



# Packed Column SFC: Impacting Project Progression from Drug Discovery through Development

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# *Outline*

- Why SFC? Educational Opportunities
- Harmonized Method Development Strategy, Achiral and Chiral
- Discovery Purification Case Studies (SFC vs. LC)
- Structure Elucidation Group (SEG) Case Studies (SFC vs. LC)
- Technology Development Opportunities



# *Chromatographic Techniques*

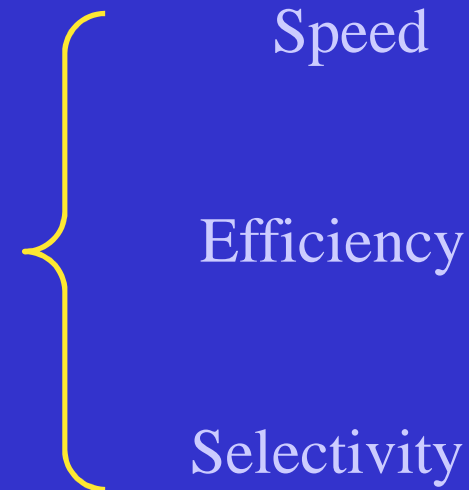
Gas Chromatography

Capillary SFC

*Packed Column SFC*

Liquid Chromatography

Capillary Electrophoresis



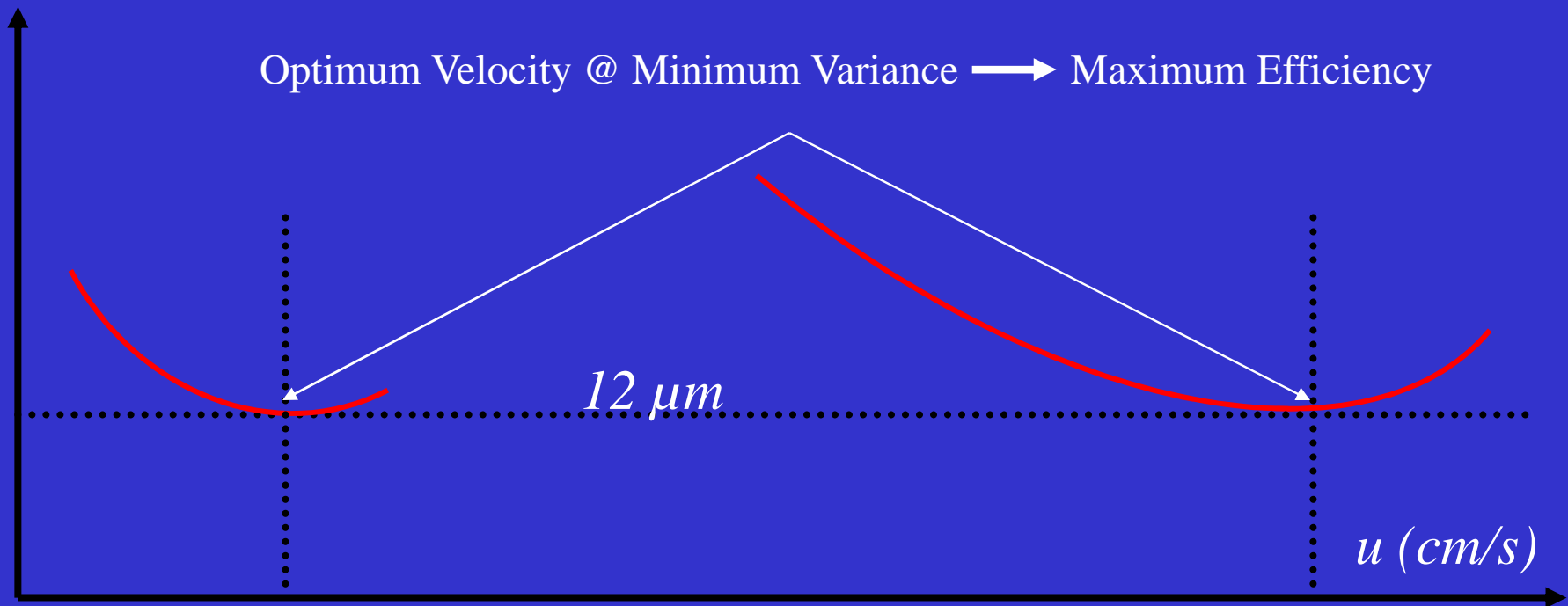


# Efficiency for *pSFC* and *LC*

*HETP* ( $\mu\text{m}$ )

$$H = A + B/u + C \cdot u$$

Optimum Velocity @ Minimum Variance  $\longrightarrow$  Maximum Efficiency



*1 mm/s*

*5 mm/s*

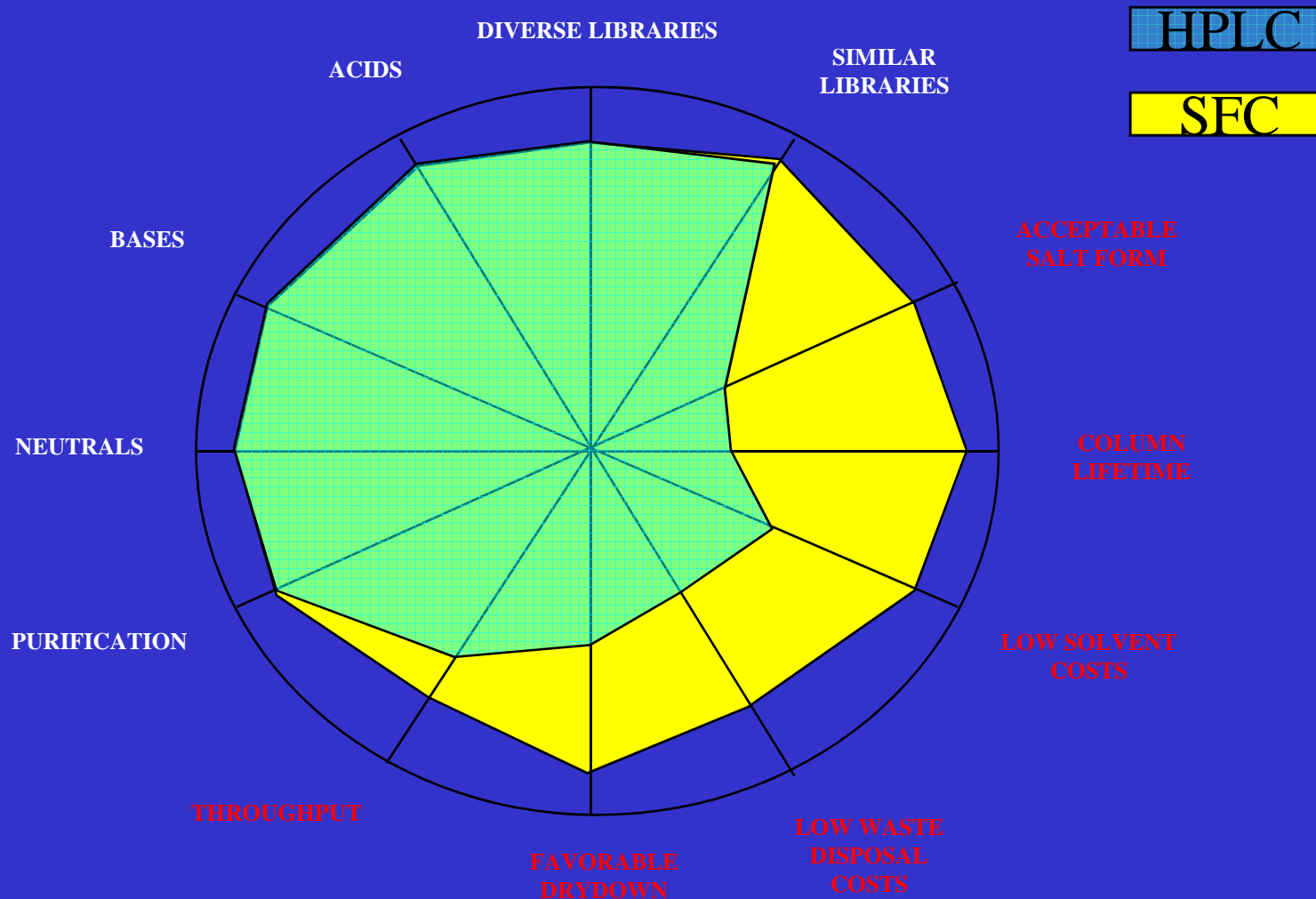
*100mm L x 4.6 mm i.d. x 5  $\mu\text{m}$*

*Hexane/IPA*

*CO<sub>2</sub>/MeOH*



# Spidergram for Pharmaceuticals



Slide courtesy of B. Farrell, Pfizer LJ



# *Advantages of Using CO<sub>2</sub>*

- Viscosity is ~100x smaller than liquids
  - Low pressure drops
  - Longer columns
  - Higher Plate counts
- Solute diffusion ~10-50x faster than liquids
  - Higher Speed
  - Fast re-equilibration
  - High throughput
- Fast mass transfer – Higher efficiency
- Inert, Safe, No Disposal Cost, **Inexpensive\$\$\$**
- Compressible liquid adjusted via Pressure and Temperature
  - \***Selective tuning opportunities**

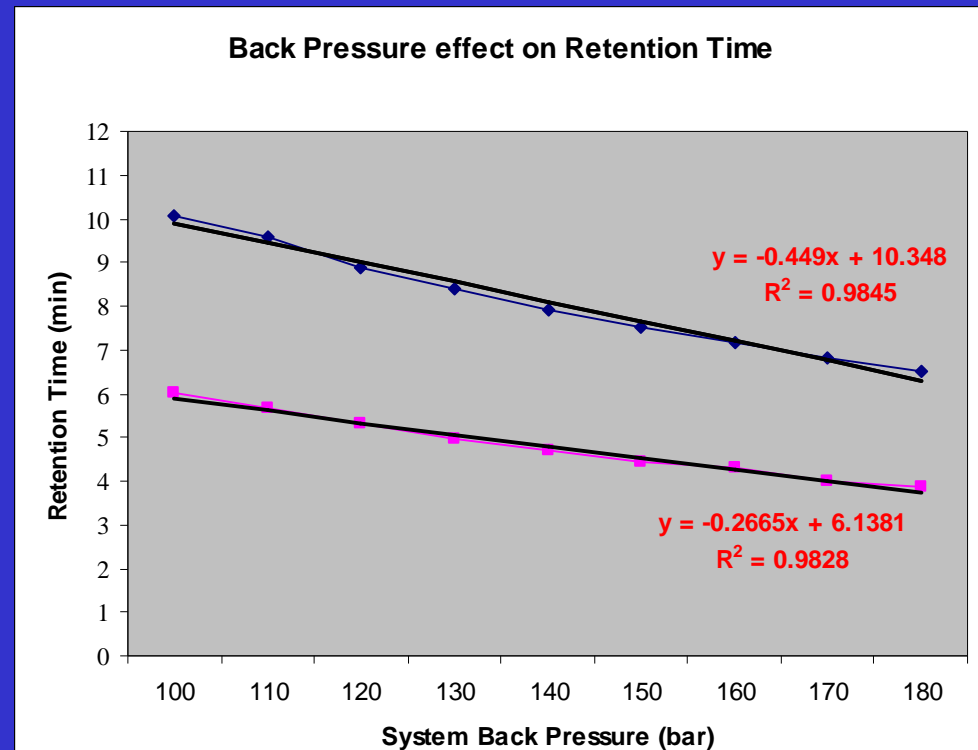


# Selective Tuning Effect on Retention - Pressure

- Can we take advantage of the compressible nature of CO<sub>2</sub> and realize chromatographic gains?

