

Optimizing Throughput and Minimizing Cost in Semi-Prep SFC

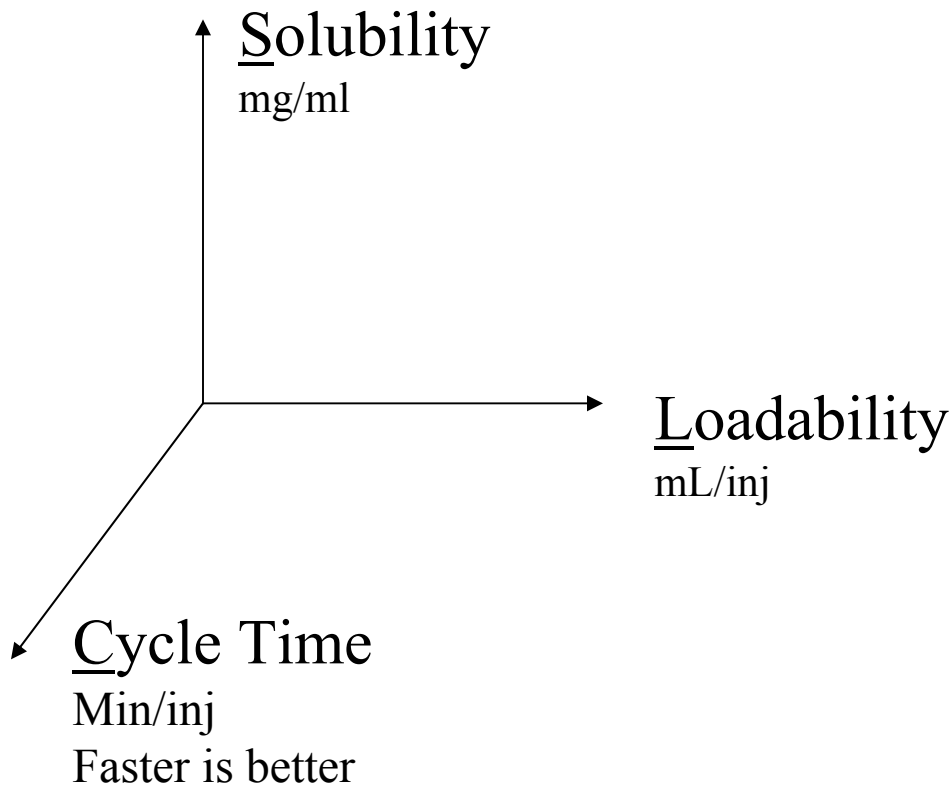
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Throughput is a Function of Solubility Loadability, and Cycle Time

$$\text{throughput} = (S \times L)/C = \text{mg/min} = \frac{(\text{mg/ml})(\text{ml/inj})}{(\text{min})/\text{inj}}$$



Method Development to Maximize Throughput-Minimize Cost

- **Solubility.** Find a good solvent for the sample that also makes a good modifier.
 - Compatible with stationary phase
 - Yields viable separation with adequate resolution at reasonable concentration : I.e., 5-40%
 - UV absorbance allows detection of solute
 - Not too high a vapor pressure for collection
 - In sample class allowed
- **Cycle Time**
 - Contaminants? Do we need to waste time avoiding them, or is it more cost effective to remove them first?
 - Trade cycle time for loadability-which yields highest throughput?
- **Loadability**-depends mostly on resolution- secondarily on sample capacity of stationary phase

SFC is Dramatically Less Expensive to Operate than HPLC

(after infrastructure is developed)

SFC produces 3-5 times more product per minute than HPLC.
Therefore:

SFC **labor** cost per gram is 1/3rd-1/5th HPLC

SFC **equipment** cost per gram is 1/2-1/4th HPLC

SFC **lab space** costs per gram is 1/3rd-1/5th HPLC

Solvent: \$196K by HPLC; \$47.3K by SFC while producing 3-5 x more product

SFC mainly uses \$0.08/Lb- \$0.18/L CO₂ vs. \$15/L heptane.

(100ml/min Heptane for 2000 hrs/yr = \$180,000; whereas, 340ml/min CO₂ costs \$7,344 (4% as much) while producing 3-5 times more product)

Modifier cost (per gram) similar (\$12K/yr in HPLC; \$40K in SFC).

Waste: HPLC must pay to dispose of **all** the mobile phase. SFC pays only to dispose of modifier (5-30% of mobile phase)

(disposal cost at least 50% of purchase price (\$96K/yr HPLC, \$20K/yr SFC))

SFC requires **7% of the dry down energy** of HPLC

SFC Direct Cost

	MiniGram	MG2	MG2	MG5
	7.8mm	20mm	30mm	50mm
	10ml/min	70ml/min	140ml/min	438ml/min
Labor ¹	\$1.00/min	\$1/min	\$1/min	\$1/min
Equip ²	\$0.25/min	\$0.50/min	\$0.50/min	\$0.60/min
maint ³	\$0.10/min	\$0.20/min	\$0.20/min	\$0.24/min
Lab ⁴	\$0.17/min	\$0.17min	\$0.17/min	\$0.17/min
Solvent ⁵				
ACN	\$0.045/min	\$0.315/min	\$0.63/min	\$1.97/min
CO ₂	\$0.0018/	\$0.0126/	\$0.0252/	\$0.079
Waste	\$0.022/min	\$0.15/min	\$0.30/min	\$0.96/min
Col ⁶	\$0.067/min	\$0.40/min	\$0.67/min	\$1/min
Tot's:	~\$1.66/min	\$2.75/min	\$3.50/min	\$6.02/min

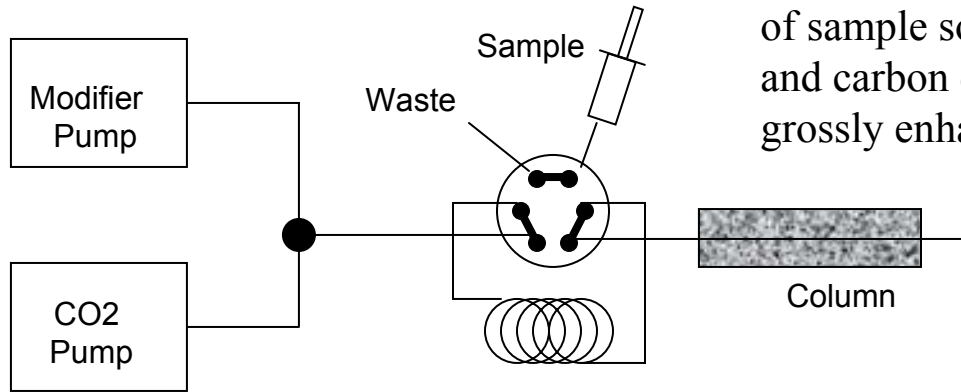
Throughput⁷

mg/min	1	6.6	13.2	41.2
\$/g cost	\$1660/g	\$416.65/g	\$265.15/g	\$146.10/g

Divide \$/g above by mg/min (actual)

