
Migrating Traditional Methods to Sub-two μm Particles

Michael Swartz, Ph. D.
Research Director
Synomics Pharma Services

mswartz@synomicspharma.com
www.synomicspharma.com



Presentation Outline

- Justifying Method Migration
- Method Parameters to Consider
- Geometric Scaling
- Case Study
- Summary



Method Conversion From HPLC to VHPLC

- Why Convert HPLC Methods to VHPLC with Sub-Two- μm Particle Chemistry?
 - Get faster results with more resolution
 - More information
 - More robust methods
 - Better situational response time (stat samples faster, research decisions with more information, process monitoring, product release)
 - More samples analyzed per system, per scientist

Increased Productivity

Migrating or Converting Methods to Sub-Two- μm Particle Chemistry

Planning for Success

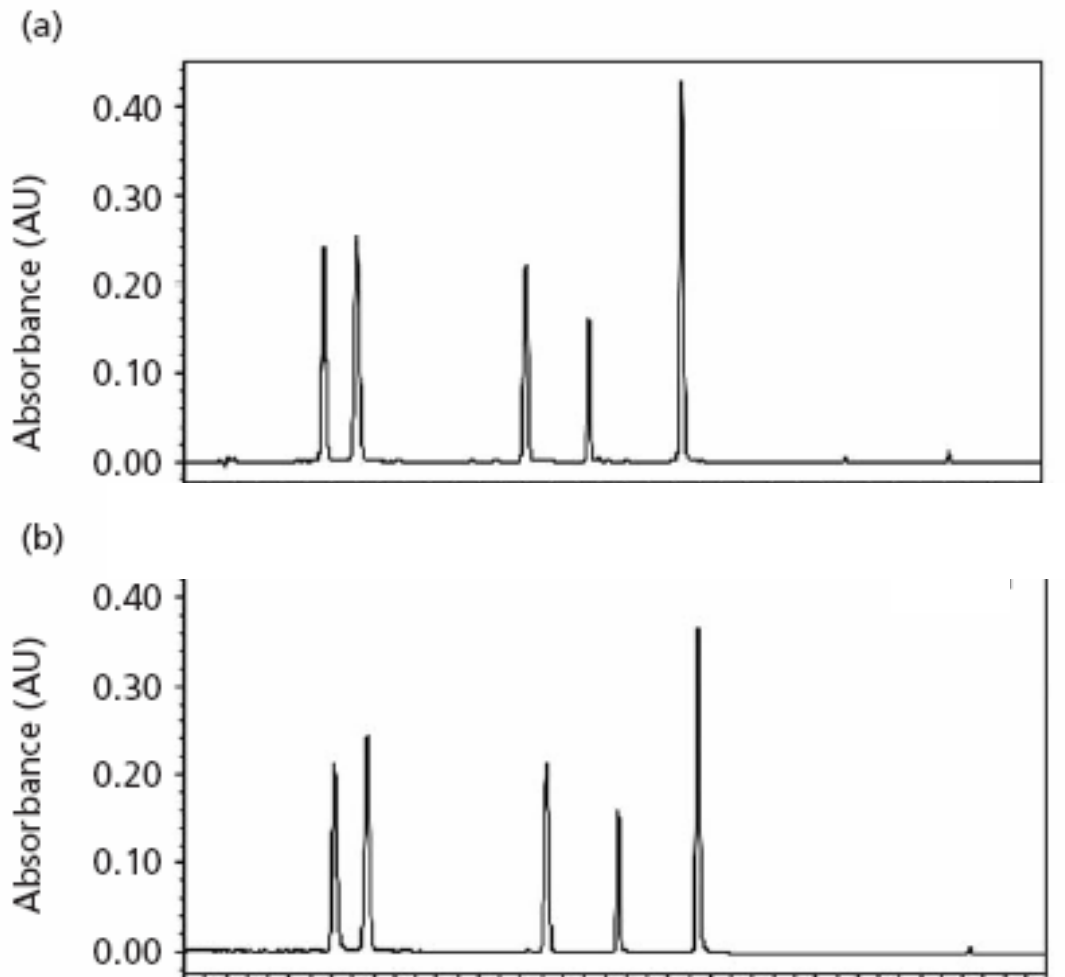
- Gather information about existing method and results
- Select new or target column
 - 5 μm to sub-2- μm particles
 - Chemistry
 - Dimensions
- Select target conditions based on geometric considerations
- Evaluate results of transfer
- Optimize as required

