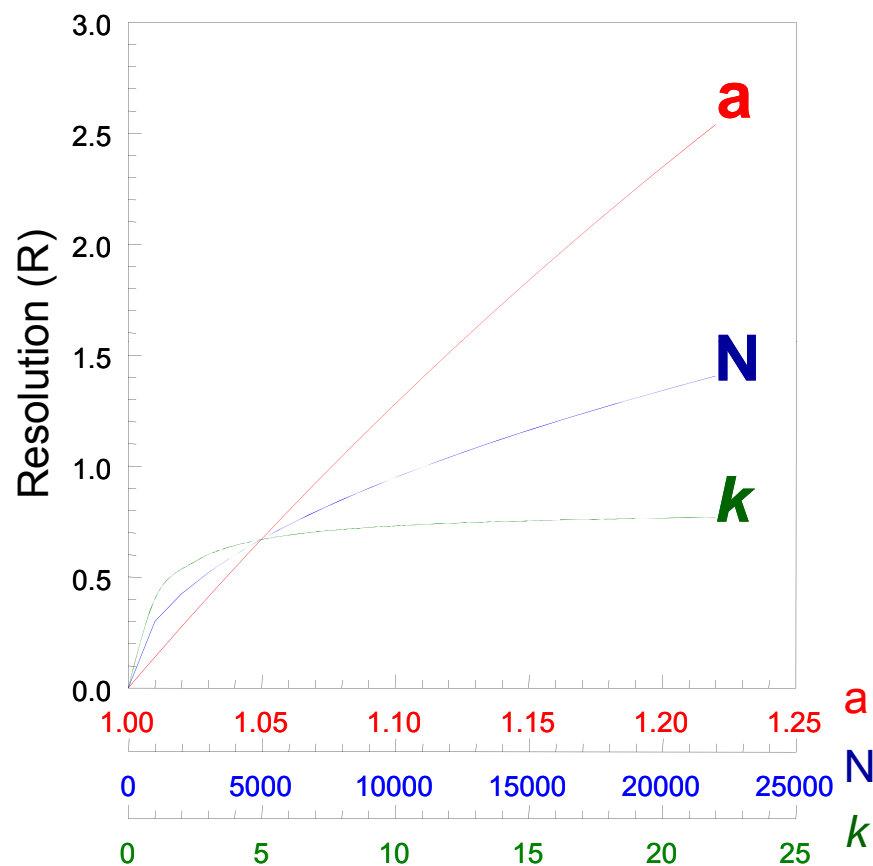


# 选择性和分离度的影响因素

柱效                  保留值          选择性

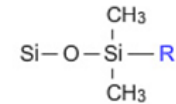
↓                          ↓                          ↓

$$R = \frac{\sqrt{N}}{4} \cdot \frac{k}{k+1} \cdot \frac{a-1}{a}$$

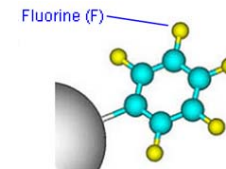
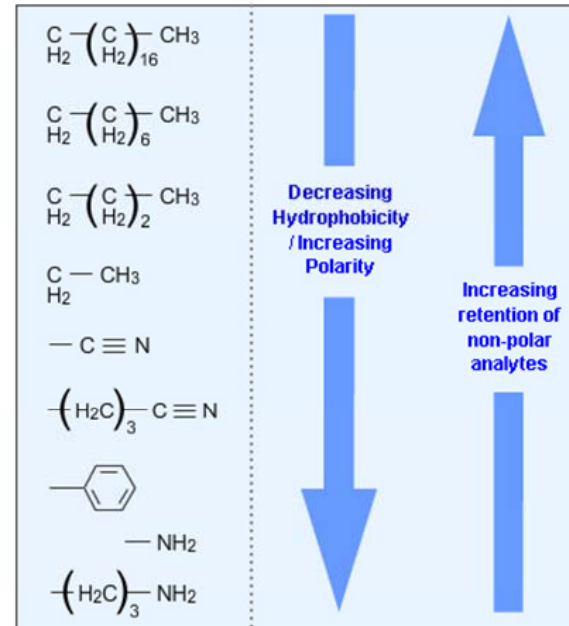


# 典型的常见键合相

- 烷基: C18, C8, C4, C1
  - 选择性相同, 疏水性/保留依次降低
  - 色散作用力
- 芳香基团
  - Phenyl, Phenyl-Hexyl, perfluorinated
  - 偶极,  $\pi$ - $\pi$ , 氢键
- 其它
  - Cyano, amino, polar embedded
  - 偶极, 氢键



Where R =

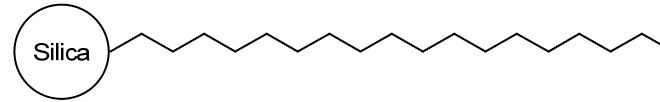


Perfluorinated

# 键合相对选择性的影响

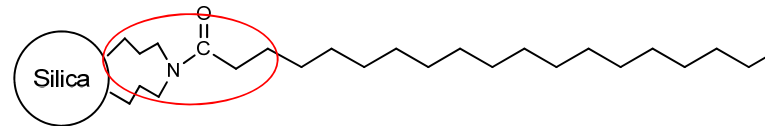
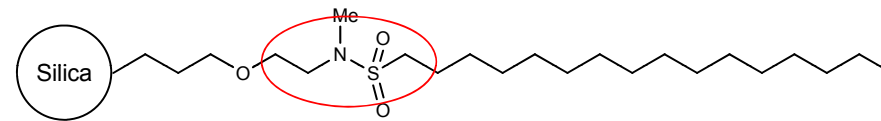
- 键合相类型

- C18



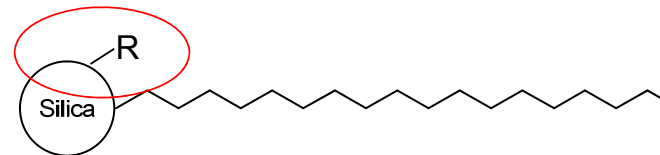
- 极性嵌入

- Acclaim PolarAdvantage
- Acclaim PolarAdvantage II
- Accucore Polar Premium



- 极性封端

- Accucore AQ
- Hypersil GOLD AQ
- Synchronis AQ

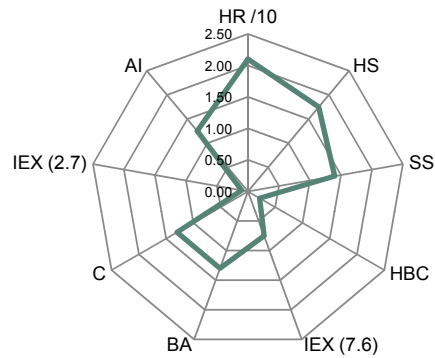




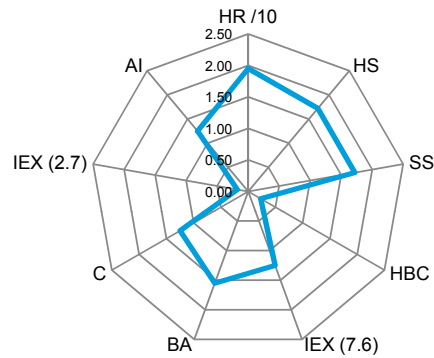


# 常见的C18键合相

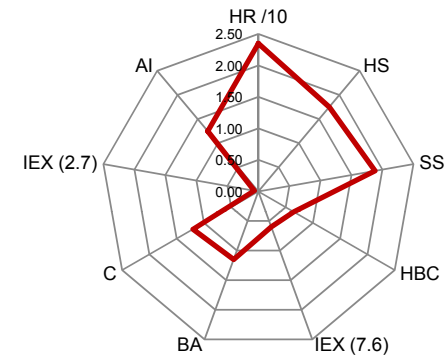
Accucore C18



Accucore aQ



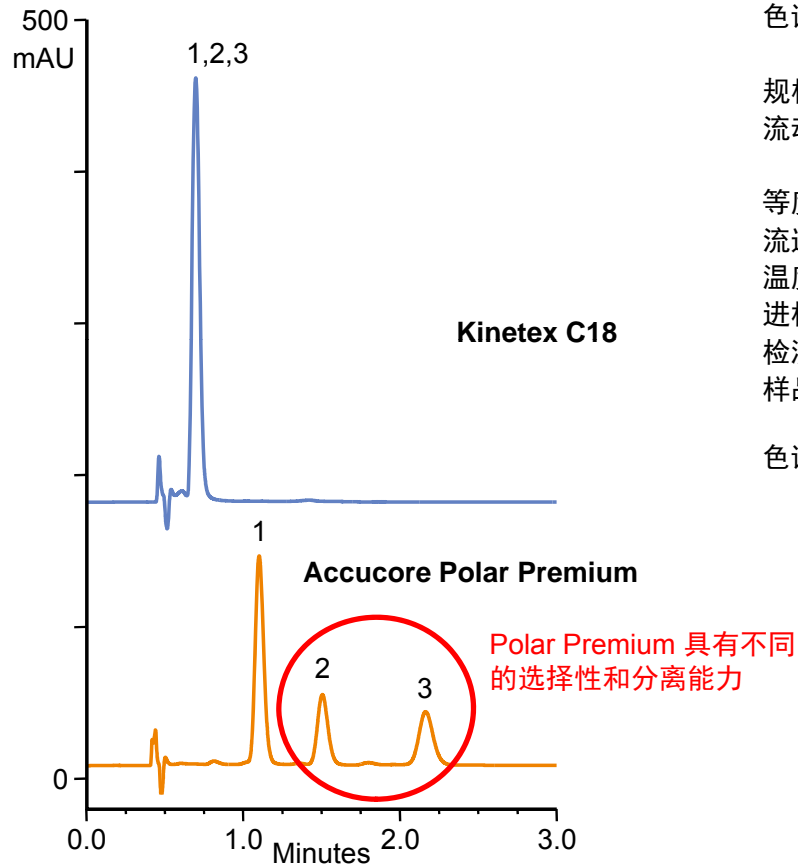
Accucore Polar Premium



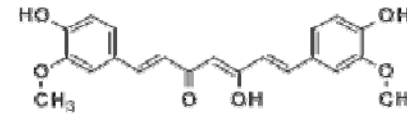
Polar Premium 的选择性与 C18 和 aQ 均不同

Polar Premium 具有更高的疏水保留性能

# 姜黄素类化合物(姜黄)

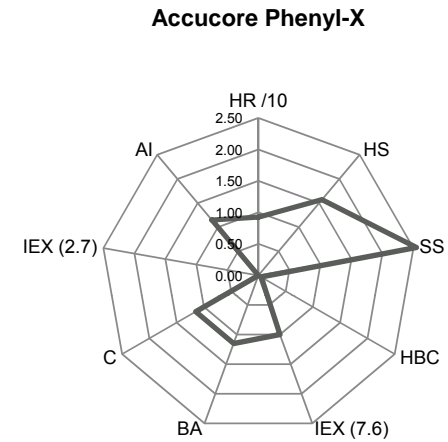
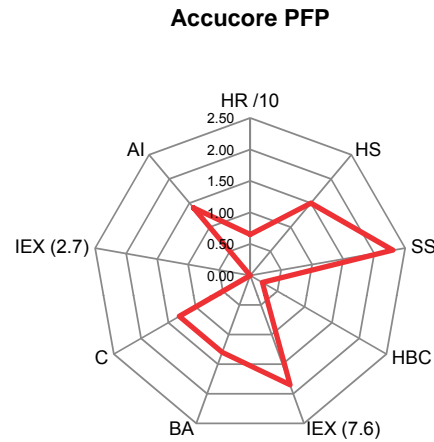
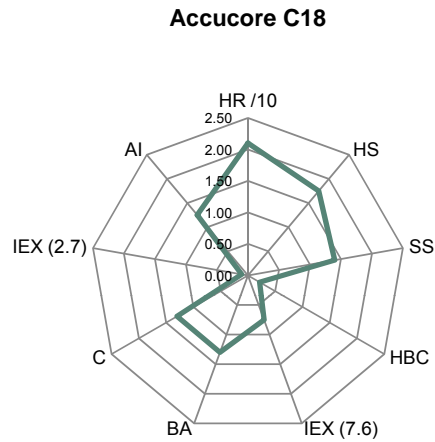


色谱柱: Accucore Polar Premium  
Kinetex C18  
规格: 2.6  $\mu\text{m}$ , 100 x 3.0 mm  
流动相: A: 甲醇  
B: 10 mM 磷酸  
等度: 80% A, 20% B  
流速: 0.800 mL/min  
温度: 40 ° C  
进样: 6  $\mu\text{L}$   
检测器: UV 428 nm  
样品: 100 mg 姜黄地下部分, 使用20mL热的70%乙醇提取; 离心, 过滤, 并用甲醇稀释10倍  
色谱峰:  
1. 姜黄素  
2. 脱甲氧基姜黄素  
3. Bis-desmethoxycurcumin



Kinetex® is a registered trademark of Phenomenex Inc., USA

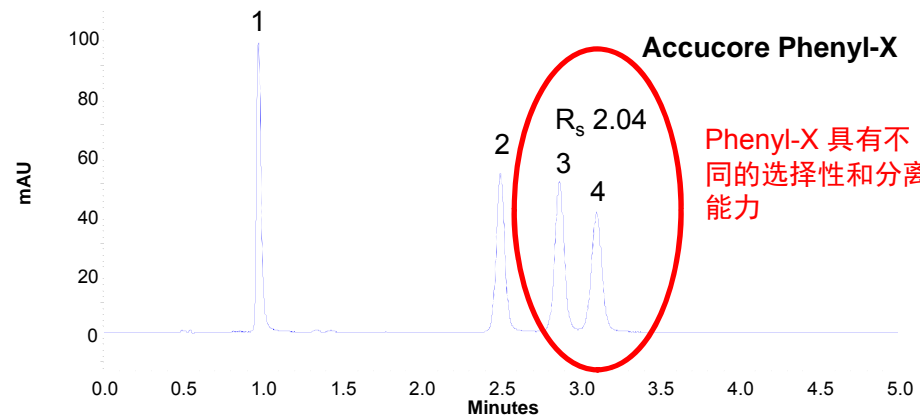
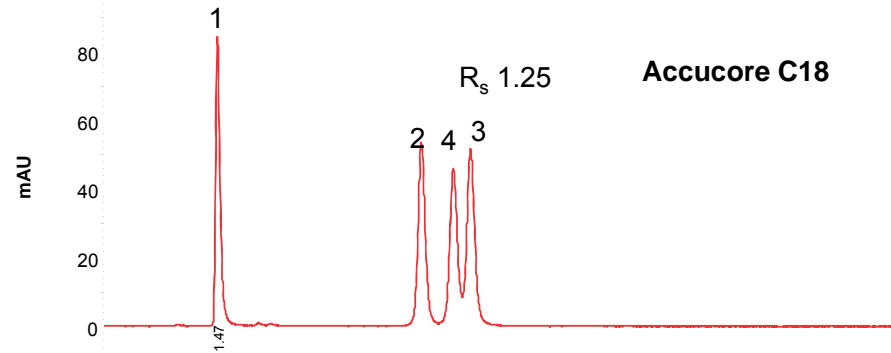
# 具有形状选择性的键合相



Phenyl-X 具有强的形状选择性(芳香选择性)

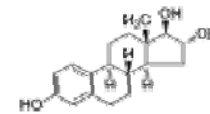
Phenyl-Hexyl 的形状选择性相反

# Phenyl-X 分离雌激素

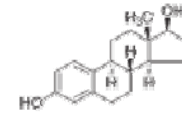


Phenyl-X 具有不同的选择性和分离能力

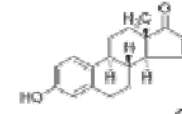
色谱柱: Accucore Phenyl-X  
 Accucore C18  
 规格: 2.6  $\mu$ m, 100 x 2.1 mm  
 流动相: A: Acetonitrile  
 B: Methanol  
 C: Water  
 等度: 15% A, 40% B, 45% C  
 流速: 400  $\mu$ L/min  
 温度: 40 °C  
 进样: 1  $\mu$ L  
 检测器: UV 220 nm



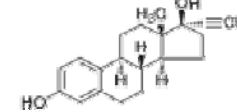
1. 雌三醇(E3)



2. 雌二醇(E2)



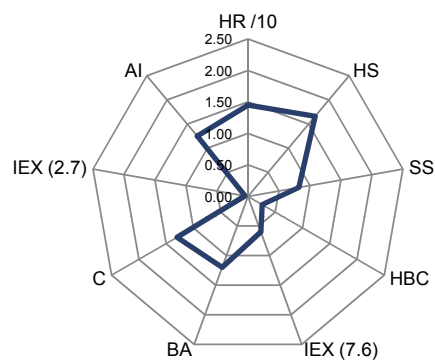
3. 雌激素酮(E1)



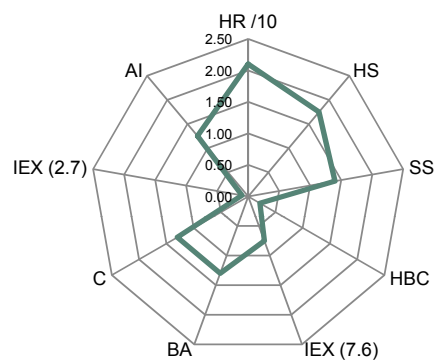
4. 炔雌醇

# 不同长度的烷基键合相

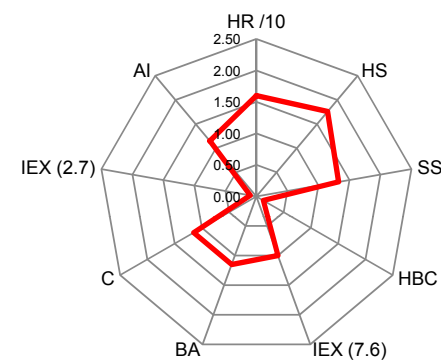
Accucore C8



Accucore C18



Accucore C30



C30 的疏水保留低于C18

形状选择性与C18类似



## 色谱柱分类工具

- **Tanaka et. al.**

K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Arki, N. Tanaka, *J. Chromatogr. Sci.* **27** (1989) 721.

- **Euerby & Petersson**

M. R. Euerby, P. Petersson, *J. Chromatogr. A* 994 (2003), p. 13 – 36

- **USP – Working Group on HPLC Columns**

Pharmacopeial Forum Vol. 31(2) [Mar.–Apr. 2005]

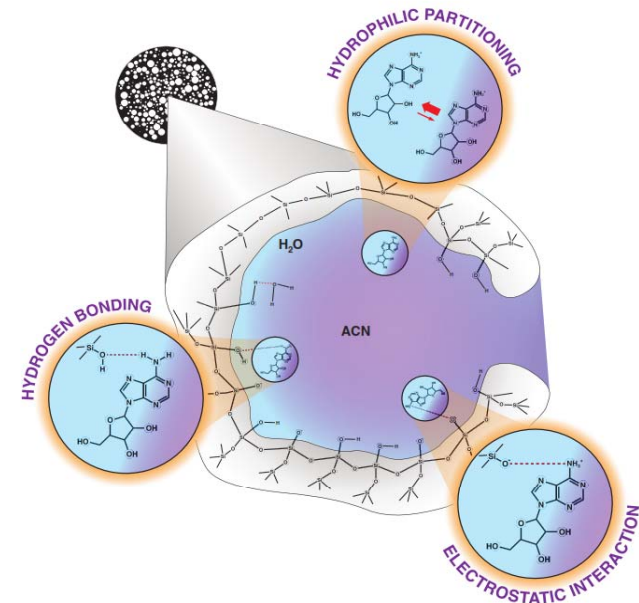
- **PQRI – Snyder, Dolan et. al.**

N.S. Wilson, M.D. Nelson, J.W. Dolan, L.R. Snyder, R.G. Wolcott, P.W. Carr, *J. Chromatogr. A* 961 (2002) 171-193.



# HILIC方法—Hydrophilic Interaction Liquid Chromatography

- 流动相含高比例有机相
  - 60 – 95% 有机溶剂 (e.g., acetonitrile)
- 固定相极性大, 适合保留极性化合物
  - 裸硅胶
  - 离子化键合相(氨基, 酰胺, 氰基)
  - 中性键合相(二醇)



固定相表面形成半固定化水层  
保留作用 分配  
氢键作用  
弱的静电吸附

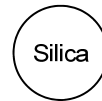
# HILIC优势

- 保留极性化合物, 与反相色谱柱相互补充
- 选择性与反相色谱柱相互补充
- 提供LC-MS 检测灵敏度
  - 高比例有机相(> 80%)
- 减少样品制备过程
  - SPE, 蛋白沉淀和液液萃取的有机相比比例较高, 反相分析需要蒸干后复溶才能进样

# HILIC Columns for Polar Analytes

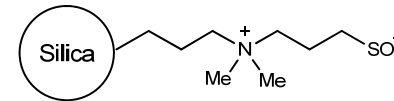
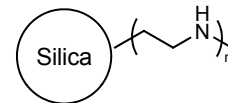
- Bare silica phases

- *Accucore HILIC*
- *Hypersil GOLD Silica*
- *Synchronis Silica*



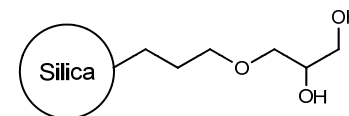
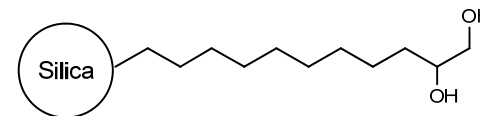
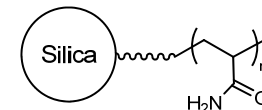
- Ionic bonded phases

- *Hypersil GOLD HILIC*
- *Synchronis HILIC*

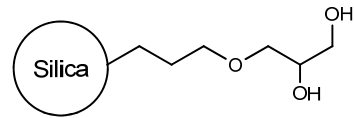
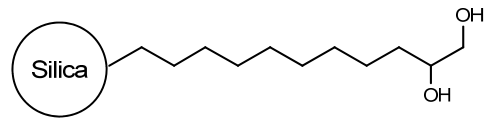
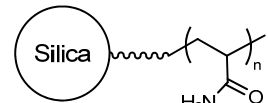
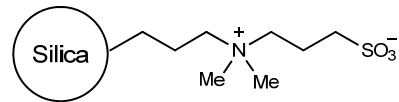
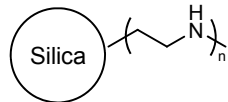


- Neutral bonded phases

- *Accucore 150-Amide-HILIC*
- *Acclaim Mixed-Mode HILIC-1*
- *BETASIL Diol*

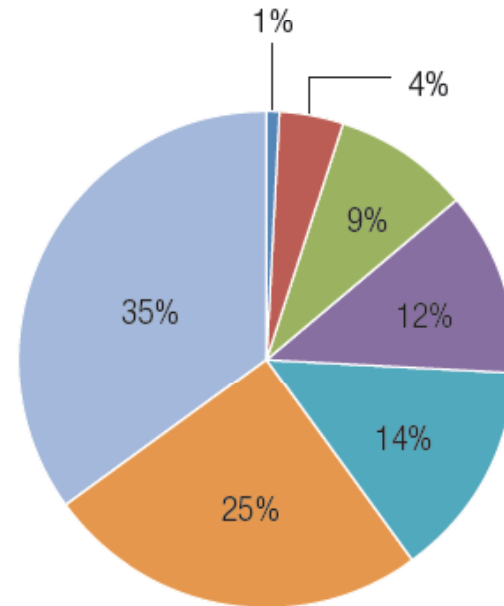


# HILIC Columns for Polar Analytes

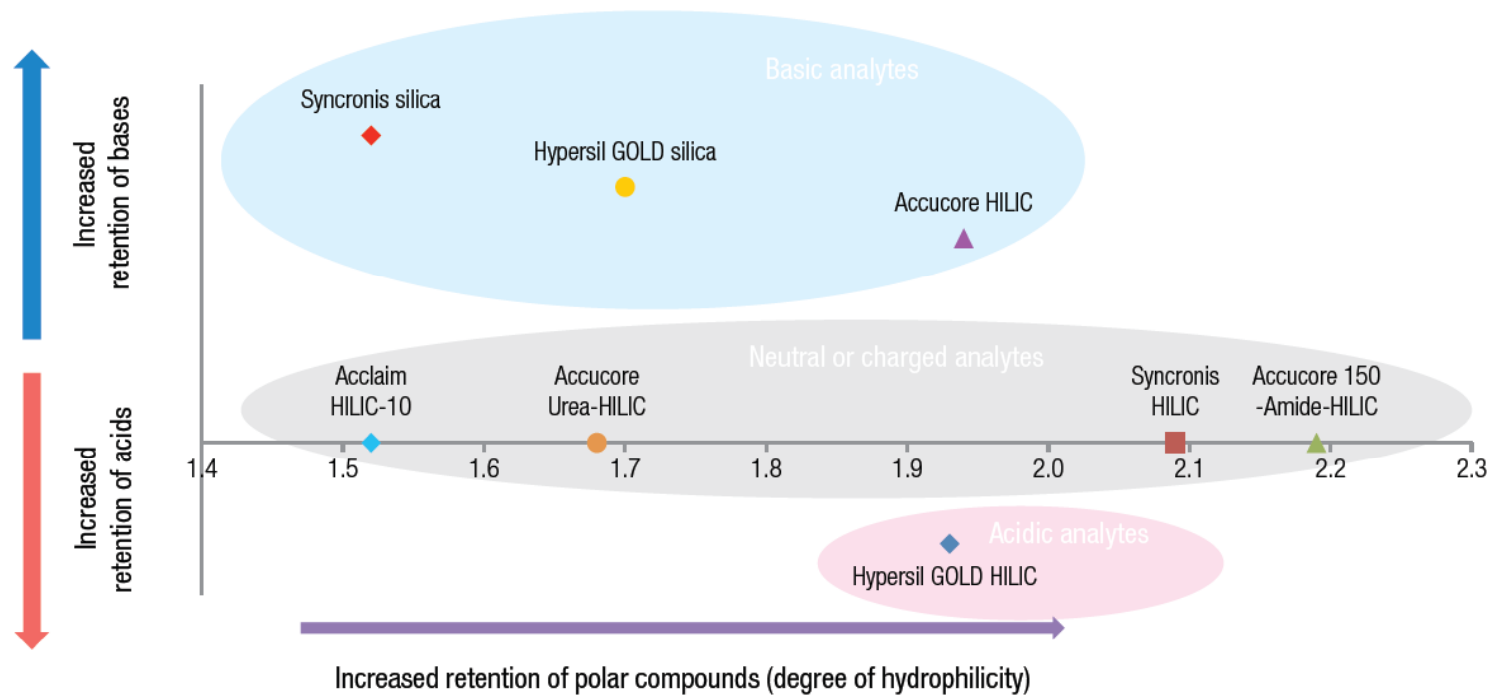


HILIC phases vs % of applications

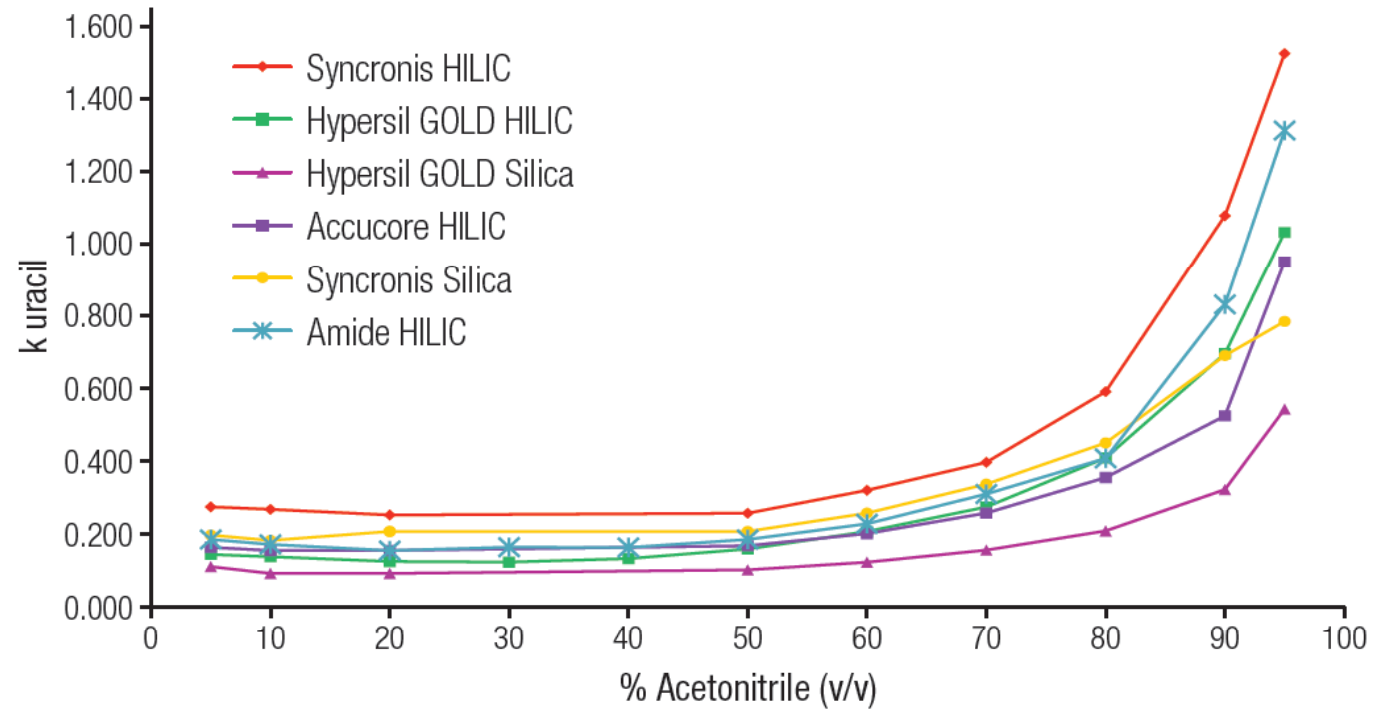
- bare/hybrid silica
- zwitterionic
- amide
- diol
- aminopropyl
- other
- cyanopropyl