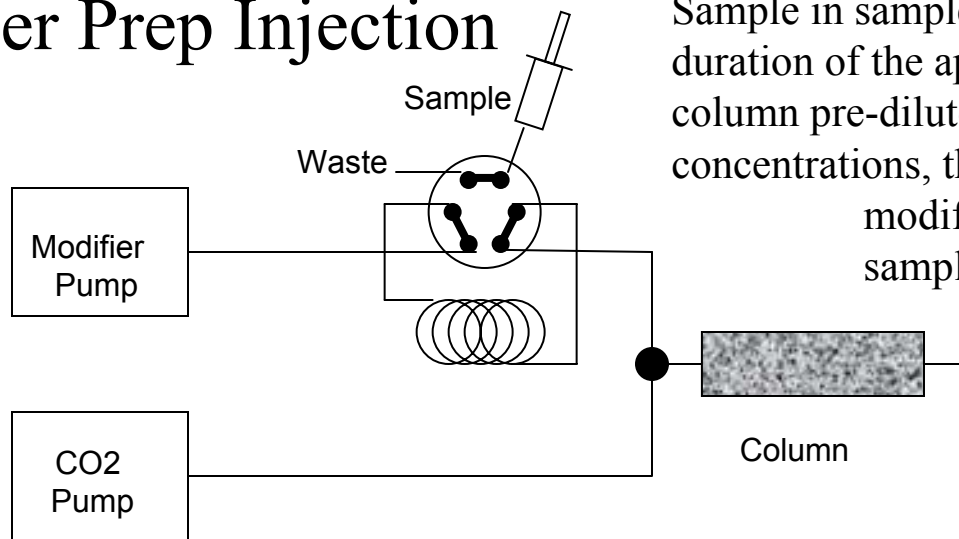
Injection Schemes: Large Volume Injection

Direct Injection



Results in the sudden introduction of a large volume of sample solvent into a stream of premixed modifier and carbon dioxide. Local solvent strength suddenly grossly enhanced. Peaks are distorted

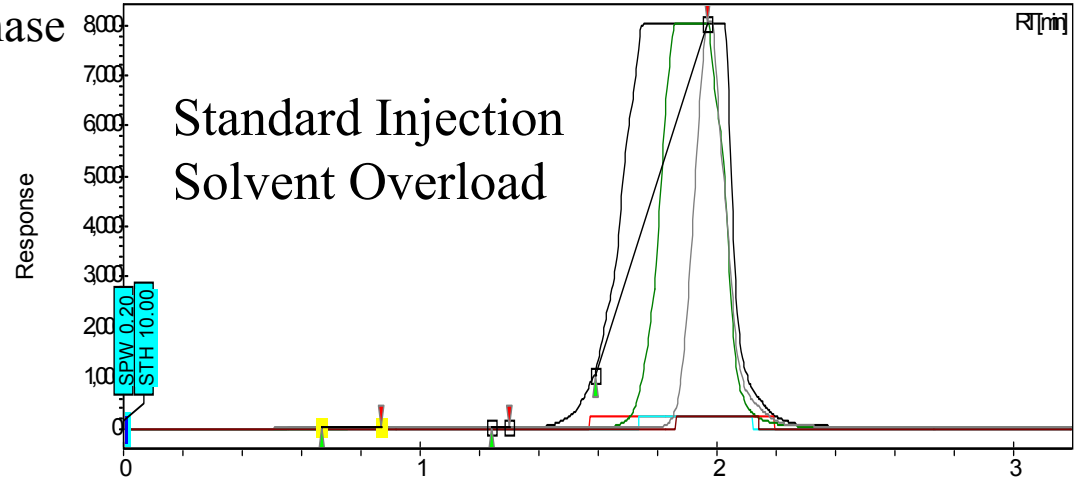
Berger Prep Injection



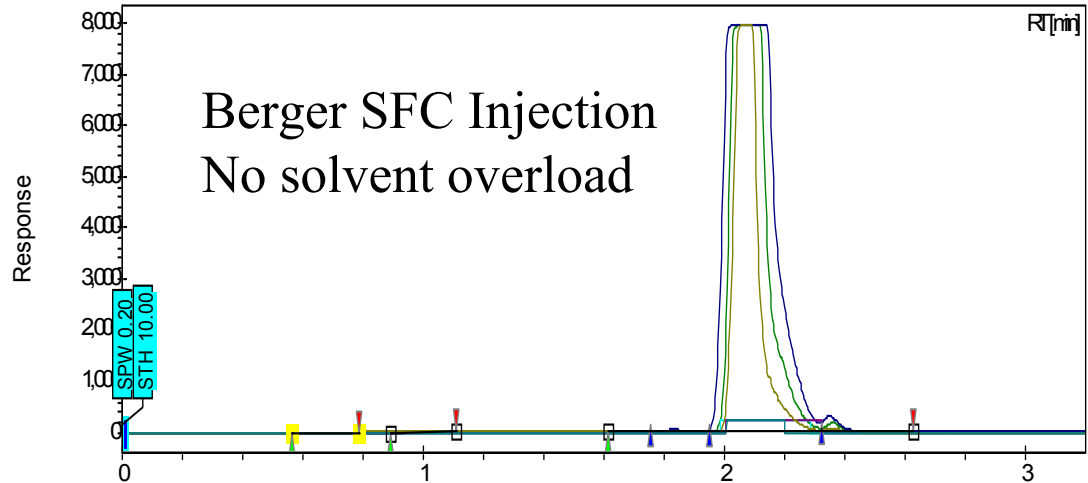
Sample in sample solvent replaces the modifier for the duration of the application time. Sample reaches column pre-diluted in mobile phase. At high solute concentrations, this actually represents a decrease in modifier concentration (focusing) during sample application.

Solvent Overloading

Injecting a large volume of too strong
a solvent into the pre-mixed mobile phase
causes a spike in the local solvent
strength at the head of the column,
resulting
in the solute being prematurely
swept down the column and fronted.