HPLC to VHPLC Conversion

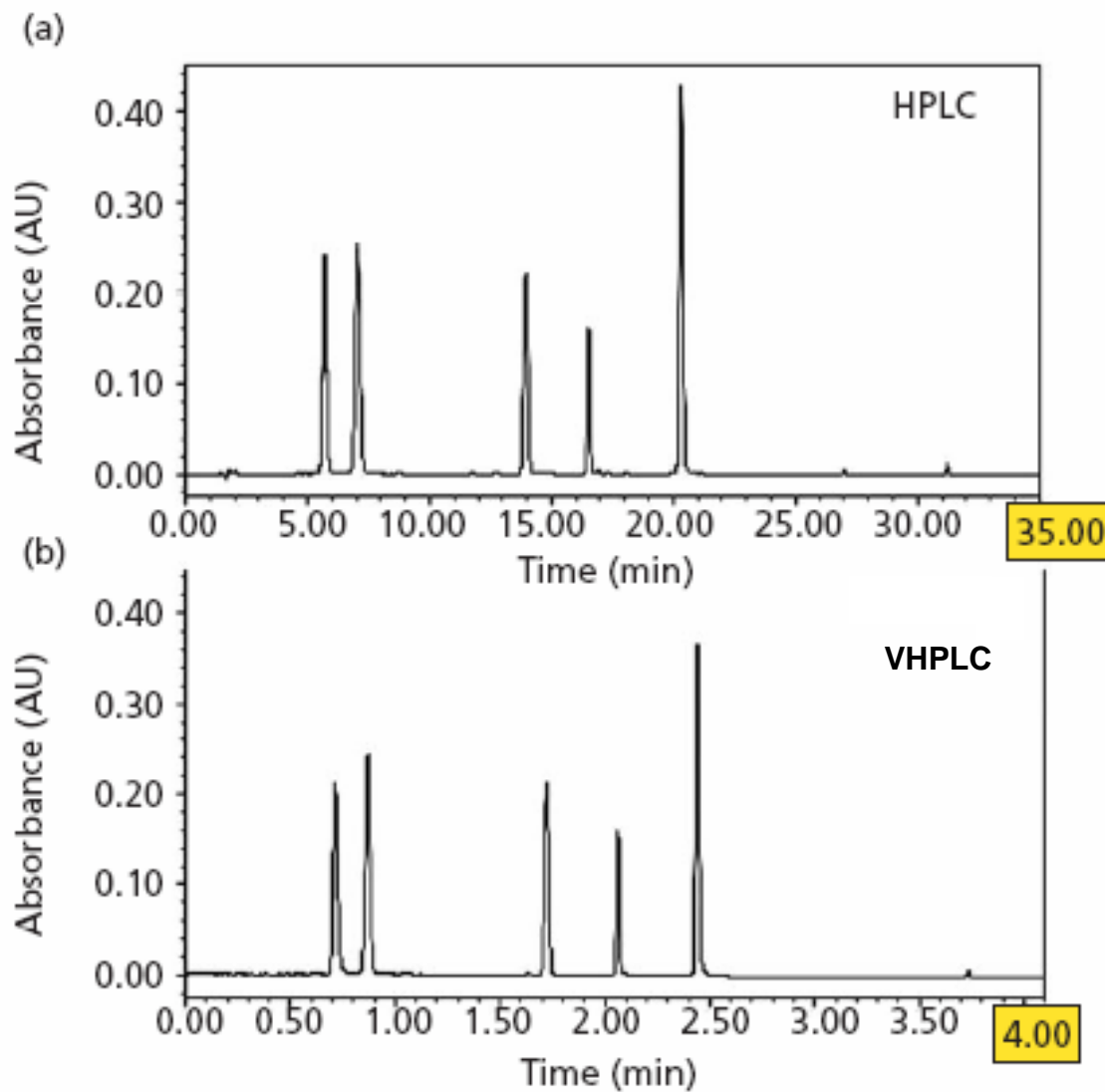
Proper Geometric Scaling is Critical For Correct Method Conversion

- Scale Injection volume
 - $V_2 = V_1 \times [(r_2^2 \times L_2)/(r_1^2 \times L_1)]$
- Scale flow rate (linear velocity)
 - $F_2 = F_1 \times (d_2)^2/(d_1)^2$
- Maintain same gradient duration and number of column volumes per segment
 - Express gradient duration in percent change per column volume (cv) units
 - Calculate each segment as a number of column volumes
 - Calculate time required to deliver the same number of column volumes to the target column at the chosen flow rate
- Adjust flow rate and gradient to optimum linear velocity

Ultimate Method Migration Goal



Ultimate Method Migration Goal



USP Column Equivalency Database

Select column for comparison:

Xbridge C18 (Waters) ▼

USP Database

CTF: CFA: TFA: BD:

Rank	F	Column	Hy	CTF	CFA	TFA	BD	USP Designation	Manufacturer
0	0	Xbridge C18	1.6	1.1	3.8	1.1	3.1	L	Waters
1	0.37	Denali C18	1.7	1.5	3.9	1.2	3.1	L	Grace Davison
2	0.44	SymmetryShield RP18	1.6	1.5	3.1	1.2	3.3	L	Waters
3	0.53	Prevail Select C18	2	1.1	4	1.1	3.2	L	Grace Davison
4	0.59	Xbridge Shield RP18	1.3	1.4	2.4	1.1	3.3	L	Waters
5	0.59	Acquity UPLC BEH Shield RP18	1.3	1.4	2.4	1.1	3.3	L	Waters
6	0.66	Hypersil BDS 18	1.5	1.5	3.5	2	3.1	L	Thermo Electron
7	0.69	Alltima HP C18	1.4	1.3	3.5	1.2	2.6	L	Grace Davison
8	0.71	ProntoSil 120-5-C18-ace-EPS	2	1.2	3.8	1.6	2.8	L	Bischoff
9	0.73	ProntoSil 200-5-C18-ace-EPS	1.1	1.2	2.2	1.2	3.2	L	Bischoff
10	0.75	Discovery C18	1.1	1.1	2.8	1.6	3	L	Supelco

Target Column Dimensions

Sub-Two- μm Particle Chemistry

- Internal Diameter
 - Generally prefer 2.1 mm
 - Only use 1 mm for specific reason
 - Severely sample limited
 - Direct flow to mass spectrometer
- Length
 - If primary goal is SPEED
 - 50 mm length to start
 - If primary goal is RESOLUTION
 - 100 mm length to start

