



Breaking the Flow Rate Barrier: Utilization of High Flow Mass-Directed SFC for Pharmaceutical Applications

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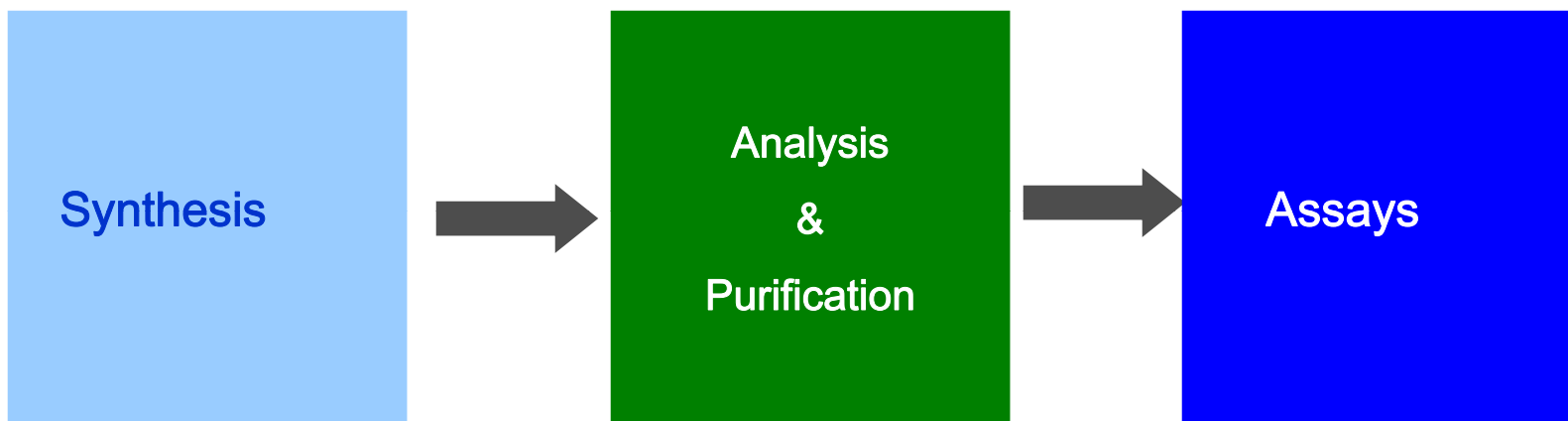


Presentation Overview

- I. Drivers for MS-Directed Purification**
- II. Challenges to MS-Directed Prep SFC**
- III. Introduction to Thar SFC/MS Prep 100**
- IV. Applications of MS-Directed Prep SFC**
- V. Next steps**



Why do we care?



Discovery

To Early
Development



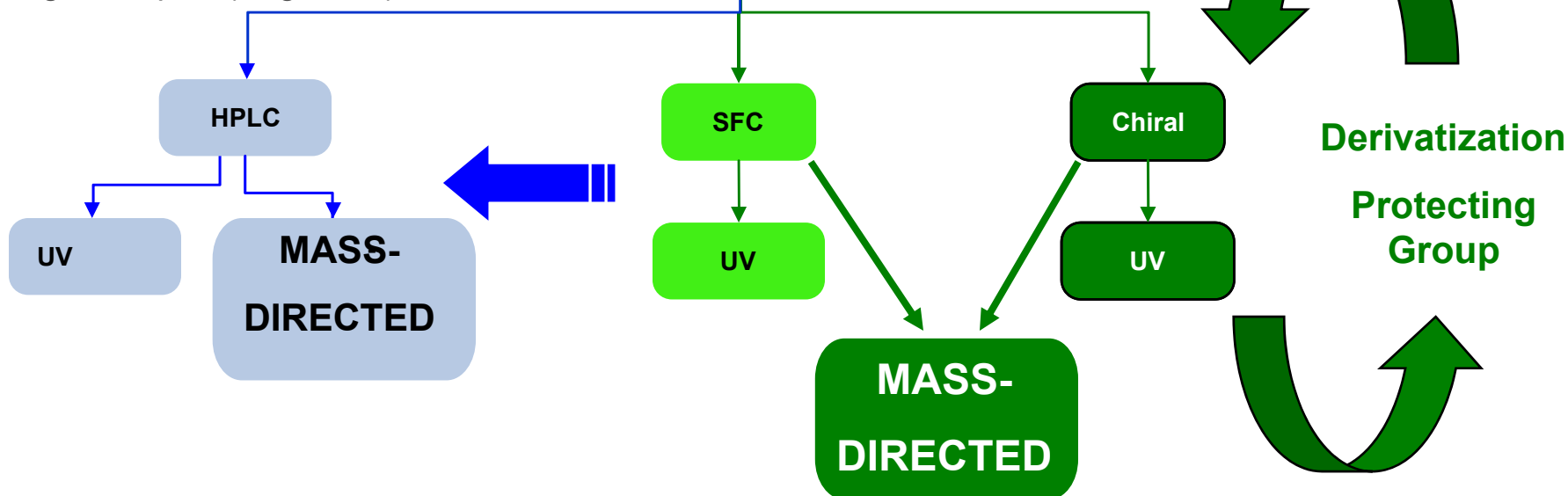
HPLC/SFC Purification

Sample Submission

Pre-prep Screening

- Targeted Libraries, 80-100µM
- Parallel synthesis (PMC) small arrays
- Single samples (singletons)

• Chiral-all scales





Racemate vs. Single Enantiomer

- **Single enantiomers of chiral active pharmaceutical ingredients (APIs) may have different:**
 - Pharmacokinetic properties in animal models
 - Absorption, distribution, metabolism and excretion
 - Pharmacological or toxicological effects
 - Biologically “active” isomer may have desirable effects
 - Biologically “inactive” isomer may be toxic
- **Increased pressure by FDA to switch from racemic to single enantiomer APIs**
- **Forces companies to take action:**
 - Develop better manufacturing controls
 - Comprehensive safety profile of both enantiomers
 - Proper clinical evaluation of these drugs



Approaches to Pure Enantiomers

- Chiral Synthetic Approach
 - Stereoselective or asymmetric syntheses
 - Biotransformation or enzymatic resolution
 - Catalytic enantioselective processes
- Racemic Approach
 - Crystallization
 - Chiral salt resolution
 - CE (capillary electrophoresis)
 - SMB (simulated moving bed technology)
 - Chromatographic separations (HPLC, SFC)



Drivers for MS-Directed SFC Development

■ Flow Rate Barrier

- Previous MS-Directed systems were limited to < 30 mL/min
- Analytical SFC run at ~5ml/min
- Using generic scale factor of the column radii from analytical (4.6mm) to prep (21mm)
 $(r_{\text{prep}}^2/r_{\text{analytical}}^2) \Rightarrow 20$
- ** Prep flow rate = 100mL/min

■ Purification of compounds with no chromophores

Merits of Purification Approach

	SFC-UV	HPLC-UV	HPLC-MS	SFC-MS
Polar compounds unretained on C18	X		X	X
Compounds too polar for CO₂/MeOH		X	X	
Incompatibility w/ H₂O but not MeOH	X			X
Incompatibility w/ MeOH but not H₂O		X	X	
Poorly-resolved chromatographic peaks			X	X
Poorly ionizing species	X	X		
Weak or non-chromophoric species			X	X
Best chance of 1:1 sample to fraction correspondence			X	X
Excludes salt forms as final products	X			X
Less post-purification evaporation times	X			X
Equipment/Maintenance costs (relative to others)		X		



Development Challenges to Mass-Directed Preparative SFC

- Requires integration of multi-vendor components
 - ↑ complexity ⇒ ↓ reliability
 - SFC and MS vendor partnerships
 - Thar SFC/MS Prep 30 not appropriate for our applications, e.g. flow rate barrier
- Splitting/Diluting/Ionization
 - Viscosity of supercritical fluid is not constant throughout gradient—split ratio not maintained
 - Affects timing
 - Injection of >100mg onto column
 - Requires appropriate amount of dilution for the MS
 - ESI needs ion source (acids) which are incompatible with separation. APCI temperatures not high enough.



