“Green” Lab Scale Chiral Chromatography: Comparison of Purification Techniques for Cost Effective and Efficient Processing of Samples While Minimizing Environmental Impact

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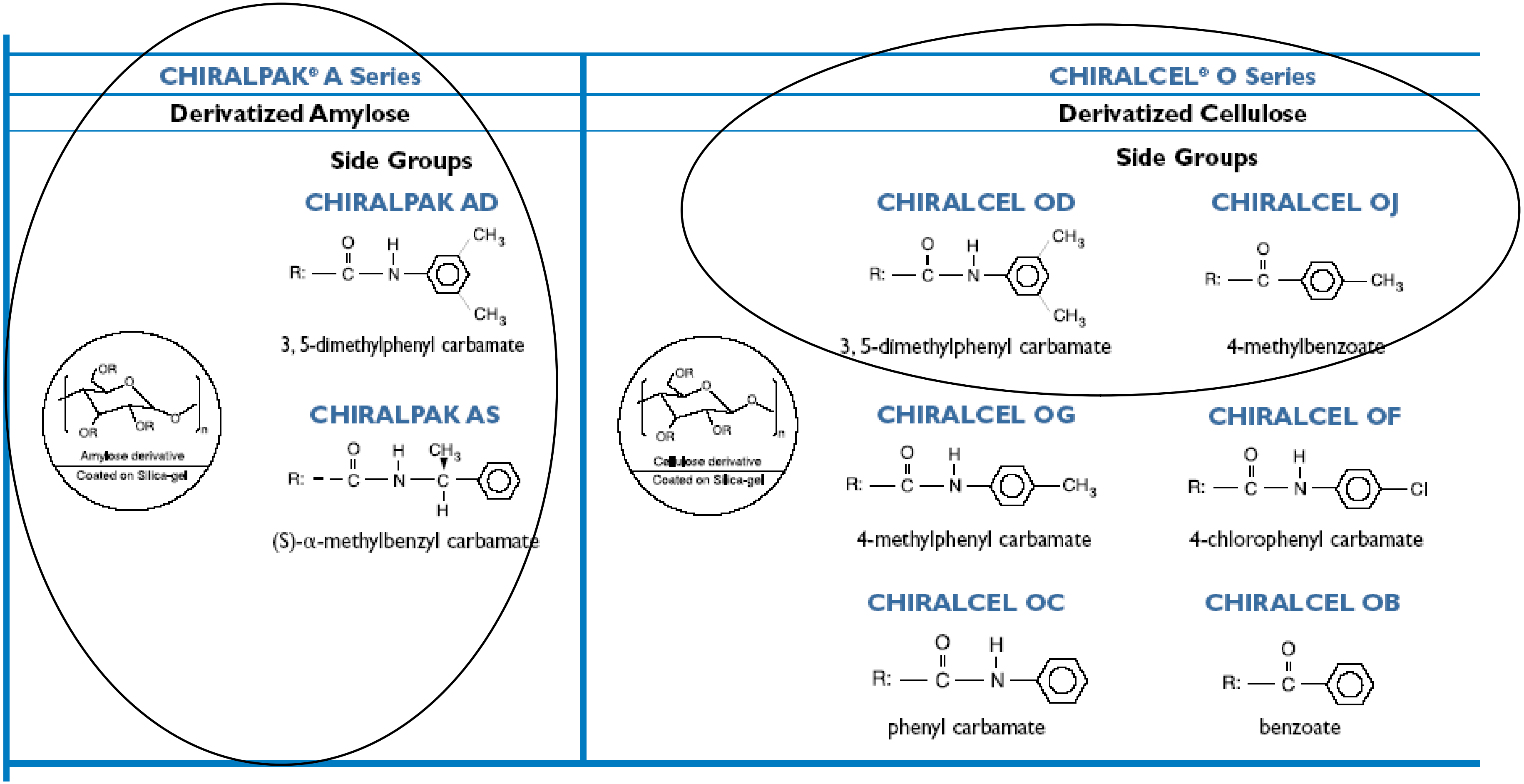
Answers That Matter.

Discussion Topics Outline

- Background of preparative chiral chromatography at Lilly -lab based technologies
- Evolution from initial lab efforts (mg scale) to full-time chiral purification global support effort (mg-kg scale w/ 1000's projects/year)
- Comparison of batch HPLC, recycling HPLC approaches (e.g. Closed Loop Recycle, Steady State Recycle), and Batch SFC technology

Answers That Matter.

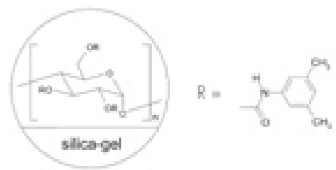
“Traditional” Daicel CSPs (Chiral Technologies)



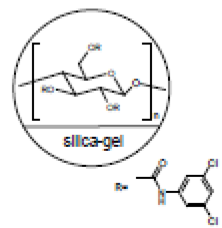
* Have been commercially available for more than 20 years now and have
the chiral purification market.

Other CSPs Actively Considered for Prep

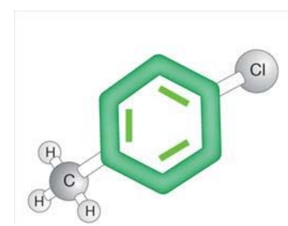
- **Chiralpak IA**



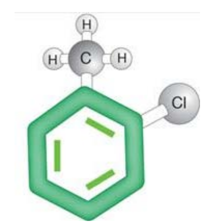
- **Chiralpak IC**



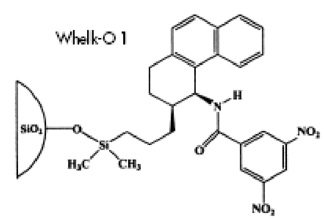
- **Chiralpak AY, Lux Amylose-2**



- **Chiralcel OZ, Lux Cellulose-2**



- **(R,R) & (S,S) Whelk-O 1**



*Lux (Phenomenex); Whelk-O (Regis)

Answers That Matter.

SFC Chiral Screen (Traditional CSPs)

5 g/min Flow rate, Isocratic, 5 minute run time

Mobile Phases: MeOH or EtOH or IPA/CO_2

Co-solvent percentages from 5 to 40% (0.2% IPAm if needed)

Columns: 4.6 x 150 mm 5μm

Chiralpak AD-H	Chiralpak AS-H
Chiralcel OD-H	Chiralcel OJ-H

~ 95% success rate

HPLC- Polar Organic Mode

HPLC- Polar Organic Mode

1 mL/min Flow rate, Isocratic, 12 minute run time

Columns 4.6 x 150 mm 5μm	Mobile Phase (with 0.2% DMEA)
Chiralpak AD-H	100% MeOH, EtOH
Chiralpak AS-H	100% MeOH, EtOH
Chiralcel OD-H	100% MeOH, EtOH
Chiralcel OJ-H	100% MeOH, EtOH

Modify with Acetonitrile if needed

~ 30% success rate

Answers That Matter.

SFC 2nd Tier Screening

Gradient: 5 g/min Flow rate, 0.5 min hold 5% Co-solvent/CO_2 ,

5.5 min 5% to 40% Co-solvent/CO_2 , 4.0 min hold 40% Co-solvent/CO_2

Co-solvents: MeOH, EtOH, IPA (0.2% IPAm if needed)

Columns: 4.6 x 150 mm 5 μm

Chiralpak IA Chiralpak IC

Lux Amylose-2 Lux Cellulose-2

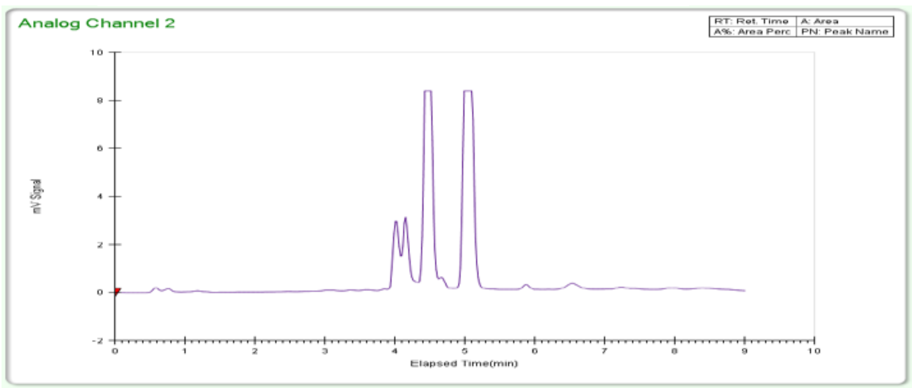
Whelk-O 1

Lilly
Answers That Matter.

