

Prodigy System: Overview and Updates

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We Simplify Science



Outline

- General System Overview
- Elevated Temperature Chromatography
- System Updates
- Case Study of Purification Workflow, with a walk-through of the Focused Gradient Calculator

Prodigy Configurations



Overview

- Preparative HPLC system, with a unique integrated heating system
- Temperatures up to 60 - 80 °C (depending on flow rate and configuration)



Standard Configuration

Up to 50 mL/min



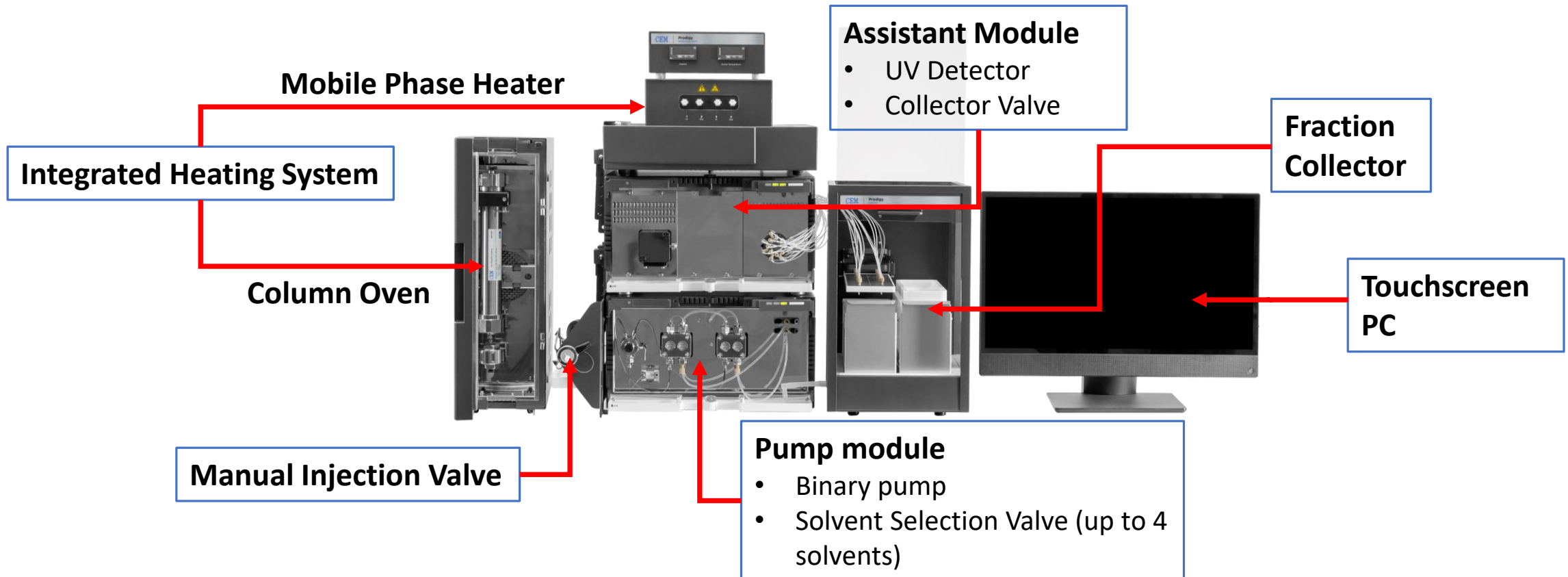
High Flow Configuration

Up to 250 mL/min

Prodigy Overview

Standard Configuration

- Flow rates up to 50 mL/min (max recommended: 40 mL/min)
- 10 – 30 mm diameter columns (19/21.2 mm are ideal) → injections up to ~ 500 mg

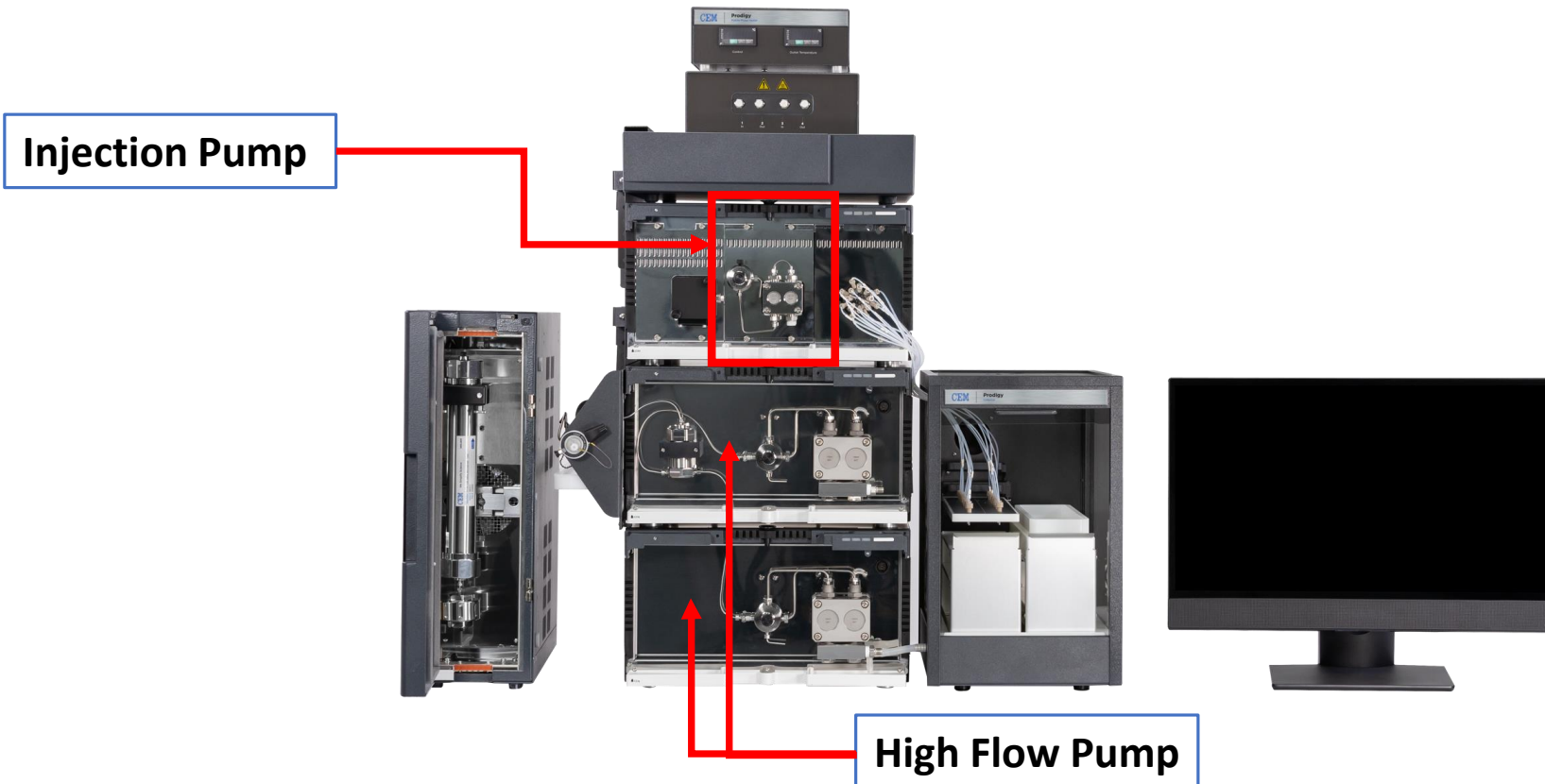


Prodigy Overview

High Flow Configuration



- Flow rates up to 250 mL/min (max recommended: 200 mL/min)
- 19 - 50 mm diameter columns → injections up to ~ 1.5 – 3 g



Why use Elevated Temperatures?

