Preparative Chiral Separations and Scale-Up

From Analytical Method Scouting to Preparative Separation

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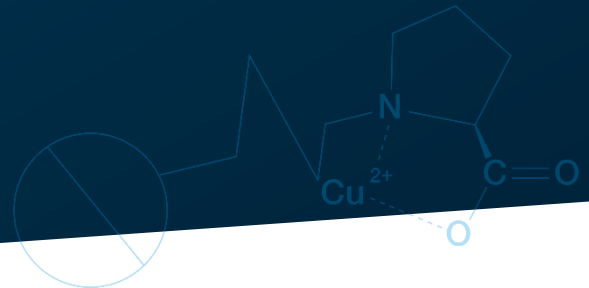
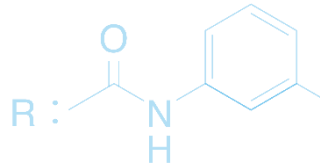
Daicel Chiral Technologies

9 December 2022



Polysaccharide Chiral Selectors



Polysaccharides: CHIRALPAK® (Coated Amylose and Immobilized Columns) and CHIRALCEL® (Coated Cellulose)

Normal, Polar Organic, and Reversed Phases, and SFC

✓First Generation COATED Columns

AD, AS, AY, AZ, OA, OB, OC, OD, OF, OG, OJ, OK, OX, OZ

✓Second Generation IMMOBILIZED COLUMNS **(Compatible with forbidden normal phase solvents)**

IA, IB, IB-N, IC, ID, IE, IF, IG, IH, IJ, IK

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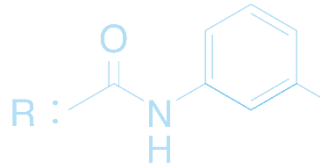
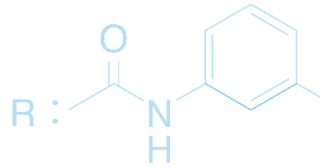
Commercial Applications



- Sertraline (Zoloft) can be resolved under *n*-hexane/2-propanol conditions with coated amylose *tris*(3,5-dimethylphenylcarbamate)
- Escitalopram (Lexapro) produced with coated amylose *tris*(3,5-dimethylphenylcarbamate)
- Levetiracetam (Keppra) produced with coated cellulose *tris*(3,5-dimethylphenylcarbamate)
- Radafaxine, which is a potent metabolite of bupropion
- (*R*)-modafinil or Armodafinil was produced for a time with coated amylose *tris*(3,5-dimethylphenylcarbamate) with 100% methanol
  - Required for toxicological and clinical studies. LC (and eventually SMB) conditions were developed on which resulted in productivity of 0.48 kg of racemate kg^{-1} of CSP and per day
  - In total, more than 600 kg of racemate were processed via this process

Start From the Beginning – Method Screening

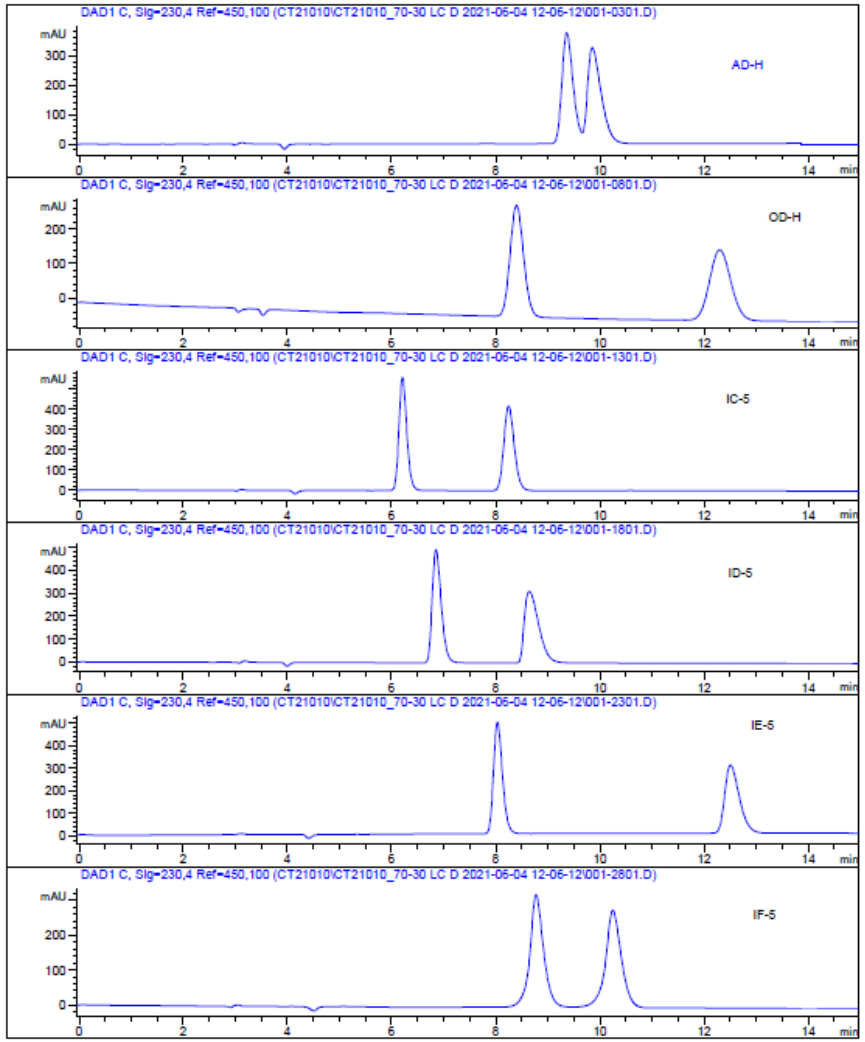


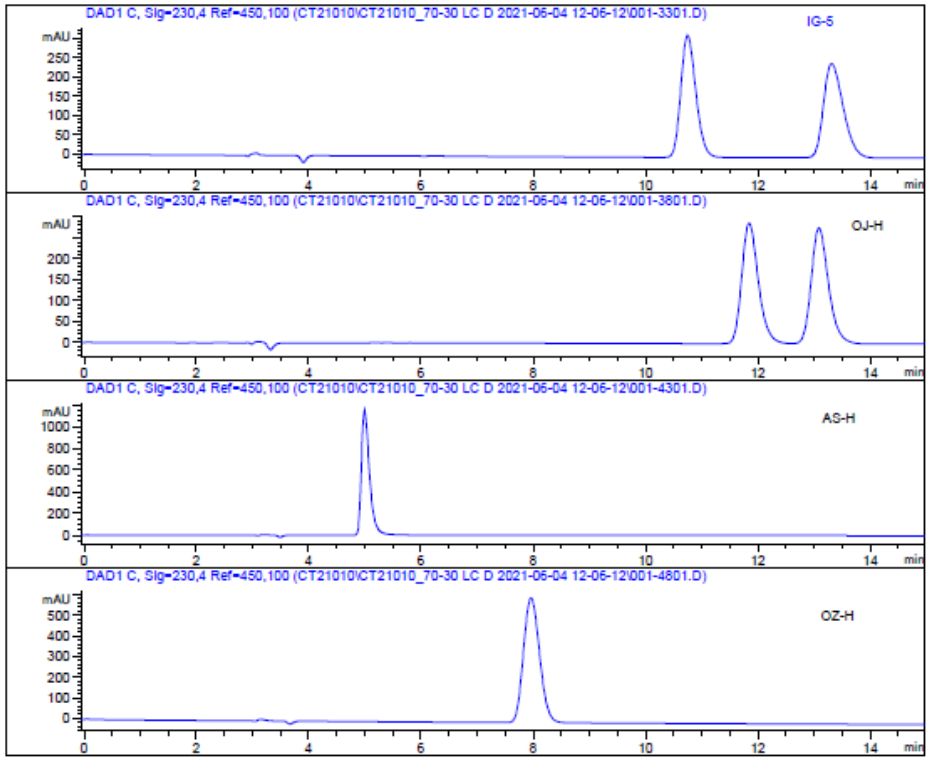
- Screening provides greatest opportunity to find appropriate separation conditions
  - Changes to the phase system have the greatest effect on selectivity
- Limited mobile phases – solubility is very important
  - High % of hexane generally negatively affects solubility, however it might be required based on selectivity
  - If required, often the addition of DCM can help increase solubility (only for immobilized CSPs)
- Screen on 5 µm (4.6x250mm) columns – 5 µm is smallest particle size for preparative separations
  - Easier transferability – more on this to come!