Challenges continued...

- Open bed fraction collection
 - Need to be able to isolate sample & solvent from depressurized CO₂
 - Requires containment of sample for ↑ recovery
 - SFC purification systems use either pressure manifolds for collections or closed containers—limits the number of collected fractions

⇒ LIMITED AUTOMATION POTENTIAL

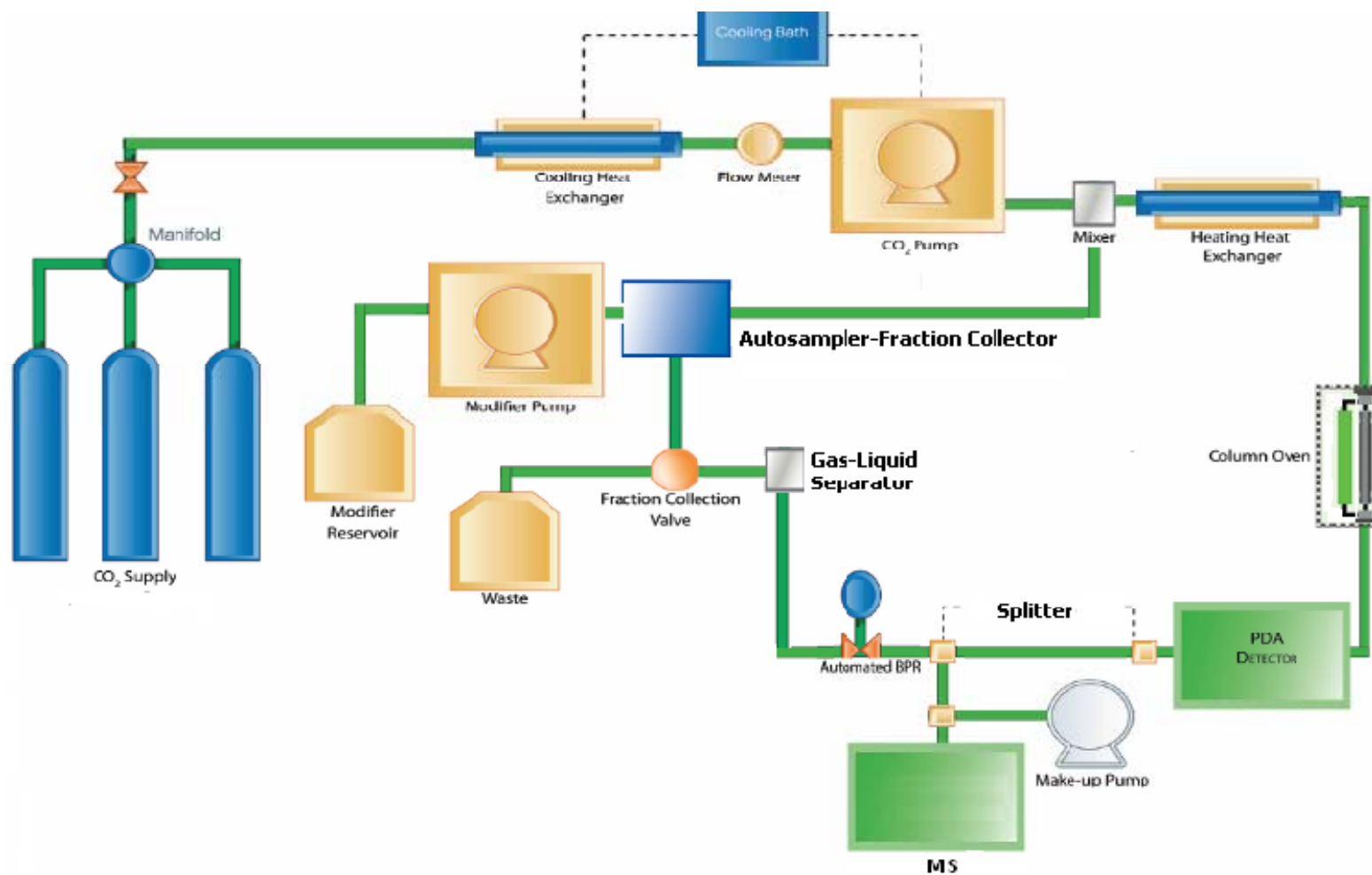


Mass-Directed Preparative SFC

- ✓ Product of a collaboration between Pfizer and Thar
- ✓ What were our goals for a Prep SFC/MS system?
 - Simplicity
 - Reliability
 - Validity
 - Open Access
- ✓ Higher Flow rates, 100 mL/min capability
- ✓ Active splitter capable of delivering constant split ratio across a gradient
- ✓ Open-bed fraction collection for automated, high throughput applications
- ✓ Ability to utilize Boolean logic for fraction collection triggering of UV and MS signals



Prep SFC/MS 100 Schematic



Thar SFC/MS Prep 100 System



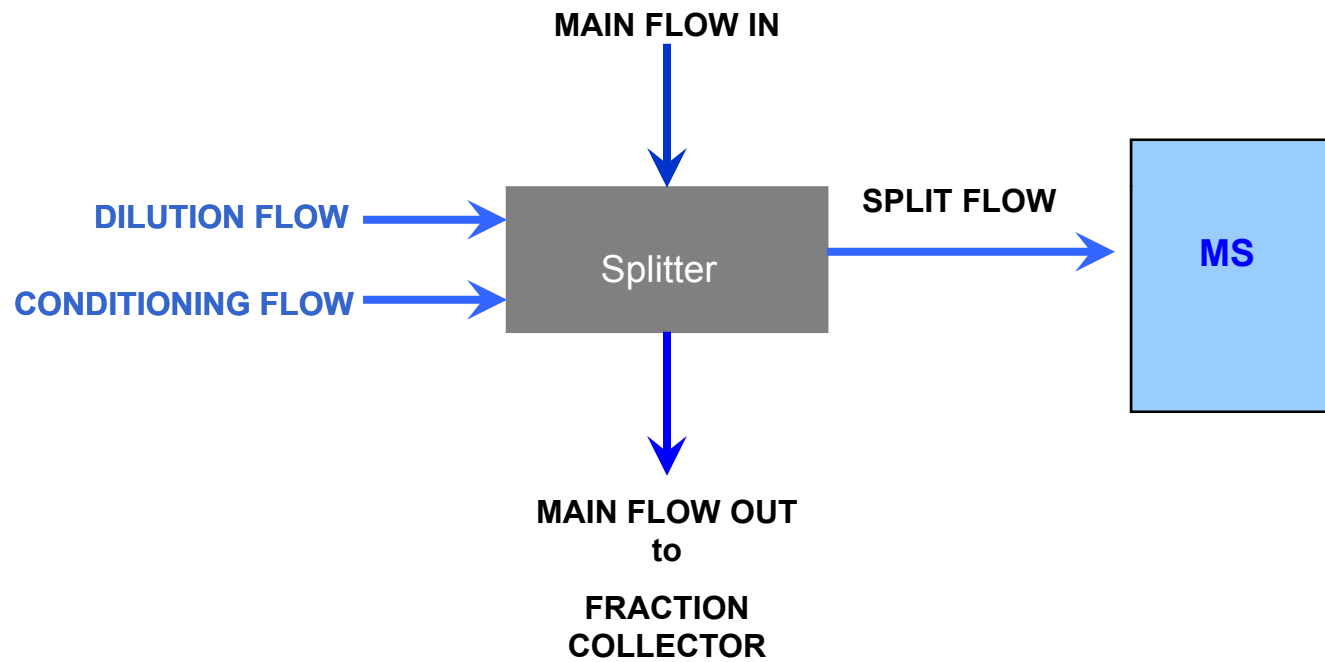
- MassLynx and FractionLynx 4.1
- Waters 3100 Mass Spec
- Waters 2996 PDA
- 2- Waters 515 pumps (conditioning and dilution)
- Waters 2767 inject/fraction collector platform
- Thar P200 CO₂ pump
- Thar P50 Co-solvent pump
- Thar ABPR
- Thar 6-Column Oven
- SSI makeup pump
- **Split diluter**
- **Gas-Liquid Separator (GLS)**

