



Unleashing the Power of SFC: A Case Study in Drug Discovery and Development

“Our Road Map to Success”

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Outline

- Discovery Purification Laboratory case studies (SFC vs. LC)
- Structure Elucidation Group (SEG) case studies (SFC vs. LC)
- Highlight solvent cost & time savings

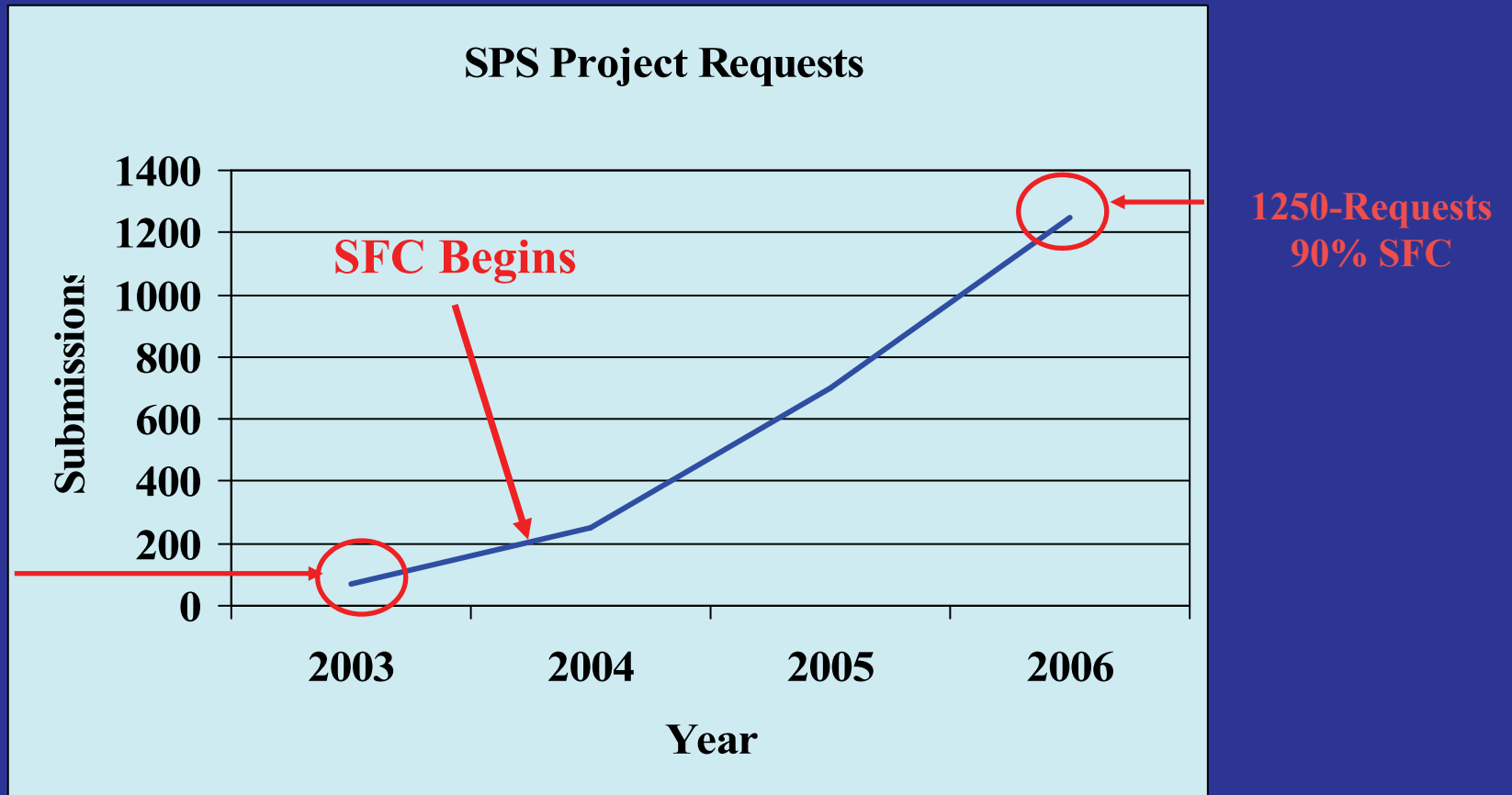


What is Success.....

- An Event That Accomplishes its Intended Purpose.
- An Attainment That is Successful.
- A State of Prosperity or Fame.
- Achiever.



Discovery Purification Group (DPG) By The Numbers.....Success



72-Requests
100% LC

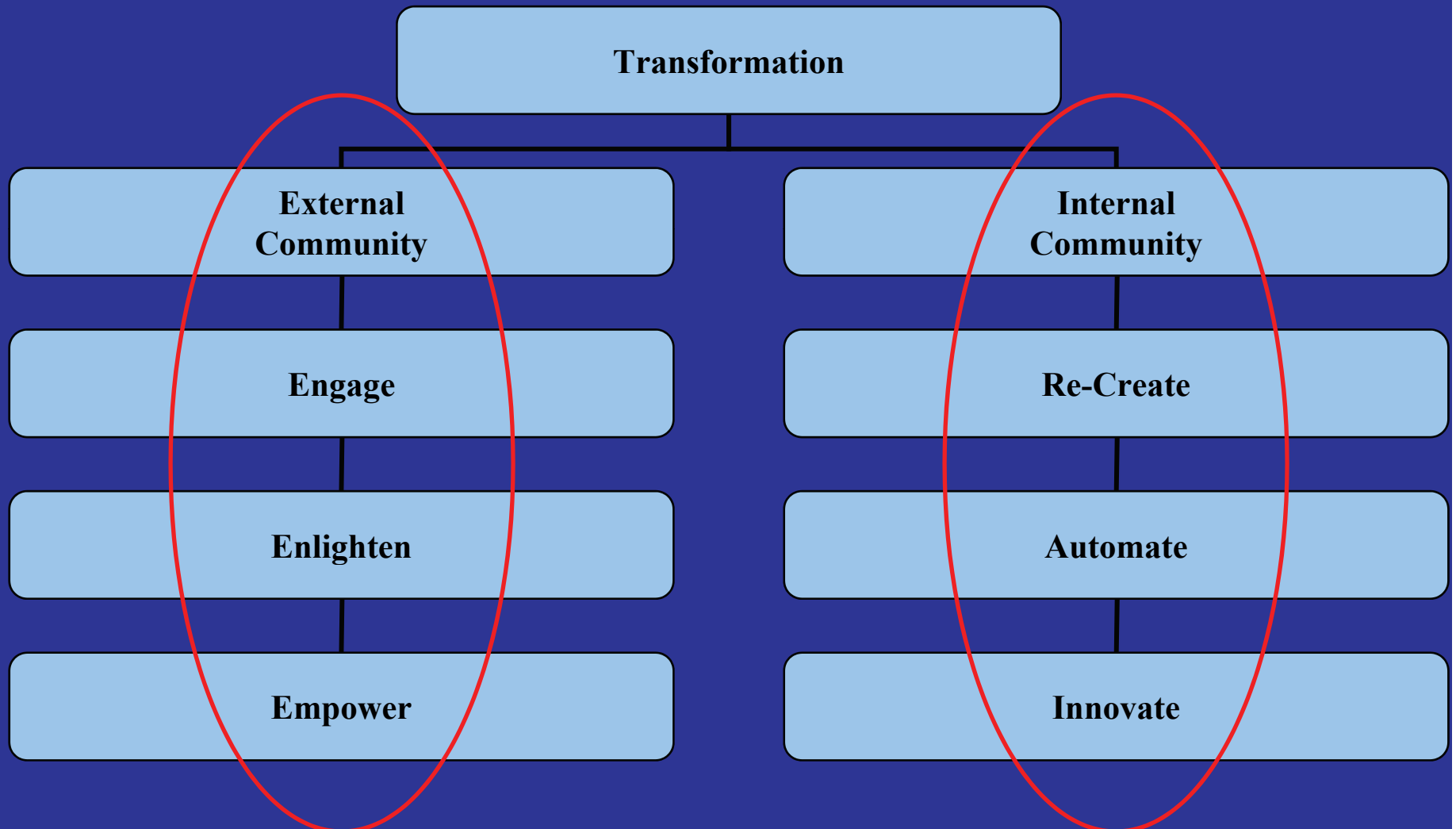
1250-Requests
90% SFC

2003: 2-FTE
1-Manager

2006: 3-FTE
1-Contractor
1-Manager



Transformational Change.....



