

# Several Uses of Mass-Directed SFC in Basic Research At Merck

---

Ray McClain & James Small  
22-Jul-2009



# Overview

---

- Introduction of Automated Synthesis and Purification Group
- Why is Mass-Directed SFC so desired
- Screening, purification, and purity assessment of libraries
- Case study: Bacterial metabolite isolation via mass-directed SFC with SIR
- Summary



# Automated Synthesis and Purification Group (ASAP)

---

- MedChem 150 people
  - ASAP – 12 member central team
    - Keep Medicinal Chemists focused



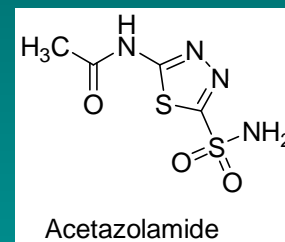
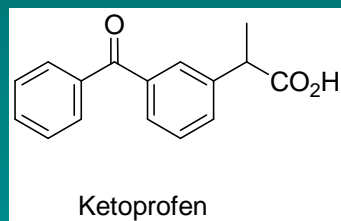
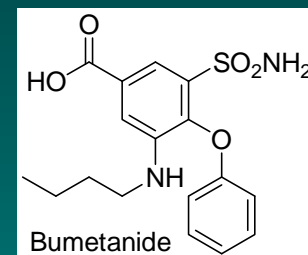
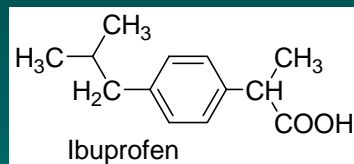
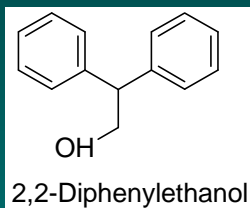
# Why Mass-Directed SFC

---

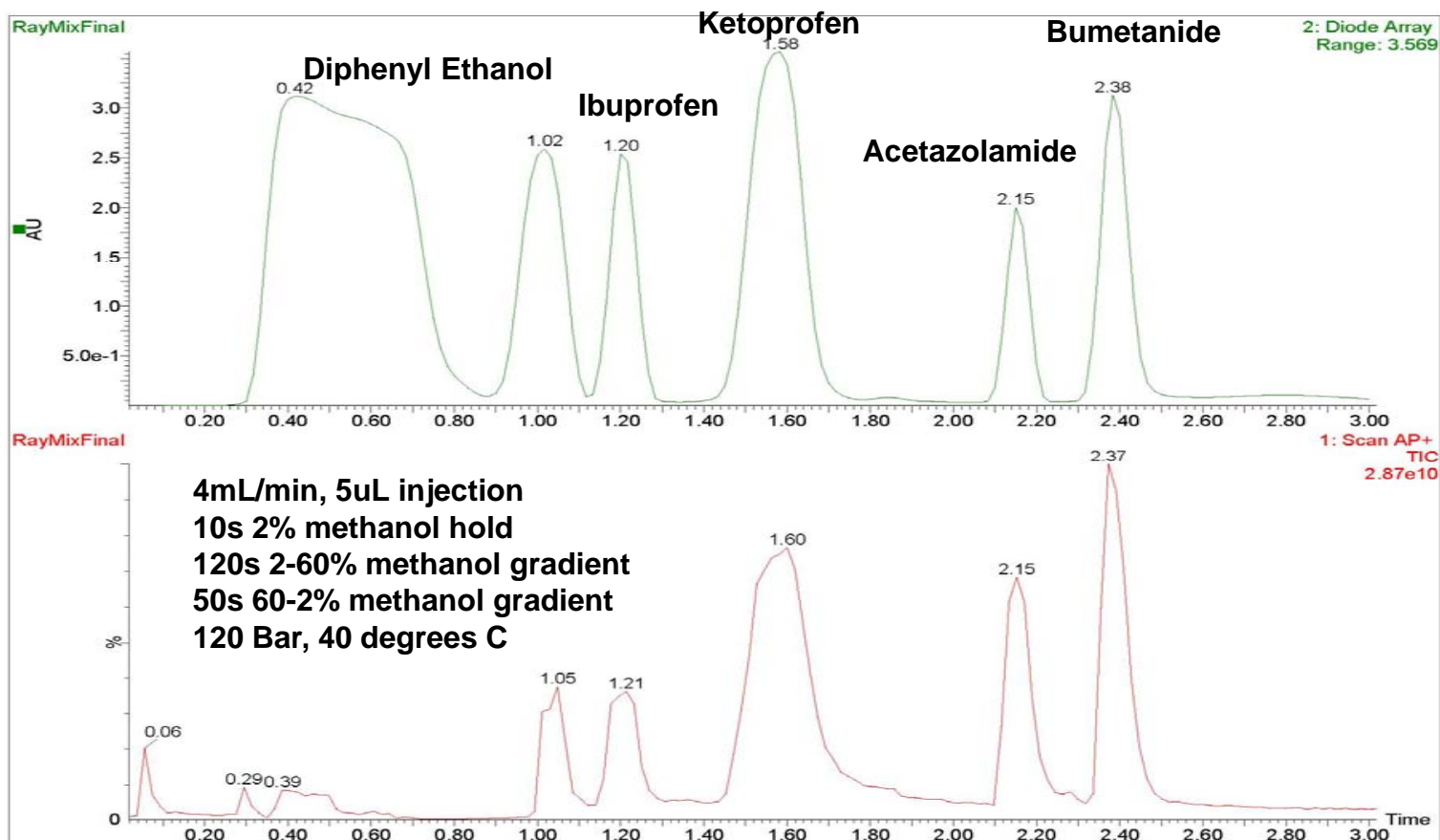
- Seeking green alternative to reversed-phase
- Approaching optimal cycle time of prep LC/MS while retaining respectable resolution
- Dry down of aqueous based fraction is slow – at least 8 hours
- Orthogonal to reversed-phase chromatography