Thar SFC/MS Prep 100 System





Tunable Splitter



↑ or ↓ MS Sensitivity

Modify Thar Mobile Phase Method

Mobile Phase Parameters

Pumping System | Additional Options

Run Isocratic Split Ratio: Low Split Run Time: 9 min

Isocratic Parameters

Total Flow: 100 ml/min

CoSolvent Percentage: 5 %

Makeup Flow Rate: 30 ml/min

Run Gradient

Gradient Parameters

Start Perc	End Perc	Start Flow	End Flow	Total Dur
5	40	100.0	100.0	10.0
40	40	100.0	100.0	470.0
40	5	100.0	100.0	30.0
5	5	100.0	100.0	5.0

Remove Gradient Add Gradient Edit Gradient

OK Cancel

- MS Sensitivity is adjusted through Inlet Method of MassLynx
 - Each splitter setting has different conditioning/dilution pump flow rates
 - Adjustments to split ratio (↑ or ↓ amount of sample to MS)

Site Acceptance Testing - Standard Runs

4-Component Standard Mixture:

- Peak A - Flavone ($m/z=223$) 7.84 mg/injection
- Peak B - Carbamazepine ($m/z=237$) 8.37 mg/injection
- Peak C - Acetaminophen ($m/z=152$) 8.00 mg/injection
- Peak D - Sulfamethazine ($m/z=279$) 8.31 mg/injection

Chromatographic Conditions:

Column: ZyroSil Pyridine/Diol, 150x21.1mm,
5 μ semi prep column from Zymor, Inc.

Modifier: Methanol

Gradient: 5-40% in 9 minutes
(Hold 5% for 5 sec, 5-40% in 480 sec,
hold 40% for 30 sec, 40-5% in 20 sec,
hold 5% for 5 sec)

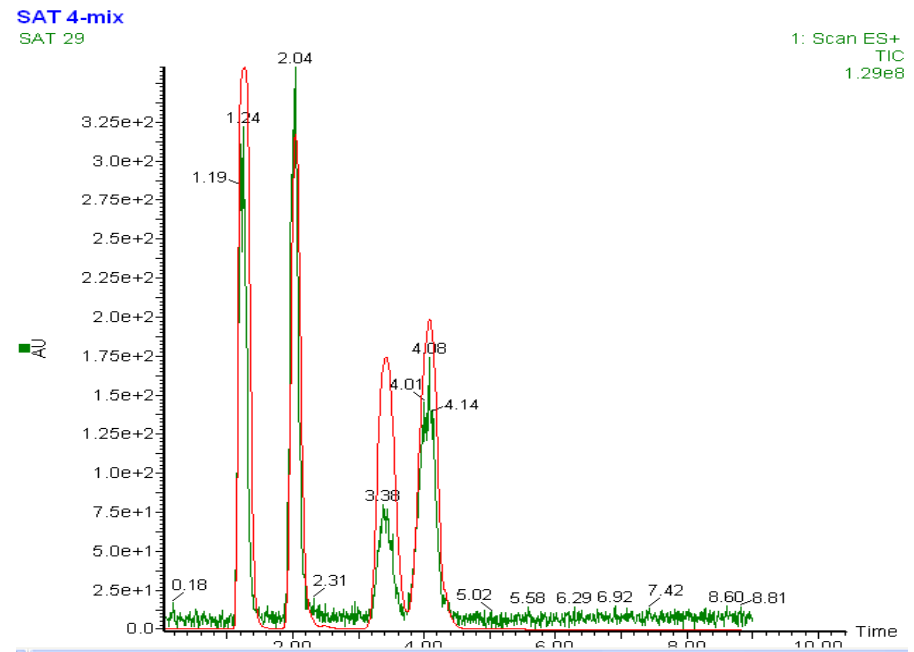
Total Flow: 100 g/min

Outlet Pressure: 120 bar

Ionization Mode: ESI+

Injection Volume: 0.9 mL

Low Split Ratio setting



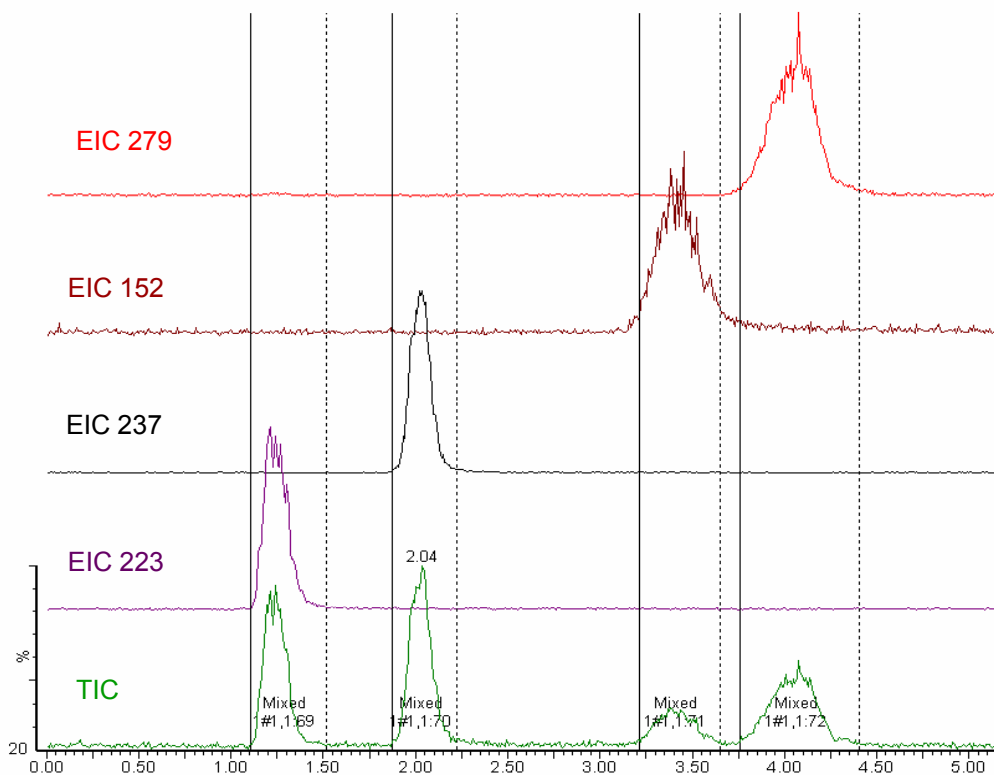
Overlaid preparative UV/TIC (ESI+) chromatograms



SAT Results:

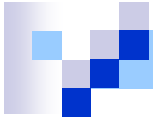
SAT 4-mix
SAT 29

	Peak	UV220 Purity	TIC Purity	Impurities (TIC)	Recovery (TIC)
Injection 1 5 sec wash	A	100	100		99
	B	100	100		98
	C	100	100		93
	D	100	99.03	0.97% C	99
Injection 2 5 sec wash	A	100	100		100
	B	100	100		97
	C	100	100		96
	D	100	98.85	1.15% C	97
Injection 3 5 sec wash	A	100	100		98
	B	100	100		98
	C	100	100		95
	D	100	99.5	0.5% C	101

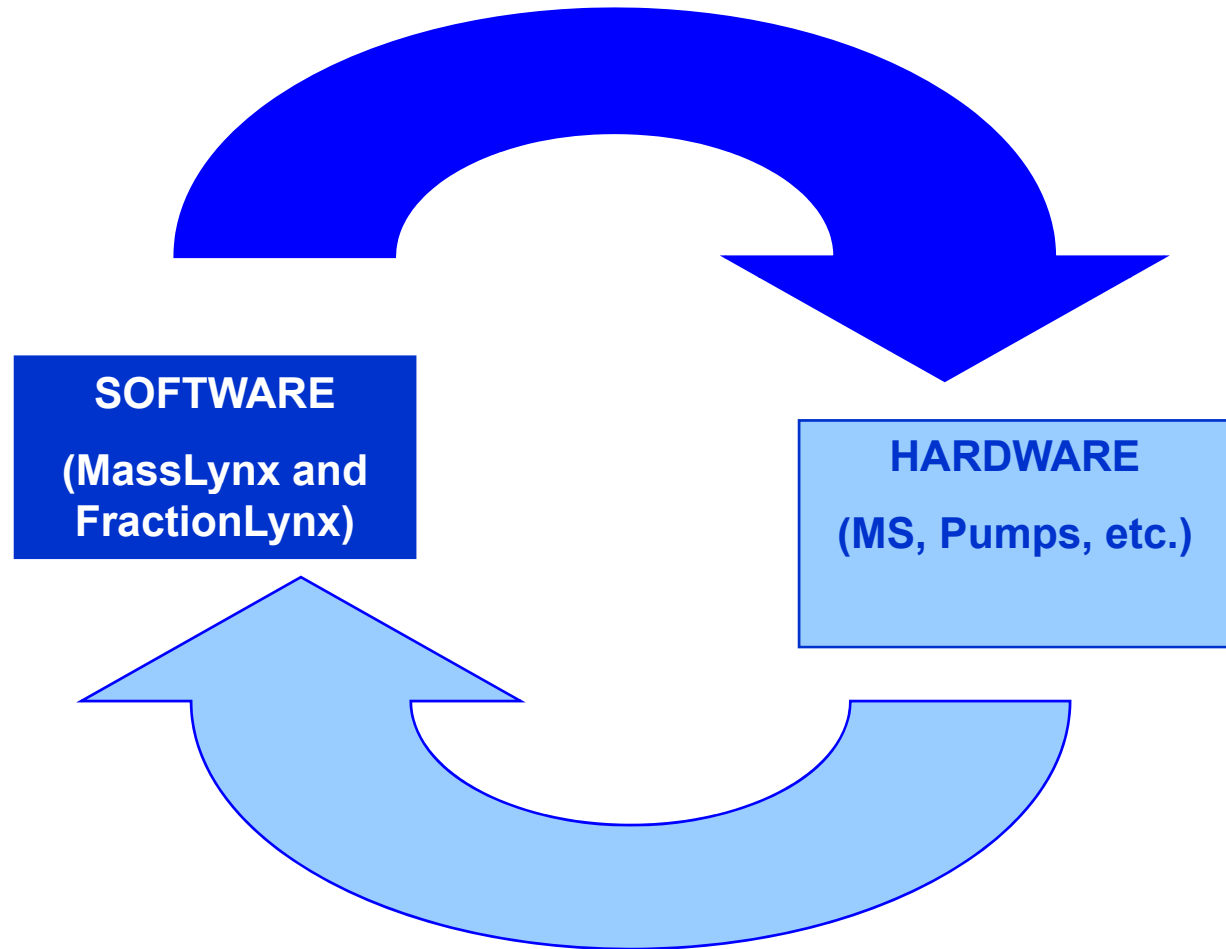


- Purity of all 4 components were >98% by UV₂₂₀
- Overall recovery of each was >90%
- Closely eluting peaks--Peak 4 contained small amounts of Peak 3 impurity
- 5 second wash between fractions minimized sample carryover





Learning Curve



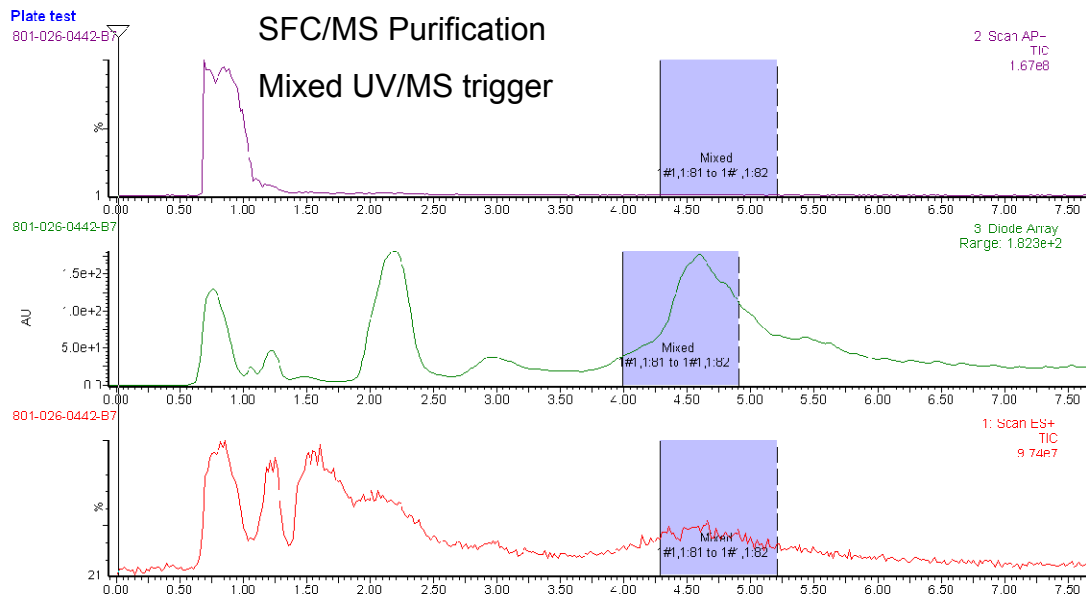
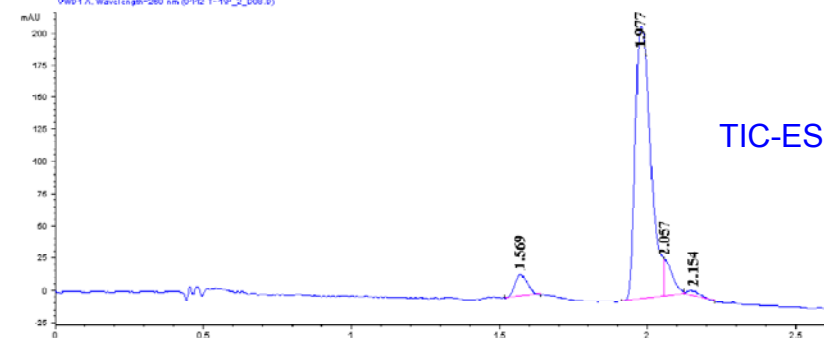
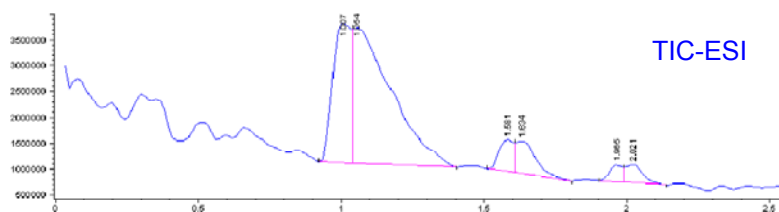
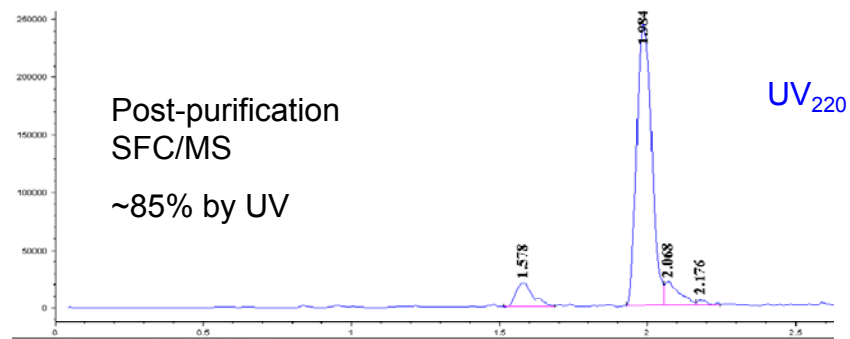
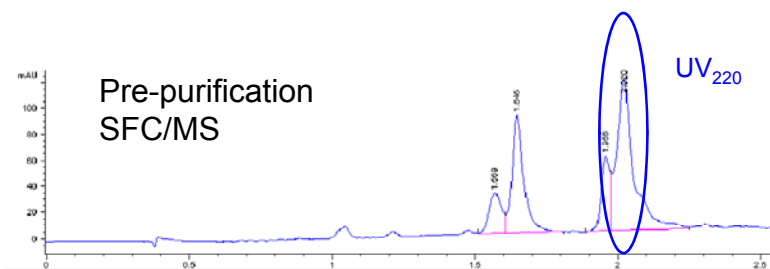