Start From the Beginning – Method Screening

Conditions

Mobile Phase = 70-30-0.1 = Hex-EtOH-DEA
Flow Rate = 1 ml/min
Sample = 1 mg/ml in EtOH





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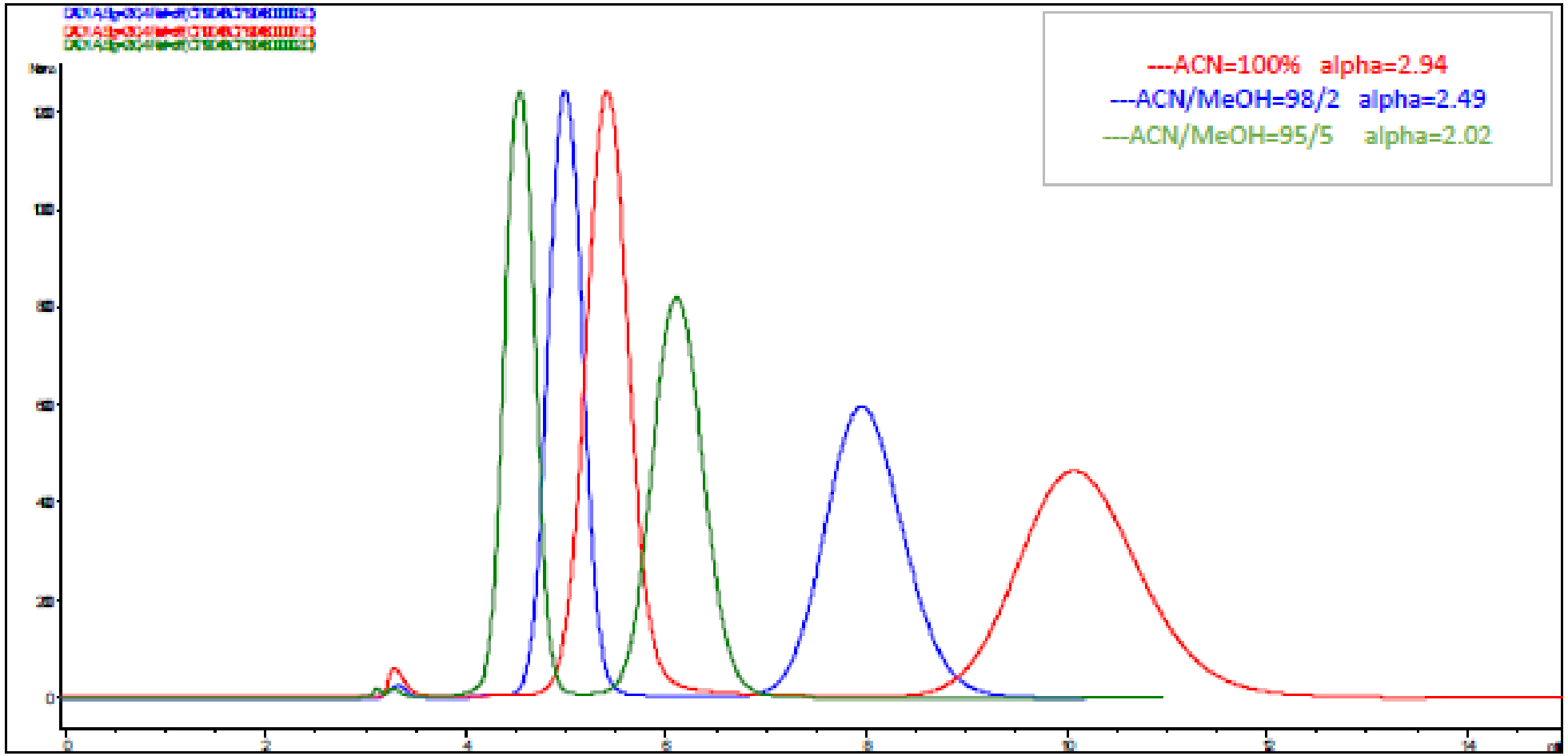
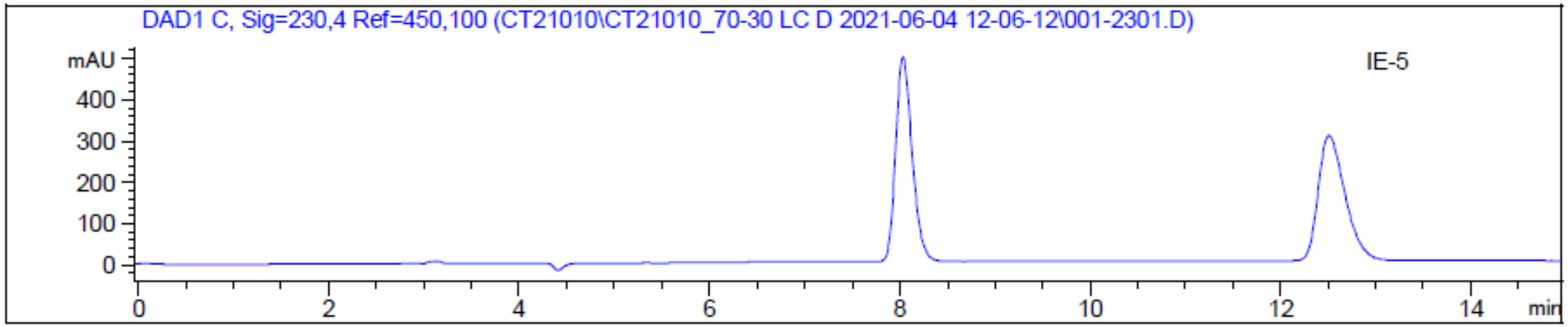


Start From the Beginning – Method Screening

- CHIRALPAK® IE-5 provided best selectivity



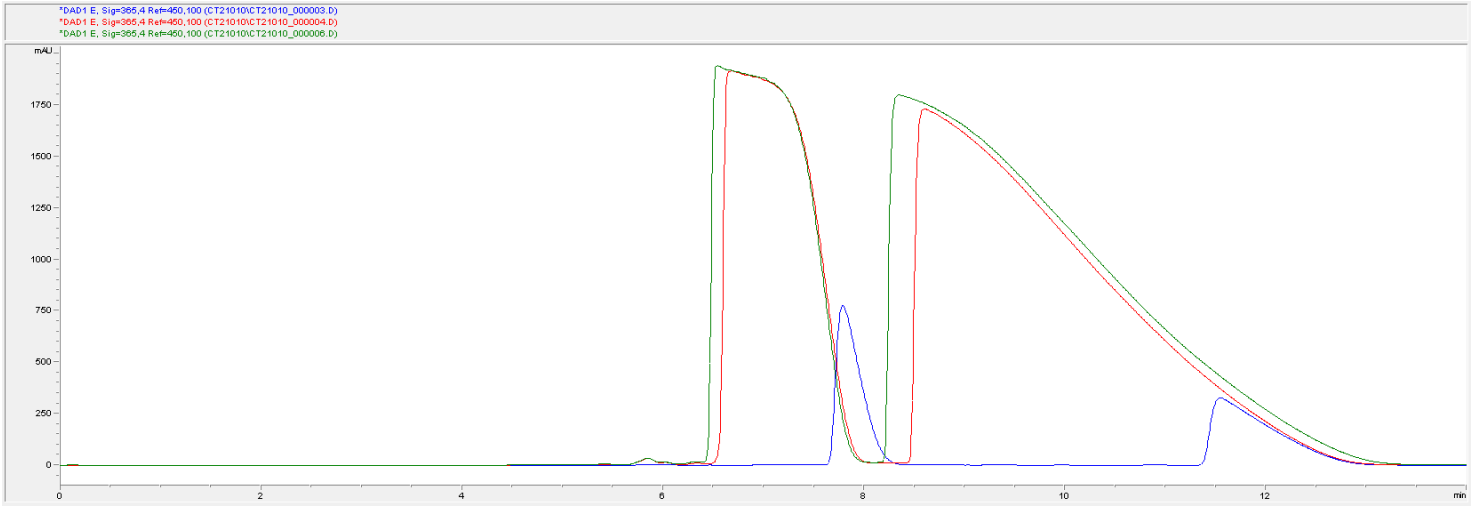
- Need to check solubility and loading to determine productivity

Solubility and Loading

- Solubility of compound in 70-30-0.1 = Hex-EtOH-DEA was 65.20 mg/ml

- Loading is performed using the “touching-band” approach
  - Using a concentrated sample, make increasingly larger injection volumes until the back of Peak 1 touches the front of Peak 2.
  - Can increase the wavelength of detection to load more material on the column – prevents the detector from being swamped

<u>Loading Injections</u>
Blue = 5 µl injection of 65.20 mg/ml
Red = 100 µl injection of 65.20 mg/ml
Green = 125 µl injection of 65.20 mg/ml