Build it and they will come....

Critical to our survival...

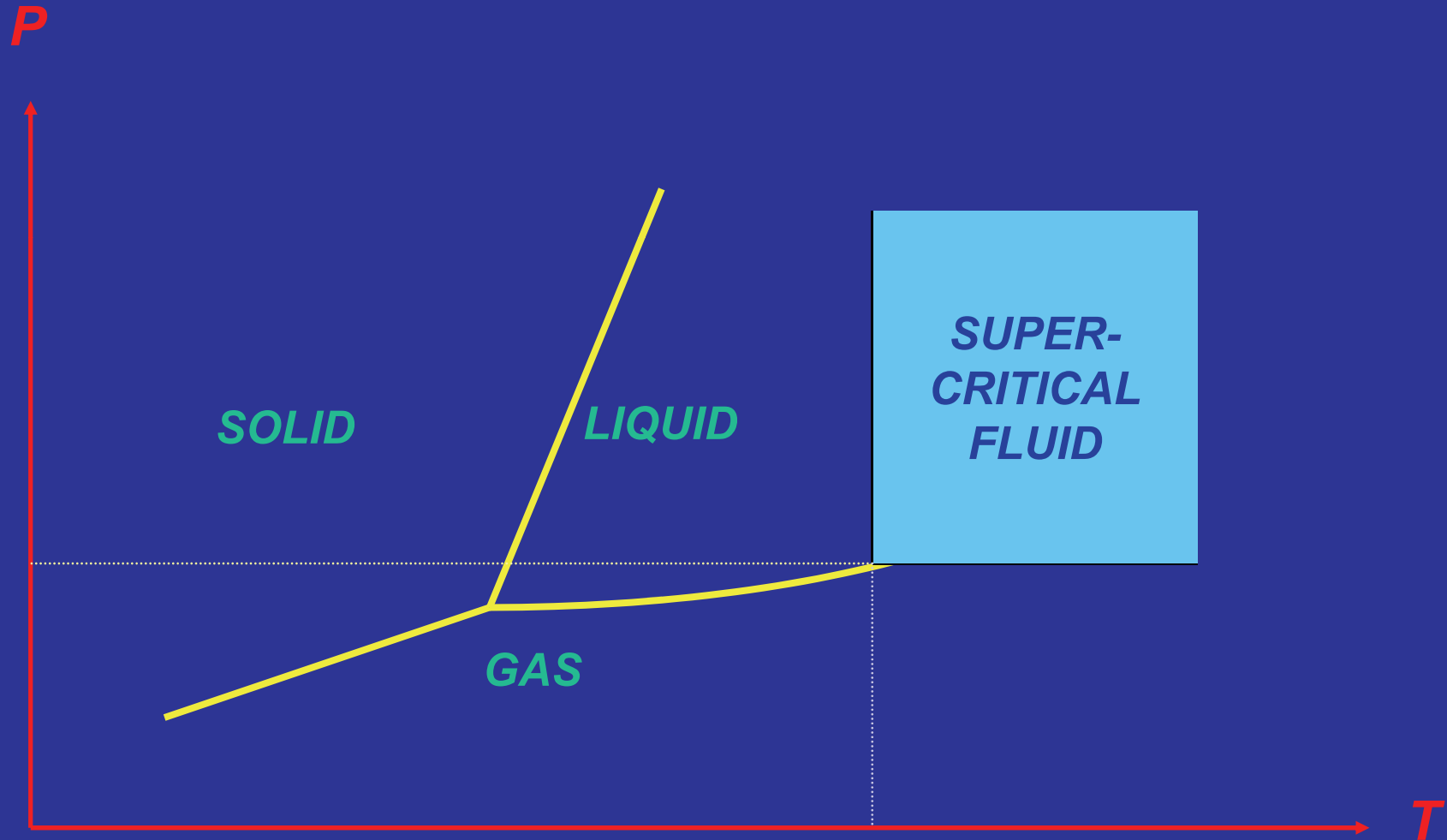


Innovation is the Answer.....





SFC is the Solution.....





DPG



High Throughput
~ 100 requests/month
40:60, achiral: chiral

Targeting major
components w/ known
structures

LC

Waters mass directed (3)
Gilson UV directed (3)

SFC

Berger MGII (1)
Thar 70 (1)
Thar 350 (1)

vs.

SEG



Not High Throughput
100% achiral

Targeting Low level
impurities of unknown
structures

LC

Waters mass directed (1)
Waters prep4000 (2)

SFC

Berger Minigram (1)

Since implementation of SFC
→ 93% of our isolations !!



Cost Analysis Theme

Substantial Operational Cost Savings:

- 1) Turnaround time savings \$\$
- 2) Significant reduction in organic solvent costs \$\$

- CO₂ purchase cost =

Cylinder:	\$2.22/L
Dewar:	\$1.00/L
Bulk delivery system:	\$0.11/L
- CO₂ disposal cost = \$ 0

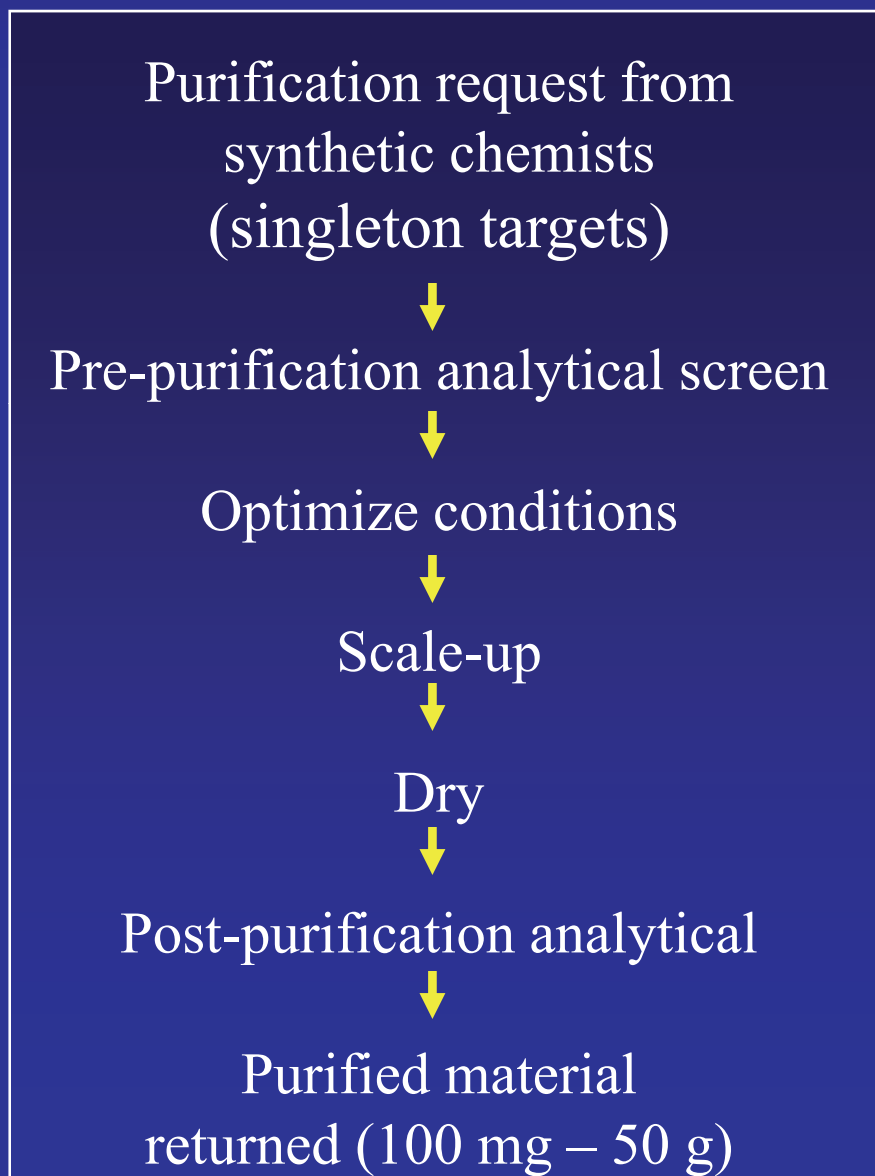
vs.