Optimizing for Library Applications

- Goal: to duplicate prep LC/MS triggering capabilities
 - ESI and APCI switching
 - Mixed mode triggering (PDA and MS)
- Installed ESCI (multimode ESI and APCI source)
- Optimization of ESCI capabilities for SFC in conjunction with Waters



Library #1: weak MS signal



Analytical:

Zymor Pyr/Diol, 150x4.6mm, 5u column
5-50%MeOH gradient, 18%/min ramp rate
5.6mL/min flow; 140 bar

Preparative:

Zymor Pyr/Diol, 150x21.1mm, 5u column
5-50%MeOH gradient, 8%/min ramp rate
100mL/min flow; 120 bar





Library #2

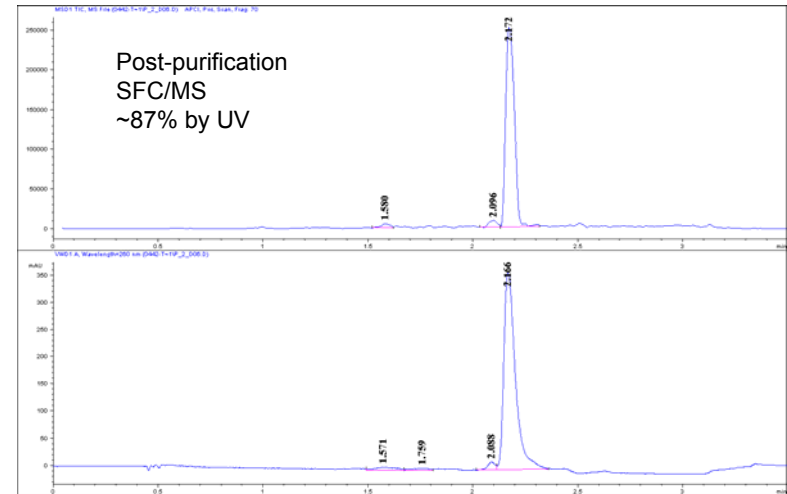
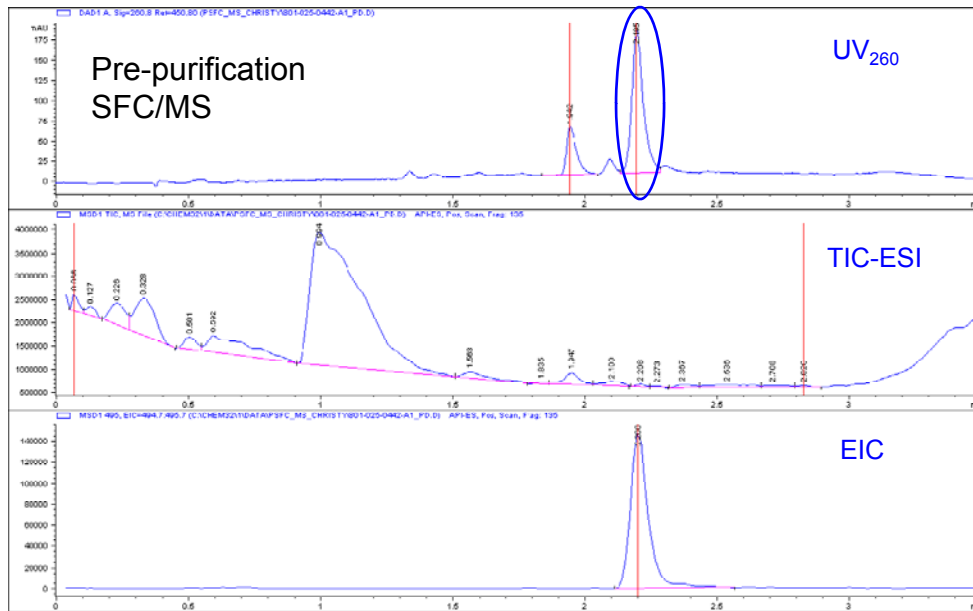
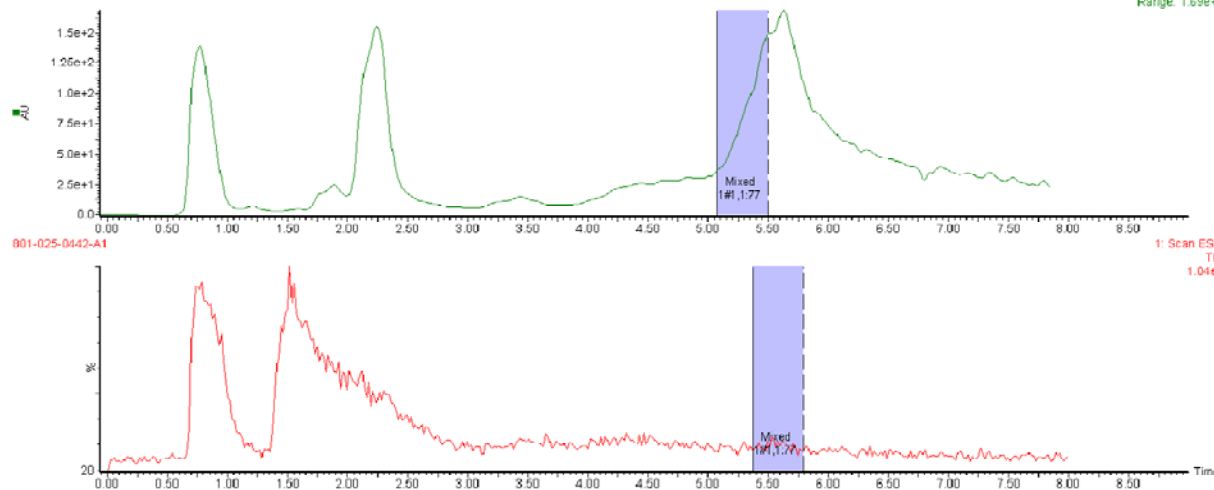