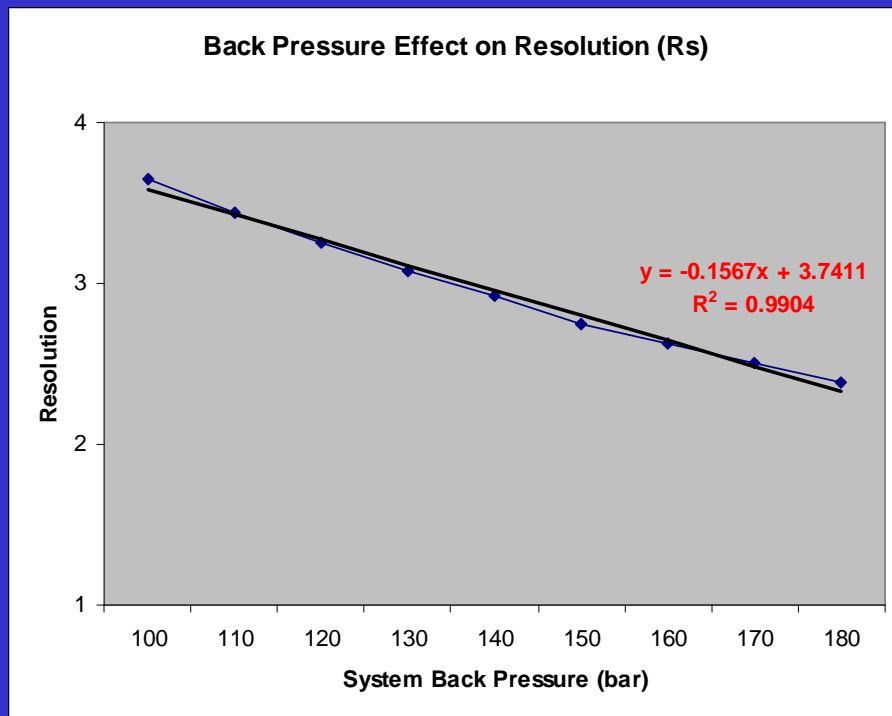
↑ Pressure ↑ Density of CO<sub>2</sub>

↑ Strength ↓ Retention Factor

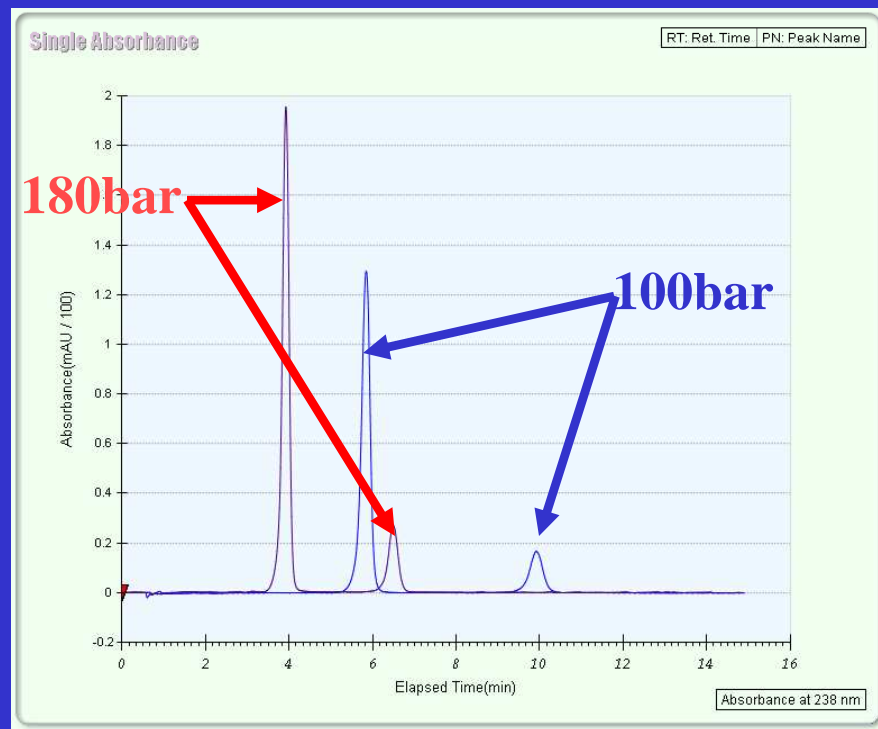




# Pressure Tuning effect on Resolution



Conditions: 2-EP, 150x4.6mm, 5um  
F: 5.0 mL/min  
T: 40C  
MP: 80:20 CO<sub>2</sub>:MeOH  
Steroid sample mix

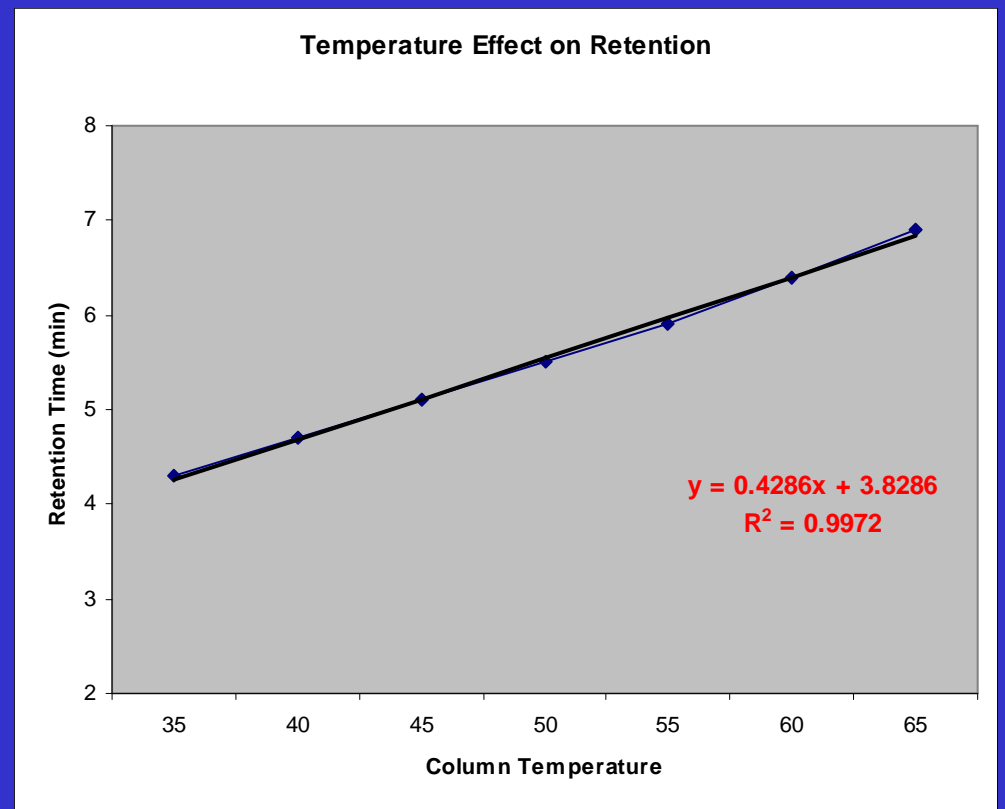




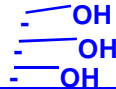
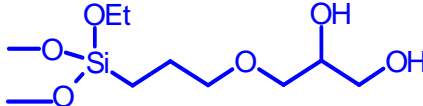
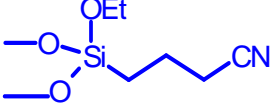
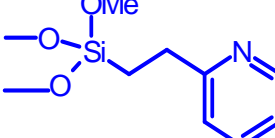
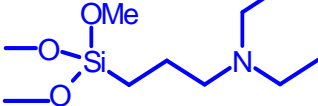
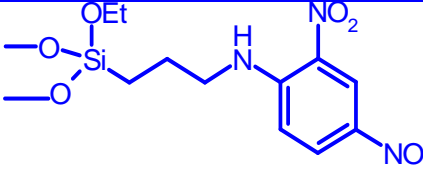
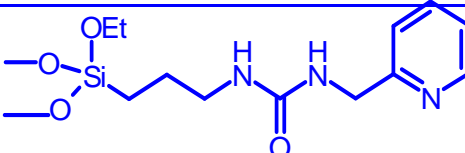
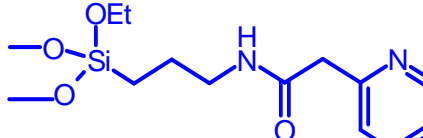
# *Selective Tuning Effect on Retention - Temperature*

- Temperature is much more sensitive in SFC than LC
- Selectivity can be effectively adjusted