Solubility and Loading

- If we don’t look at a higher wavelength, we might falsely believe we’re overloaded

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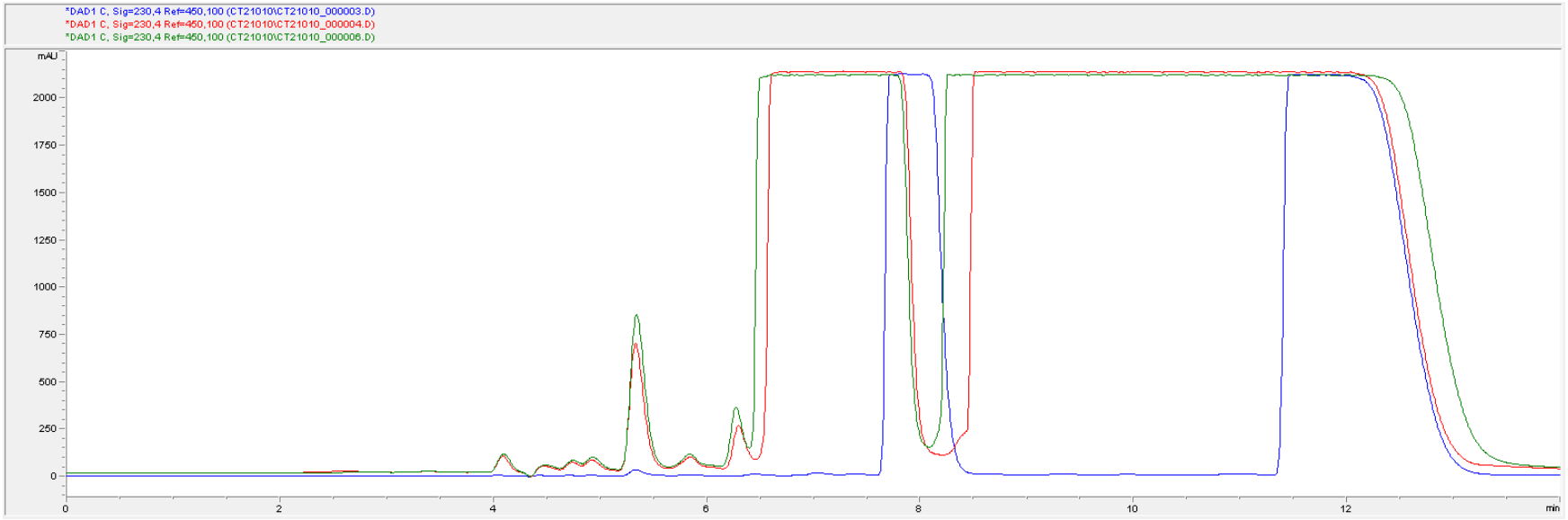
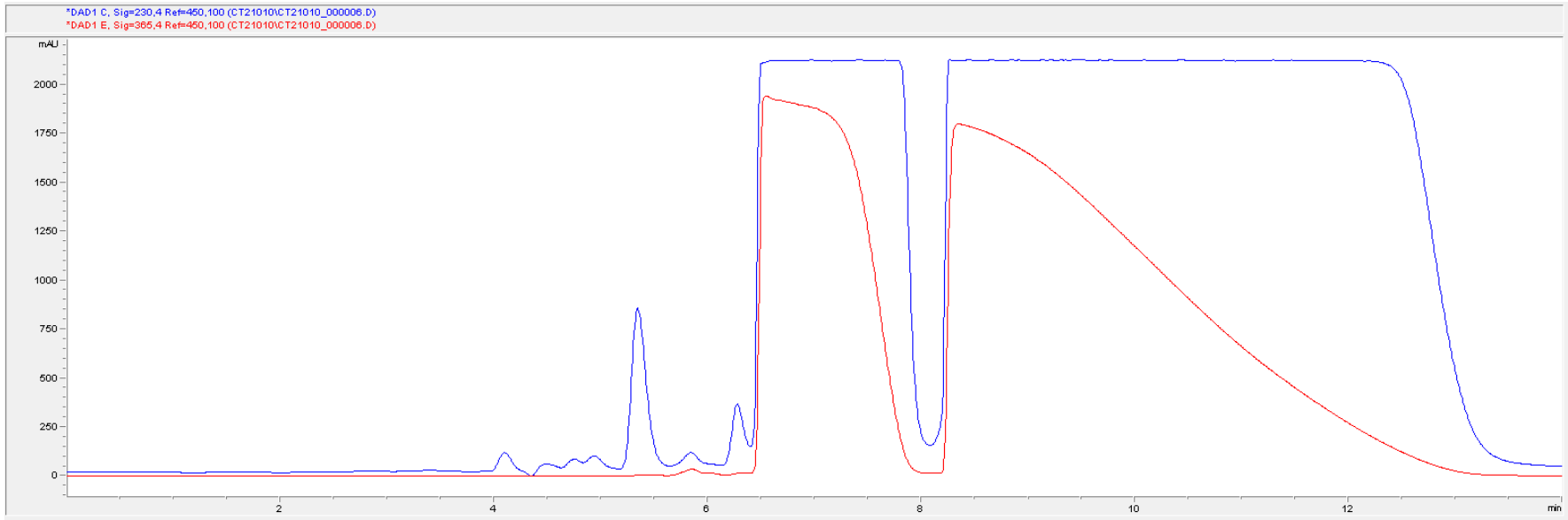


Solubility and Loading

- If we don’t look at a higher wavelength, we might falsely believe we’re overloaded

Loading Injections

Blue = 125 µl injection at 230 nm
Red = 125 µl injection at 365 nm



Productivity

- With solubility, injection volume, and cycle time, we can calculate productivity

CT	21010		
		Customer	
		Sample name	
		Separation Scale	
		Target peak	

Solubility

MP Composition

Hex	EtOH	DEA	
70.00	30.00	0.10	

Weight, mg	32.60
Volume, ml	0.50

Loading

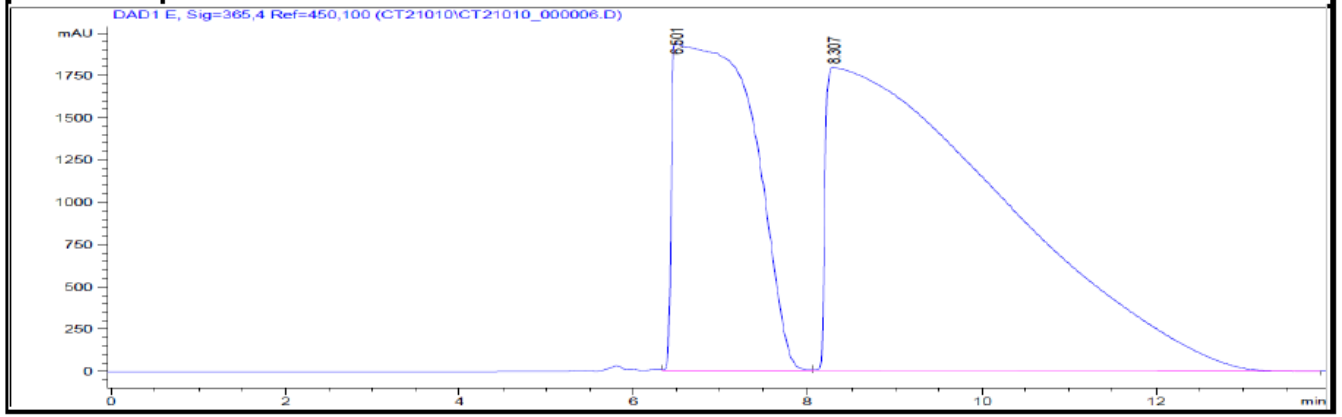
CSP	Particle Size, µm	Column Length, mm	Column ID, mm
IE	5	250	4.6

Cycle Start, min	6.20
Cycle End, min	13.50

	Cycle Time, min	7.3
Injection Volume,	µl	125.00
	Sample Concentration, mg/ml	65.2

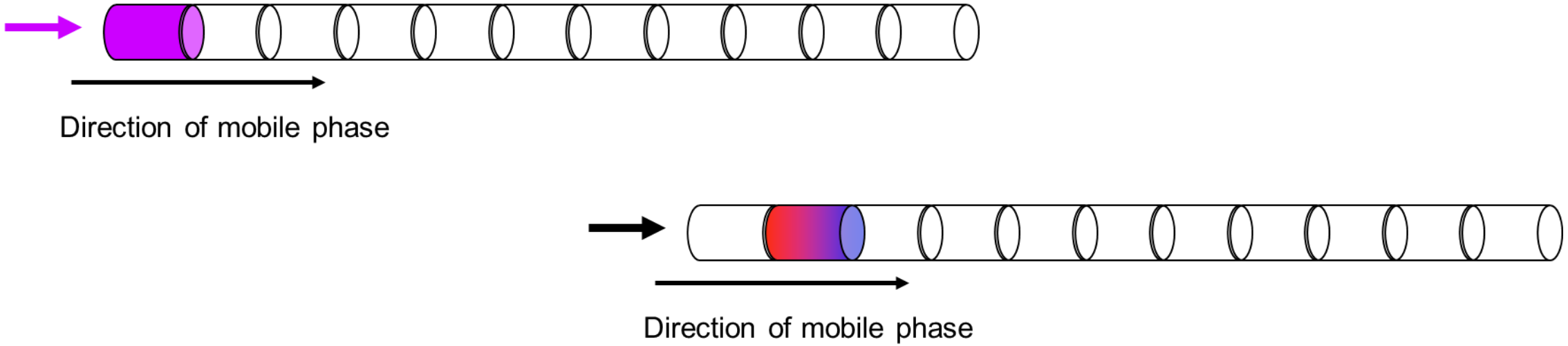
Solubility, mg/ml	65.20	Productivity, mg/hr	66.99

Comments: Solubility could be higher



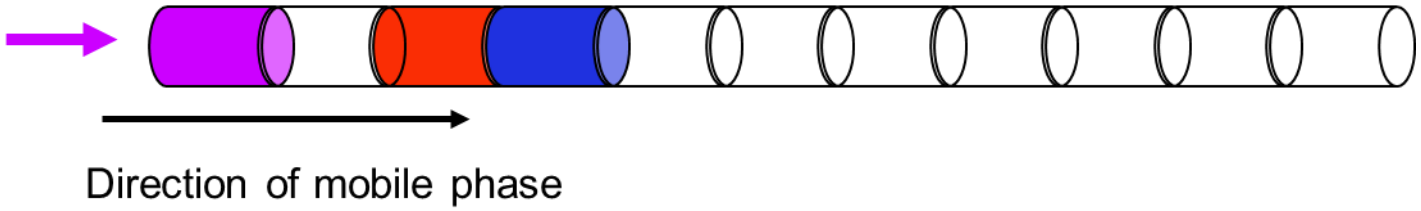
Stacked Injections

First injection

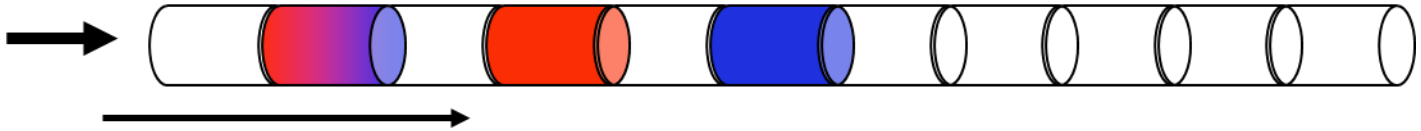


Stacked Injections

Second injection



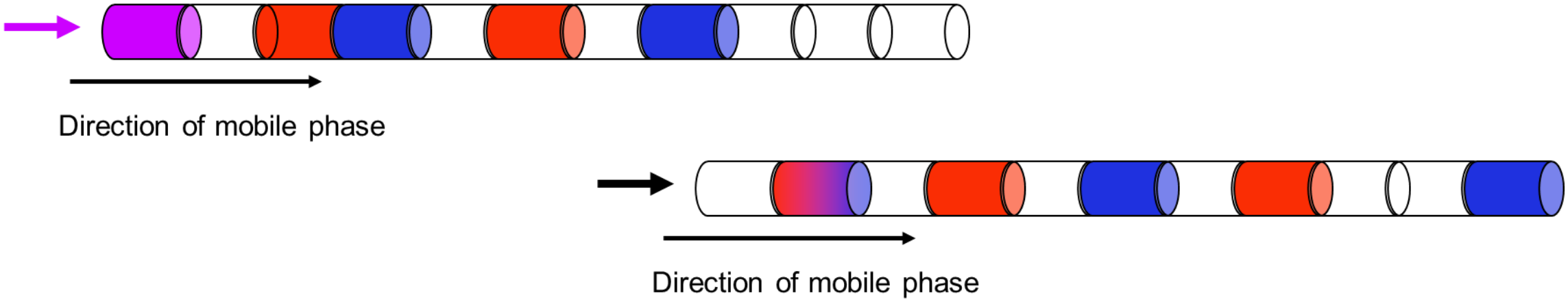
Direction of mobile phase



Direction of mobile phase

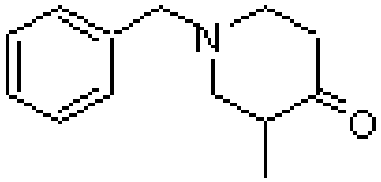
Stacked Injections

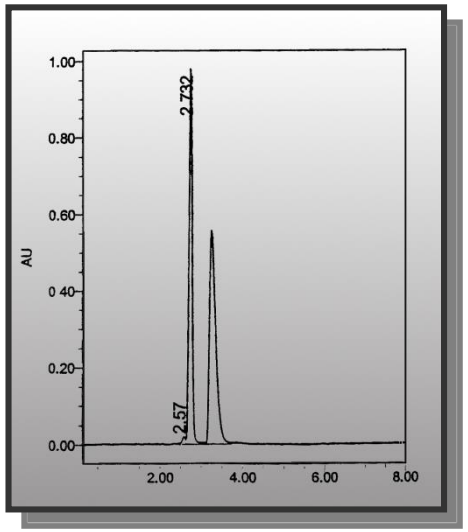
Third injection



Great – but will it work in the real world?