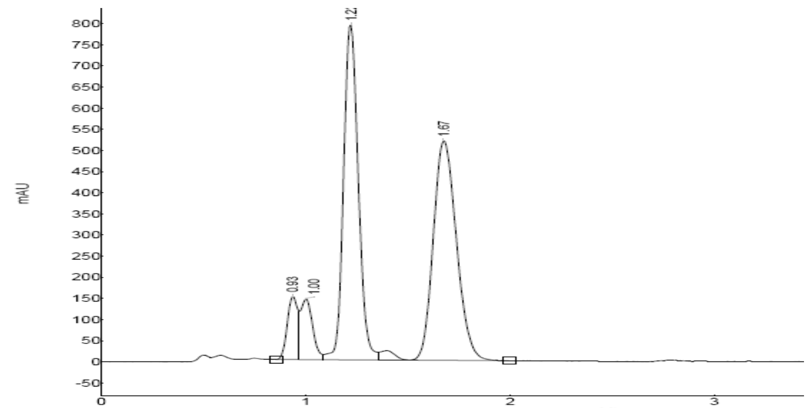
Thar X5 Parallel UV SFC instrument

Gradient: 5 mL/min each column
5% hold 0.5 min
5% to 40% over 5.5 min
40% hold 2.0 minute
40% to 5% 0.5 minute
5% hold 1 minute
9.5 minute cycle time

Columns
4.6 x 150 mm 5mm
1: Chiralpak IA
2: Chiralpak IC
3: Lux Amylose-2
4: Lux Cellulose-2
5: (S,S) Whelk-O



Gradient screening result
Chiralpak IC MeOH



Optimized isocratic chromatogram
Chiralpak IC 30% MeOH/CO_2 5 mL/min

- **reducing chiral method development time with smaller columns w/ smaller particle CSP also being utilized as needed when available**

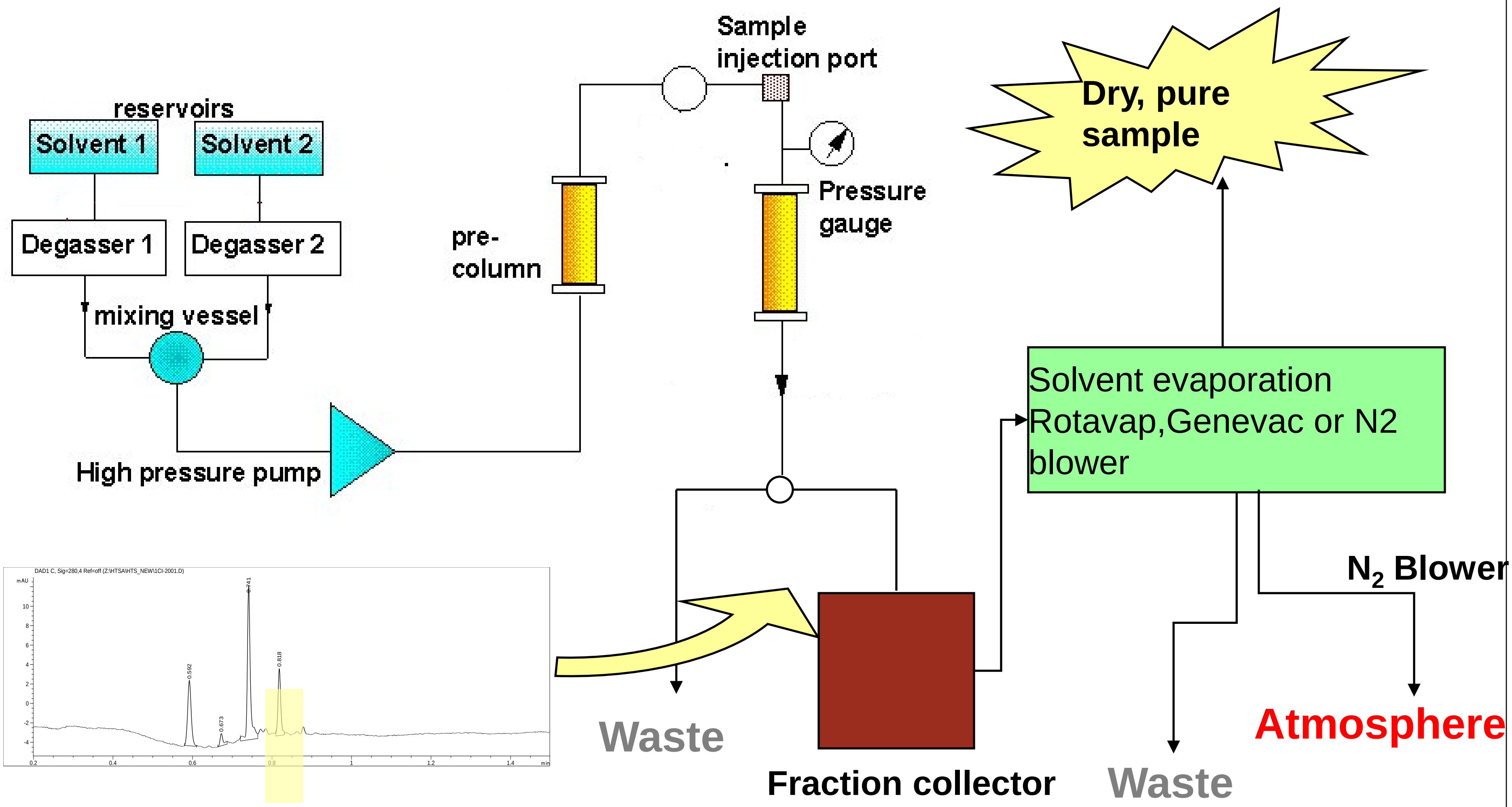
Answers That Matter.

HPLC- Low Polar Mode

- Same CSPs as traditional and 2nd tier
- EtOH and IPA/heptane (0.2% amine or 0.1% TFA as needed)
- Included in evaluation for larger scale projects and others that fail SFC evaluation

Answers That Matter.

Traditional Batch HPLC purification

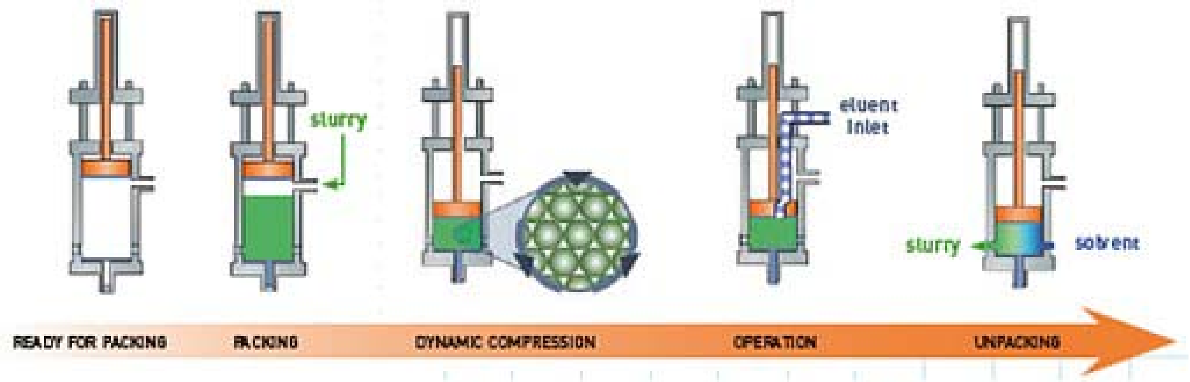


Development of Recycling HPLC Technologies

- Recycling HPLC described more than 50 yrs. ago
- Simulated Moving Bed (SMB) invented in the 1960's also; applied to chiral chromatography in the 1990's
- Steady State Recycle (SSR) introduced and applied to chiral in the 1990's (same instrumentation allows for closed loop recycling)
- SSR chromatography trademarked as "SteadyCycle" (CYBA Technologies LCC- P Grill) or "CYCLOJET" (Novasep)

Answers That Matter.

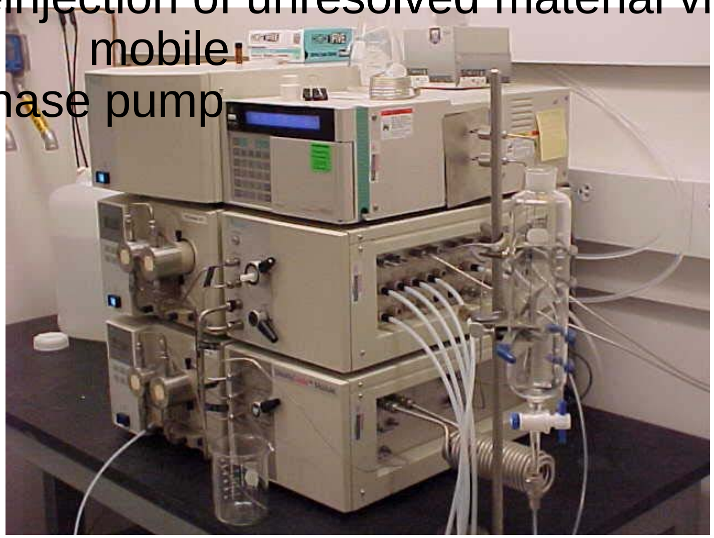
ProChrom HPLC Equipment Utilized at Lilly in the 1990's