# HILIC 模式的选择



# HILIC Retention Behaviour of Polar Analytes

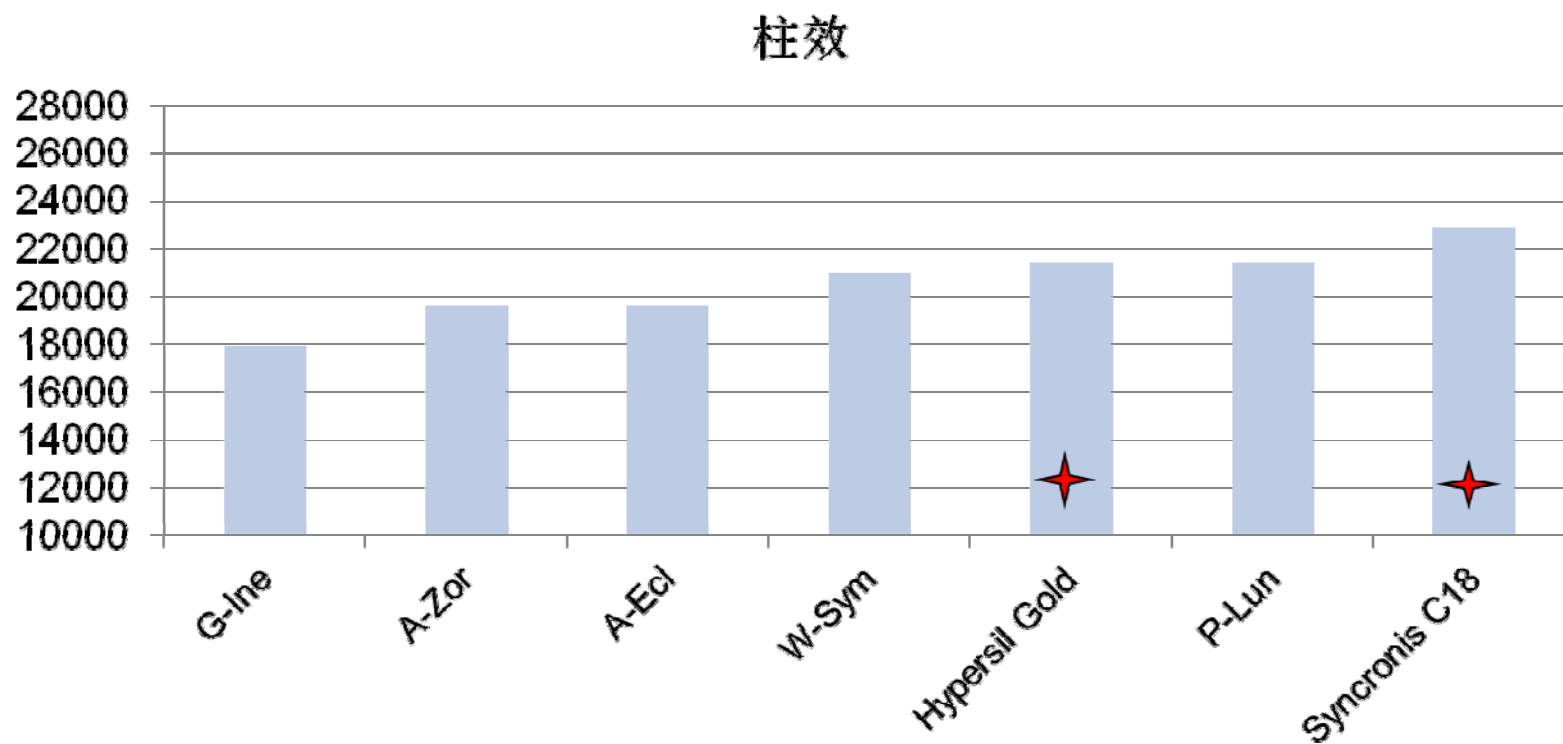


# Thermo 提供全面的键合相

		AQUASIL	Aclaim	Accucore	BetaBasic	BETASIL	BioBasic	Hypersil	Hypersil BDS	Hypersil GOLD	HY PURITY	Synchronis	Hypercarb
Reversed-phase	C30		X										
	C18		X	X	X	X	X	X	X	X	X	X	
	C8		X		X	X	X	X	X	X	X	X	
	C6					X							
	C4				X		X			X			
	C1					X		X					
	RP-MS			X									
	Phenyl		X		X	X		X	X	X		X	
	Polar endcapped	X		X						X	X	X	
	Polar embedded (PA)		X										
	Polar embedded (PA2)		X								X		
	PFP			X						X			
	Phenyl / hexyl			X		X							
	HILIC / Normal phase	Cyano				X	X		X	X	X		
Amino								X		X		X	
HILIC			X	X						X		X	
Silica						X		X		X		X	
Diol						X							
IEX	Anion Exchange						X	X		X			
	Cation Exchange						X						
Mixed mode	Trinity P1		X										
	Mixed Mode HILIC-1		X										
	Mixed Mode WAX-1		X										
	Mixed Mode WCX-1		X										
	Hypercarb												X

- 有机酸专用柱
- 表面活性剂专用柱
- 爆炸物专用柱
- 汽车尾气专用柱
- 表面活性剂专用柱
- 氨基甲酸酯专用柱
- 敌草快、百草枯专用柱
- 卤乙酸专用柱
- .....

## 柱效测试结果-基于Tanaka 方法





## 中国药典品种—盐酸曲马多

- 测试条件:

样品: 盐酸曲马多片剂

流动相: 甲醇/水 (醋酸-醋酸钠pH4.5) =35/65

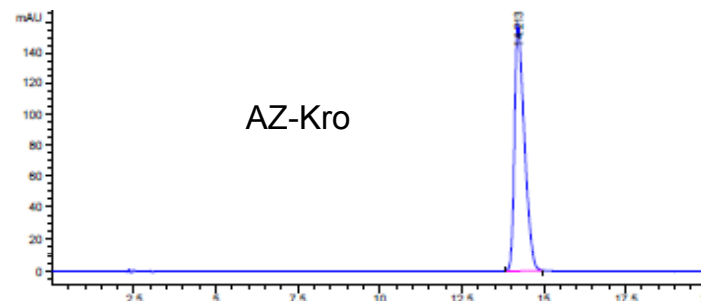
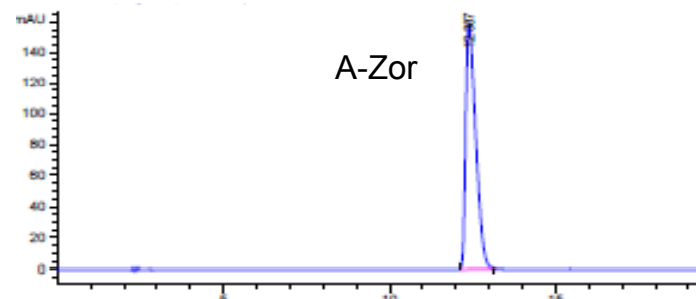
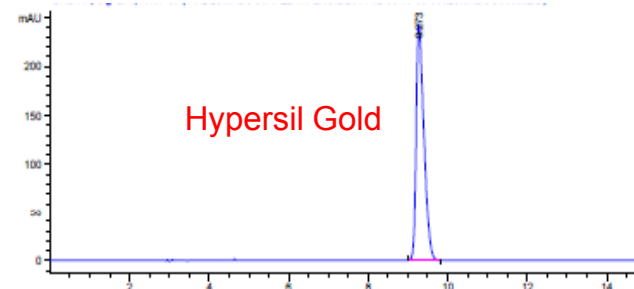
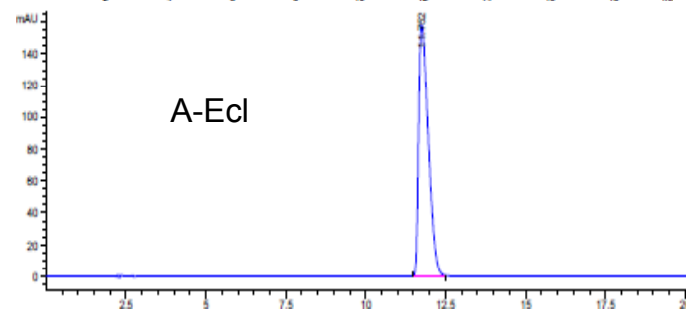
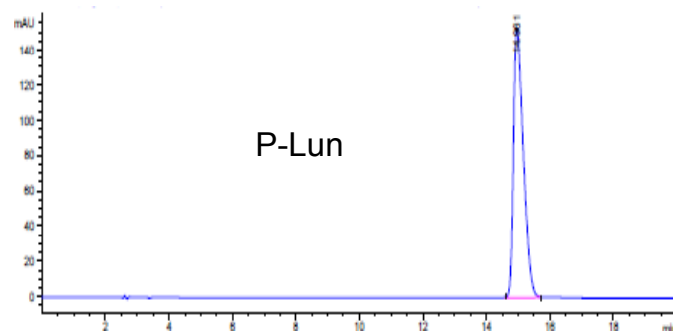
流速: 1.0mL/min;

检测波长: 271nm;

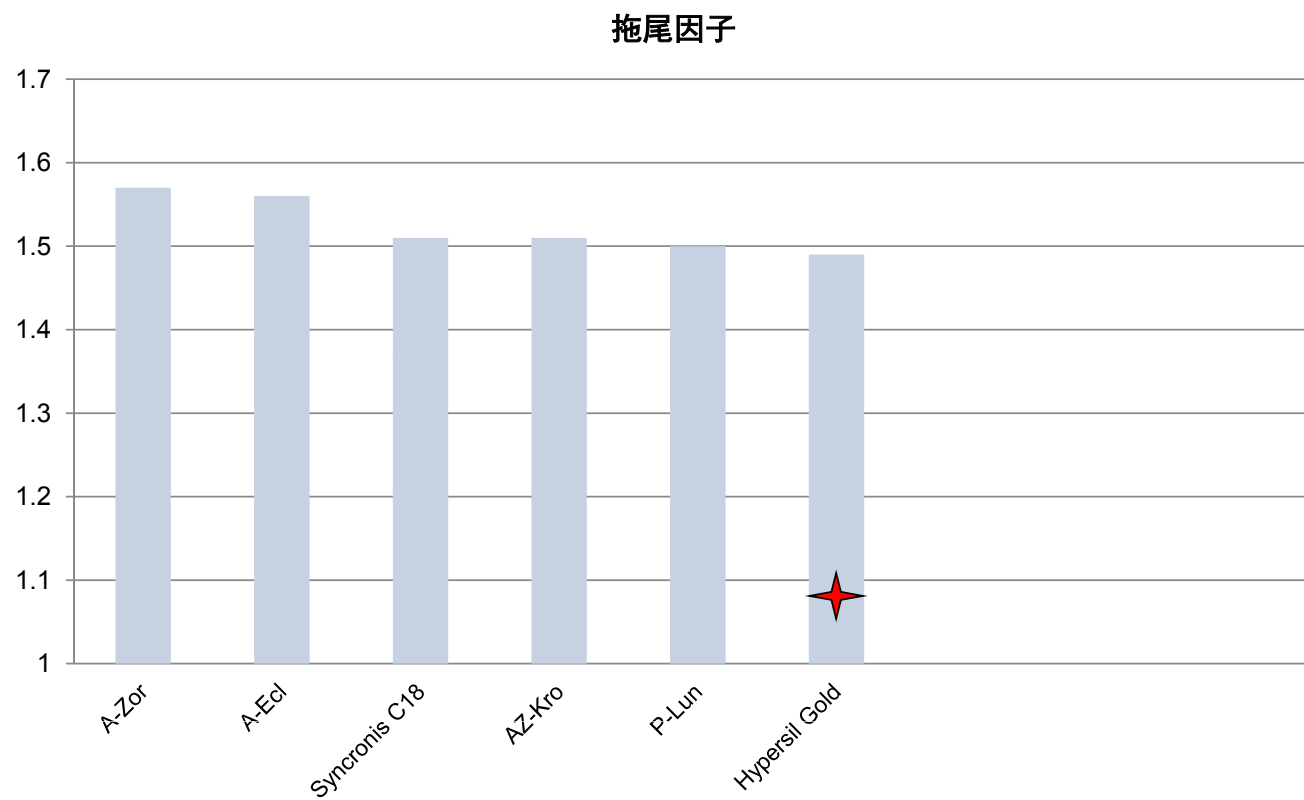
柱温: 30° C

进样体积: 20uL

# 测试结果—盐酸曲马多



# 实际样品测试结果-盐酸曲马多



## 中国药典品种—布洛芬

- 测试条件:

样品: 布洛芬片剂

流动相: 乙腈/水 (醋酸-醋酸钠pH2.5) =60/40

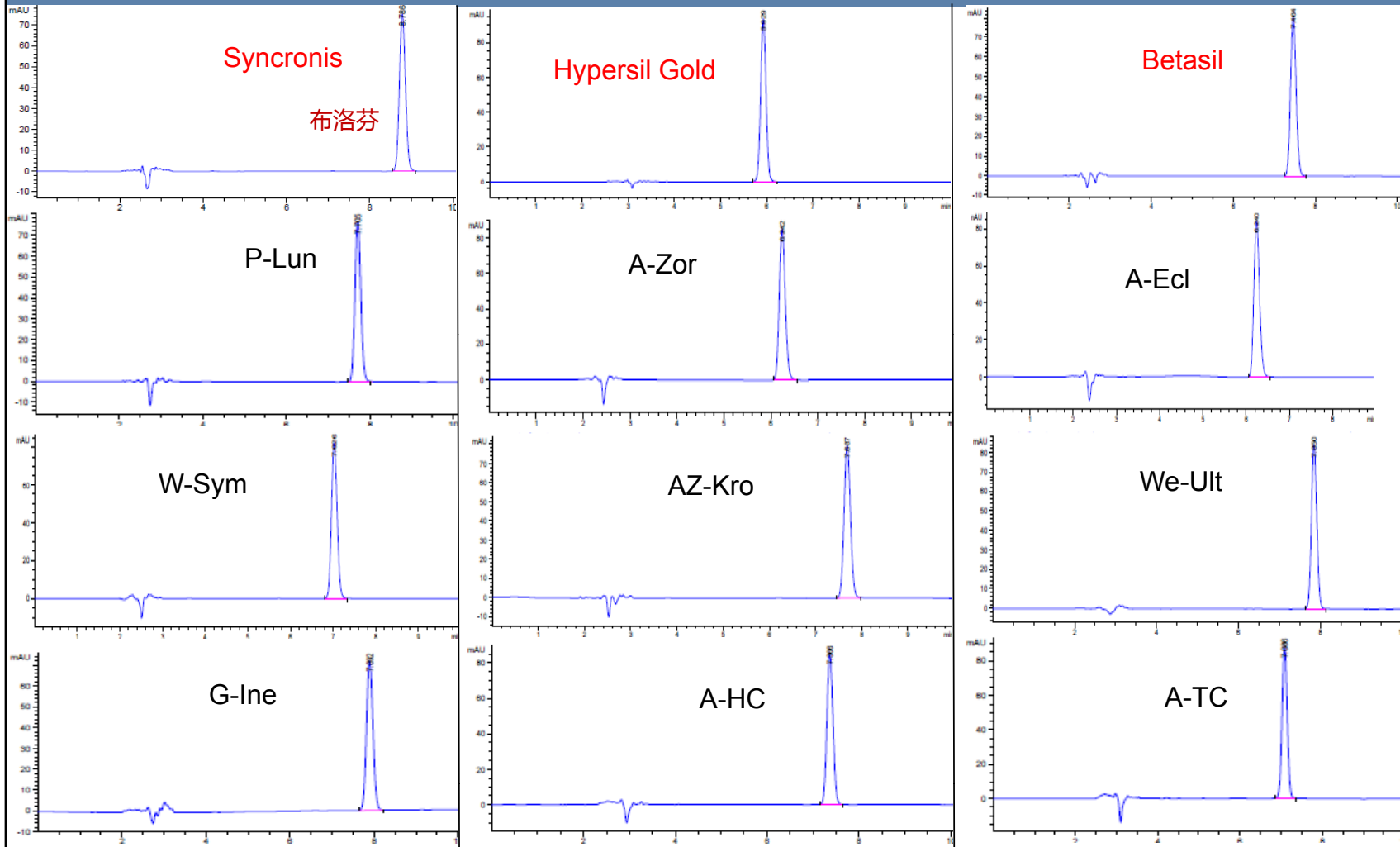
流速: 1.0mL/min;

检测波长: 263nm;

柱温: 30° C

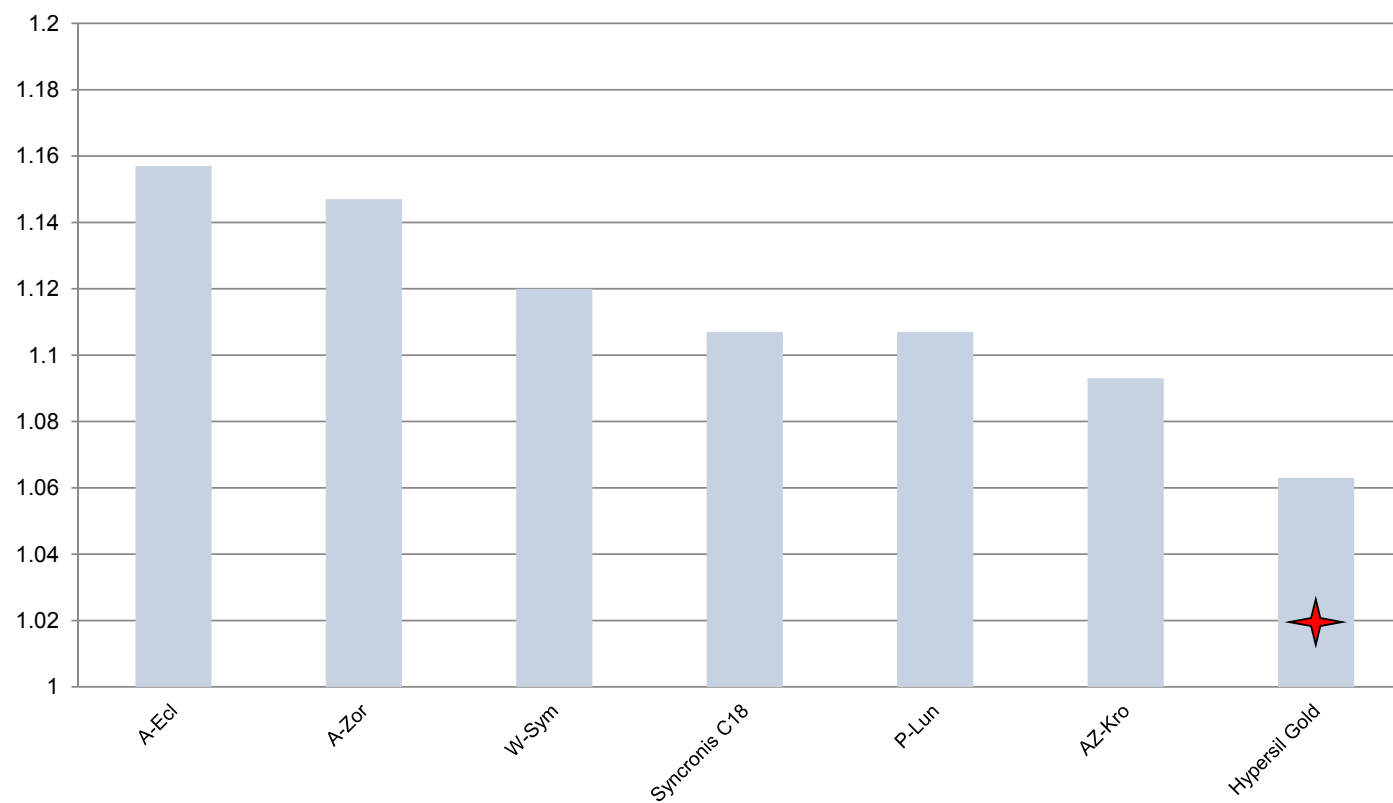
进样体积: 20uL

# 测试结果—布洛芬



# 实际样品测试结果-布洛芬

拖尾因子



## 2015 ChP项目

阿司匹林肠溶片  
奥卡西平片  
奥拉西坦  
奥美拉唑肠溶胶囊  
倍他司汀  
吡拉西坦  
布洛芬  
茶苯海明片  
茶碱缓释片  
碘海醇注射液  
对乙酰氨基酚  
法莫替丁  
富马酸比索洛尔片  
格列吡嗪  
左氧氟沙星  
硫酸吗啡缓释片  
头孢硫脒  
螺内酯片  
米非司酮片  
尼莫地平注射液  
尼群地平片  
双氯芬酸钠肠溶片  
维生素B2片  
维生素E  
戊四硝酯片  
硝苯地平片  
硝酸甘油片  
辛伐他汀片  
盐酸二甲双胍肠溶胶囊  
叶酸片

多西他赛  
氯雷他定片  
盐酸格拉司琼注射液  
盐酸雷尼替丁胶囊  
盐酸纳洛酮注射液  
布地奈德  
非洛地平片  
茴拉西坦分散片  
单硝酸异山梨酯缓释片  
卡马西平片

硝酸甘油片

-替代Venuisil

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## 有关物质—方法

### 样品制备:

取本品20片,精密称定,研细,精密称取适量(约相当于硝酸甘油2.5mg),置25mL量瓶中,加流动相适量,超声约3分钟,振摇约30分钟,使硝酸甘油溶解,用流动相稀释至刻度,摇匀,滤过,取续滤液作为供试品溶液,照硝酸甘油溶液含量测定项下的方法测定,即得。

取硝酸甘油对照品适量,加0.1mol/L盐酸溶液溶解并稀释制成每1ml中约含0.5mg的溶液,置水浴中加热10分钟,放冷,用0.1mol/L氢氧化钠溶液调节pH值至中性,取20 $\mu$ L注入液相色谱仪,记录色谱图,在**相对保留时间约0.4处应出现两个降解产物峰(1, 2-二硝酸甘油峰和1, 3-二硝酸甘油峰)**,两峰之间的分离度应大于1.0。理论板数按硝酸甘油峰计算不低于2000。

### 检测方法:

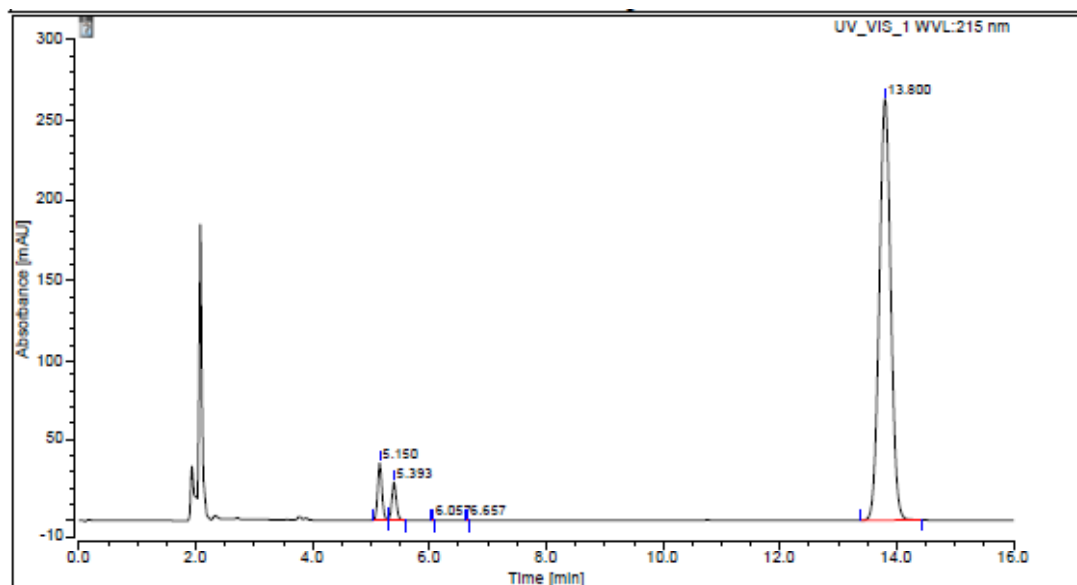
流动相: 乙腈-水(50:50)

流速: 1.0mL/min

检测: 215 nm

柱温: 30 $^{\circ}$ C

# 含量测定—Synchronis C18图谱



## Integration Results

Peak Name	Retention Time min	Peak Area mAU*min	Resolution USP	Tailing	Theoretical	Amount	Units
1	5.150	2.89041	n.a.	1.10	24793	n.a.	n.a.
2	5.393	1.98300	1.82	1.13	25116	n.a.	n.a.
3	6.057	0.00116	7.95	0.87	620789	n.a.	n.a.
4	6.657	0.00117	18.95	0.85	668321	n.a.	n.a.
n.a. C1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	mg/ml
5	13.800	59.68309	36.44	1.03	23577	n.a.	n.a.