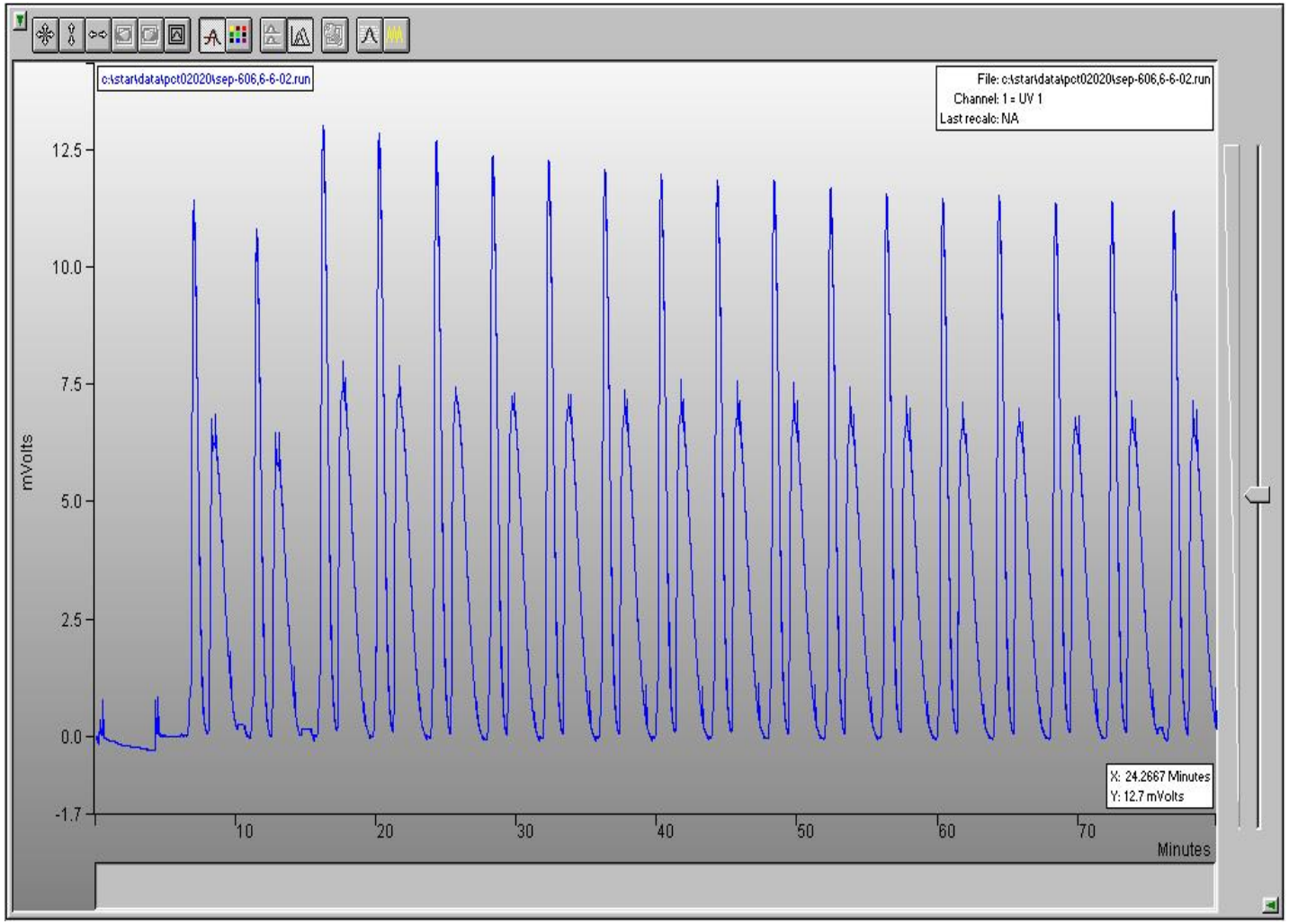
Stacked Injections



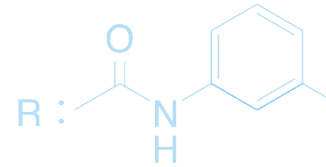


150 ml/min
675 mg / injection
Acetonitrile
Cycle time 4 min
T = 25°C
10.1 g/hr

Column : CHIRALPAK AD
20 μm, 500 x 50 mm



Analytical Productivity Scaled to Preparative Dimensions



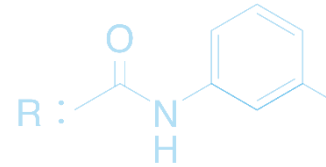
- We choose 5 µm 4.6x250 mm length columns for screening and loading because the productivity of a method scales proportionally to the ratio of the column internal diameters (assuming the lengths are the same).

- Scaling Factor = $(\text{Column i.d. \#1})^2/(\text{Column i.d. \#2})^2$

- For a 1 cm prep column, scaling factor is 4.7
  - $(1\ \text{cm})^2/(0.46\text{cm})^2$

- A 2 cm = 21.2, a 3 cm = 42.5, a 5 cm = 118.1, and an 11 cm = 571.8

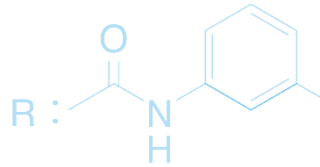
Analytical Productivity Scaled to Preparative Dimensions



- For the given method example, the productivity on a 4.6x250 mm analytical column was 66.99 mg/hr, or 621 days for 1 kg

- For a 1 cm prep column, one could achieve 314.8 mg/hr, or 132 days for 1 kg
- For a 2 cm prep column, one could achieve 1.42 g/hr, or 29 days for 1 kg
- For a 3 cm prep column, one could achieve 2.85 g/hr, or 14.6 days for 1 kg
- For a 5 cm prep column, one could achieve 7.91 g/hr, or 5.3 days for 1 kg
- For a 11 cm prep column, one could achieve 38.3 g/hr, or 1.1 days for 1 kg

System Requirements

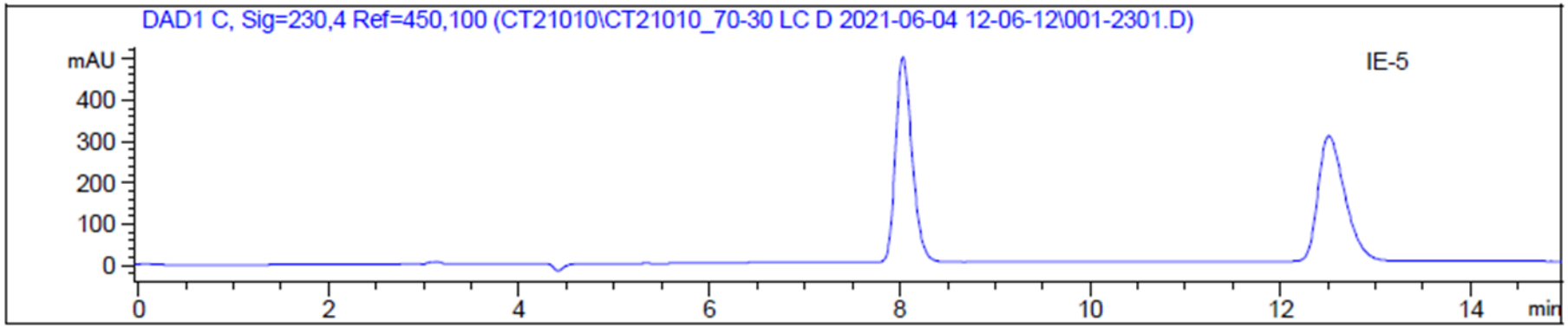


- Flow rates and injection volumes scale by the same scaling factors – need a system that can achieve the equivalent flow rate and injection volume of the desired column i.d.
- 11 cm column would produce/require 570 ml/min of mobile phase – typically requires large infrastructure to remove solvent from resulting fractions
- 5 cm column would be 118 ml/min, which might be manageable on 20 L rotavaps
- Smaller columns likely manageable on 20 L rotavaps, or something smaller

System Requirements

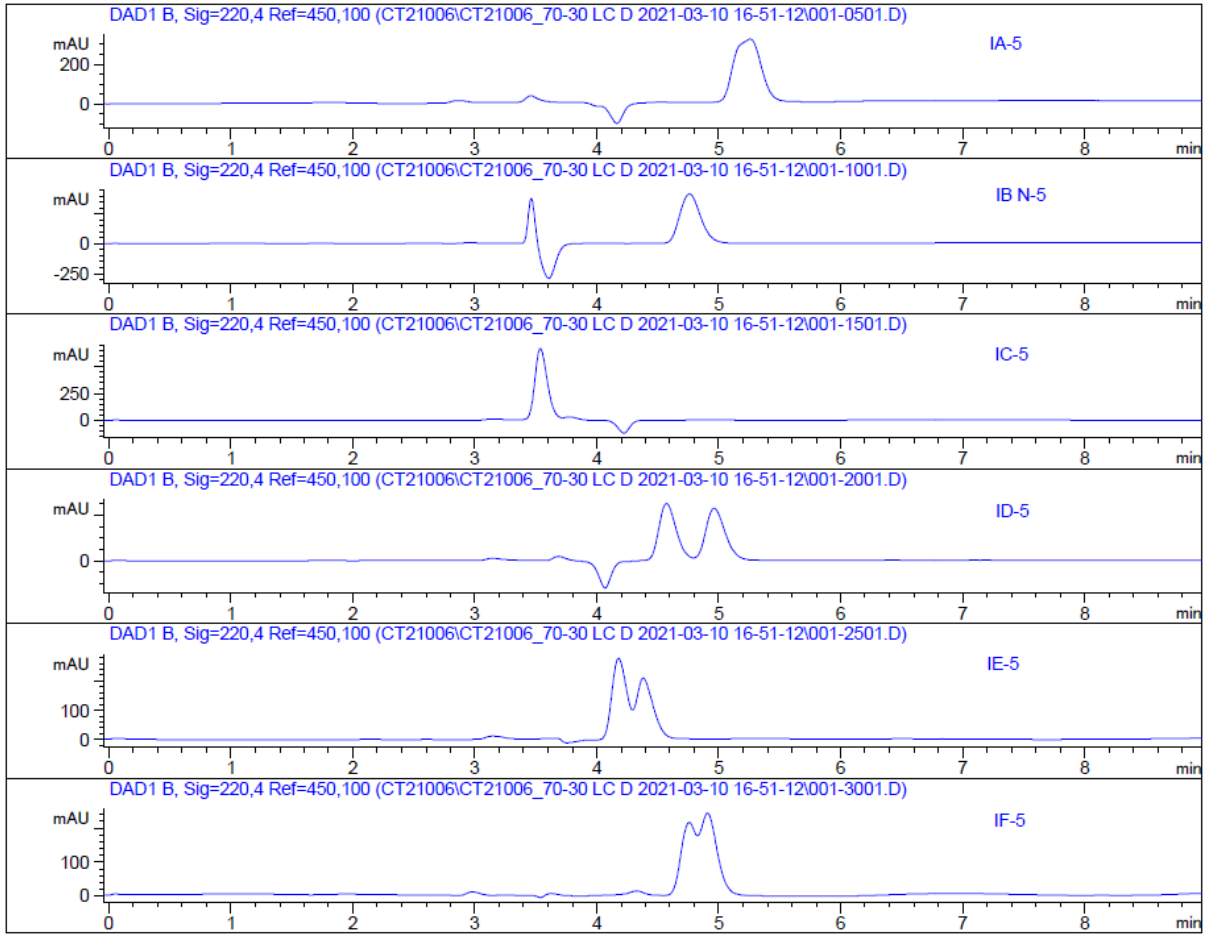
- A system that can achieve ~120 ml/min can will cover 1 cm prep columns up to 5 cm prep columns.