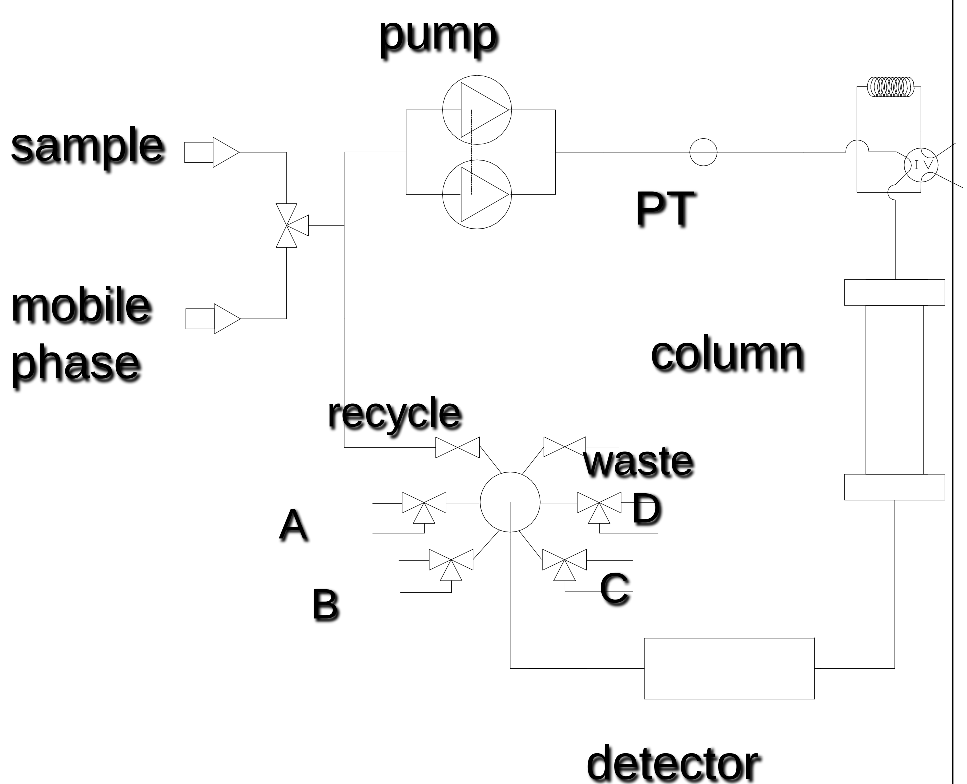
Answers That Matter.

Closed Loop Peak Shave/Recycle Technology

Isocratic batch technique that allows for stacked injection and selective removal of sample profile with closed loop reinjection of unresolved material via mobile phase pump



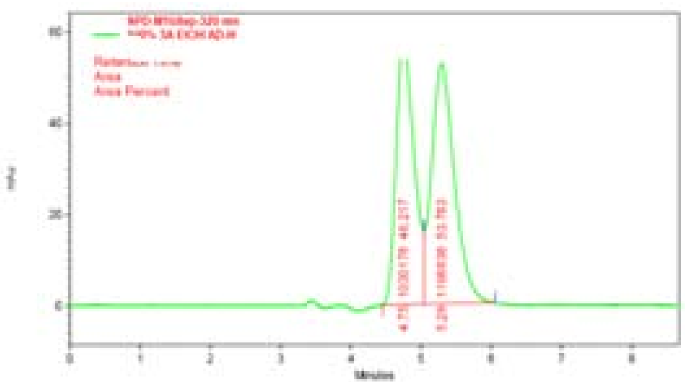
•Varian 800 ml/min R&S…zonator w/ SteadyCycle (Hitachi SSR license

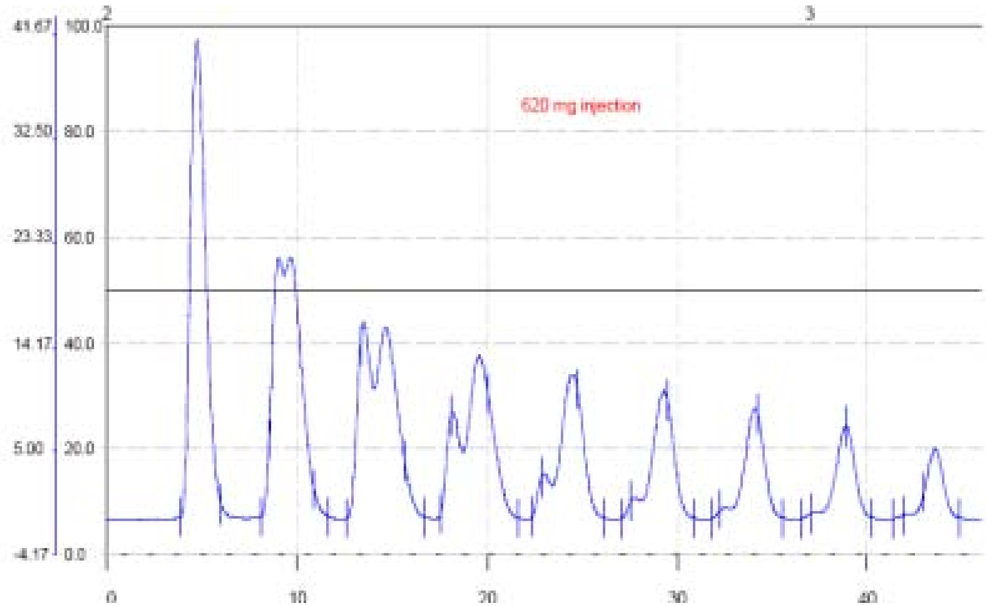


•Diagram courtesy of Charles "Pete" Grill

Answers That Matter.

Closed Loop Peak Shave/Recycle Example





Analytical chromatogram

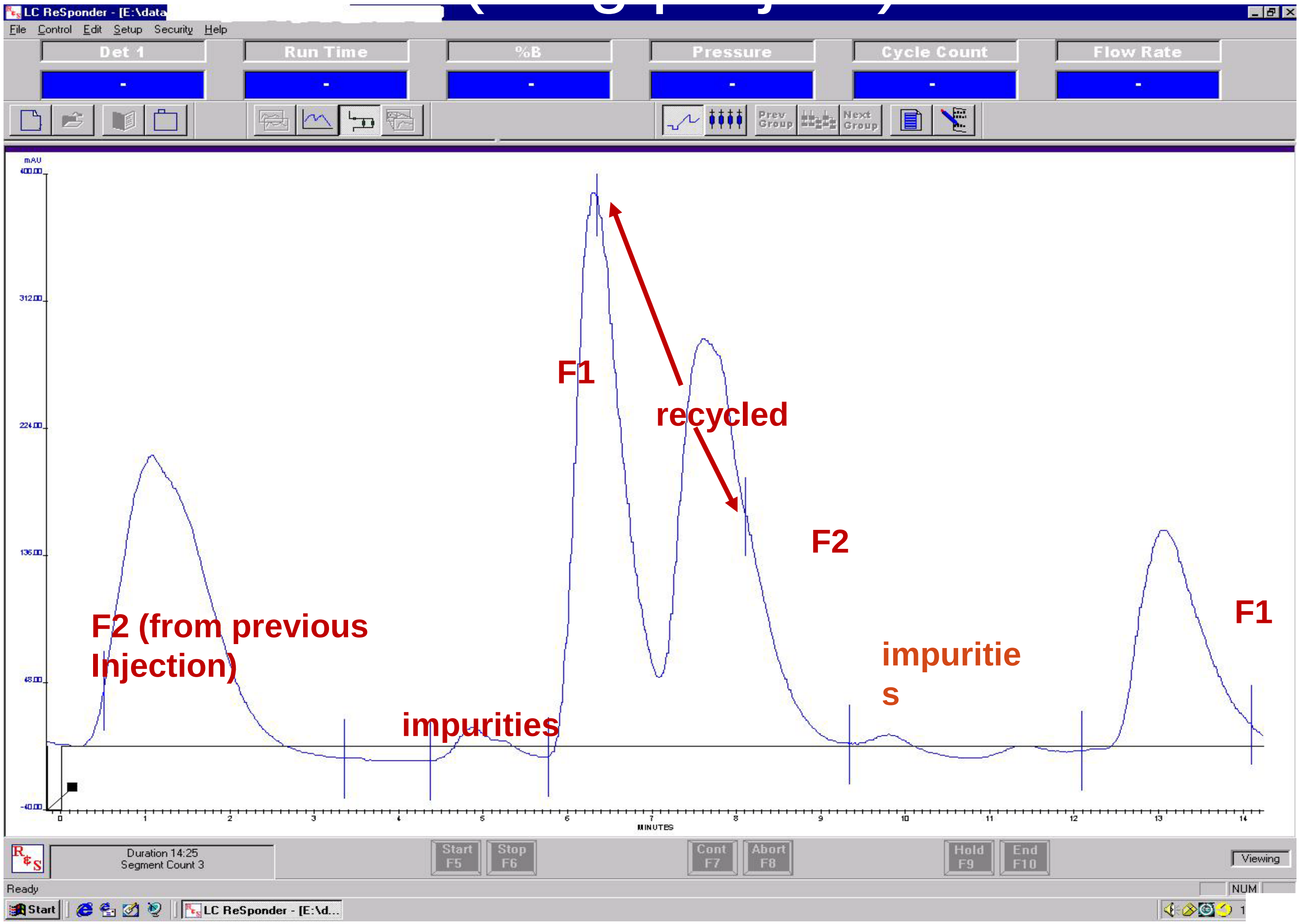
Preparative chromatogram

Stacked Peak Shave/Recycle Technique Discussion

- Isocratic technique that can be quickly optimized and automated for repetitive injection
- Requires fast cycle time for elution of mixture components
- Solvent consumption reduced (typically 2-3x) over traditional batch approach (only solvent being used is in fraction or waste stream)
- Column can be overloaded with no need for offline reinjection of material

Answers That Matter.

Stacked Recycle Chiral Prep HPLC Example (73 g project)



Answers That Matter.