↑ Temperature ↓ Density of CO<sub>2</sub>  
↓ Strength ↑ Retention Factor



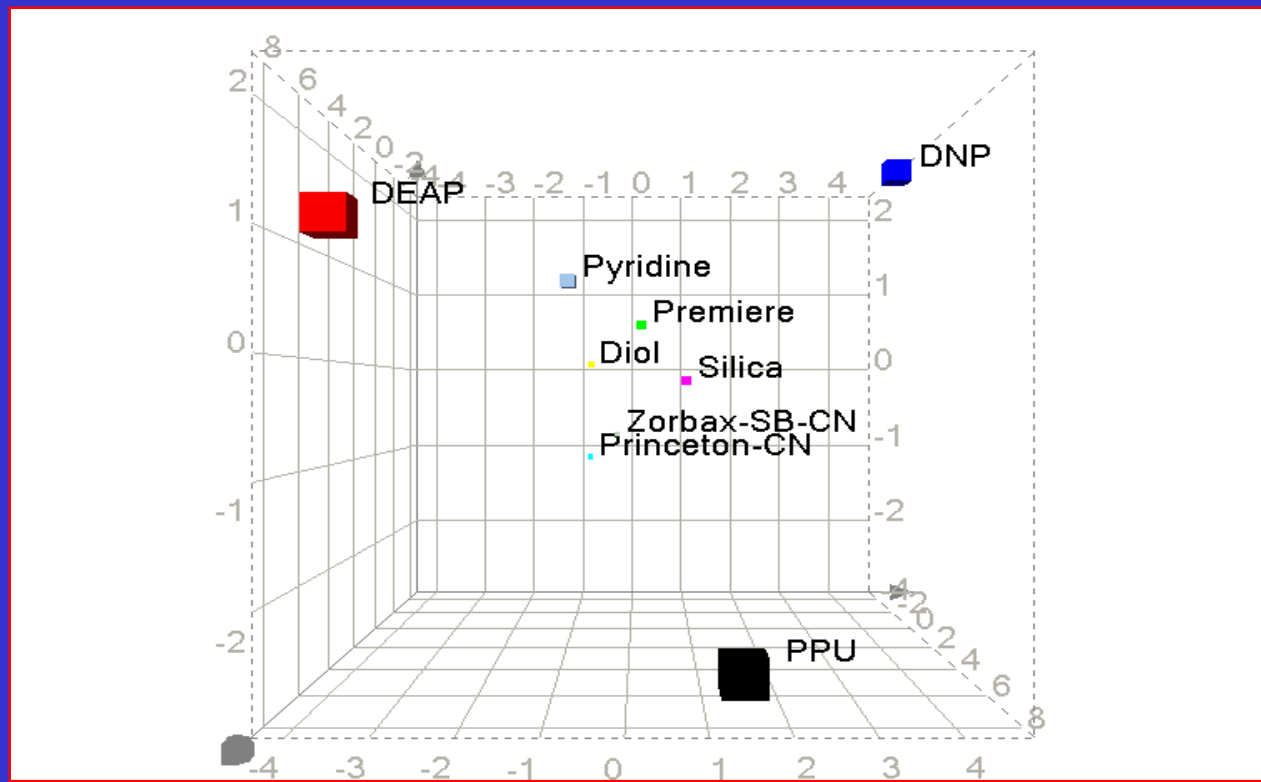
# Achiral SFC Stationary Phase Selection

<b>Silica</b>	
<b>Diol</b>	
<b>Cyano (CN)</b>	
<b>2-ethylpyridine</b>	
<b>N,N'-diethylaminopropyl (DEAP)</b>	
<b>2,4-dinitrophenyl (DNP)</b>	
<b>2-pyridylpropylurea (PPU)</b>	
<b>2-pyridyl-propylamide (Premier)</b>	



# Orthogonal Selectivity

- Generic screening method approach
- Selectivity of new stationary phases are evaluated using principal component evaluation (vector space transform to reduce multi-dimensional data sets)
- Pharmaceutical compounds (neutral apolar, neutral polar, bases, acids)





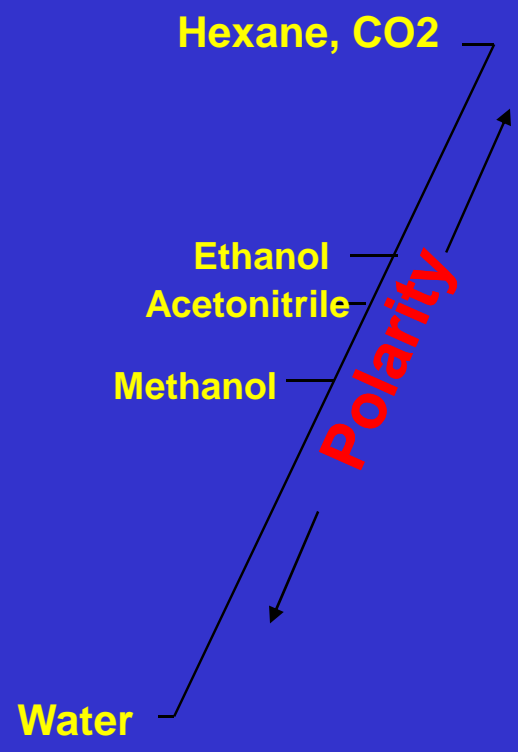
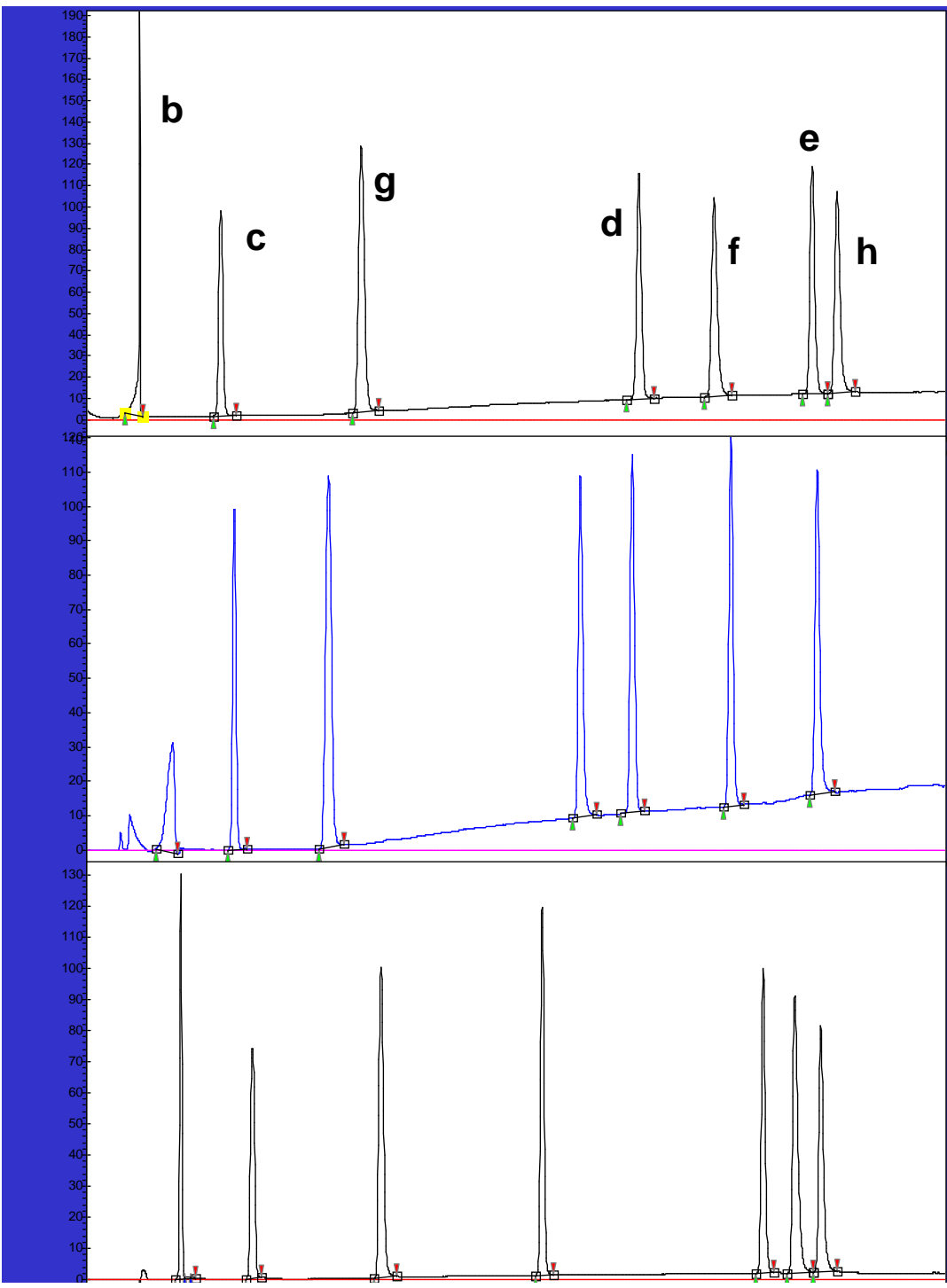
# *Harmonized Achiral Method Development Strategy*

## •Tier-1 SFC Approach

- Columns: PPU, DNP, DEAP 250x4.6mm, 5um
- Mobile Phase: CO<sub>2</sub> with 5-60% modifier, linear gradient in 8-min, 1.5-min pre-equilibration, 9.5-min injection cycle
- Modifier:
  - 1: MeOH w/ 20mM Ammonium Acetate
  - 2: MeOH 0.2% IPAm
  - 3: MeOH
- Back Pressure: 150bar
- Temperature: 40C
- Flow Rate: 5.0 mL/min

\*90% of proprietary compound success with Ammonium Acetate, elute highly polar compounds

- a: Benzene
- b: Acetophenone
- c: Benzophenone
- d: *p*-Toluic Acid
- e: Methyl Paraben
- f: Acetanilide
- g: Caffeine
- h: Uracil





# *Harmonized Chiral Method Development Strategy*

## •Tier-1 SFC Approach

- Columns: AD-H, OD-H, AS-H, OJ-H, IC 150x4.6mm, 5um
- Mobile Phase: CO<sub>2</sub> with 5-50% modifier, linear gradient in 5-min, 1.0-min pre-equilibration, 6.0-min injection cycle
- Modifier:
  - 1: 1:1 MeOH:EtOH
  - 2: 1:1 MeOH:EtOH w/ 0.2% IPAm
  - 3: 1:1MeOH:EtOH w/ 0.1% TFA and 0.1% IPAm
- Back Pressure: 150bar
- Temperature: 40C
- Flow Rate: 5.0 mL/min
- Total Screen Time: 1.5-hrs, 15-chromatograms, manual review... time...