# Structures Of Test Components



# Analytical SFC/MS Screen



# Benefit of Focused Gradient Prep

---

- Focused gradients
  - Don't waste time evenly spacing all peaks with the entire gradient range (5-50)
  - Focus the gradient range around desired compound
    - Combines and elutes weaker retained species immediately after void
    - Maximizes resolution resulting in accurate fractionation of single (or multiple) peak(s) of interest
    - Combines and elutes stronger retained species during the 100% organic blow-off period



# Narrow Gradient Table in FractionLynx

100gram\_autopurify.flp - AutoPurify

File Edit View Help

Walk-up | MS+ Data | MS- Data | DAD Data | Instrument | MS Process | Spectrum Test | Printing | Chromatogram Test  
 Purification Strategy | Analytical Interpretation | Generic Method | Narrow Method | File Creation Options | Automatic Stages

Narrow Method Settings  
 The Narrow Method is selected using the Retention Time of the peak from the Analytical Run.

Method	Start RT	End RT	Inlet Method	Switch Method	Pre Run Method	MIT File
NarrowA	0.00	1.15	Mrk 5-10% Low 1	-None-	Prep prerun 5	APCI 5-10 MIT
NarrowB	1.15	1.35	Mrk 7-12% Low 1	-None-	Prep prerun 7	APCI 7-12 MIT
NarrowC	1.35	1.55	Mrk 10-15% Low	-None-	Prep prerun 10	APCI 10-15 MIT
NarrowD	1.55	1.71	Mrk 12-18% Low	-None-	Prep prerun 12	APCI 12-18 MIT
NarrowE	1.71	1.90	Mrk 15-20% Low	-None-	Prep prerun 15	APCI 15-20 MIT
NarrowF	1.90	2.10	Mrk 18-23% Low	-None-	Prep prerun 18	APCI 18-23 MIT