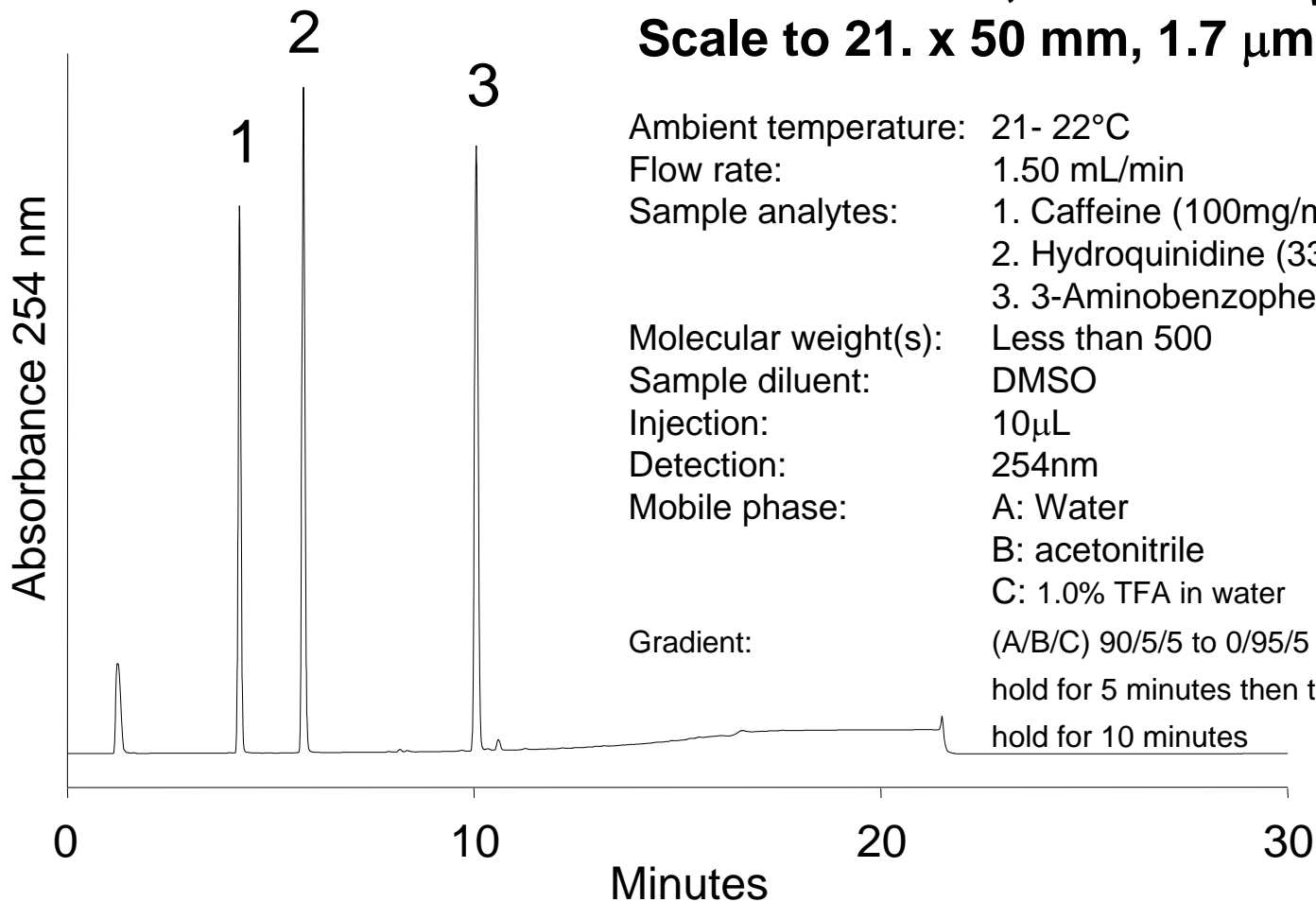
Method Conversion:

Original HPLC Method

Resolution (1,2) = 12

Resolution (2,3) = 28

Original Column: 4.6 x 150 mm, 5 μ m
@ 1.5 mL/min, 30 minute cycle time
Scale to 21. x 50 mm, 1.7 μ m Column



Ambient temperature: 21- 22°C
 Flow rate: 1.50 mL/min
 Sample analytes: 1. Caffeine (100mg/mL),
 2. Hydroquinidine (33mg/mL),
 3. 3-Aminobenzophenone (39mg/mL)
 Molecular weight(s): Less than 500
 Sample diluent: DMSO
 Injection: 10 μ L
 Detection: 254nm
 Mobile phase: A: Water
 B: acetonitrile
 C: 1.0% TFA in water
 Gradient: (A/B/C) 90/5/5 to 0/95/5 in 15 minutes (Curve 6)
 hold for 5 minutes then to initial conditions (curve 11)
 hold for 10 minutes



Minutes
 SMITHERS

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Target Conditions

Injection Volume Considerations

- Geometrically scale injection volume to volume of column
- Capacity proportional to surface area and internal solvent volume
- Suggested minimum injection volume on VHPLC instruments is 0.5 – 1 μL
 - If calculated volume too small for injection, dilute 5 - 10x with initial strength mobile phase
 - Typically 5 μL maximum injection on 2.1 x 50 mm