- **Run-to-run reproducibility**
- **Separation performance**
 - Improved solubility of hydrophobic sequences
 - Improved peak shape
 - Mass transfer kinetics
 - Less peak tailing
 - Can affect selectivity
- **Workflow and process considerations**
 - Scale-up from elevated temperature analytical runs
 - Reduced mobile phase viscosity (lower backpressures) enables higher flow rates and shorter methods
 - Reduced fraction volume and number of fractions

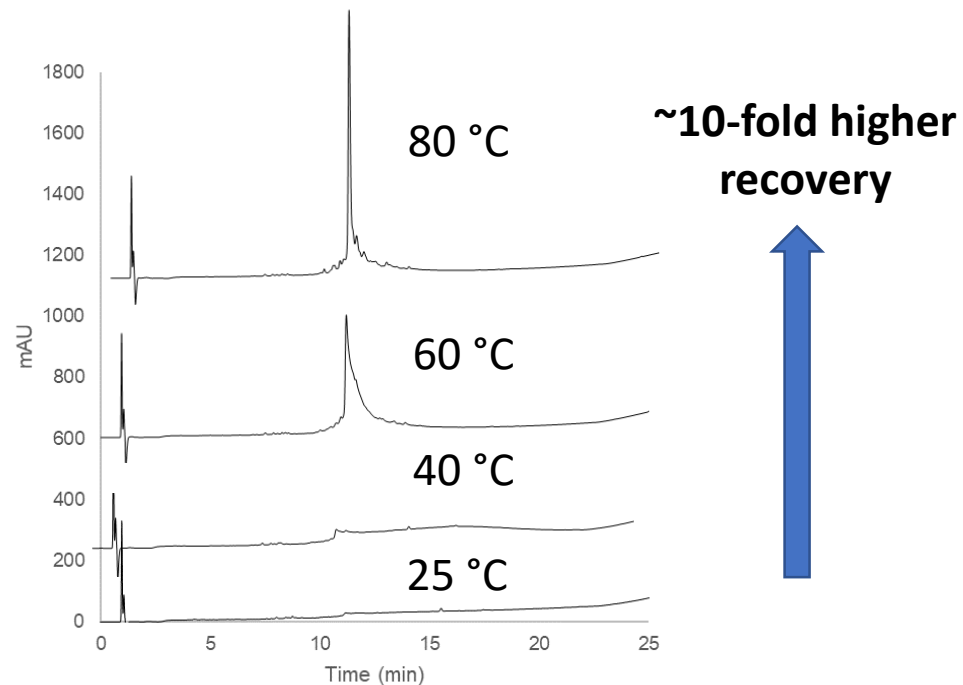
Why use Elevated Temperatures?



Purification of Hydrophobic and Long Sequences

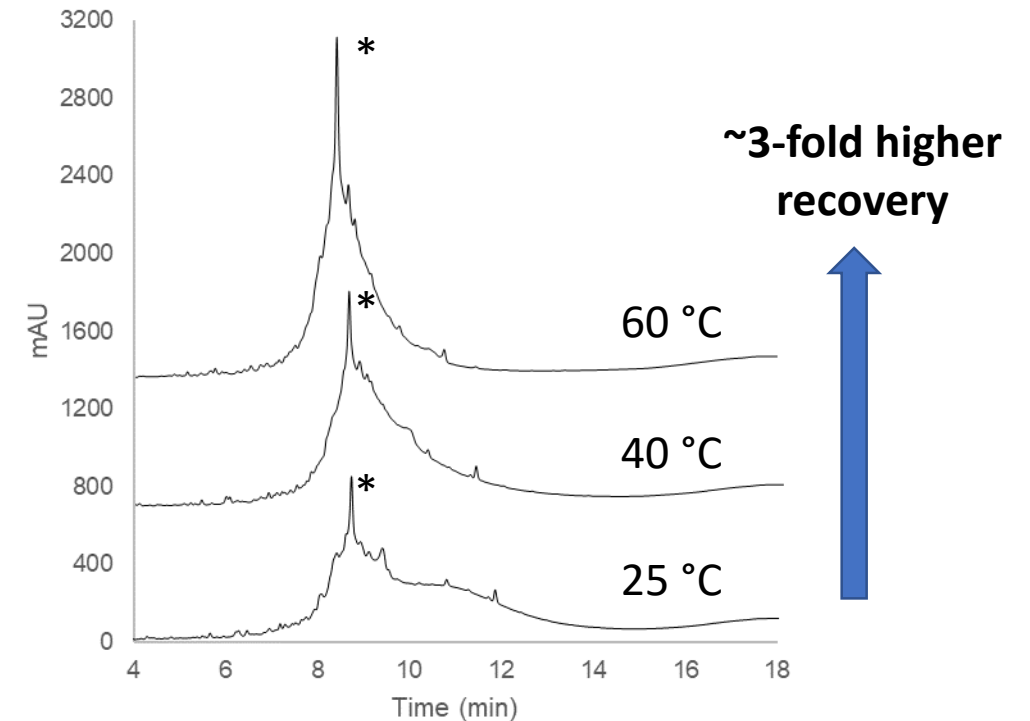
1-42 β -amyloid (42-mer)

- Particularly hydrophobic, and tends to stick to columns
- Low recovery in ambient conditions



Proinsulin (86-mer)

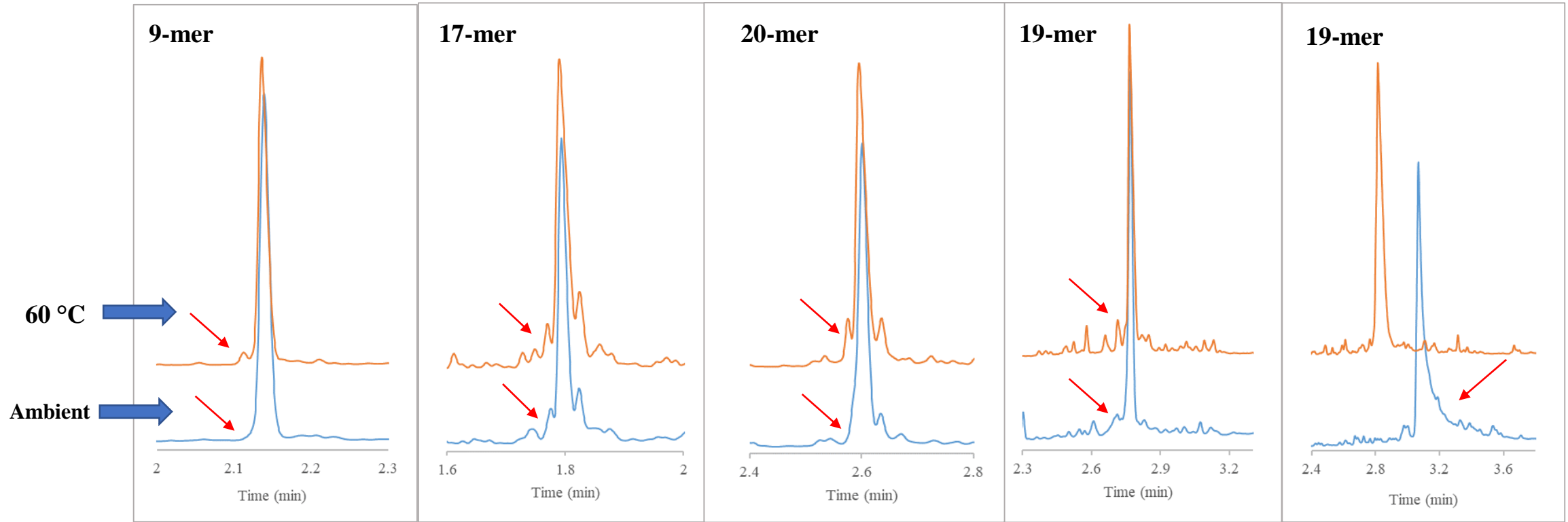
- Long sequence, with complex impurity profile
- Poor solubility