- Should be able to perform stacked injections to maximize cycle times



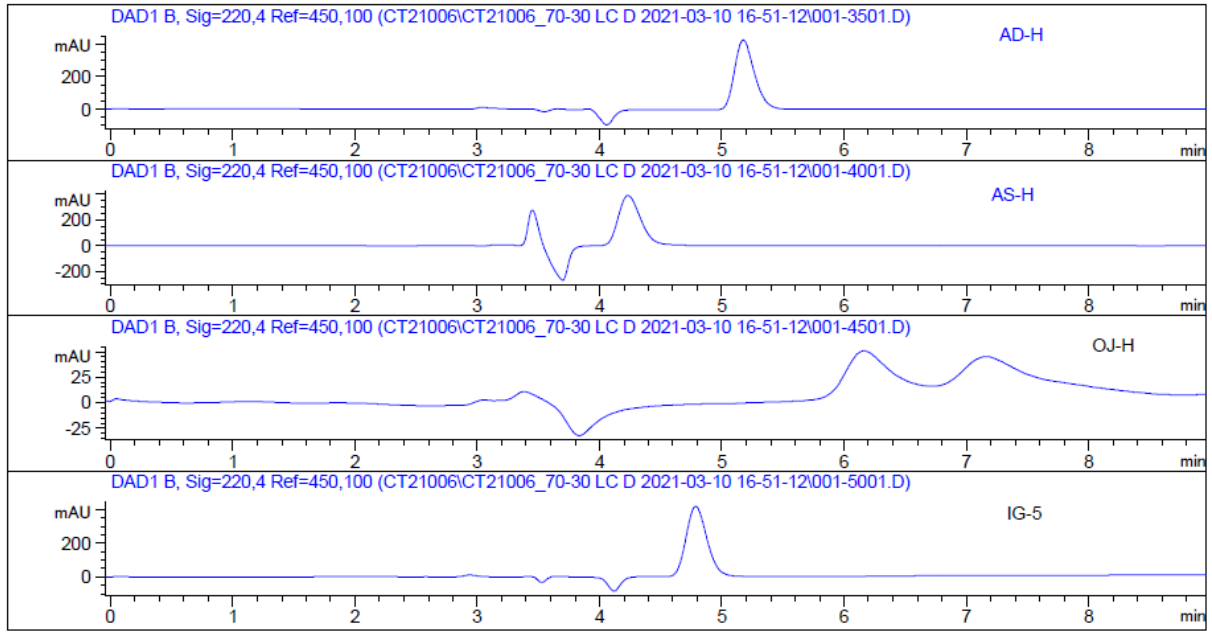
- Should be able to inject ~15 ml of sample feed

Start From the Beginning – Method Screening



Conditions

Mobile Phase = 70-30-0.1 = Hex-IPA-DEA
Flow Rate = 1 ml/min
Sample = 1 mg/ml in EtOH



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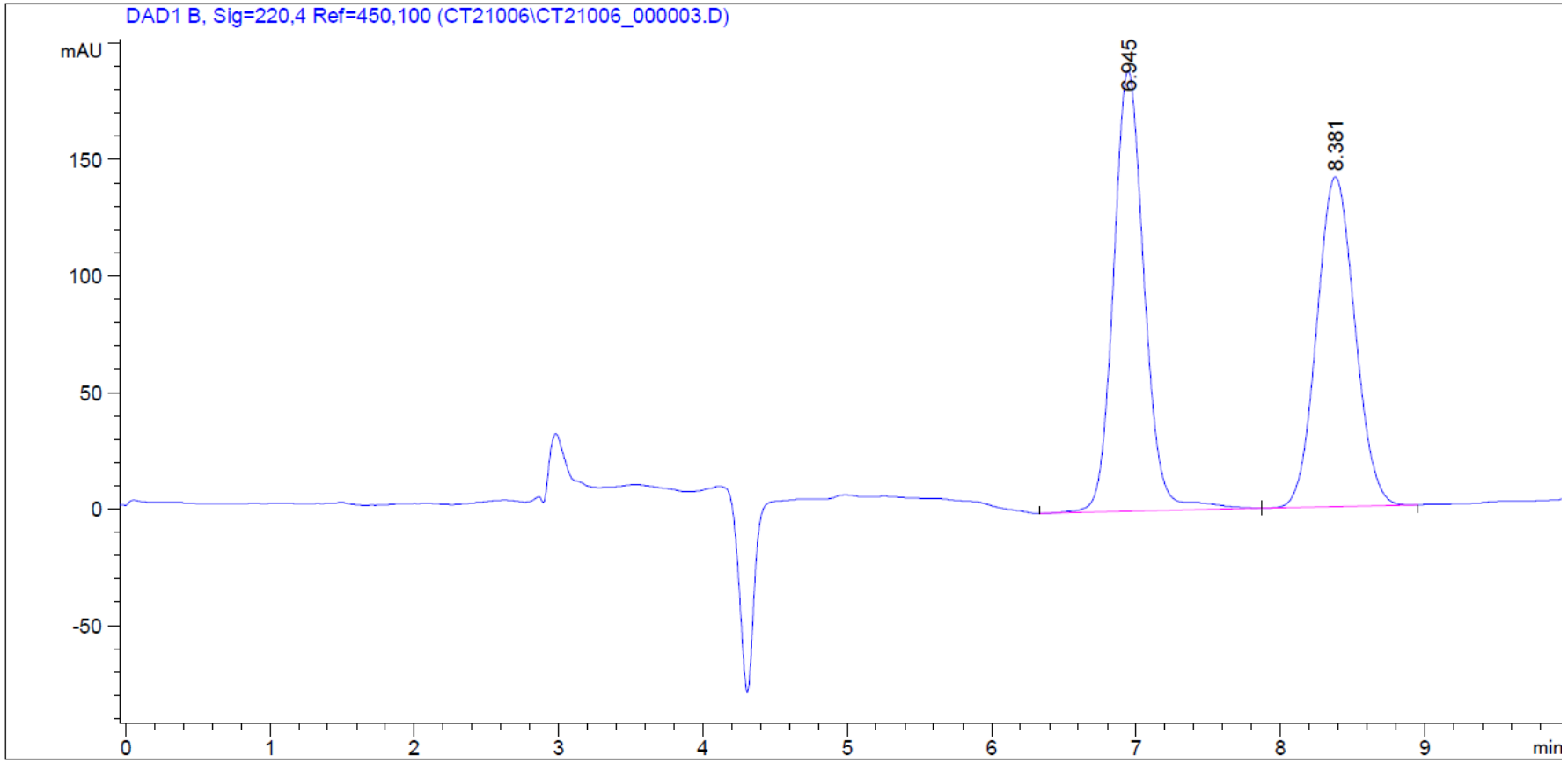
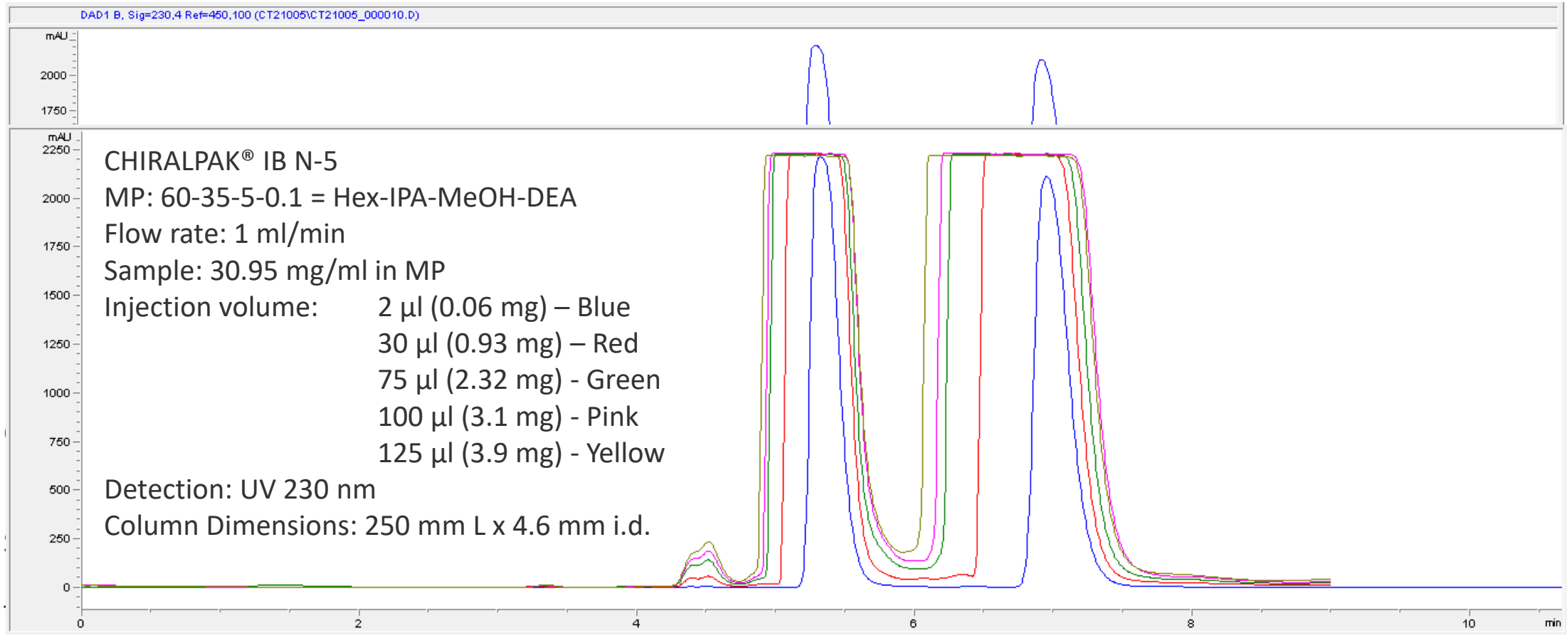