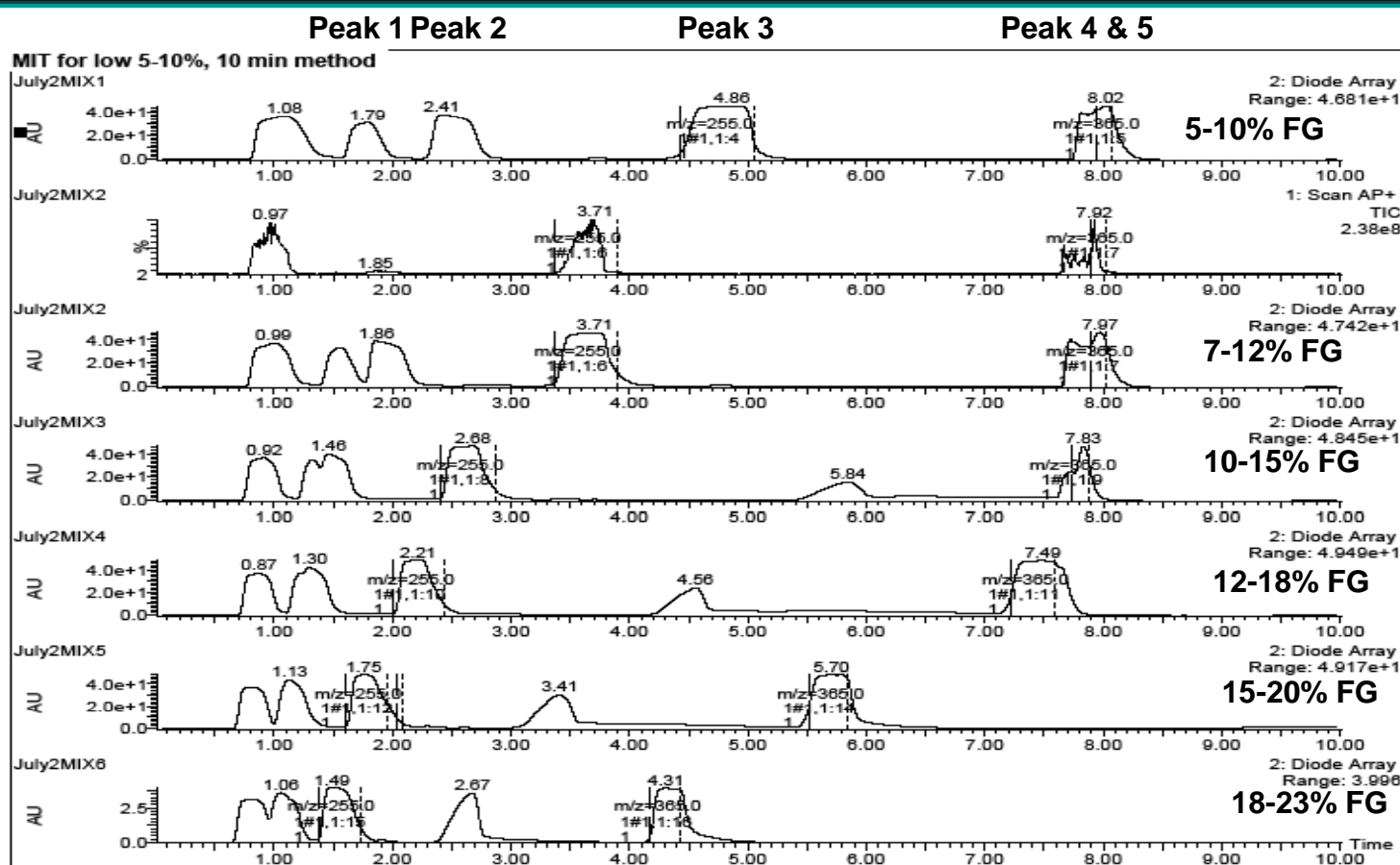
Common Settings for All Narrow Methods

Method	MS Method	Tune File	Fraction File
Settings	Prep APCI MS	Prep APCI Tune	Prep Fraction