Steady State Recycle (SSR) Technology

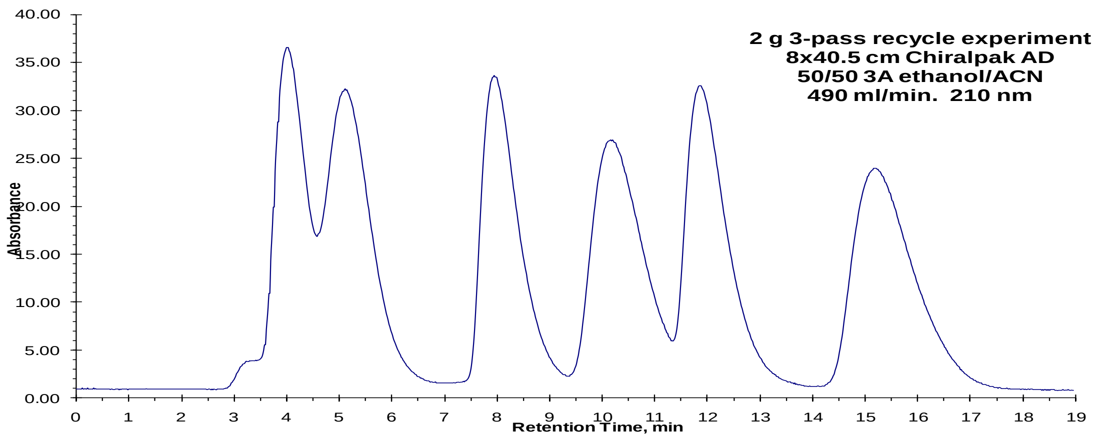
Binary, isocratic, single column, fixed flow, semi-continuous separation technique (for comparison, SMB is a multicolumn continuous technique with flow rate parameter variations available)

Modification of closed loop peak shave/recycle technique with additional sample injection pump for continuous injection and collection of sample (equal amounts of each isomer removed and injected when profile reaches steady state)

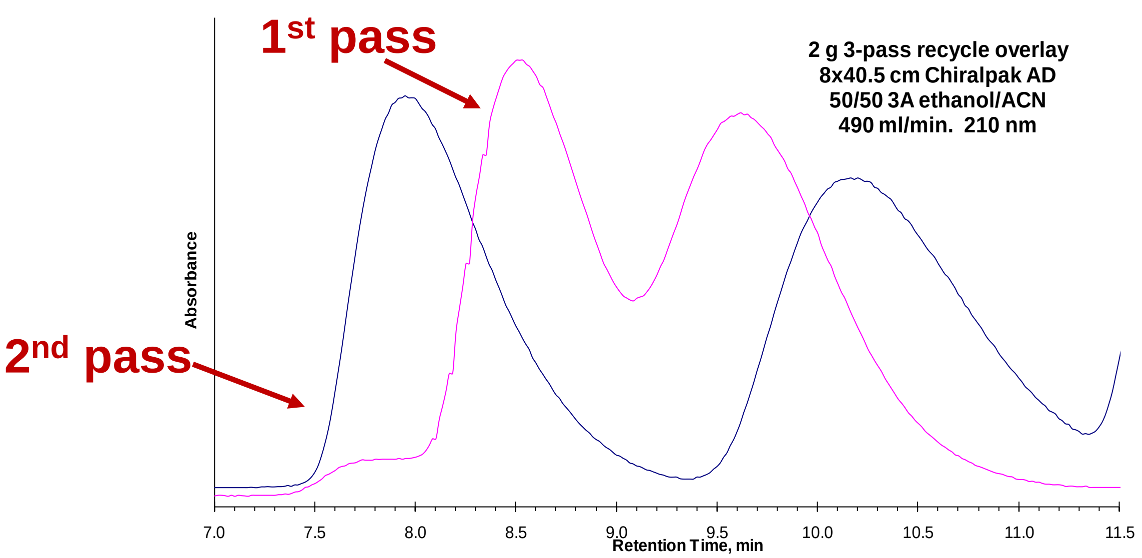
Requires additional experiments to set up but more efficient than peak shave/recycle for production of larger amounts of compound and uses less solvent (typically requires solubility in mobile phase and fast cycle time with selectivity >1.1)

Answers That Matter.

SSR Method Development



Closed loop recycle experiment



Answers That Matter.

SSR Method Development

File	C:\Dow_Demo-01-07-2003\Recycle-008_Dow
Run Name	Recycle-
Points	1,364.00000

Time	Time min	Det 2 V	Overlap
0:00	0.0000	0.96	
0:01	0.0167	0.96	
0:02	0.0333	0.96	
0:03	0.0500	0.96	
0:04	0.0667	0.96	
0:05	0.0833	0.96	
0:06	0.1000	0.96	
0:07	0.1167	1.07	
0:08	0.1333	0.96	
0:09	0.1500	0.96	
0:10	0.1667	1.01	
0:11	0.1833	0.96	
0:12	0.2000	0.96	
0:13	0.2167	1.07	
0:14	0.2333	0.96	
0:15	0.2500	0.96	
0:16	0.2667	0.96	
0:17	0.2833	0.96	
0:18	0.3000	0.96	
0:19	0.3167	0.96	
0:20	0.3333	1.07	
0:21	0.3500	0.96	
0:22	0.3667	1.01	
0:23	0.3833	0.96	
0:24	0.4000	0.96	
0:25	0.4167	0.96	
0:26	0.4333	0.96	
0:27	0.4500	0.96	
0:28	0.4667	0.96	

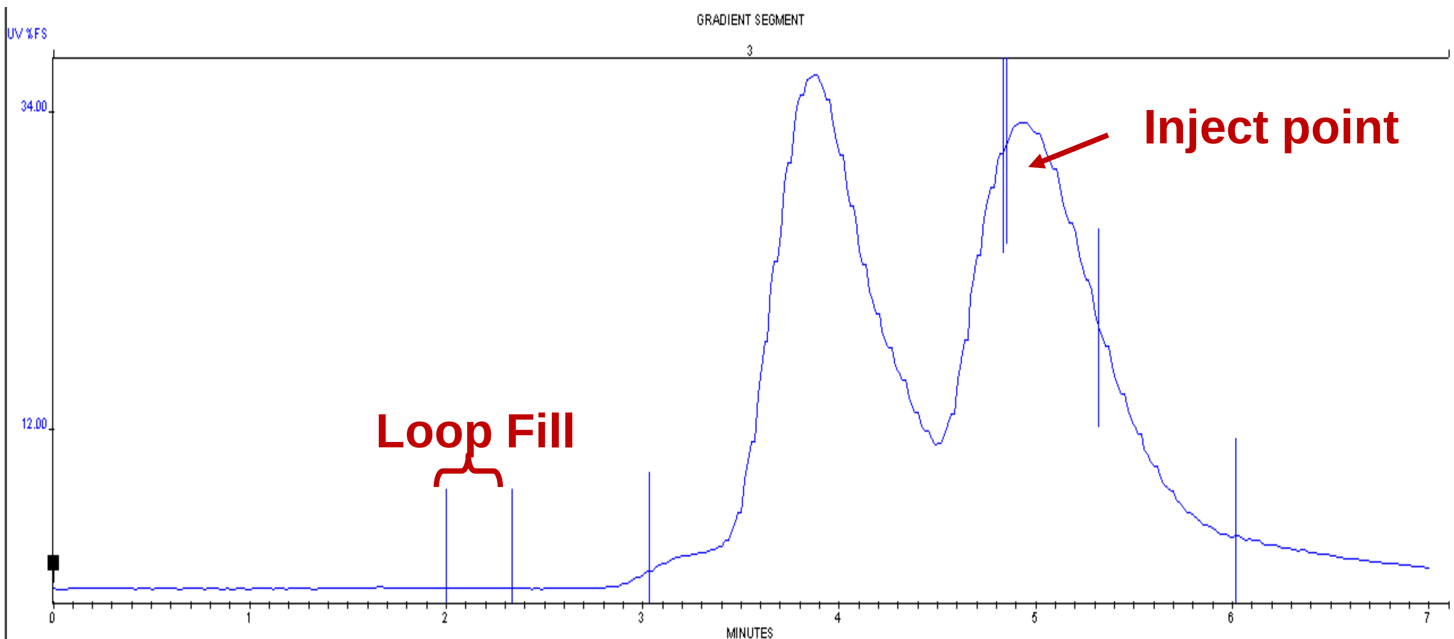
Retention Time

Cycle	Peak 1 min	Peak 2 min	Midpoint min	Cycle Time min
1	4.02	5.1	4.56	--
2	7.95	10.17	9.06	4.50
3	11.87	15.19	13.53	4.47

Event	Plot Time min	Relative Time min
F-1	7.5	--
End F-1	8.05	0.6
Inject	9.2	1.7
F-2	9.8	2.3