Method Conversion

Column Comparison: Injection Volumes

- Scale target injection volume to match column volume

4.6 x 150mm



$20\mu\text{L injection} / 2.49\text{mL} = 0.8\%$

2.1 x 50mm



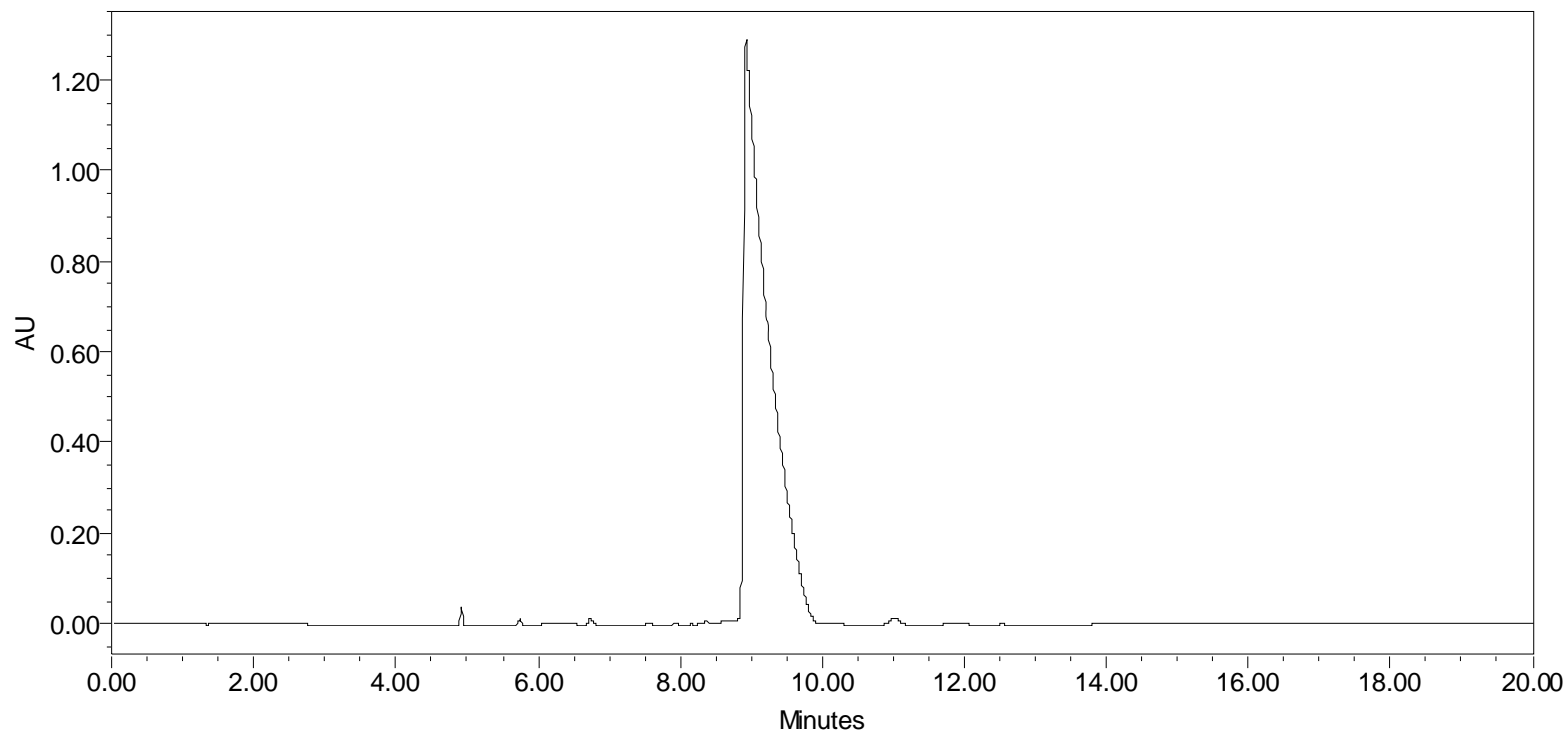
$20\mu\text{L injection} / 0.19\text{mL} = 11\%$

**Sample volume too large
for smaller column volume**

Method Conversion

Column Comparison: Injection Volumes

Column transfer from 4.6mm to 2.1mm i.d.
No injection volume scaling



Method Conversion

Calculate Injection Volume

Target injection volume =

$$\text{Original injection volume} \times \frac{\text{Target Column Volume}}{\text{Original Column Volume}}$$

Scaling a **10 μ L** injection on 4.6 x 150mm to 2.1 x 50mm

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

$$10\mu\text{L} \times \frac{0.19}{2.49} = 10\mu\text{L} \times 0.076$$

$$= \mathbf{0.8\mu\text{L}}$$

Method Conversion

Scale the Flow Rate to Column Geometry

Scaling a **1.5mL/min** flow rate on 4.6x150mm to 2.1x50mm*

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{\pi \times r^2 \text{ of Target}}{\pi \times r^2 \text{ of Original}}$$

This reduces to:

$$\text{Target Flow Rate} = \text{Original Flow Rate} \times \frac{d^2_{\text{Target}}}{d^2_{\text{Original}}}$$

So:

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

*Note: this assumes same particle size

Gradient Segments

Express as Number of Column Volumes

For 15 min at 1.5mL/min on a 4.6 x 150mm column

$$\text{Gradient Volume} = \text{Flow Rate} \times \text{Time} = 1.5\text{mL/min} \times 15\text{min} = 22.5\text{mL}$$

$$\text{Column Volume} = \pi \times r^2 \times L = 3.14 \times 0.23^2 \times 15.0 = 2.49\text{mL}$$

$$\text{Gradient Duration (\#cv)} = \frac{\text{Gradient Volume}}{\text{Column Volume}}$$

$$\text{Gradient Duration} = \frac{22.5\text{mL}}{2.49\text{mL}} = 9.03 \text{ cv}$$

Original Gradient Profile for Scaling

Step	Time Since Injection	Flow Rate	%A	%B	Curve	Segment Duration (min)	Segment Duration (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

Scaling Gradient Step Time to 2.1 by 50 mm Column *Maintaining Gradient Step Duration (cv)*

Original Step 2: 15 min @ 1.5 mL/min with duration of **9.03cv**

Calculate Target Step 2: (keeping duration @ **9.03cv**)

$$\text{Column Volume} = \pi \times r^2 \times L = 3.14 \times 0.105^2 \times 5.0 = 0.17\text{mL}$$

$$\text{Gradient Step Volume} = \text{Duration (cv)} \times \text{Target Column Volume}$$

$$= \mathbf{9.03cv} \times 0.17\text{mL} = 1.54\text{mL}$$

$$\text{Gradient Step Time} = \text{Gradient Step Volume} / \text{Flow Rate}$$

$$= 1.54\text{mL} / 0.31 \text{ mL/min} =$$

5 min

Scaled Gradient Profile

2.1x50mm Column

Adjust time for same number of column volumes
per gradient segment

Gradient Step	Time Since Injection	Flow Rate	% A	% B	Curve	Segment Duration (min)	Segment Duration (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	0.31	95	5	1	3.33	6.02