- solvent purchase cost =

ACN :	\$ 7.33/L	EtOAc:	\$ 6.49/L
MeOH:	\$ 2.42/L	EtOH:	\$ 8.38/L
Water:	\$ 2.75/L	Hexanes:	\$ 3.88/L
- solvent disposal cost = \$5.28/L



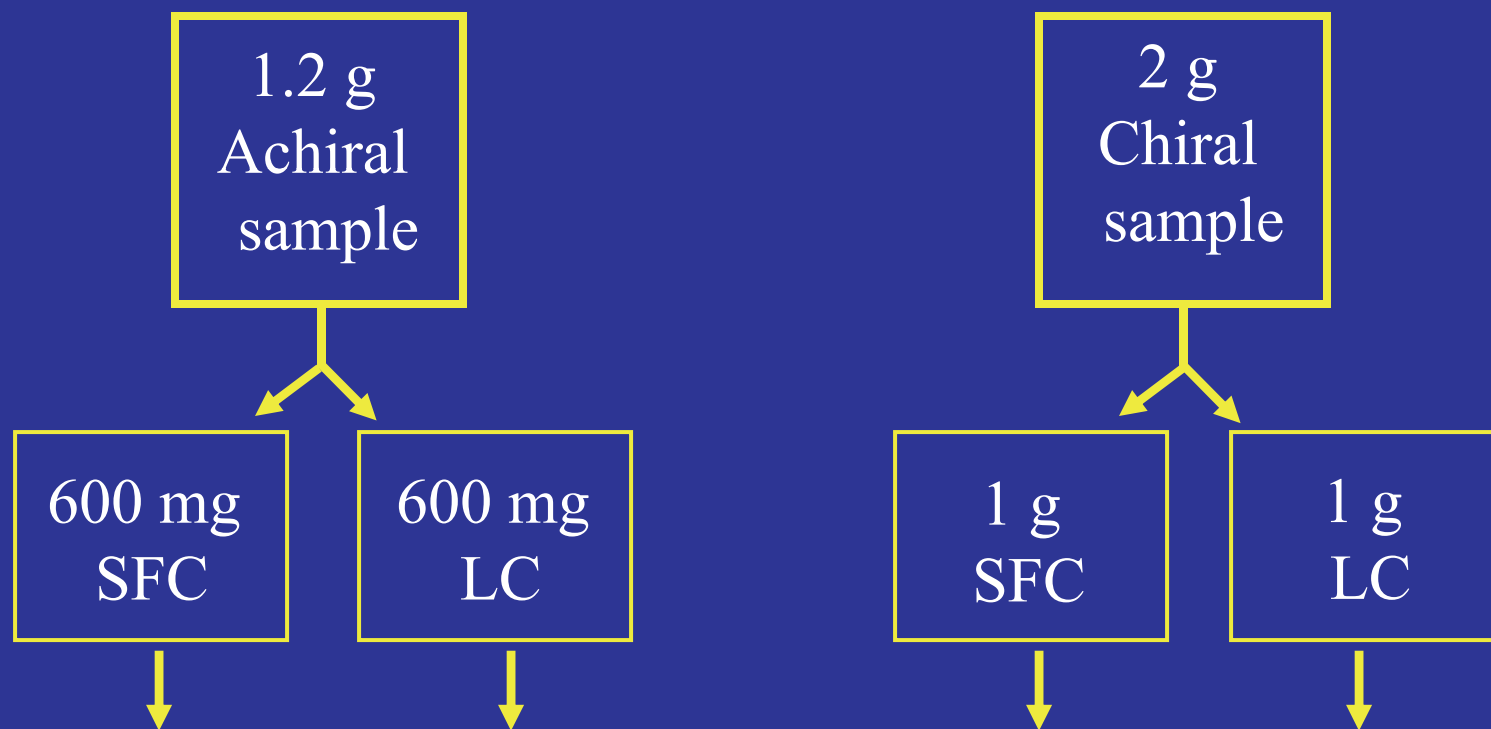
Discovery Purification Group





Discovery Purification Group

case studies

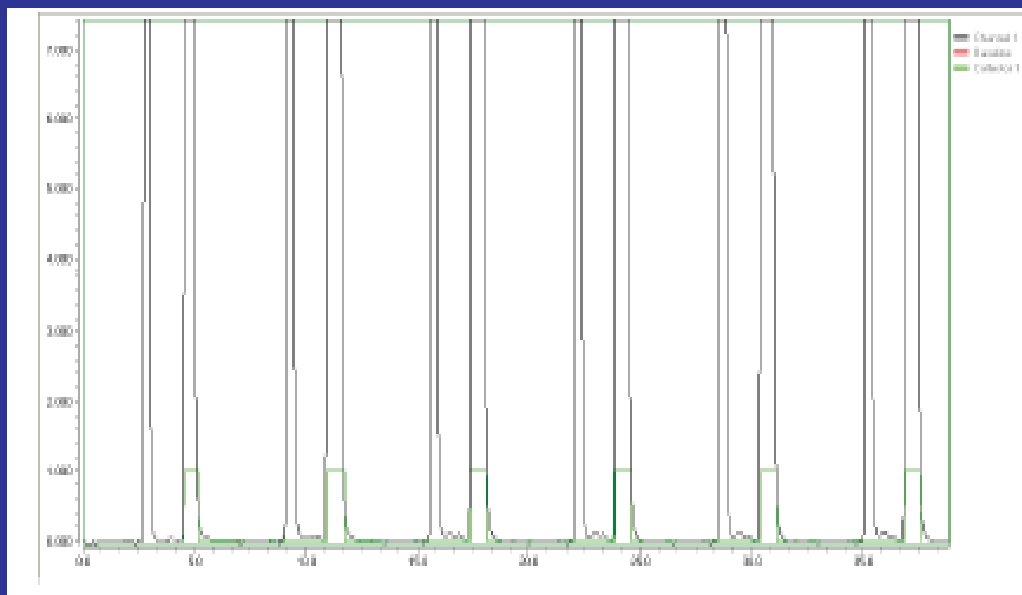


Compared turnaround time and solvent cost



Discovery Purification Group

Achiral case study



SFC Purification

Thar 70

Zymor, Pyrid. 21x250mm, 5um

70:30, CO₂:MeOH

Flow: 60 mL/min

Sample: 600 mg

Load = 100 mg

Total run: 40 min (6 injs.)