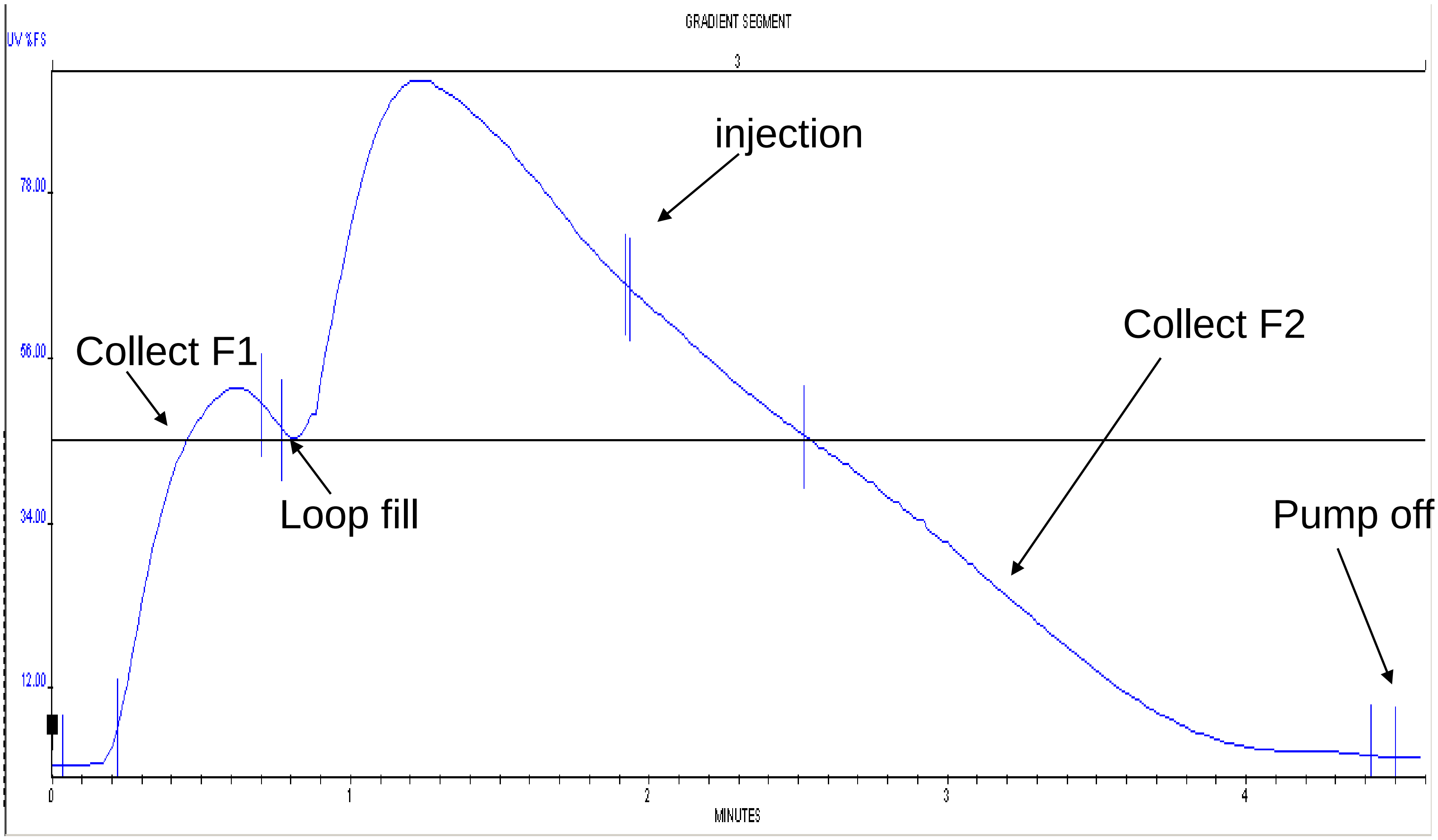
Parameters Determined from Overlay Experiment

Icycle experiment

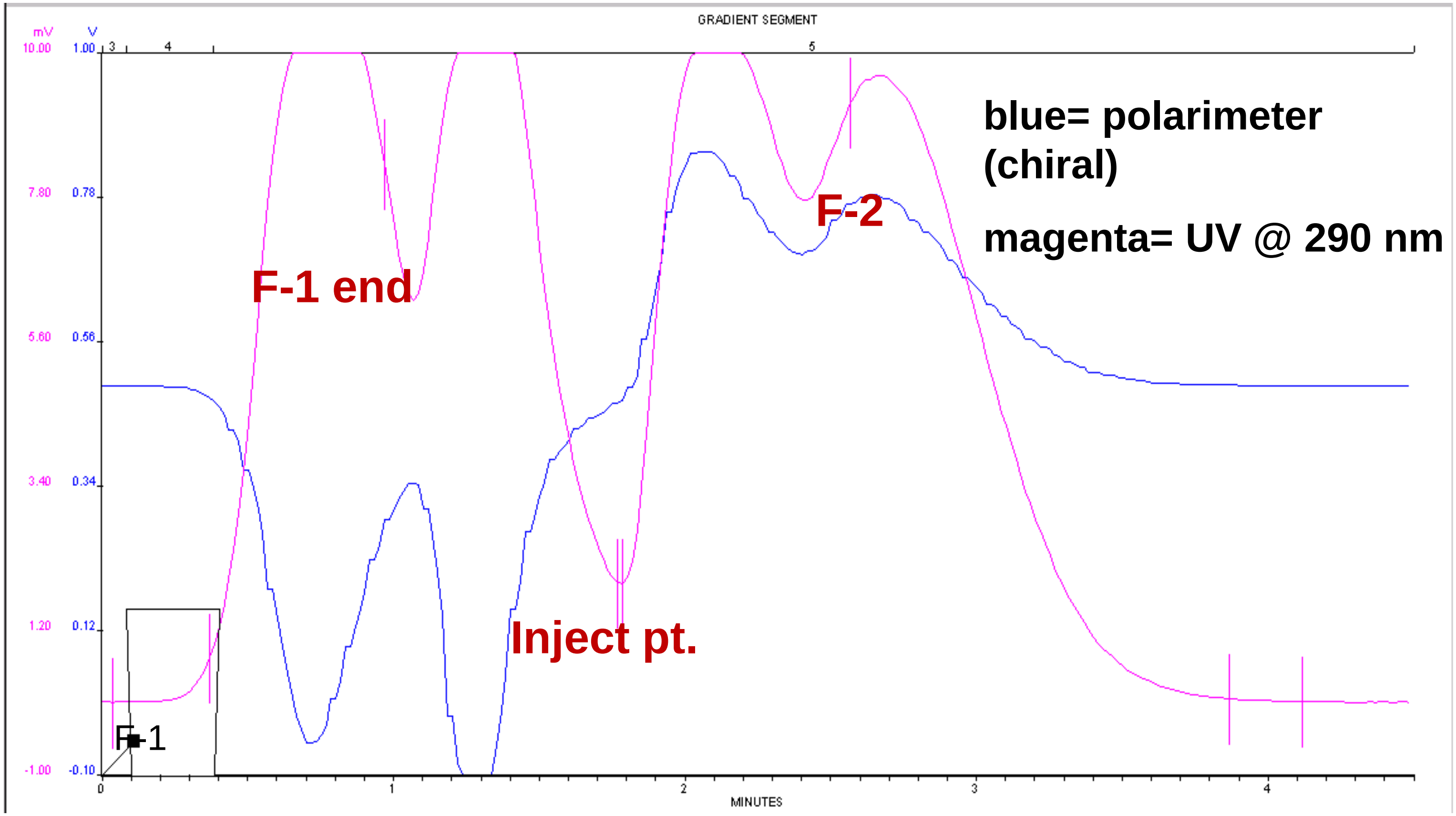


Answers That Matter.

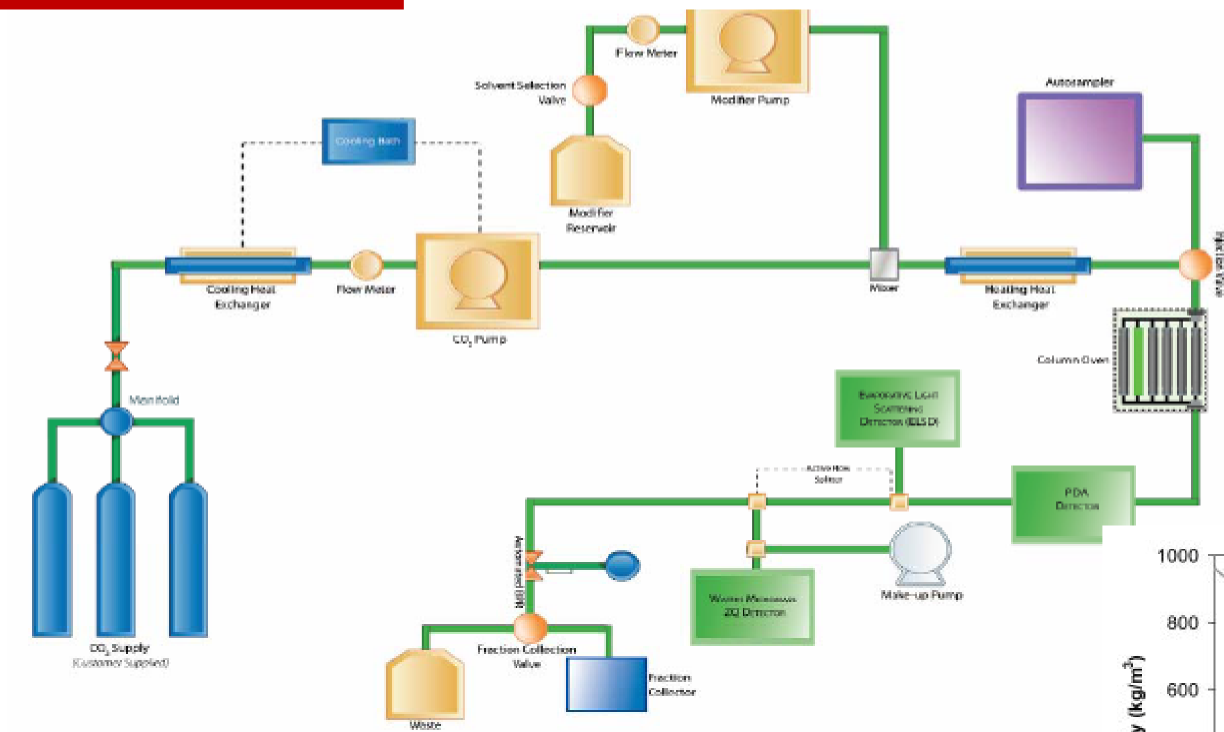
Steady State Chromatogram (6 g loading)