Conditions

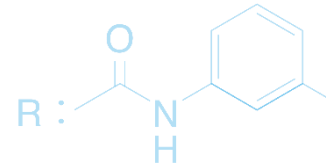
Mobile Phase = 60-35-5-0.1 = Hex-IPA-MeOH-DEA
Flow Rate = 1 ml/min
Sample = 1 mg/ml in EtOH

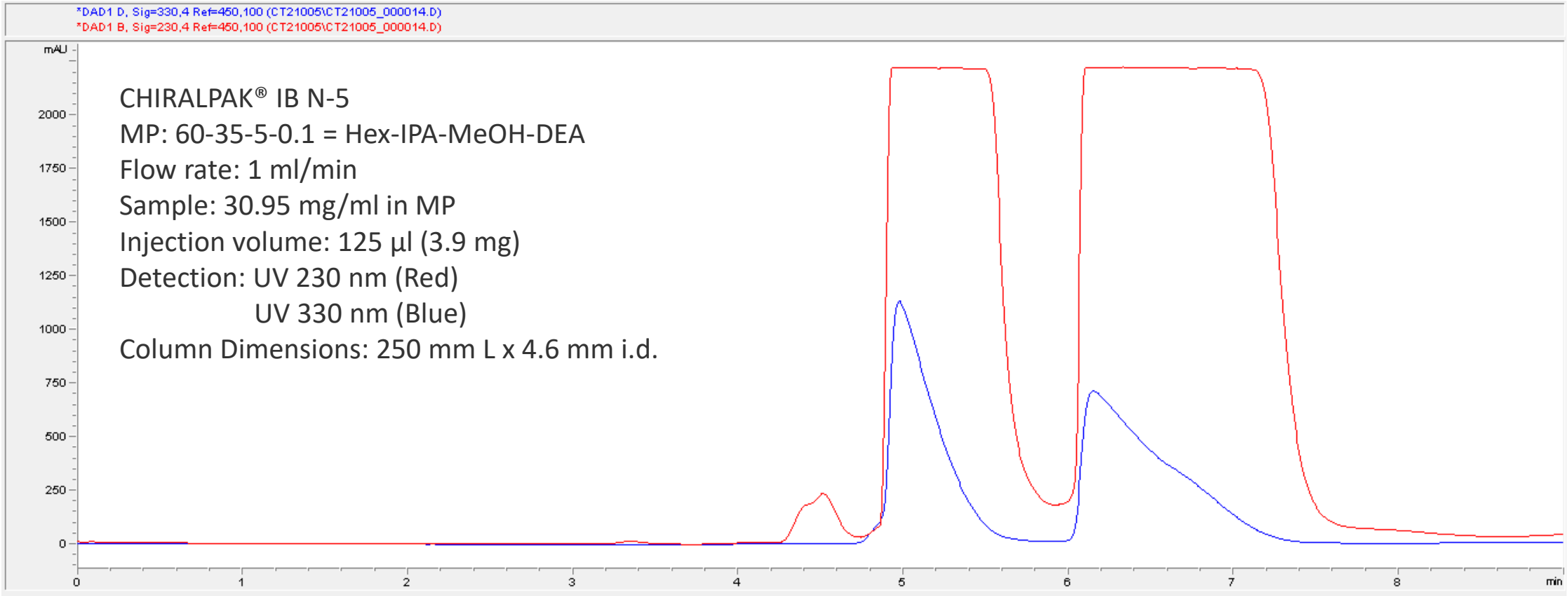
Analytical Scaling to Preparative Separations



Detection: UV 230 nm
Column Dimensions: 250 mm L x 4.6 mm i.d.

Analytical Scaling to Preparative Separations





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Analytical Scaling to Preparative Separations

CT	21005

Customer	
Sample name	
Separation Scale	100 mg to 1 gram
Target peak	Unknown

Solubility

MP Composition

Hex	i-PrOH	MeOH	DEA
60.00	35.00	5.00	0.10

Weight, mg	30.95
Volume, ml	1.00

Solubility, mg/ml	30.95

Loading

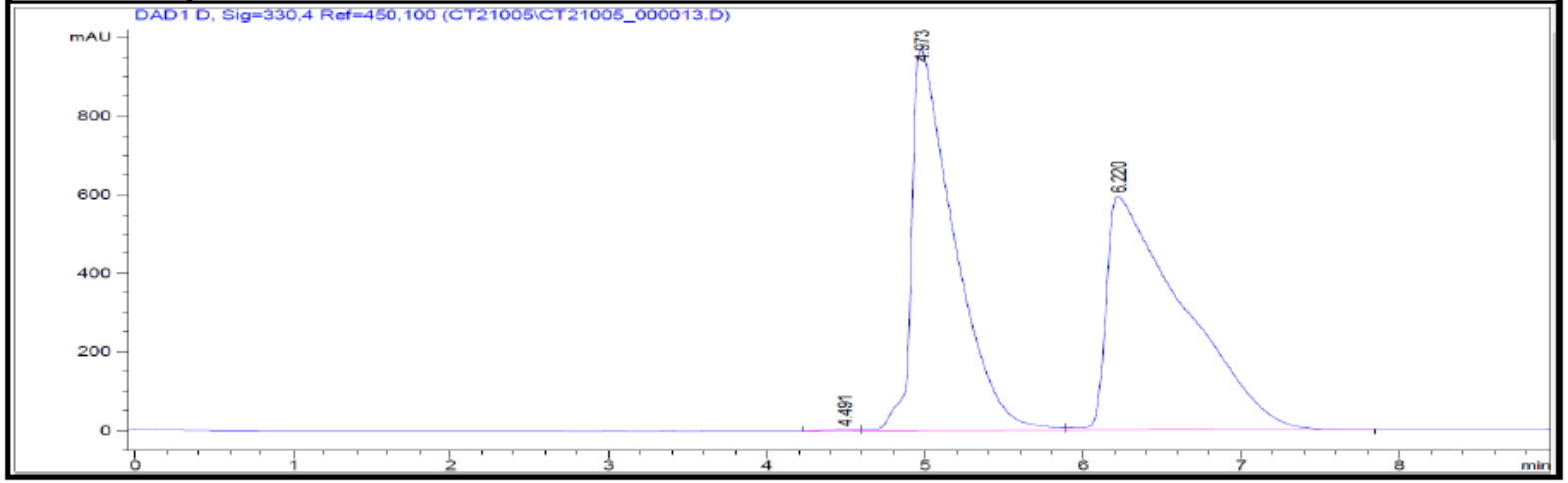
CSP	Particle Size, µm	Column Length, mm	Column ID, mm
IB N-5	5	250	4.6

Cycle Start, min	4.60
Cycle End, min	7.60

Cycle Time, min		3
Injection Volume,	µl	125.00
Sample Concentration, mg/ml		30.95