Peak Lies in OverLap between Narrow Methods

Use early running method     Use late running method

# Std Mix on Focused Gradients

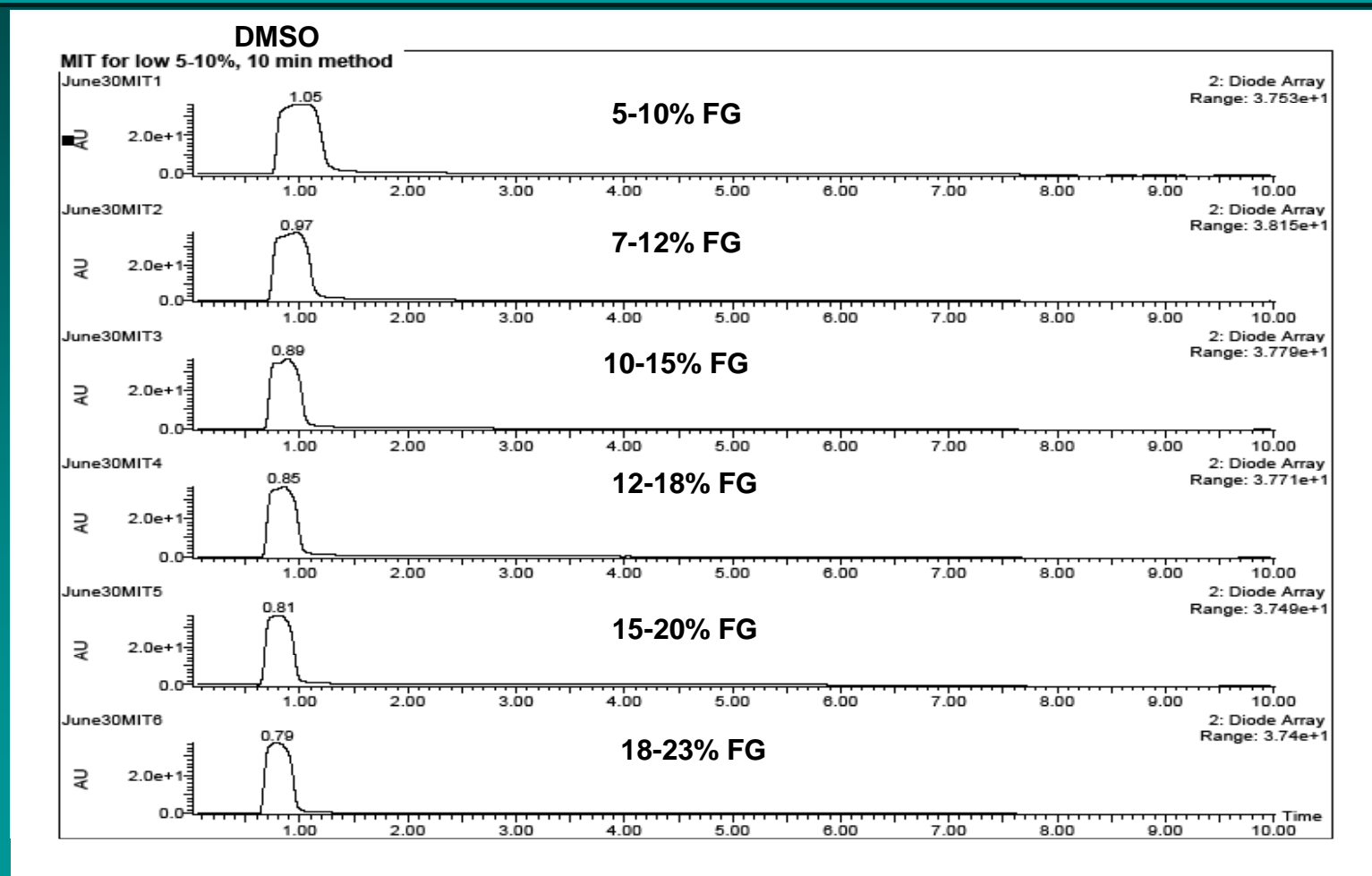


3cm i.d. x 10cm, 5µm, Ethylpyridine  
100mL/min, 120 Bar, 40 degrees C

7 min methanol focused gradient  
2 min 50% methanol flush  
1 min 50-5% reverse gradient