Plate test
801-025-0442-A1

SFC/MS Purification

3: Diode Array
Range: 1.69e+2

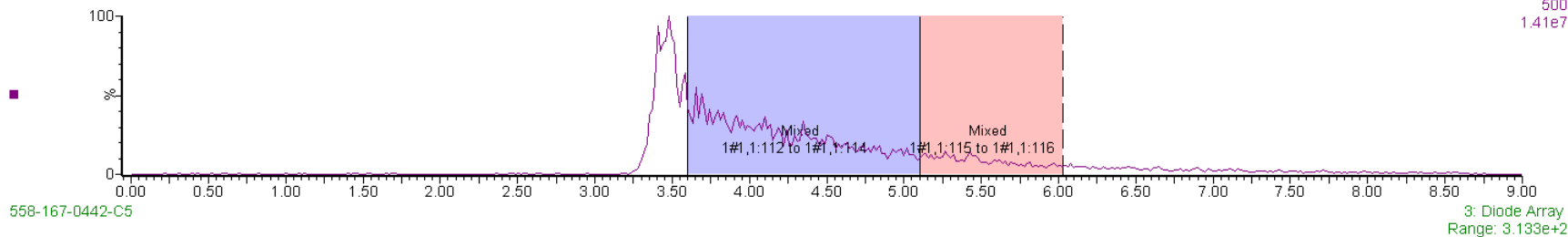


Using Mixed Triggering

Plate test

558-167-0442-C5

1: Scan ES+
500
1.41e7



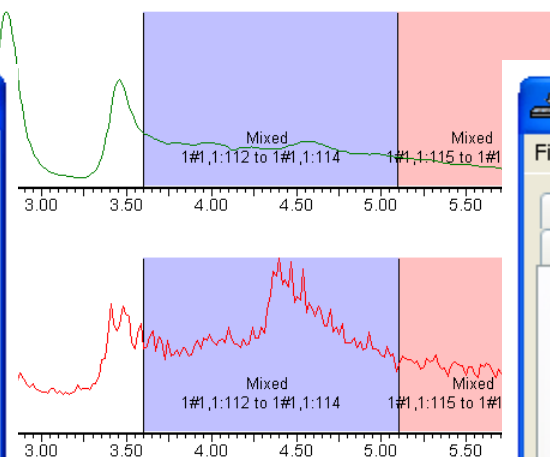
FractionLynx Method - 100ml_16_9_delay_...

File View Help

APCl- APCl+ PDA Timed Events
General Timing Volume ES- ES+

Trace Monitoring
Solvent Front Delay (secs): 60

Peak Timing
Split/Collector Delay (secs): 16.9
MS/Analog Delay (secs): 0
MS/DAD Delay (secs): 8.7



FractionLynx Method - 100ml_16_9_delay_...

File View Help

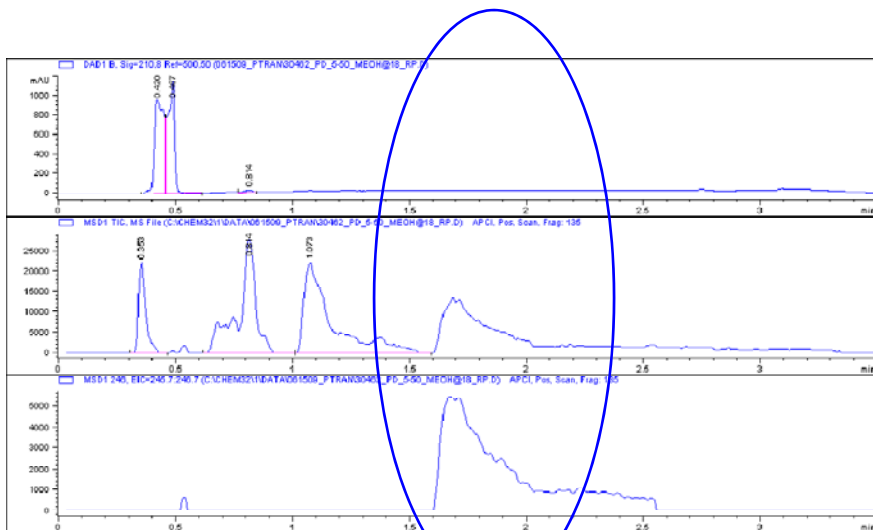
APCl- APCl+ PDA Timed Events
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Trace Monitoring
Solvent Front Delay (secs): 60

Peak Timing
Split/Collector Delay (secs): 16.9
MS/Analog Delay (secs): 0
MS/DAD Delay (secs): -8.7



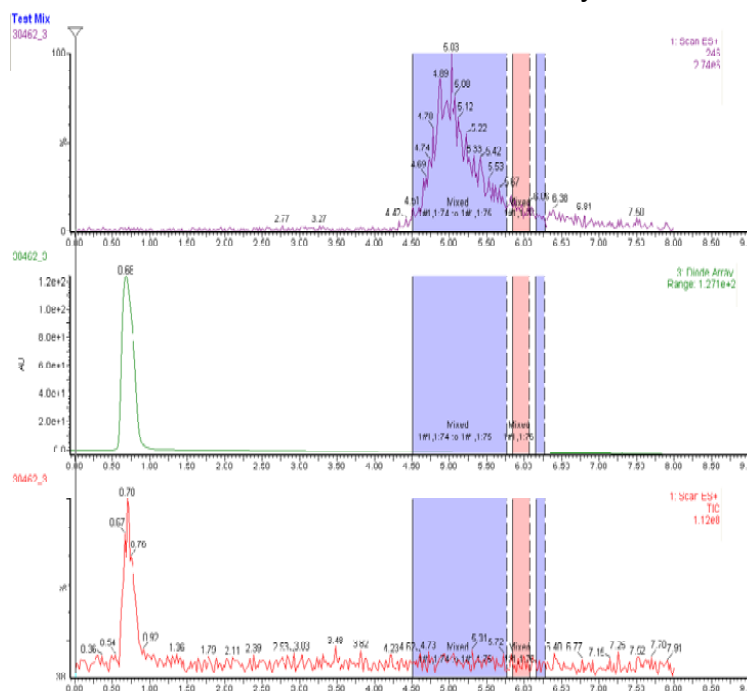
Non-Chiral Singleton



Pre-purification
SFC/MS

Challenge:
No UV signal

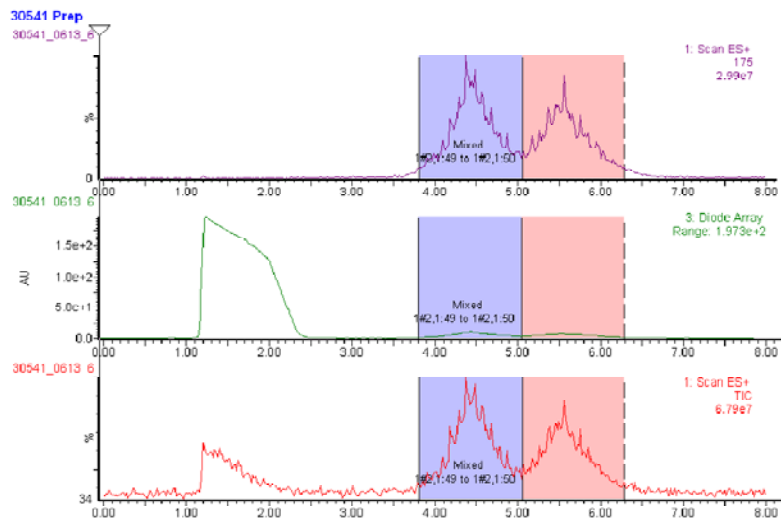
SFC/MS Purification – MS only





Chiral Purification

SFC/MS Purification



Analytical:

PhenomenexLux Cellulose-2,
250x4.6mm, 5u column

15%MeOH, 2.0mL/min flow;
140 bar

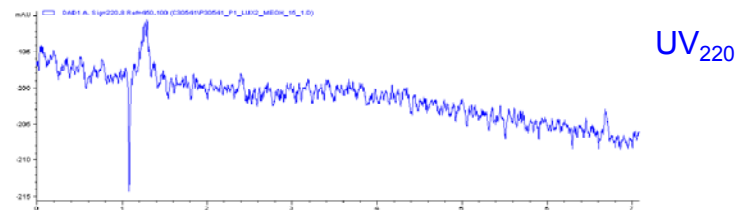
Preparative:

Phenomenex Lux Cellulose-2,
150x21.1mm, 5u column

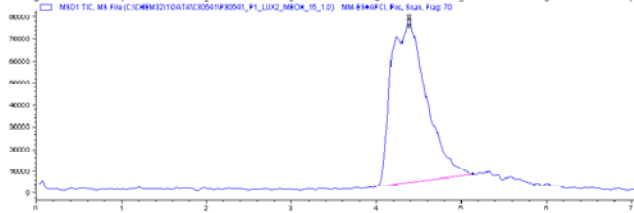
8%MeOH, 100mL/min flow;
120 bar

MS only triggering

Post-purification SFC/MS

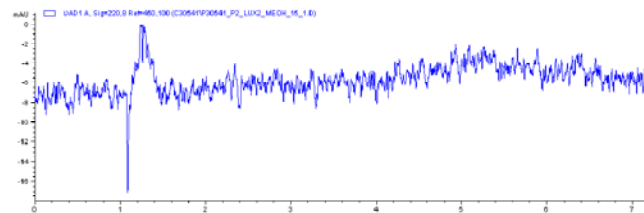


UV₂₂₀

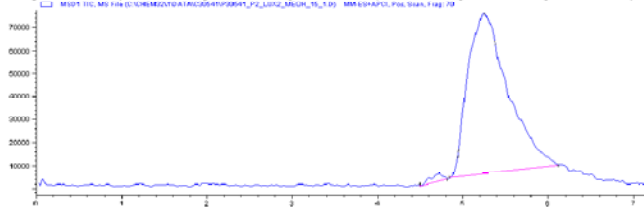


TIC

ENANTIOMER 1
98.5% ee



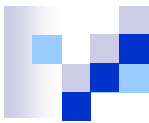
UV₂₂₀



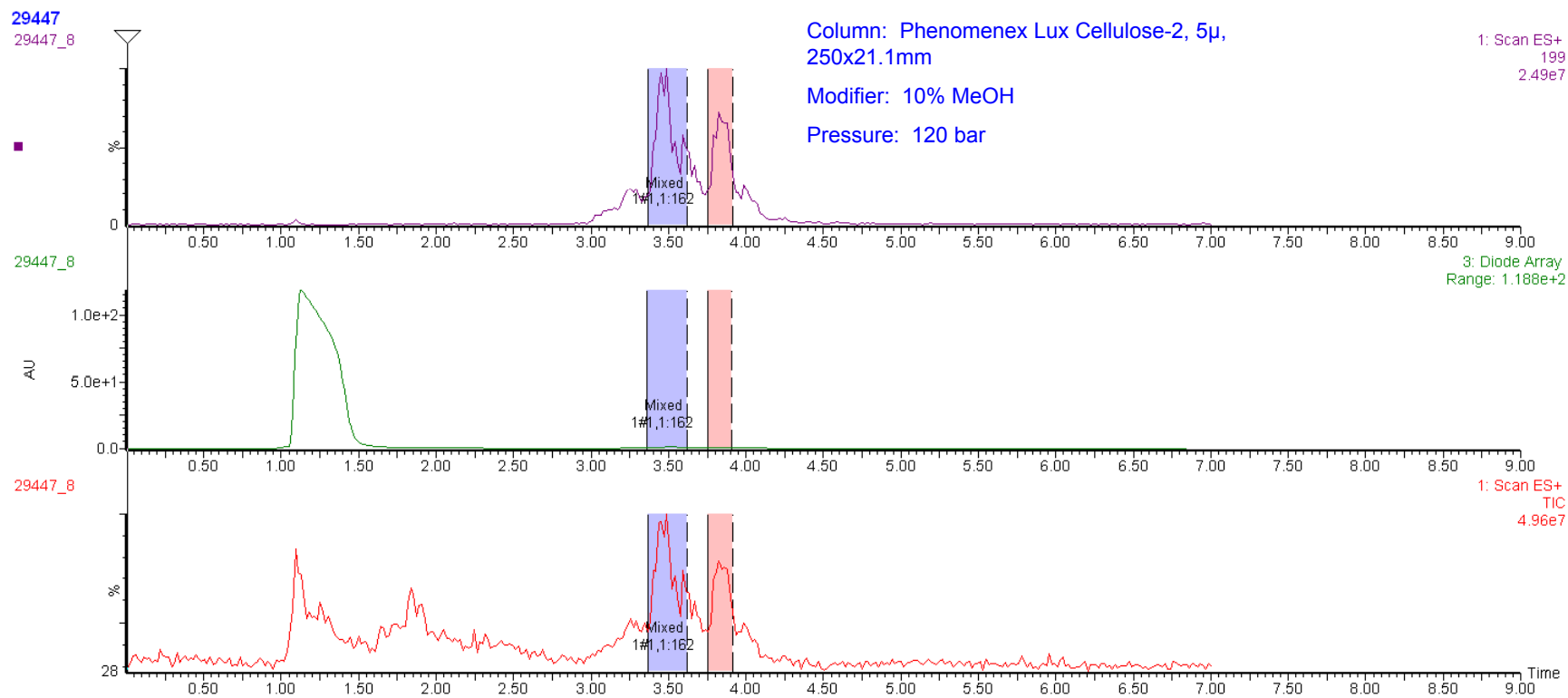
TIC

ENANTIOMER 2
~97% ee





Chiral Example #2



Challenge: Little to no UV chromophore





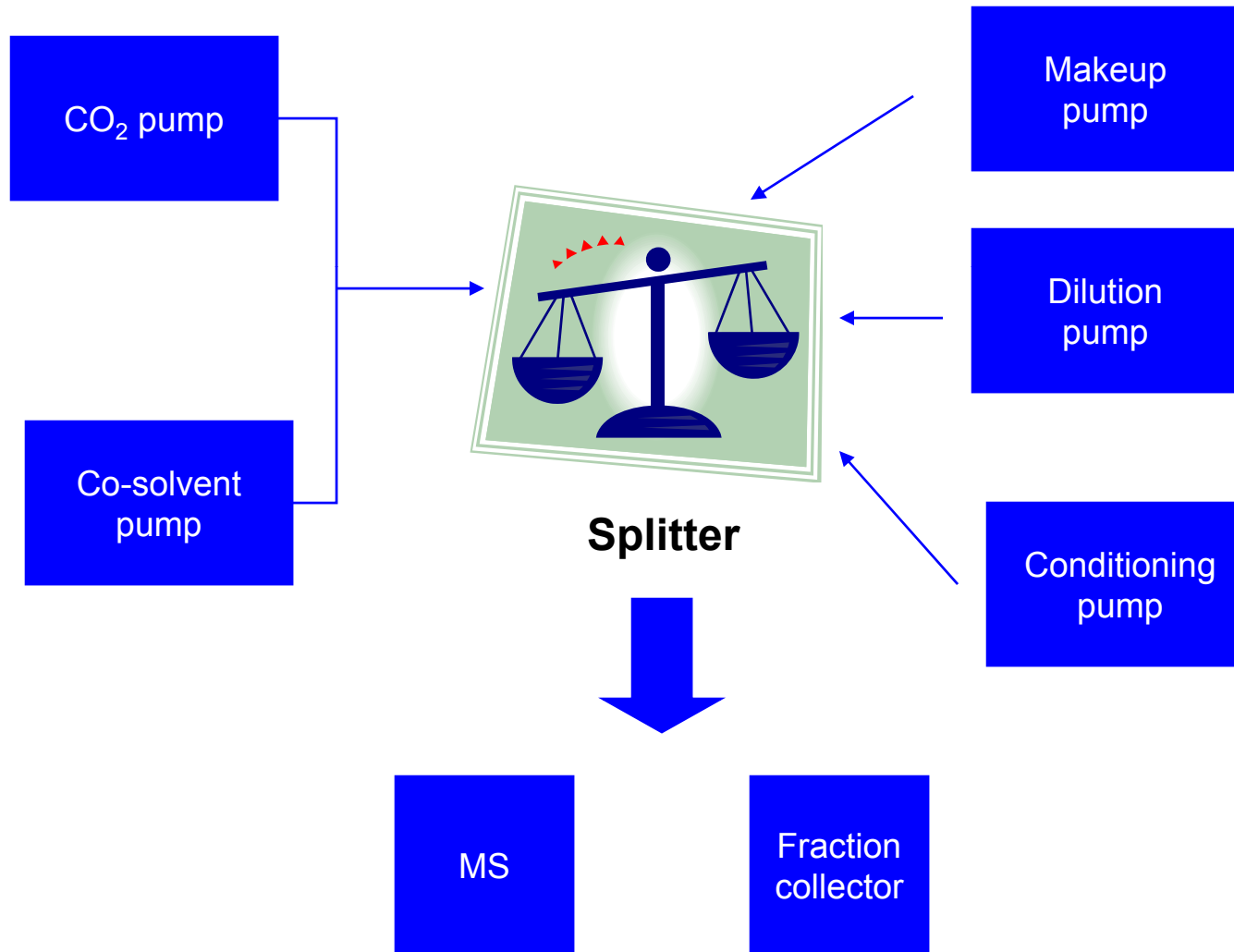
Prep SFC/MS 100 Realities

■ Hurdles

- Multiple pumps, from different vendors
- Requires frequent recovery studies to ensure system performance
- Splitter requires 5 components in order to achieve the proper dilution/split ratio;
 - heavy dependence on proper working order of cond and dilution pumps, and splitter not clogging



System complexities

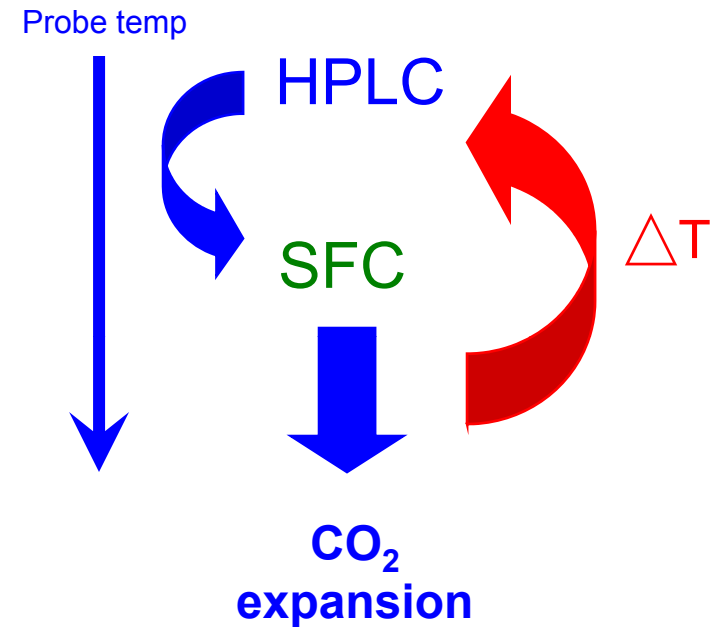