Solvent consumed: 720 mL MeOH

Fraction volume: ~ 90 mL MeOH

Evaporation Time: ~ 60 min

Cost analysis:

Solvent cost = \$ 5.72

3 hrs. for project completion



Discovery Purification Group

Achiral case study

LC, Purification

Waters MS-directed

Auto-Purification Platform

Phenom. Luna C-18(2)

21.2x150mm, 5um

A: 0.1%FA (aq.); B: 0.1%FA ACN

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

Flow: 27 mL/min

Sample: 600 mg

Load = 100 mg

Total run: 75 min (6 inj.)

Solvent consumption: 2 L

Fraction volume: ~130 mL

Evaporation Time: ~360 min

Cost analysis:

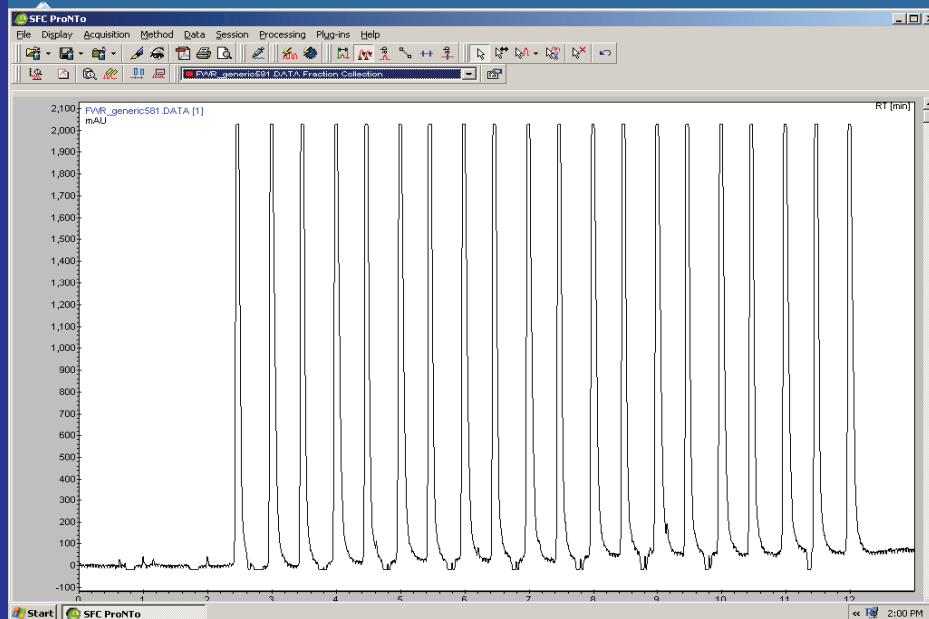
Solvent cost = \$ 20.91

8-9 hrs. for project completion



Discovery Purification Group

Chiral case study



SFC, Purification

Berger MG-II

Chiralcel AD-H, 21x250mm, 5 μ m

80:20, CO₂:MeOH

Flow: 60 mL/min

Sample: 1 g racemic material

Load = 100 mg

Total run: 14 min, 10 stacked inj.

Solvent consumed: 170 mL MeOH

Fraction_T volume: ~ 30 mL MeOH,
each racemate

Evaporation Time: ~ 60 min

Cost analysis:

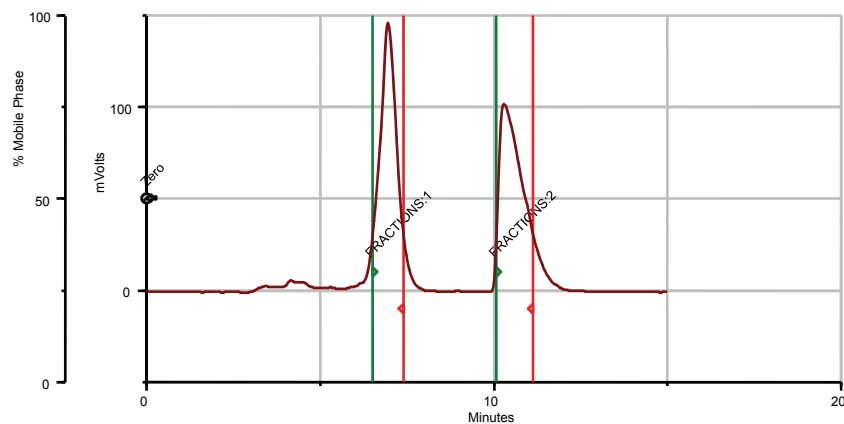
Solvent cost = \$ 1.37

2-3 hrs. for project completion



Discovery Purification Group

Chiral case study



Injection Volume: 0.5 mL (load=25 mg)

LC, Purification

Gilson Purification Module

Chiralcel OD-H,
21x250mm, 5 μ m

70:30, Hexane:EtOH

Flow: 12 mL/min

Sample: 1 g racemic material

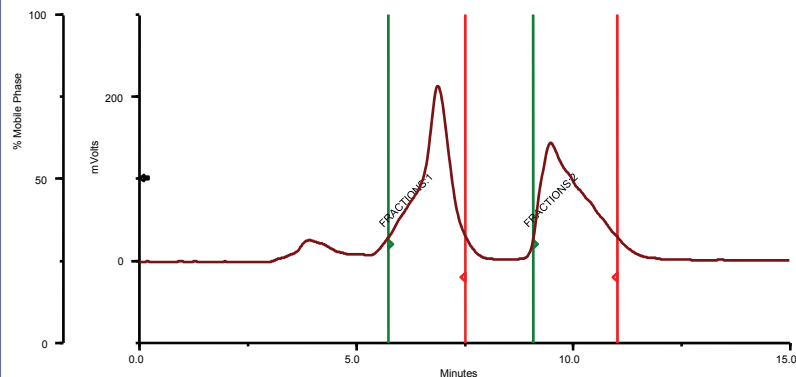
Total run: 260 min, (19X)

40 sequential injs.

Solvent consumption: 3.1 L

Fraction_T volume: ~ 250 mL,
each racemate (8X)

Evaporation time: ~ 240 min (4X)



Injection Volume: 1.0 mL (load=50 mg)



Discovery Cost/Time Analysis

	SFC		LC		
	Solvents	Time spent (FTE)	Solvents	Time spent (FTE)	Cost Analysis
<u>Achiral</u>	\$5.72	< 3hrs.	\$20.91	8-9 hrs.	72% solvent 65% time
<u>Chiral</u>	\$1.37	2-3 hrs.	\$32.78	9-10 hrs.	96% solvent 82% time



Structure Elucidation Group (SEG)

Isolation request from
ARD & CRD



Reproduce chromatography



Develop analytical method



Scale-up



Dry (0.3 – 5 mg)



Confirm target/purity



MS + NMR data/interpretation



Report



Structural Elucidation Group

LC isolation case study

Background:

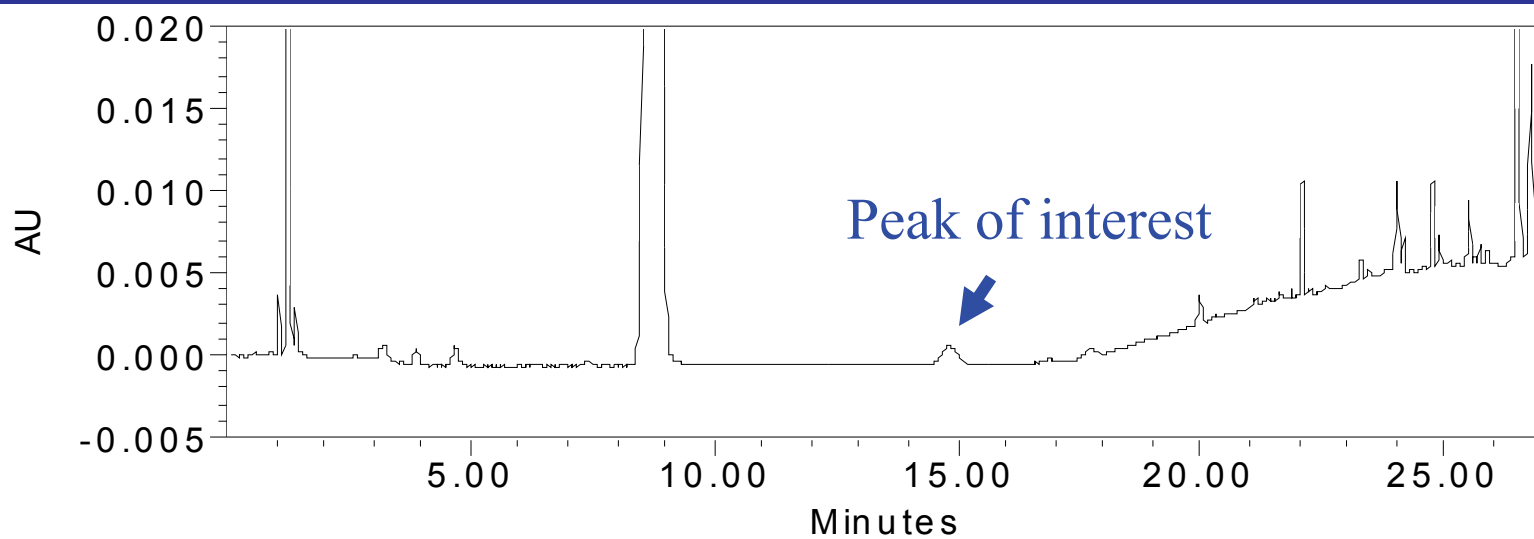
- 0.3% level PRI
- request was for isolation and full structure elucidation of target peak

Column: Ace 3 C8,

4.6x100mm

Mobile: 50mM NaClO₄

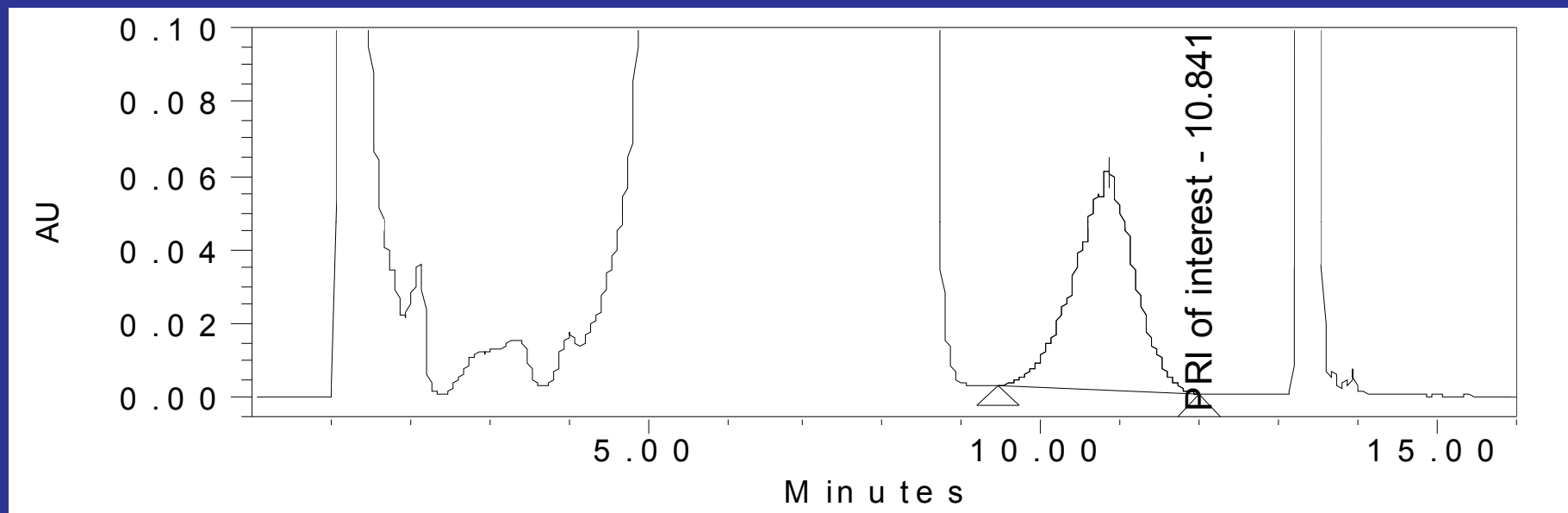
0.1% H₃PO₄ /ACN





Structural Elucidation Group

LC isolation case study



Prep scale:

25x100 mm, 59 mL/min



Structural Elucidation Group

LC isolation case study

- ✓ Dev. Method for scale-up (< 1 day)
- ✓ Scale-up to prep. LC (< 1 day)
- ✓ Load 330mg over 6 inj. via 0.1% TFA/water, ACN mobile
- ✓ Collect fractions (~ 2hrs.)
- ✓ Pool fractions (750 mL)
- ✓ Rotovap (1-2 hrs.)
- ✓ Lyophilize (2 days)
- ✓ ~ 5.7 L solvents consumed
- ✓ ~ 500 µg to NMR
- ✓ Solvent Cost: \$ 49.37
- ✓ Turnaround time: ~ 3 days

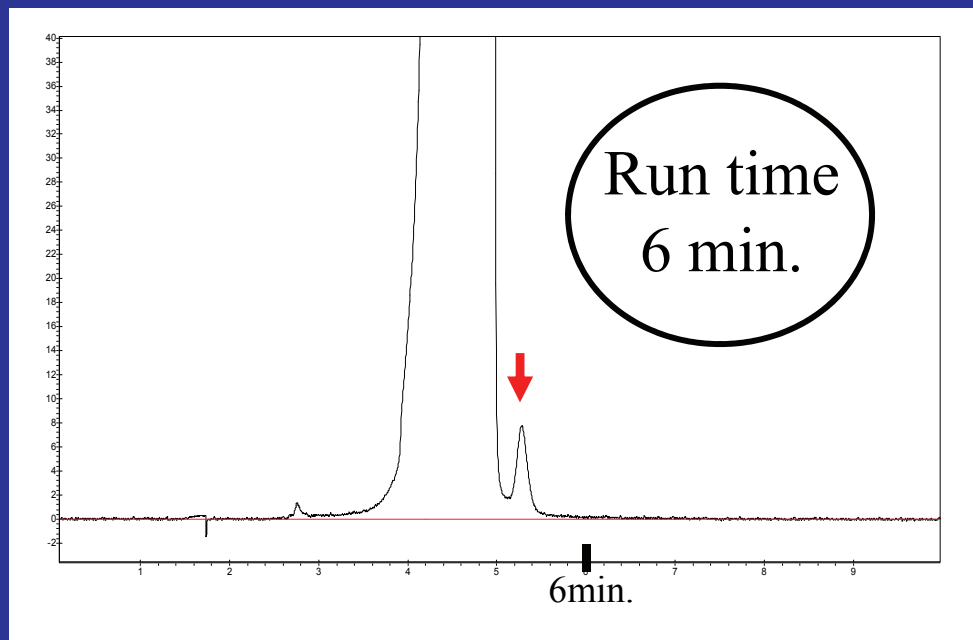


Structural Elucidation Group

SFC stacked case study

Background:

- 0.3% level PRI
- request was for isolation and full structure elucidation of target peak