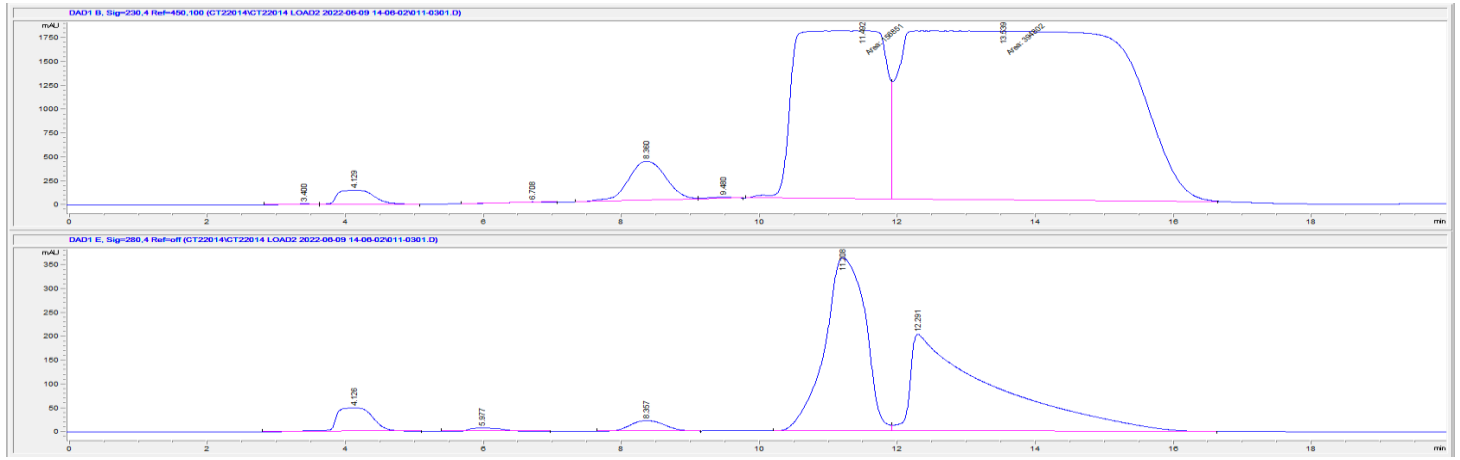
Productivity, mg/hr	77.38

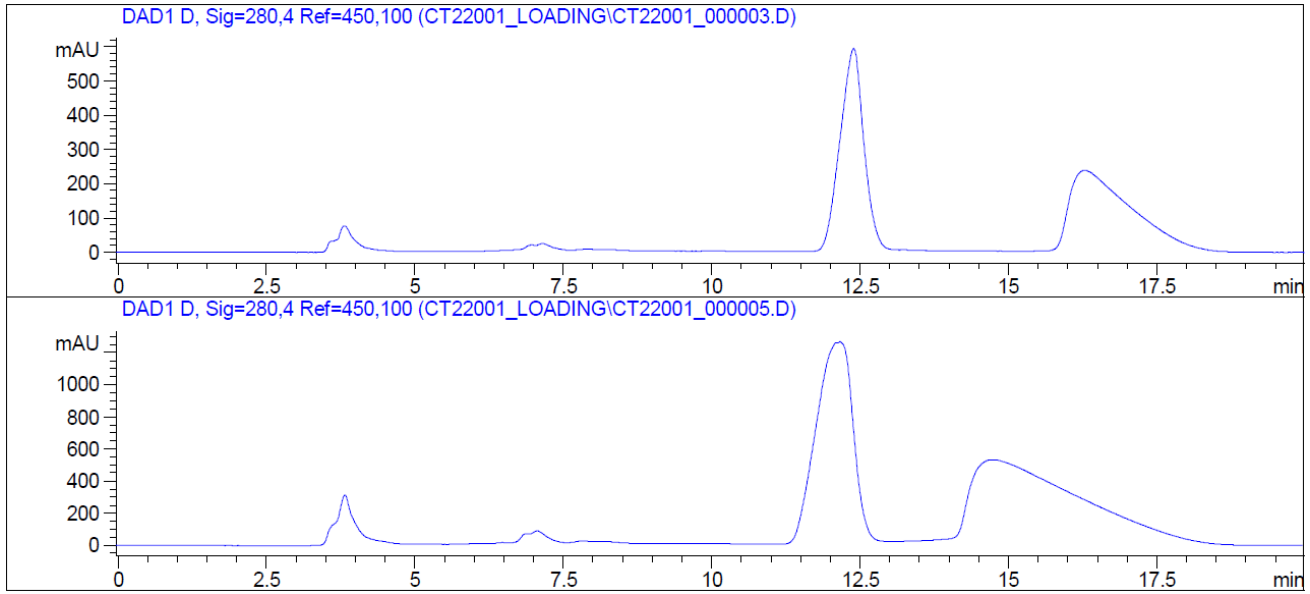
Comments: Note late eluting impurities



- 77.38 mg/hr on a 4.6 mm i.d. analytical column
- 363.7 mg/hr on a 1 cm i.d. semi-prep column
- 1.64 g/hr on a 2.1 cm i.d. prep column
- 3.28 g/hr on a 3 cm i.d. prep column
- 9.2 g/hr on a 5 cm i.d. prep column

Not All Preparative Loadings Are the Same!





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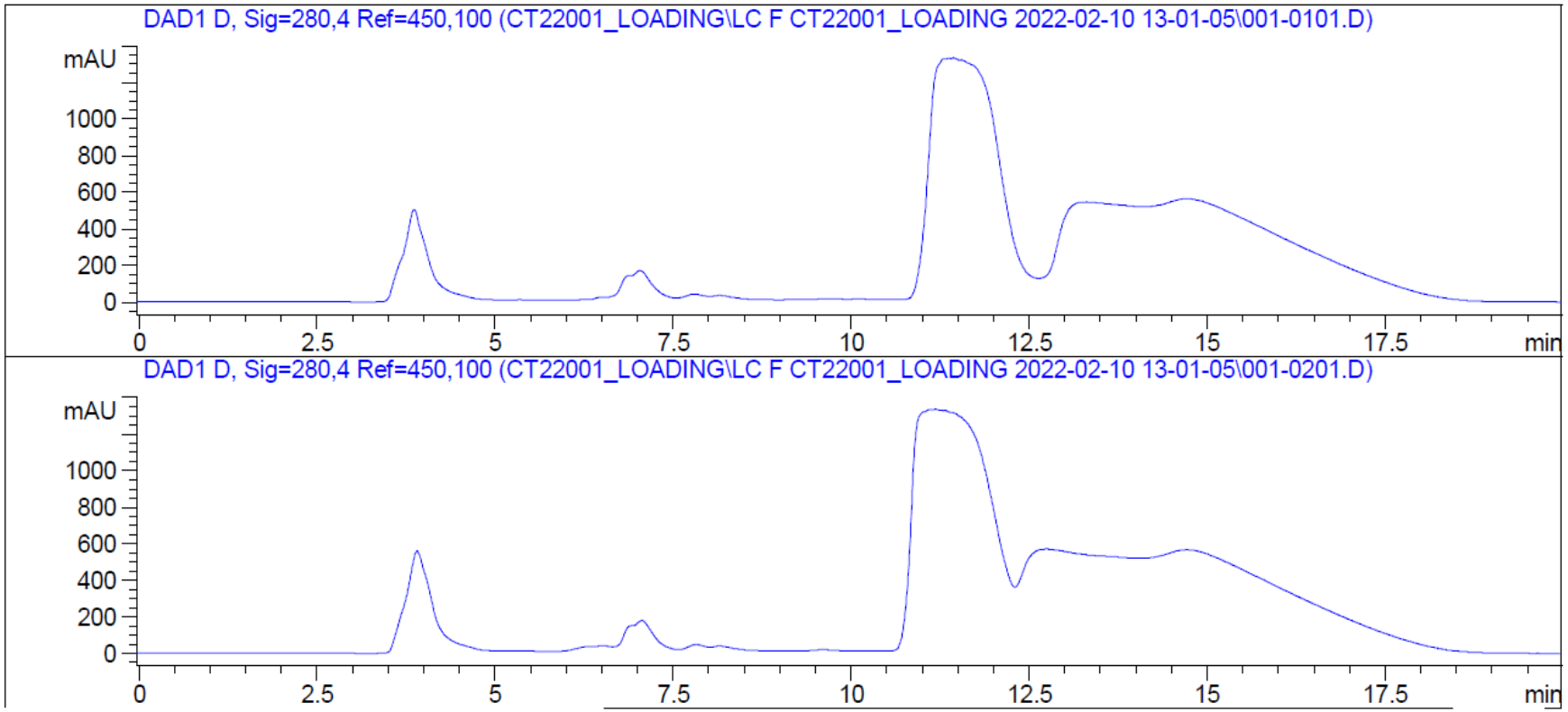

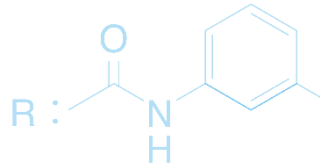

Questions?