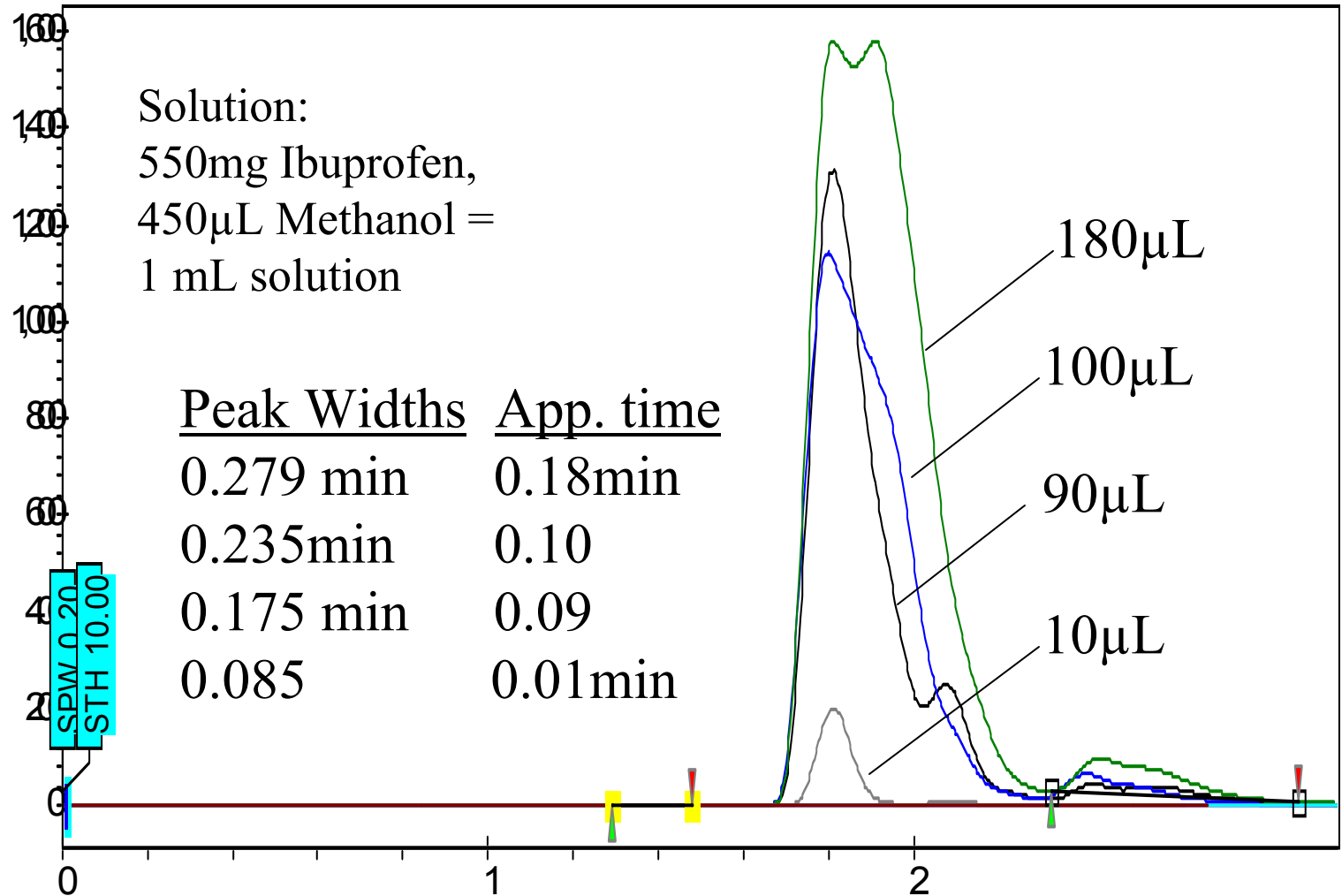


The same amount of sample is
introduced in both cases
we used high surface area silica's
($>350\text{m}^2/\text{g}$)(1/3rd surface coverage)



Conditions: Sulfanilamide 5
mg/mL in methanol; 0.5, 1.0, 1.8
mL inj. 20% to 50% MeOH at
10%/min; 50 mL/min; 100 bar;
6 μm , 60A Diol, 21.2 x 150 mm

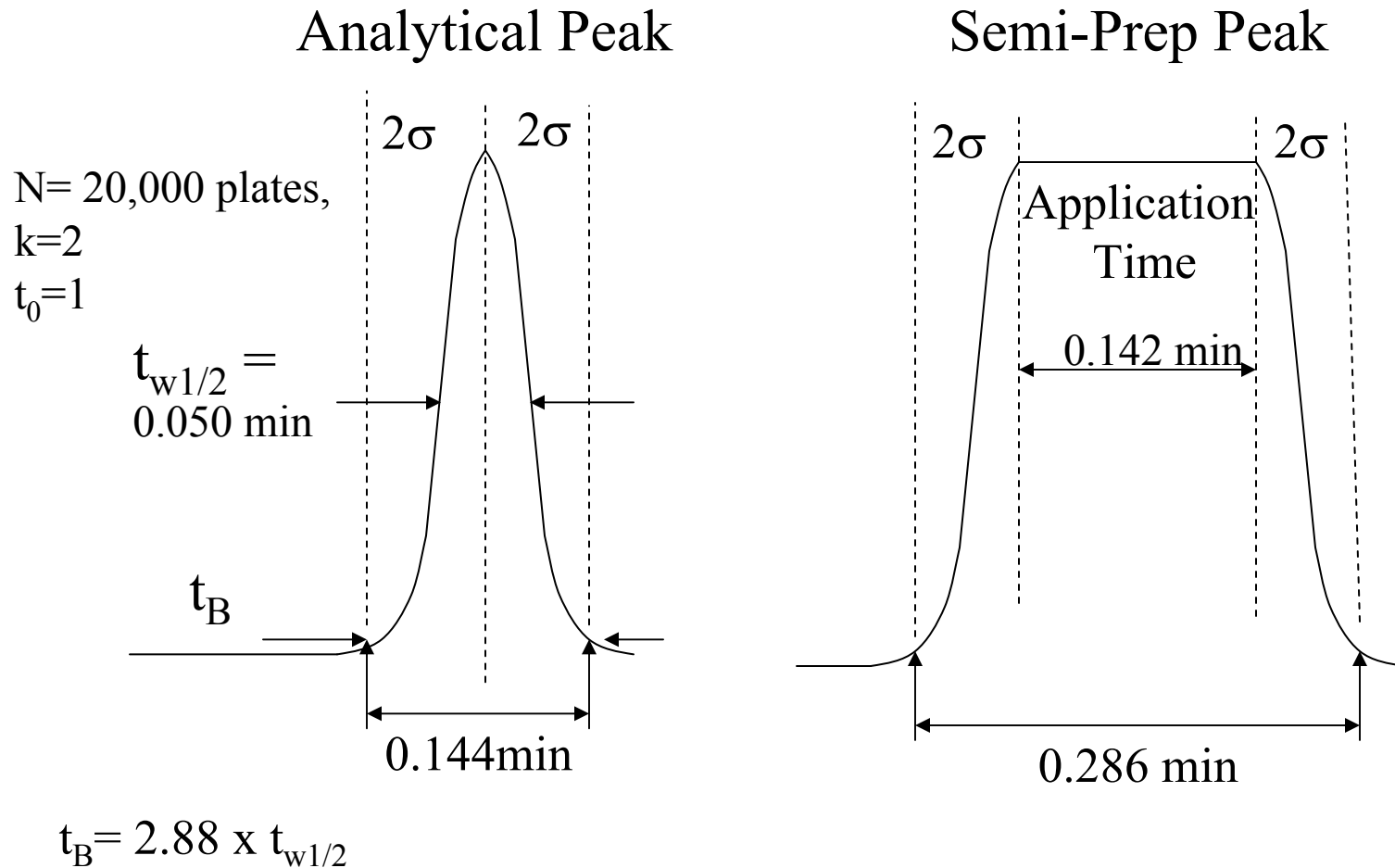
Loadability on a 10 x 250mm, 6 μ Ethyl-pyridine Column



Peak width appears to be the sum of band broadening (bb) (the width an impulse injection reaches through diffusion and physical phenomena) and the application time

<u>bb</u> + <u>App. Time</u> = expected	<u>Obs Pk Wd</u>
0.085 + 0.18min = 0.265	0.279 min
0.085 + 0.10 = 0.185	0.235min
0.085 + 0.09 = 0.175	0.175 min
0.01 = 0.085	0.085