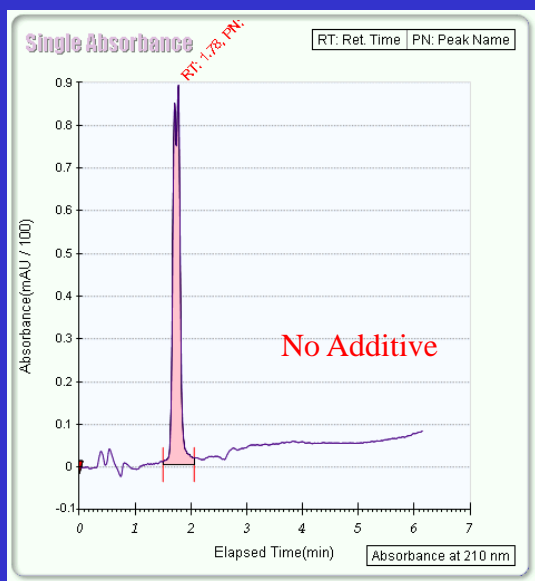
## *Chiral Screening Results*

- 120 Novel Compounds: acidic, basic, neutral
- 95.6% observable separation\*
- Column Success:
  - AD>OD>OJ>IC>AS
- Additive Success:
  - TFA/IPAm\*>IPAm>MeOH

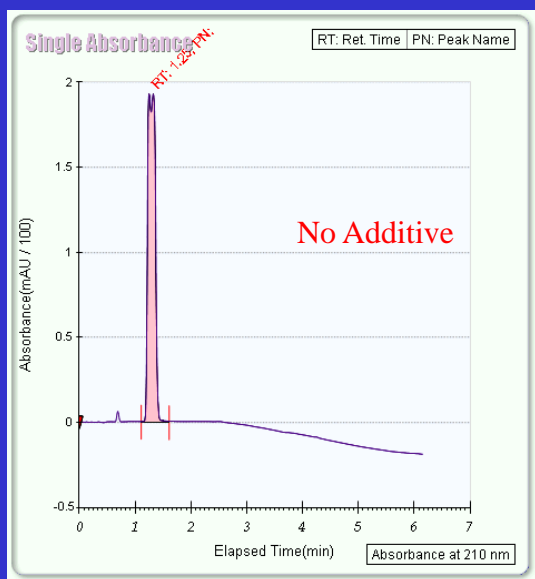
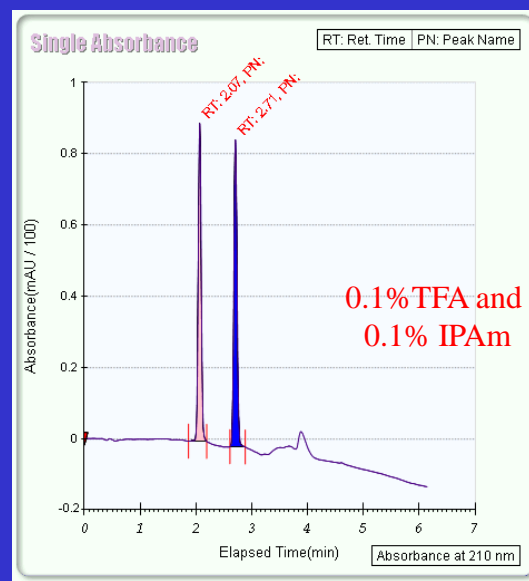
\*Positive effect on basic/neutral compounds