Estimate Optimum Flow Rate

VHPLC

- Consider 1.7 μ m target particle (2.1mm ID column)
- Assume same temperature and viscosity
- Adjust flow rate based on van Deemter curve and approximate molecular weight
 - ~0.6 mL/min for smaller molecules
 - average 500 dalton (molecular weight) molecules
 - ~0.1 mL/min for larger molecules because diffusion is slower
 - e.g., ~2,000 dalton peptides

Scaling for VHPLC Flow Rate

Step Time to Maintain Duration (cv)

Original Step 2: 15 min. @ 1.5 mL/min with Duration of **9.03cv**

Calculate Target Step 2: (keeping duration @ **9.03cv**)

Target Column Volume (2.1 x 50) = 0.17mL

Gradient Step Volume = Duration (cv) x Target Column Volume

= 9.03cv x 0.17mL = 1.54mL

Gradient Step Time = Gradient Step Volume / VHPLC Flow Rate

= 1.54mL / 0.60 mL/min. =

2.6min

Optimized Gradient Profile

Select VHPLC flow rate and adjust time to maintain same number of column volumes per segment

Gradient Step	Time Since Injection	Flow Rate	% A	% B	Curve	Segment Duration (min)	Segment Duration (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

Method Migration Process:

Steps for Success

Quick Review

- Refer to current chromatography
 - Observe system volumes, solvents and detection technique
 - Define objectives/room for improvement
- Select column dimensions – scale flow for linear velocity
 - 50 mm length for speed
 - 100mm length for complex samples/resolution
- Scale injection volume to column dimensions
- Use a gradient and flow scaled from current method
 - Adjust gradient to accommodate system differences, column differences and particle size differences
- Use a gradient and flow rate scaled for smaller particles and column dimensions

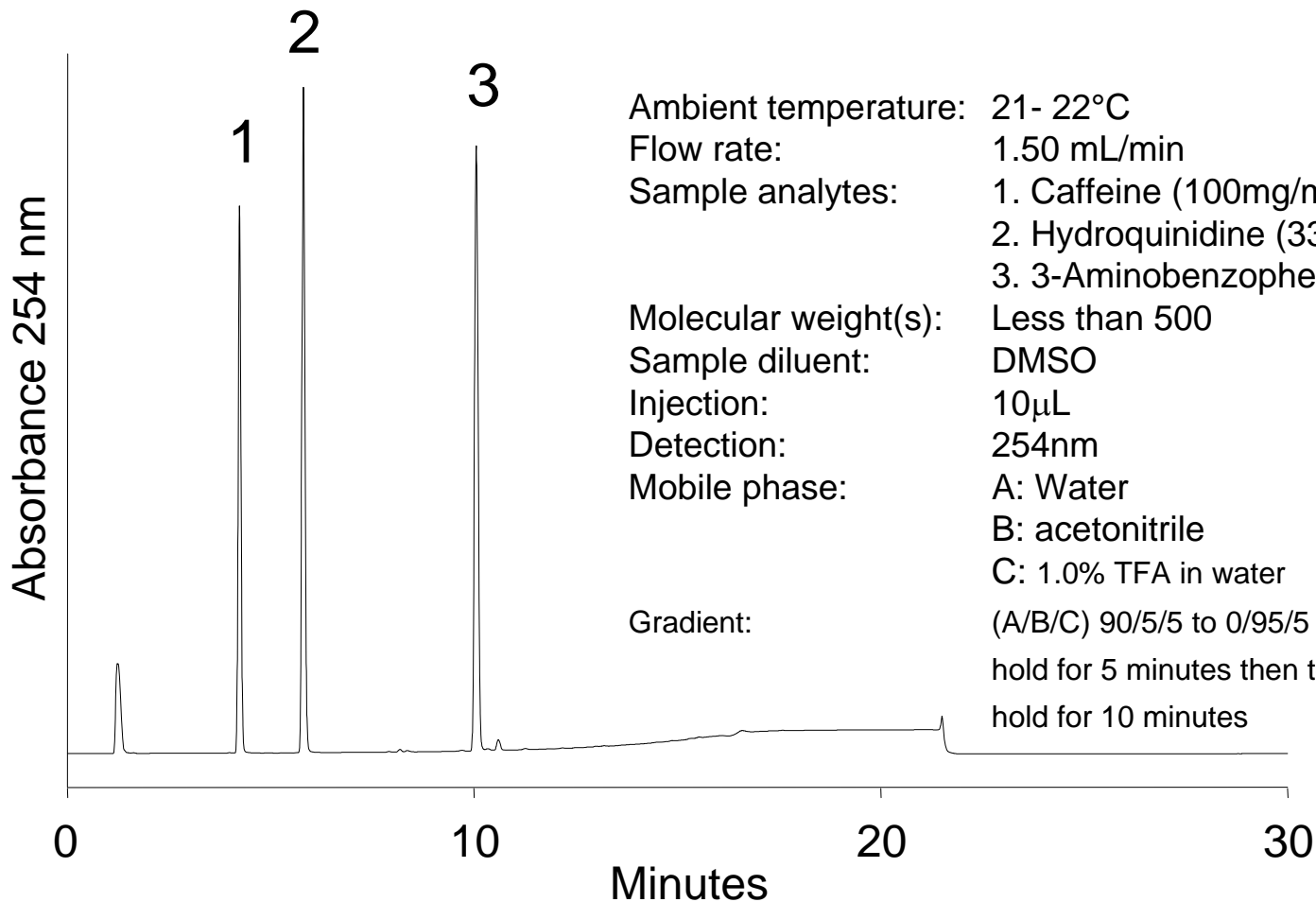
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Flow rate: 1.50 mL/min
Sample analytes: 1. Caffeine (100mg/mL),
2. Hydroquinidine (33mg/mL),
3. 3-Aminobenzophenone (39mg/mL)