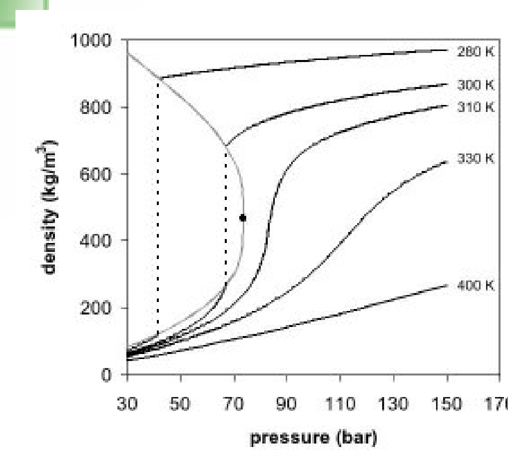


Another SSR Project (UV & Polarimeter Chromatograms)



What is SFC?





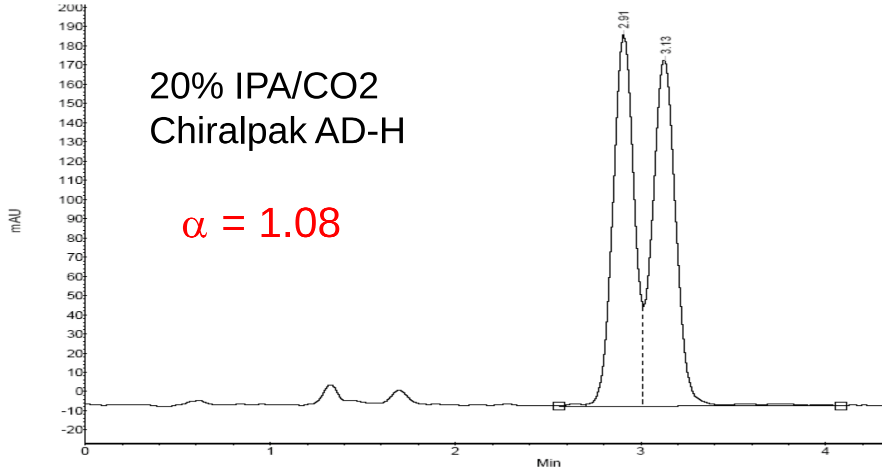
- *A chromatographic technique similar to HPLC*
- *Uses CO_2 as the main component of the mobile phase*
- *Uses gradient as well as isocratic*
- *Physical parameters such as T and P can be used to control retention*

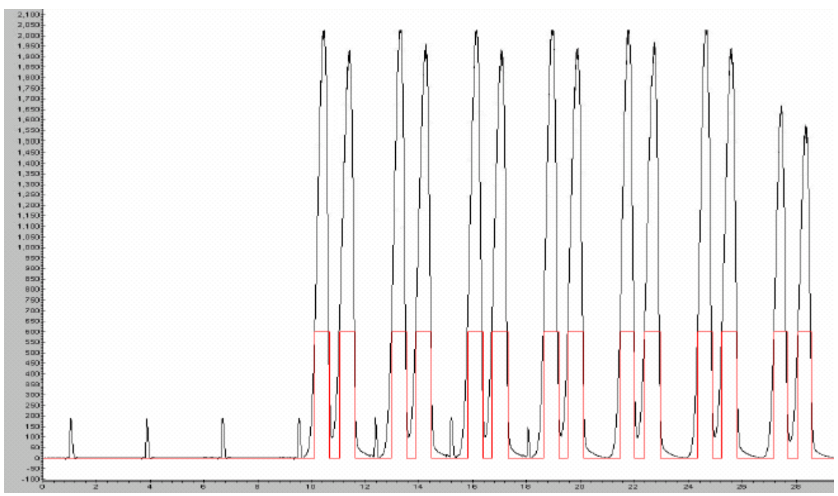
Answers That Matter.

Berger Minigram and AutoPrep SFC Instruments (introduced at Lilly nearly 10 years ago)

Answers That Matter.

More Difficult Berger MultiGram II SemiPrep Purication



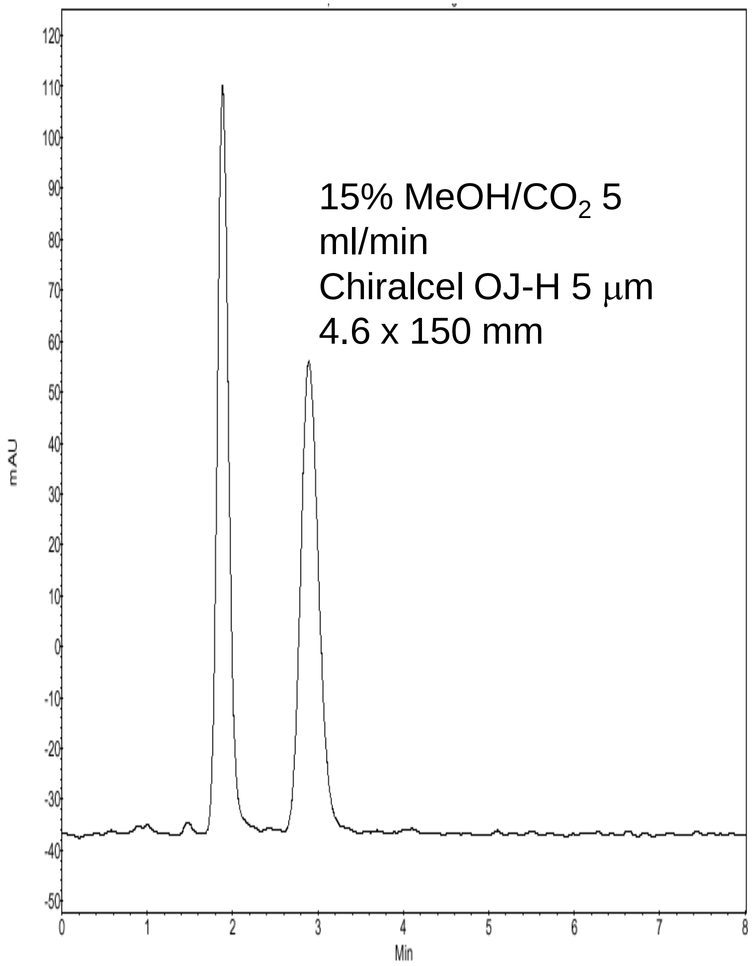


11 mg/injection every 3 min
Difficult solubility
No HPLC separation
89% Total recovery (44.5% for each enantiomer)
Both enantiomers 98% EE

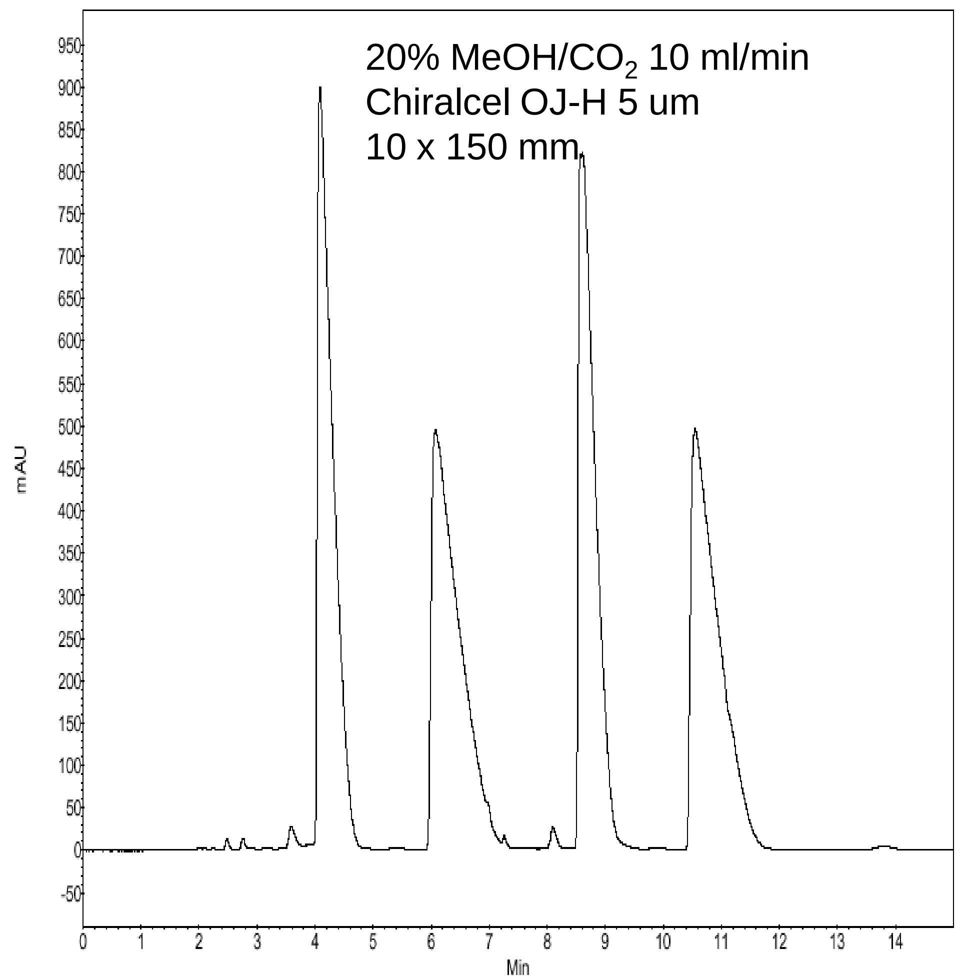
Preparative separation of seven stacked injections