● 盐酸雷尼替丁胶囊  
-替代Kromasil

● The world leader in serving science

# 有关物质—方法

## 样品制备:

取装量差异项下的内容物,混合均匀,精密称取适量(约相当于雷尼替丁 20mg),置200mL量瓶中,加水溶解并稀释至刻度,摇匀,作为供试品溶液,精密量取10 $\mu$ L注入液相色谱仪,记录色谱图;另取盐酸雷尼替丁对照品约22mg,精密称定,置200mL量瓶中,加水溶解并稀释至刻度,摇匀,同法测定。按外标法以峰面积计算,并将结果乘以0.8961,即得。取盐酸雷尼替丁约0.1g,置100mL量瓶中,加50%氢氧化钠溶液1mL,加水约60mL,振摇使溶解,用水稀释至刻度,摇匀,室温放置1小时后,取10 $\mu$ L注入液相色谱仪,记录色谱图。

## 检测方法:

流动相: A:磷酸盐缓冲液(取磷酸6.8 mL置1900 mL水中,加入50%氢氧化钠溶液8.6mL,加水至2000mL,用磷酸或50%氢氧化钠溶液调节pH 值至7.1 $\pm$ 0.05)-乙腈(98 :2 )

B:磷酸盐缓冲液-乙腈(78 :22 )

流速: 1.5mL/min

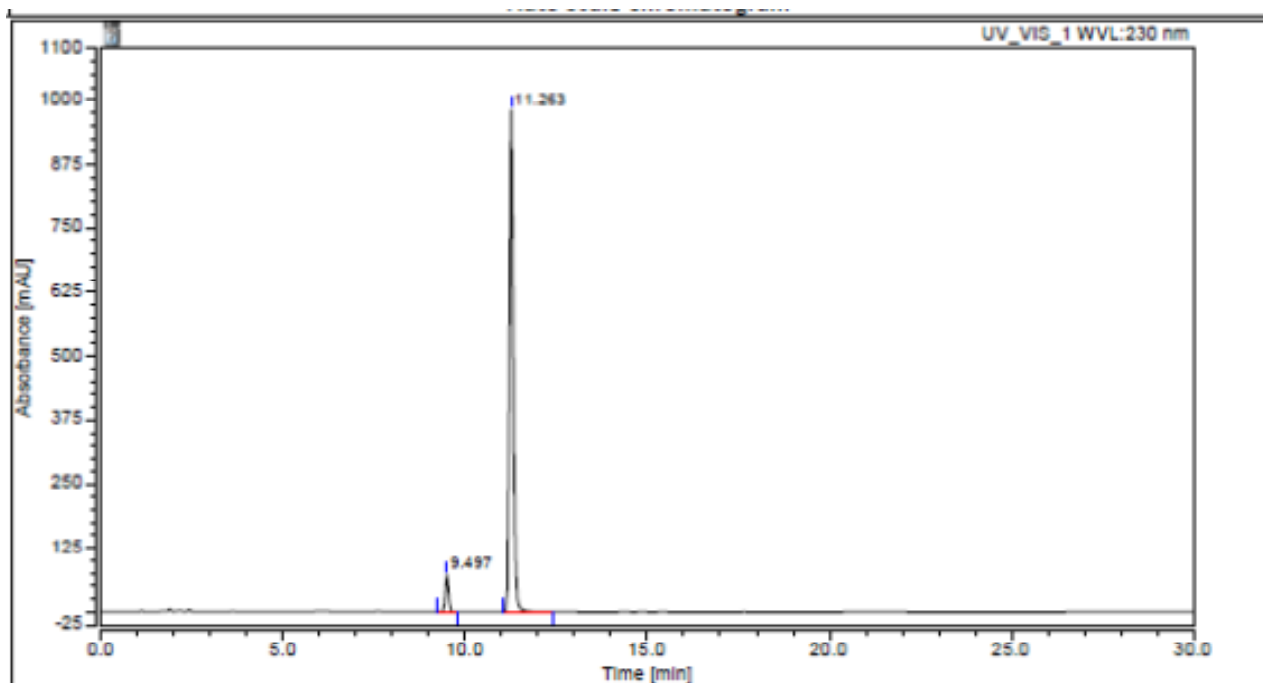
检测: 230 nm

柱温: 35 $^{\circ}$ C

时间(分钟)	流动相A (%)	流动相B (%)
0	100	0
15	0	100
23	0	100
24	100	0
30	100	0

调节流速或流动相比比例,使主成分色谱峰的保留时间约为12分钟,杂质 I 的相对保留时间约为0.85,雷尼替丁峰与杂质 I 峰的分离度应大于4.0。

# 含量测定—HypersilGold图谱



## Integration Results

Peak Name	Retention Time min	Peak Area mAU*min	Resolution USP	Tailing	Theoretical	Amount	Units
1	9.497	7.39678	n.a.	1.00	54089	n.a.	n.a.
2	11.263	123.03797	9.92	1.18	54490	n.a.	n.a.

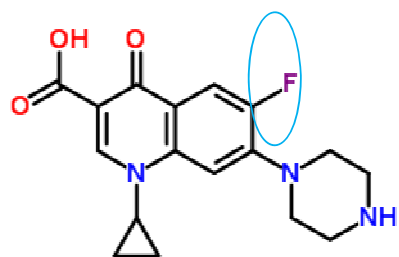
● **USP/EP项目**

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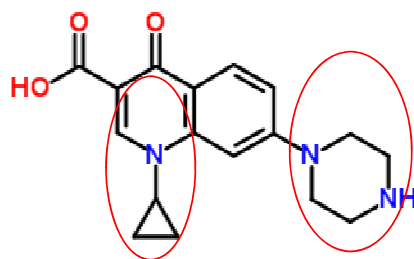
## USP/EP—原料药项目

1	Amoxicillin	USP
2	Amoxicillin	EP
3	Ampicillin	USP
4	Ampicillin	EP
5	Ceftriaxone Sodium	USP
6	Ceftriaxone Sodium	EP
7	Tetracycline hydrochloride	USP
8	Sulfadiazine	EP
9	Trimethoprim	USP
10	Trimethoprim- (For Impurities C, E, A, D, G, B, J and F)	EP
11	Trimethoprim- (For Impurities H & I)	EP
12	Ciprofloxacin hydrochloride	USP
13	Ciprofloxacin hydrochloride	EP
14	Ofloxacin	USP
15	Ofloxacin	EP
16	Levofloxacin	USP
17	Ibuprofen	USP
18	Ibuprofen (Assay)	USP
19	Ibuprofen	EP
20	Metronidazole	USP
21	Metronidazole	EP
22	Caffeine	USP
23	Caffeine	EP
24	Carbamazepine	USP
25	Piracetam	EP

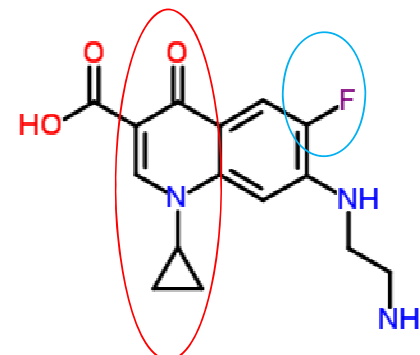
## 环丙沙星(Ciprofloxacin)---EP System Suitability Mix Chromatograms



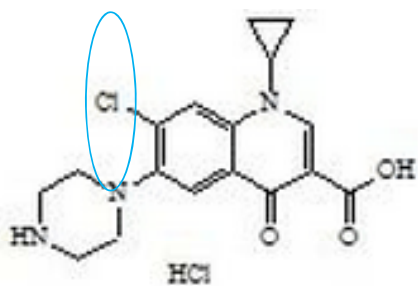
环丙沙星 LogP=0.65  
LogD=-2.98



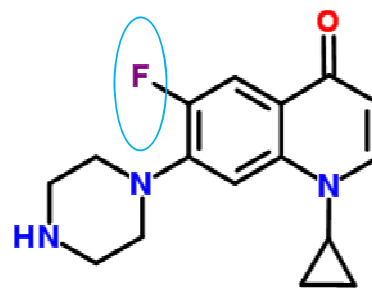
B LogP=0.29  
LogD=-3.05



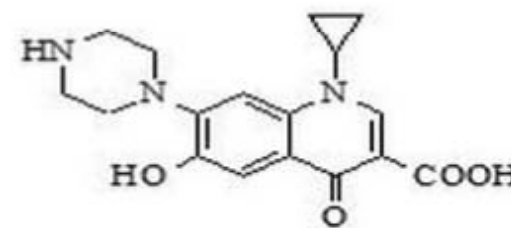
C LogP=-0.14  
LogD=-2.99



D



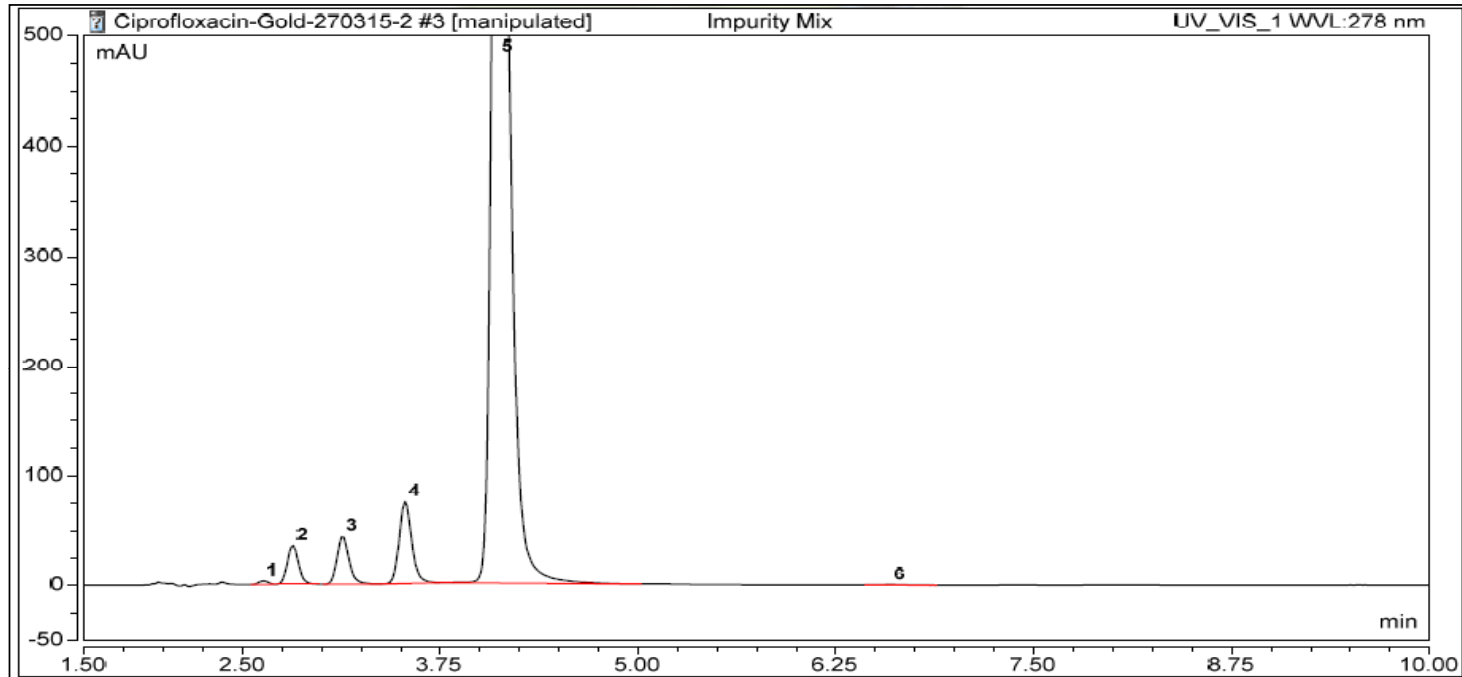
E LogP=0.06  
LogD=-2.39



F

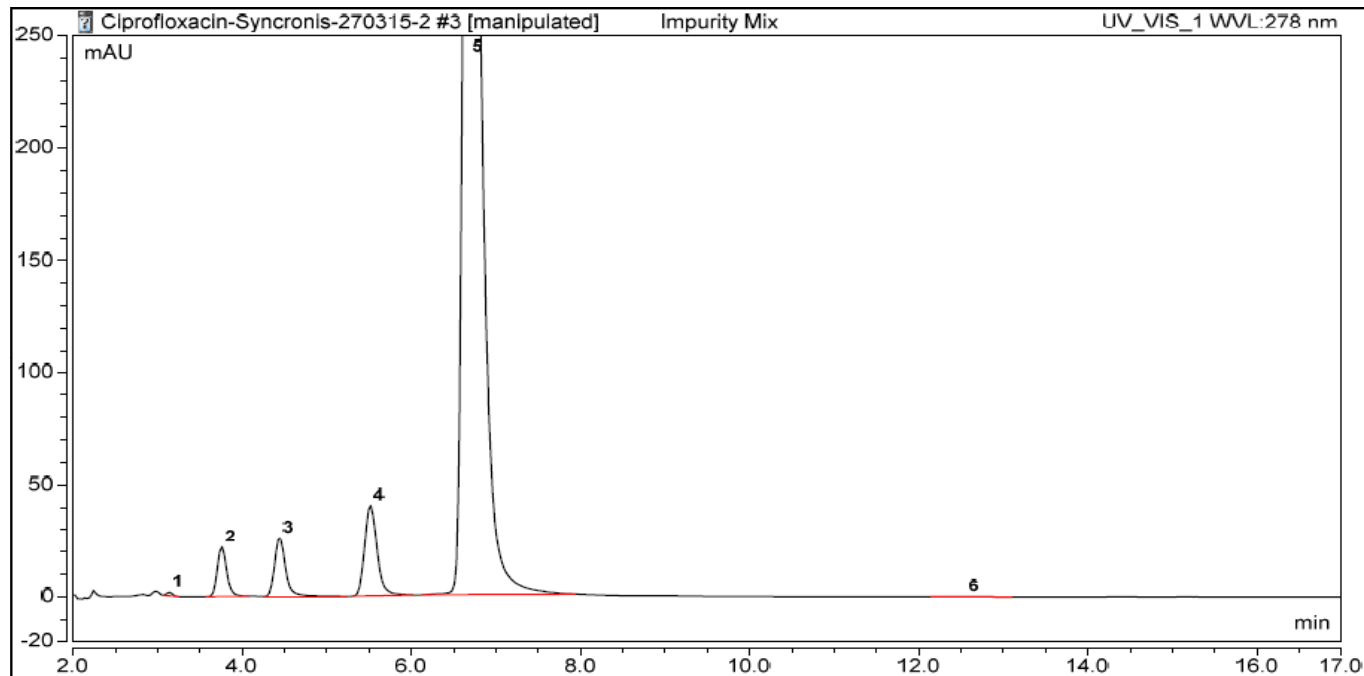


## 环丙沙星(Ciprofloxacin)---EP System Suitability Mix Chromatograms



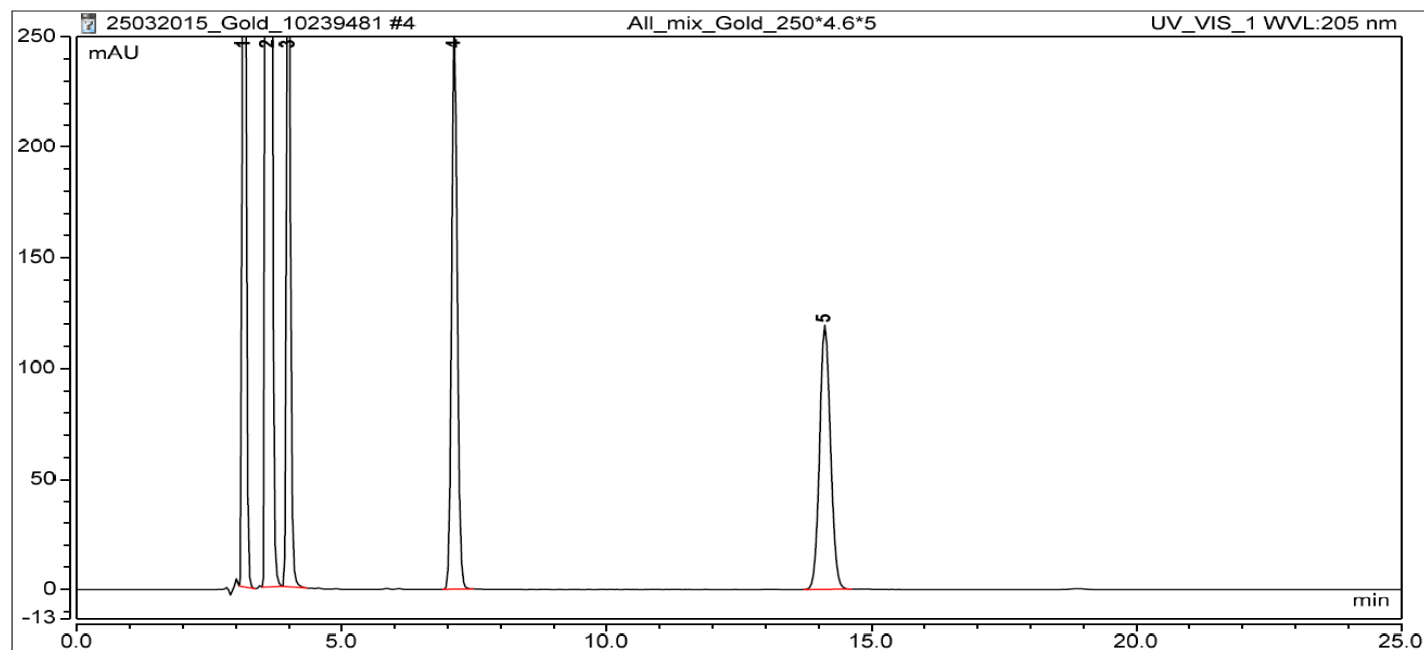
Peak Results							
No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Impurity-E	2.633	0.229	0.16	n.a.	0.97	8815
2	Impurity-F	2.820	2.910	2.08	1.57	1.08	8077
3	Impurity-B	3.133	3.891	2.78	2.44	1.20	9047
4	Impurity-C	3.526	6.957	4.97	2.89	1.17	9921
5	Ciprofloxacin	4.120	126.037	89.98	3.74	1.38	8732
6	Impurity-D	6.600	0.042	0.03	10.82	1.11	8696

## 环丙沙星(Ciprofloxacin)---EP System Suitability Mix Chromatograms



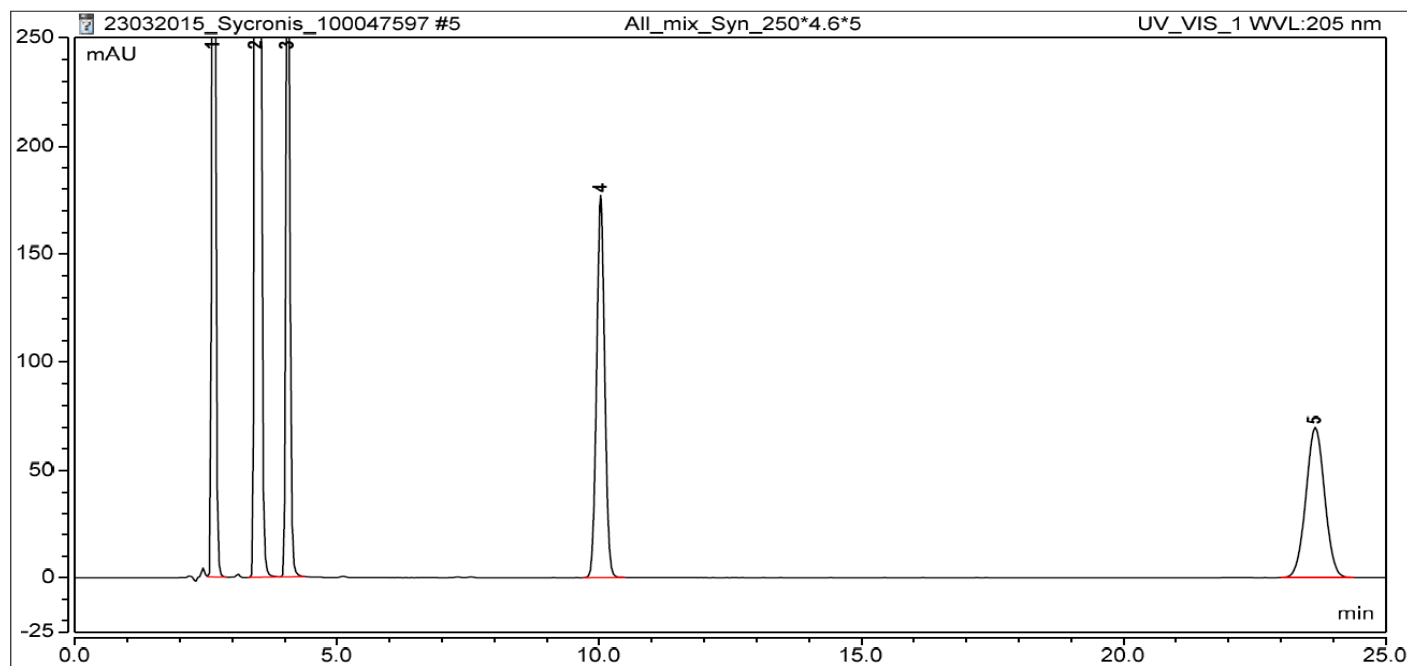
Peak Results							
No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Impurity-E	3.133	0.115	0.08	n.a.	1.17	9642
2	Impurity-F	3.760	2.830	2.03	3.87	1.14	5841
3	Impurity-B	4.440	3.916	2.81	3.27	1.30	6481
4	Impurity-C	5.513	6.915	4.95	4.46	1.24	7110
5	Ciprofloxacin	6.693	125.779	90.11	3.94	1.52	6253
6	Impurity-D	12.560	0.035	0.03	12.59	1.04	7103

# 吡拉西坦(Piracetam)---EP System Suitability Mix Chromatograms



Peak Results							
No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Impurity D	3.155	40.269	11.83	3.62	1.19	13361
2	Piracetam	3.608	210.494	61.86	0.00	1.29	10307
3	Impurity A	3.991	29.612	8.70	2.98	1.22	19004
4	Impurity B	7.125	31.170	9.16	20.97	1.09	21560
5	Impurity D	14.118	28.751	8.45	40.35	1.07	22068

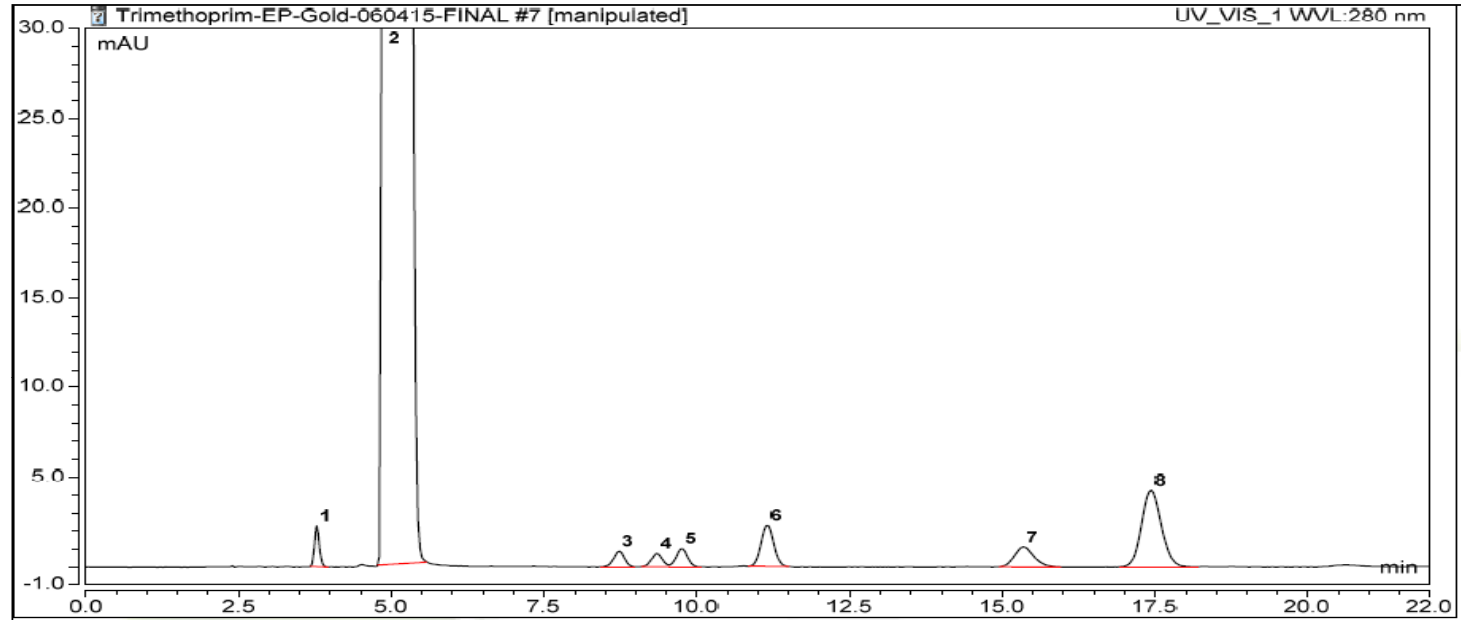
# 吡拉西坦(Piracetam)---EP System Suitability Mix Chromatograms