# Variable Timing of Modifier Injection

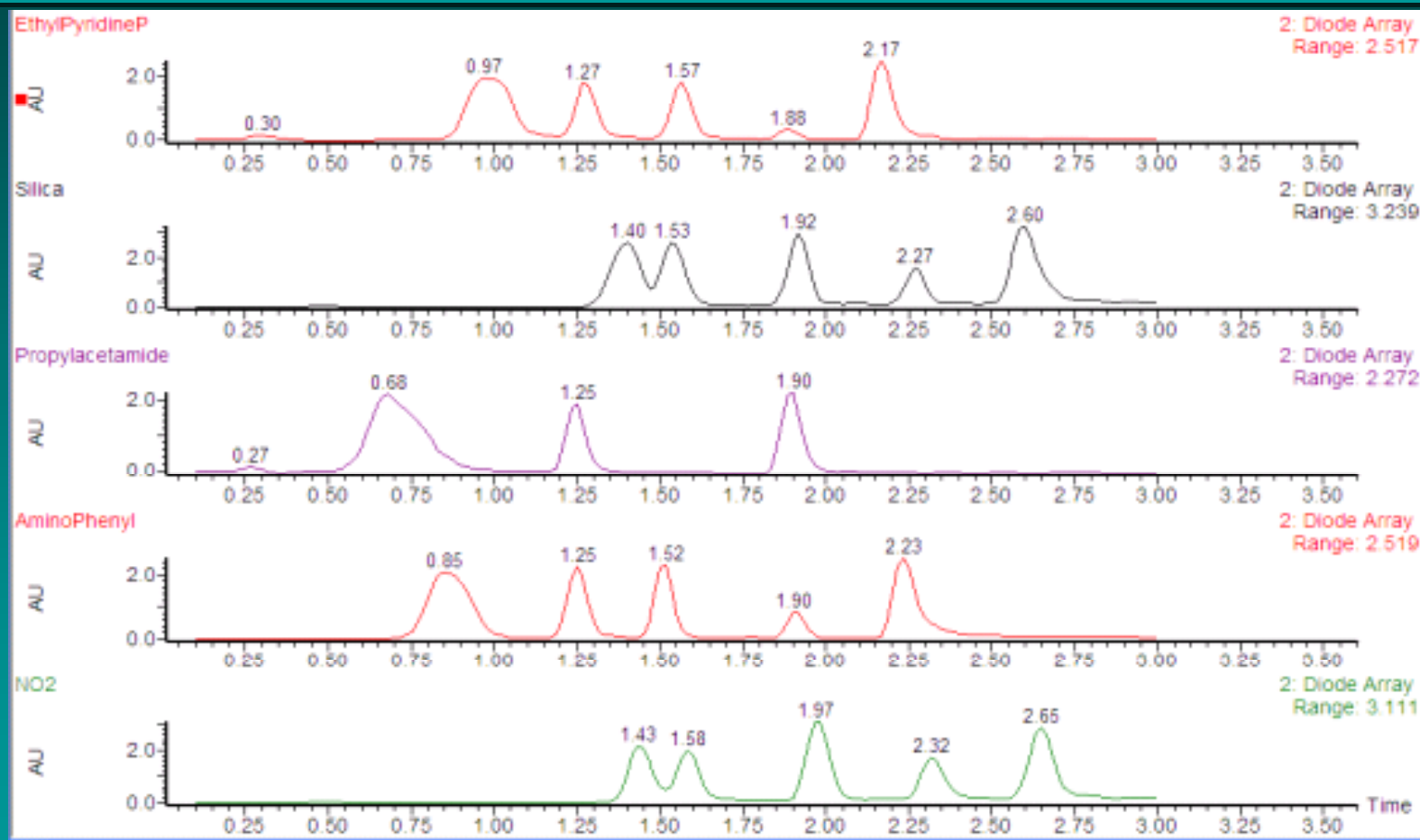


3cm i.d. x 10cm, 5 $\mu$ m, Ethylpyridine  
100mL/min, 120 Bar, 40 degrees C

7 min focused gradient  
2 min 50% flush  
1 min 50-5% reverse gradient