Performance Improvements for Shorter Sequences



Improved Peak Shape and Alternative Selectivity



Neoantigens¹ Case Study: 9 to 20-mer Sequences



Ambient vs 60 °C

| # | Sequence | Crude Purity | Ambient Conditions | | 60 °C | |
|----------------|----------------------|--------------|--------------------|------------|--------------|------------|
| | | | Final Purity | % Recovery | Final Purity | % Recovery |
| 1 | GWVKPIIIGHHAYGDQYRAT | 69 | 93 | 36 | 92 | 42 |
| 2 | TLYEQEIEV | 81 | 94 | 35 | 95 | 54 |
| 3 | HGSRKNITDMVEGAKKANG | 74 | 94 | 41 | 99 | 34 |
| 4 | SLLNQPKAV | 76 | 97 | 63 | 96 | 68 |
| 5 | EDPYLFELPVLKYLDMGTT | 54 | 98 | 70 | 98 | 76 |
| 6 | ALAVLSNYDA | 78 | 95 | 67 | 98 | 74 |
| 7 | TMEDKIYDQQVTKQCLCF | 53 | 91 | 39 | 96 | 60 |
| 8 | YSYPETPLYMQTASTSYE | 55 | 94 | 23 | 95 | 45 |
| 9 | KVGYTERQRWDFLSEASIM | 60 | 91 | 20 | 95 | 41 |
| 10 | RLRMREHMMKNVDTNQD | 70 | 95 | 26 | 94 | 18 |
| 11 | VYEKNGYIYF | 85 | 96 | 67 | 96 | 76 |
| 12 | ALAVLCNYDA | 77 | 96 | 37 | 97 | 63 |
| 13 | ALVPPSKRKMWVSPAeka | 71 | 99 | 21 | 99 | 19 |
| 14 | ISTPTPTIVHPGSLPLHLG | 62 | 94 | 47 | 95 | 58 |
| 15 | IVQENNTPGTYLSSVSARD | 70 | 93 | 32 | 91 | 43 |
| 16 | RFHMKVSVYLLAPLREALS | 55 | 96 | 61 | 96 | 77 |
| 17 | ENLKQNDISAEFTYQTKDA | 72 | 87 | 29 | 95 | 40 |
| 18 | YMMPVNSEV | 74 | 97 | 45 | 98 | 54 |
| 19 | TNDVKTLADLNGVIEEFT | 63 | 71 | 10 | 93 | 27 |
| 20 | SAWLFRMWYIFDHNYLKPL | 44 | 93 | 57 | 95 | 71 |
| Average | | 67 | 93 | 41 | 96 | 52 |

60 °C vs Ambient Conditions

11% increase in % recovery

3% increase in final purity



¹Hilf, N. et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature* 565 (2019), 240-245.

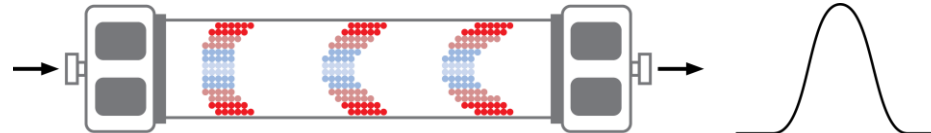
Instrument and Column Considerations



Optimal Operation at Elevated Temperatures

- **Stationary Phase stability***
 - Bonded phase type
 - Base particle type
- **Instrument Considerations**
 - Thermal Mismatch: column vs mobile phase temperature

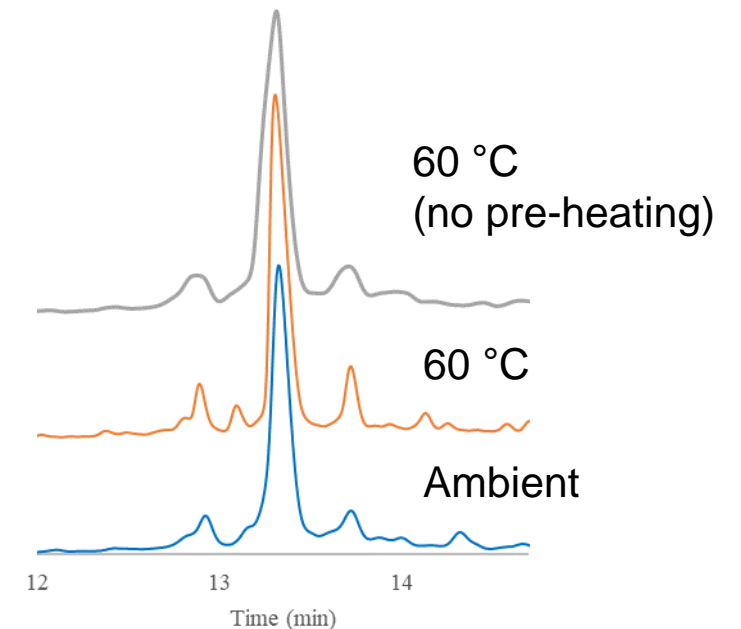
No mobile phase pre-heating



With mobile phase pre-heating



Neoantigen 5 (04-M01)

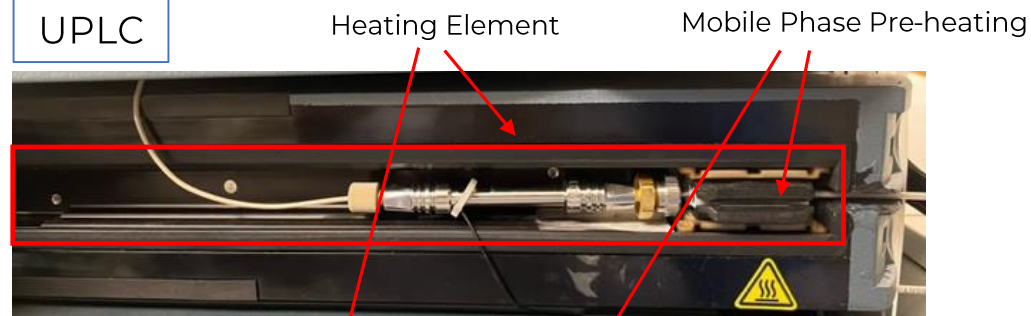


*Teutenberg, T. et al. Temperature and pH-Stability of Commercial Stationary Phases. *J. Sep. Sci.* 32 (2009), 1262-1274.