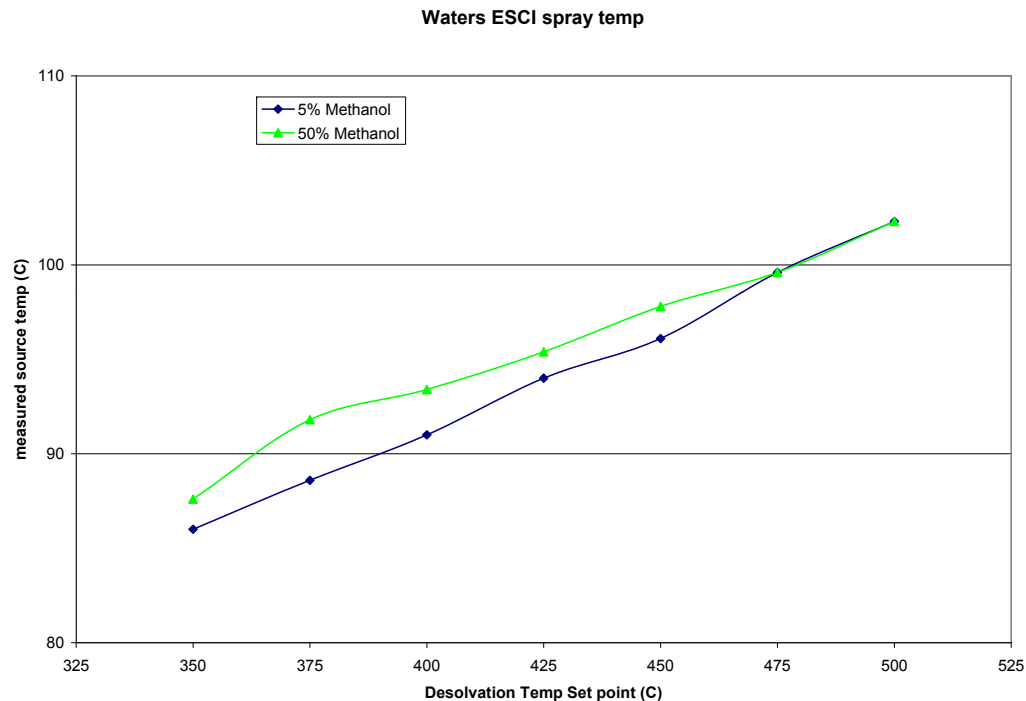




New Challenges & Next Steps

- System has only been optimized for 100 mL/min
 - Flexibility of lower flow rates is desired vs. increasing column size (\$\$)
 - Operation at lower flows will require studies to determine the correct timing delays; may be system-specific
- Open Access uptake
- Temperature of the MS spray is significantly less than set point
 - Better MS signal if temperature was higher

Spray Temp Studies



Results:

- Source temperature – 140°C
- Actual temperatures ~ 40° less than setpoint
- Direct heating of conditioning and dilution fluids had no effect



Conclusions

With the Thar SFC/MS 100:

- We now have the first high flow mass-directed SFC
- Can demonstrate high recovery and purity with standards
- Can be utilized in either ESI, APCI, or both (ESCI) modes
- Collections based on UV only, MS only or a combination of triggers
- Requires a considerable learning curve
- Requires more characterization to increase flow rate flexibility





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