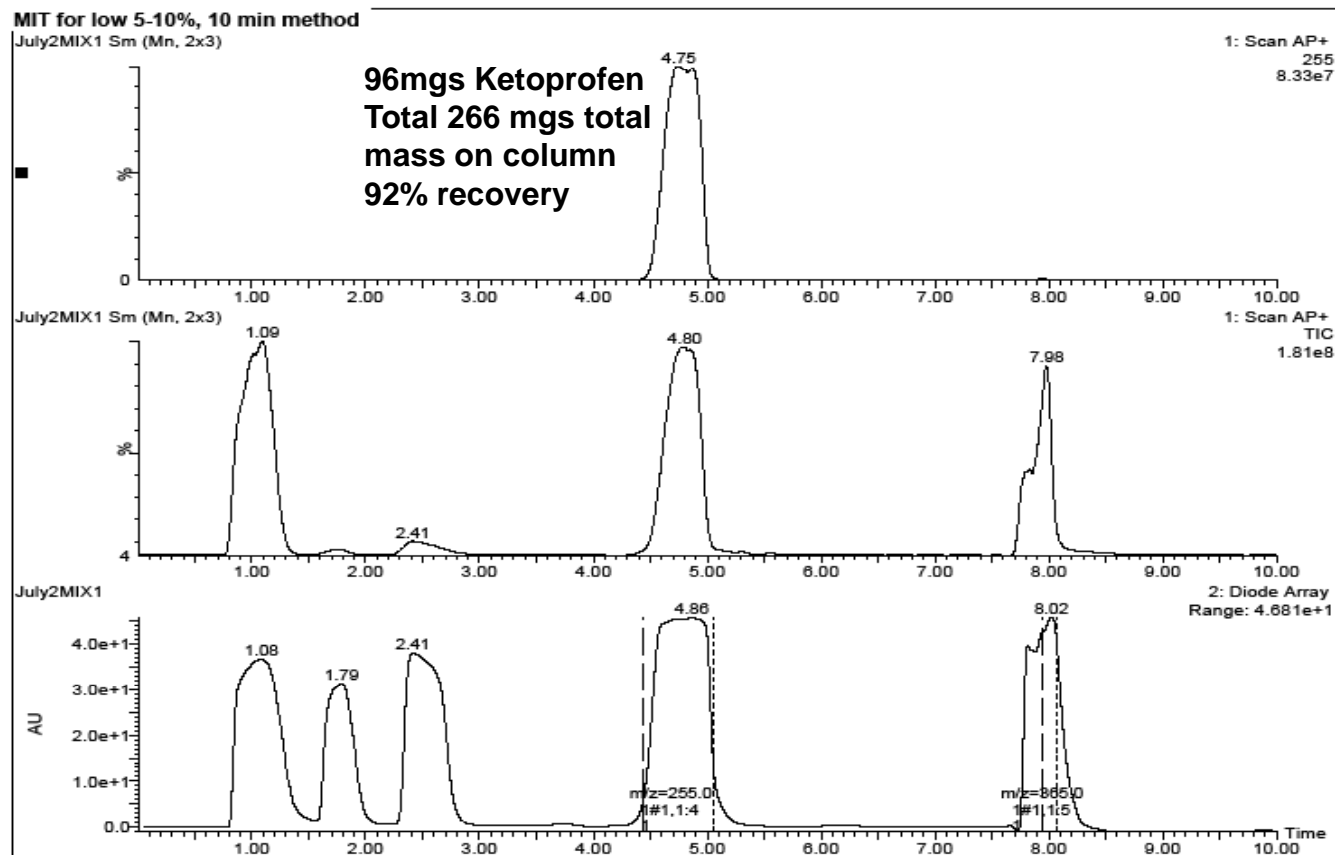
# Changing Selectivity / Different Columns



50mm x 4.6mm, 5µm, Stationary phase inside.  
4mL/min  
10s 2% methanol hold  
120s 2-60% methanol gradient  
50s 60-2% methanol gradient