Sample: 70mg/mL

Column: CYANO (Princeton)

250mm x 10 mm, 5 μ m

$P_0 = 140$ bar

$T = 35^\circ\text{C}$

$F = 10.0$ ml/min

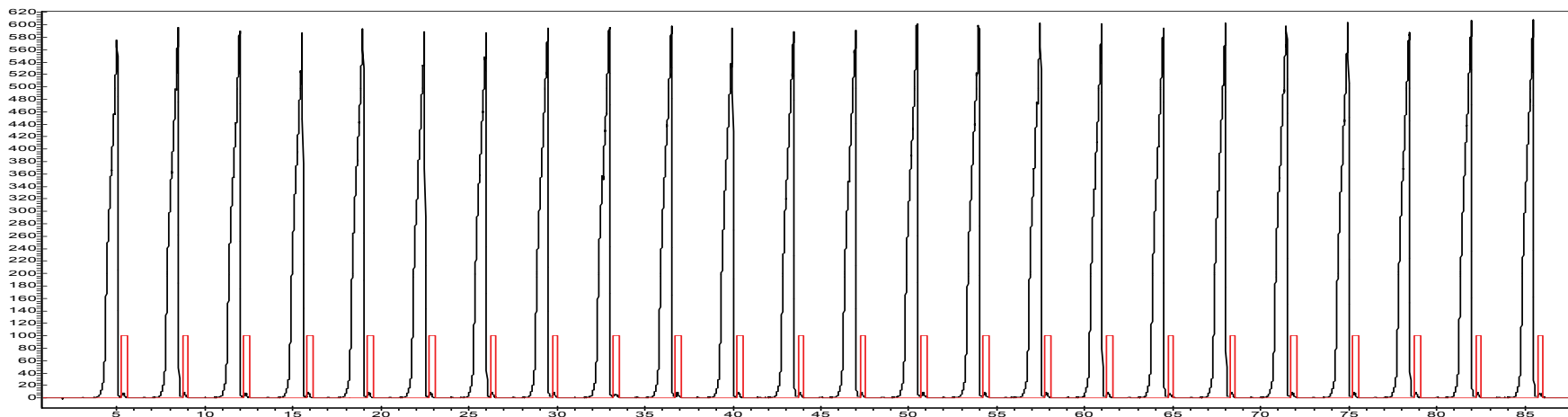
Isocratic: 15/85, MeOH/ CO_2

Additives: 0.2% DEA, FA

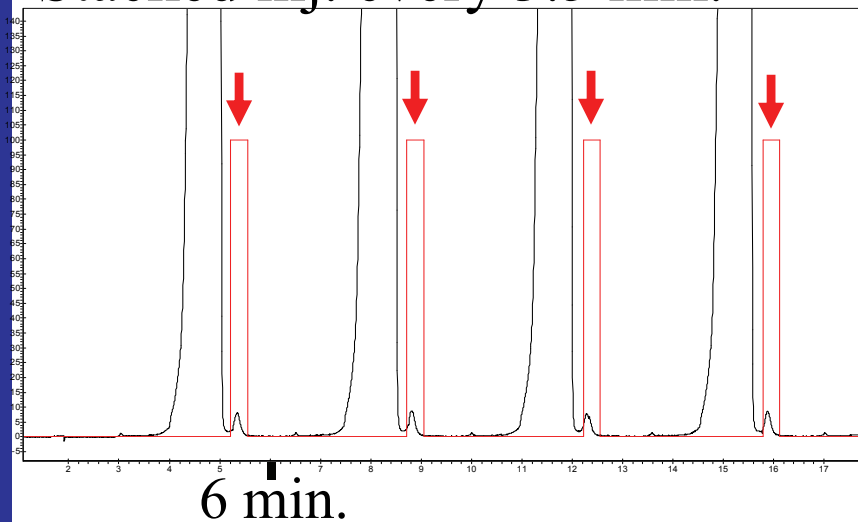


Structural Elucidation Group

SFC stacked case study



Stacked inj. every 3.5 min.

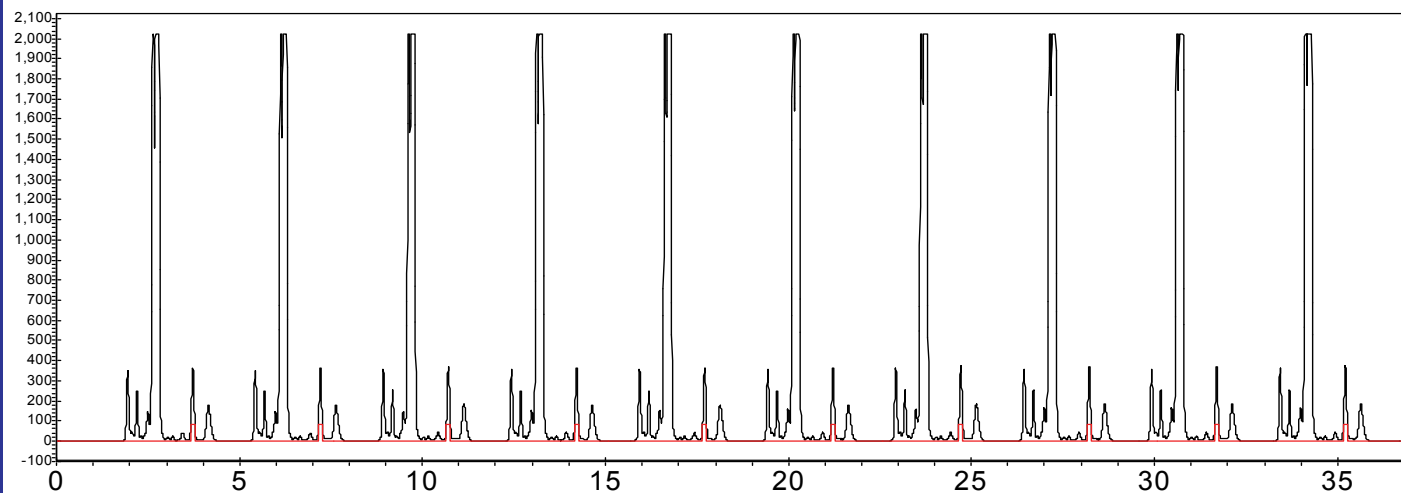


- Method Development (3 hrs.)
- 41 injections
- stacked over 2.4 hrs.
- 350 μ g obtained \rightarrow NMR
- Solvent Cost : \$4.38
- \$4.38 vs. \$49.37 \rightarrow 91% savings
- Time spent: ~ 6 hrs.