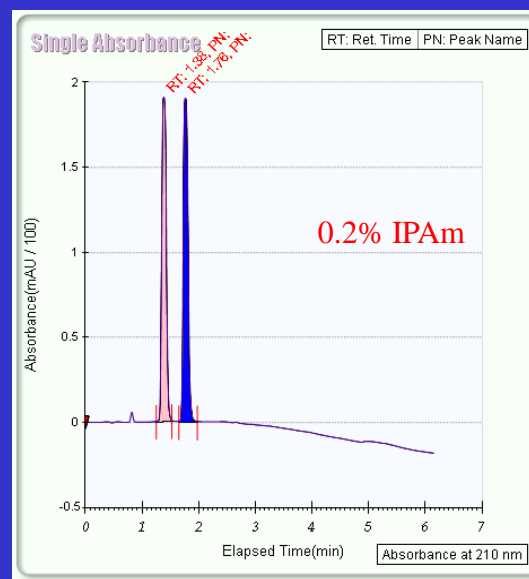
# Chiral Additive Effect



Neutral Compounds



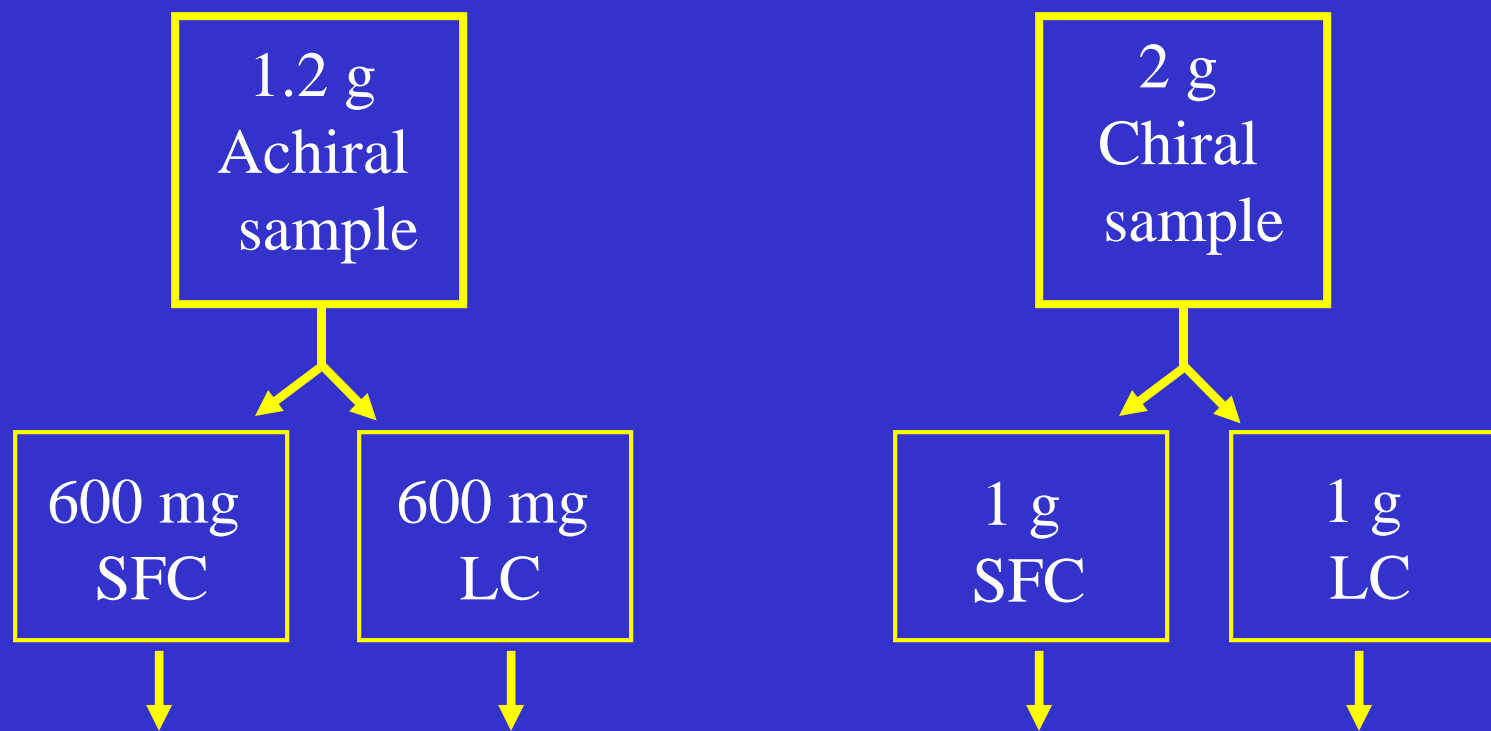
Basic Compounds





# *Discovery Purification*

## *case studies*

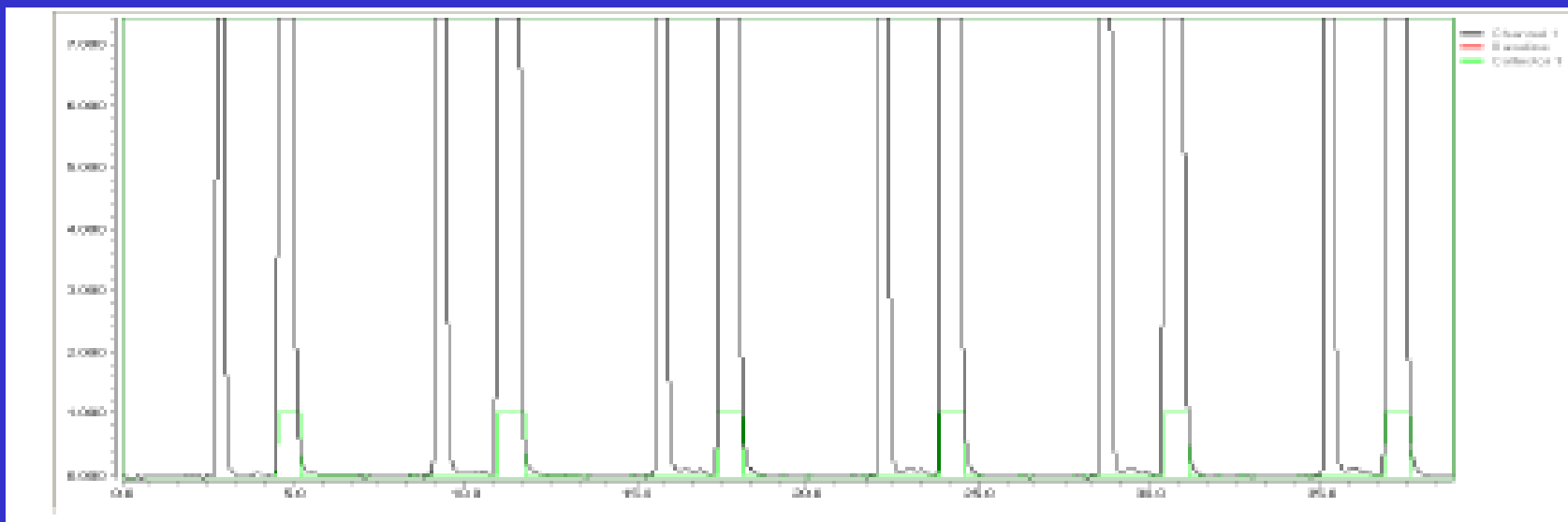


Compare time and solvent cost



# Discovery Purification

## Achiral case study



Thar 70

Pyridine 21x250mm, 5 $\mu$ m

70:30 CO<sub>2</sub>:MeOH, 60mL/min

Sample: 600 mg, 6-injections

Total run: 40 min

Solvent consumed: 720 mL MeOH

Fraction volume: ~ 90 mL MeOH

Evaporation Time: ~ 60 min

### Cost analysis:

Solvent cost = \$ 3.59

Project Time: 3 hours



# *Discovery Purification*

## *Achiral case study*

### LC, Purification

Waters MS-directed

Auto-Purification Platform

Luna C-18(2), 21.2x150mm, 5um

A: H<sub>2</sub>O w/ 0.1%FA

B: CH<sub>3</sub>CN w/ 0.1%FA

Gradient: 70% A to 30% A in 10 min

Flow: 27 mL/min

Sample: 600 mg, 6-injections

Total run: 75 min

Solvent consumption: 2 L

Fraction volume: ~130 mL

Evaporation Time: ~360 min

### Cost analysis:

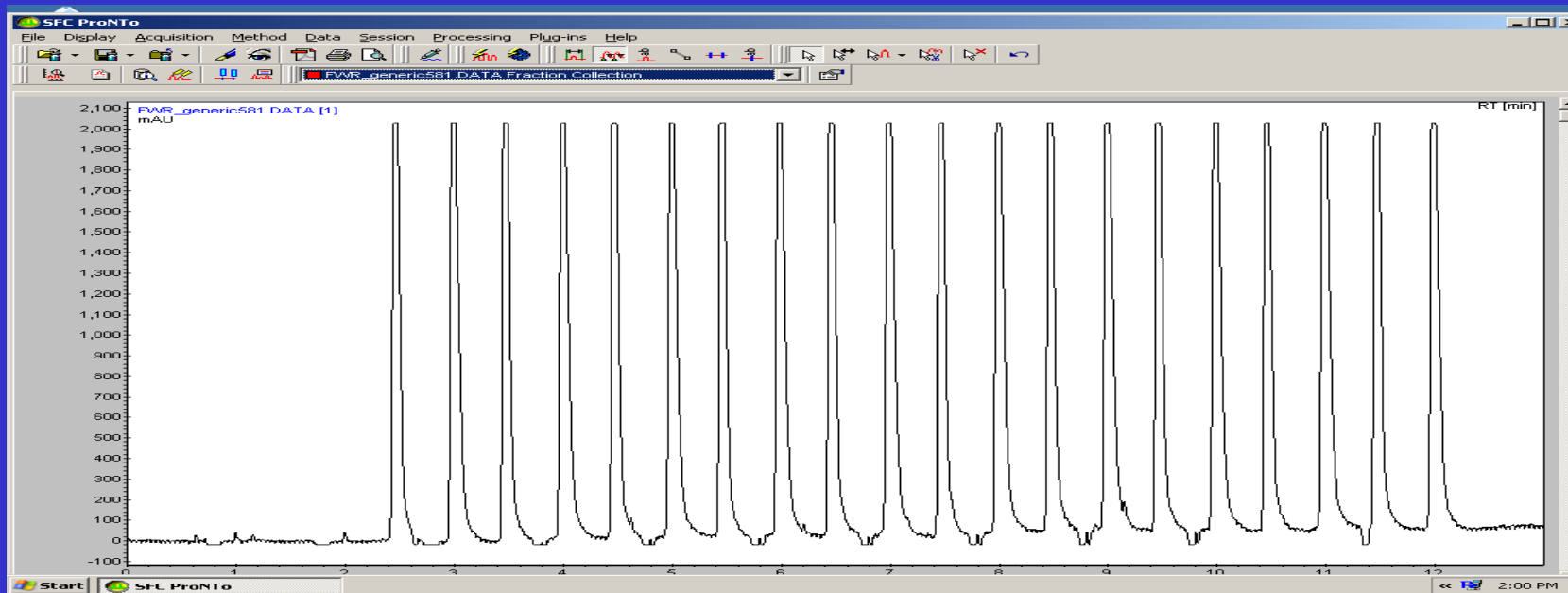
Solvent cost = \$ 20.91

8-9 hrs. for project completion



# Discovery Purification

## Chiral case study



Berger MG-II

Chiralcel AD-H, 21x250mm, 5um

80:20, CO<sub>2</sub>:MeOH, 60mL/min

Sample: 1 g racemic material, 10-injections

Total run: 14 min

Solvent consumed: 170 mL MeOH

Fraction volume: ~ 30 mL MeOH/enantiomer

Evaporation Time: ~ 60 min

Cost analysis:

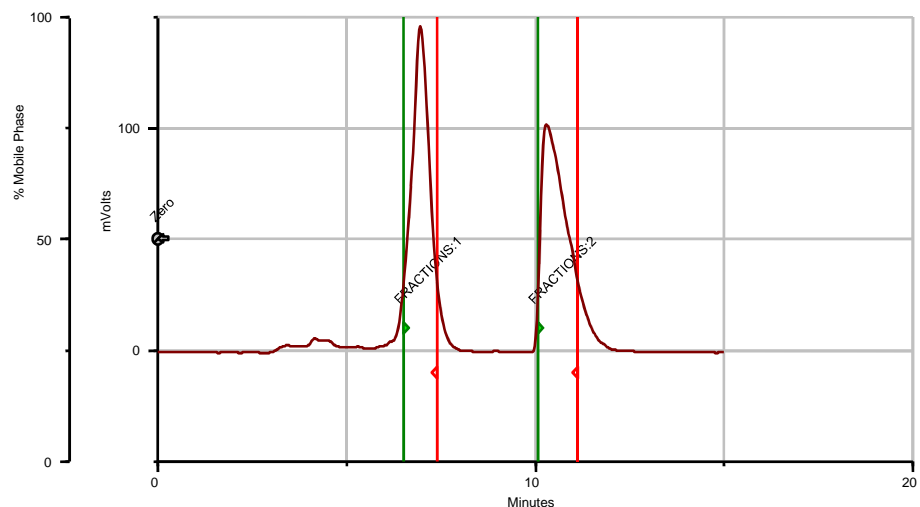
Solvent cost = \$ 1.15

2-3 hrs. for project completion



# Discovery Purification

## Chiral case study



Injection Volume: 0.5 mL (load=25 mg)

Gilson Purification Module  
Chiralcel OD-H,  
21x250mm, 5 $\mu$ m  
70:30, Hexane:EtOH, 12mL/min  
Sample: 1 g racemic material, 40injs  
Total run: 260 min, (19X)  
Solvent consumption: 3.1 L  
Fraction volume: ~ 250 mL,  
each enantiomer (8X)  
Evaporation time: ~ 240 min (4X)



# *Discovery Cost/Time Analysis*

	SFC		LC		
	Solvents	Time spent (FTE)	Solvents	Time spent (FTE)	Cost Analysis
<u>Achiral</u>	\$3.59	< 3hrs.	\$20.91	8-9 hrs.	83% solvent 65% time
<u>Chiral</u>	\$1.15	2-3 hrs.	\$32.78	9-10 hrs.	96% solvent 82% time



# *Structural Elucidation Group*

## *LC isolation case study*

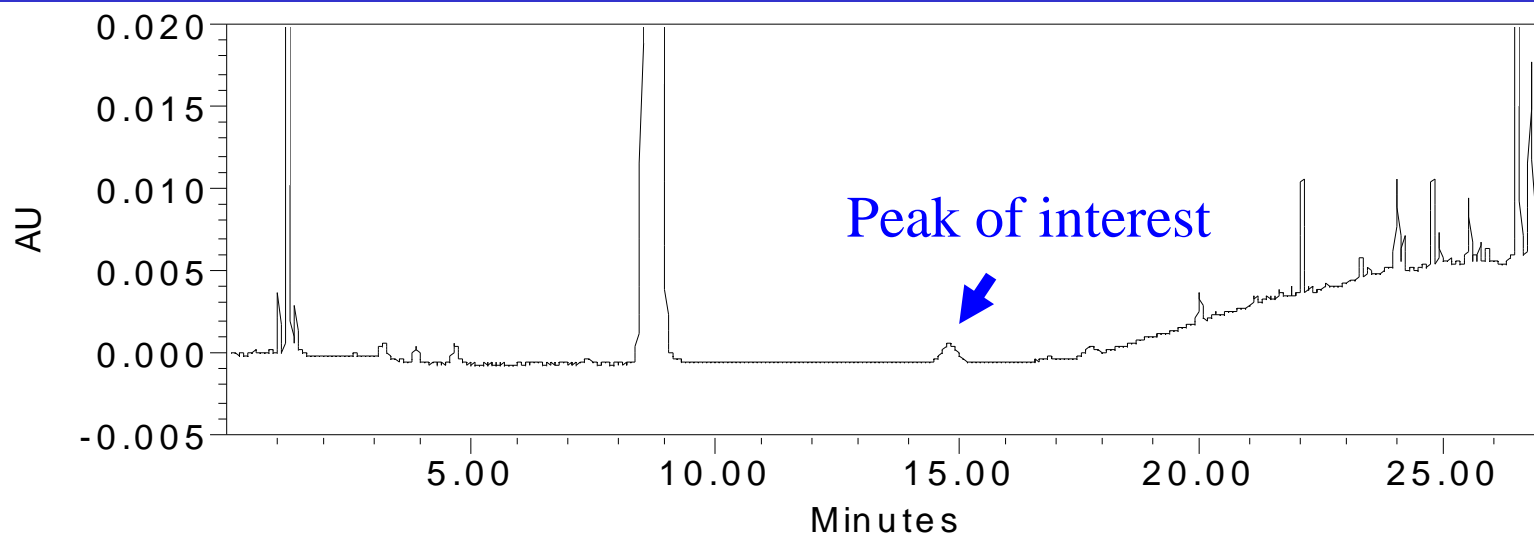
- 0.3% level impurity of interest
- Isolation and full structure elucidation of target peak