Peak Results							
No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Impurity D	2.641	40.879	12.08	6.89	1.27	9979
2	Piracetam	3.475	207.935	61.43	0.00	1.32	10351
3	Impurity A	4.061	29.686	8.77	4.61	1.14	18756
4	Impurity B	10.028	31.260	9.24	31.71	1.03	20853
5	Impurity C	23.653	28.725	8.49	51.35	1.02	21098

# 甲氧苄啶

## System Suitability and Impurity Mix Chromatograms

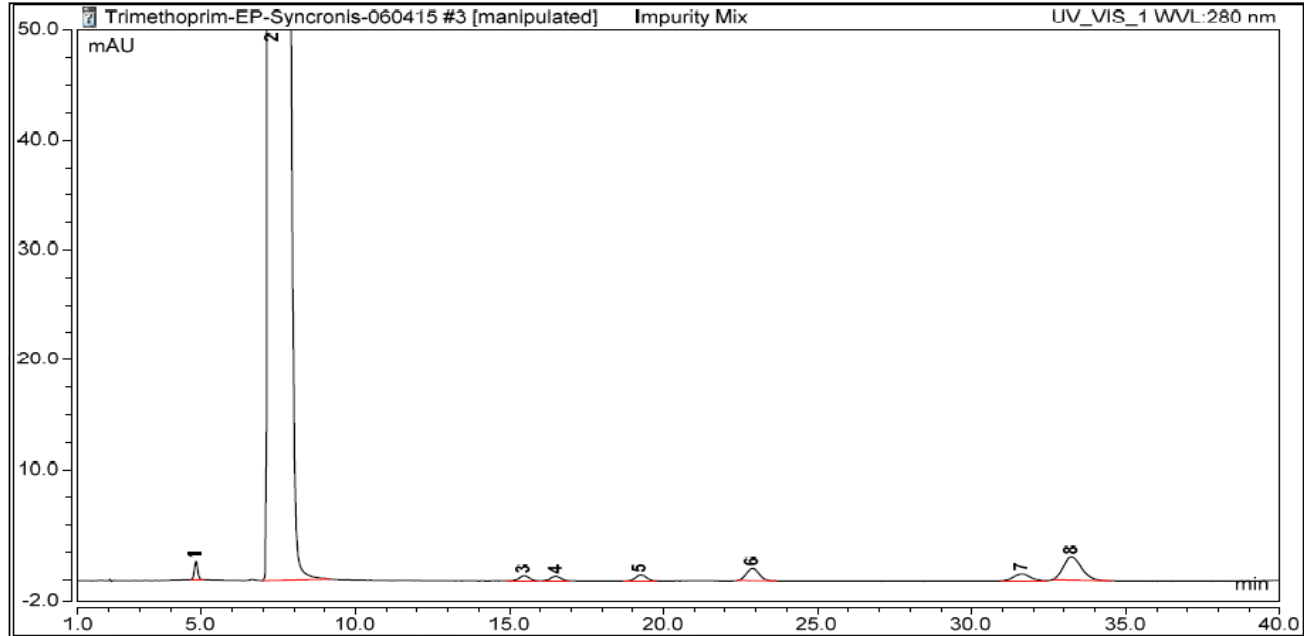


Peak Results							
No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Impurity-E	3.780	0.210	0.08	3.02	1.13	10995
2	Trimethoprim	4.902	245.165	98.65	0.00	3.97	1062
3	Impurity (Unknown)	8.732	0.180	0.07	8.40	0.94	12502
4	Impurity-D	9.345	0.159	0.06	9.58	n.a.	12980
5	Impurity-G	9.758	0.218	0.09	10.41	n.a.	13638
6	Impurity-B	11.153	0.562	0.23	12.71	1.05	13461
7	Impurity-J	15.343	0.418	0.17	17.84	1.19	11513
8	Impurity-F	17.435	1.608	0.65	21.00	1.09	13714

Impurity Mix

# 甲氧苄啶

## System Suitability and Impurity Mix Chromatograms

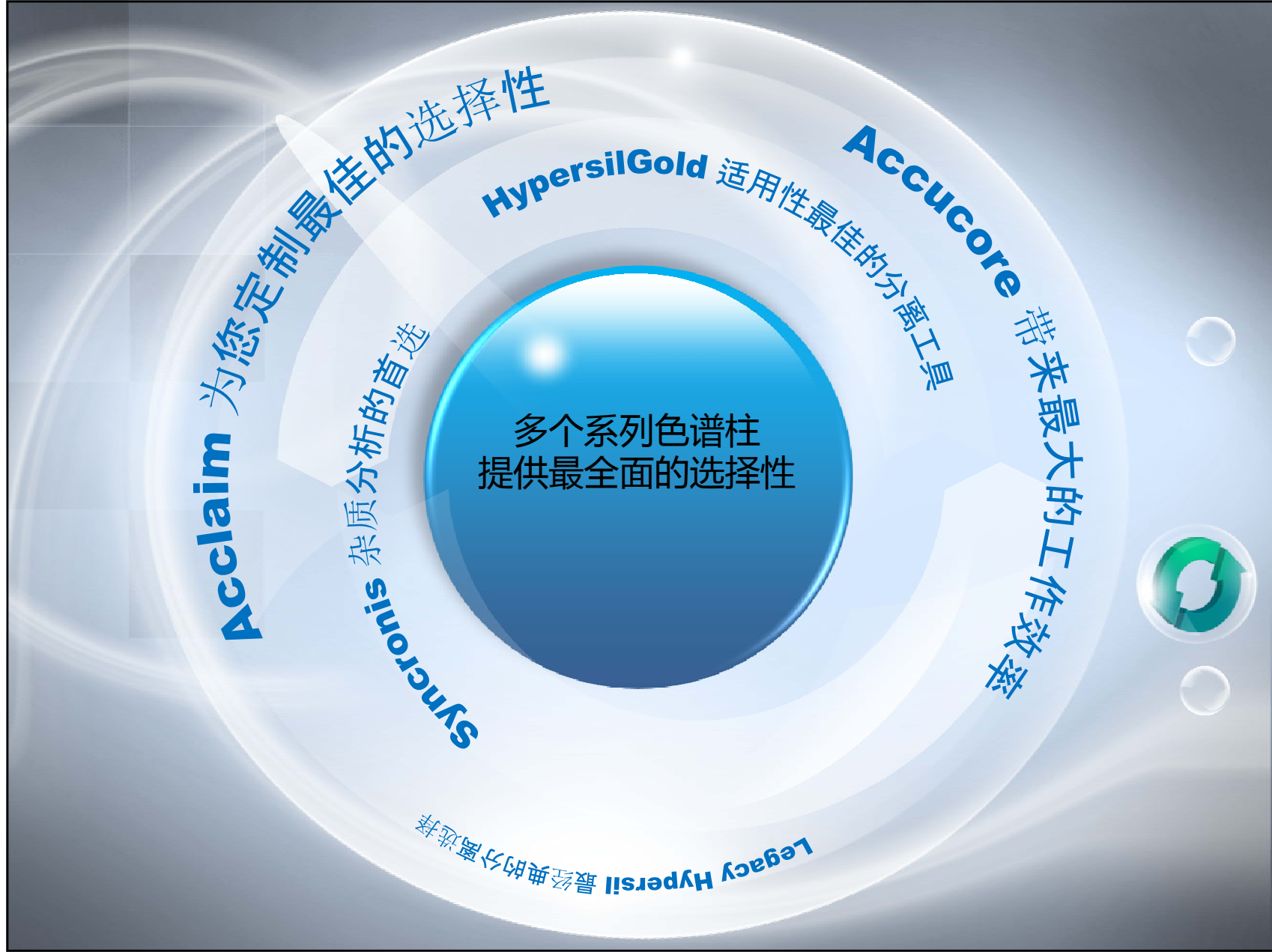


Peak Results							
No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (EP)	Asymmetry (EP)	Plates (EP)
1	Impurity-E	4.827	0.209	0.08	5.02	1.09	9882
2	Trimethoprim	7.310	243.869	98.74	0.00	2.31	1344
3	Impurity (Unknown)	15.480	0.183	0.07	12.04	0.99	12065
4	Impurity-D	16.513	0.151	0.06	13.39	0.94	12915
5	Impurity-G	19.252	0.227	0.09	16.65	1.11	14422
6	Impurity-B	22.887	0.532	0.22	20.11	1.08	14672
7	Impurity-J	31.622	0.378	0.15	27.29	1.04	16363
8	Impurity-F	33.242	1.422	0.58	27.74	1.22	15246

Impurity Mix

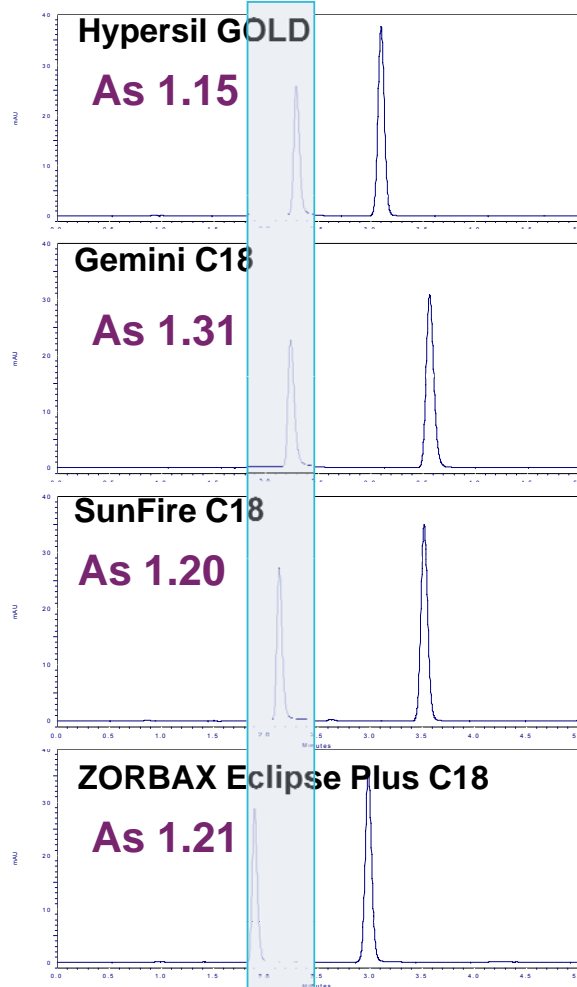
● 色谱柱的针对性选择

——关键色谱柱性能





# Hypersil GOLD - 超纯硅胶色谱柱



与其它C18色谱柱保留相近, 而表现出更好的对称性

色谱柱: 150 x 4.6mm, 5 $\mu$ m

流动相: H<sub>2</sub>O/MeCN, 60/40

流速: 1.0mL/min

柱温: 25° C

检测器: UV@ 254nm

进样量: 5 $\mu$ L

色谱峰: 1. 吡啶 2. 苯酚

# Hypersil GOLD – 超纯硅胶色谱柱

- 超纯硅胶

	Mg	Fe	K	Na	Ca	Ti	Al
Hypersil	30	240	10	2000	30	60	250
BDS	10	90	10	1350	10	30	130
GOLD	<1	<10	<1	<5	<5	<1	<1

- 全新的键合和端基封尾工艺
  - 减少硅醇基
  - 减少峰型拖尾
  - 硅胶颗粒球型对称，分布窄



# 制药行业首选色谱柱——Synchronis

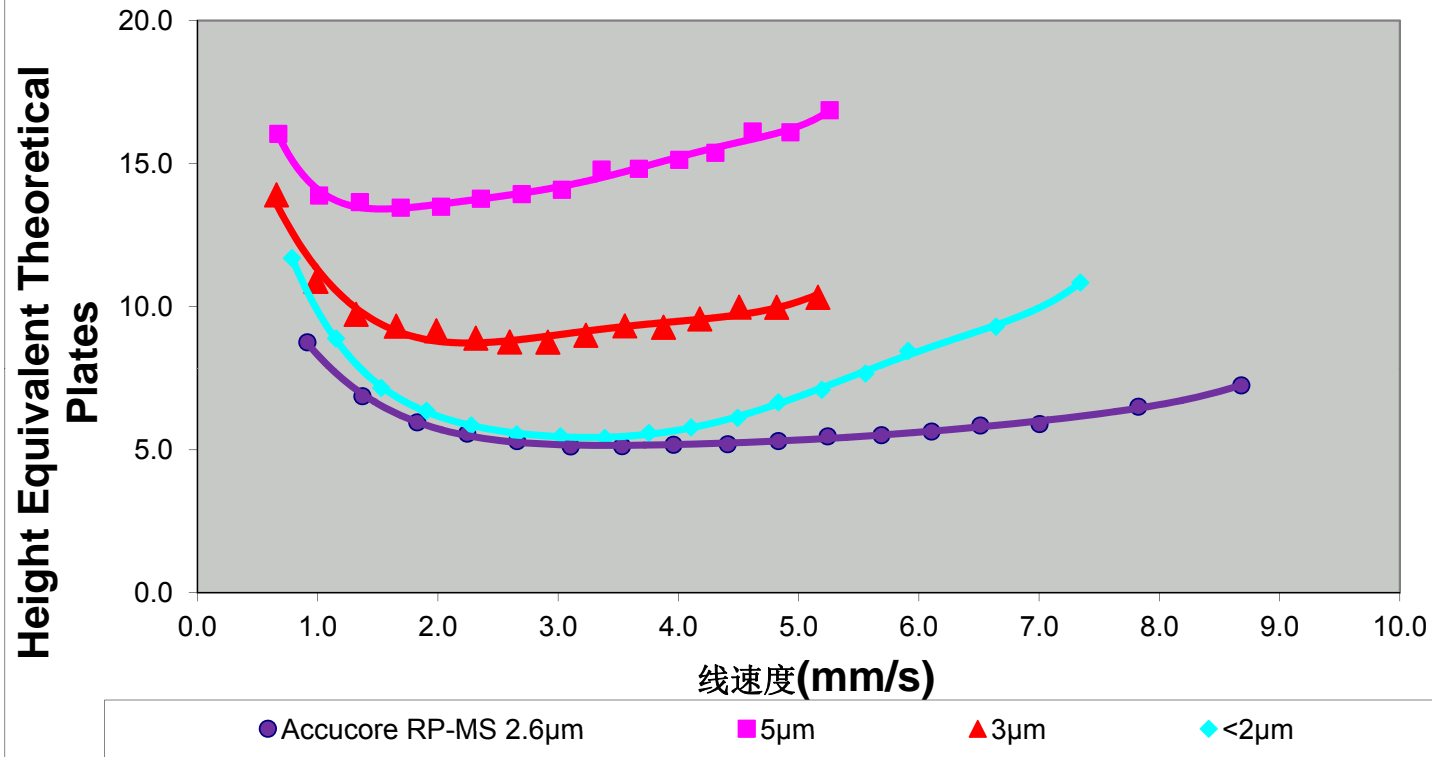
- 全新超纯硅胶为基质，同类硅胶中**金属含量最低**

金属杂质含量 (ppm)	钠	镁	铝	钙	铁	钴
超纯硅胶 (Hypersil GOLD, Zorbax, Luna等)	10	10	10	10	10	10
<b>Synchronis</b>	<b>0.9</b>	<b>0.3</b>	<b>1</b>	<b>0.5</b>	<b>0.9</b>	<b>0.5</b>

- 采用致密键合技术和双重封尾技术：更高柱效
- **高比表面积，提高载样量：尤其适用于有关物质分析**
- 高的键合密度（碳载量），具有更强的分离能力
- 改良自动化装填：更好的批内、批间重现性



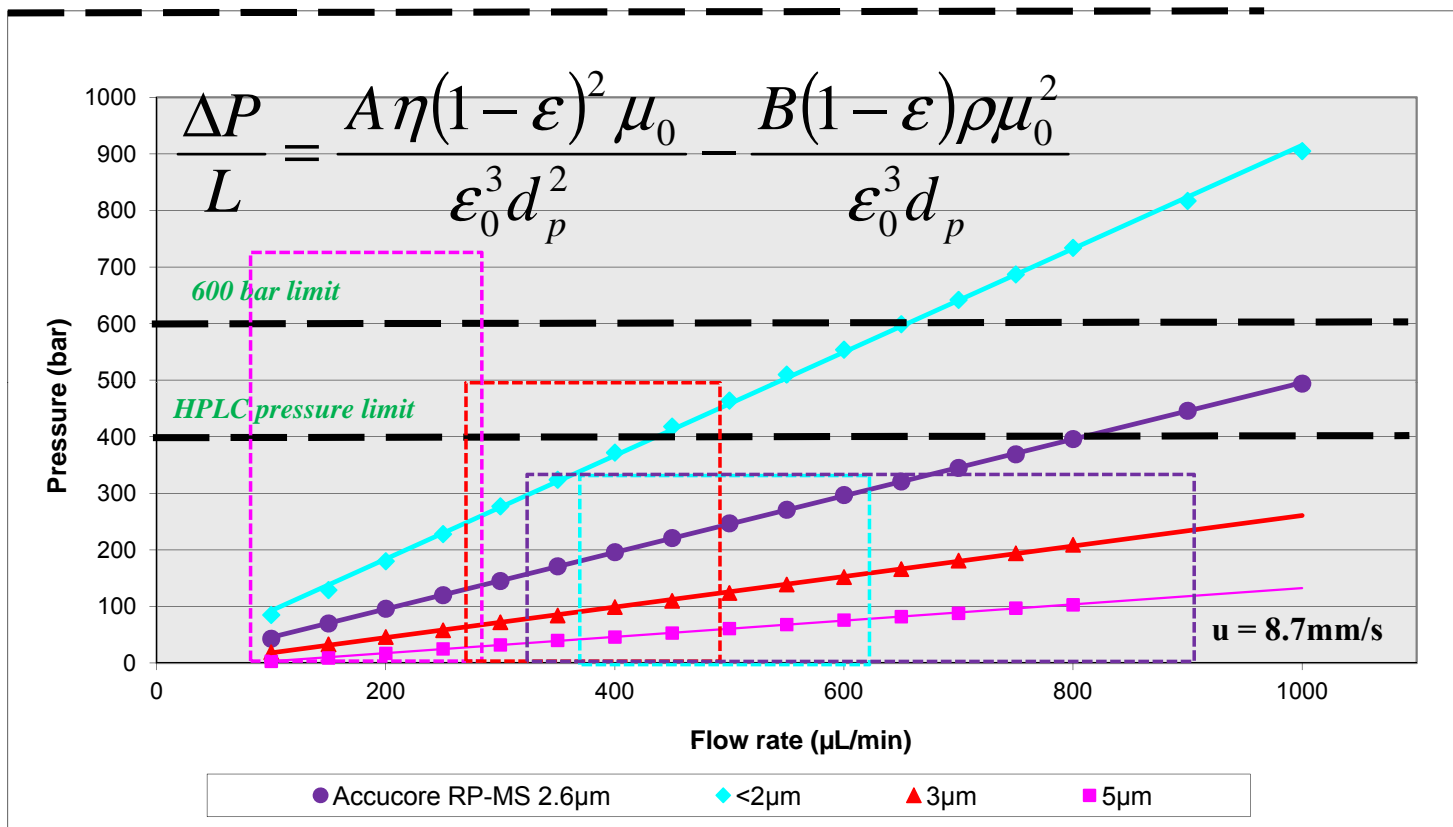
# Accucore—兼具高效快速的色谱柱



Columns: 100 x 2.1 mm  
Mobile phase: H<sub>2</sub>O / ACN (1:1)  
Temperature: 30 ° C  
Detection: UV at 254 nm  
Flow rate range: 0.1 to 1.0 mL/min  
Analyte: o-xylene

**核壳填料柱效最高，流速变化损失的柱效最小**

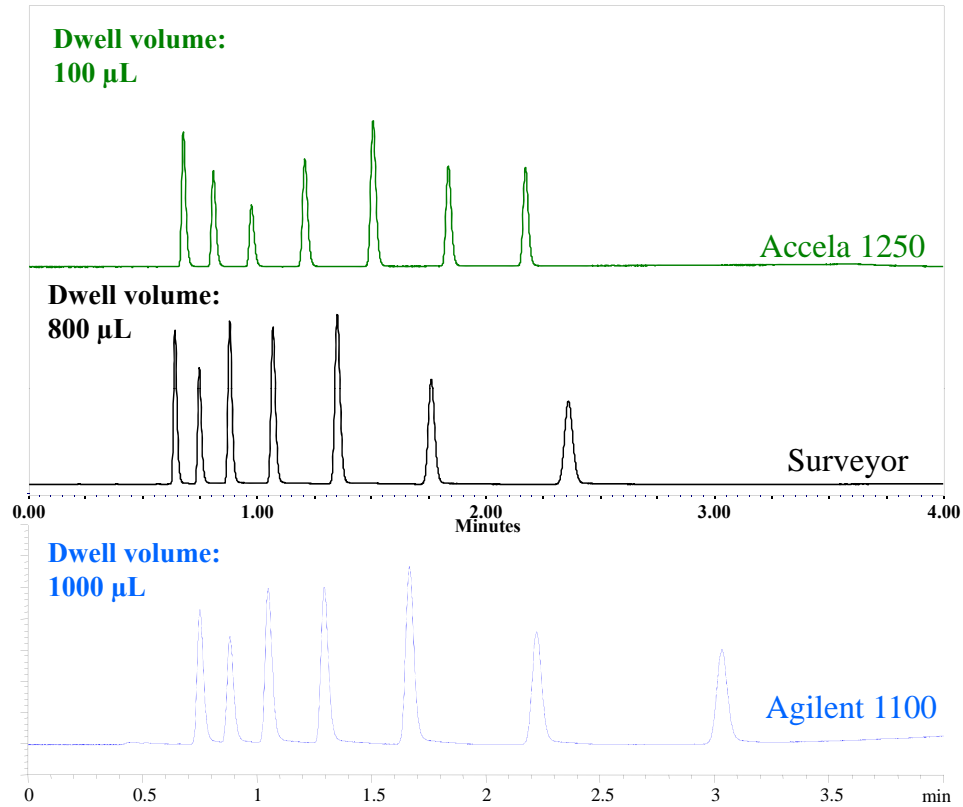
# 压力对比



Columns: 100 x 2.1 mm  
 Mobile phase: H<sub>2</sub>O / ACN (1:1)  
 Temperature: 30 ° C

P < 600 bar, 流速变化范围广

# 系统相关

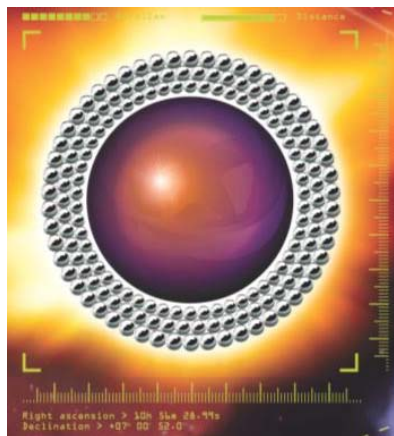


- Column: Accucore RP-MS 2.6 µm, 100 x 2.1 mm
- Gradient: 65 - 95 % B in 2.1 min  
95 % B for 0.4 min
- Flow rate: 400 µL/min

	Accela 1250	Surveyor	Agilent 1100
Run time (min)	2.5	3.0	3.5
Average PW (1/2 Height)	0.02	0.02	0.04