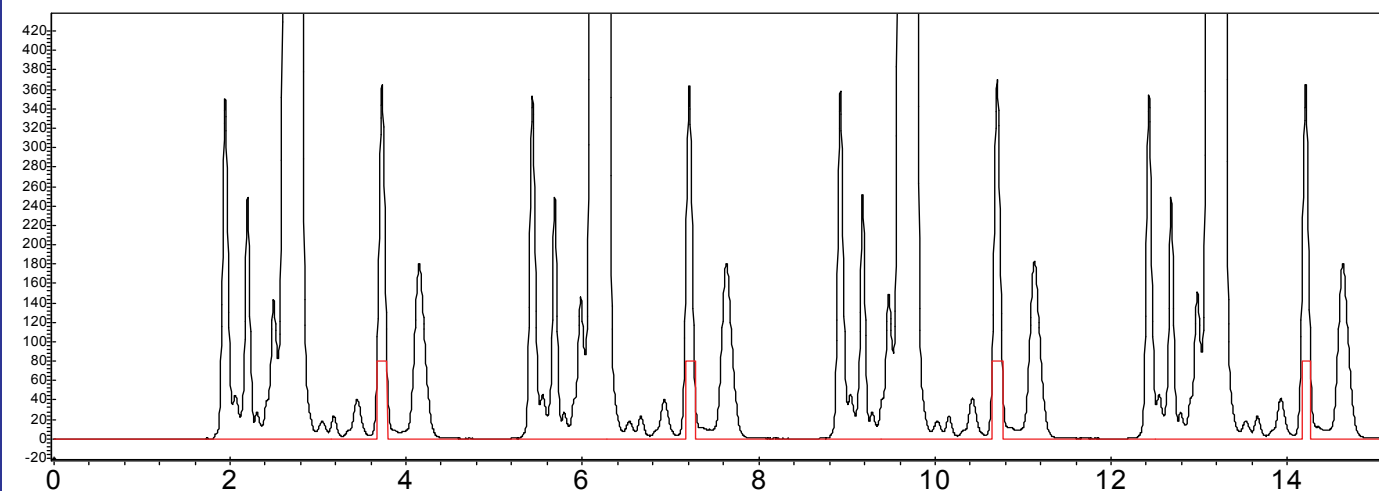


Structural Elucidation Group

SFC stacked case study



3.8% target
impurity



Run time
5.5 min.

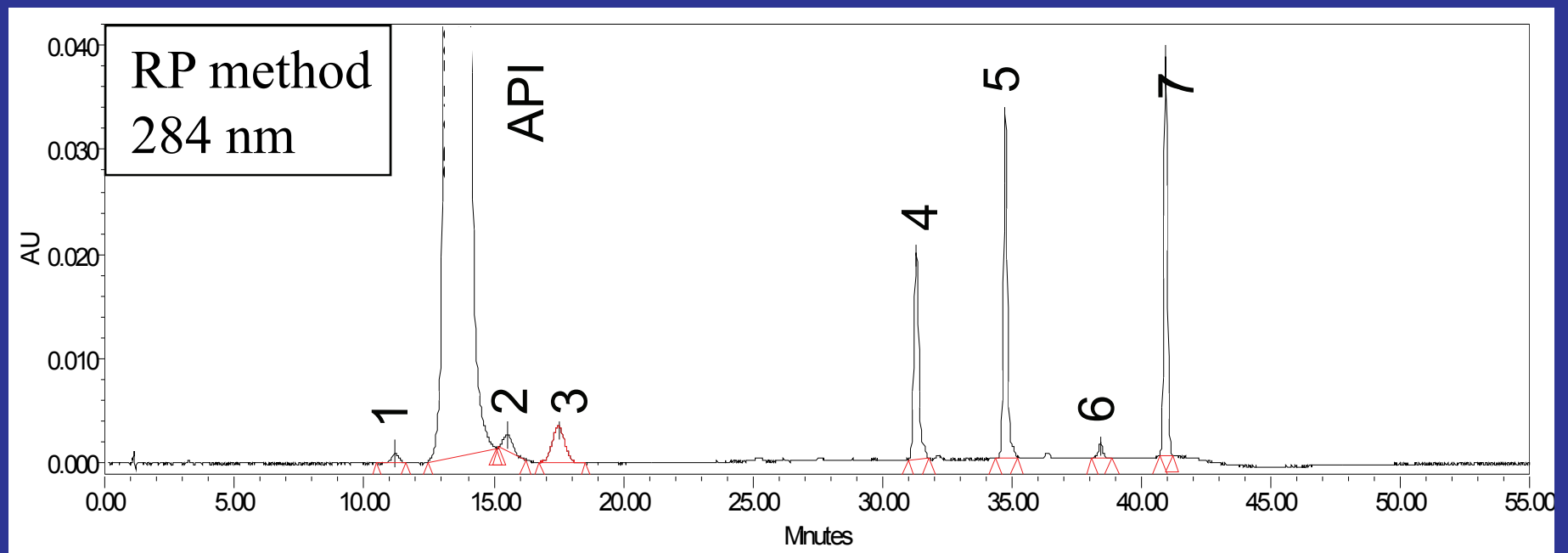


Stacked inj.
every 3.5 min.