Scale-up of a Carboxylic Acid by SFC- No TFA Needed

Analytical



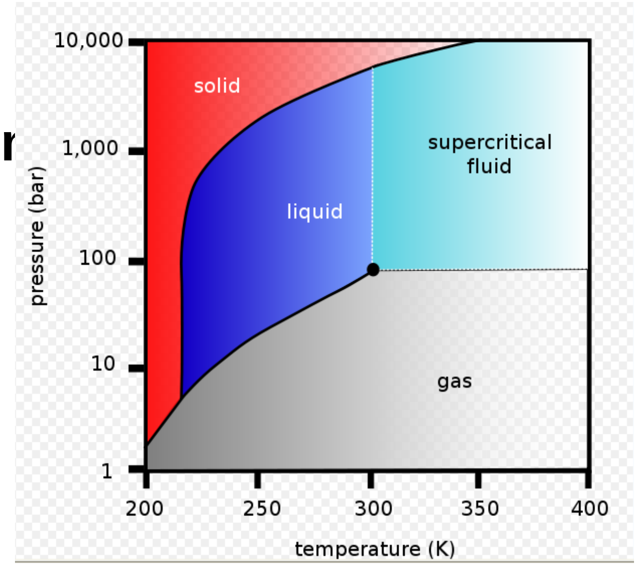
Stacked Injection (Prep)



Answers That Matter.

Lean Six Sigma- Global SFC Initiative

- **Aim to move 80 - 85% of purifications to SFC by 2010**
- **$2 Million Investment (2009)**
- **Significant solvent savings per year**
- **Greater efficiency, shorter timeline**
- **Less energy used and a significant tir reduction at sample drying stage**

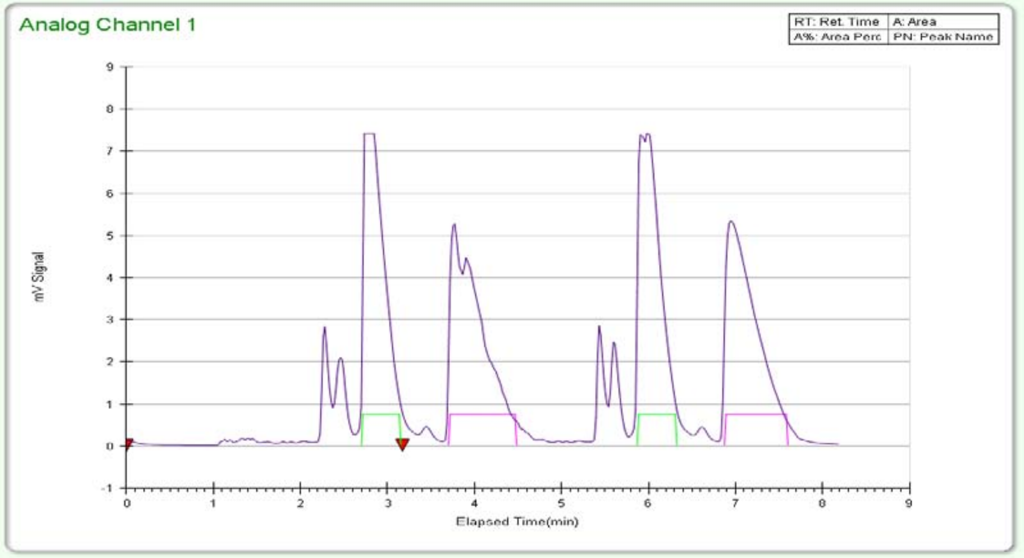


Major component is Carbon Dioxide in a Supercritical Liquid state

Project black belt (Craig White- Lilly UK)
Project sponsor (Keith Burton- Lilly US)

Answers That Matter.

Purification using the Thar SFC 80

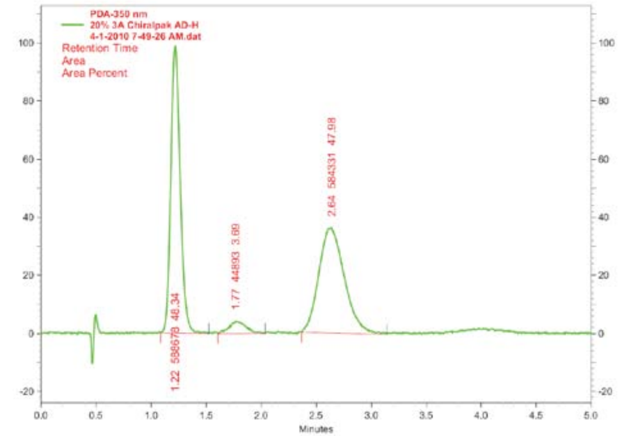


- 30% MeOH/CO_2 70 mL/min Chiralpak IC 21 x 250 mm 5µm
- 68 mg/injection every 3 min (impurities didn't allow faster cycle time)

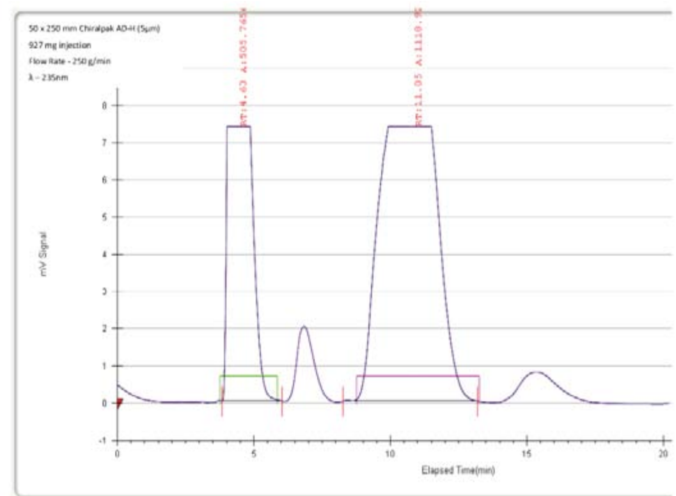
Answers That Matter.

Scale up from mg to 10 g

Jasco
Analytical SFC

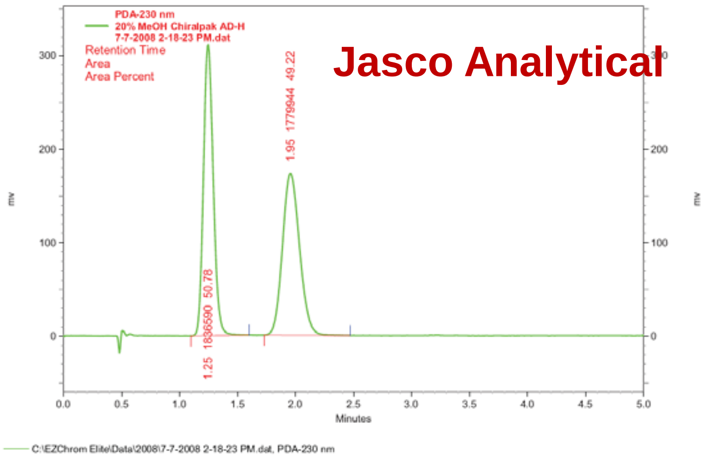


Thar 350
Prep SFC



Answers That Matter.