**不同系统中都有出色的表现**

# Accucore 发展历程



**Accucore 初次发布**

**Jun-2011**

- 2.6  $\mu\text{m}$ , 80  $\text{\AA}$
- 6-phases



**Apr-2012**

- 2.6  $\mu\text{m}$ , 80  $\text{\AA}$
- 1-phase

**Accucore C8**



**Jun-2012**

- 2.6  $\mu\text{m}$ , 150  $\text{\AA}$
- 2-phases

**Accucore Bio分析柱**



**Sep-2012**

- 4  $\mu\text{m}$ , 80  $\text{\AA}$
- 2-phases

**Accucore XL**



**Jan-2013**

- 2.6  $\mu\text{m}$ , 80/150  $\text{\AA}$
- 5-phases

**Accucore 系列键合相**

**July-2014**

- 1.5  $\mu\text{m}$ , 80  $\text{\AA}$

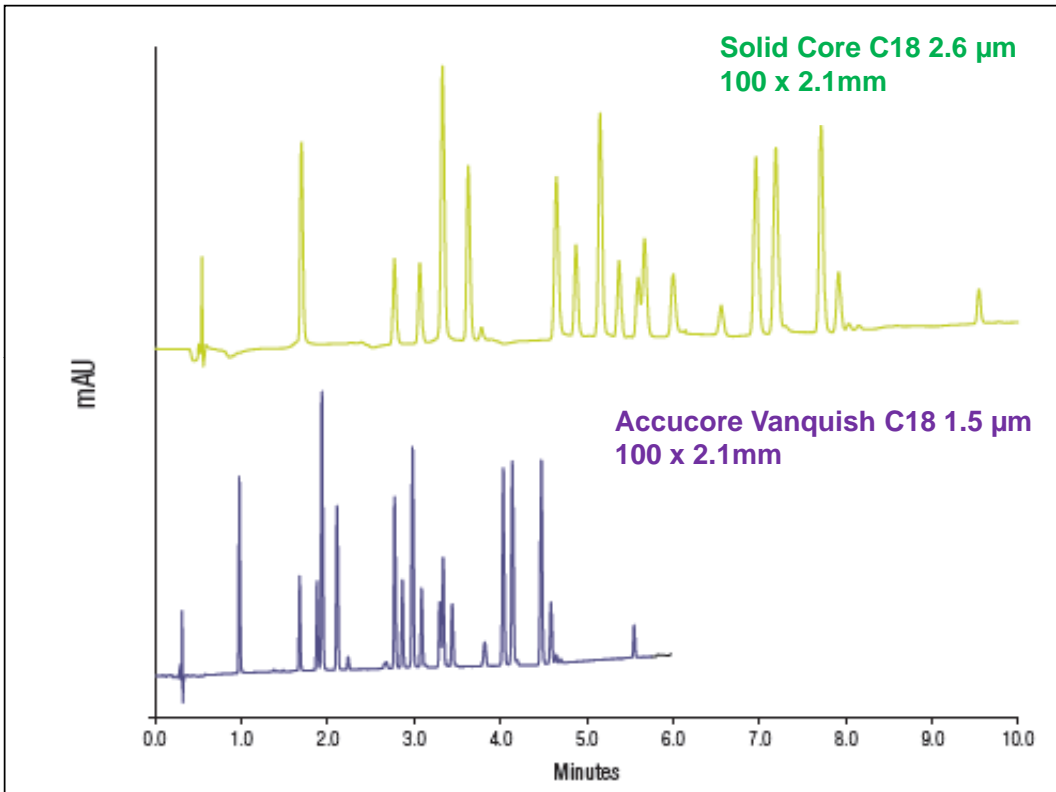
**Accucore Vanquish**



**CHROMELEON 72**  
SingleSolvent



## 核增强技术优势: **更多结果**

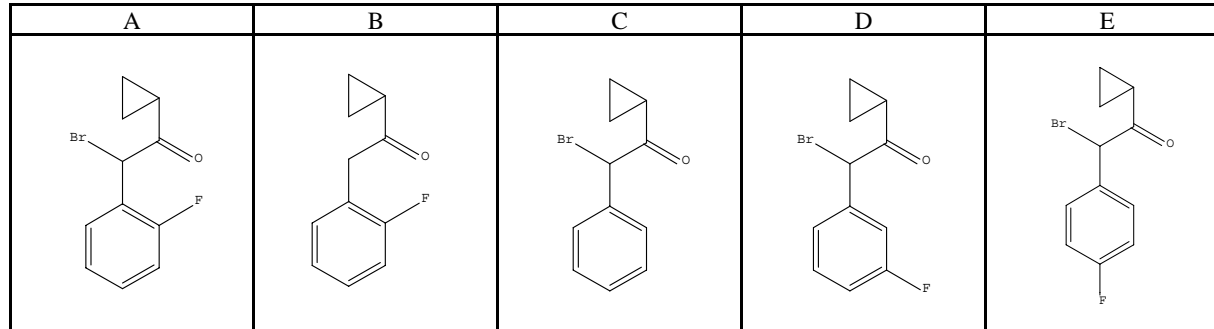
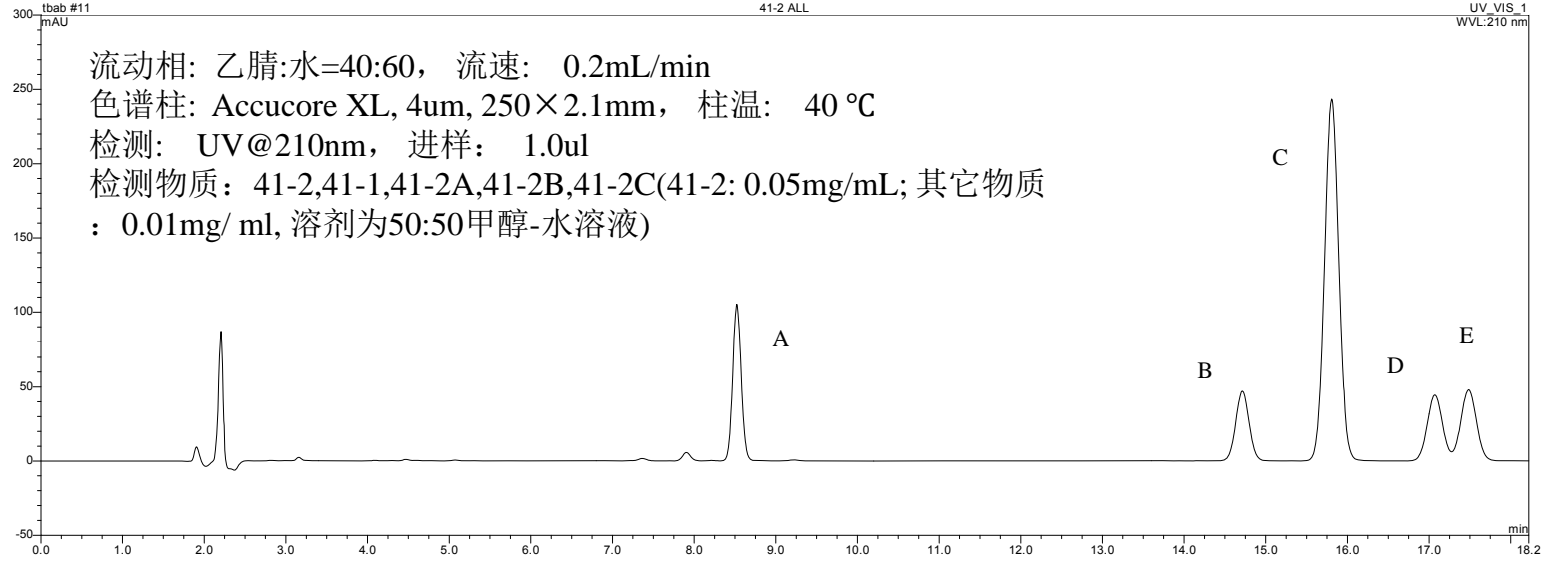


**节省40%时间, 分离度更高**

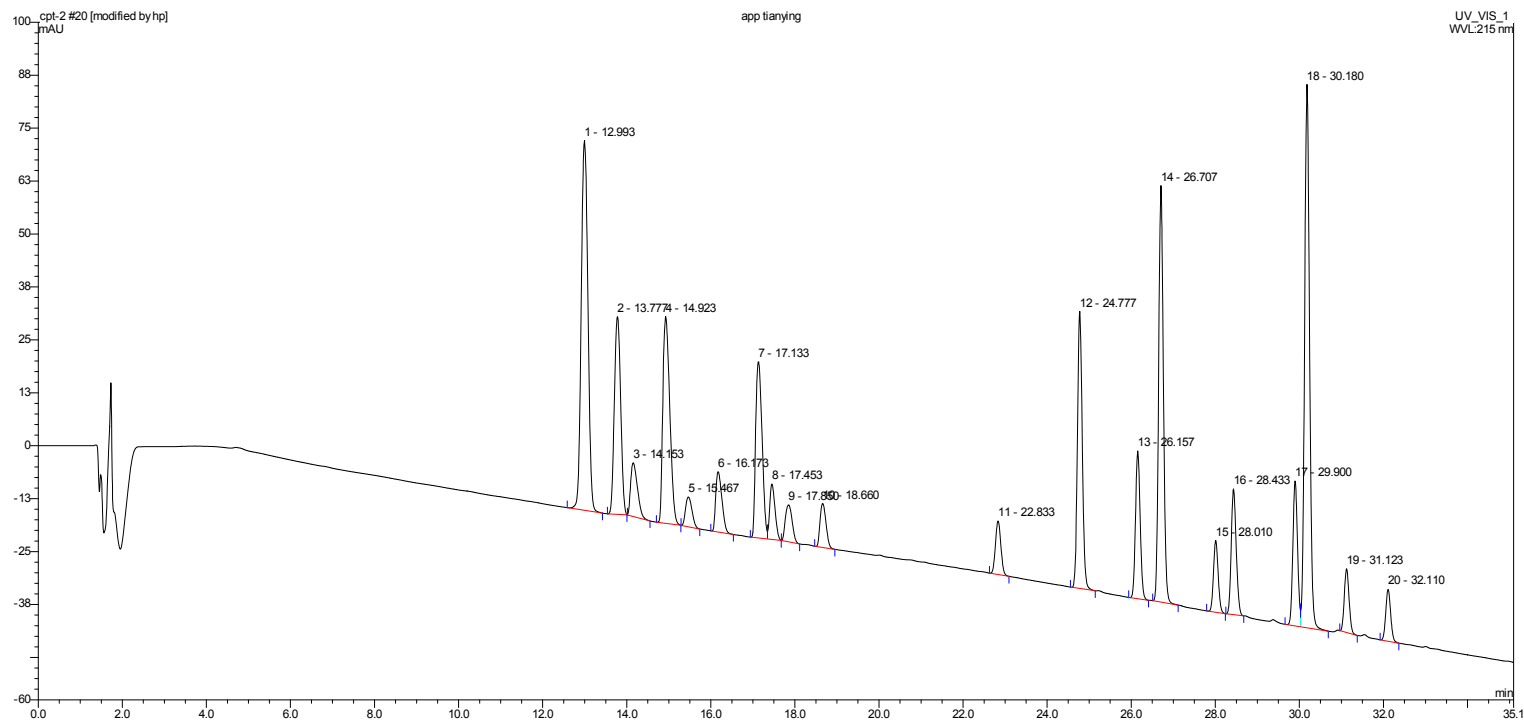
Mobile phase A:	water	
Mobile phase B:	acetonitrile	
Gradient:	Accucore Vanquish C18 1.5 µm 100 × 2.1 mm	
Time (min)	%B	
0	20	
4	40	
7	80	
Solid core C18 2.6 µm 100 × 2.1 mm		
Time (min)	%B	
0	20	
6.9	40	
12.1	80	
Flow Rate:	Solid Core C18 2.6 µm 100 × 2.1 mm = 380 µL/min	
	Accucore Vanquish C18 1.5 µm 100 × 2.1 mm = 650 µL/min	
Inj. Volume:	0.5 µL	
Temp.:	43 °C	
Detection:	UV at 230 nm (0.1s rise time, 50 Hz)	
Analytes:	1. Desethylatrazine	10. Diuron
	2. Metoxuron	11. Isoproturon
	3. Hexazinone	12. Metobromuron
	4. Simazine	13. Metazachlor
	5. Cyanazine	14. Sebutylazin
	6. Methabenzthiazuron	15. Propazine
	7. Chlorotoluron	16. Terbutylazine
	8. Atrazine	17. Linuron
	9. Monolinuron	18. Metolachlor



# 药物中间体多组分检测

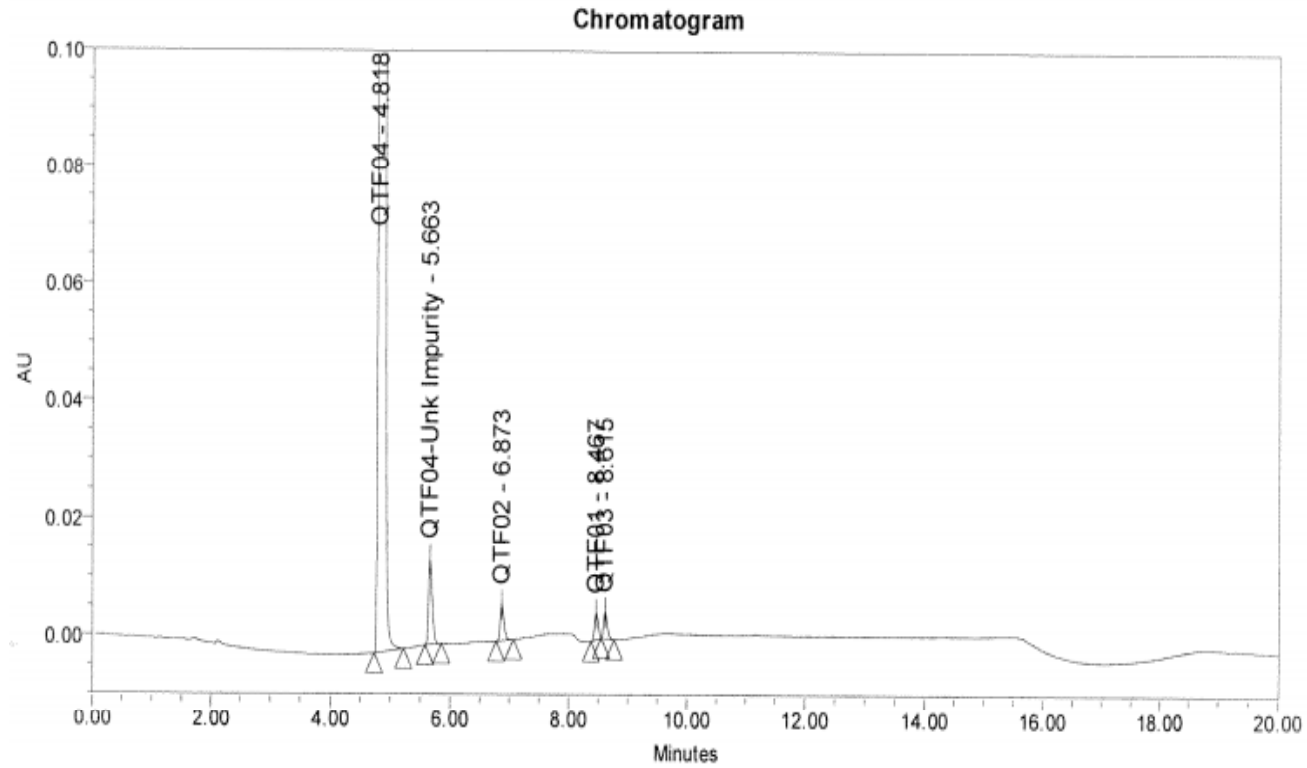


# 药物中间体多组分检测



色谱柱: Accucore XL, 4um, 250×2.1mm, PN: 74104-252130  
柱温: 30 °C  
检测: UV@215nm  
进样: 3.0 ul  
检测物质: 客户提供样品溶液  
流动相: A: 12%乙腈/10mM 醋酸铵, B: 60%乙腈/10mM 醋酸铵  
流速: 0.3mL/min

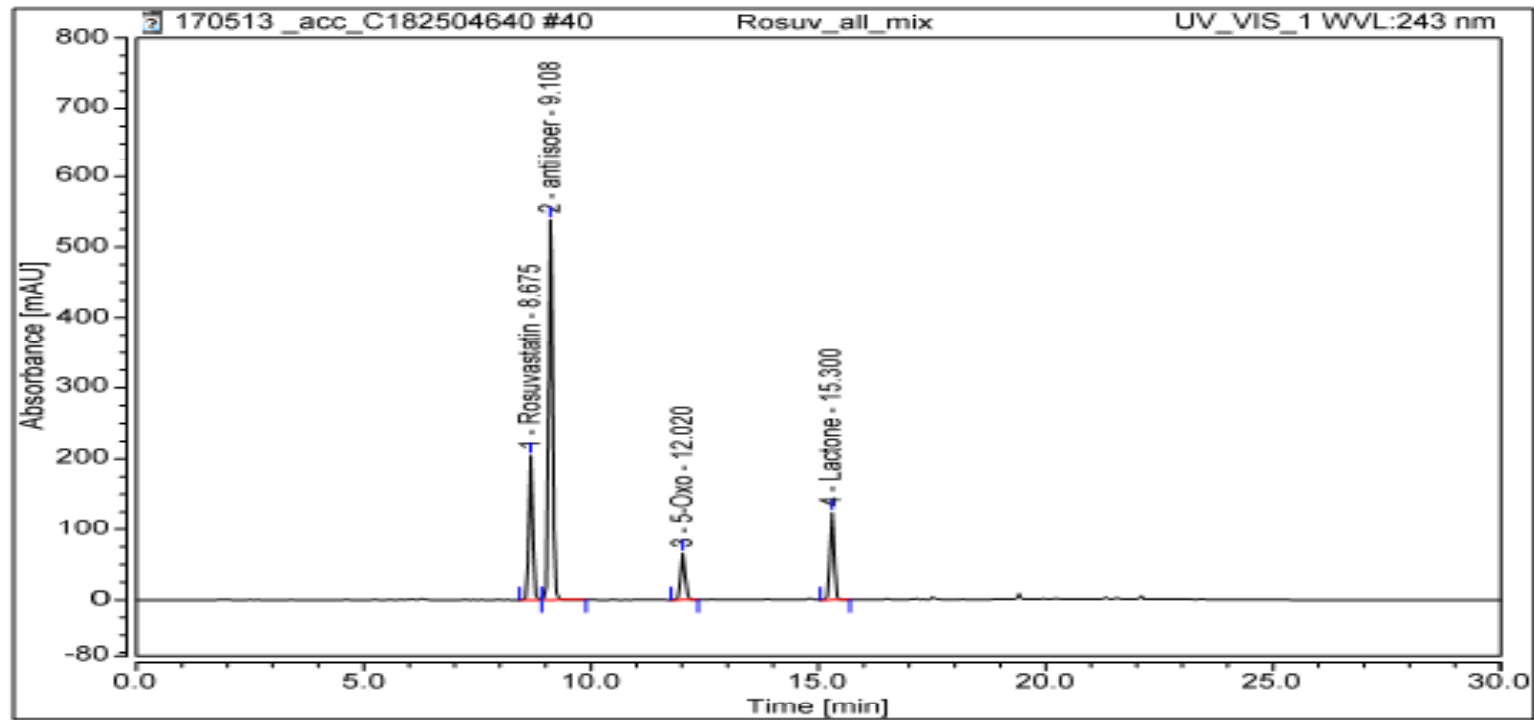
# 奎的平分析



系统适用性实验

# 洛伐他汀系统适用性试验

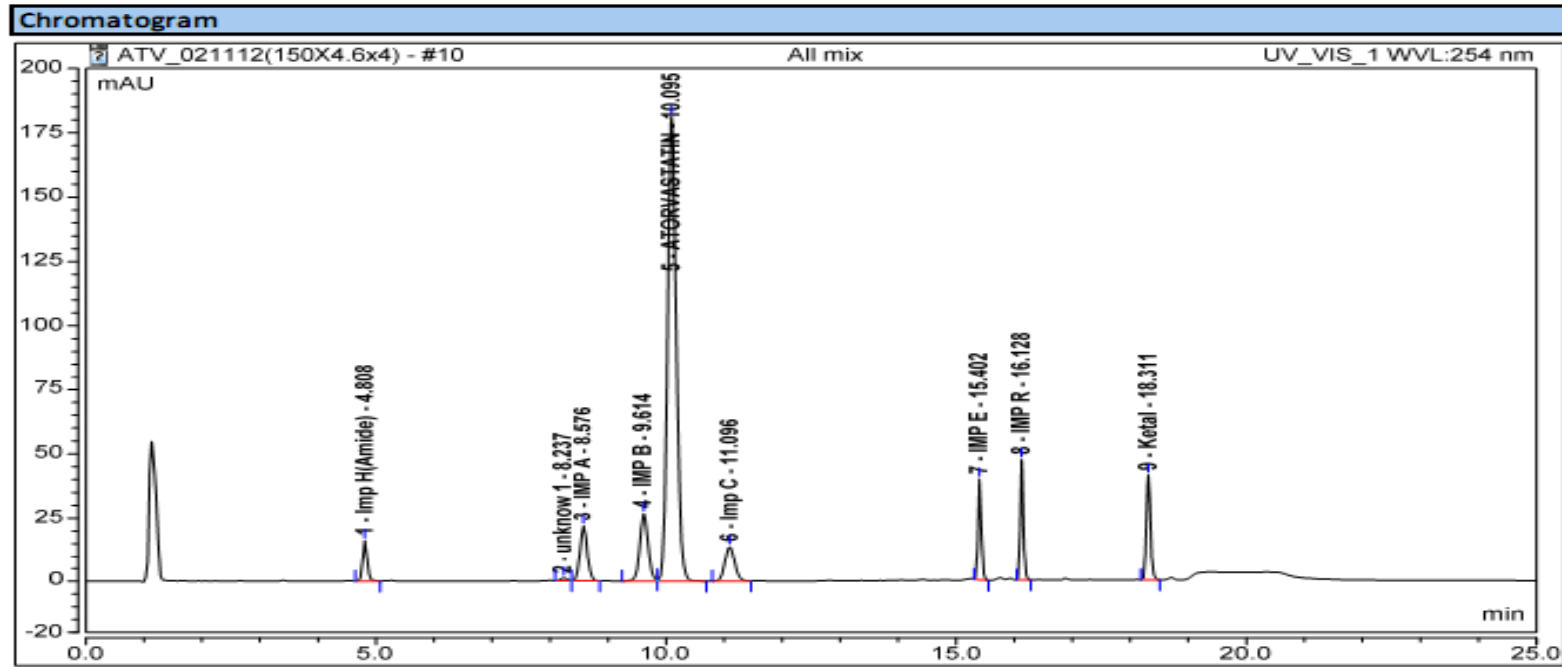
## Chromatogram



## Integration Results

No.	Peak Name	Retention Time min	Area mAU*min	Relative Area %	Resolution (USP)
1	Rosuvastatin	8.675	19.154	20.89	n.a.
2	anti isoer	9.108	52.164	56.88	2.92
3	5-Oxo	12.020	7.524	8.20	17.74
4	Lactone	15.300	12.863	14.03	19.33

# 阿托伐他汀杂质分析—Accucore C8 XL



No.	Peak Name	Retention Time min	Area mAU*min	Rel.Area %	Resolution (USP)	Asymmetry (USP)	Plates (USP)
1	Imp H(Amide)	4.808	2.168	2.80	n.a.	1.08	19153
2	unknow 1	8.237	0.025	0.30	19.27	1.07	23070
3	IMP A	8.576	10.455	6.15	1.51	1.10	21869
4	IMP B	9.614	19.865	8.48	4.23	1.07	22003
5	ATORVASTATIN	10.095	998.578	60.09	1.80	1.13	21611
6	Imp C	11.096	7.140	5.08	3.48	1.08	21848
7	IMP E	15.402	7.153	5.09	21.25	1.11	343881
8	IMP R	16.128	8.654	5.59	7.22	1.17	449182
9	Ketal	18.311	11.412	6.42	19.55	1.15	332711

检测成分	色谱柱	部件号
阿托伐他汀	Accucore C8 XL 4 $\mu$ m , 4.6 $\times$ 150 mm	74204-154630
阿托伐他汀, 氨氯地平	Accucore C8 XL 4 $\mu$ m , 4.6 $\times$ 150 mm	74204-154630
阿托伐他汀API	Accucore PFP 2.6 $\mu$ m , 4.6 $\times$ 150 mm	17426-154630
阿托伐他汀中间体	Accucore PFP, 2.6 $\mu$ m, 3 $\times$ 100 mm	17426-103030
比马前列素	Accucore Phenyl hexyl, 2.6 $\mu$ m 3 $\times$ 100 mm	17926-103030
醋氯芬酸	Synchronis C18 5 $\mu$ m, 3mm $\times$ 100 mm	97105-103030
地西台宾	Accucore HILIC 3mm , 150 mm	17526-153030
蒿甲醚	Accucore XL C18/Accucore C18	74104-254630/17126-153030
蒿乙醚	Accucore XL C18, 4 $\mu$ m, 4.6 $\times$ 150 mm	74104-154630
喹硫平杂质	Accucore PFP, 2.6 $\mu$ m, 3 $\times$ 100 mm	17426-103030
氯苯那敏, 假麻黄碱, 布洛芬	Accucore PFP, 3 $\times$ 150 mm	17426-153030
孟鲁司特钠	Accucore RPMS 2.6 $\mu$ m , 3 $\times$ 100 mm	17626-103030
帕立骨化醇	Accucore C18, 2.6 $\mu$ m, 3 $\times$ 150 mm	17126-153030
瑞舒伐他汀	Accucore C18 XL, 4.0 $\mu$ m 4.6 $\times$ 250 mm	74104-254630
伊曲康唑	Accucore C18 3 $\times$ 100 mm	17126-103030

● **Acclaim 系列色谱柱**

● The world leader in serving science

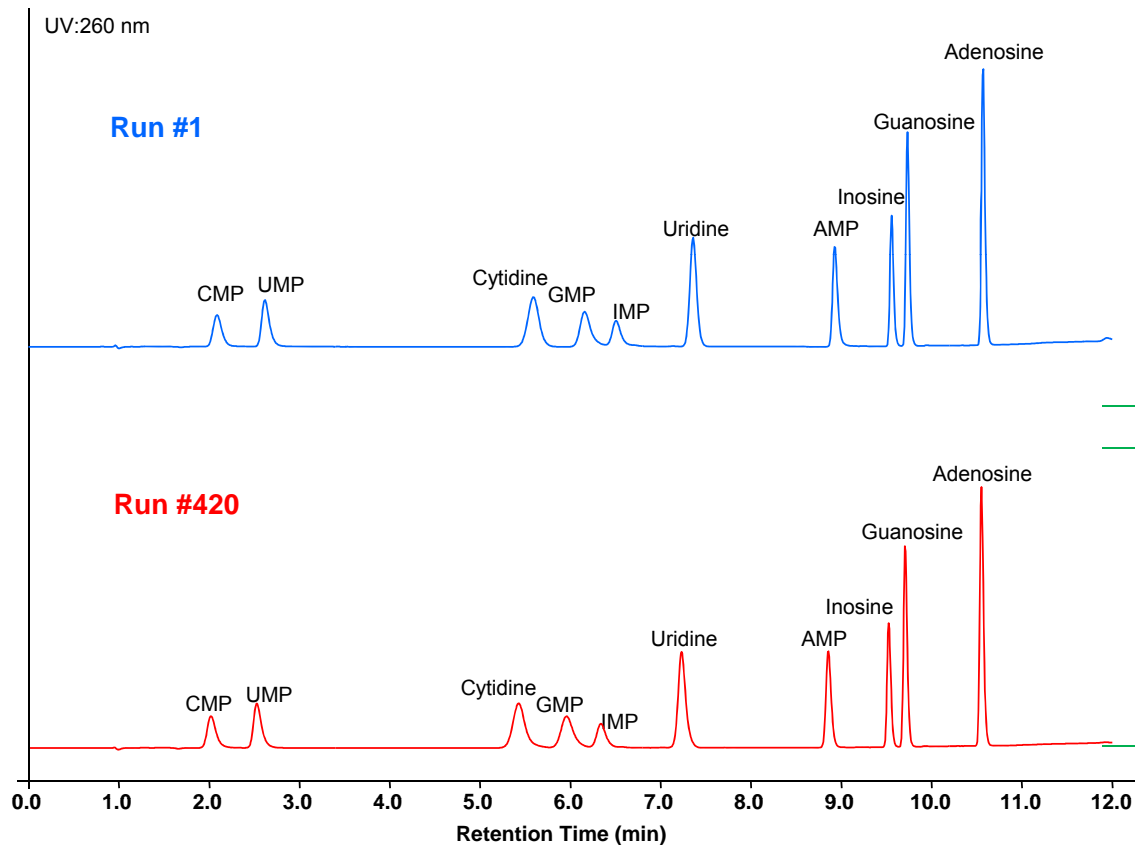
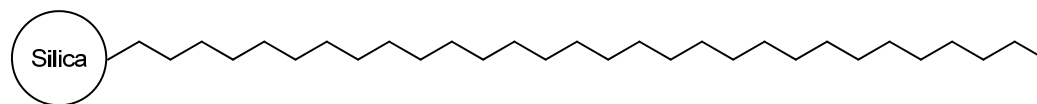
## Acclaim 反相色谱柱-极佳的分离和稳定性