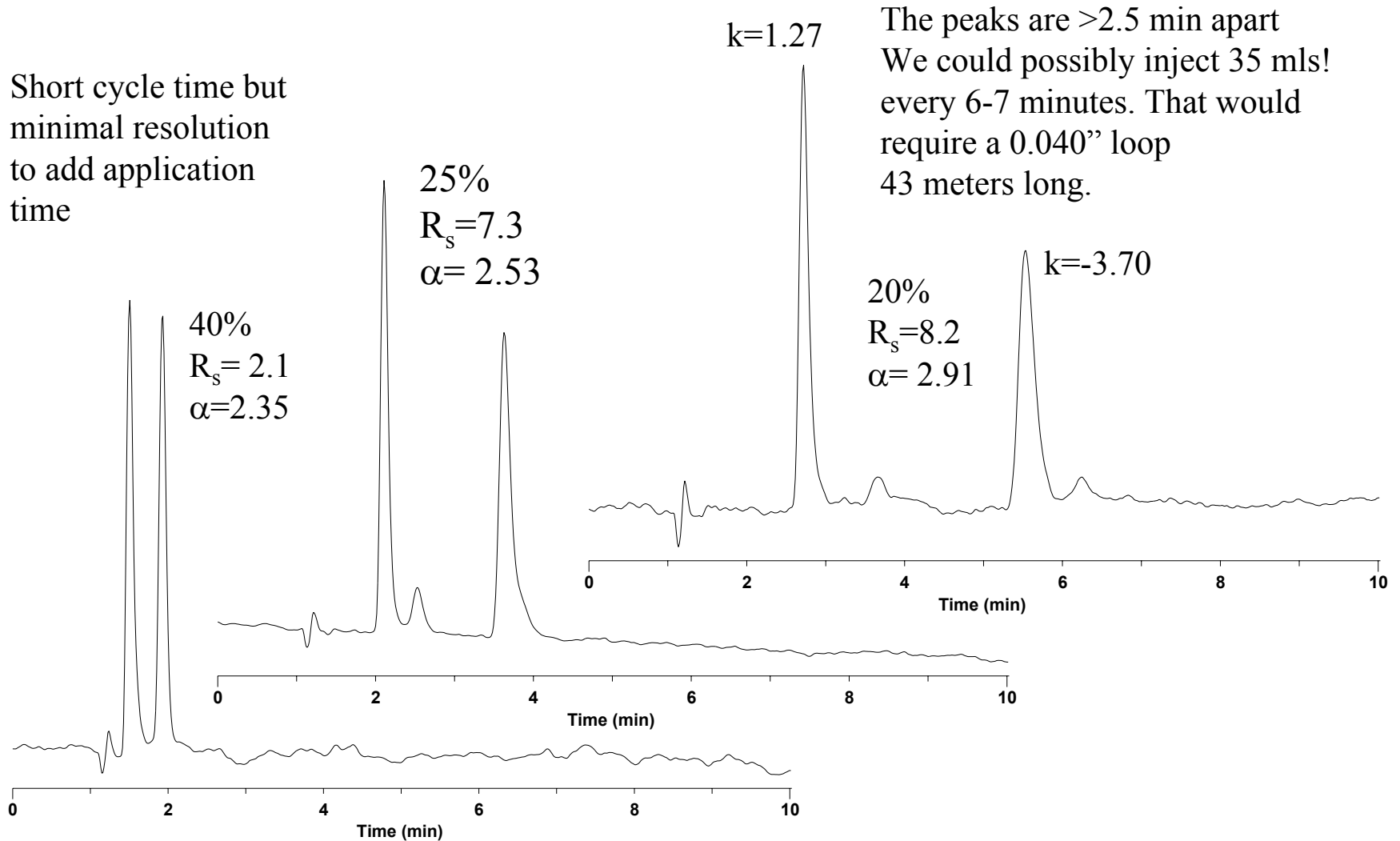
Semi-Prep Peaks are broadened by Diffusion AND by the Application time of the Sample. (other band broadening ignored in this example)



At 20% mod, 50 ml/min on a 20mm column; modifier flow is 10ml/min. Application time of 0.142min equals 1.42mls injected

Which separation is better for Prep?

Short cycle time but minimal resolution to add application time



$k=1.27$

The peaks are >2.5 min apart
We could possibly inject 35 mls!
every 6-7 minutes. That would
require a 0.040" loop
43 meters long.

40%
 $R_s=2.1$
 $\alpha=2.35$

25%
 $R_s=7.3$
 $\alpha=2.53$

20%
 $R_s=8.2$
 $\alpha=2.91$

$k=-3.70$

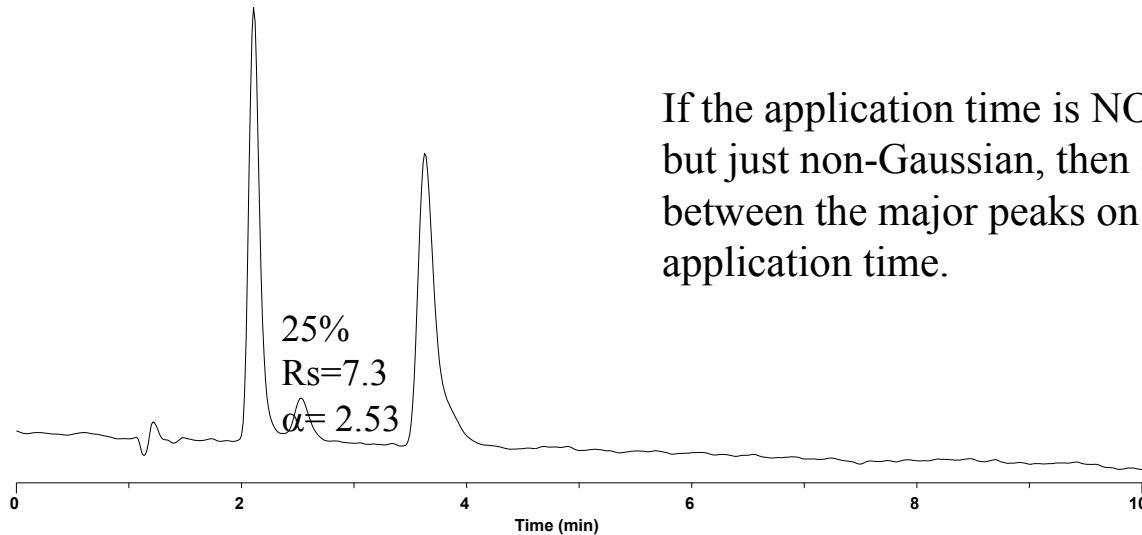
Time (min)

Time (min)

Time (min)

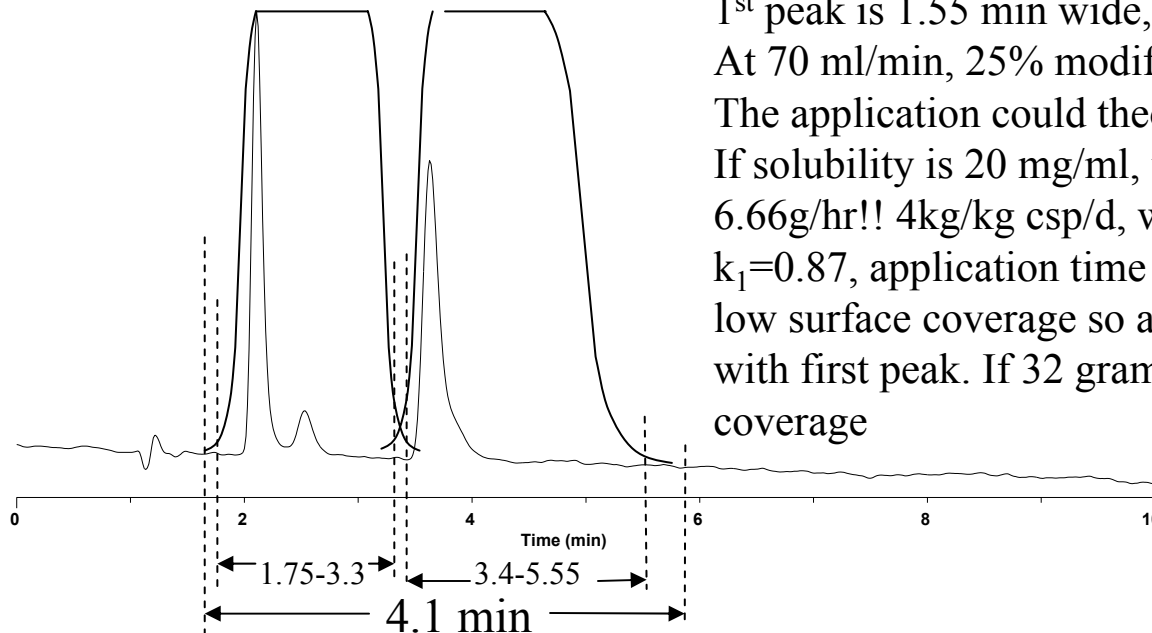
Understanding the “Berger” Injection Scheme

If the application time is NOT a form of “overload,” but just non-Gaussian, then as a worst case, the time between the major peaks on the baseline could be the application time.