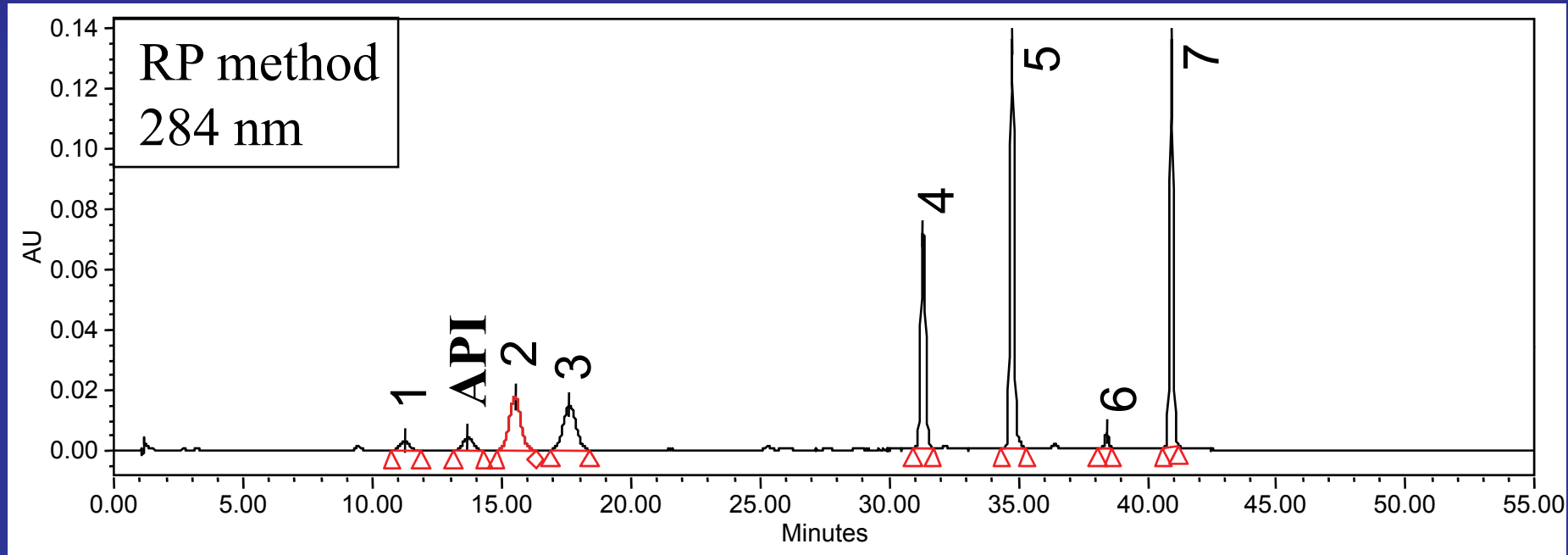
SFC - API extraction

- ❑ 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





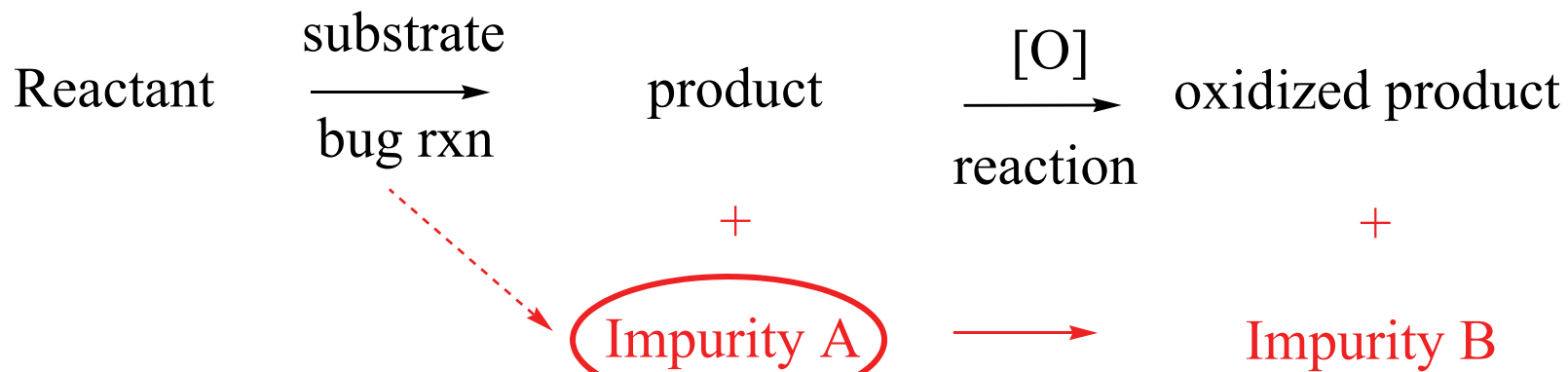
SFC - API extraction



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

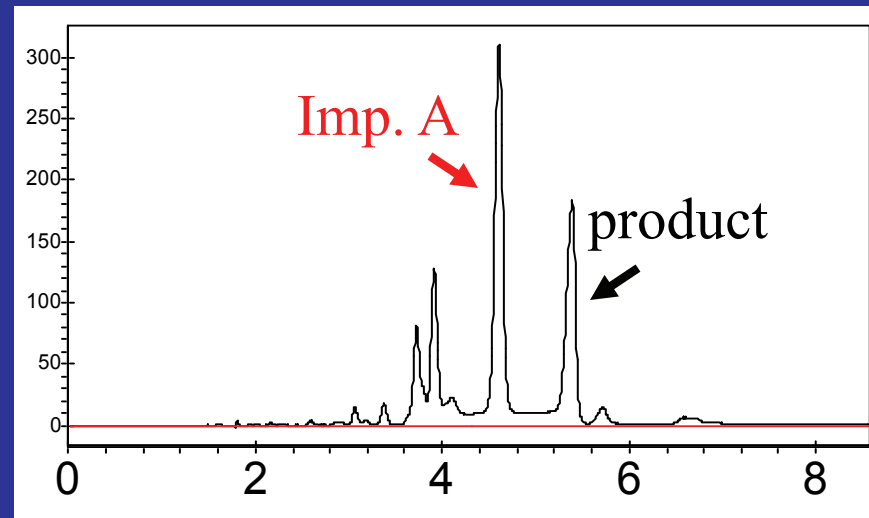
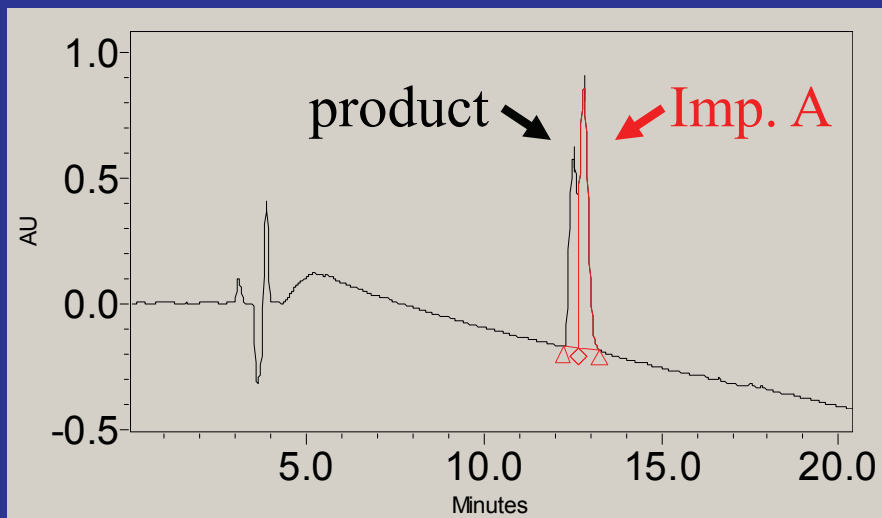
Significantly increase concentration (40X) and loadability of target impurities

SFC case study: Biocatalysis Impurity A



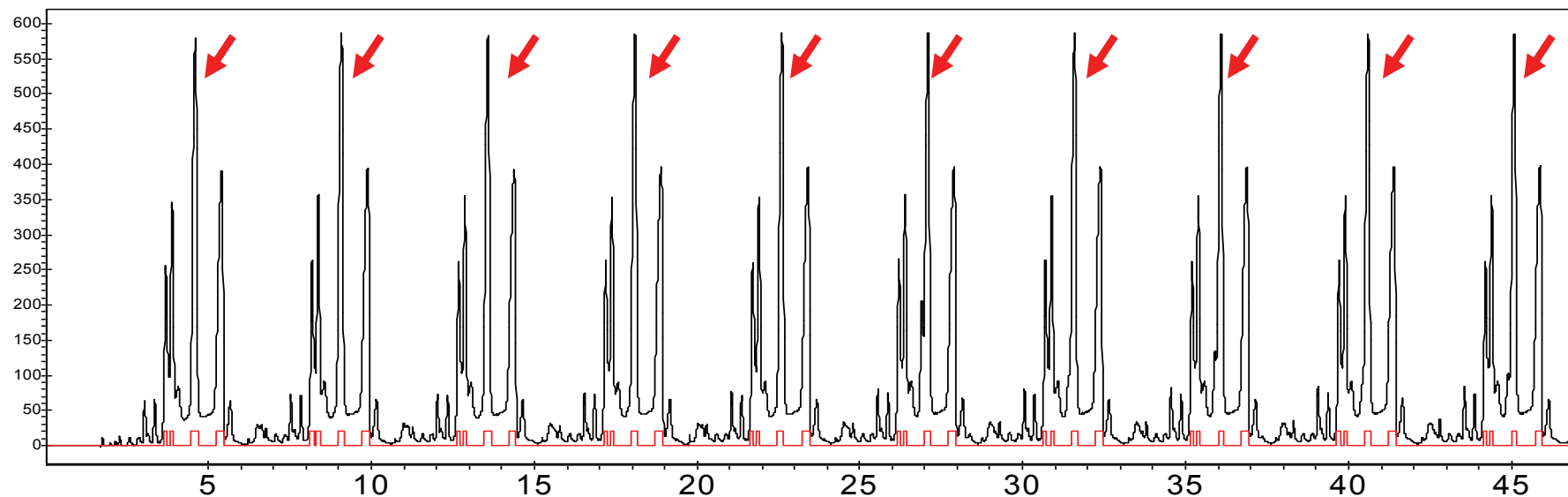
product/Impurity A sample
via CRD LC Method

product/Impurity A sample
via SFC Isolation Method





SFC case study: Biocatalysis Impurity A



- 1hr. to dev. SFC method
- Stacked injs. over 47 minutes
- Fraction dry down = 1 hr.
- 8 mg for NMR
- Solvent Cost: \$1.43
- CRD sample → NMR sample = 3hrs.!

Princeton 2-ethyl pyridine,
5 μ m, 10x250 mm
10 mL/min
85/15, CO₂/MeOH
P₀: 140 bar

○ *Conclusions*

- Packed column SFC using CO₂ has many advantages over LC for DPG & SEG purification applications
- Solvent cost savings - purchase, disposal
- Time savings - project turnaround times decreased
- decrease in labor costs
- Selectivity impact – crucial for impurity isolations
- CO₂ has low toxicity, green chemistry



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