SFC Scale up from analytical to prep (400 g)

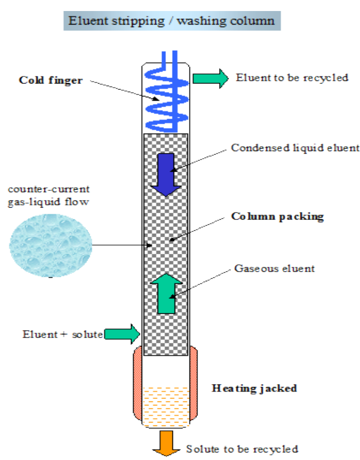


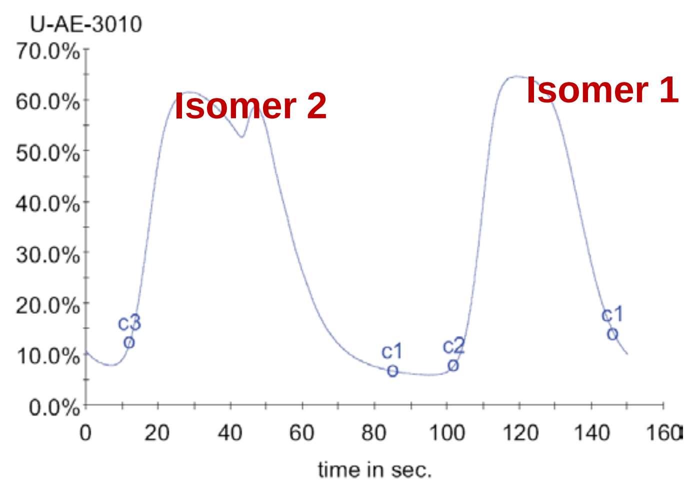
Jasco Analytical

```
Record 664:
Cycle: 0          Duration: 149.95 sec.
  Start date: 2009-05-01 14:25:28
  End date:   2009-05-01 14:27:57
 Avg. Flow                    Avg. Temp.                  Avg. Pres.
 U-FT-3010 : 499.9 g/min     U-TT-3010 : 35.0 °C         U-PT-3040 : 100.0 Bar
 U-FT-3020 : 0.0 ml/min
 Feed setpoint : 50.0 ml/min
Reference point: 8   Collection point: 4   Opening time:
  R1 : 0.20 sec.
  R2 : 3.00 sec.
  R3 : 10.60 sec.
                       C3 : 12.10 sec.      C1 : 12.10 sec.
  R4 : 34.70 sec.
  R5 : 44.95 sec.
  R6 : 49.00 sec.
                       C1 : 85.10 sec.      C3 : 73.00 sec.
                       C2 : 102.10 sec.     C1 : 17.00 sec.
  R7 : 102.15 sec.
  R8 : 126.20 sec.
                       C1 : 146.05 sec.     C2 : 43.95 sec.
                                            C1 : 3.90 sec.
Comment:
```

5x33 cm Chiralpak AD
20:80 MeOH/CO_2
220 nm UV detection
10 mL (600 mg) injections in MeOH
- approx. 650 injections made to process the 400 g as shown
- peak drifting observed for the first two days of operation but settled down after the pump check valves were cleaned/replaced and a 40 deg. C setting on the cyclones was used

Novasep SuperSep 50





stacked injection

Answers That Matter.

Increasing Efficiency for Purification in Support of Pharmaceutical Chemistry

- **Direct comparison of chromatographic technologies (optimization) for larger scale projects**
- **Chromatographic resolution vs. other resolution techniques and/or enantioselective synthesis**

Considerations for Preparative Chromatography

- What is your screening data telling you (e.g. selectivity, peak resolution, etc.)?
- Is existing option(s) "scalable" (e.g. how well does it load, cycle time, etc.)?
- Time and "Cost"
- Method optimization with "production" in mind- HPLC vs. SFC
- Sample Purity and Solubility
- Cost reduction- controlling variables such as solvent consumption
- Quality and recovery expectations

Answers That Matter.

Possible Scenarios for Technique Choice Based on Initial Screening Data

- SFC superior to LC (selectivity and peak resolution, <five column volume cycle time)
- LC superior to SFC (selectivity and peak resolution, <three column volume cycle time)
- SFC and LC similar; availability of prep instruments w/capacity, solubility, and scale-up comparison considered for larger samples
- Poor SFC and LC options- additional CSP evaluation, alternative molecules

Fixed and Variable Cost Factors

- Approximate initial lab instrument capital costs

  Batch HPLC → SSP → → SFC SMB (not deployed at Lilly)

  2x 2x 2x

  (depreciation and maintenance contributes approx. \$2/g material purified based on aggregate of purification and supporting instrumentation in our lab operation vs. material processed year to year)

- FTE and/or contractor costs and associated lab overhead (up to \$1000/day/individual)

- Supplies (CSP < \$1/g material purified, solvents the main cost, see later slide)

Typical Solvent Consumption per Technique

- Batch HPLC (0.5L-25L/g sample processed; in practice <5L/g when selected for larger scale)

- Closed Loop/Recycle HPLC (0.2L-10L/g sample processed; in practice <2L/g when selected for larger scale)

- SSR (0.15L-5L/g sample processed; in practice <1L/g when selected for larger scale)

- SFC (typical 50-500 mg loading on 5 cm ID column/injection with 2-5 min. cycle time)

  10:90 modifier/CO_2 mobile phase (0.12L-3L co-solvent/g sample processed)

  20:80 modifier/CO_2 mobile phase (0.24L-6L co-solvent/g sample processed)

  40:60 modifier/CO_2 mobile phase (0.48L-12L co-solvent/

  processed)

SSR vs. Batch HPLC Comparison Example

Summary of Optimized Batch Conditions (Single pass stacked injection using 70/30 MeOH/ACN):

8 x 40.5 cm Chiralpak AD (1 kg packing)

Loading: 2 gram (batch optimized loading)

Cycle Time: 10 minutes

Productivity: 20 gram /hour,

0.48 kg/kg CSP/day (day = 24 hour)

Solvent usage: 29.4 liter /hour

706 liter /day

1.47 liter /gram material

Total solvent usage (theoretical, for 391 g): 575 liters 70/30 (403 L MeOH x $2.75/L + 172 L ACN x $3.35/L = ~$1684)

Summary of Final SSR Development Conditions (50/50 3A ethanol/ACN):

8 x 40.5 cm Chiralpak AD (1 kg packing)

Loading: 6 gram (60 ml injection) -- optimized

Cycle Time: 4.6 minutes

Productivity: 78 gram /hour,

1.87 kg/kg CSP/day (day = 24 hour)

Solvent usage: 16.3 liter /hour

391 liter /day

*0.21 liter /gram material

Total solvent usage (for 391 g): ~82 liters 50/50 3A/ACN (41 L 3A x $2.17/L + 41 L ACN x $3.35/L = ~$226)

* Similar to solvent/feed ratios observed with assorted large scale SFC campaigns

Answers That Matter.

Actual Comparison to SFC- "Ideal" Racemic Intermediate for Large Scale

Quantity	technique	Column	Mobile Phase	Loading	Through put (g/hour)	Production Rate (kg/kg CSP/day)	Solvent Consumption (l/g)
391 g	SSR	8x40.5 cm Chiralpak AD	50:50 3A/ACN	6 g	78	*1.87	0.21
	Batch HPLC	8x40.5 cm Chiralpak AD	70:30 MeOH/ACN	2	20	0.48	1.47
	SFC	5x15 cm Chiralpak AD-H	20:80 3A/CO_2	600 mg	27.7	2.95	0.13

* Threshold for SMB consideration is typically > 1 kg/kg CSP/day

Another Larger Scale Campaign Comparison- Racemic Intermediate

Quantity	technique	Column	Mobile Phase	Loading	Through put (g/hour)	Production Rate (kg/kg CSP/day)	Solvent Consumption (l/g)
1520 g	SSR	8x38 cm Chiralpak AD	100% MeOH	3 g	41.5	0.996	0.31
	Batch HPLC	8x38 cm Chiralpak AD	100% MeOH	1.43 g	6.6	0.158	2.5
	SFC	5x25 cm Chiralpak AD-H	20:80 MeOH/CO2	500 mg	8.2	0.596	0.44
3312 g (same cpd.)	SSR	11x33 cm Chiralpak AD	100% MeOH	6.8 g	102	1.224	0.3

Answers That Matter.

CSP and Solvent Costs- Chiral Purification

(key inputs include solubility, resolution, automation, and type of solvent used)

Batch HPLC

$5/g $60/g

(applied on mg-500 g scale; larger scale only if the other more cost effective techniques not viable)

Batch SFC

$1/g $16/g

currently applied on mg- 1 kg scale when viable

SSR

$0.75/g $10/g

currently applied on 10 g-5 kg scale when viable

SMB (outsourced to external partners)

$0.10/g $10/g

metric ton scale lab scale

Solvent and CSP costs lower due to bulk usage in LSCL
(SFC prep CSP 2-4x higher when 5 micron used)

Costs due to 4L and 20 L solvent container usage are up to 5x higher/L so
should only be used for smaller scale or low solvent consumption projects

Supplies and materials cost/gram of material produced

$0.01/g $1/g $10/g $25/g $50/g $75/g $100.00/g

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Phase Appropriate Technology

	Discovery	Early Development	Full Development	Manufacturing
Quantity	1mg - 50g	50g - 5kg	5 -100kg	Tons
Appropriate Technology	HPLC (15%) SFC (80%) SSR (5%)	HPLC (10%) SFC (50%) SSR (40%)	HPLC SFC SSR SMB	SFC? SMB
Cost importance	Minor	Significant	Very Significant	Critical
Scale-up feasibility	Minor importance	Middle importance	Major importance	Prerequisite

Answers That Matter.

Conclusions

We believe the use of newer SFC technologies in conjunction with strategies to make LC separations more efficient will increase our productivity and decrease the overall cost of operations for purification at Lilly.

- Well known advantages of green chromatography.

- Reduced method development time by the use of parallel column screening as well as smaller particle CSP and columns.

- Even though >95% success rates can be achieved with either an HPLC or SFC approach, better outcomes through available complementary techniques and column chemistries is important.

- The scale of preparative purification support that can be accomplished in a regular laboratory in 2011 is remarkable when compared to the not-so-distant past.

- Use of efficiency strategies in large scale purification and technologies like SSR and SFC have driven these improvements.

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