Molecular weight(s): Less than 500
Sample diluent: DMSO
Injection: 10 μ L
Detection: 254nm
Mobile phase: A: Water
B: acetonitrile
C: 1.0% TFA in water

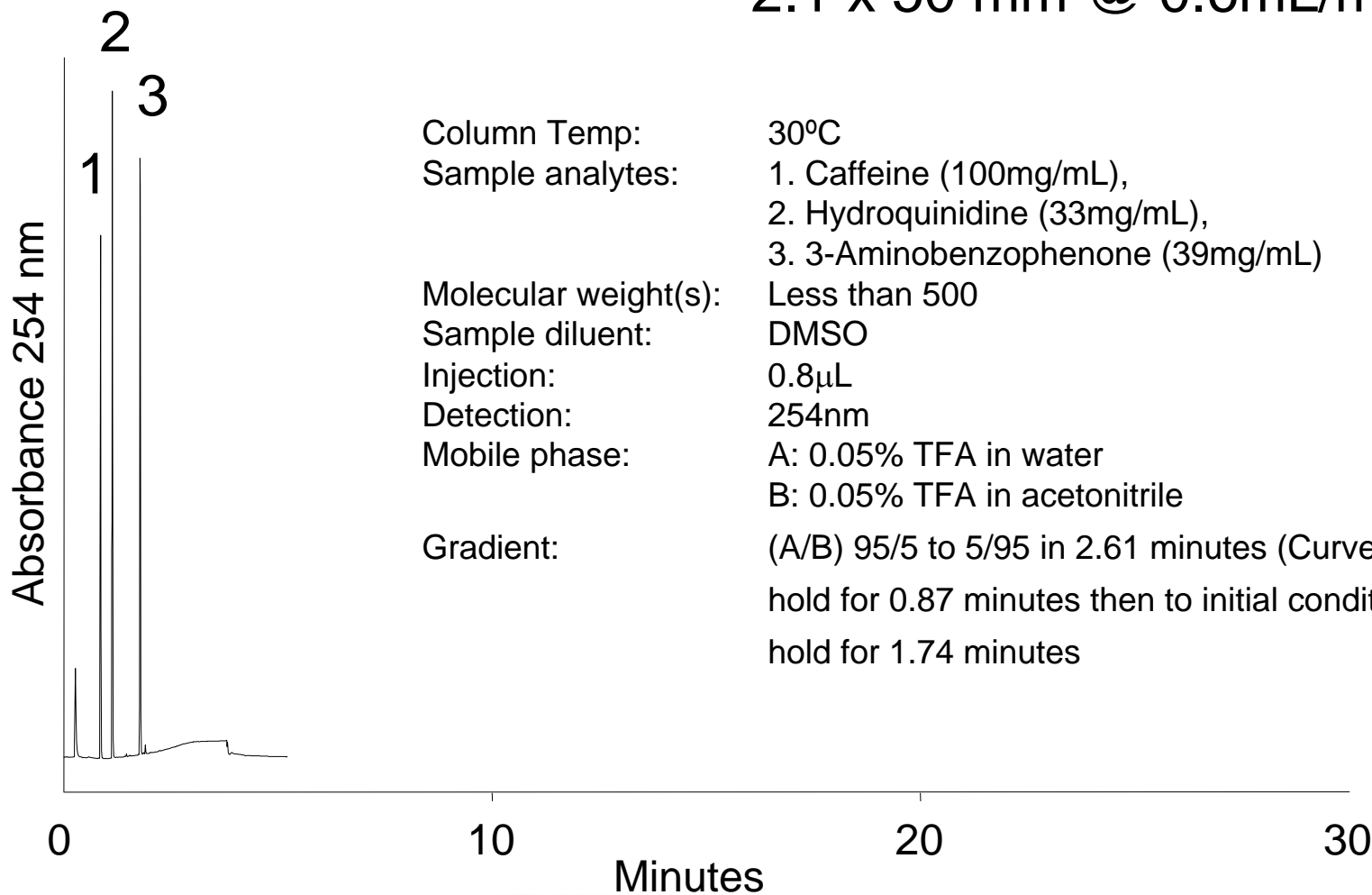
Gradient: (A/B/C) 90/5/5 to 0/95/5 in 15 minutes (Curve 6)
hold for 5 minutes then to initial conditions (curve 11)
hold for 10 minutes

Method Conversion

Scaled VHPLC

Resolution 1,2 = 11
Resolution 2,3 = 26

C18 Column 1.7 μm ,
2.1 x 50 mm @ 0.6mL/min



Column Temp: 30°C
 Sample analytes: 1. Caffeine (100mg/mL),
 2. Hydroquinidine (33mg/mL),
 3. 3-Aminobenzophenone (39mg/mL)
 Molecular weight(s): Less than 500
 Sample diluent: DMSO
 Injection: 0.8 μL
 Detection: 254nm
 Mobile phase: A: 0.05% TFA in water
 B: 0.05% TFA in acetonitrile
 Gradient: (A/B) 95/5 to 5/95 in 2.61 minutes (Curve 6)
 hold for 0.87 minutes then to initial conditions (curve 11)
 hold for 1.74 minutes