Heating Approaches: Analytical vs Preparative Scale

Analytical Scale

UPLC



HPLC



Preparative Scale

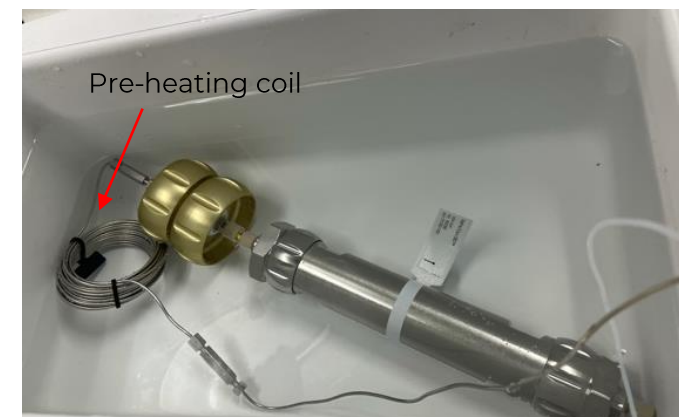


Column Heating Jacket

- Column specific jackets needed
- Separate mobile phase heater required

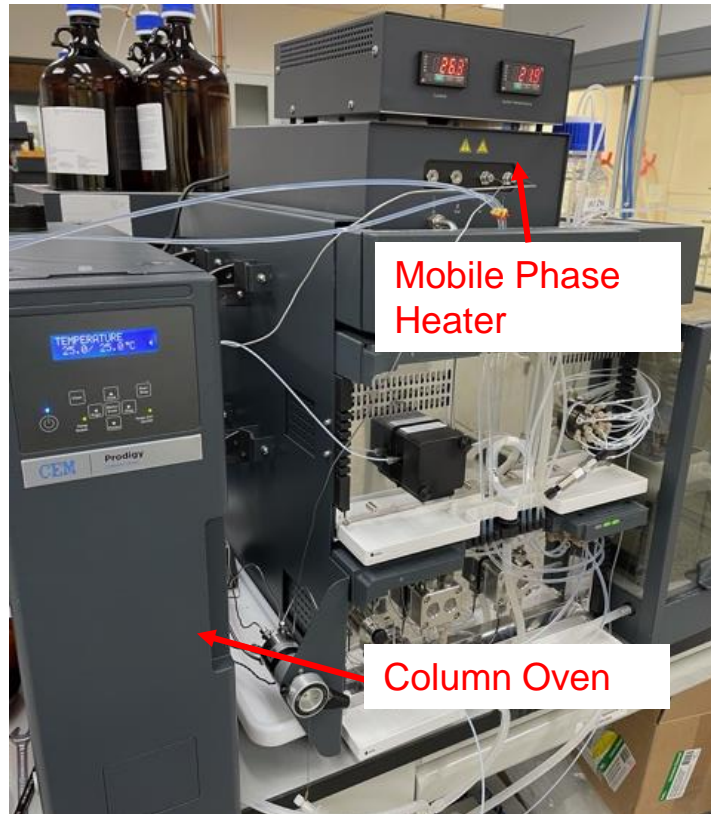
Water Bath

- Long heating and equilibration times
- No Leak detection and potential contamination of the bath



Fully Integrated Heating System

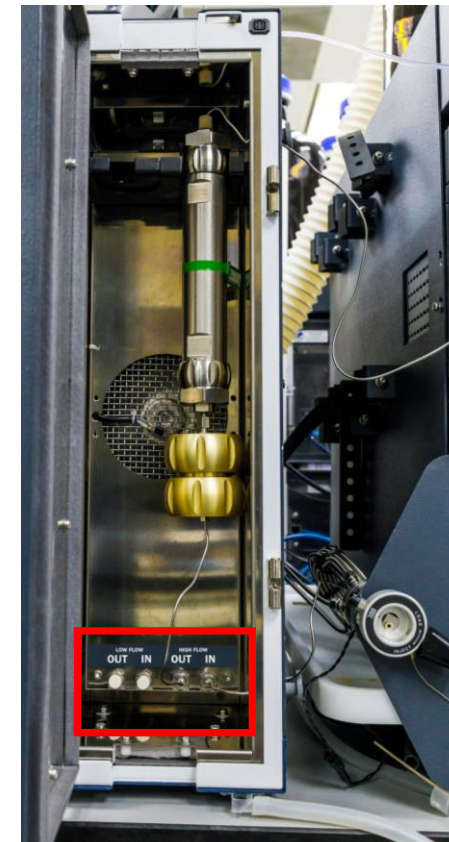
Updated Approach



Updated Approach



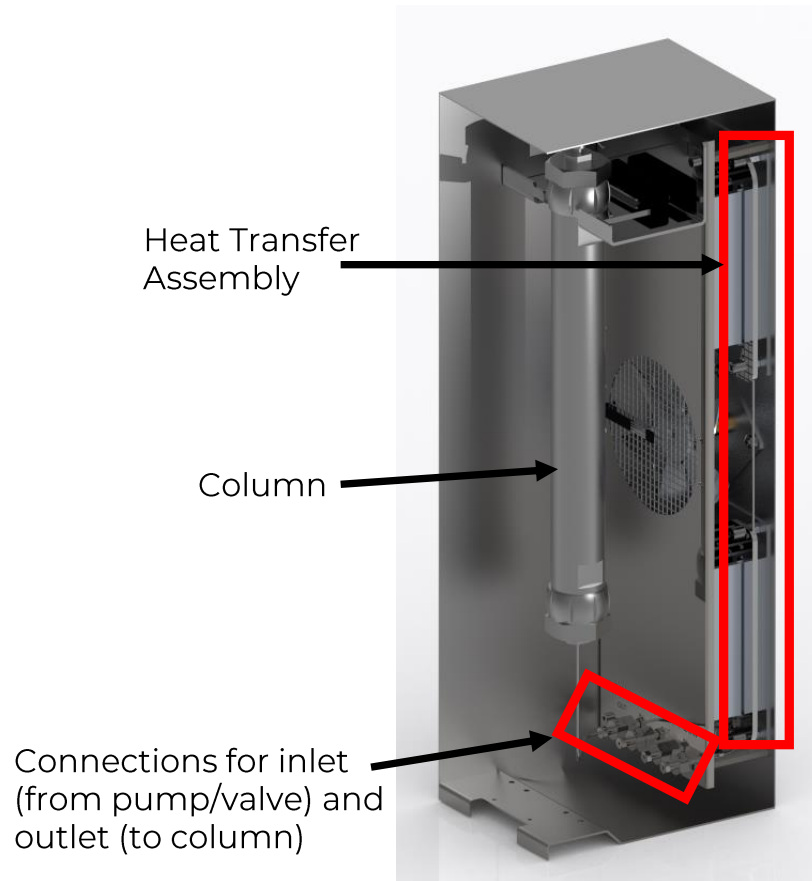
*Expected Release
between August
and October



Fully Integrated Heating System



Overview



- Integrate mobile phase heating into column oven
- Eliminate separate Mobile Phase Heater for standard configuration (reduction in Prodigy system price)
- Active Heating and Cooling
- Does not occupy space containing column
- High performance operation from 25 – 70 °C and flow rates from 4 – 40 mL/min
- Enables complete control of heating system solely through column oven
- Rapid temperature equilibration