Name	USP	Chemistry	Bonding	Particle Size (µm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	pH Range	Aqueous Compatibility
120 C18	L1	C18	Monomeric End-capped	2.2, 3.0, 4.5	120	300	2.0 – 8.0	90%
300 C18	L1	C18	Monomeric End-capped	3.0	300	100	2.0 – 8.0	95%
120 C8	L7	C8	Monomeric End-capped	2.2, 3.0, 4.5	120	300	2.0 – 8.0	90%
C30	L62	C30	Monomeric End-capped	3.0, 4.5	200	200	2.0 – 8.0	100%
PolarAdvantage (PA)	L60	Sulfonamide-embedded	Monomeric End-capped	2.2, 3.0, 4.5	120	300	2.0 – 8.0	100%
PolarAdvantage II (PA2)	L60	Amide-embedded	Multi-point bonding End-capped	2.2, 3.0, 4.5	120	300	1.5 – 10.5	100%
Phenyl-1	L11	Alkyl aromatic	Multi-point bonding End-capped	3.0, 4.5	120	300	2.0 – 8.0	100%

**中国药典200个西药项目和300多个中药项目**



# Nutritional Nucleotides & Nucleosides on **C30** phase

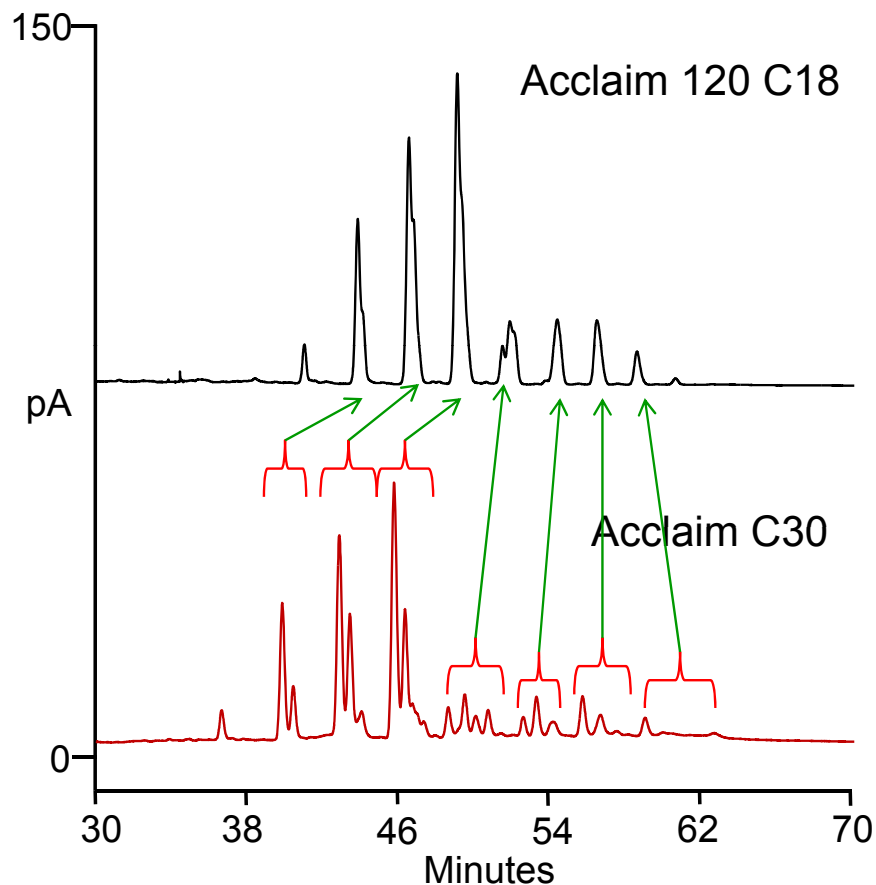


Column: **Acclaim C30**, 3  $\mu$ m  
 Dimension: 2.1  $\times$  150 mm  
 Mobile Phase: A) DI water  
 B) NH<sub>4</sub>OAc (100 mM, pH5)  
 C) CH<sub>3</sub>OH  
 Temperature: 15  $^{\circ}$ C  
 Flow Rate: 0.4 mL/min  
 Injection: 10  $\mu$ L, 1 ppm of each analyte  
 Detection: UV at 260 nm

Gradient:

Time	%A	%B	%C
-5.0	80	20	0
0	80	20	0
3.0	80	20	0
5.9	70	20	10
8.5	40	20	40
11.9	40	20	40
12.0	80	20	0

# Analysis of Cooking Oil: C30 vs. C18

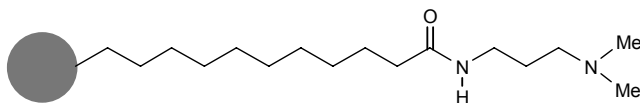


Column: **Acclaim C30** or Acclaim 120 C18, 5  $\mu$ m  
 Dimension: 4.6x150 mm  
 Mobile Phase: Acetonitrile (MeCN)/Iso-propanol (IPA)/  
 Ammonium Acetate (0.1 M, pH5.0)  
 (Buffer)  
 Temperature: 40° C  
 Flow Rate: 1.0 mL/min  
 Inj. Volume: 2  $\mu$ L  
 Detection: Corona *ultra* (Gain = 100 pA; Filter =  
 medium; Neb. Temp = 25° C)  
 Sample: Peanut oil (5 mg/mL in iso-propanol)

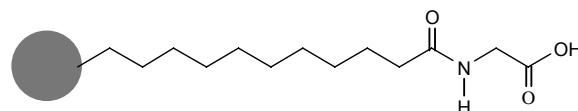
## Gradient

Time (min)	MeCN	IPA	Buffer
-15	90	5	5
0	90	5	5
0.1	90	5	5
60	0	95	5
70	0	95	5

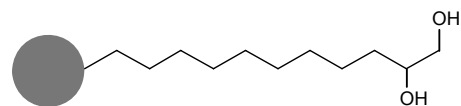
## 混合模式色谱柱



Acclaim Mixed-Mode WAX-1



Acclaim Mixed-Mode WCX-1



Acclaim Mixed-Mode HILIC-1

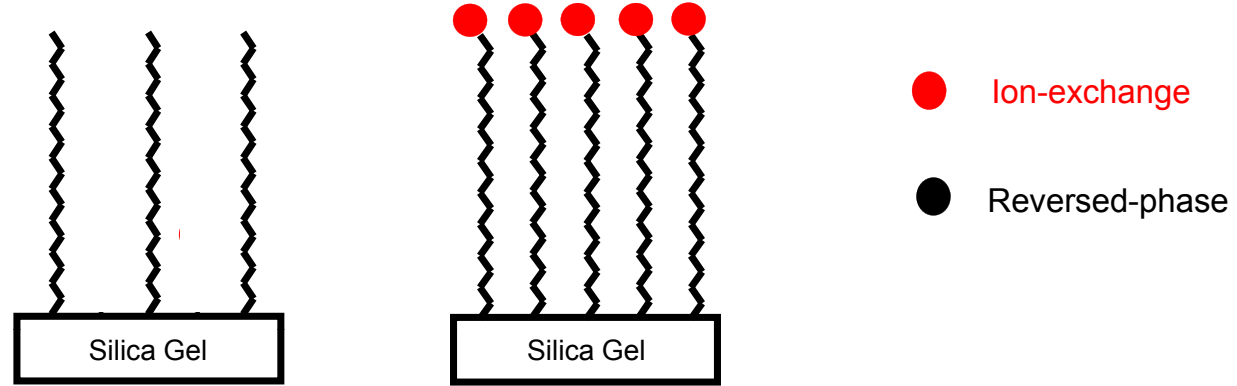
Name	USP	Chemistry	Particle Size (μm)	Pore Size (Å)	Surface Area (m <sup>2</sup> /g)	pH Range	Aqueous Compatibility
Trinity P1	N/A	Alkylamino bonded silica coated with sulfonated nano-polymer beads	3.0	300	100	2.5 – 7.5	100%
Trinity P2	N/A	Carboxylated bonded silica coated with quat nano-polymer beads	3.0	120	300	2.5 – 7.5	100%
Mixed-Mode WAX-1	L78	Alkyl tertiary Amine	3.0, 4.5	120	300	2.5 – 7.0	100%
Mixed-Mode WCX-1	L85	Alkyl carboxylic	3.0, 4.5	120	300	2.5 – 7.0	100%
Mixed-Mode HILIC-1	N/A	Alkyl diol	3.0, 4.5	120	300	2.5 – 7.5	100%

# Application-Specific Columns

Name	Targeted Applications	Retention Mechanism	Particle Size (µm)	pH Range	Aqueous Compatibility
Acclaim Trinity P1	Pharmaceuticals and counterions	Mixed-mode	3.0	2.5 – 7.5	100%
Acclaim Trinity P2	Pharmaceuticals and counterions	Mixed-mode	3.0	2.5 – 7.5	100%
Acclaim Organic Acid	Organic acids	Reversed-phase	3.0, 4.5	2.0 – 8.0	100%
Acclaim Surfactant Plus	Various types of surfactants	Mixed-mode	3.0, 4.5	2.5 – 7.5	100%
Acclaim Carbamate	Carbamates pesticides/herbicides in EPA Method 531.2	Reversed-phase	2.2, 3.0, 4.5	2.0 – 8.0	90%
Acclaim Carbonyl C18	Ketones & aldehydes in U.S. EPA Methods 1667, 554, OT-11; CARB 1004	Reversed-phase	2.2	2.5 – 8.0	90%
Acclaim Explosives E2	Explosives related compounds in EPA Method 8330	Reversed-phase	2.2, 3.0, 4.5	2.5 – 8.0	90%
Acclaim Trinity Q1	Diquat and paraquat in EPA Method 549.2	Mixed-Mode	2.2	2.5 – 7.5	100%
Acclaim HAA	Haloacetic acids analysis in drinking water by LC/MS	Mixed-Mode	3.0	2.5 – 7.5	100%

# 特殊选择性—混合基质色谱柱

- 键合相



- 用途

非极性化合物：反相 C18

例-大部分药物

极性化合物：aQ, HILIC

例-阿卡波糖

离子化合物：离子交换柱

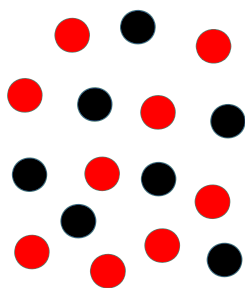
例-二甲双胍

既有极性化合物又有非极性化合物：??

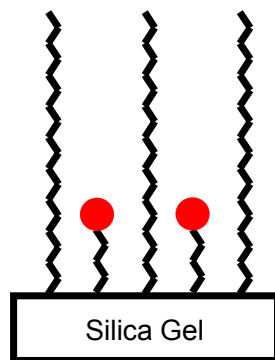
例-药物及其对离子

# RP/IEX 混合基质填料

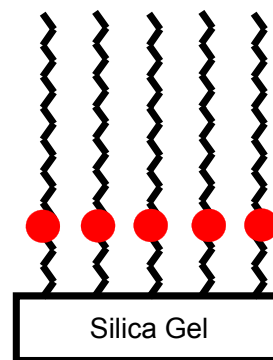
颗粒混合



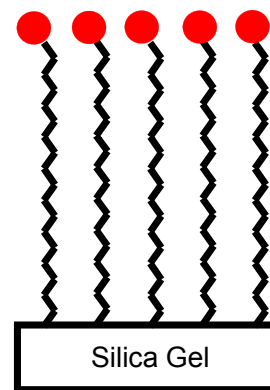
基团混合



单链键合  
(嵌入基团)



单链键合  
(官能团在末端)

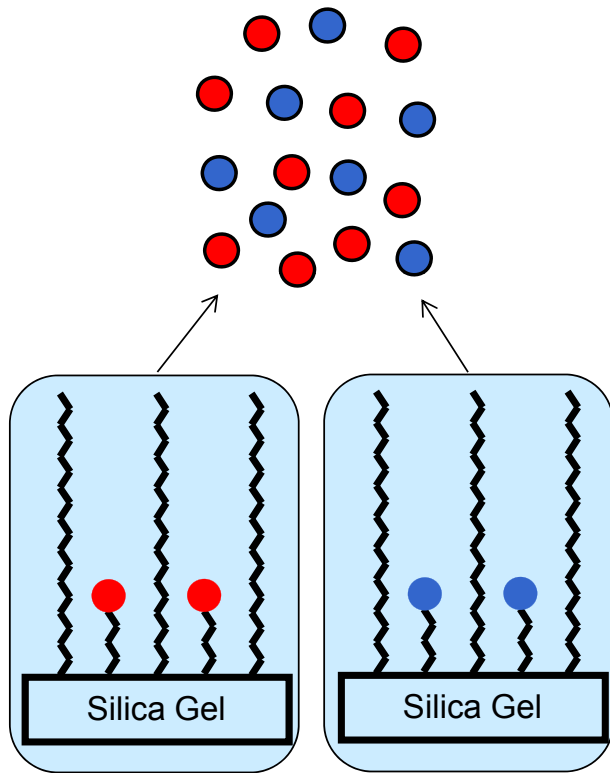


● Ion-exchange

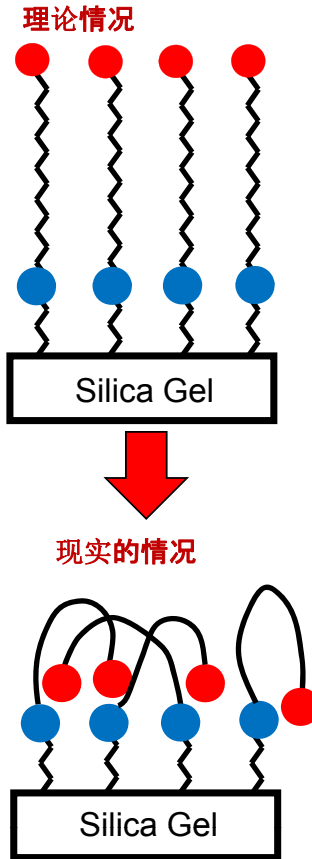
● Reversed-phase

# RP/AEX/CEX 三重机制键合相

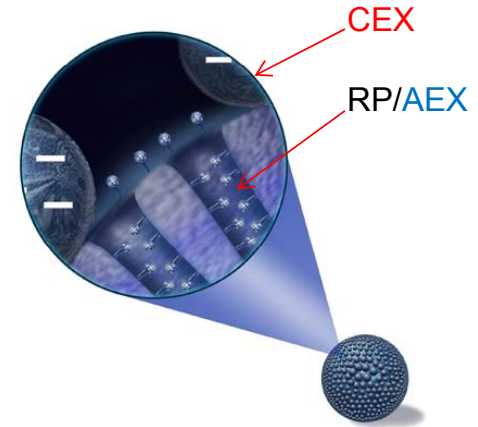
颗粒混合



单链键合(两性)

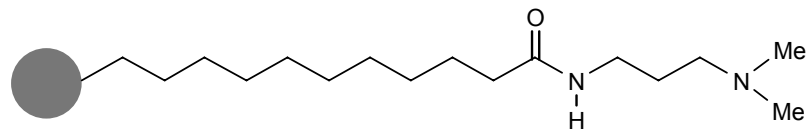
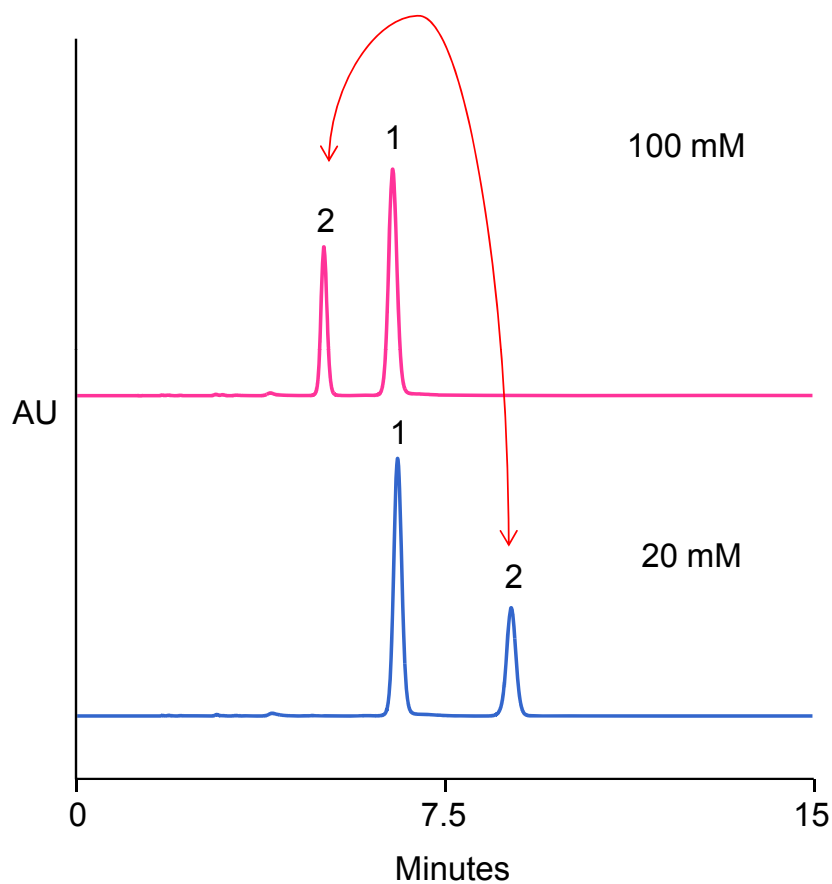


纳米微球-硅胶键合相(NSH)



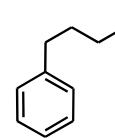
- Ion-exchange
- Reversed-phase

# 通过缓冲液浓度调节选择性

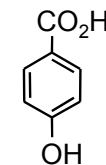


色谱柱: Acclaim 混合基质 WAX-1, 5  $\mu\text{m}$   
规格: 150x4.6 mm  
流动相: 50/50 v/v acetonitrile/phosphate buffer, pH6  
温度: 30 ° C  
流速: 1 mL/min  
进样量: 2  $\mu\text{L}$   
检测: UV (210 nm)

色谱峰: 1. 丁基苯 (0.1 mg/mL)  
2. 4-水杨酸 (0.5 mg/mL)



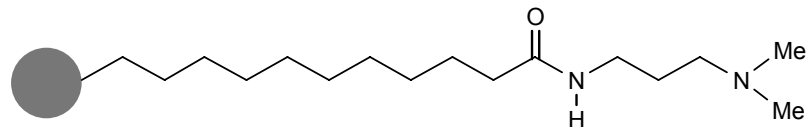
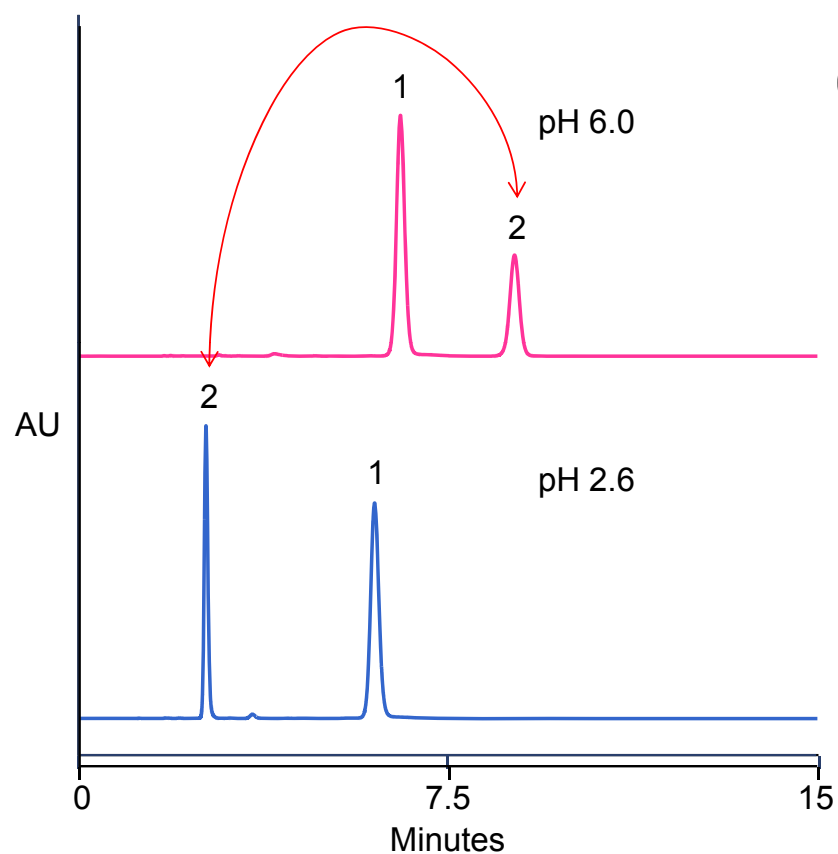
Butylbenzene



4-Hydroxybenzoic acid

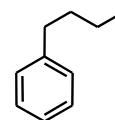


# 通过pH调节选择性

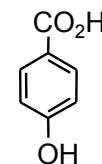


色谱柱: Acclaim 混合基质 WAX-1, 5  $\mu\text{m}$   
规格: 150x4.6 mm  
流动相: 50/50 v/v acetonitrile/ 20 mM phosphate buffer  
温度: 30 ° C  
流速: 1 mL/min  
进样量: 2  $\mu\text{L}$   
检测: UV (210 nm)  
色谱峰:

1. Butylbenzene (0.1 mg/mL)
2. 4-Hydroxybenzoic acid (0.5 mg/mL)

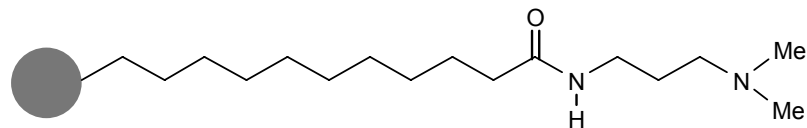
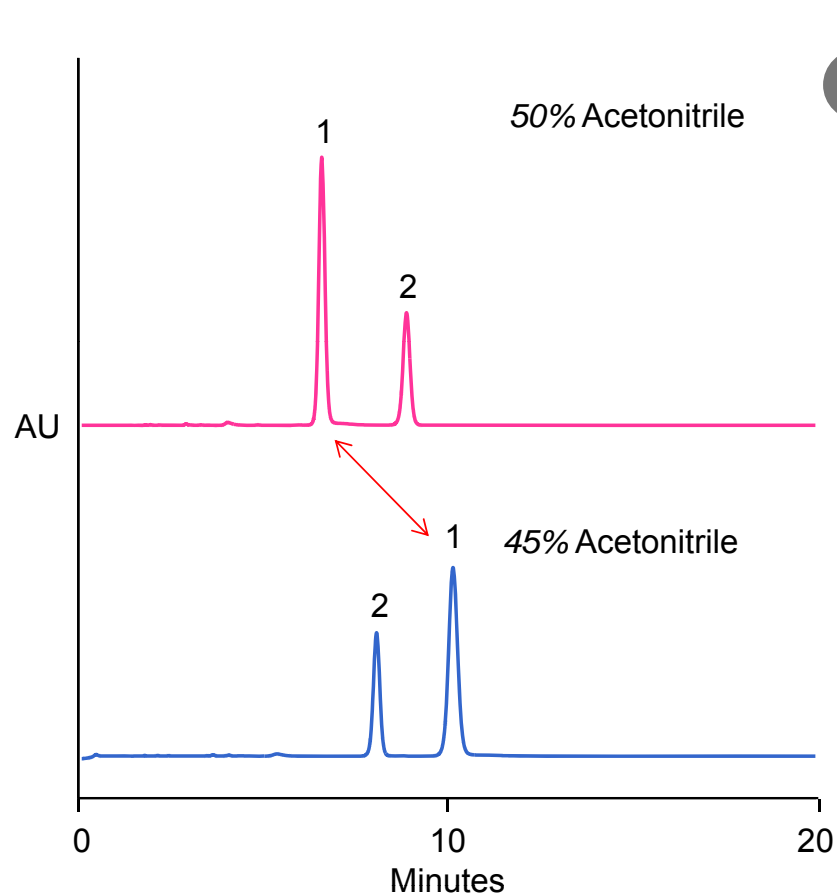


Butylbenzene



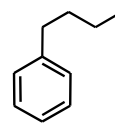
4-Hydroxybenzoic acid

# 通过有机相比例调节选择性

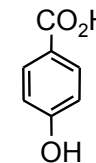


色谱柱: Acclaim 混合基质 WAX-1, 5  $\mu$ m  
规格: 150x4.6 mm  
流动相: Acetonitrile/ 20 mM phosphate buffer, pH6  
温度: 30 ° C  
流速: 1 mL/min  
进样量: 2  $\mu$ L  
检测: UV (210 nm)

色谱峰: 1. Butylbenzene (0.1 mg/mL)  
2. 4-Hydroxybenzoic acid (0.5 mg/mL)

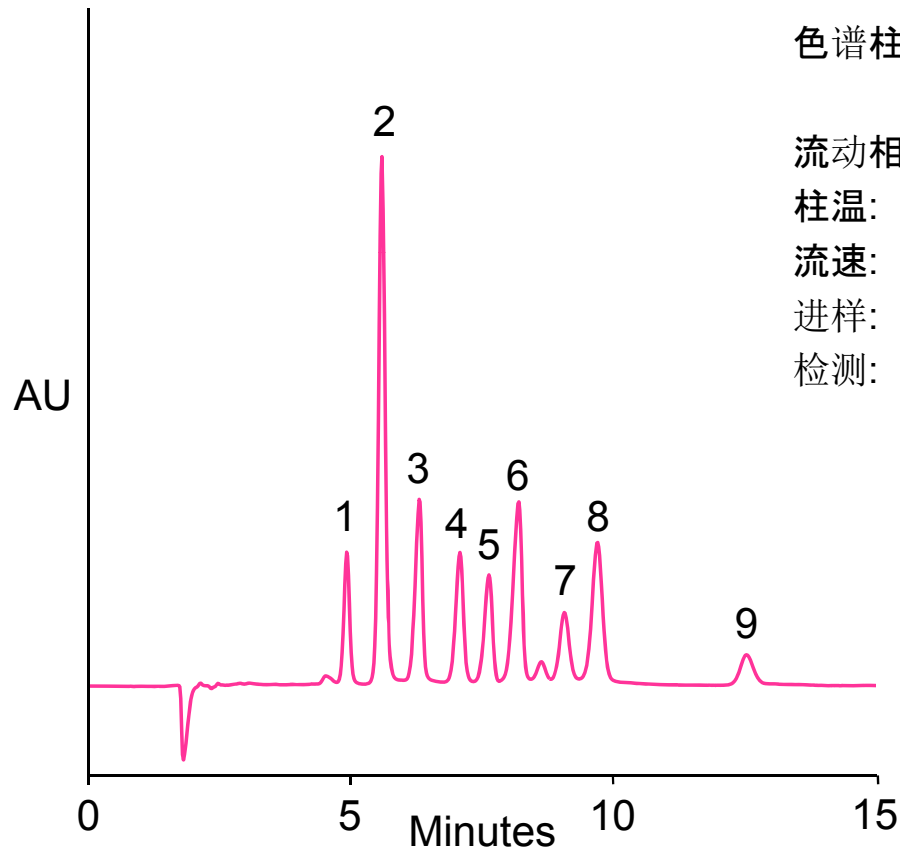
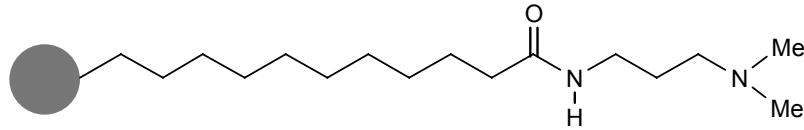