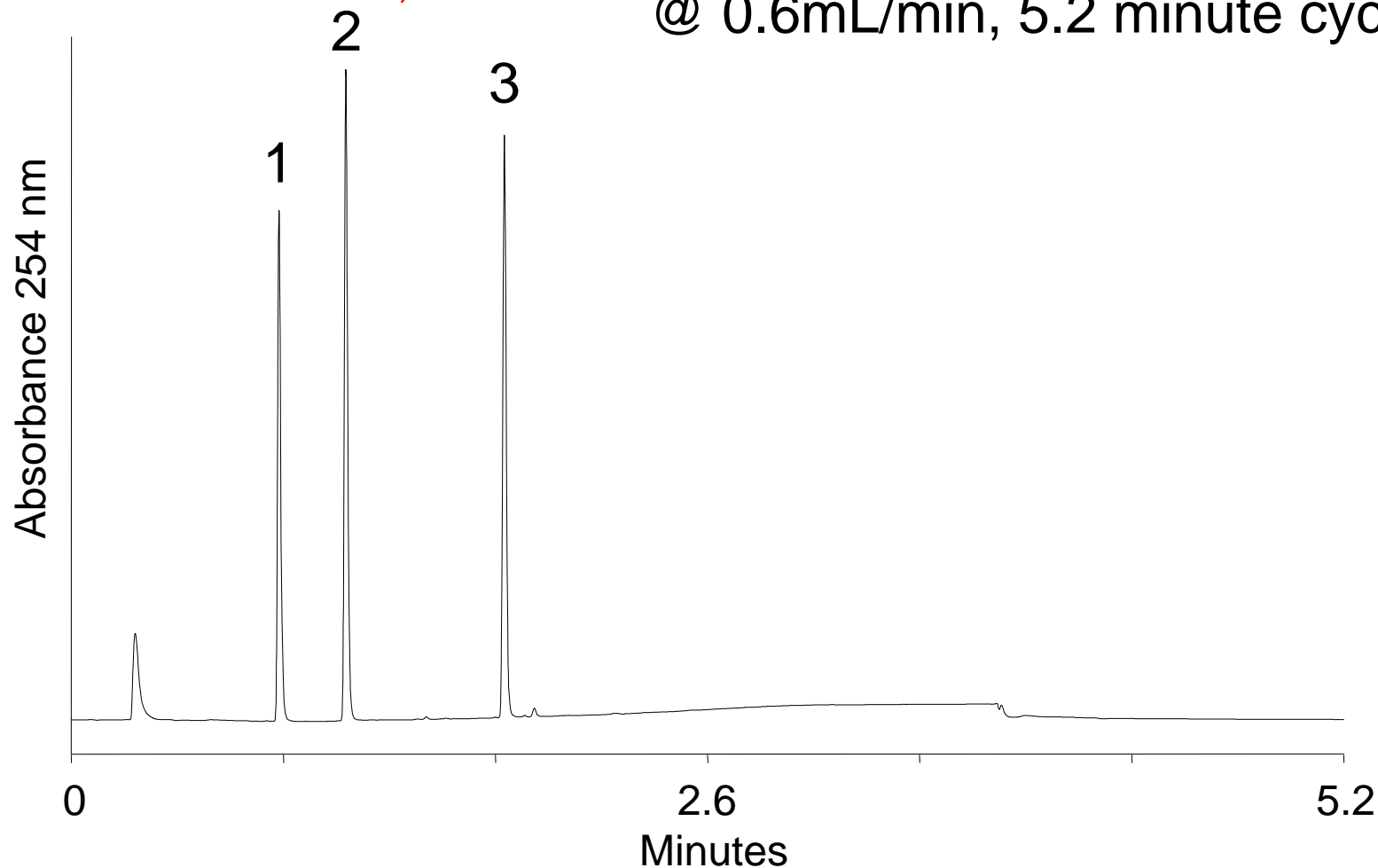
Method Conversion

Scaled VHPLC Magnified

Resolution 1,2 = 11

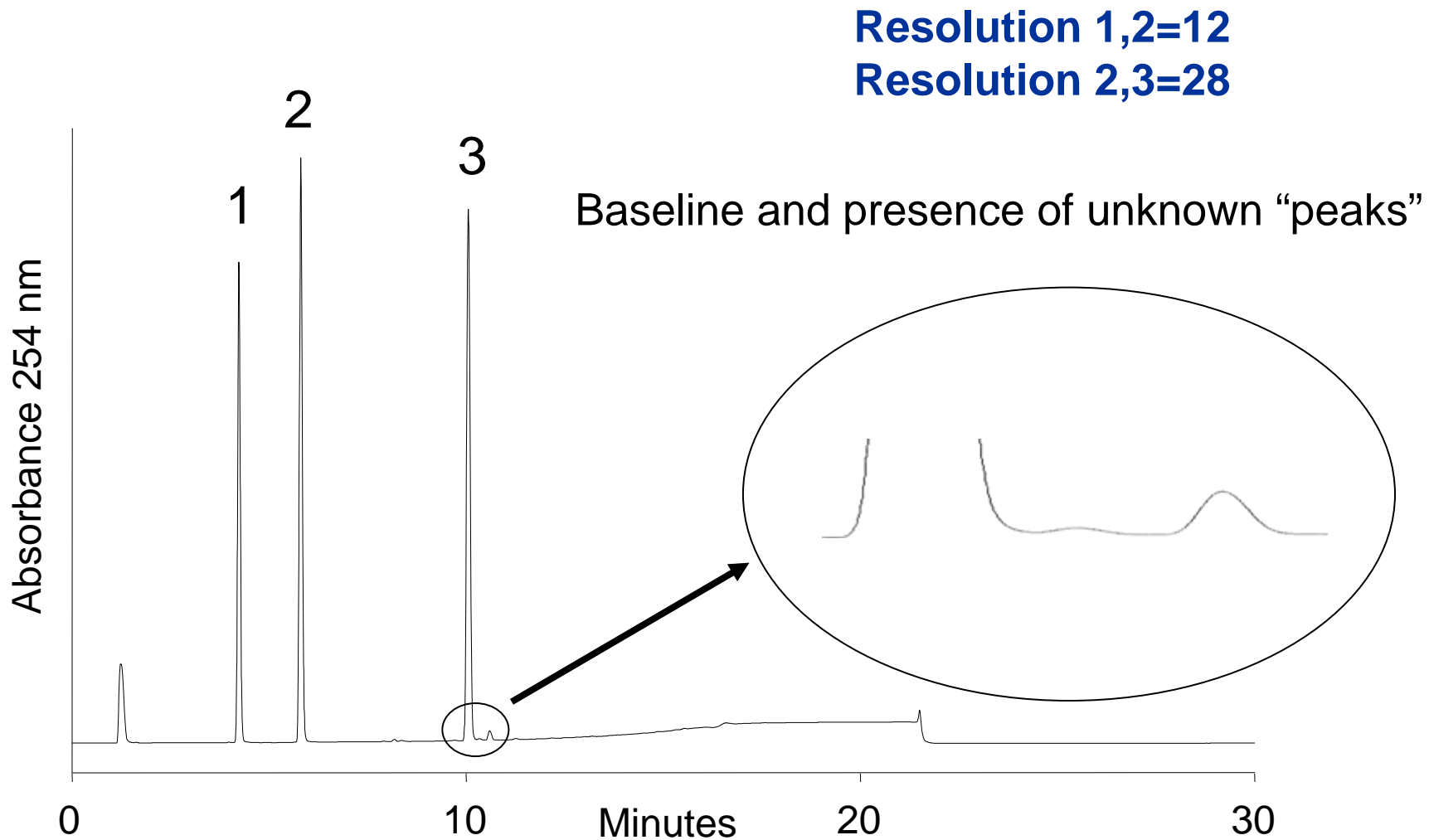
Resolution 2,3 = 26

VHPLC: 2.1 x 50 mm column, 1.7 μ m
@ 0.6mL/min, 5.2 minute cycle time



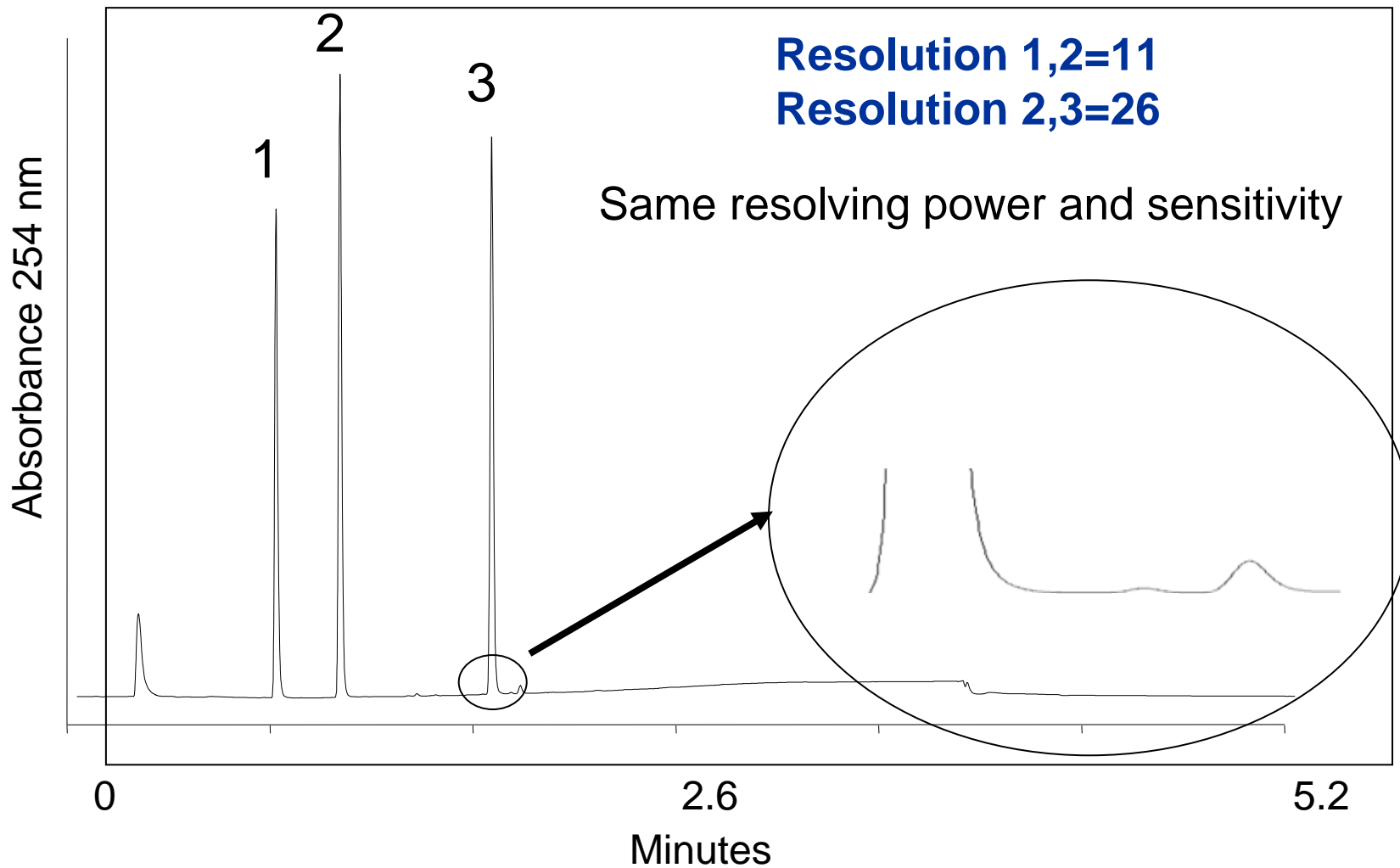
Original HPLC Method

Critical resolution



VHPLC Magnified View

Critical resolution



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Acknowledgements

mswartz@synomicspharma.com

- Colleagues @ Synomics Pharma
 - Mark Emanuele
 - Amber Awad
- Former Colleagues @ Waters
 - Tanya Jenkins
 - Andy Aubin

And:

