



Acquity **H**
UPLC® CLASS

培训教程 H-Class 基础



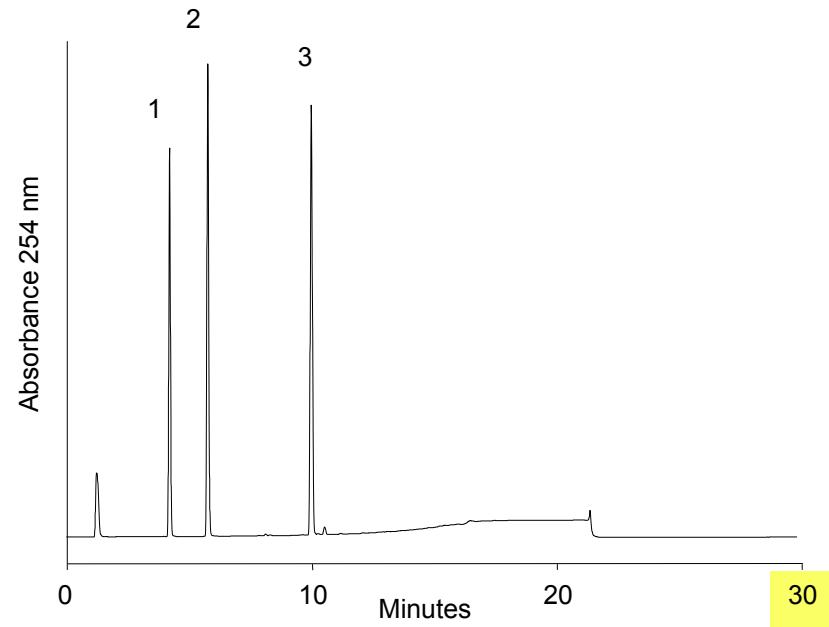
方法转换考虑因素

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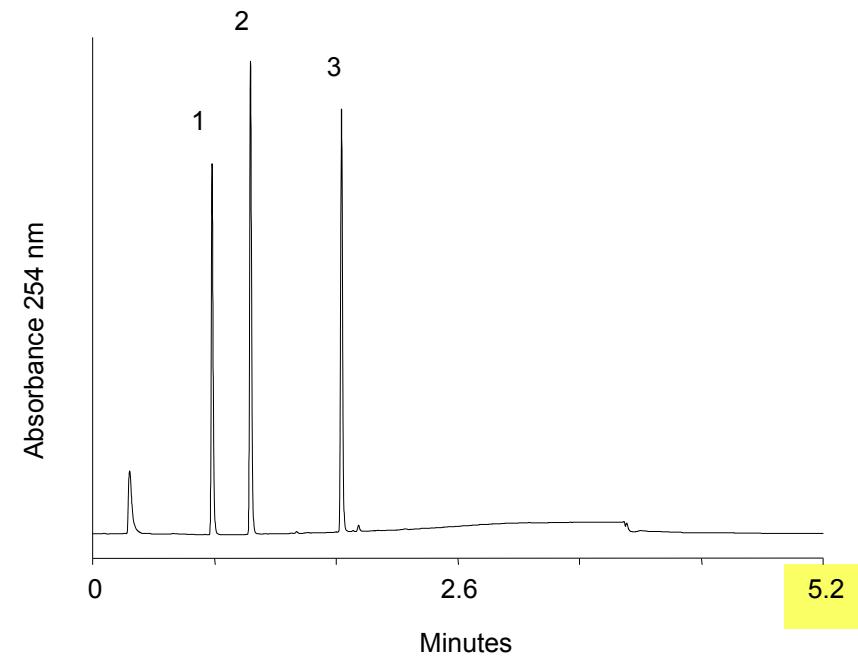
- 四种类别的 HPLC 方法转换包括：
 - 长柱转换为短柱
 - 从一个色谱柱品牌转换到另一个品牌
 - 从一个系统转换为另一个不同的系统
 - 同时转换系统和色谱柱
- 方法转换到 UPLC 系统且使用 UPLC 柱时,UPLC的好处才能够完全体现
 - 需要同时转换系统和色谱柱

从HPLC 转换到 UPLC

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原始 **30** 分钟 **HPLC**图



转换后 **5.2** 分钟 **UPLC**图

实现方法转换：HPLC 到 UPLC

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- UPLC将会同时提高速度、灵敏度和分离度
- 详细地描述HPLC方法将会大大简化转换过程
- 系统地转换会得到最好的结果

关于方法转换的特别提示

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- 新的**UPLC**方法可能会与原来的**HPLC**方法有所不同....
 - 操作条件, 比如, 流速
 - 运行时间
 - 表现
- 但是, 新的**UPLCTM**方法必须达到**HPLC**方法的目标要求
 - 相关被分析物的完全分离
 - 峰纯度
 - 确切地鉴别峰
 - 定量的准确度和精密度

成功进行方法转换的步骤

- 得到现有方法和结果的信息
- 仪器比较
- 选择新的或是目标色谱柱
 - 色谱柱化学
 - 直径
- 在几何放大的基础上选择目标条件
- 评估转换的结果
- 如需要再进行优化

所需的信息: 原始方法

- 色谱柱
 - 色谱柱化学(键合配体, 品牌, 粒径大小)
 - 柱内径
- 色谱条件
 - 流动相
 - 流速
 - 梯度表: 包括再生和再平衡
 - 温度
- 样品
 - 稀释
 - 浓缩
 - 分子量
 - 进样体积

所需的信息: 原始结果

- 色谱图
 - 峰数目
 - 保留
 - 分离度
- 定量
 - 检测限
 - 定量限
 - 线性范围
 - 准确度
 - 精密度

成功进行方法转换的步骤

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梯度与等度

- 等度方法
 - 不同仪器之间的系统体积差异影响较小
 - 可以直接通过几何缩放的形式转换
- 梯度方法
 - 不同仪器之间的系统体积差异对转换影响很大

仪器比较:溶剂输送

- 我们假定:
 - 溶剂总是以程序所设定的流速流动
 - 百分比组成总是以设定值变化
 - 梯度按照所设定的梯度表而运行
 - 溶剂组成可以按照所设定的时间达到色谱柱

仪器比较:溶剂输送

- 然而, 色谱系统的不同会影响:
 - 保留时间
 - 灵敏度
 - 重新平衡的时间

所需的信息:原始方法

- 梯度形成的模式
 - 配有梯度比例阀的单泵
 - 双泵
 - 品牌和型号
- 系统体积 (死体积或滞后体积)
 - 值和测量所用方法
- 进样机理
- 检测模式

例:原始方法

- 室温: 21- 22°C
- 流速: 1.50 mL/min
- 分析样品:
 - 咖啡因 (100 µ g/mL)
 - 氢化奎尼丁 (33 µ g/mL)
 - 3-氨基二苯酮(39 µ g/mL)
- 分子量: 小于 500 amu
- 样品稀释剂: 二甲基亚砜(DMSO)
- 进样体积: 10 µ L
- 检测波长: 254nm
- 流动相:
 - A: 0.05% TFA 水溶液
 - B: 0.05% TFA 乙腈溶液

例:原始的梯度表

梯度段	时间	流速	%A	%B	曲线
起始	0	1.5	95	5	*
2	15	1.5	5	95	6
3	20	1.5	5	95	1
4	30	1.5	95	5	1

例:原始的色谱柱

- 固定相: Xterra® MS C₁₈
- 粒径: 5 μm
- 内径: 4.6 mm
- 长度: 150 mm



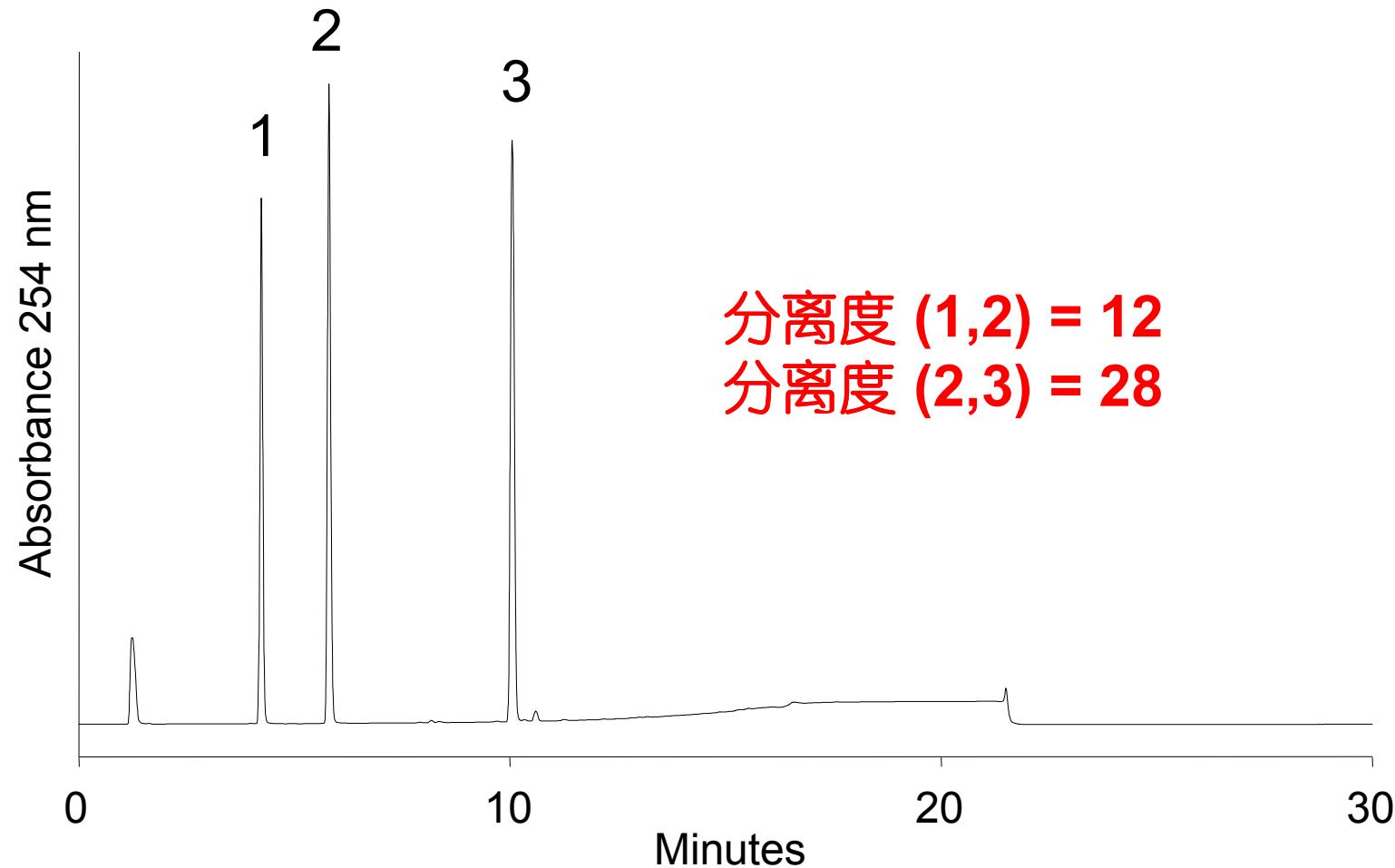
计算: $\frac{L}{dp} = \frac{150,000 \mu\text{m}}{5 \mu\text{m}} = 30,000$

例：原始的仪器

- Waters Alliance® 2695 溶剂管理器
 - 低压混合的单泵梯度
- Waters Alliance® 2695 样品管理器
- Waters 2487 TUV / 254nm



例：原始的结果



系统滞后测试结果

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- Alliance® 2695 = 1.04 ml
- ACQUITY UPLC™ = 0.109 ml

- 这将会给方法转换带来什么不同吗?
 - Alliance 2695 使用 4.6 × 150 mm 色谱柱
 - ACQUITY UPLC™ 使用 2.1 × 50 mm 色谱柱

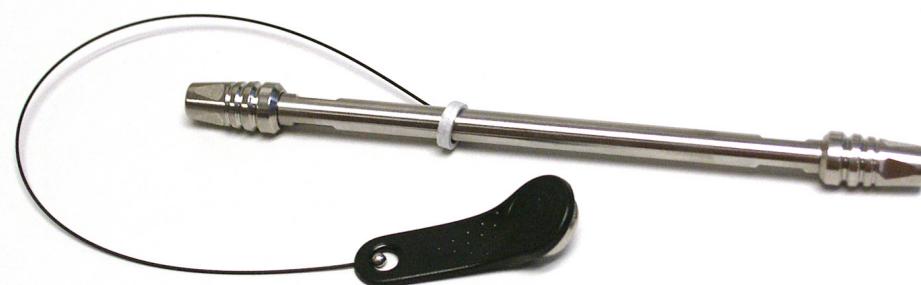
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目标柱 :ACQUITY UPLC 柱

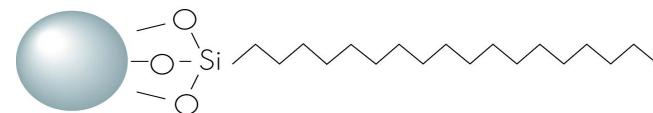
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- 键合相
 - ACQUITY UPLC BEH C₁₈
 - ACQUITY UPLC BEH Shield RP₁₈
 - ACQUITY UPLC BEH C₈
 - ACQUITY UPLC BEH Phenyl
- 查看色谱柱选择性表找到与之前的柱子最相近的

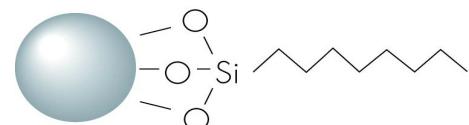


ACQUITY UPLC 柱

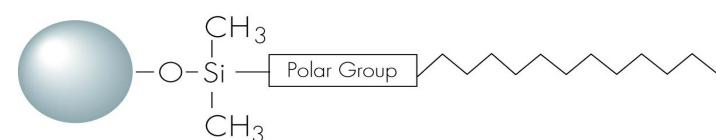
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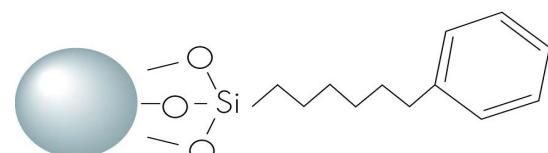
ACQUITY UPLC BEH C₁₈



ACQUITY UPLC BEH C₈



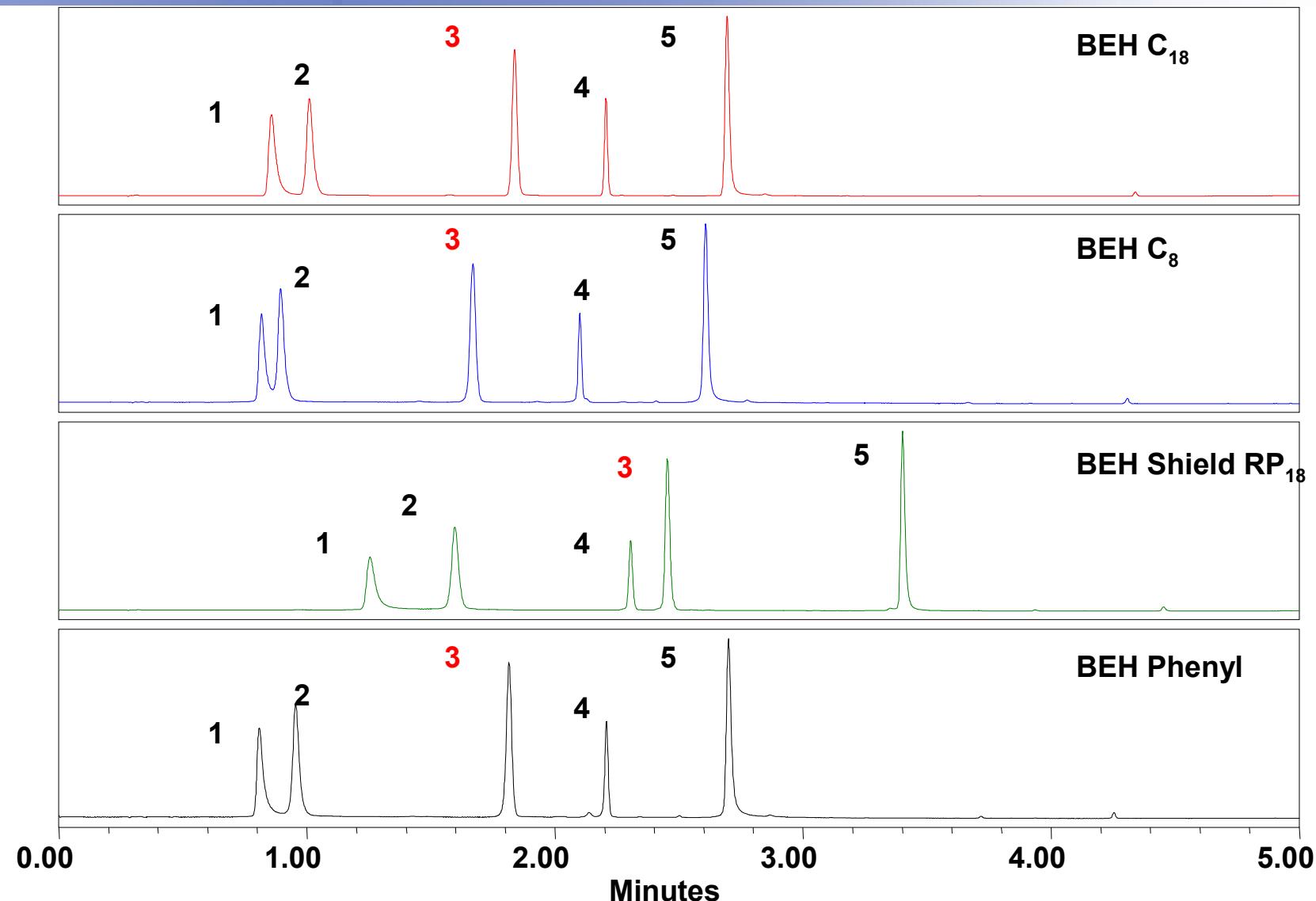
ACQUITY UPLC BEH Shield RP₁₈
Waters 专利技术
(氨基甲酸酯极性基团嵌入)



ACQUITY UPLC BEH Phenyl

配体选择性 紫海胆中的咖啡酸衍生物

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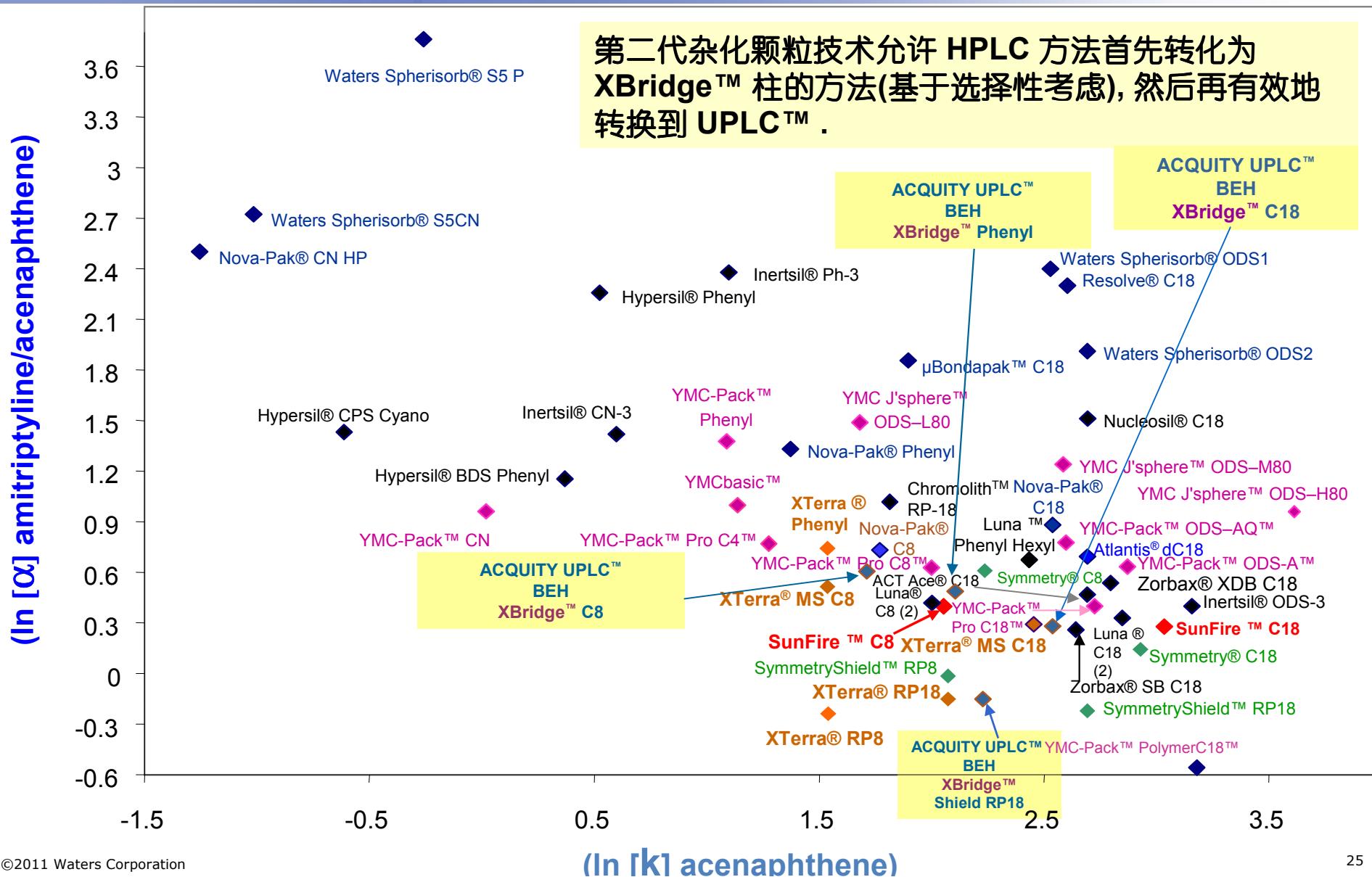


用来测量残留硅羟基活性的组分

- 二氢苊(中性组分) 的保留取决于填料中化学键合相的疏水性
 - 填料的疏水性越高, 保留因子(k)值越高.
- 阿米替林(碱性组分, $pK_a=9.4$)的保留取决于两个因素: 化学键合相的疏水性和残留硅羟基的活性及浓度(碱性化合物较难分析的一个很好的例子)

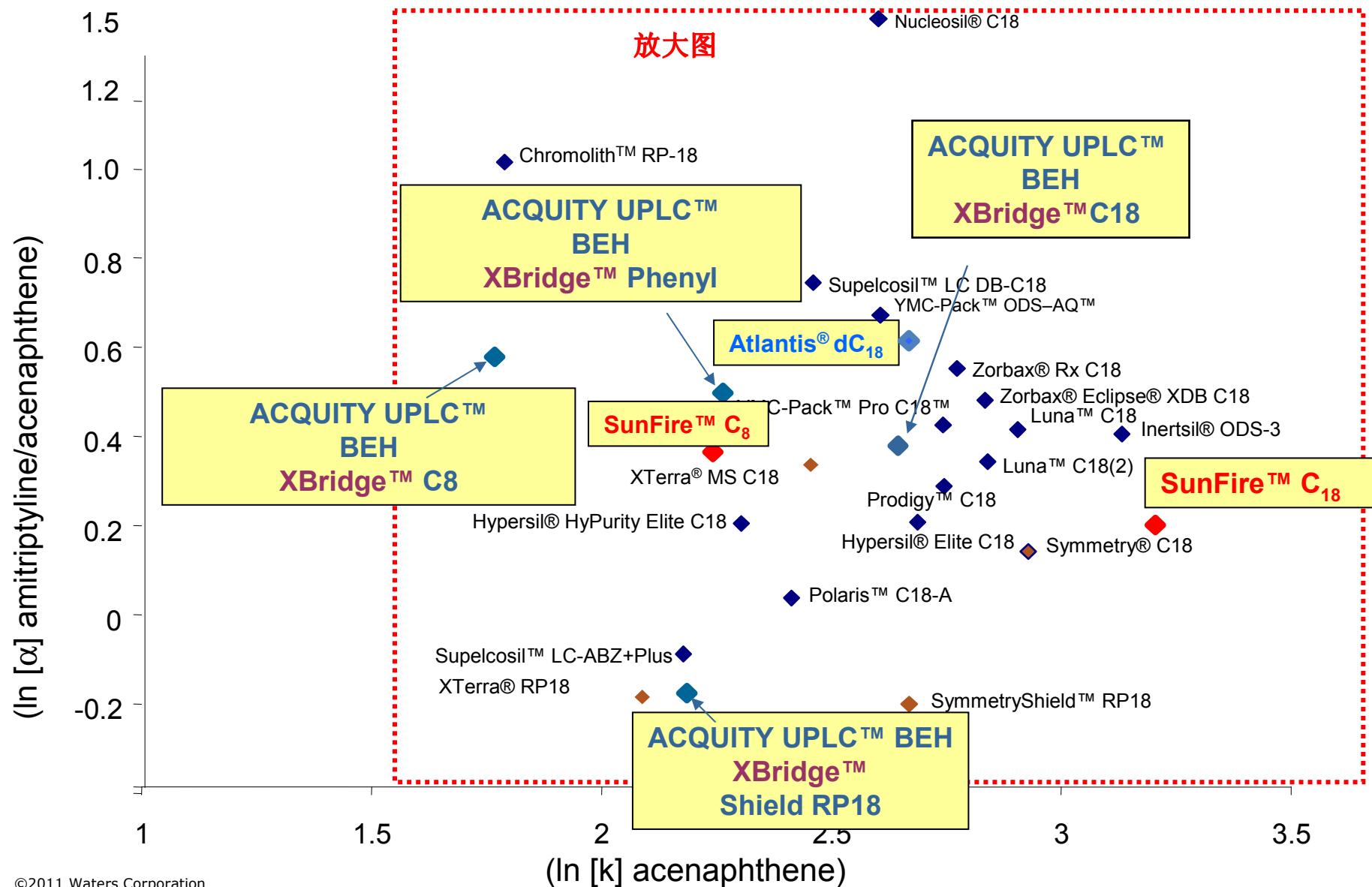
反相柱选择性表

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现代“C₁₈ 区域”