1st peak is 1.55 min wide, application time would be 1.3 min
 At 70 ml/min, 25% modifier, we deliver 17.5 ml/min.
 The application could theoretically be 22.75 mls!

If solubility is 20 mg/ml, throughput is 111 mg/min!
 6.66g/hr!! 4kg/kg csp/d, with a solute with poor solubility
 $k_1=0.87$, application time is longer than t_0 . There must be
 low surface coverage so as not to distort second peak
 with first peak. If 32 grams csp in col; $0.049 \times \text{mg/inj} = \% \text{surface coverage}$



Which Separation gives the Highest Throughput?

<u>%Mod</u>	<u>app. Time</u>	<u>Inj vol</u>	<u>Cycle time</u>	<u>g/min*</u>
40%	0.1min	4 ml	1	80mg/min
25%	1.3min	32.5 ml	3	217mg/min
20%	2.3 min	46 ml	6	153mg/min

*at 20mg/ml

Sample Overload effects: Fronting

Flurbiprofen shows classic fronting due to solute overload.

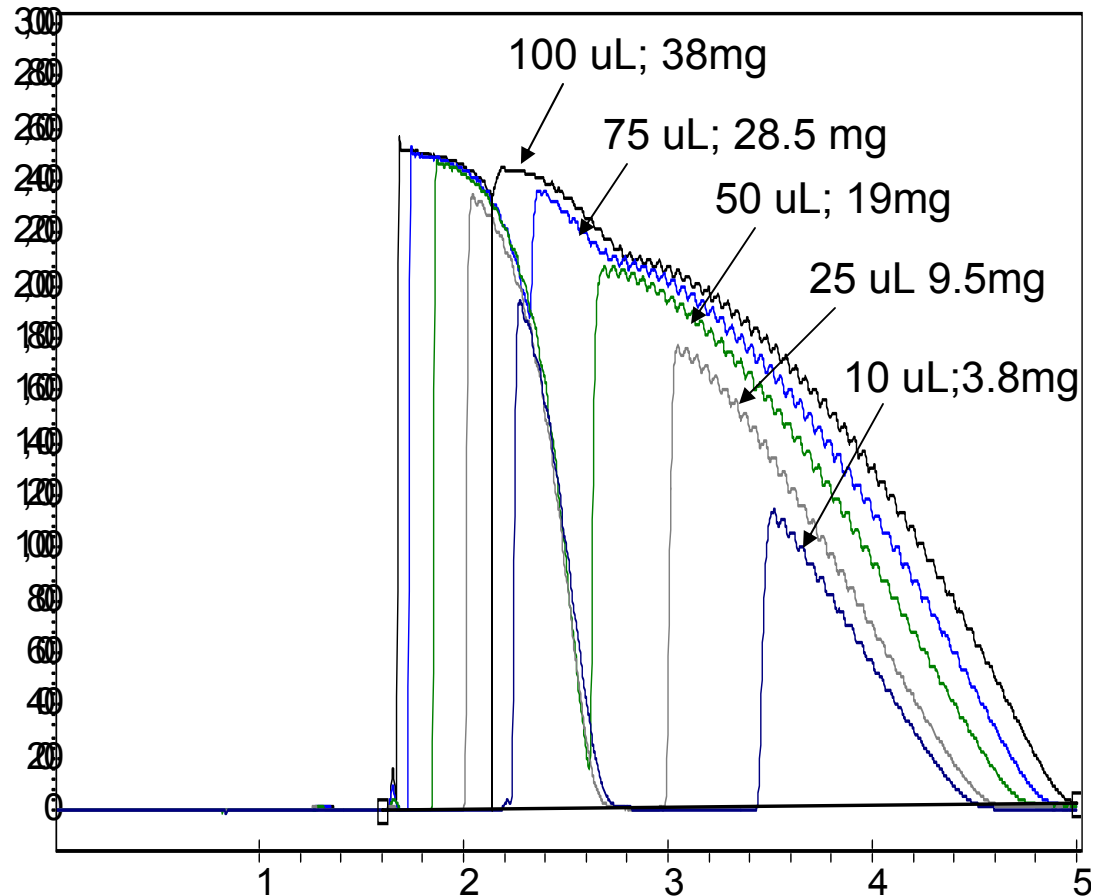
Conditions:

Sample is **378 mg/mL Flurbiprofen** in MeOH/ChiralPak AD-H, **4.6 x 250 mm**; 15% MeOH @ 3.7 mL/min, 30C; 290 nm observation

The column has ~ 1.9 grams packing. If $\sigma = 0.134$ cm, then there is 0.010g packing/ σ . If there are 20m²/g of packing, then 1 $\sigma = 0.2$ m². If there are 8 μ M/m² of sites on the packing, then the column has a theoretical analytical capacity w/o fronting of (4 σ) = 6.4 μ M of molecules on 6.4 μ M of sites (100% coverage). If molecular weight is 400, then we could theoretically apply 2.6mg (at 100% surface coverage). The smallest injection put 3.8mg on-column and the peaks are severely fronted.