Butylbenzene



4-Hydroxybenzoic acid

# 特殊色谱柱—混合基质WAX分离有机酸



色谱柱: Acclaim Mixed-Mode WAX-1, 5  $\mu$ m  
4x150 mm

流动相: 25 mM PBS, pH=6.0

柱温: 30  $^{\circ}$  C

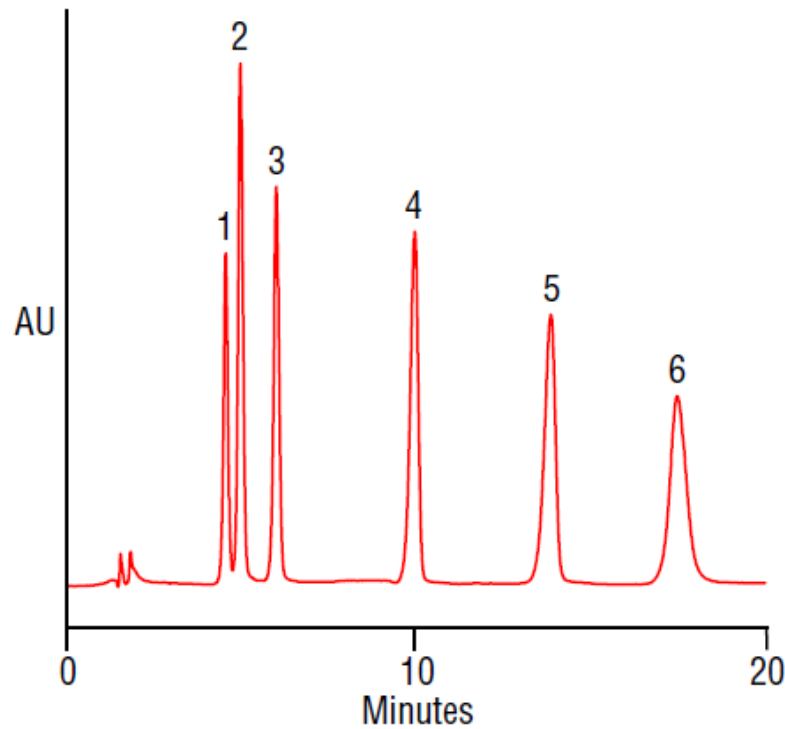
流速: 0.50 mL/min

进样: 10  $\mu$ L

检测: UV @ 210 nm

1. 奎宁酸
2. 莽草酸
3. 羟基乙酸
4. 乳酸
5. 乙酸
6. 甲酸
7. 抗坏血酸(Vit. C)
8. 异抗坏血酸
9. 丙酸

# 氨基酸分析



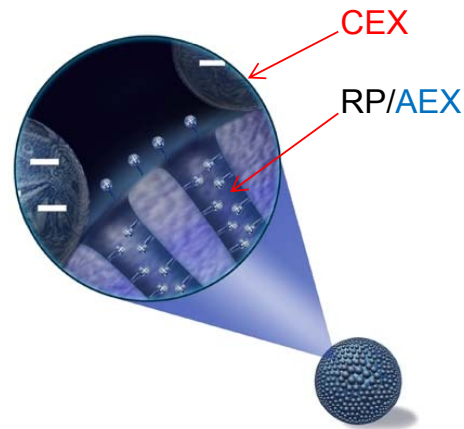
Column: Acclaim Mixed-Mode WAX-1, 5  $\mu$ m  
Dimensions: 4.6  $\times$  150 mm  
Mobile Phase: 25/75 v/v 25 mM phosphate buffer, pH 6.0/acetonitrile

Temperature: 30°C  
Flow Rate: 1 mL/min  
Inj. Volume: 10  $\mu$ L  
Detection: UV, 210 nm

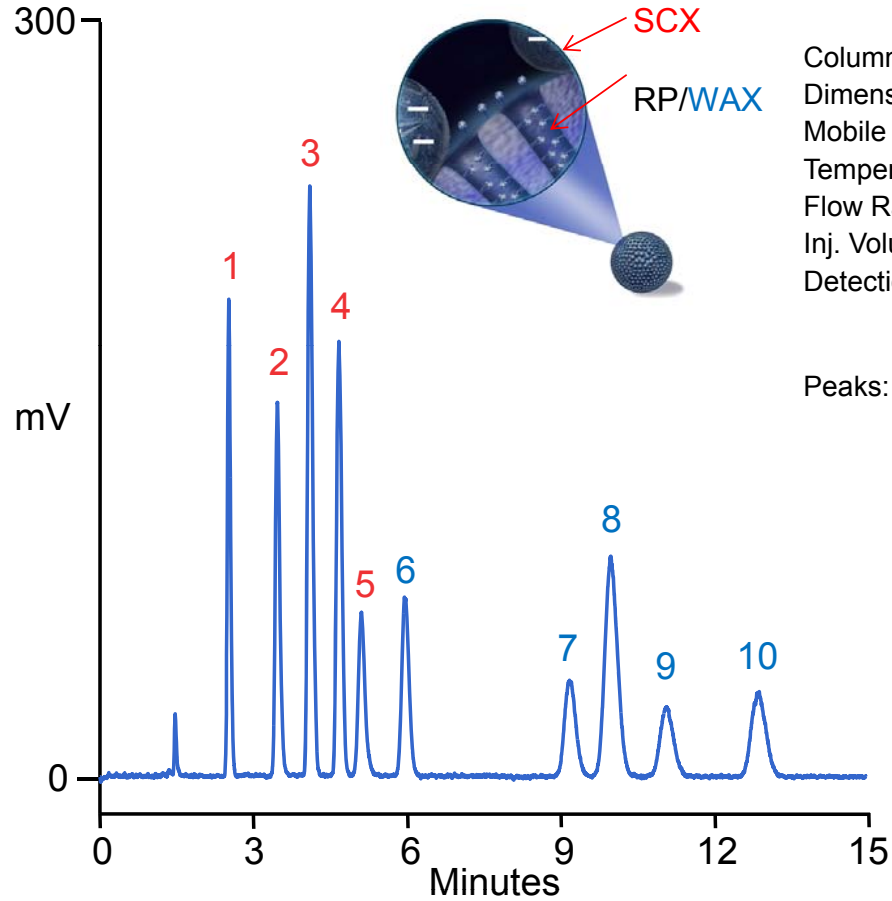
Peaks:  
1. Leucine  
2. Isoleucine  
3. Valine  
4. Alanine  
5. Serine  
6. Glycine

# Trinity P1 分离药物和对离子

- 药物开发中，成盐是一个重要的步骤
  - 50% 药物以盐的形式存在
- 挑战
  - RP: 对离子没有保留, 或保留很弱; 对API有弱的保留、或足够强的保留
  - IEX: 需要AEX 和CEX 色谱柱; 不适用于中性API
  - HILIC: 仅限于极性化合物
- 解决方案
  - Acclaim Trinity P1 – RP/AEX/CEX 三重键合相, 用于阳离子、阴离子、中性药物分析。



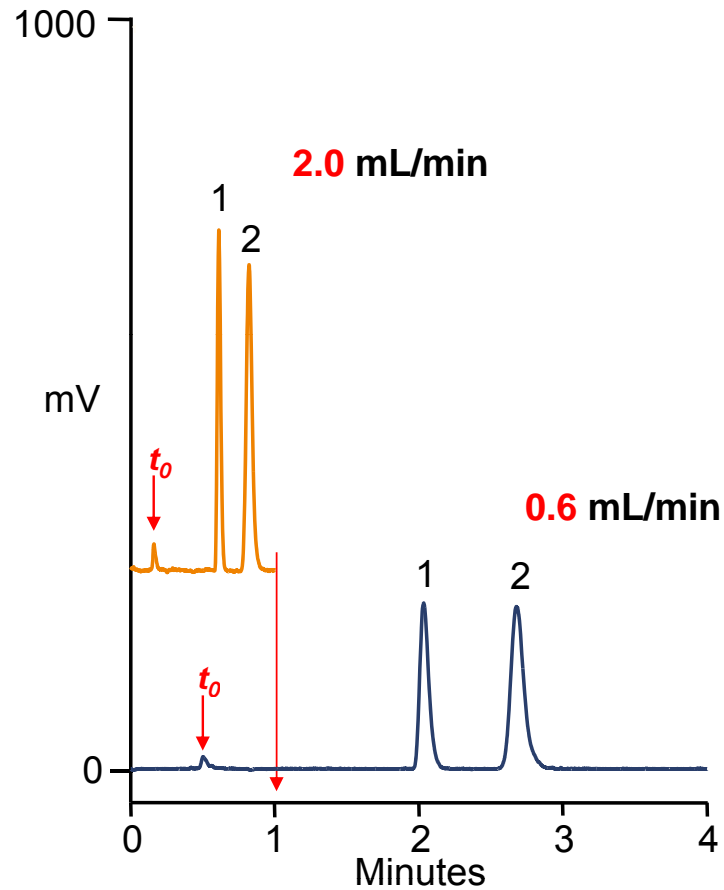
# 对离子检测



Column: **Acclaim Trinity P1**, 3  $\mu\text{m}$   
Dimensions: 3.0  $\times$  100 mm  
Mobile Phase: 60/40 v/v  $\text{CH}_3\text{CN}/20$  mM (total)  $\text{NH}_4\text{OAc}$ , pH5  
Temperature: 30  $^\circ\text{C}$   
Flow Rate: 0.5 mL/min  
Inj. Volume: 2  $\mu\text{L}$   
Detection: Corona *ultra* (Gain = 100 pA; Filter = med; Neb Temp = 30  $^\circ\text{C}$ )

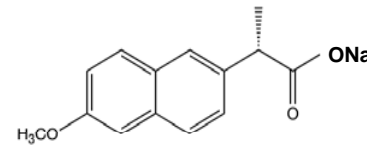
Peaks: (50 to 100 ppm)  
1. Choline  
2. Tromethamine  
3. Sodium  
4. Potassium  
5. Meglumine  
6. Mesylate  
7. Nitrate  
8. Chloride  
9. Bromide  
10. Iodide

# 亲水性药物及对离子

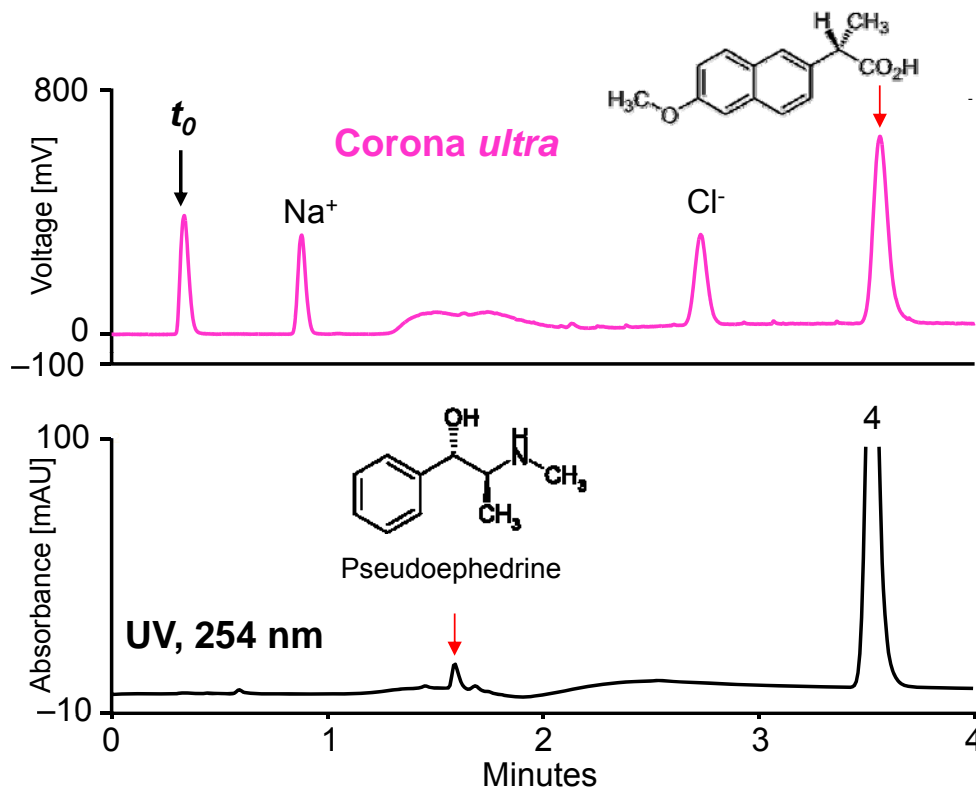


Column: **Acclaim Trinity P1**, 3  $\mu\text{m}$   
Dimensions: 3.0 x 50 mm  
Mobile Phase: 80/20 v/v  $\text{CH}_3\text{CN}/20 \text{ mM}$  (total)  $\text{NH}_4\text{OAc}$ , pH5  
Temperature: 30 ° C  
Flow Rate: 0.6 and 2.0 mL/min  
Inj. Volume: 2.5  $\mu\text{L}$   
Detection: Corona *ultra*  
(Gain = 100pA; Filter = med; Neb Temp = 30° C)  
Sample: Na, Naproxen (0.2 mg/mL in mobile phase)  
Peaks:

1.  $\text{Na}^+$
2. Naproxen



# 不同性质药物的同时检测



Column: **Acclaim Trinity P1**, 3  $\mu\text{m}$

Dimensions: 3.0 x 50 mm

Mobile Phase: A:  $\text{CH}_3\text{CN}$

B: D.I.  $\text{H}_2\text{O}$

C: 0.1 M  $\text{NH}_4\text{OAc}$ , pH 5.2

Temperature: 30 ° C

Flow Rate: 0.6 mL/min

Inj. Volume: 1  $\mu\text{L}$

Detection: UV at 254 nm and a Corona ultra detector in series

Corona ultra settings:

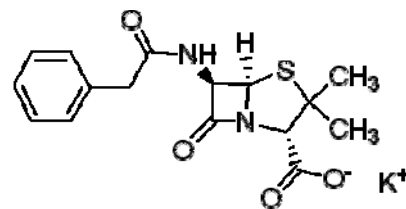
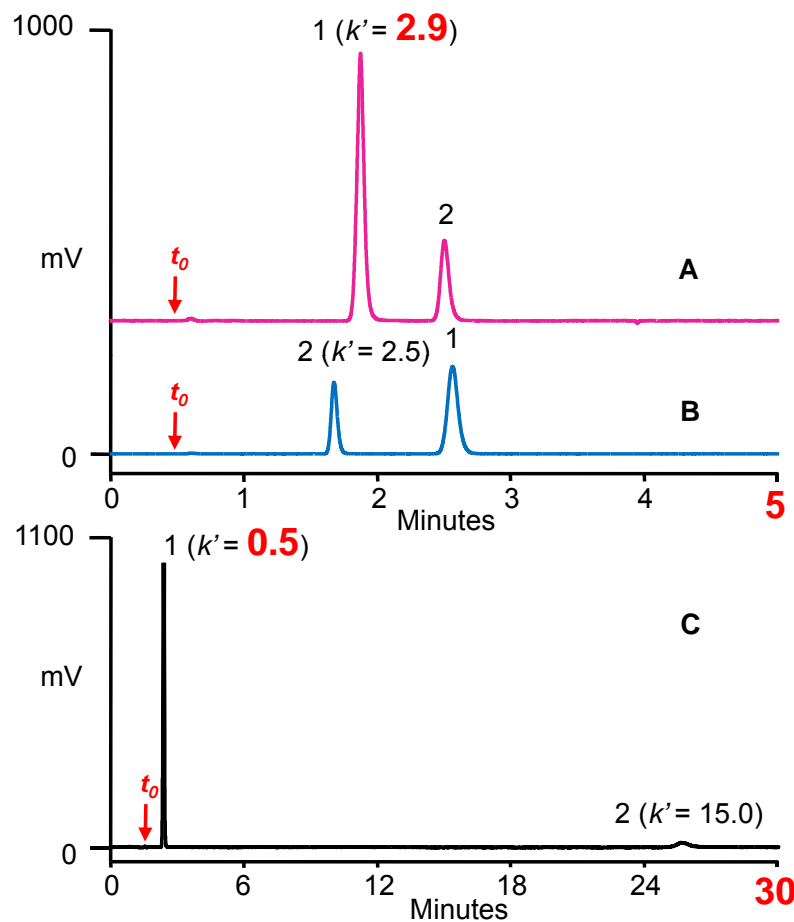
Gain = 100 pA; Filter = med; Neb. Temp = 30° C

Gradient:

Time (min)	% A	% B	% C	Curve
-4	20	65	15	5
0	20	65	15	5
0.1	20	65	15	5
1	80	0	20	5
4	80	0	20	5



# Hydrophilic Acidic API & Counterion – Penicillin G Potassium



## A & B

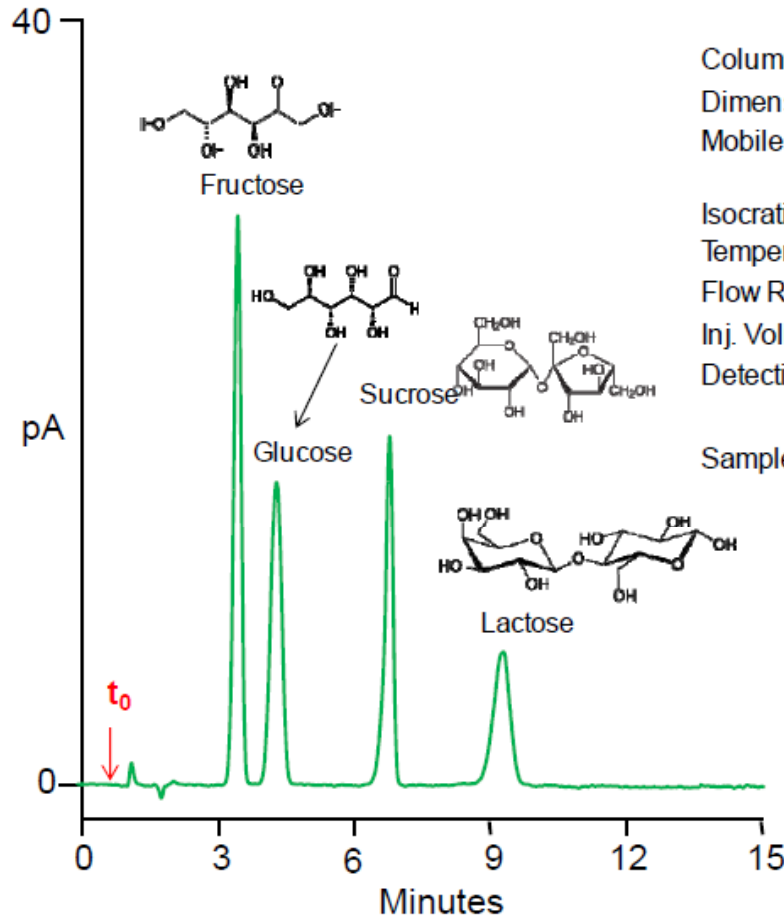
Column: Acclaim Trinity P1, 3  $\mu$ m  
 Dimensions: 3.0 x 50 mm  
 Mobile Phase: A: 80/20 v/v CH<sub>3</sub>CN/ 20 mM (total) NH<sub>4</sub>OAc, pH5.2  
 B: 60/40 v/v CH<sub>3</sub>CN/ 20 mM (total) NH<sub>4</sub>OAc, pH5.2  
 Temperature: 30 °C  
 Flow Rate: 0.6 mL/min  
 Inj. Volume: 2  $\mu$ L

## C

Column: Zwitterionic HILIC, 5  $\mu$ m  
 Dimensions: 4.6 x 150 mm  
 Mobile Phase: 85/15 v/v CH<sub>3</sub>CN/ 15 mM (total) NH<sub>4</sub>OAc, pH5.2  
 Temperature: 30 °C  
 Flow Rate: 1.0 mL/min  
 Inj. Volume: 5  $\mu$ L

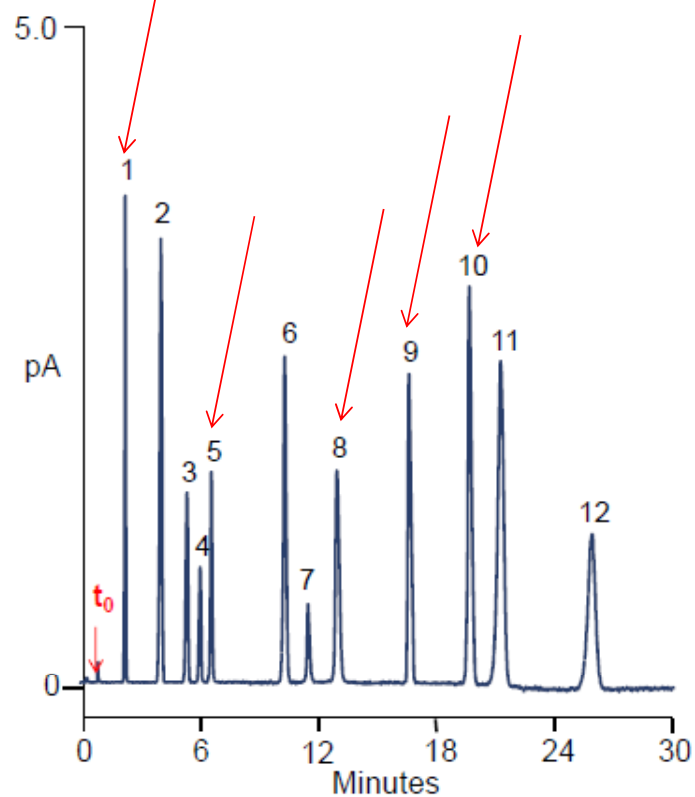
Detection: Aerosol based detector  
 Sample: Penicillin G, K salt (0.2 mg/mL in mobile phase)  
 Peaks:  
 1. Penicillin G  
 2. K<sup>+</sup>

# Trinity P2分析糖



Column: **Acclaim Trinity P2, 3  $\mu$ m**  
Dimensions: 3.0 x 100 mm  
Mobile Phases: A: Acetonitrile  
B: 100 mM Ammonium formate, pH 3.65  
Isocratic: 80%A/ 20%B  
Temperature: 60 ° C  
Flow Rate: 0.50 mL/min  
Inj. Volume: 5  $\mu$ L  
Detection: Corona Veo: evaporator 55 ° C, data rate 5 Hz, filter 2 sec, power function 1.50  
Samples: 0.25 mg/mL each in mobile phase

# Trinity P2分离药物和对离子



Column: Acclaim Trinity P2, 3  $\mu$ m  
Dimensions: 3.0 x 100 mm  
Mobile Phases: A: Water  
B: 100 mM Ammonium formate, pH 3.65  
Gradient: -10.0 0.0 2.0 22.0 30.0  
%A: 90 90 90 0 0  
%B: 10 10 10 100 100  
Temperature: 30° C  
Flow Rate: 0.60 mL/min  
Inj. Volume: 1  $\mu$ L  
Detection: Corona Veo: evaporator 55° C, data rate 5 Hz, filter 2 sec, power function 1.50  
Samples: 0.02 – 0.10 mg/mL each in D.I. water  
Peaks:

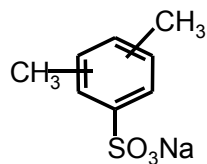
1. Phosphate
2. Sodium
3. Potassium
4. Chloride
5. Malate
6. Bromide
7. Nitrate
8. Citrate
9. Fumarate
10. Sulfate
11. Magnesium
12. Calcium

# Acclaim Surfactant Plus

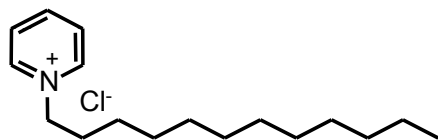
- **目的:** 高效分离各种表面活性剂
- **原理:** 反相作用/阴离子交换
- **产品主要特色:**
  - 广泛的选择性, 能同时分离阴离子, 阳离子, 非离子和两性离子型表面活性剂
  - 完全符合阳离子表面活性剂的检测需求
  - 对聚氧乙烯型表面活性剂有非常好的分离效
  - 低流失, 适合多种检测器联用
    - Charged aerosol detector (**CAD**)
    - Mass spectrometer (**MS**)
    - Evaporative light scattering detector (**ELSD**)
    - Suppressed conductivity detector (**SCD**)
    - UV-Vis detector (**UV**)