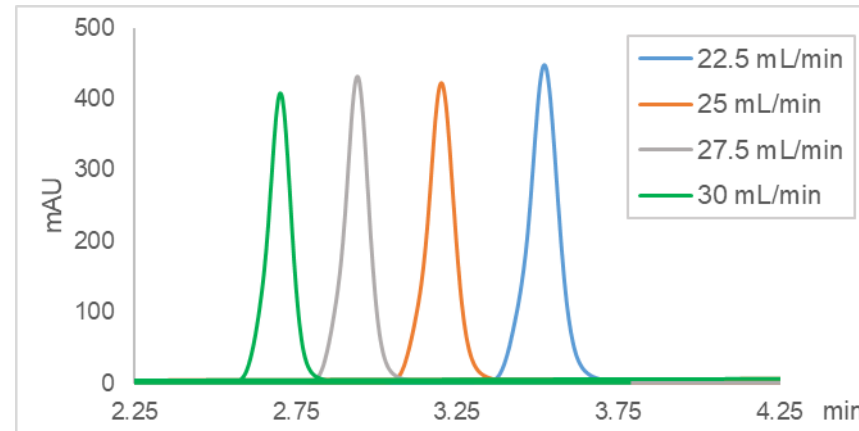
Fully Integrated Heating System



Performance Comparison at 60 °C

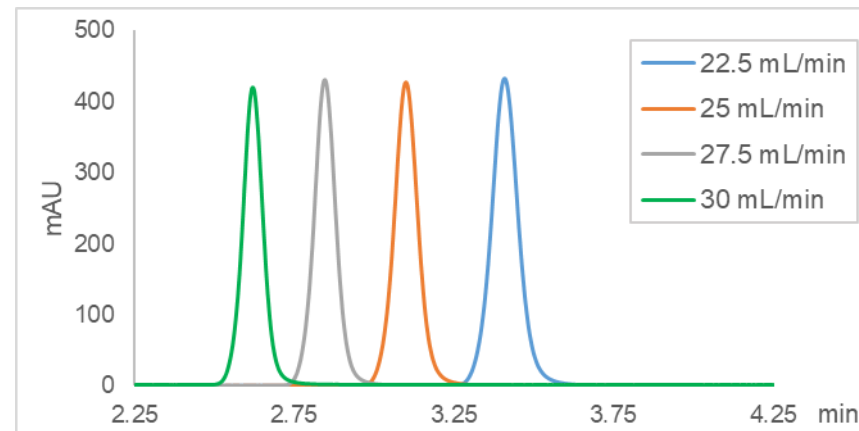
Separate Mobile
Phase Heater

Internal Volume: ~2.5 mL



Fully Integrated
Heating System

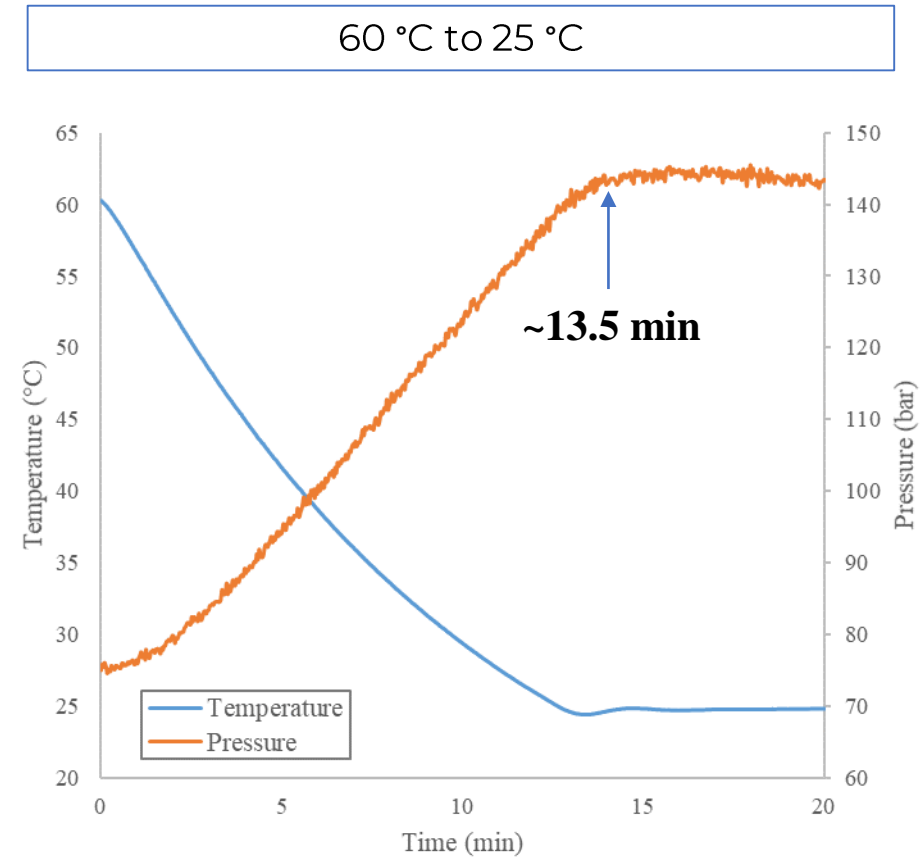
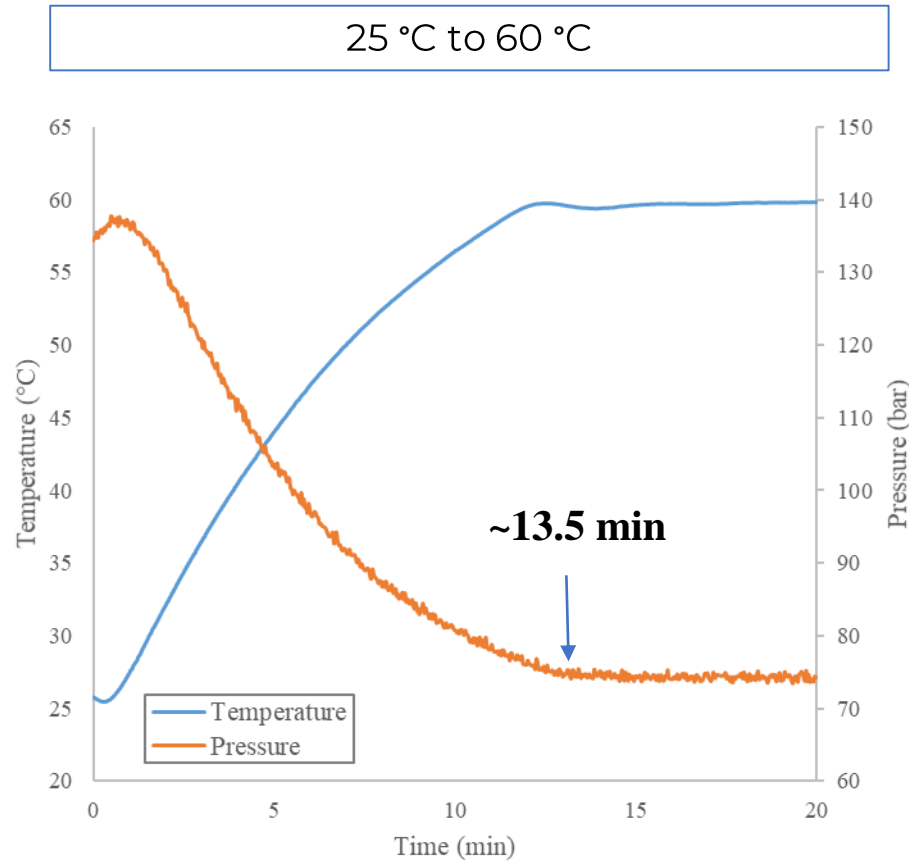
Internal Volume: ~0.5 mL



Fully Integrated Heating System



Temperature Equilibration Time



Dual Column Option (August-October 2024 Release)

Applications

- Columns with different phases/dimensions
- Allow installation of shorter columns for quick scouting runs

Optional upgrade for Column oven

- Dual Column Mount
- Manual Switching Valve and mount
- Interior Oven Panel Kit

Manual Switching Valve (2-position)



Robotic Fraction Collector (August-October 2024 Release)



Updated Approach



Rack Options: 99 x 15 mL tubes or 30 x 50 mL centrifuge tubes

*Enclosure still in development

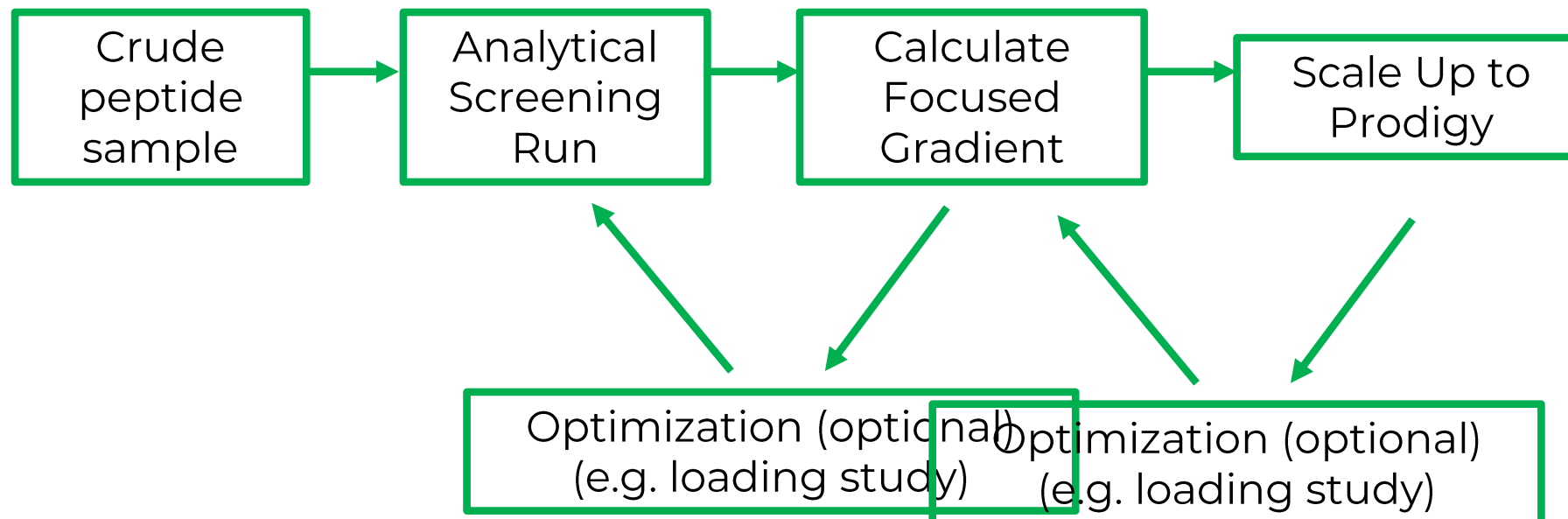
Software Updates



Expected Release in Late June/Early July

- Implementation of full oven control within Prodigy software
- Expanded user access controls (e.g. password strength requirements, automatic logouts after idle period, etc)
- Expanded logging (system logging, audit trail updates)
- Flow rate acceleration, enabling automatic and settable flow ramping (reduce column pressure shocks)
- New colors for live traces, improving color contrast
- Ability to select custom trace colors

Prodigy Purification Workflow



Screening Runs

UPLC



2.1-3 mm ID columns
< 2 μ m particle size

2.1 x 50 mm; 6-8 minute runs

(U)HPLC



3-4.6 mm ID columns
3 – 10 μ m particle size

4.6 x 100/150/250 mm; 20-90 min runs

Preparative HPLC



10 – 50 mm ID columns

Case Study – Neoantigen Peptide (19-mer)

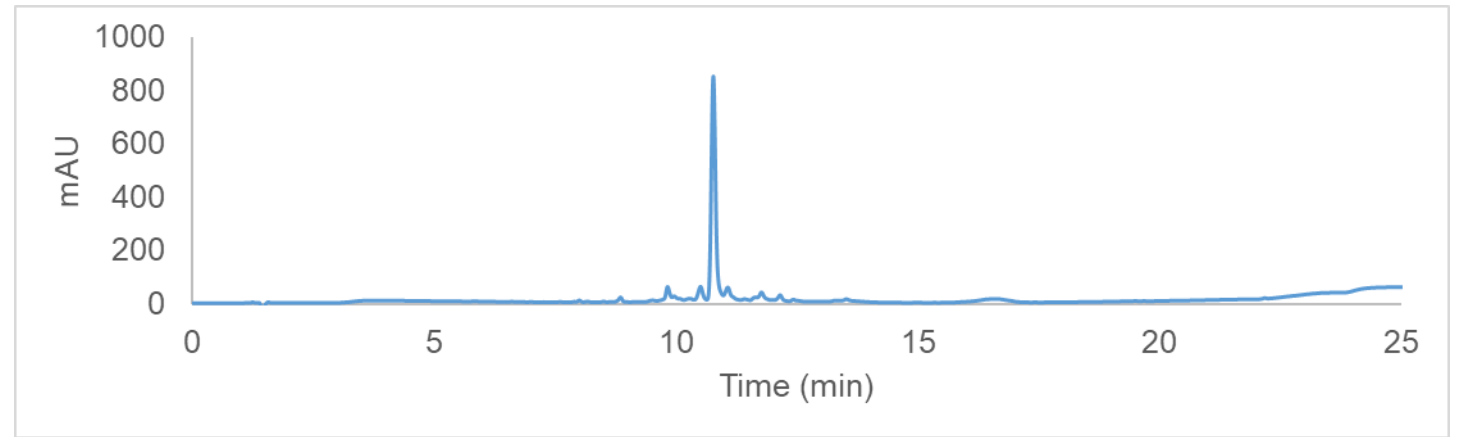


Screening Runs

Sample: 5 mg/mL in 95% Water, 5% ACN

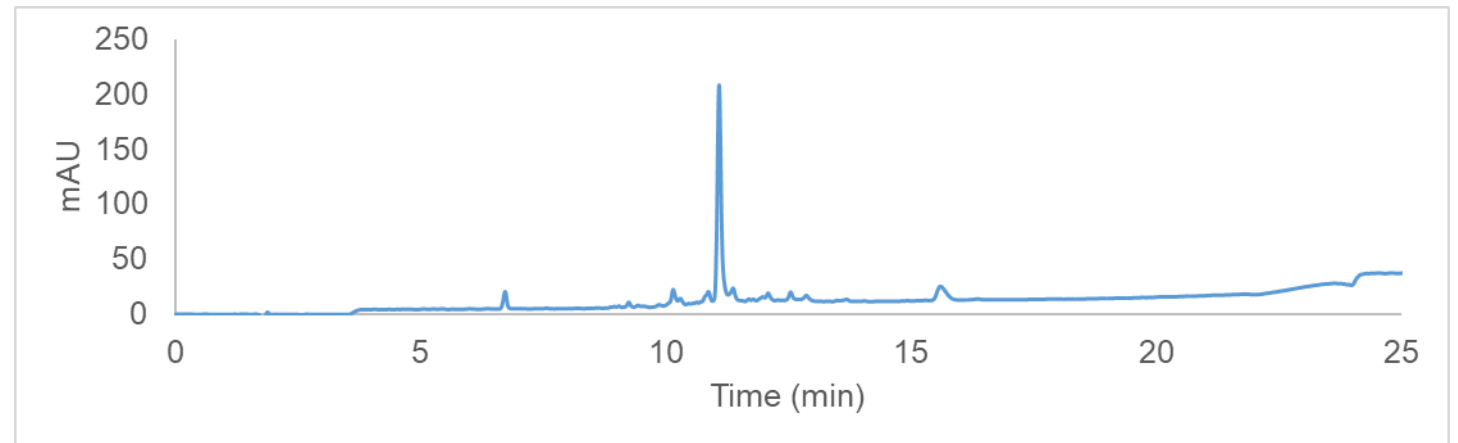
Analytical HPLC System

- Column: 4.6 x 150 mm C18, 5 μ m
- Flow Rate: 1.3 mL/min
- Temperature: **40°C**
- Method: 5-70% ACN (0.1% TFA) over 19 min
- Injection Volume: 15 μ L



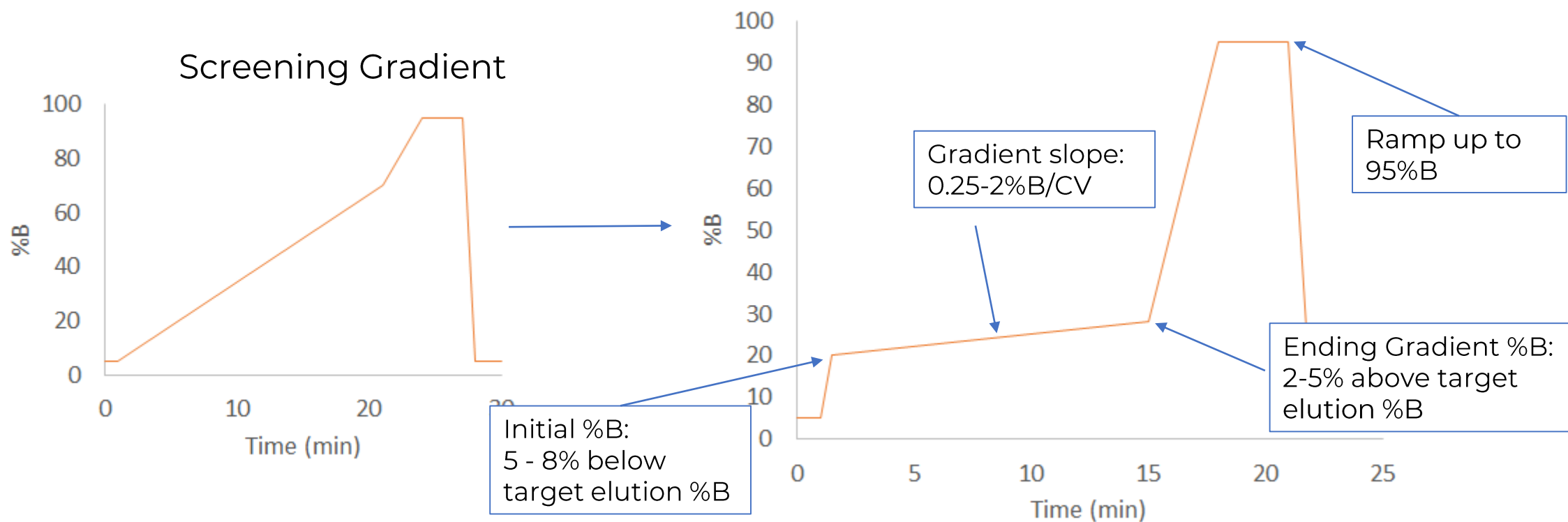
Prodigy System

- Column: 21.2 x 150 mm C18, 5 μ m
- Flow Rate: 27.5 mL/min
- Temperature: **40°C**
- Method: 5-70% ACN (0.1% TFA) over 19 min
- Injection volume: 300 μ L



Focused Gradients

Create shallow gradient centered around peak of interest to increase resolution without increasing overall run time

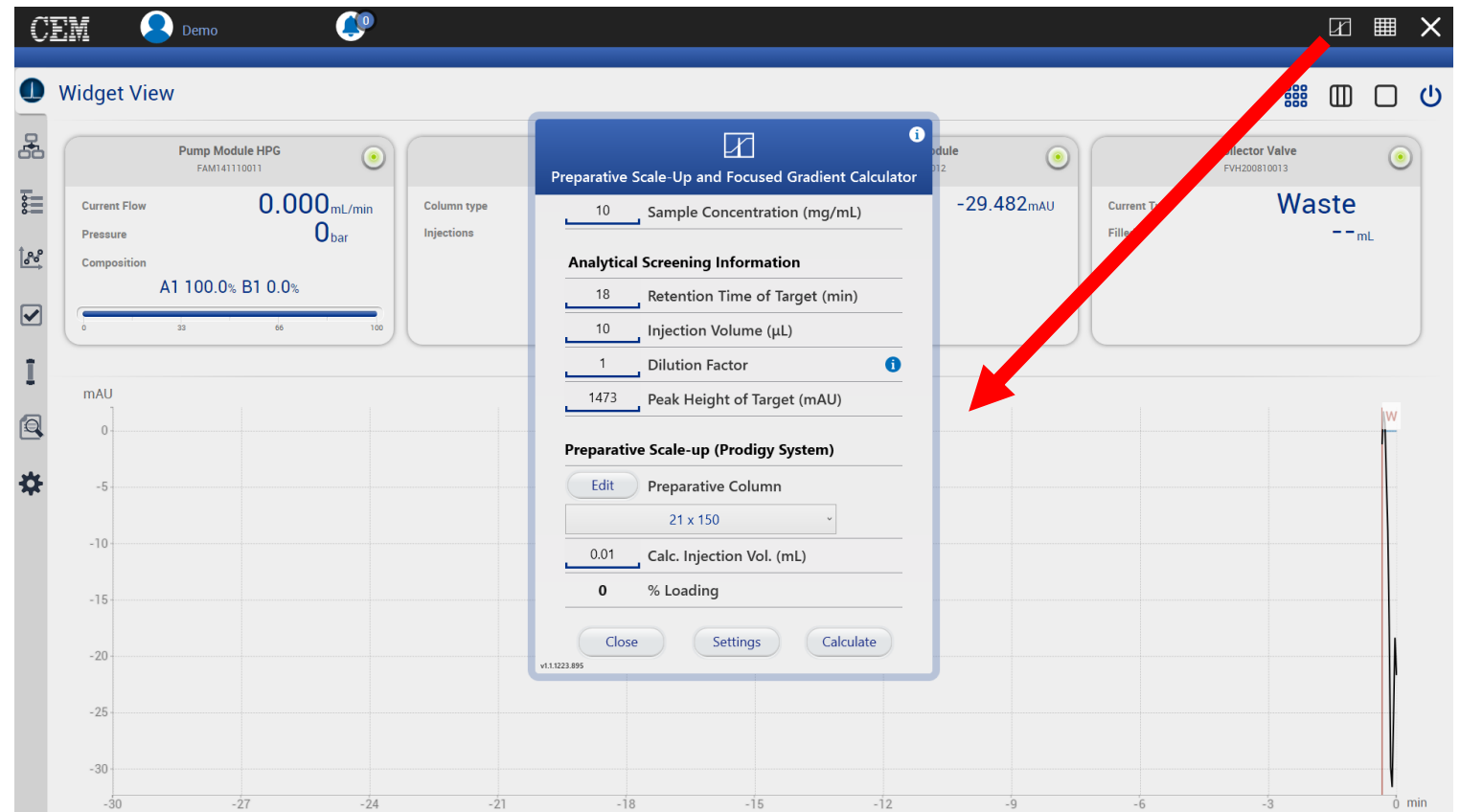


Case Study – Neoantigen Peptide (19-mer)



Determine Focused Gradient

- 1.) Open Prodigy Software
- 2.) Open Focused Gradient Calculator

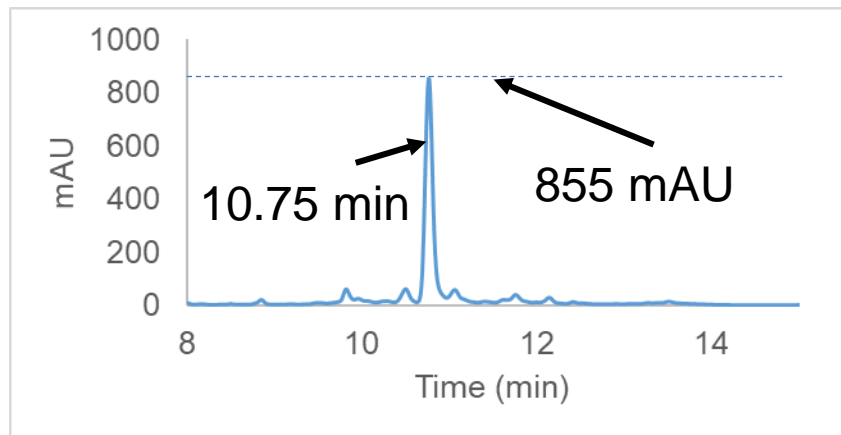


Case Study – Neoantigen Peptide (19-mer)



Determine Focused Gradient

Enter the retention time and peak height from the analytical screening run into the Focused Gradient Calculator



Sample Concentration →
Retention Time →
Injection Volume →
Dilution Factor →
Peak Height →

Choose Prep Column →
Desired Prep Injection Volume →

The calculator interface includes the following fields and controls:

- Sample Concentration (mg/mL): 5
- Analytical Screening Information:
 - Retention Time of Target (min): 10.75
 - Injection Volume (μL): 15
 - Dilution Factor: 1
 - Peak Height of Target (mAU): 855
- Preparative Scale-up (Prodigy System):
 - Preparative Column: 21 x 150
 - User Injection Vol. (mL): 4
 - % Loading: 0.07
- Buttons: Close, Settings, Calculate
- Version: v1.1.1087.1212

Case Study – Neoantigen Peptide (19-mer)



Determine Focused Gradient

Preparative Scale-Up and Focused Gradient Calculator

5 Sample Concentration (mg/mL)

Analytical Screening Information

10.75 Retention Time of Target (min)

15 Injection Volume (µL)

1 Dilution Factor

855 Peak Height of Target (mAU)

Preparative Scale-up (Prodigy System)

Edit Preparative Column
21 x 150

4 User Injection Vol. (mL)

0.07 % Loading

Close Settings **Calculate**

v1.1.1087.1212



Calculated Focused Gradient Method

27.6 Suggested flow rate

8 Calc. Duration (min.)

23.8 Beginning %B

35.8 Ending %B

0.90 Isocratic Hold (min.)

196 UV Trigger Threshold (mAU)

Estimated Solvent Consumption

182 Eluent A (mL)

238 Eluent B (mL)

Done Re-Calculate

Case Study – Neoantigen Peptide (19-mer)

Focused Gradient Run



The screenshot displays the CEM software interface for configuring a method. The main window is titled "Methods & Sequences" and shows a list of methods on the left, including "purge_fraction_collection" and "8minGrad". The right side of the interface is divided into several sections: "Variables", "Start Options", and "Peak Recognition".

The "Variables" section includes the following parameters:

- Flow Rate: 27.6 mL/min
- Gradient A B: A 76.2%, B 23.8%
- GradStart: A 64.2%, B 35.8%
- Collect to Next: Start: 196 Units, Stop: 196 Units

The "Start Options" section shows the sample ID as "NeoantigenPeptide_5mgml_4n" and the column as "21.2 x 150 (1234)".

A "Calculated Focused Gradient Method" dialog box is open in the foreground, displaying the following parameters:

| Parameter | Value |
|----------------------------|-------|
| Suggested flow rate | 27.6 |
| Calc. Duration (min.) | 8 |
| Beginning %B | 23.8 |
| Ending %B | 35.8 |
| Isocratic Hold (min.) | 0.90 |
| UV Trigger Threshold (mAU) | 196 |

The "Estimated Solvent Consumption" section shows:

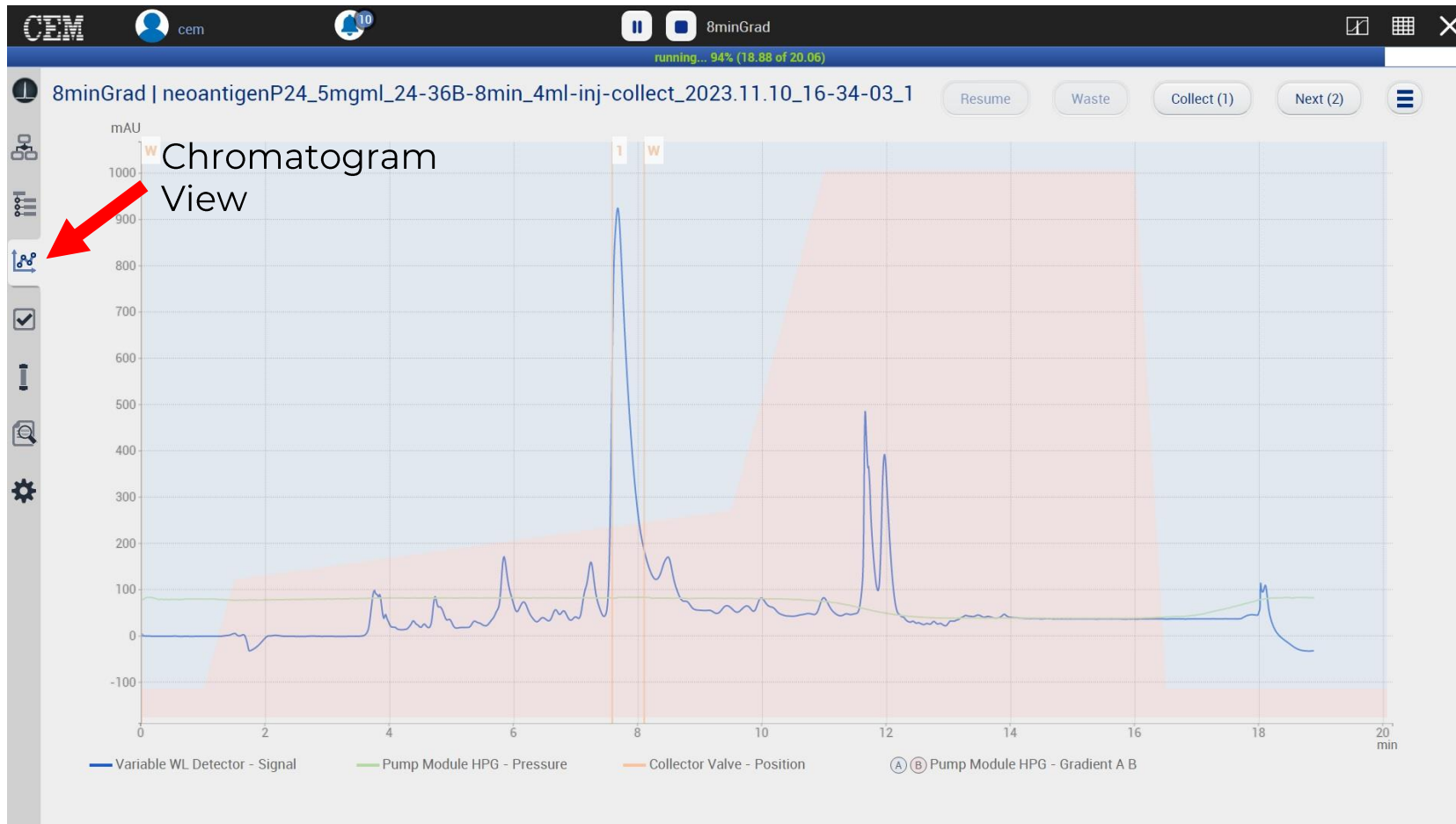
| Eluent | Volume (mL) |
|----------|-------------|
| Eluent A | 182 |
| Eluent B | 238 |

The "Peak Recognition" section shows a UV Threshold of 196 units and a peak at 21.799 min.

Red boxes highlight the "Start" button in the top right corner, the "Calculated Focused Gradient Method" dialog box, and the "Variables" section. A red arrow points from the "Suggested flow rate" value in the dialog box to the "Flow Rate" value in the "Variables" section.

Case Study – Neoantigen Peptide (19-mer)

Focused Gradient Run



Focused Gradient Calculator

Settings



Preparative Scale-Up and Focused Gradient Calculator

5 Sample Concentration (mg/mL)

Analytical Screening Information

10.75 Retention Time of Target (min)

15 Injection Volume (µL)

1 Dilution Factor

855 Peak Height of Target (mAU)

Preparative Scale-up (Prodigy System)

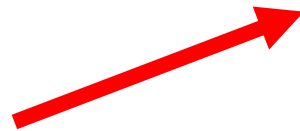
21 x 150 Preparative Column

4 User Injection Vol. (mL)

0.07 % Loading

Close **Settings** Calculate

v1.1.1087.1212



Settings

Analytical System

Separate Analytical System

0.38 Dwell Volume (mL)

4p6x150 Scouting Column

10-30B_15mlmin_20min Scouting Method

Prodigy System

20.7 Dwell Volume (mL)

Enable AutoThreshold Calculation

10 Min. Threshold

25 Peak Height %

2000 Max. Threshold

4p6x150to21x150_27mlmin AutoThreshold Calibration

Focused Gradient Preferences

8 Starting %B Offset

4 Ending %B Offset

Crude Purity

High Purity

Default

Low Purity

High Purity

Cancel

Improved Performance using Elevated Temperatures

- **Faster**
 - Ability to use higher flow rates, leading to reduced run times
 - Can often use shorter columns due to higher resolution
- **More Efficient**
 - Directly scale-up from heated analytical runs
 - Higher recovery (and often higher purity) with improved peak shape
 - Fewer fractions and reduced fraction volume



Thanks for Your Attention



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