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目标柱的规格

- 内径
 - 通常用 2.1mm
 - 只有在特殊情况下用 1.0mm
 - 严格的样品限量
 - 流路直接接至MS
- 柱长
 - 如果主要目标是速度
 - 以50 mm 柱长为起点 ($L/dp = 29,500$)
 - 如果主要目标是分离度
 - 以100 mm 柱长为起点 ($L/dp = 58,900$)

目标柱规格 ACQUITY UPLC柱

- 从 4.6 x 150 mm XTerra MS C18 (5 μm 颗粒) 柱缩放到 2.1 x 50 mm ACQUITY UPLC BEH C18 柱 (1.7 μm 颗粒)

成功进行方法转换的步骤

- 得到现有方法和结果的信息
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目标条件:参数

- 参数
 - 流动相
 - 温度
 - 几何缩放
 - 进样量
 - 流速
 - 等度方法只需要调整流速和进样量
 - 梯度曲线
- 几何缩放的目的是为了减少评估和优化转换方法所带来的变化

目标条件:流动相

- 用完全相同的流动相
 - 添加剂
 - pH
 - 离子强度
 - 有机溶剂
 - 组成百分比
- 只有在评估几何转换之后需要优化时再做修改

目标条件:温度

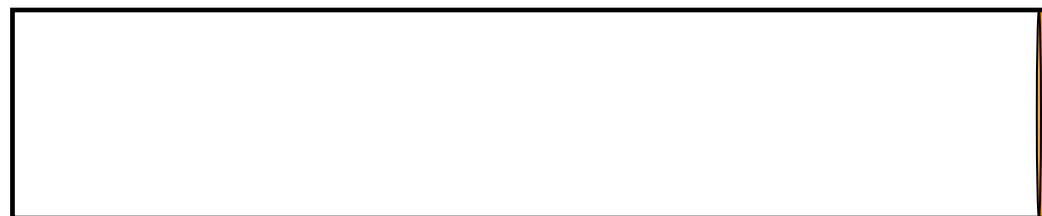
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- 温度直接影响每一个色谱机理
- 在方法转换当中温度一定要保持一致
- 溶剂预热是必需的
 - 溶剂在原来柱中的时间是**1.66分钟**
 - 溶剂在优化的目标柱中的时间是**15秒**
 - 热传递的时间很少
- ACQUITY UPLC TM 柱稳定装置可用于调谐大约**0.5 到 0.75 mL/min**的流速

目标条件:进样量

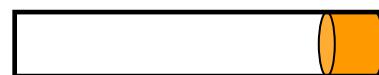
- 用完全相同的样品
 - 同样的浓度
 - 同样的稀释倍数
- 计算对应于柱体积的进样体积

4.6 x 150 mm



20 μL 进样量/2.49 mL = 0.8%

2.1 x 50 mm



20 μL 进样量/0.17 mL = 12%

计算进样体积

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$$\text{目标进样体积} = \text{原来的进样体积} \times \frac{\text{目标柱体积}}{\text{原始柱体积}}$$

缩放 10 μL 进样量从 4.6 x 150 mm 到 2.1 x 50 mm

$$10 \mu\text{L} \times \frac{3.14 \times 1.1^2}{3.14 \times 2.3^2} \times \frac{50}{150} =$$

$$10 \mu\text{L} \times \frac{0.17}{2.49} = 10 \mu\text{L} \times 0.068$$

$$= 0.7 \mu\text{L}$$

目标条件：进样量

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- 用同样的样品
 - 同样的浓度
 - 同样的稀释倍数
- 计算对应于柱体积的进样体积
 - 建议在ACQUITY UPLCTM 上的最小进样体积为0.5 - 1μL
 - 如果计算出的进样体积太小
 - 用起始强度的流动相稀释5-10倍
 - 在2.1 x 50mm 柱上最大进样体积为5μL

目标条件：流速

- 调节流速,由于线速度一定,因此流速与柱内径的平方成比例
- 对于小颗粒的填料可调节线速度

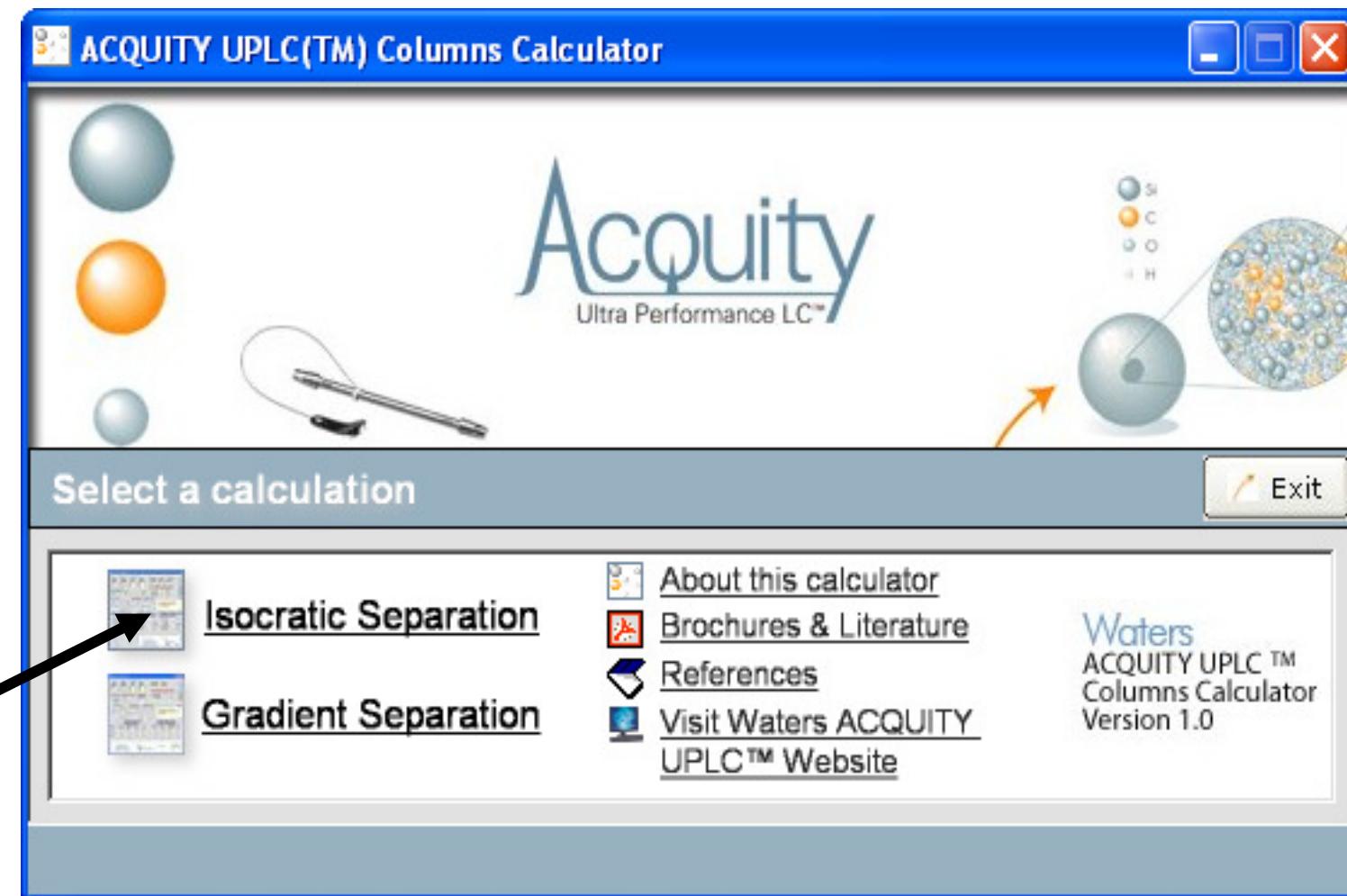
$$\text{目标流速} = \text{原始流速} \times \frac{\pi r^2}{\pi r^2} \frac{\text{目标}}{\text{原始}}$$

简化为：

$$\text{目标流速} = \text{原始流速} \times \frac{d^2}{d^2} \frac{\text{目标}}{\text{原始}}$$

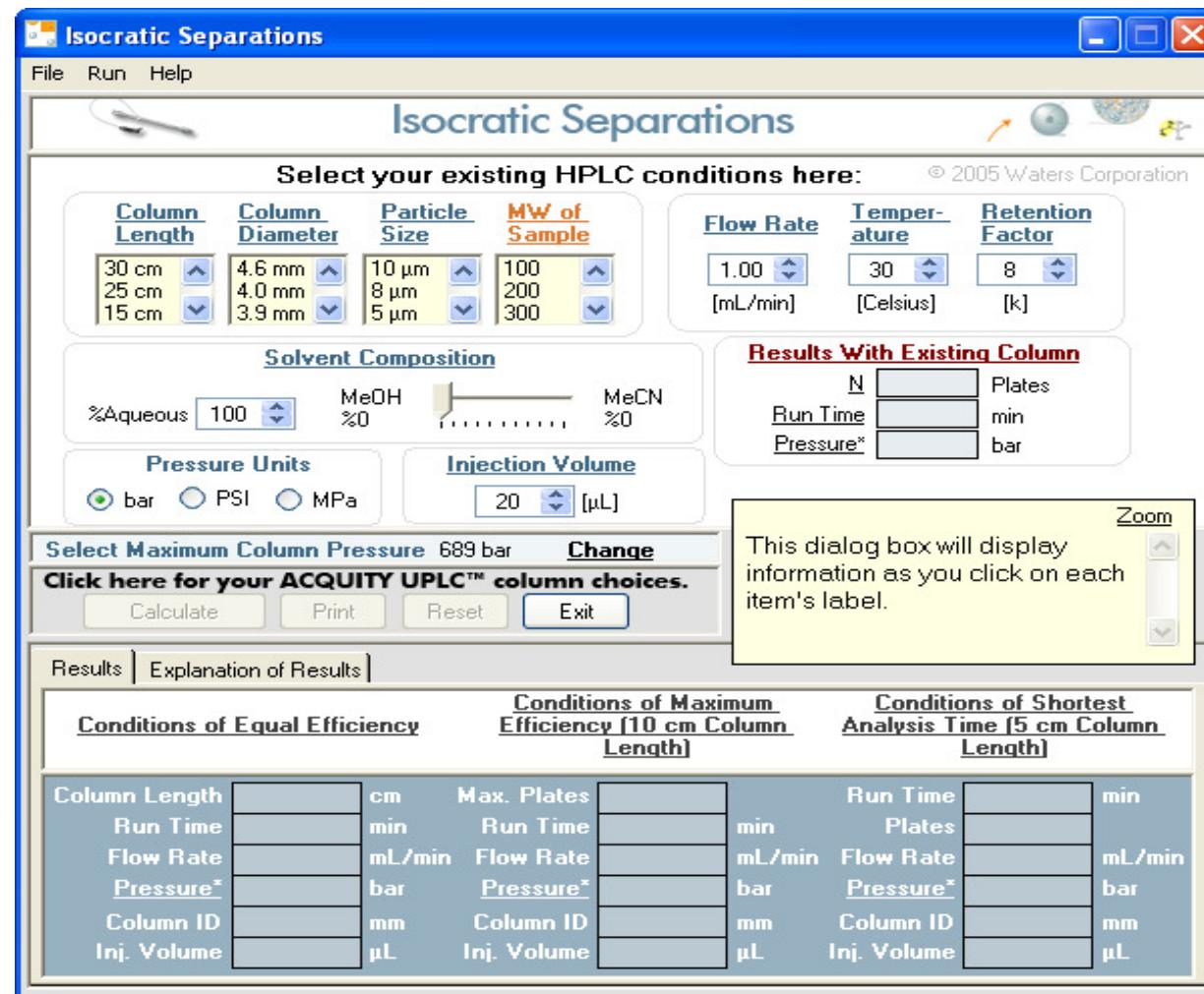
缩放 1.5 mL/min 流速从4.6x150mm 到2.1x50mm

$$1.5 \text{ mL/min.} \times \frac{2.1^2}{4.6^2} = 0.31 \text{ mL/min.}$$



等度分离

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Isocratic Separations

File Run Help

Isocratic Separations

Select your existing HPLC conditions here:

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Column Length	Column Diameter	Particle Size	MW of Sample	Flow Rate	Temperature	Retention Factor
30 cm	4.6 mm	10 μ m	100	1.50	23	8
25 cm	4.0 mm	8 μ m	200	[mL/min]	[Celsius]	[k]
15 cm	3.9 mm	5 μ m	300			

Solvent Composition

%Aqueous: 60 MeOH MeCN: %40

Results With Existing Column

N: Plates
Run Time: min
Pressure*: PSI

Pressure Units: bar, PSI, MPa
Injection Volume: 10 μ L

Select Maximum Column Pressure: 10000 PSI [Change](#)

Click here for your ACQUITY UPLC™ column choices.

Calculate Print Reset Exit

Zoom

Isocratic retention factor, maximum value = 20. The retention factor is the analysis time divided by the column dead

计算

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Isocratic Separations

Select your existing HPLC conditions here:

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Column Length	Column Diameter	Particle Size	MW of Sample	Flow Rate	Temperature	Retention Factor
30 cm	4.6 mm	10 μ m	100 200 300	1.50 [mL/min]	23 [Celsius]	8 [k]
25 cm	4.0 mm	8 μ m				
15 cm	3.9 mm	5 μ m				

Solvent Composition

%Aqueous: 60 MeOH: 0 MeCN: 40

Pressure Units: bar, PSI, MPa

Injection Volume: 10 μ L

Results With Existing Column

N: Plates
Run Time: min
Pressure*: PSI

Select Maximum Column Pressure: 10000 PSI Change

Click here for your ACQUITY UPLC™ column choices.

Calculate Print Reset Exit

Results | Explanation of Results

Conditions of Equal Efficiency Conditions of Maximum Efficiency (10 cm Column Length) Conditions of Shortest Analysis Time (5 cm Column Length)

Column Length	cm	Max. Plates	Run Time	Plates	min
Run Time	min	Run Time	Run Time	Plates	min
Flow Rate	mL/min	Flow Rate	mL/min	Flow Rate	mL/min
Pressure*	PSI	Pressure*	PSI	Pressure*	PSI
Column ID	mm	Column ID	mm	Column ID	mm
Inj. Volume	μ L	Inj. Volume	μ L	Inj. Volume	μ L

Isocratic retention factor, maximum value = 20. The retention factor is the analysis time divided by the column dead

Zoom

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等度 UPLC 条件

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Results		Explanation of Results					
Conditions of Equal Efficiency		Conditions of Maximum Efficiency (10 cm Column Length)		Conditions of Shortest Analysis Time (5 cm Column Length)			
Column Length	5 cm	Max. Plates	21025	Run Time	1.3 min		
Run Time	1.6 min	Run Time	8.7 min	Plates	8393		
Flow Rate	0.649 mL/min	Flow Rate	0.242 mL/min	Flow Rate	0.782 mL/min		
Pressure*	8290 PSI	Pressure*	6096 PSI	Pressure*	10000 PSI		
Column ID	2.1 mm	Column ID	2.1 mm	Column ID	2.1 mm		
Inj. Volume	0.7 μL	Inj. Volume	1.4 μL	Inj. Volume	0.7 μL		

比传统的“双倍”流速略高一点

原始的梯度表

梯度	时间	流速	%A	%B	曲线	梯度段 (min)	梯度段 (cv)
Initial	0	1.5	95	5	*	0	0
2	15	1.5	5	95	6	15	
3	20	1.5	5	95	1	5	
4	30	1.5	95	5	1	10	

梯度段:用柱体积来描述

对于 1.5mL/min 在 $4.6 \times 150\text{mm}$ 柱上运行 15分钟

$$\text{梯度体积} = \text{流速} \times \text{时间} = 1.5\text{mL/min} \times 15\text{min} = 22.5\text{mL}$$

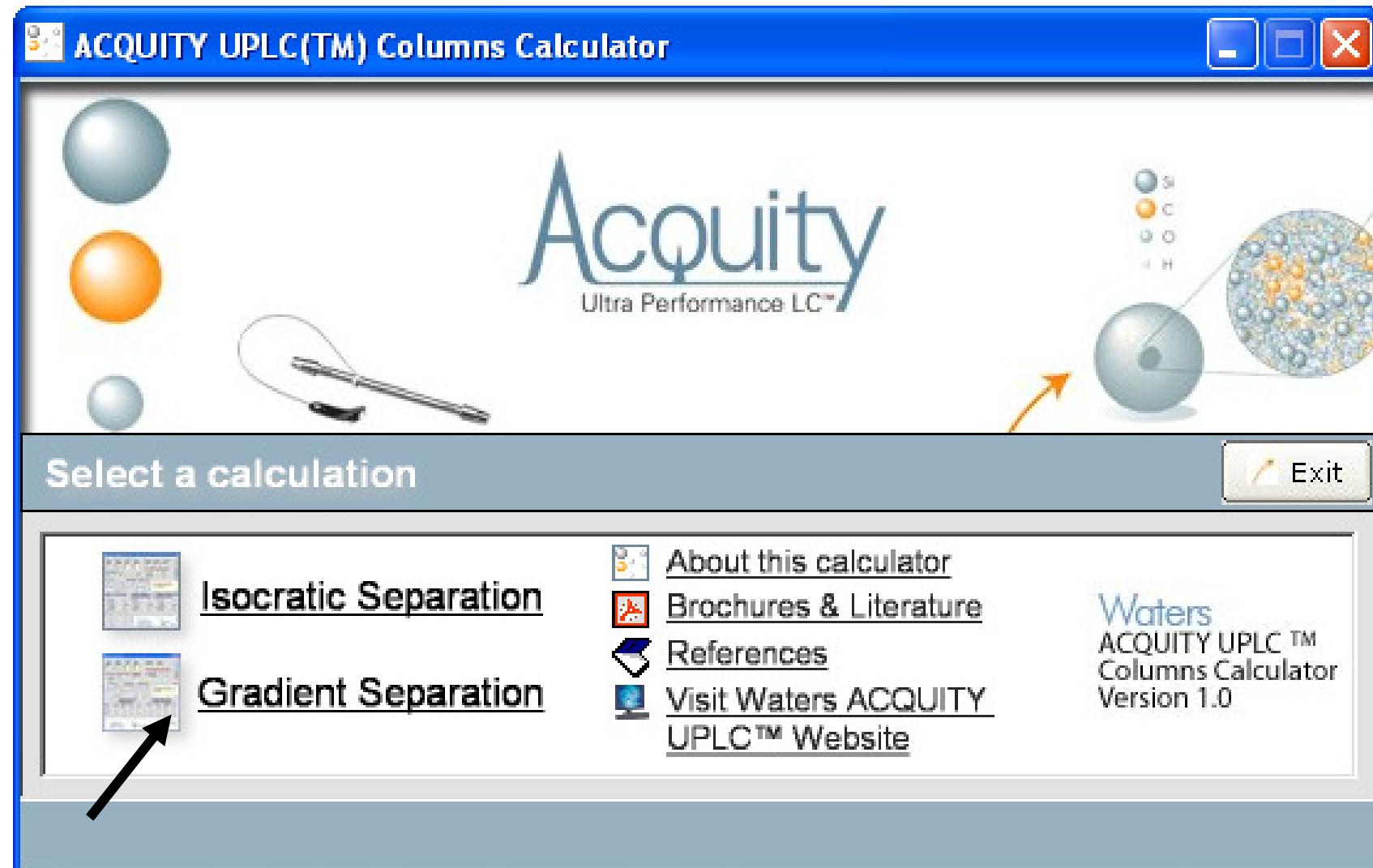
$$\text{柱体积} = \pi \times r^2 \times L = 3.14 \times 2.3^2 \times 150 = 2.49\text{mL}$$

$$\text{梯度段 (cv)} = \frac{\text{梯度体积}}{\text{柱体积}}$$

$$\text{梯度段} = \frac{22.5 \text{ mL}}{2.49 \text{ mL}} = 9.03 \text{ cv}$$

原始的梯度表

梯度	时间	流速	%A	%B	曲线	梯度段 (min)	梯度段 (cv)
Initial	0	1.5	95	5	*	0	0
2	15	1.5	5	95	6	15	9.03
3	20	1.5	5	95	1	5	3.01
4	30	1.5	95	5	1	10	6.02



UPLC 梯度分离

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Gradient Separations

Select your existing HPLC conditions here: © 2005 Waters Corporation

Column Length	Column Diameter	Particle Size	MW of Sample	Flow Rate	Temperature	Injection Volume	Instrument Delay
30 cm	4.6 mm	10 μ m	100	1.00	30	20 μ L	1.00
25 cm	4.0 mm	8 μ m	200	[mL/min]	[Celsius]	[μ L]	[mL]
15 cm	3.9 mm	5 μ m	300				

Organic Modifier in B

MeCN %100 MeOH %0

Pressure Units

bar PSI MPa

Results With Existing Column

Peak Capacity Pressure*

Step **Time** **Flow** **%A** **%B** **Time Segment (min)** **Column Volumes**

Init Cond.	0.00	1.00	100	0	0.00	-
Init Hold						
3						
4						
5						
6						
7						
8						
9						
10						

Select Maximum Column Pressure 689 bar [Change](#)

Click here for your ACQUITY UPLC™ column choices. [Calculate](#)

Acquity Columns Calculator

HPLC 梯度方法

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Gradient Separations

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Column Length	Column Diameter	Particle Size	MW of Sample	Flow Rate	Temperature	Injection Volume	Instrument Delay
30 cm	4.6 mm	10 μ m	100	1.50	23	10	1.00
25 cm	4.0 mm	8 μ m	200	[mL/min]	[Celsius]	[μ L]	[mL]
15 cm	3.9 mm	5 μ m	300				

Organic Modifier in B Pressure Units

MeCN %100 MeOH %0 bar PSI MPa

Results With Existing Column

Peak Capacity Pressure*

Step Time Flow %A %B Time Segment [min] Column Volumes

Init Cond.	0.00	1.50	95	5	0.00	-
Init Hold	0.00	1.50	95	5	0.00	0.00
3	15.00	1.50	5	95	15.00	13.68
4	20.00	1.50	5	95	5.00	4.56
5	30.00	1.50	95	5	10.00	9.12
6						
7						
8						
9						
10						

Select Maximum Column Pressure 10000 PSI [Change](#)

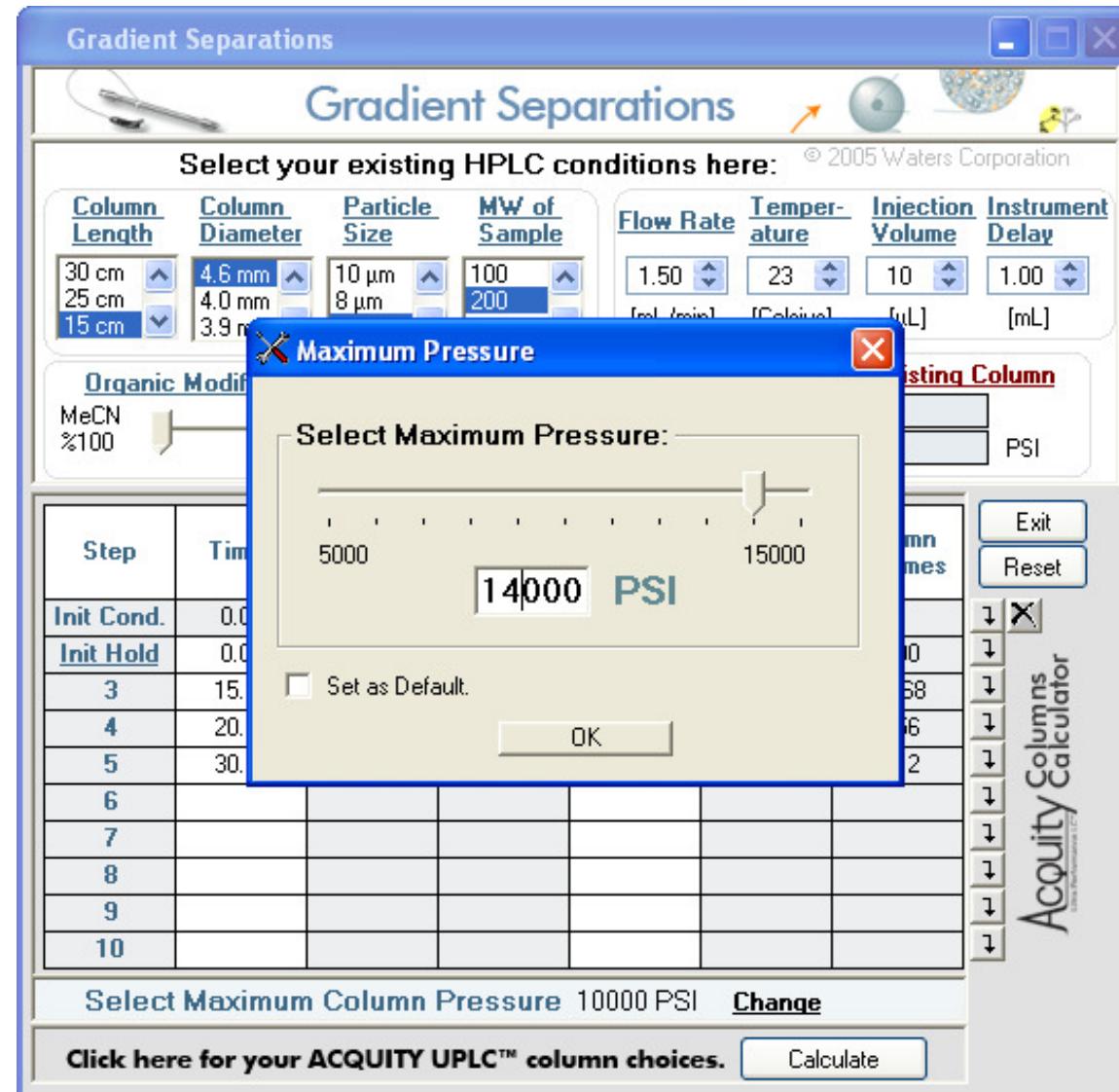
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Acquity Columns Calculator

HPLC 梯度方法

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计算

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Column Length	Column Diameter	Particle Size	MW of Sample	Flow Rate	Temper-ature	Injection Volume	Instrument Delay
30 cm	4.6 mm	10 μ m	100	1.50	23	10	1.00
25 cm	4.0 mm	8 μ m	200	[mL/min]	[Celsius]	[μ L]	[mL]
15 cm	3.9 mm	5 μ m	300				

Organic Modifier in B

MeCN %100 MeOH %0

Pressure Units

bar PSI MPa

Results With Existing Column

Peak Capacity 119
Pressure* 1407 PSI

Step	Time	Flow	%A	%B	Time Segment (min)	Column Volumes
Init Cond.	0.00	1.50	95	5	0.00	-
Init Hold	0.00	1.50	95	5	0.00	0.00
3	15.00	1.50	5	95	15.00	13.68
4	20.00	1.50	5	95	5.00	4.56
5	30.00	1.50	95	5	10.00	9.12
6						
7						
8						
9						
10						

Select Maximum Column Pressure 14000 PSI [Change](#)

[Click here for your ACQUITY UPLC™ column choices.](#) [Calculate](#)

Acuity Columns Calculator

UPLC 方法

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2.1 mm ID

1.0 mm ID

Gradient Results

Results With Existing Column

Peak Capacity:	119	Column Length:	15 cm	Particle Size:	5 μ m
Pressure* [PSI]	1407	Column Diameter:	4.6 mm	MW of Sample:	200

Geometrically Scaled UPLC(TM) Method Choices | Optimally Scaled UPLC(TM) Method Choices | Print

Scaled Gradient 2.1 mm Column
(HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow [mL/min]	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [μ L]	Detailed Gradient Profile
5 cm	2.1	0.313	124	5.1	3619	0.7	View
10 cm	2.1	0.313	180	10.1	7237	1.4	View

Change Flow Calculate HPLC Linear Velocity UPLC(TM) Linear Velocity

Scaled Gradient 1.0 mm Column
(HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow [mL/min]	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [μ L]	Detailed Gradient Profile
5 cm	1.0	0.071	74	5.1	3619	0.2	View
10 cm	1.0	0.071	129	10.1	7237	0.3	View

Change Flow Calculate HPLC Linear Velocity UPLC(TM) Linear Velocity

Geometrically Scaled UPLC(TM) Method Choices

几何缩放到 UPLC 方法

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↓

Geometrically Scaled UPLC(TM) Method Choices | Optimally Scaled UPLC(TM) Method Choices | Print

Scaled Gradient 2.1 mm Column
(HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow [mL/min]	Peak Capacity	Run Time [min]	Pressure [*] [PSI]	Injection Volume [μL]	Detailed Gradient Profile
5 cm	2.1	0.313	124	5.1	3619	0.7	View
10 cm	2.1	0.313	180	10.1	7237	1.4	View

Change Flow Calculate HPLC Linear Velocity UPLC(TM) Linear Velocity

Geometrically Scaled Gradient: 2.1 X 50 mm

Step	Time (min)	Flow mL/min	%A	%B	Time Segment [min]	Column Volumes
Init Cond.	0.00	0.313	95	5	0.00	-
Init Hold	0.00	0.313	95	5	0.00	0.00
3	5.00	0.313	5	95	5.00	13.68
4	6.67	0.313	5	95	1.67	4.56
5	10.00	0.313	95	5	3.33	9.12
6						
7						
8						
9						
10						

Injection Volume: 0.7 μL

Pmax = 3619 PSI

Print

调整梯度表到2.1x50 mm柱

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在相同线速度下调节流速到 2.1 x 50mm柱

梯度	时间	流速	%A	%B	曲线	梯度段(分)	梯度段(cv)
Initial	0	0.31	95	5	*	0	0
2	5	0.31	5	95	6	5.0	9.03
3	6.67	0.31	5	95	1	1.67	3.01
4	10.00	0.31	95	5	1	3.33	6.02

计算



Gradient Separations

Select your existing HPLC conditions here:

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Column Length	Column Diameter	Particle Size	MW of Sample	Flow Rate	Temperature	Injection Volume	Instrument Delay
30 cm	4.6 mm	10 μm	100	1.50	23	10	1.00
25 cm	4.0 mm	8 μm	200	[mL/min]	[Celsius]	[μL]	[mL]
15 cm	3.9 mm	5 μm	300				

Organic Modifier in B Pressure Units

MeCN %100 MeOH %0 bar PSI MPa

Results With Existing Column

Peak Capacity: 119 Pressure*: 1407 PSI

Step	Time	Flow	%A	%B	Time Segment (min)	Column Volumes
Init Cond.	0.00	1.50	95	5	0.00	-
Init Hold	0.00	1.50	95	5	0.00	0.00
3	15.00	1.50	5	95	15.00	13.68
4	20.00	1.50	5	95	5.00	4.56
5	30.00	1.50	95	5	10.00	9.12
6						
7						
8						
9						
10						

Select Maximum Column Pressure 14000 PSI Change

Click here for your ACQUITY UPLC™ column choices. Calculate

Acuity Columns Calculator

UPLC 方法

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2.1 mm ID

1.0 mm ID

Gradient Results

Results With Existing Column

Peak Capacity:	119	Column Length:	15 cm	Particle Size:	5 μ m
Pressure* [PSI]	1407	Column Diameter:	4.6 mm	MW of Sample:	200

Geometrically Scaled UPLC(TM) Method Choices | Optimally Scaled UPLC(TM) Method Choices | Print

Scaled Gradient 2.1 mm Column
(HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow mL/min	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [μ L]	Detailed Gradient Profile
5 cm	2.1	0.313	124	5.1	3619	0.7	View
10 cm	2.1	0.313	180	10.1	7237	1.4	View

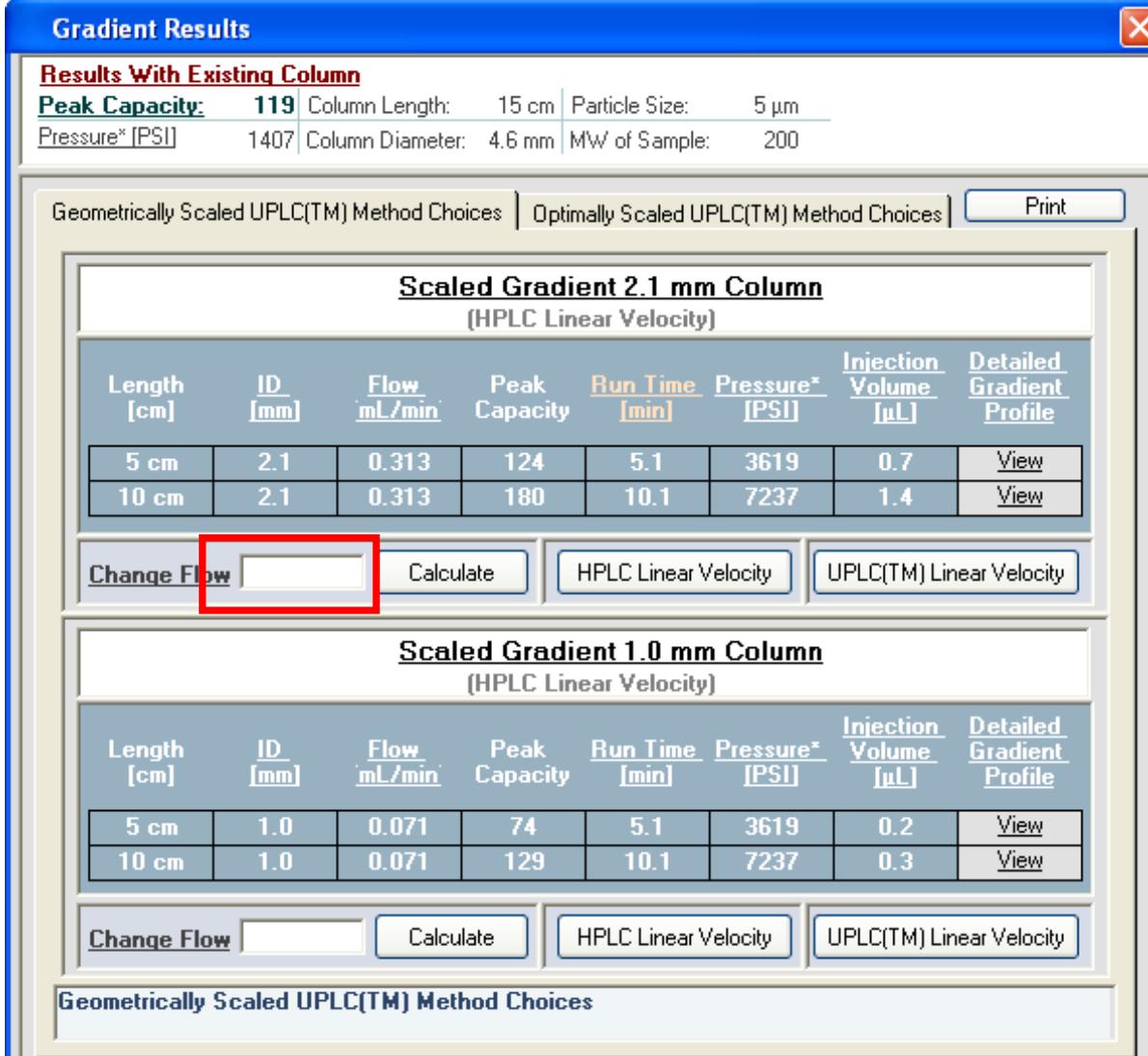
Change Flow Calculate HPLC Linear Velocity UPLC(TM) Linear Velocity

Scaled Gradient 1.0 mm Column
(HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow mL/min	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [μ L]	Detailed Gradient Profile
5 cm	1.0	0.071	74	5.1	3619	0.2	View
10 cm	1.0	0.071	129	10.1	7237	0.3	View

Change Flow Calculate HPLC Linear Velocity UPLC(TM) Linear Velocity

Geometrically Scaled UPLC(TM) Method Choices



用传统的“加倍”法转到UPLC方法

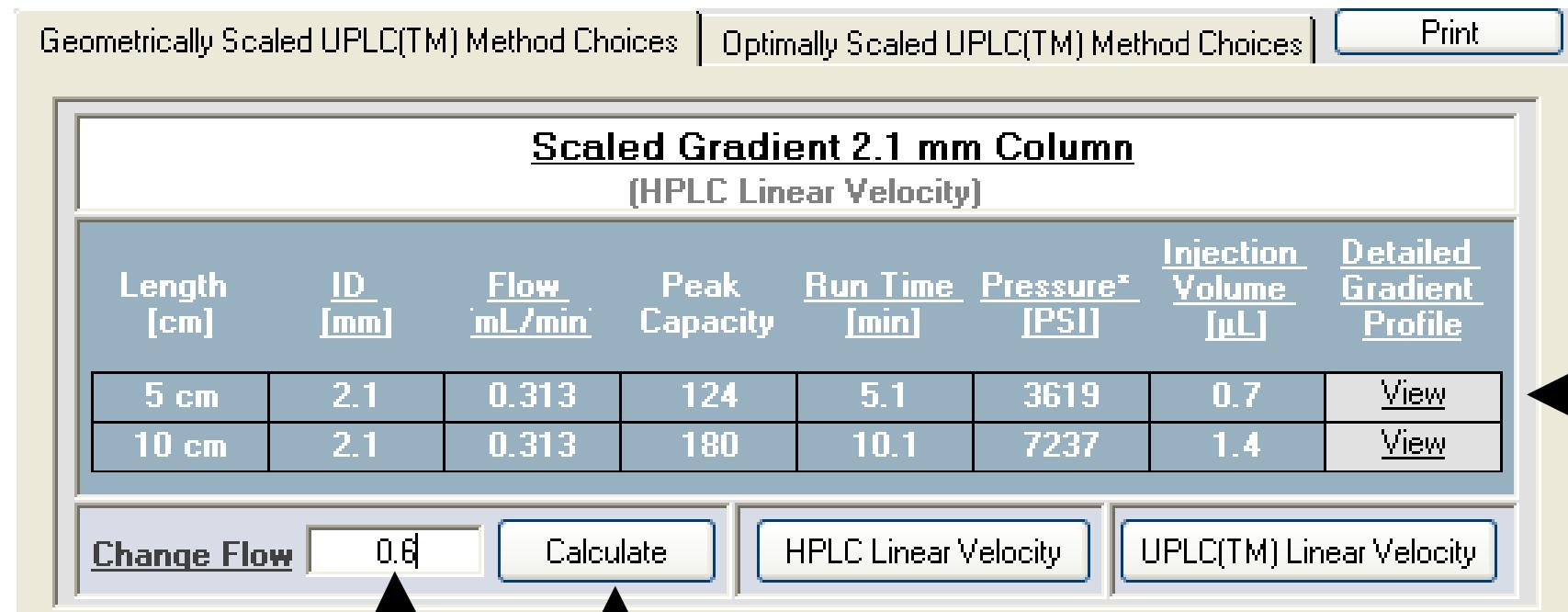
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Geometrically Scaled UPLC(TM) Method Choices | Optimally Scaled UPLC(TM) Method Choices | Print

Scaled Gradient 2.1 mm Column
(HPLC Linear Velocity)

Length [cm]	ID [mm]	Flow [mL/min]	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [μL]	Detailed Gradient Profile
5 cm	2.1	0.313	124	5.1	3619	0.7	View
10 cm	2.1	0.313	180	10.1	7237	1.4	View

Change Flow **Calculate** **HPLC Linear Velocity** **UPLC(TM) Linear Velocity**



UPLC 流速 0.6 ml/min

用传统的“加倍”法转到UPLC方法

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Geometrically Scaled Gradient: 2.1 X 50 mm

<u>Step</u>	<u>Time [min]</u>	<u>Flow mL/min</u>	<u>%A</u>	<u>%B</u>	<u>Time Segment [min]</u>	<u>Column Volumes</u>
Init Cond.	0.00	0.600	95	5	0.00	-
Init Hold	0.00	0.600	95	5	0.00	0.00
3	2.61	0.600	5	95	2.61	13.68
4	3.47	0.600	5	95	0.87	4.56
5	5.21	0.600	95	5	1.74	9.12
6						
7						
8						
9						
10						

Injection Volume: 0.7 μL Pmax = 6945 PSI

转换到 2.1 x 50 mm 柱 UPLC 流速 0.6 mL/min

梯度	时间	流速	%A	%B	曲线	梯度段 (分)	梯度段 (cv)
Initial	0	0.6	95	5	*	0	0
2	2.61	0.6	5	95	6	2.61	9.03
3	3.48	0.6	5	95	1	0.87	3.01
4	5.22	0.6	95	5	1	1.74	6.02

优化到 UPLC:方法选择

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Gradient Results

Results With Existing Column

Peak Capacity:	119	Column Length:	15 cm	Particle Size:	5 μ m
Pressure* [PSI]	1407	Column Diameter:	4.6 mm	MW of Sample:	200

Geometrically Scaled UPLC(TM) Method Choices Optimal Scaled UPLC(TM) Method Choices Print

Maximum Peak Capacity at Equal Run Time
(Optimized Linear Velocity)

Length [cm]	ID [mm]	Flow mL/min	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume μ L	Detailed Gradient Profile
5 cm	2.1	0.591	179	15.0	6845	0.7	View
10 cm	2.1	0.616	231	15.0	14001	1.4	View

Shortest Analysis Time at Equal Peak Capacity
(Optimized Linear Velocity)

Length [cm]	ID [mm]	Flow mL/min	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume μ L	Detailed Gradient Profile
5 cm	2.1	0.998	120	2.3	11490	0.7	View
10 cm	2.1	0.618	120	2.6	14001	1.4	View

Optimal Scaled UPLC(TM) Method Choices

优化到UPLC:相同峰容量时的最短分析时间

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Gradient Results

Results With Existing Column

Peak Capacity	119	Column Length:	15 cm	Particle Size:	5 µm
Pressure* [PSI]	14001	Column Diameter:	4.6 mm	MW of Sample:	200

Geometrically Scaled UPLC(TM) Method Choices Optimally Scaled UPLC(TM) Method Choices Print

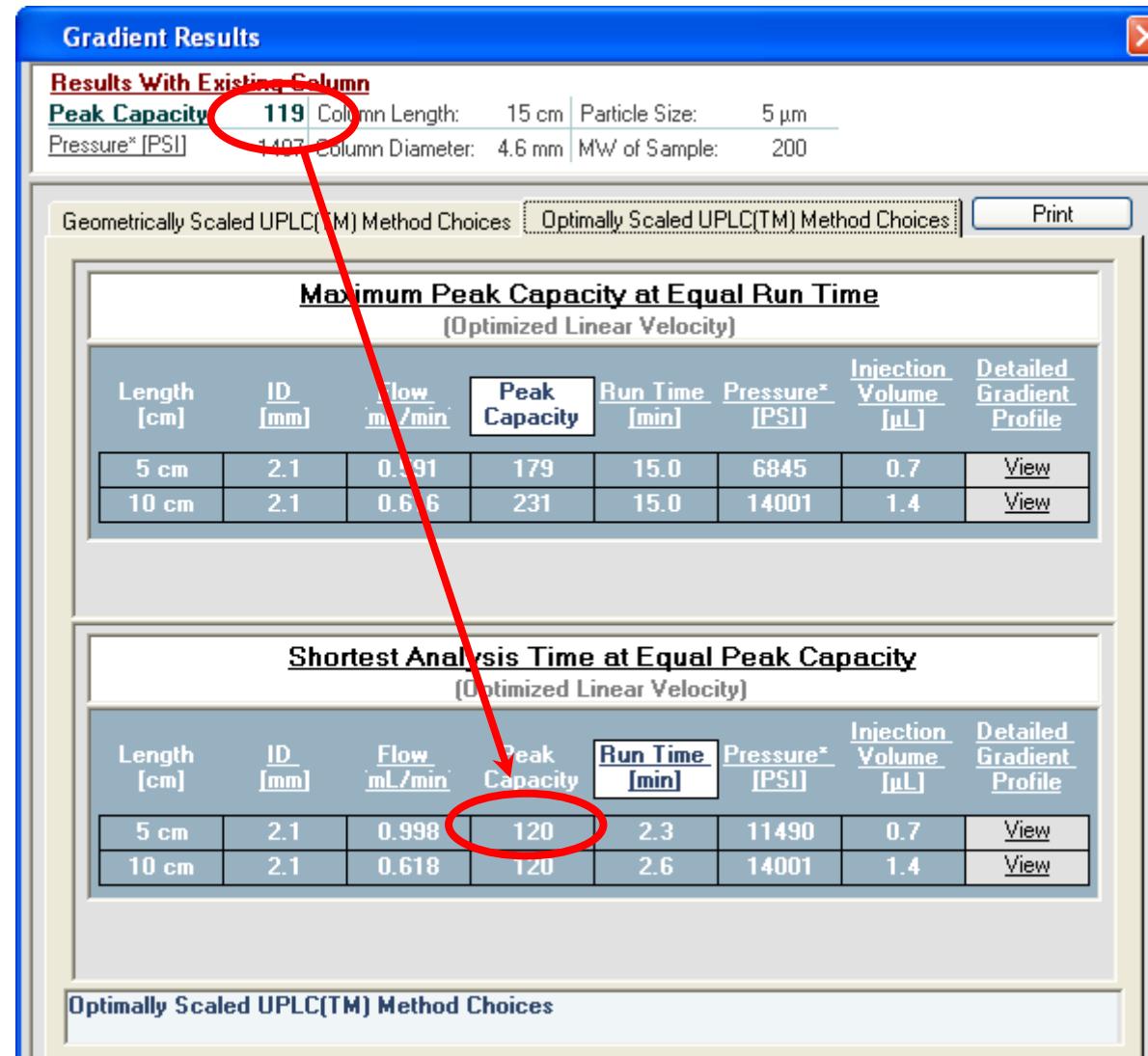
Maximum Peak Capacity at Equal Run Time
(Optimized Linear Velocity)

Length [cm]	ID [mm]	Flow mL/min	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [µL]	Detailed Gradient Profile
5 cm	2.1	0.991	179	15.0	6845	0.7	View
10 cm	2.1	0.618	231	15.0	14001	1.4	View

Shortest Analysis Time at Equal Peak Capacity
(Optimized Linear Velocity)

Length [cm]	ID [mm]	Flow mL/min	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [µL]	Detailed Gradient Profile
5 cm	2.1	0.998	120	2.3	11490	0.7	View
10 cm	2.1	0.618	120	2.6	14001	1.4	View

Optimally Scaled UPLC(TM) Method Choices



相同峰容量的最短分析时间

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Shortest Analysis Time at Equal Peak Capacity: 2.1 X 50 mm

Step	Time [min]	Flow mL/min	%A	%B	Time Segment [min]	Column Volumes
Init Cond.	0.00	0.998	95	5	0.00	-
Init Hold	0.00	0.998	95	5	0.00	0.00
3	2.27	0.998	5	95	2.27	19.79
4	3.02	0.998	5	95	0.76	6.60
5	4.53	0.998	95	5	1.51	13.19
6						
7						
8						
9						
10						

Injection Volume: 0.7 μL

Pmax = 11490 PSI

Print

优化到 UPLC 相同时间内最大峰容量

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Gradient Results

Results With Existing Column

Peak Capacity:	119	Column Length:	15 cm	Particle Size:	5 μ m
Pressure* [PSI]	1407	Column Diameter:	4.6 mm	MW of Sample:	200

Geometrically Scaled UPLC(TM) Method Choices Optimally Scaled UPLC(TM) Method Choices Print

Maximum Peak Capacity at Equal Run Time
(Optimized Linear Velocity)

Length [cm]	ID [mm]	Flow mL/min	Peak Capacity	Run Time [min]	Pressure* [PSI]	Injection Volume [μ L]	Detailed Gradient Profile
5 cm	2.1	0.591	179	15.0	6845	0.7	View
10 cm	2.1	0.616	231	15.0	14001	1.4	View

相同时间内最大峰容量

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Maximum Peak Capacity at Equal Run Time: 2.1 X 50 mm



Step	Time (min)	Flow mL/min	%A	%B	Time Segment [min]	Column Volumes
Init Cond.	0.00	0.591	95	5	0.00	-
Init Hold	0.00	0.591	95	5	0.00	0.00
3	15.00	0.591	5	95	15.00	77.61
4	20.00	0.591	5	95	5.00	25.87
5	30.00	0.591	95	5	10.00	51.74
6						
7						
8						
9						
10						

Injection Volume: 0.7 μ L

Pmax = 6845 PSI

Print

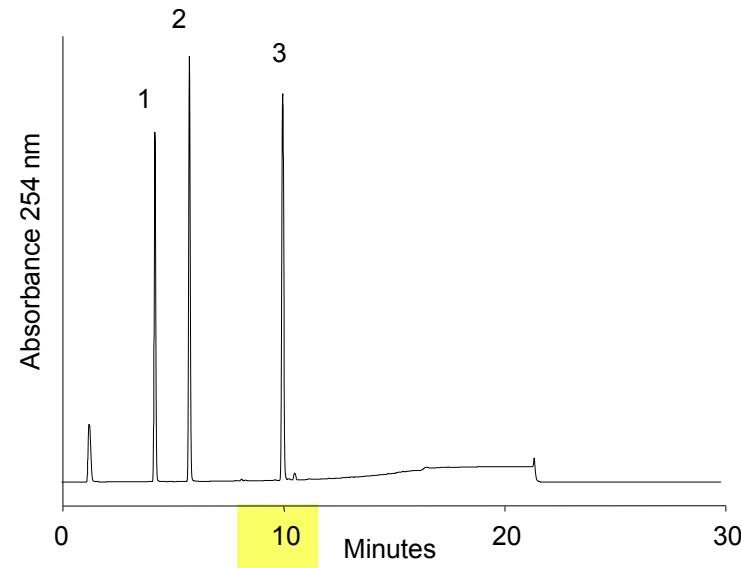
目标条件:几何缩放和 UPLC™ 线速度调整

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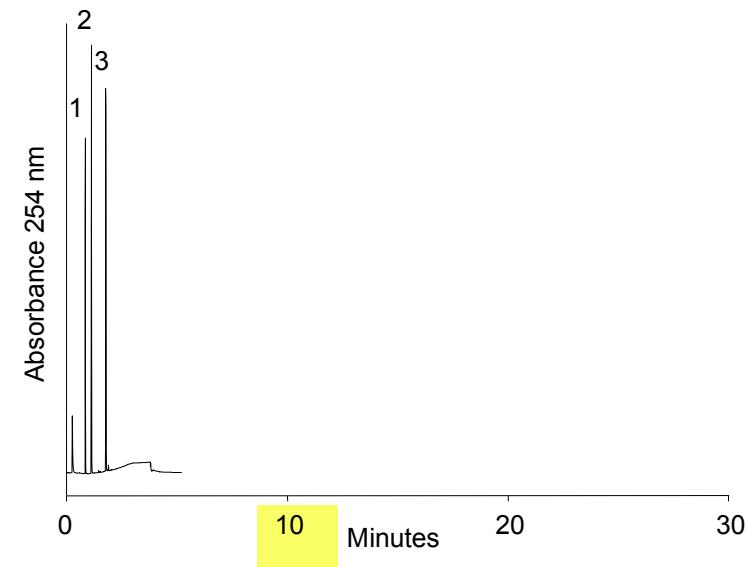
- 参数
 - 流动相
 - 温度
 - 几何缩放
 - 进样量
 - 流速
 - 等度方法只需调整流速和进样量
 - 梯度曲线
- 几何缩放的目的是为了减少评估和优化转换方法所带来的变化
- 调整到 UPLC 的线速度

HPLC 转换到 UPLC

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原始 30 分钟 HPLC



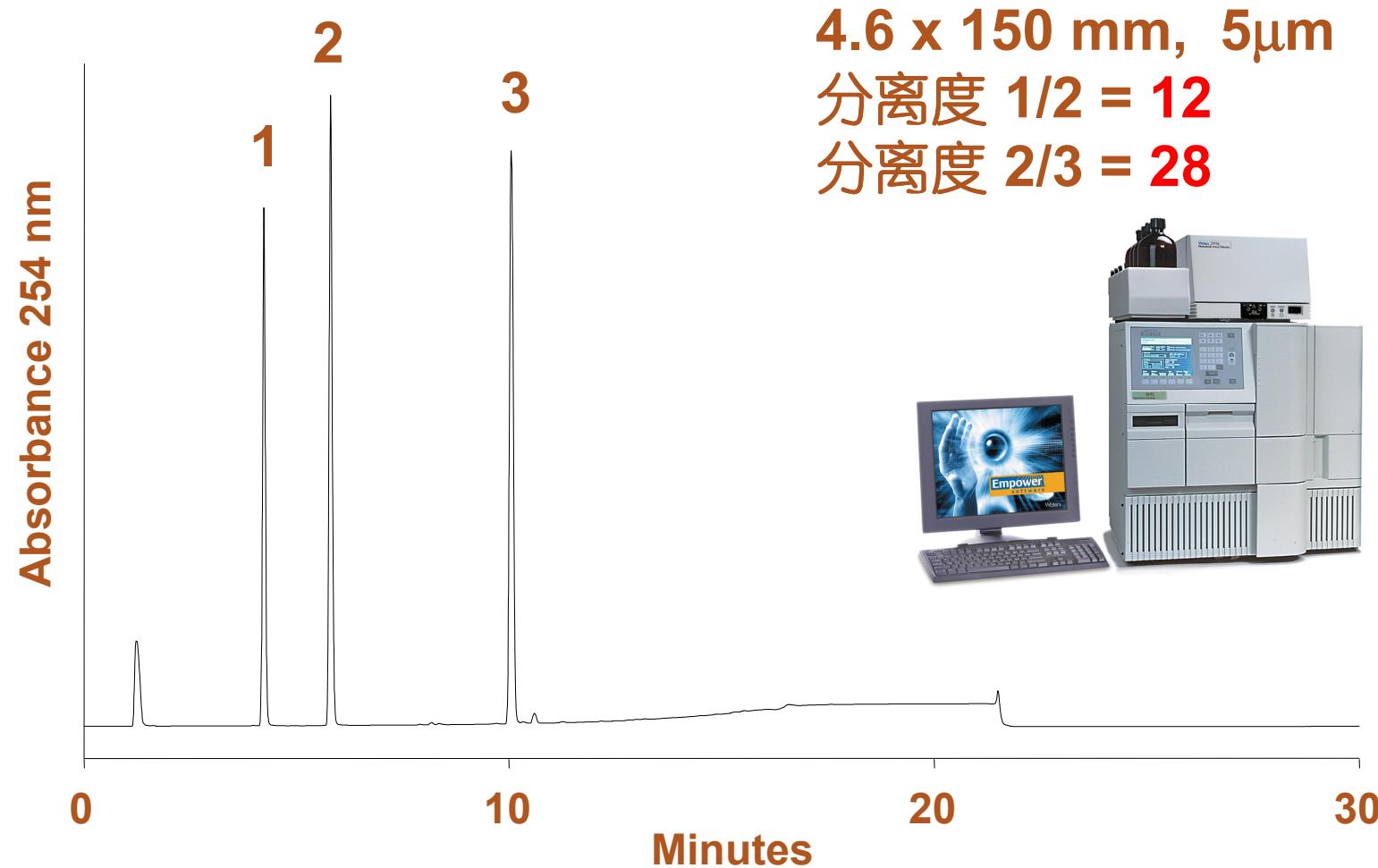
转换到 5.2 分钟 UPLC

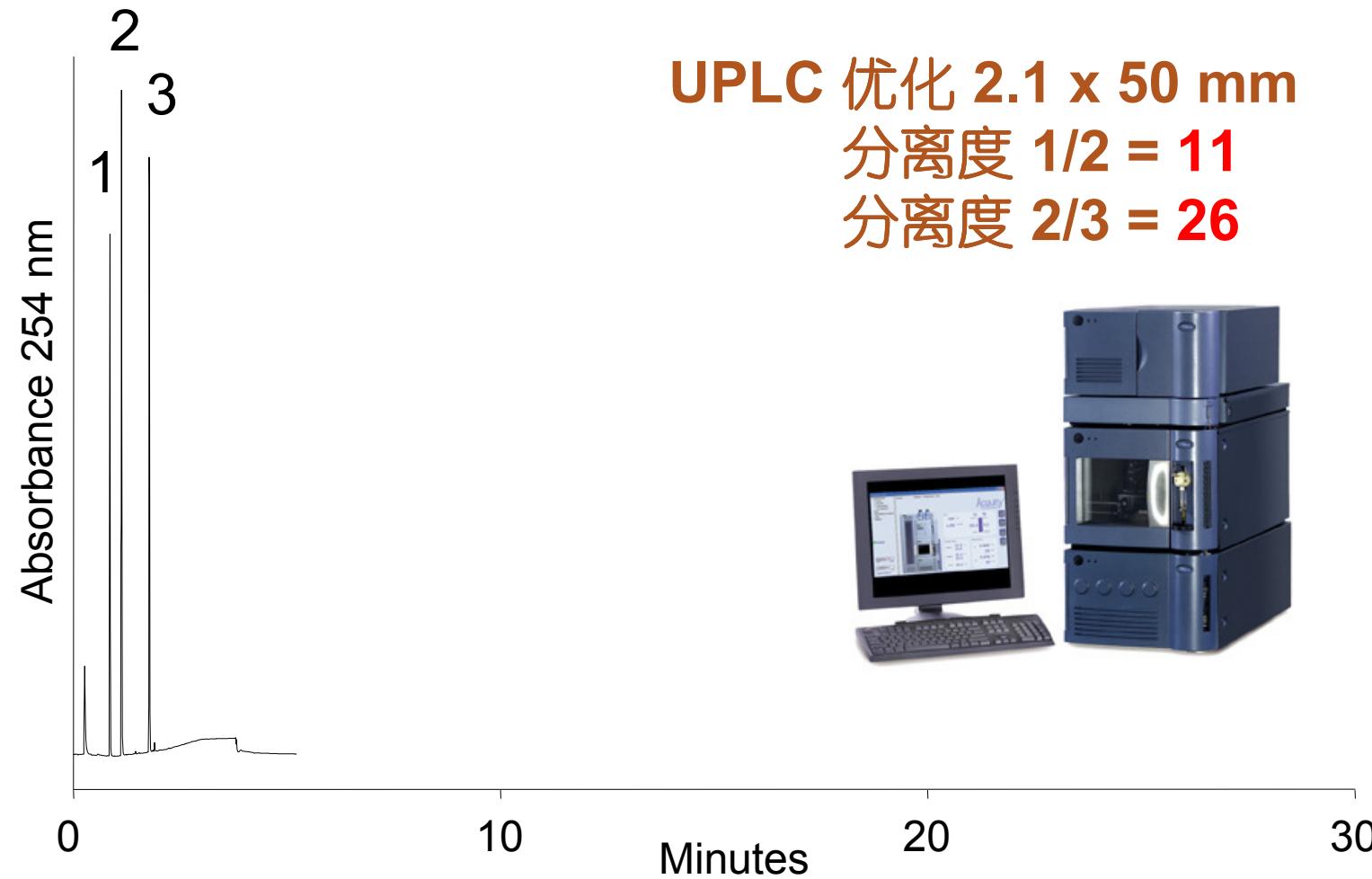
成功进行方法转换的步骤

- 得到现有方法和结果的信息
- 仪器比较
- 选择新的或是目标柱
 - 色谱柱化学
 - 直径
- 在几何放大的基础上选择目标条件
- **评估转换的结果**
- 如需要再进行优化

评价结果

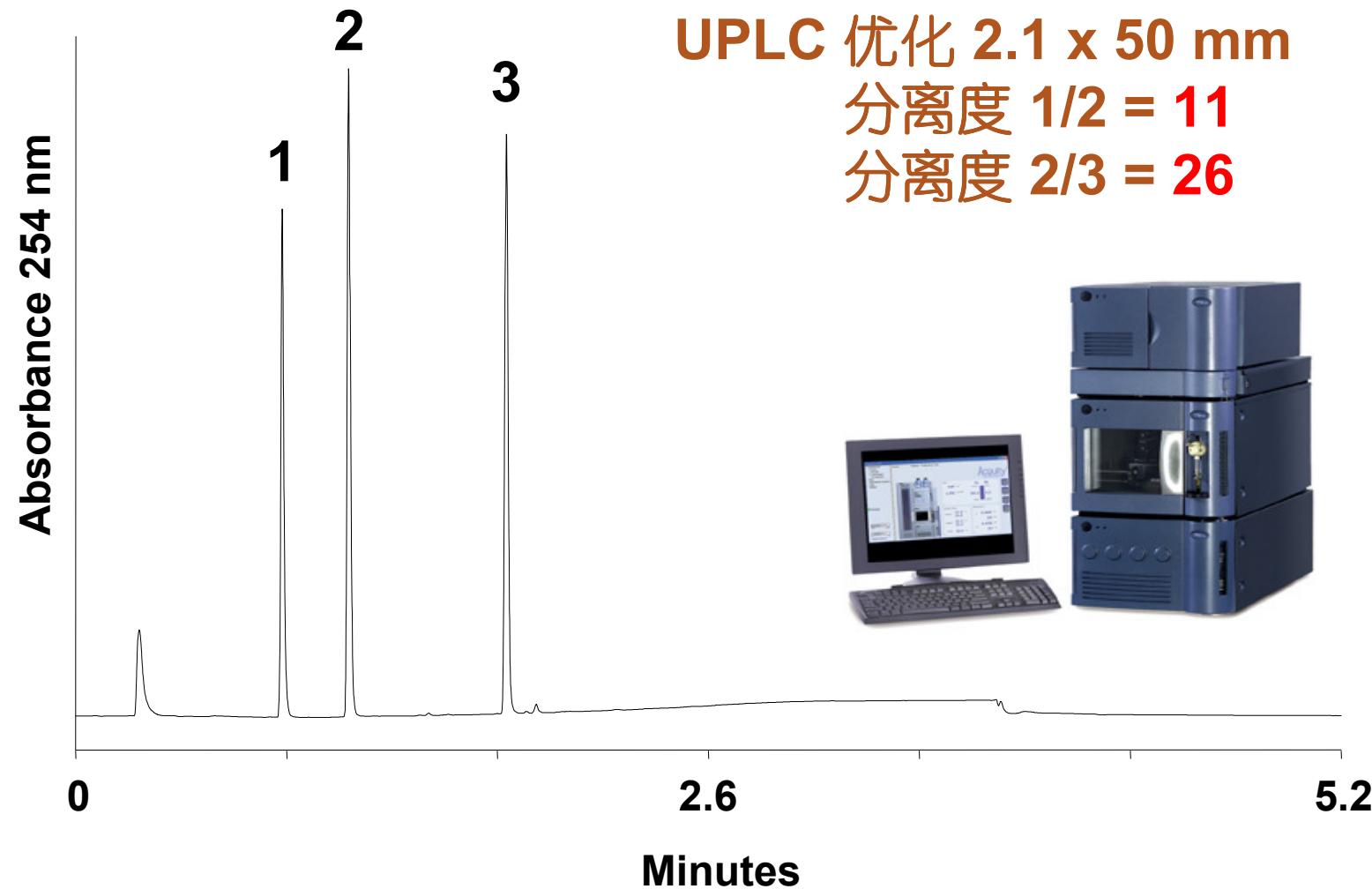
- 峰匹配
 - 数峰个数
 - 考察峰间距
 - 严格考察基线中出现的和缺少的小峰
 - 匹配洗脱顺序和分离度
- 进一步进行通常的定量评估
 - 分离度
 - 检出限 (LOD)
 - 定量限 (LOQ)





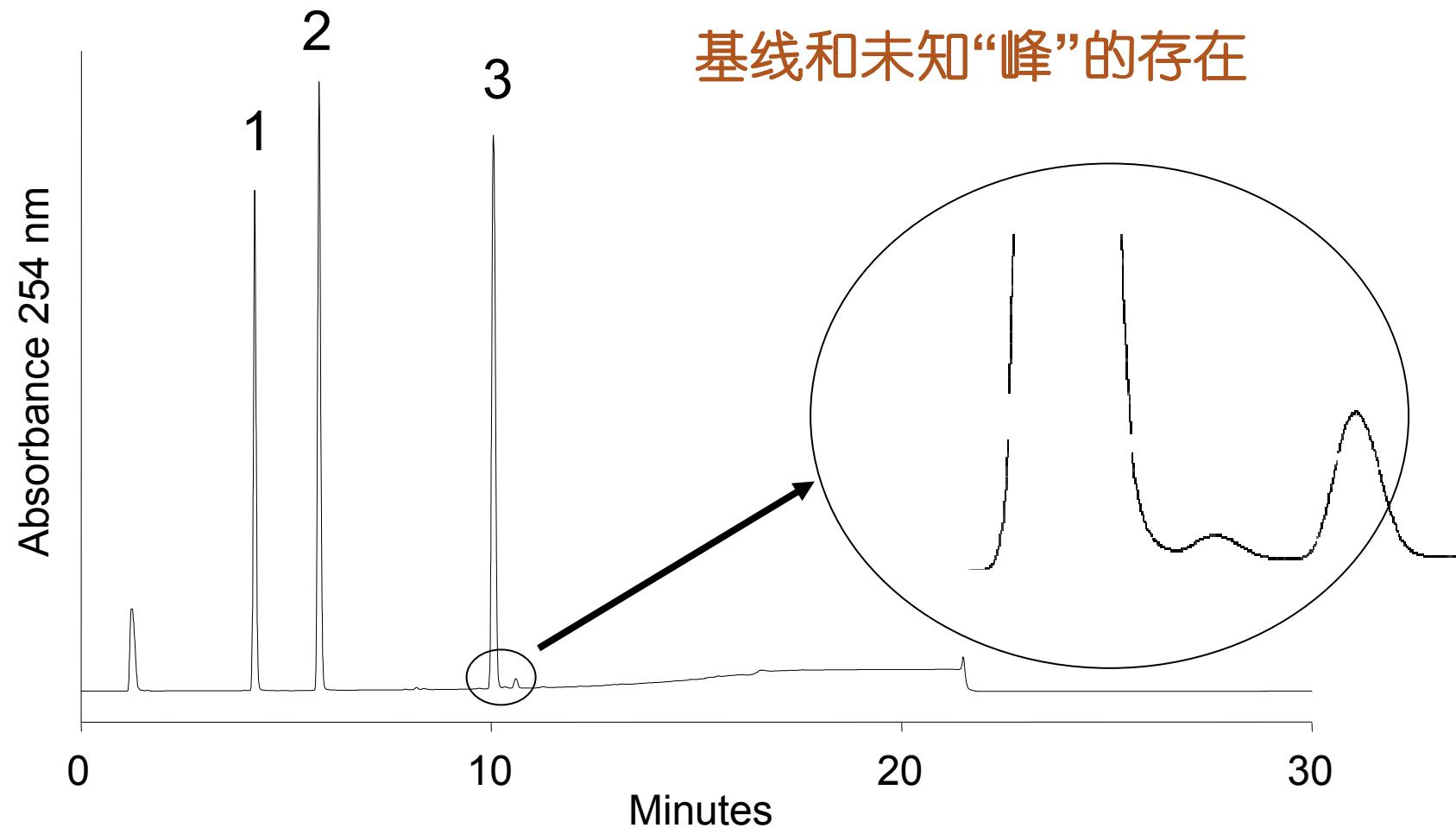
按比例放大图

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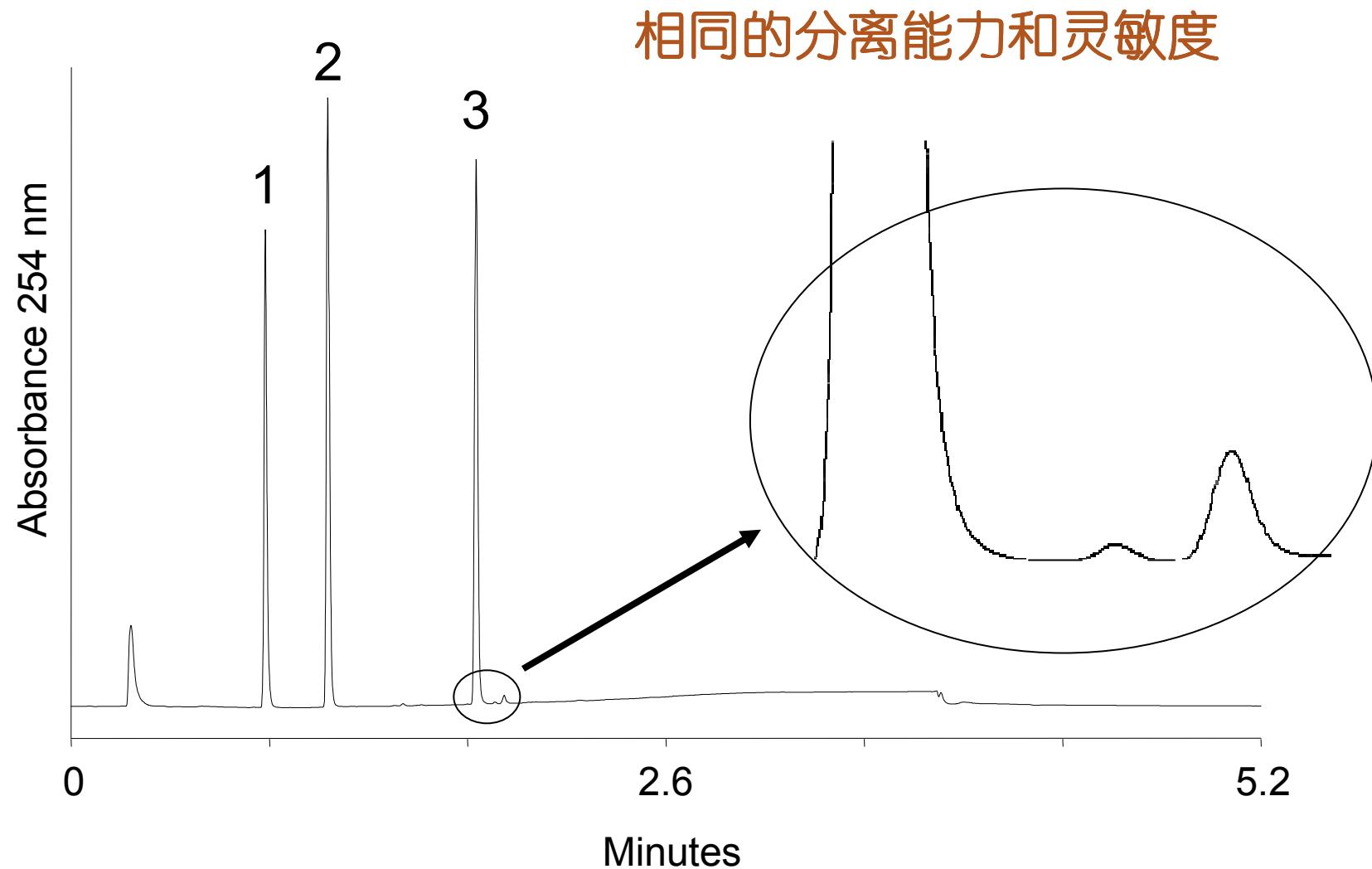
原始的 30分钟 HPLC方法
微量组分

Waters
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5.2 分钟 UPLC 微量组分的放大图

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成功进行方法转换的步骤

- 得到现有方法和结果的信息
- 仪器比较
- 选择新的或是目标柱
 - 色谱柱化学
 - 直径
- 在几何放大的基础上选择目标条件
- 评估转换的结果
- 如需要再进行优化

优化转化的方法



- ACQUITY UPLC™ 和反相HPLC运用的是同样的理论
- 之前所用的一切理论仍然适用
 - 所有的化学操作仍然适用
 - 所有的策略仍然与之前相同
 - 仿真软件在一些例子中已经验证过是很有用的，就像是在通常的HPLC上一样
- 优化总是要进行的，**但过程会大大加快**

总结



- 方法可以从HPLC直接转换至ACQUITY UPLC™
 - 分离度增加
 - 速度增加
 - 检测灵敏度增加
- 许多参数可以也必须做一定的处理以保证原来的结果
- 注意通往成功的细节