*Column: Ace 3 C8,*

*4.6x100mm*

*Mobile: 50mM NaClO<sub>4</sub>*

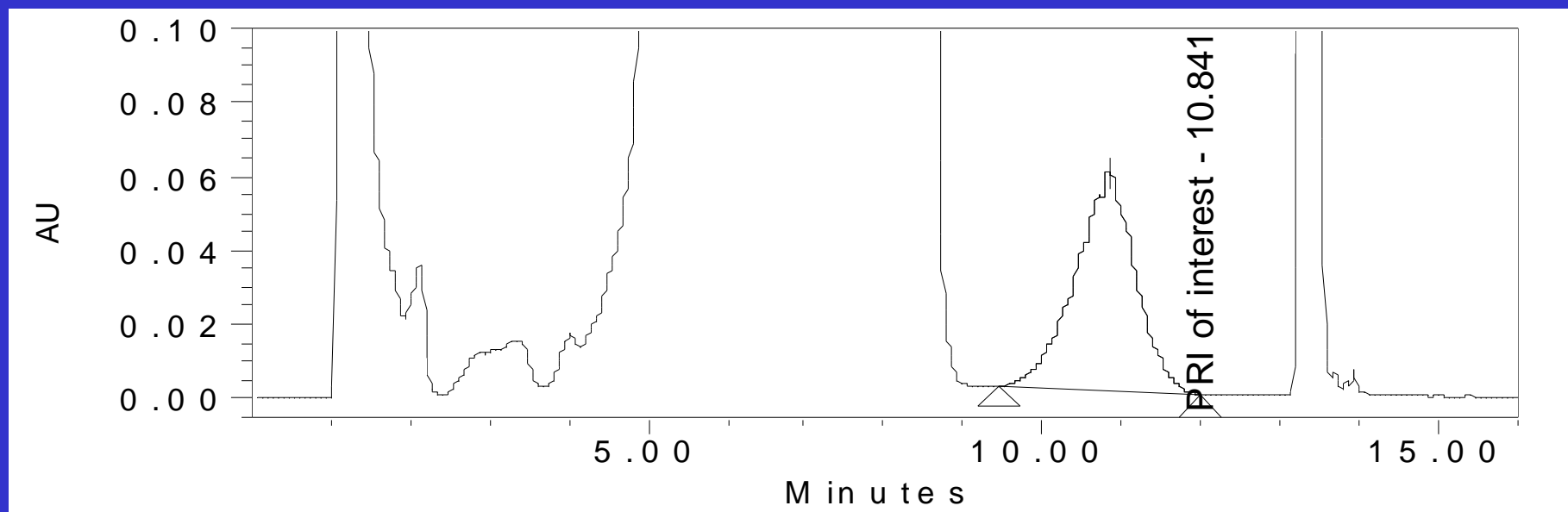
*0.1% H<sub>3</sub>PO<sub>4</sub> /ACN*





# Structural Elucidation Group

## LC isolation case study



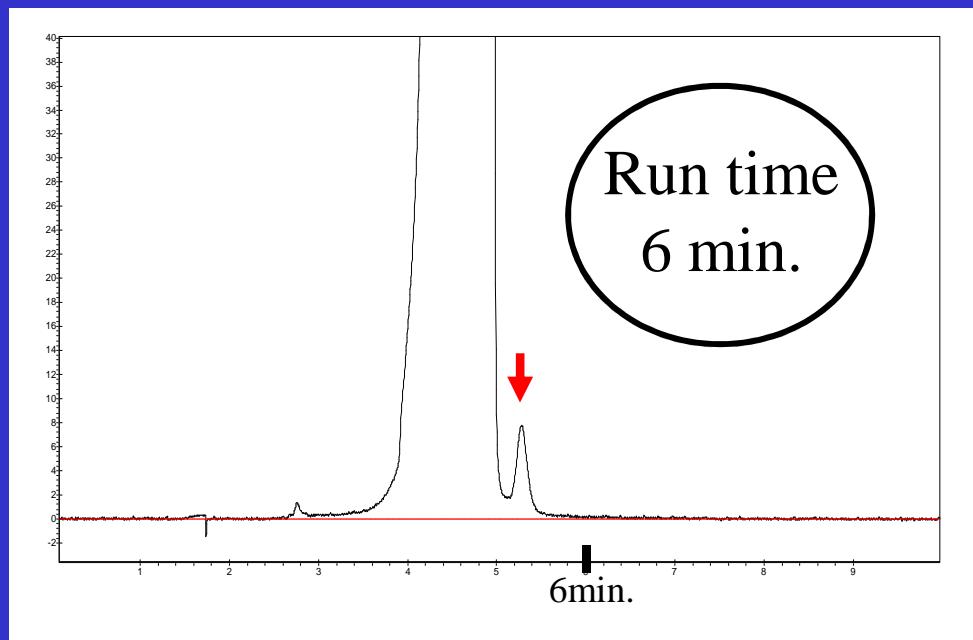
Method Development/Isolate – 1.5 days  
25x100mm, 59 mL/min, H<sub>2</sub>O:CH<sub>3</sub>CN w/ 0.1% TFA  
6-injections, 330-mg load/injection  
Dry Down – 1.5 days  
500 µg obtained → NMR  
Solvent Cost : \$49.37  
Turnaround: ~ 3 days.



# *Structural Elucidation Group*

## SFC stacked case study

- 0.3% level impurity of interest
- Isolation and full structure elucidation of target peak

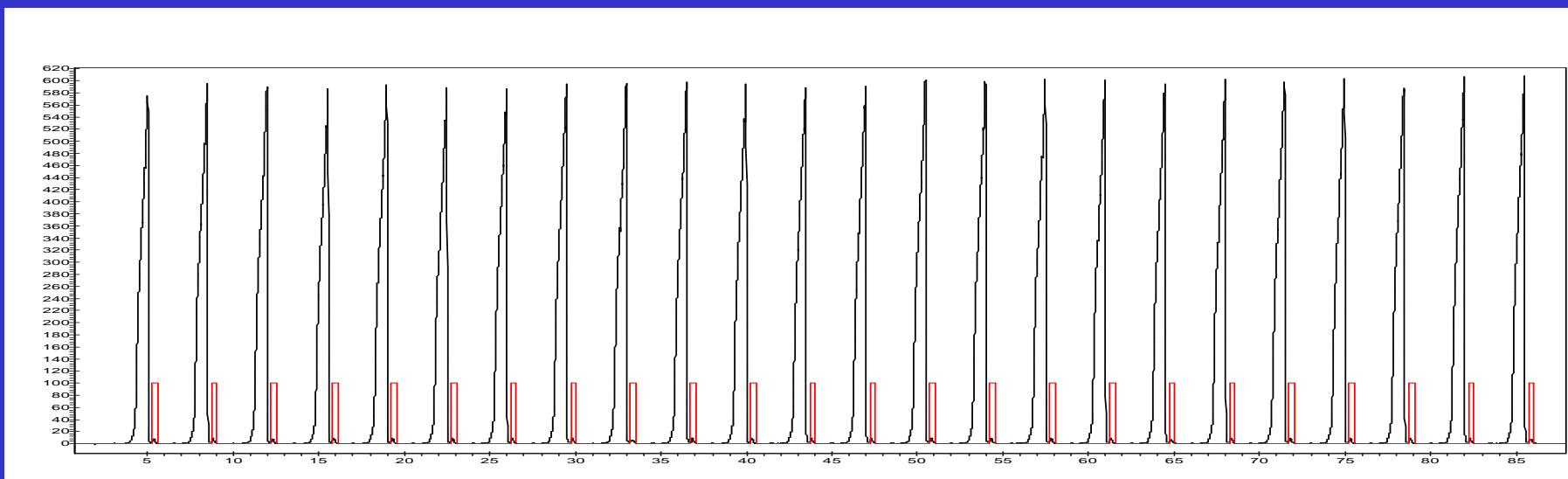


Column: PPU (Princeton)  
250mm x 10 mm, 5 $\mu$ m  
P = 140bar  
T = 35 $^{\circ}$ C  
F = 10.0ml/min  
Isocratic: 85:15 CO<sub>2</sub>:MeOH  
w/0.2% IPAm

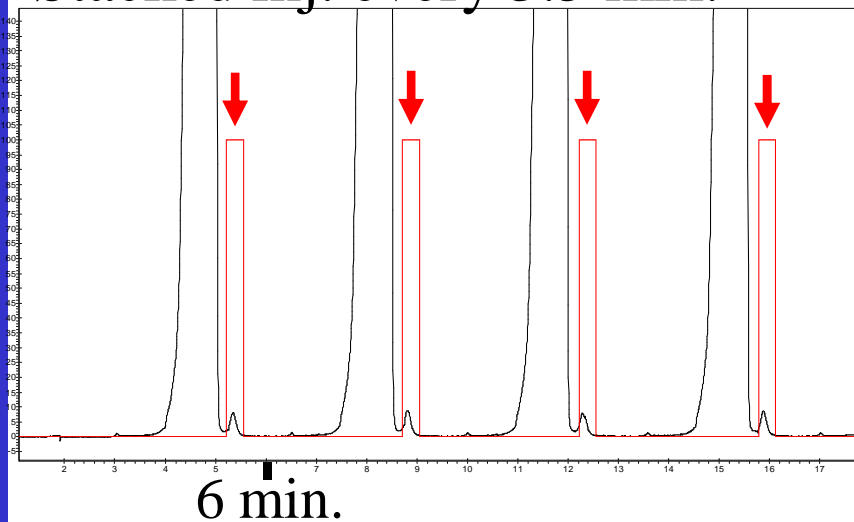


# Structural Elucidation Group

## SFC stacked case study



Stacked inj. every 3.5 min.

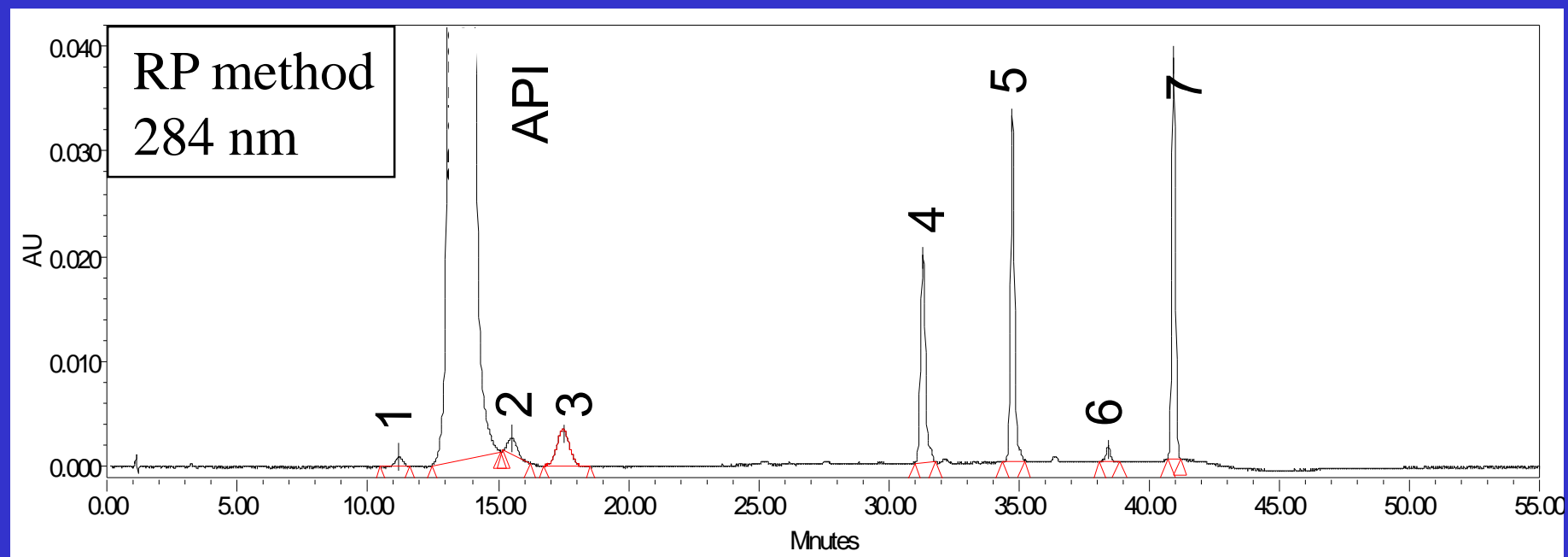


- Method Development (3 hrs.)
- 41 injections, 2.4 hours
- 350  $\mu$ g obtained  $\rightarrow$  NMR
- Solvent Cost : \$4.38
- \$4.38 vs. \$49.37  $\rightarrow$  91% savings
- Time spent: ~ 6 hrs.