Daicel Columns Overload Easily

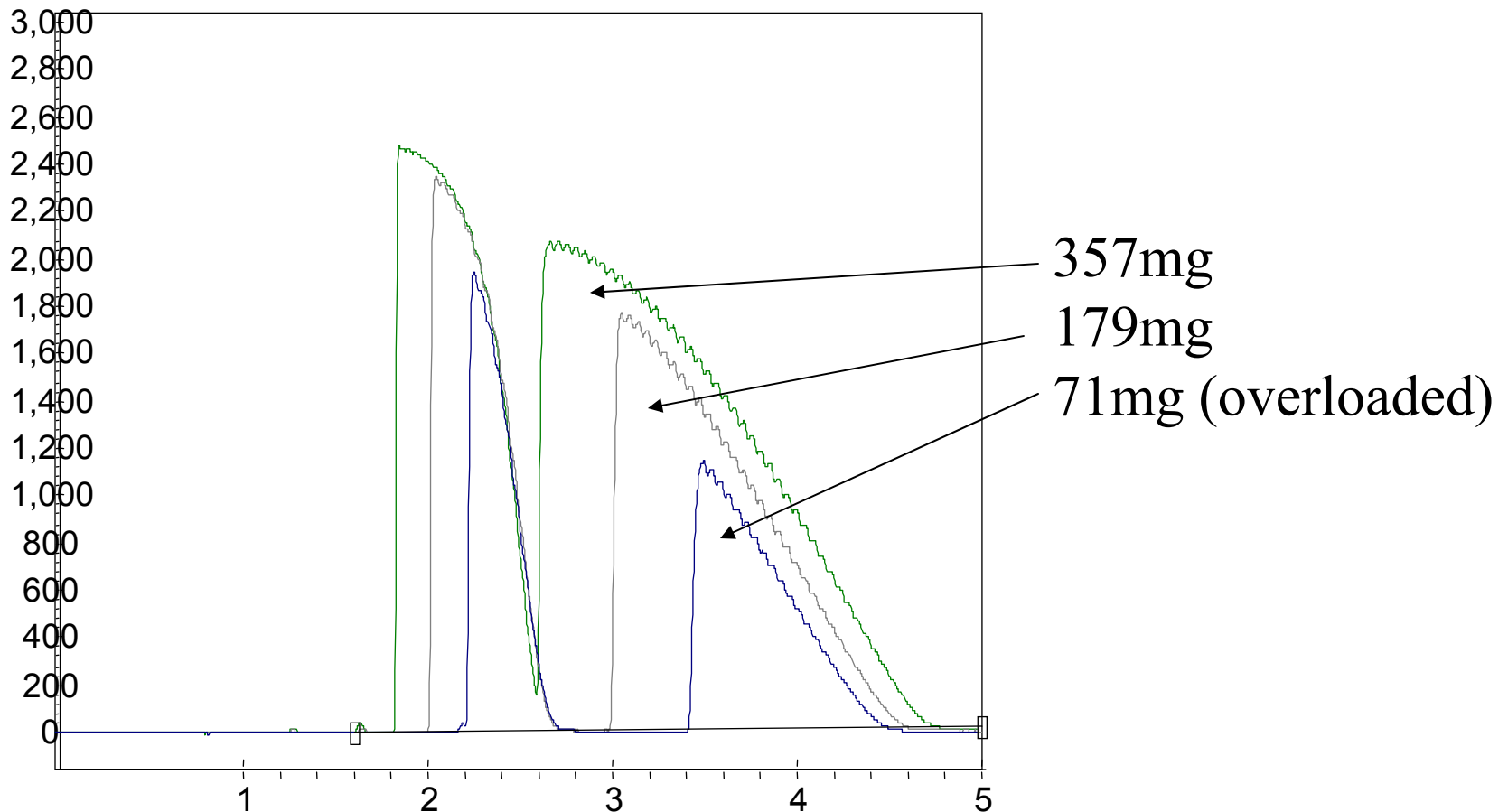
It is NOT the volume, it's the mass



Daicel Loadability

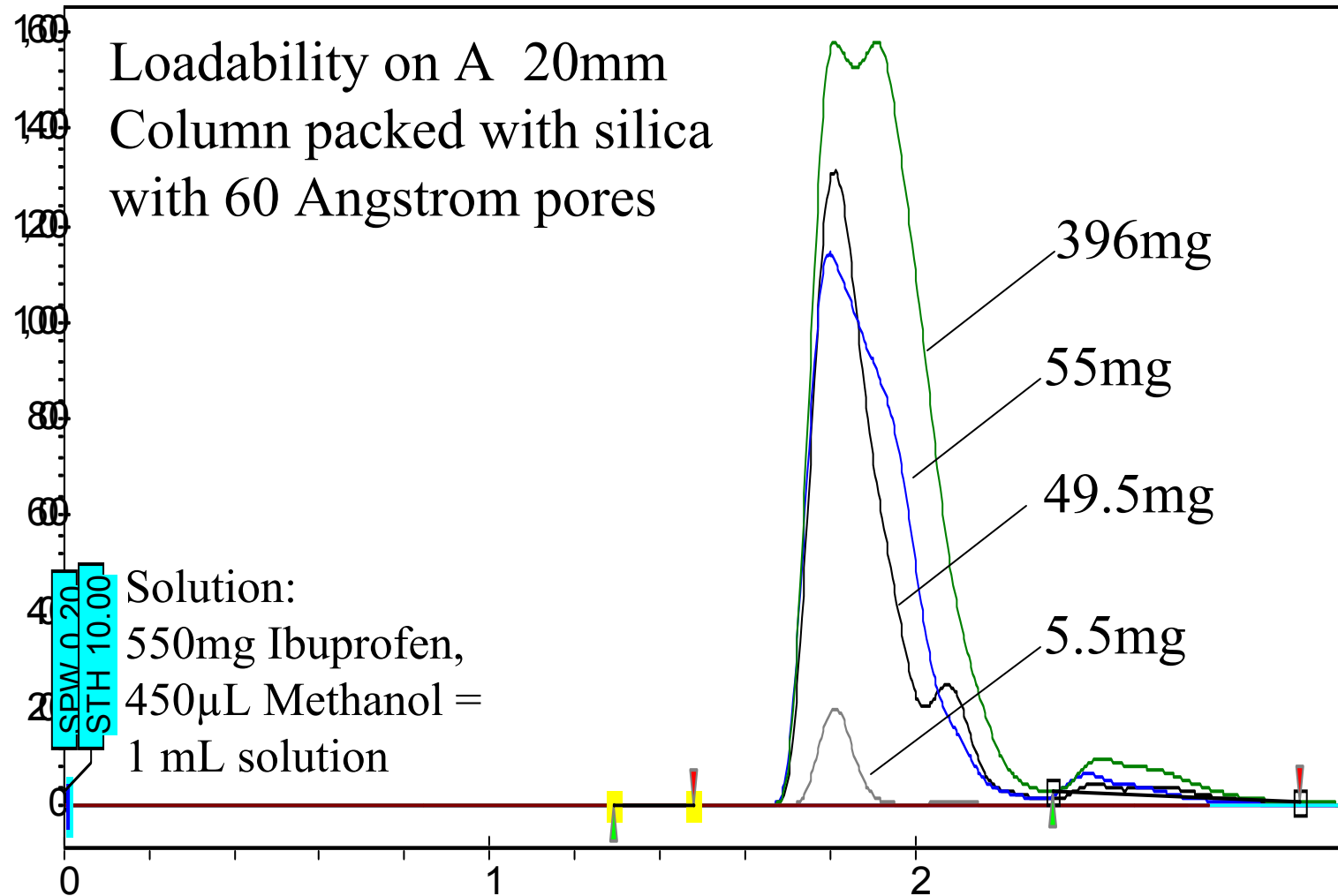
Results from 4.6mm column Scaled to 20mm

20mm col $k=4$ 24.9m^2 82Mg MAX



But What About The Previous Example?

There is NO FRONTING AT ALL



How Much Surface Area Do we Need in order to Avoid Overload?

$$8\mu\text{Moles}/\text{m}^2 \text{ sites} = 4.8 \times 10^{18} \text{ sites}/\text{m}^2$$

If a solute has MW 400,
 $1\text{mg} = 1.51 \times 10^{18}$ molecules

If one molecule sat on one site,
It would require:

0.313m^2 of surface area per mg injected
(for 100% surface coverage!)

Totally Porous Silica's have Huge Range of Surface Areas

• 60 Angstrom pores	500m ² /g	
• 100Angstrom	350m ² /g	
• 120Angstrom	300m ² /g	
• 300 Angstrom	100m ² /g	
• 500 Angstrom	35m ² /g	Daicel columns
• 4000Angstrom	10m ² /g	←

Chiral Technologies (Daicel) packings are apparently on 1500Angstrom silica. We can guess they have maybe 20m²/g of surface area.