## 不同类型的表面活性剂

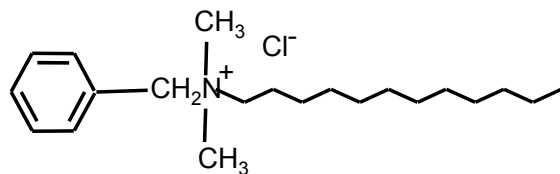
二甲苯磺酸钠



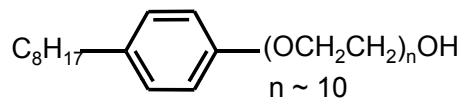
十二烷基盐酸吡啶



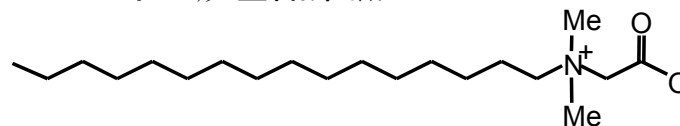
十二烷基二甲基苄基溴铵



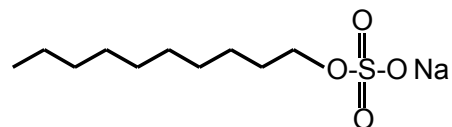
Triton X-100



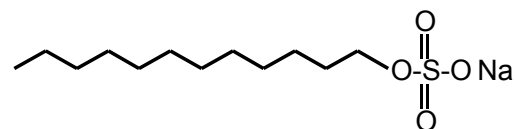
十六烷基甜菜碱



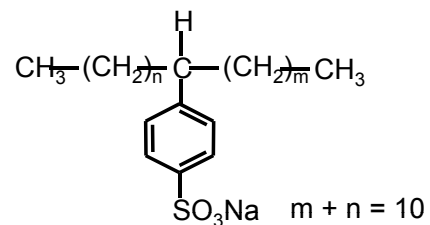
癸烷磺酸钠



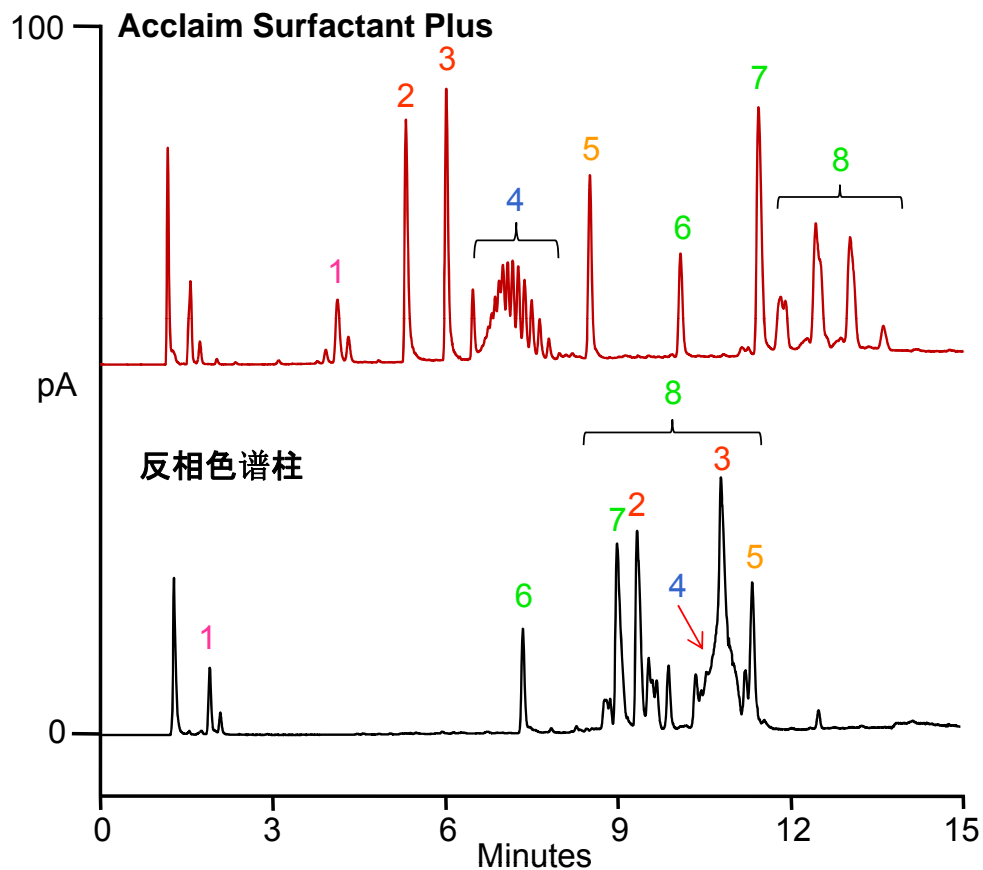
十二烷基磺酸钠(SDS)



十二烷基苯基磺酸钠 (LAS)



# 阴离子，阳离子，非离子和两性离子型表面活性剂的同时分离



色谱柱: Acclaim Surfactant Plus, 3  $\mu$ m  
 规格: 3.0 x 150 mm  
 梯度:

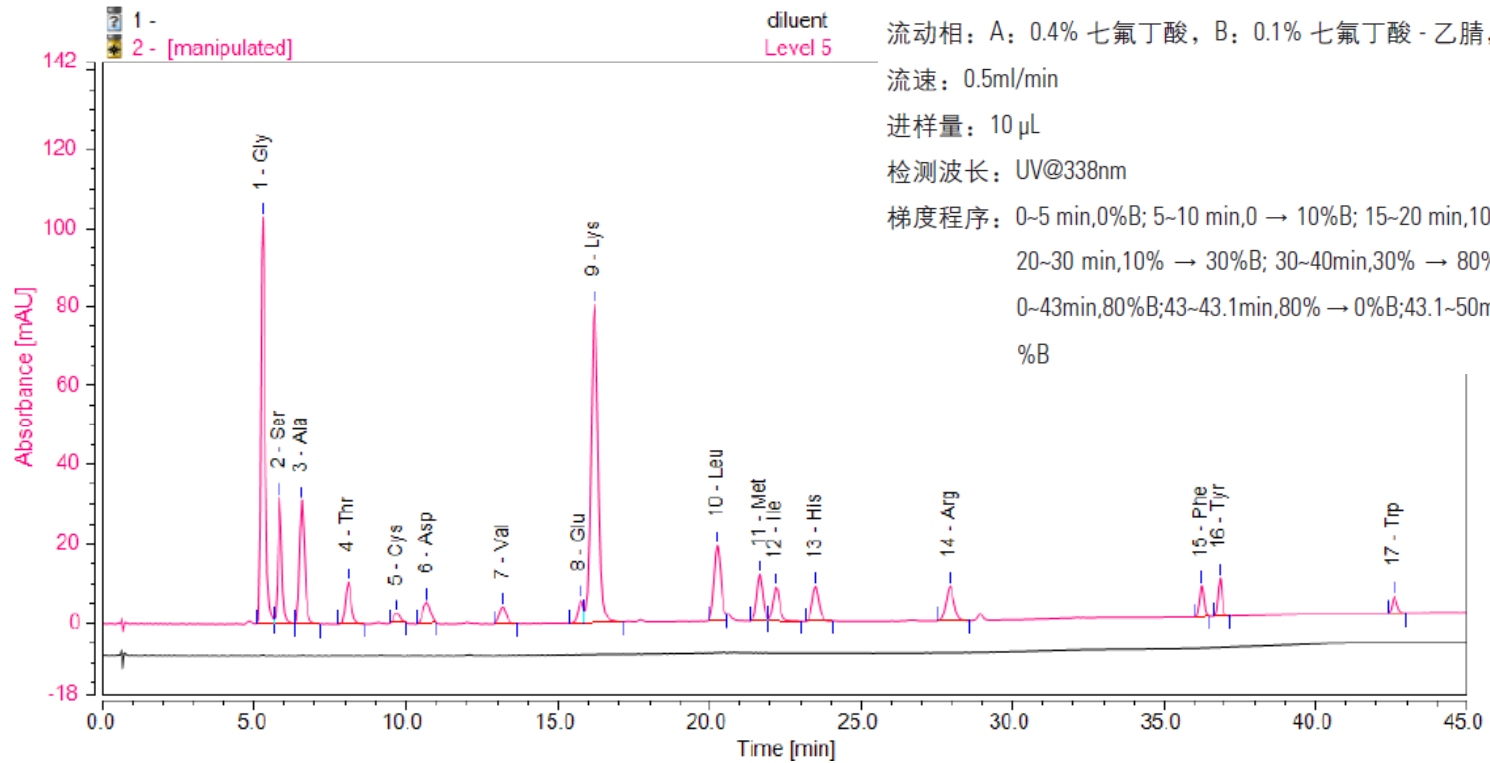
Time (min)	Acetonitrile	0.1 M Ammonium Acetate, pH5
-8	25	75
0	25	75
10	80	20
15	80	20

柱温: 30 ° C  
 流速: 0.6 mL/min  
 进样: 5  $\mu$ L  
 检测: Corona *ultra* (Gain = 100 pA; Filter = med; Neb Temp = 20 ° C)

色谱峰: (100 - 400  $\mu$ g/mL)

1. 二甲苯磺酸盐
2. 十二烷基盐酸吡啶
3. 十二烷基二甲基苯扎溴铵
4. Triton X-100
5. 十六烷甜菜碱
6. 葵烷磺酸盐
7. 十二烷磺酸盐
8. 十二烷基苯基磺酸钠 (LAS)

# Hypercarb分离氨基酸



## 色谱条件

色谱柱: Hypercarb, 4.6 × 150 mm, 5 μm(PN: 35005-154630)

柱温: 30°C

流动相: A: 0.4% 七氟丁酸, B: 0.1% 七氟丁酸 - 乙腈,

流速: 0.5ml/min

进样量: 10 μL

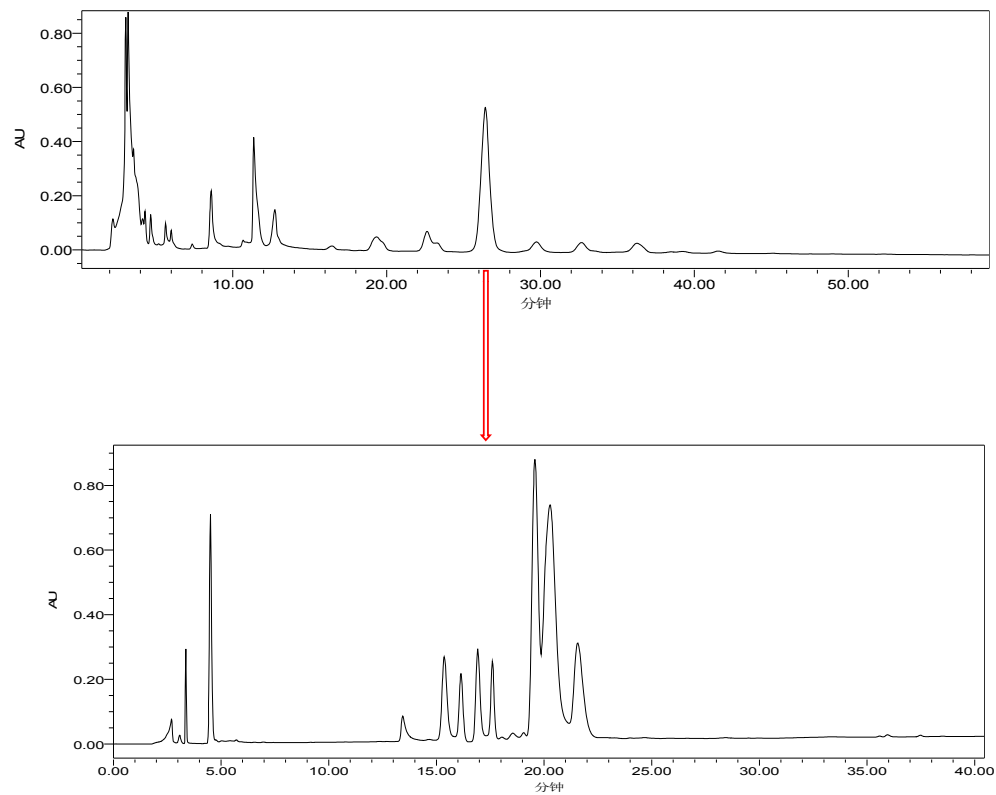
检测波长: UV@338nm

梯度程序: 0-5 min,0%B; 5-10 min,0 → 10%B; 15-20 min,10%B;  
20-30 min,10% → 30%B; 30-40min,30% → 80%B;4  
0-43min,80%B;43-43.1min,80% → 0%B;43.1-50min,0  
%B





# Hypercarb分离氨基酸



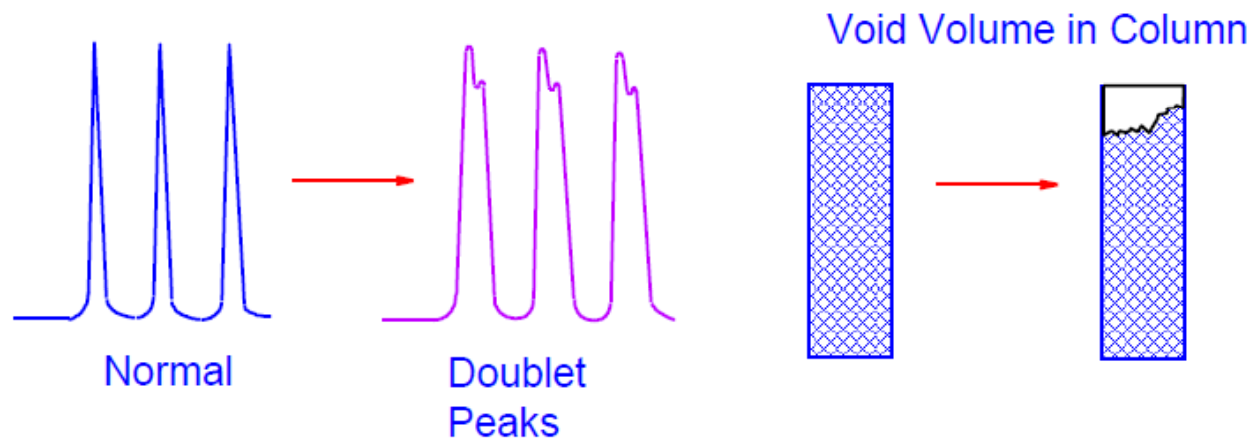
● 色谱柱的常见问题与维护

## 常见问题

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- 1. 空洞问题
- 2. 污染问题
- 3. 批次差异问题

# 填料塌陷



- 死体积增加
- 保留时间短的峰更易受影响

# 填料塌陷



# 压力高

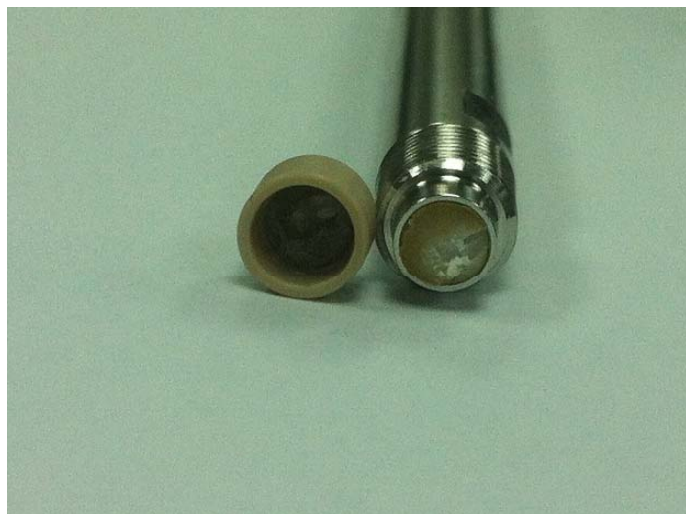
- 现象
  - 突然升高
- 原因
  - 系统压力
  - 色谱柱污染
  - 筛板堵塞
- 解决方案
  - 清洗
    - 大分子物质或吸附强化合物
    - 样品沉淀
  - 反冲 – 乙腈, 乙腈/异丙醇
  - 样品纯化;
  - 保护柱

## 峰形问题

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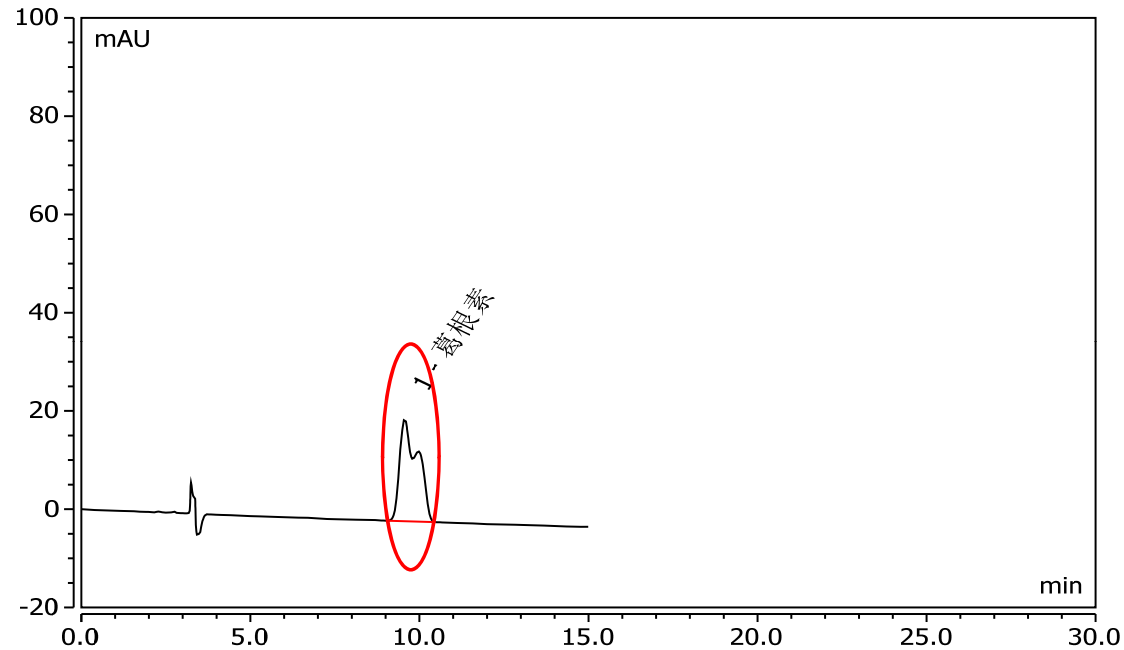
- 分叉峰
- 拖尾峰
- 展宽峰

# 污染





## 待测物对照品的色谱峰形异常（前沿甚至分叉）



- 问题解析：溶剂效应。

流动相极性（25%甲醇）< 样品溶剂极性（50%乙醇）

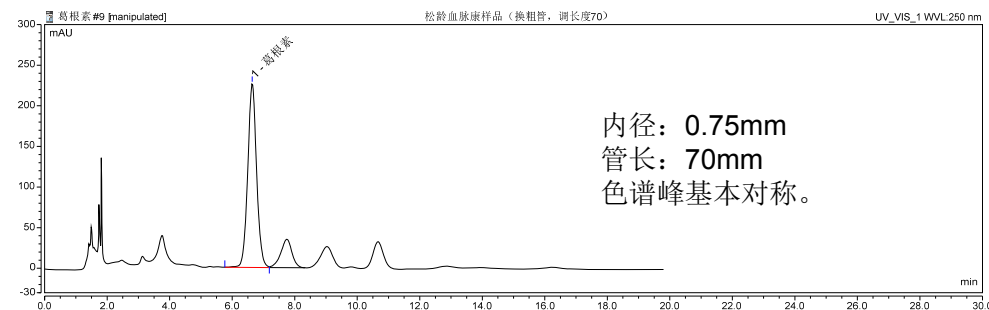
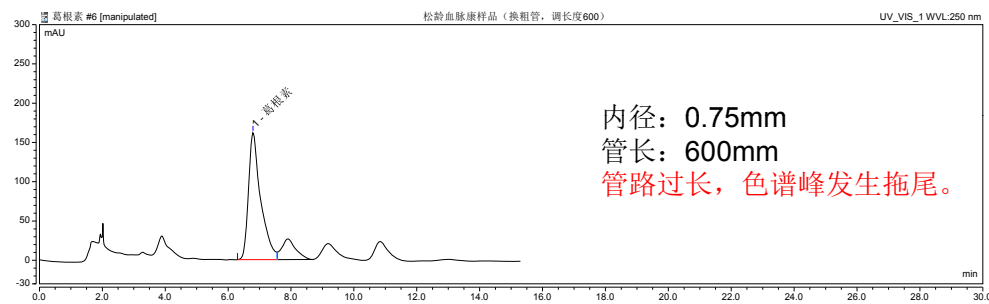
样品溶液进样后，未能与流动相完全混合即被带入色谱柱，色谱柱头处的流动相受到样品溶剂的干扰，导致RT靠前的色谱峰发生变形。

# 解决办法提高样品进柱前的混合效率。

## ① 增大进样器和色谱柱之间管路的内径

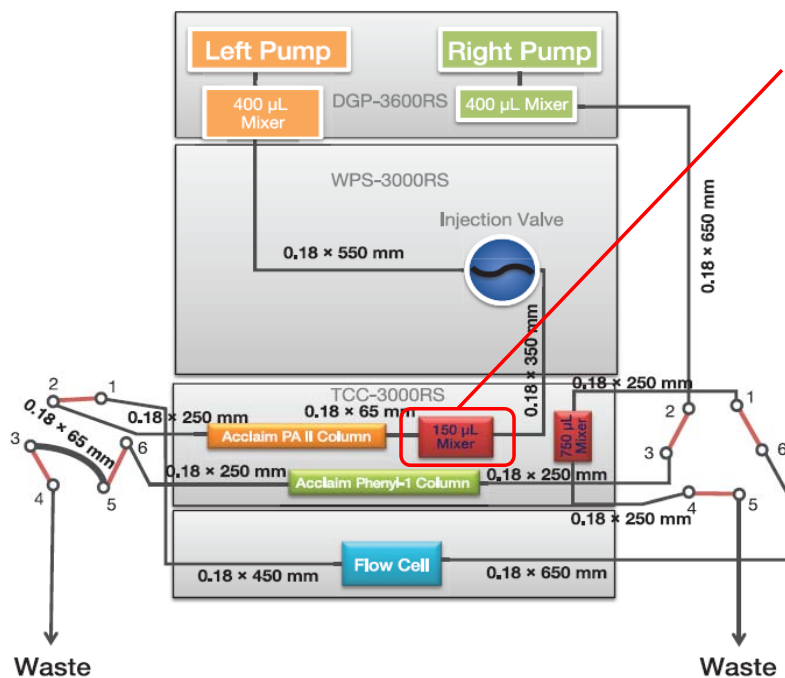
优点：简单易行，立竿见影；（用户原来使用的Agilent 1200即为粗内径连接管）

缺点：可能导致峰行展宽，柱效下降。（需对管路长度进行适当优化）



## 解决办法提高样品进柱前的混合效率。

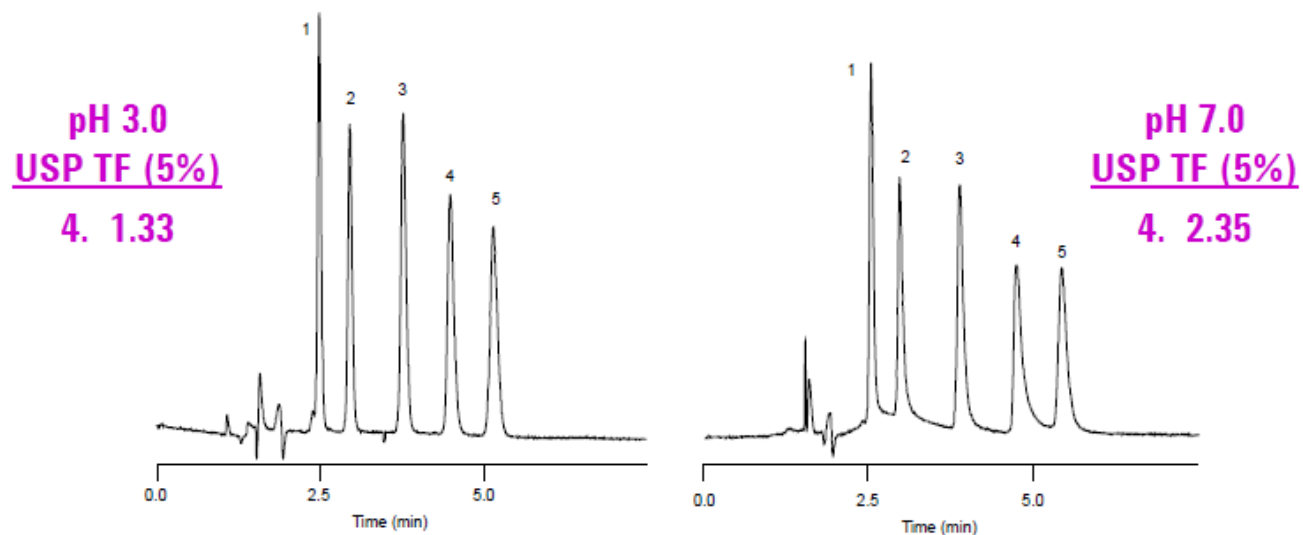
② 在进样器和色谱柱间增设静态混合器<sup>[1]</sup>



供试品溶液为纯乙腈；  
流动相初始浓度为20%乙腈。  
增加150 $\mu\text{L}$ 混合器可降低溶剂效应。

[1] AN-1023 咖喱酱中苏丹红-IV检测

# 次级相互作用



- 色谱柱活化:

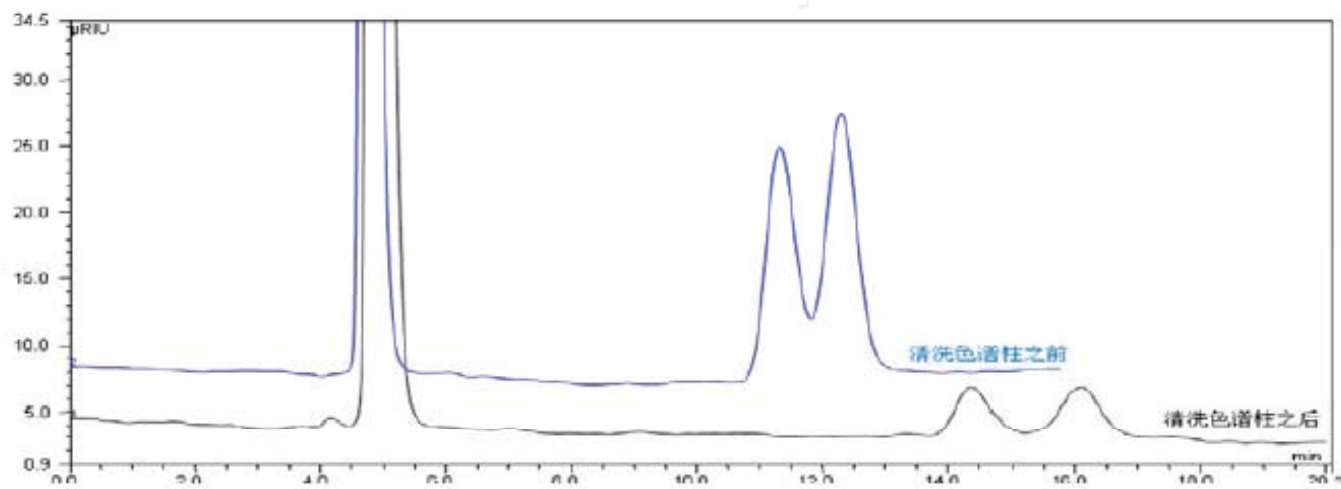
1. 反相— 甲醇/乙腈5倍柱体积
2. 正相— 异丙醇, 低流速过夜

- 色谱柱再生

反冲是针对污染的有效方法

0.2ml/min, 异丙醇-乙腈(1:1), 2小时以上或过夜。

## 氨基柱再生



乙腈:H<sub>2</sub>O=50:50, 含0.5%氨水。30倍柱体积

谢谢!