# *SFC - API extraction*

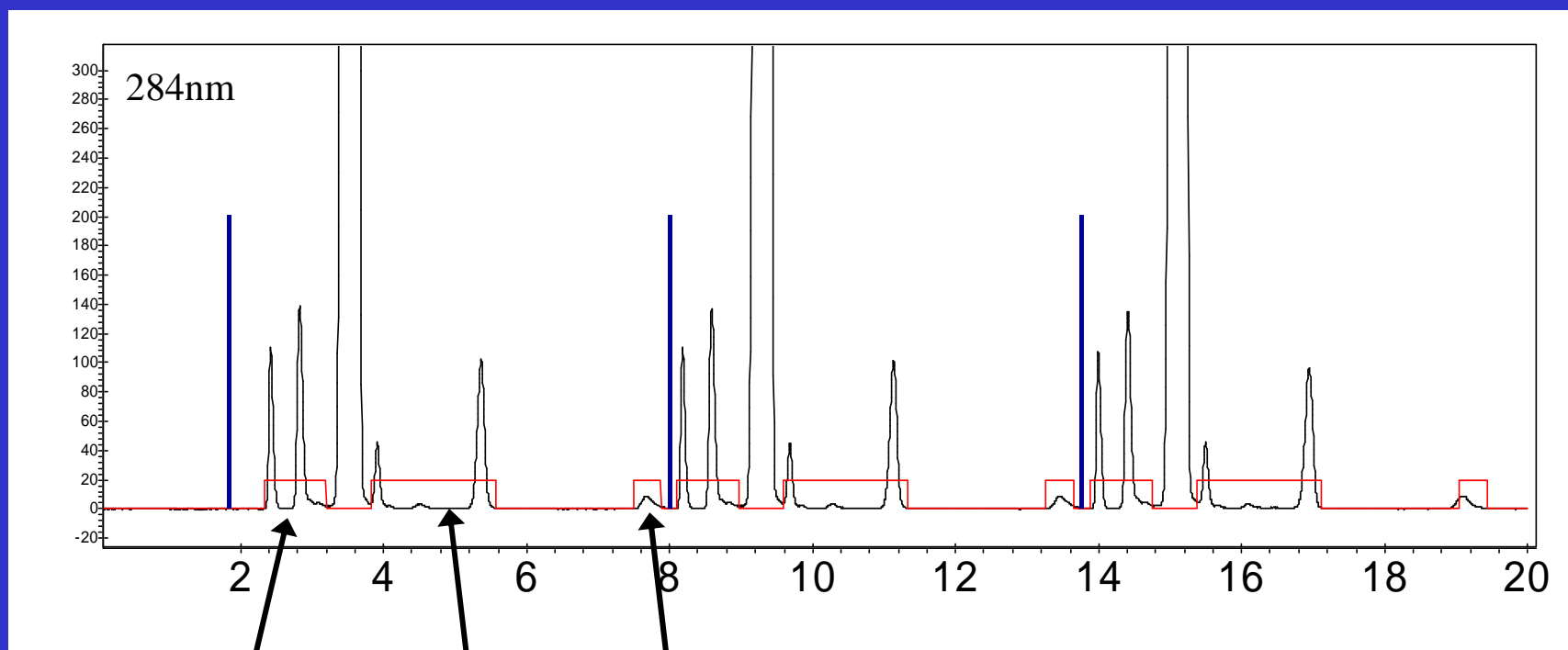
- Extracting API → enriching impurities → LC-NMR
- LC-NMR is very difficult when trying to park a low level impurity (0.1-0.2%) in the flow cell
- Minimal time investment
- LC-NMR possible now even on lowest level impurities





# *SFC – MBE/API extraction*

SFC chromatogram stacked injections



Fraction 1:

Peaks  
4, 6, 7

Fraction 2:

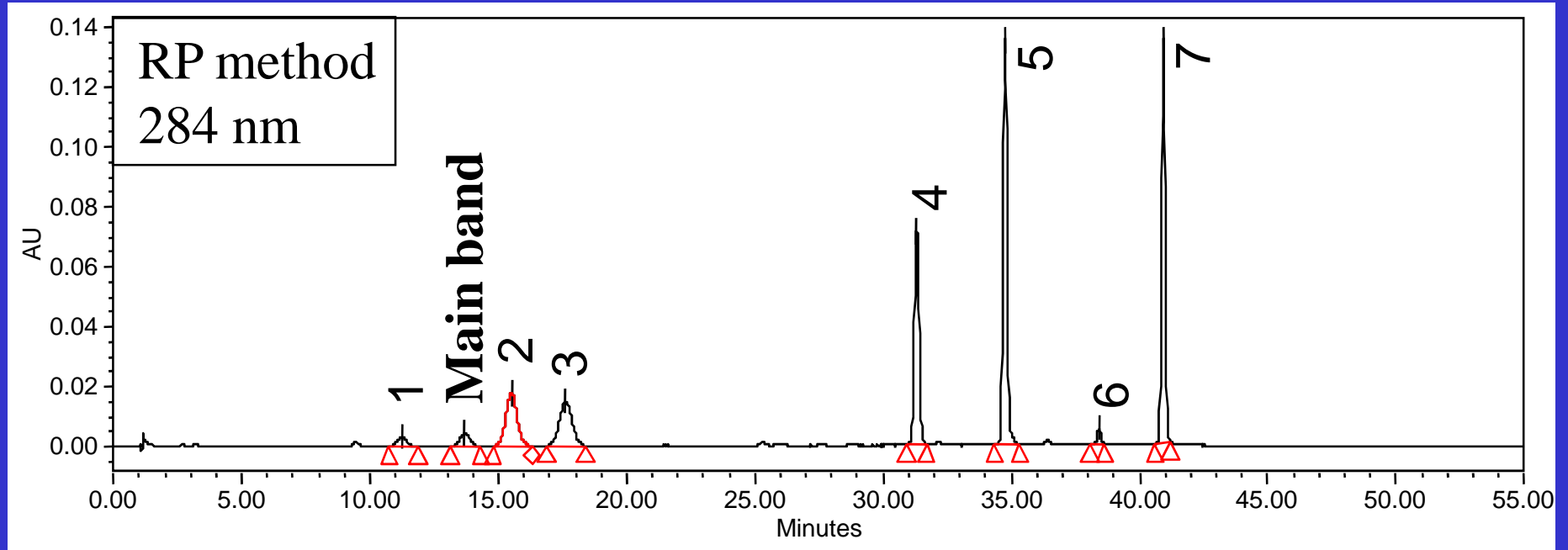
Peaks  
1, 3, 5

Fraction 3:

Peak  
2



# SFC – MBE/API extraction



peak	%Area		peak	%Area	
1	0.05	1.5	4	0.7	18.3
API	97.1	2.4	5	0.9	29.1
2	0.1	11.0	6	0.04	1.11
3	0.3	10.5	7	0.9	25.7

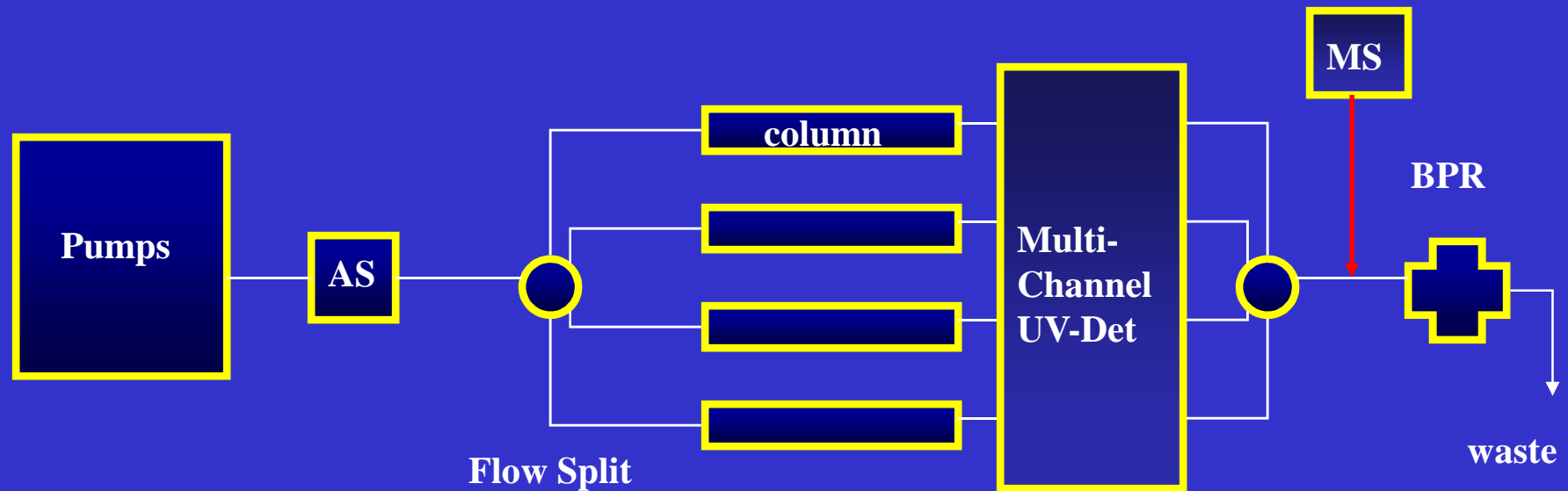
Significantly increase concentration (40X) and loadability of target impurities