Calculated vs. Observed

Maximum possible loading with 100% surface area = **0.082g = 82 mg**

We Measured overload at 71mg

Measured agrees with calculated surprisingly well since calculated assumes 100% surface coverage by solute which is generally thought to be unrealistic!

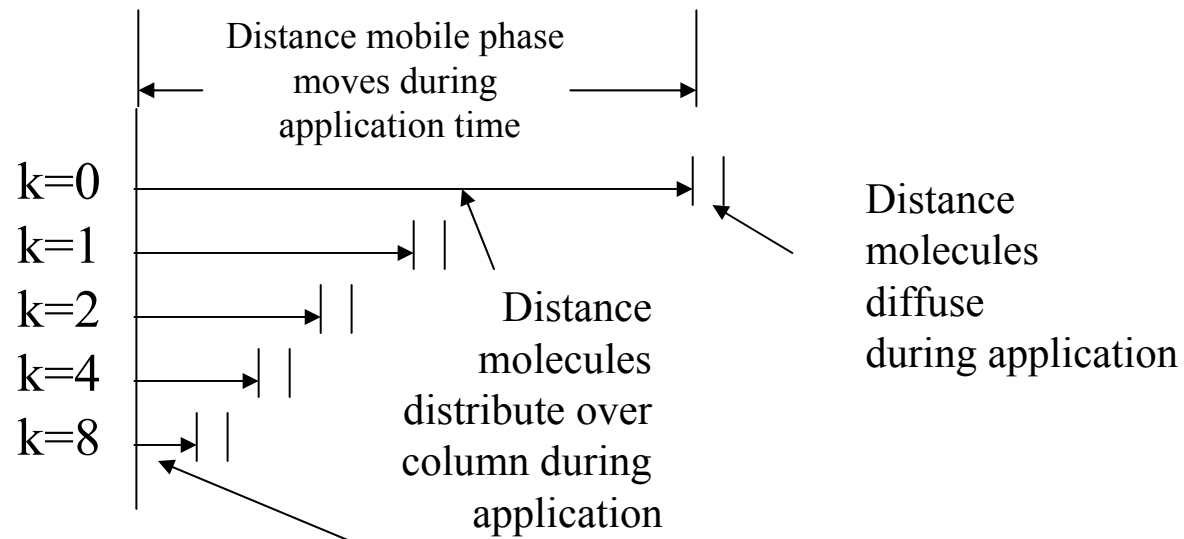
Effect of k on Loadability with Long Application Times

Concentration on column surface dependent on k.

i.e., $\frac{1}{2}$ max at $k=1$; $\frac{2}{3}$ max. at $k=2$; $\frac{4}{5}$ max. at $k=4$; $\frac{8}{9}$ max. at $k=8$

Peaks should be less overloaded at lower k

**You should be able to put
more concentrated samples on at lower k!**



The shorter the distance the sample is distributed over,
the higher the surface concentration

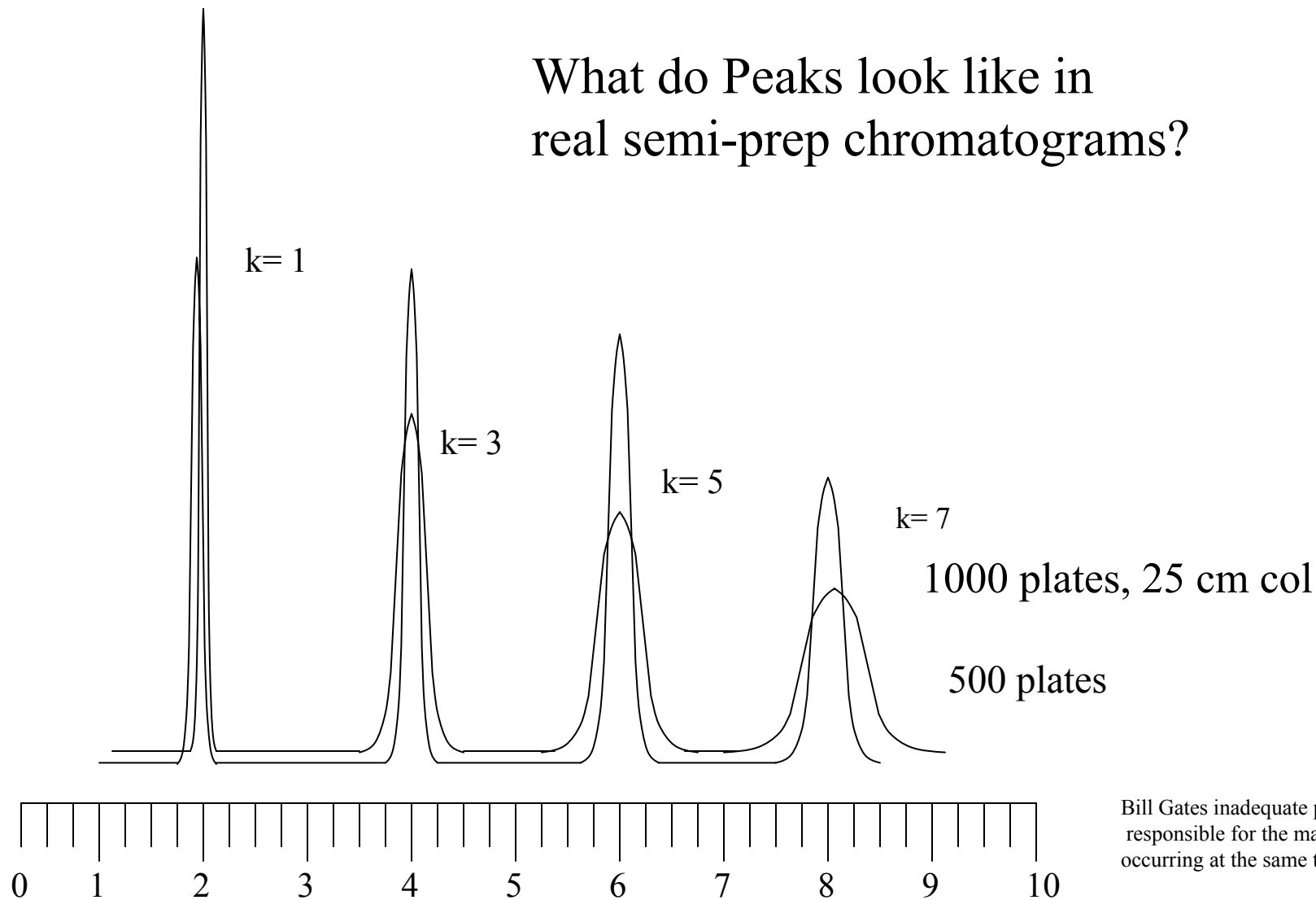
It Should Be a Goal To Make Smaller Pore Columns that Work

all 20mm ID

	Surface Areas	max inj mass
<u>A Daicel Column with 20 m²/g 1500Angstrom pores</u>		
k= 0	124.7m ²	0.41g
k= 1	62.4m ²	0.20g
k= 4	24.9m²	0.082g
k= 8	13.9m ²	0.046g
<u>With 300Angstrom pores</u>		
k= 0 yields	625m ² of surface	2 grams
k= 1	313m ²	1 gram
k= 4	125m ²	0.4 g
k= 8	69.4m ²	222mg
<u>With 120 Angstrom pores</u>		
k= 0	1875m ²	6g
k= 1	938m ²	3g
k= 4	375m ²	1.25g
k= 8	208m ²	0.67 g

We need 0.313m²/mg injected. Assumes sample replaces modifier

What do Peaks look like in real semi-prep chromatograms?



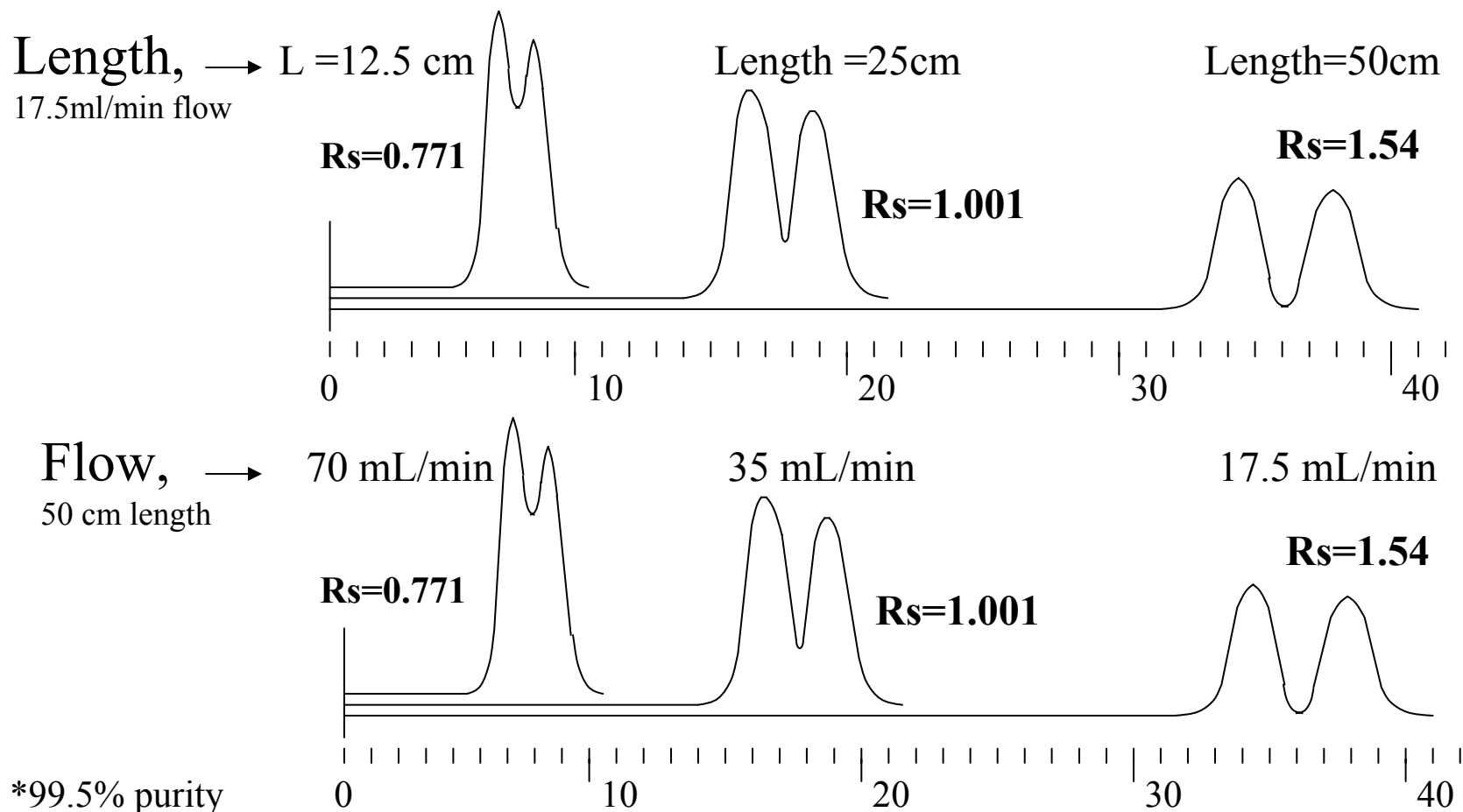
Bill Gates inadequate products are responsible for the maxima not occurring at the same time

Using a Shorter Column gives the same Result as increasing Flow on a Long Column

0.95g/h
Recovery* = 19%

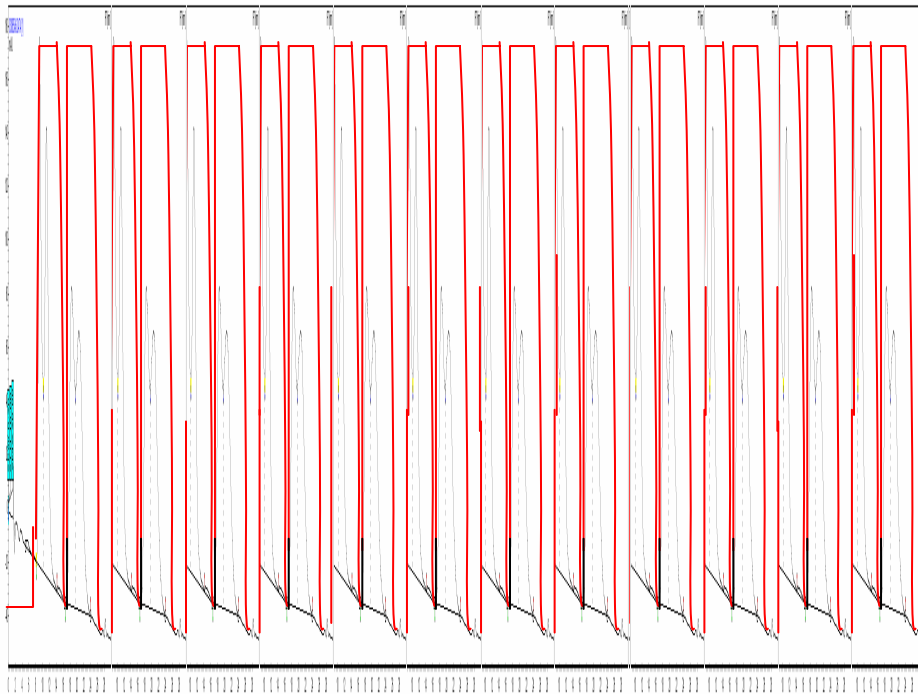
2.22g/h
Recovery* = 80%

1.7g/h
Recovery* = 99.5%



We can Get Huge Throughput if we have Excess Resolution
With low solubility IF there is enough surface area

If we expand the bottom example on the previous slide
We can inject 36 mls every 5.33 minutes, broadening the
Peaks to nearly 1.5 minutes wide each. We can then make
12 injections of 36 mls in one hour!



20 x 250 mm Chirapac AS-H
120ml/min;. 20% EtOH
200 bar, 30°C
36 mls injected

12x36 ml @30 mg/ml
12.96 grams/hour

“Good Enough” can be Expensive!

- If one intends to run one, ten, or even 100 stacked injections any old separation may be “good enough”.
- One to 100 injections may take less than 1 to more than 16 hours.
- Semi-prep SFC costs \$60-\$600/hr.
- If throughput is half optimum, it could cost an additional \$5 to \$4800.

If this were routine, add as much as \$832,000/year to the cost of separations.

It appears “good enough” is good enough IF you don’t care how much it costs (somebody else’s budget?).

Summary

- For bigger jobs, extra method development time can save a great deal of money.
- Try to use highest surface area packings
- Use the smallest particles available
- Try to stay super-optimum in flow
- Try to avoid long retention times
- A very large column at much below optimum flow does not produce higher throughput if the smaller column is run at the same flow rate, but it does cost a lot more.