*SFC Technology Development  
Opportunities*



# *SFC Parallel Screening*

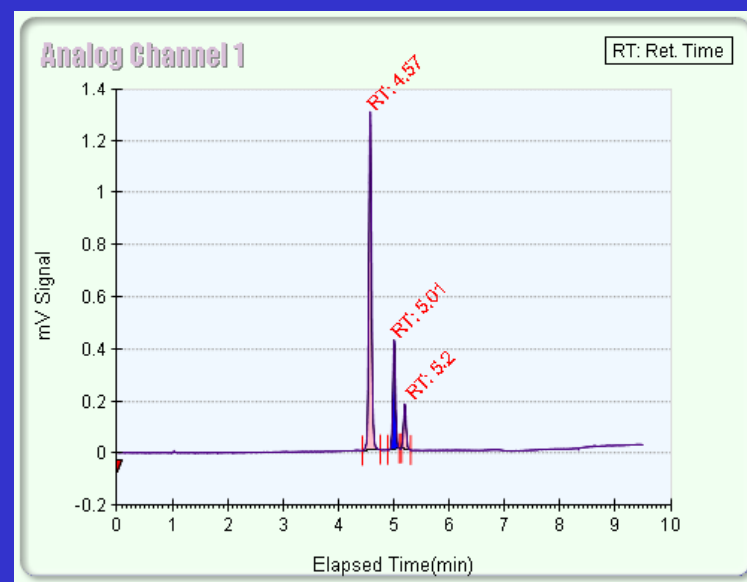
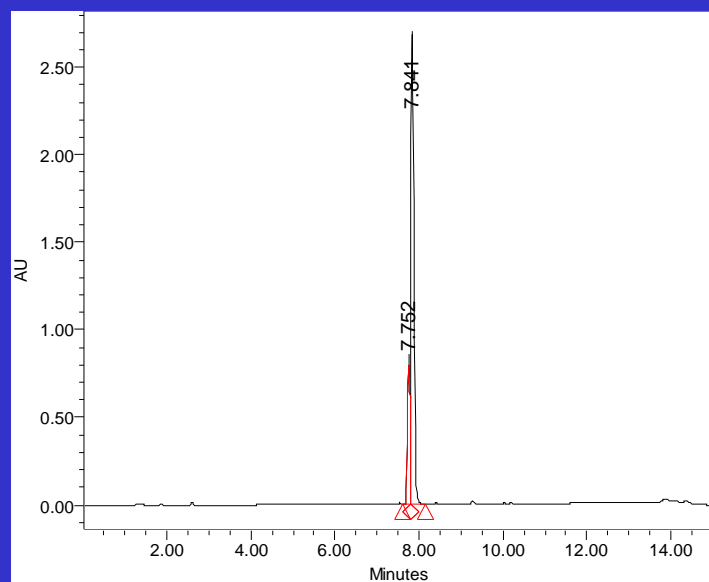
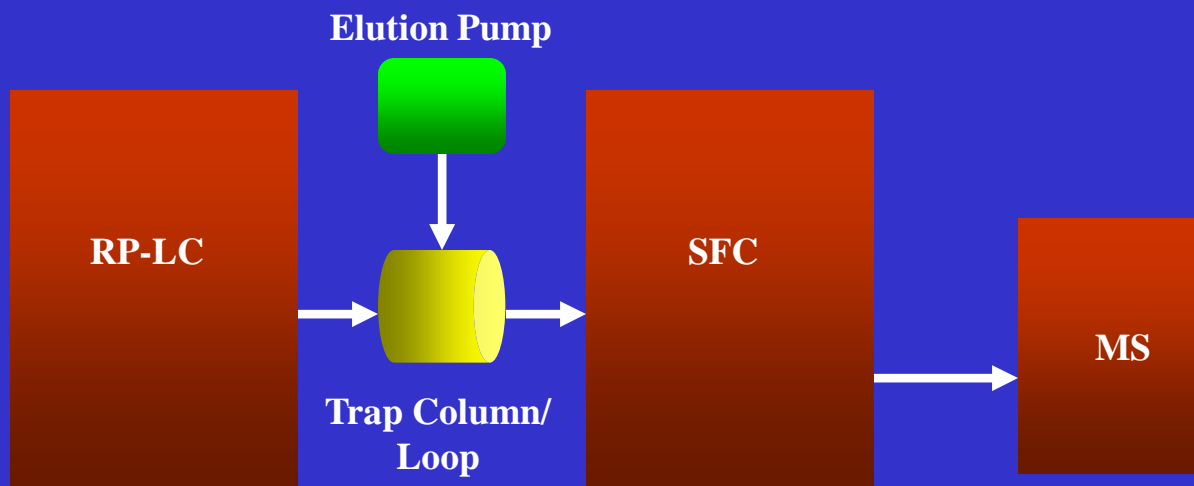


- Potential Technology Collaboration
- Breadboard platform built in AA, LC
- Connection with chromatographic database
- Multi-channel UV detector
- MS interface – MUX expensive...
- Low Cost MS – De-convolution “Smart” Software



## *2D LC-SFC-MS*

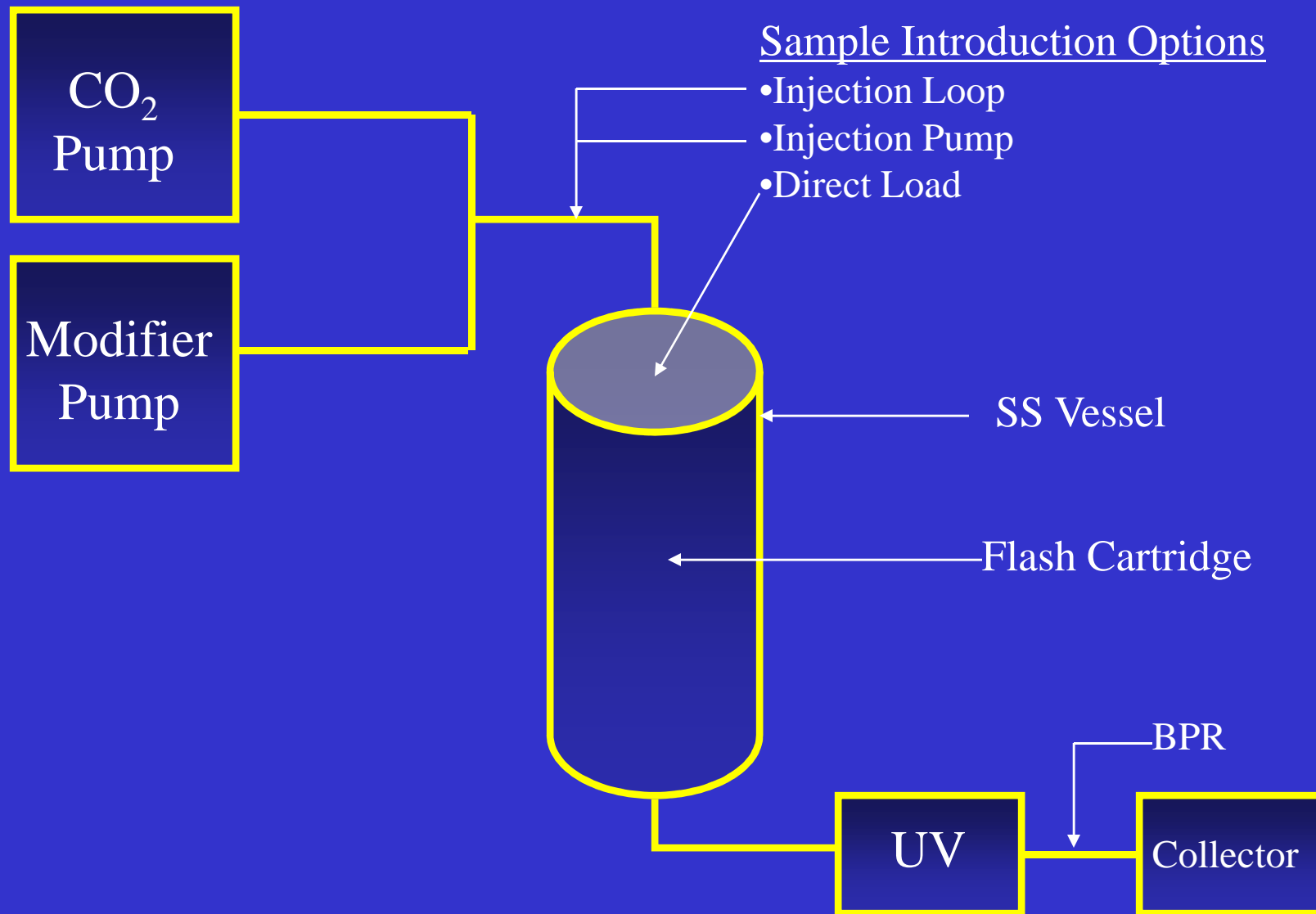
- 1<sup>st</sup> dimension is insufficient to separate analytes
- Direct a series of unresolved peaks onto a second column with different physico-chemical properties
- Due diligence main-band impurity detection
- 1<sup>st</sup> dimension non-MS friendly technique – impurity identification
- Fast method development for SFC impurity isolation
- Capitalize on 2<sup>nd</sup> dimension SFC speed



*Full-automation not yet achieved*



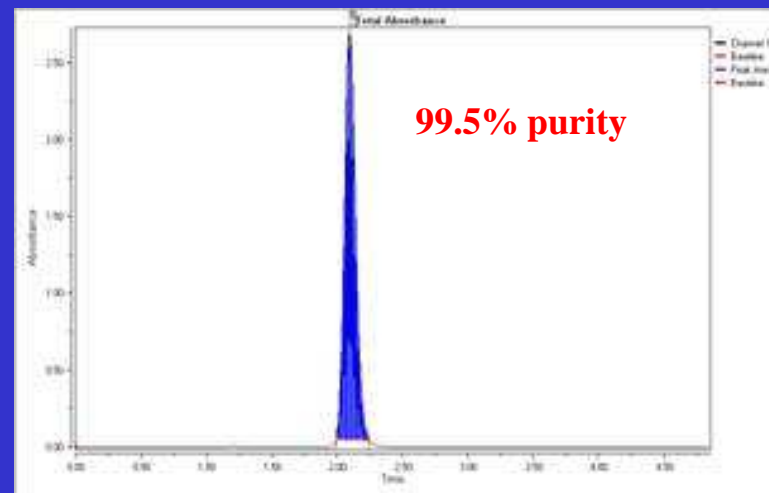
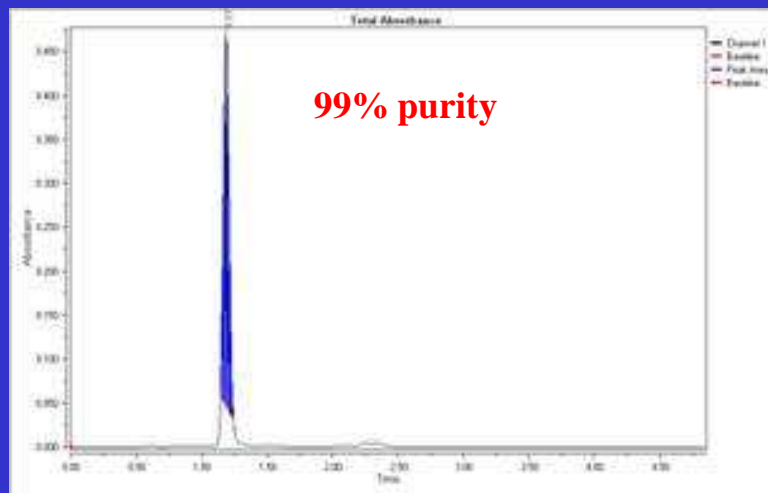
# Green Flash - Possibilities





# Green Flash - Results

- 1.3g of Acetaminophen and 2.2g of Benzoic Acid dissolved in 50-mL of MeOH
- Direct load on column
- Vessel pressurized with CO<sub>2</sub> at 150 g/min
- Modifier: 5-35% MeOH in 10-min, hold at 35% MeOH
- BP: 100 bar



*Data courtesy of Harbaksh Sidhu, Patent Pending, Thar Instruments*



# *Next Generation SFC-MS Directed Purification*

## What Do We Want:

- Preparative SFC/MS purification system compliant with workflow software for libraries and parallel singletons
- System with open access, walk-up functionality for med chem mg-scale and multi-gram scale purification of singletons
- Universal active flow splitter
- Universal open bed SFC fraction collector
- Flow rate up to 100 mL/min



# *Conclusions*

- Packed column SFC using CO<sub>2</sub> has many advantages over LC for analytical/purification applications
- Solvent cost savings - purchase, disposal
- Time savings - project turnaround time decreased  
- decrease in labor costs
- Selectivity impact – crucial for purification/impurity isolations
- CO<sub>2</sub> has low toxicity, green chemistry
- *Analytical Chemistry/Innovation hold the key to the Future!*



# Acknowledgements.....

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Ziqiang Wang

Rui Chen



*Thank you!*

*And*

*Questions?*