# Single Peak Fractionation

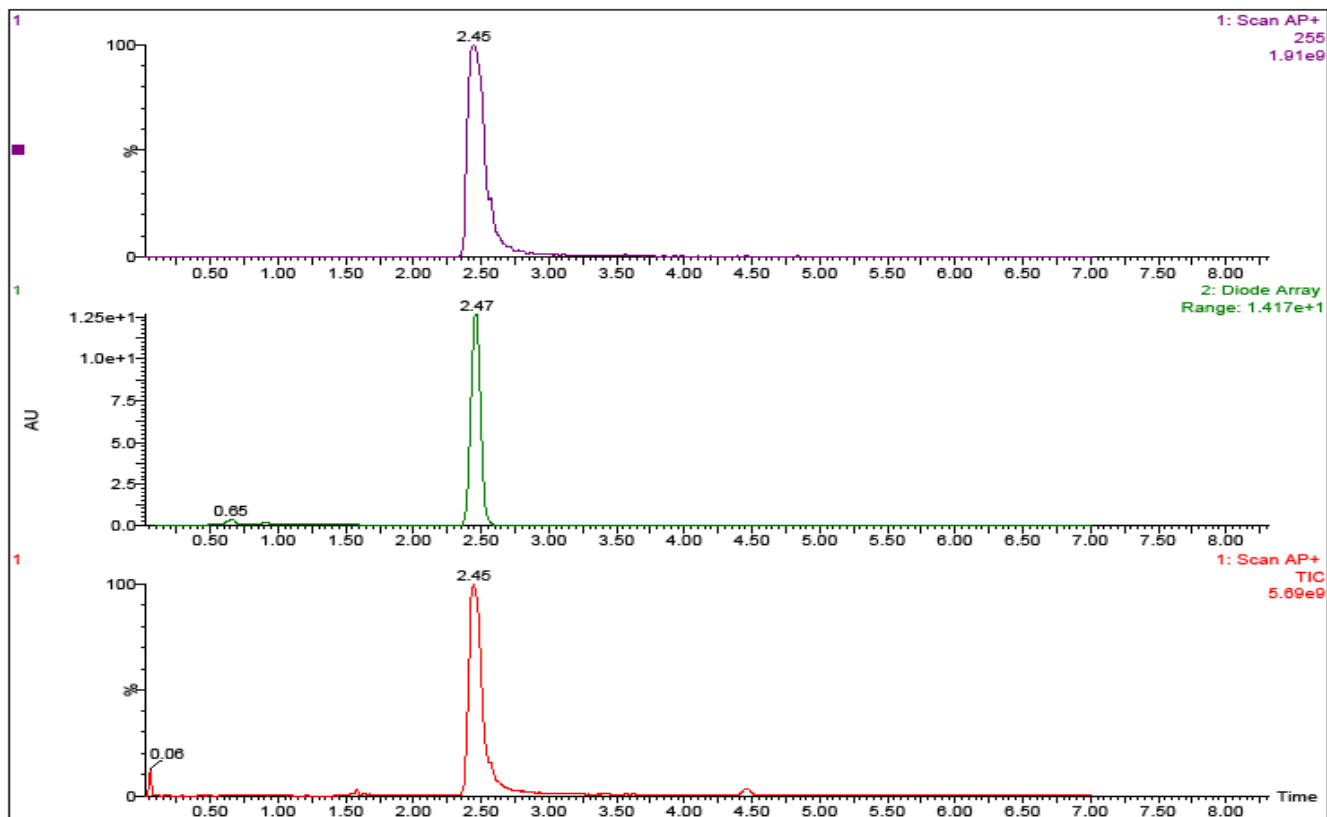


3cm i.d. x 10cm, 5um, Ethylpyridine  
100mL/min, 120 Bar, 40 degrees C

7 min 5-10% methanol  
2 min 50% methanol  
1 min 50-5% reverse gradient



# 100% Purity of Ketoprofen



100mm x 4.6mm, 5 $\mu$ m, Ethylpyridine Column

4mL/min

10s 2% methanol hold

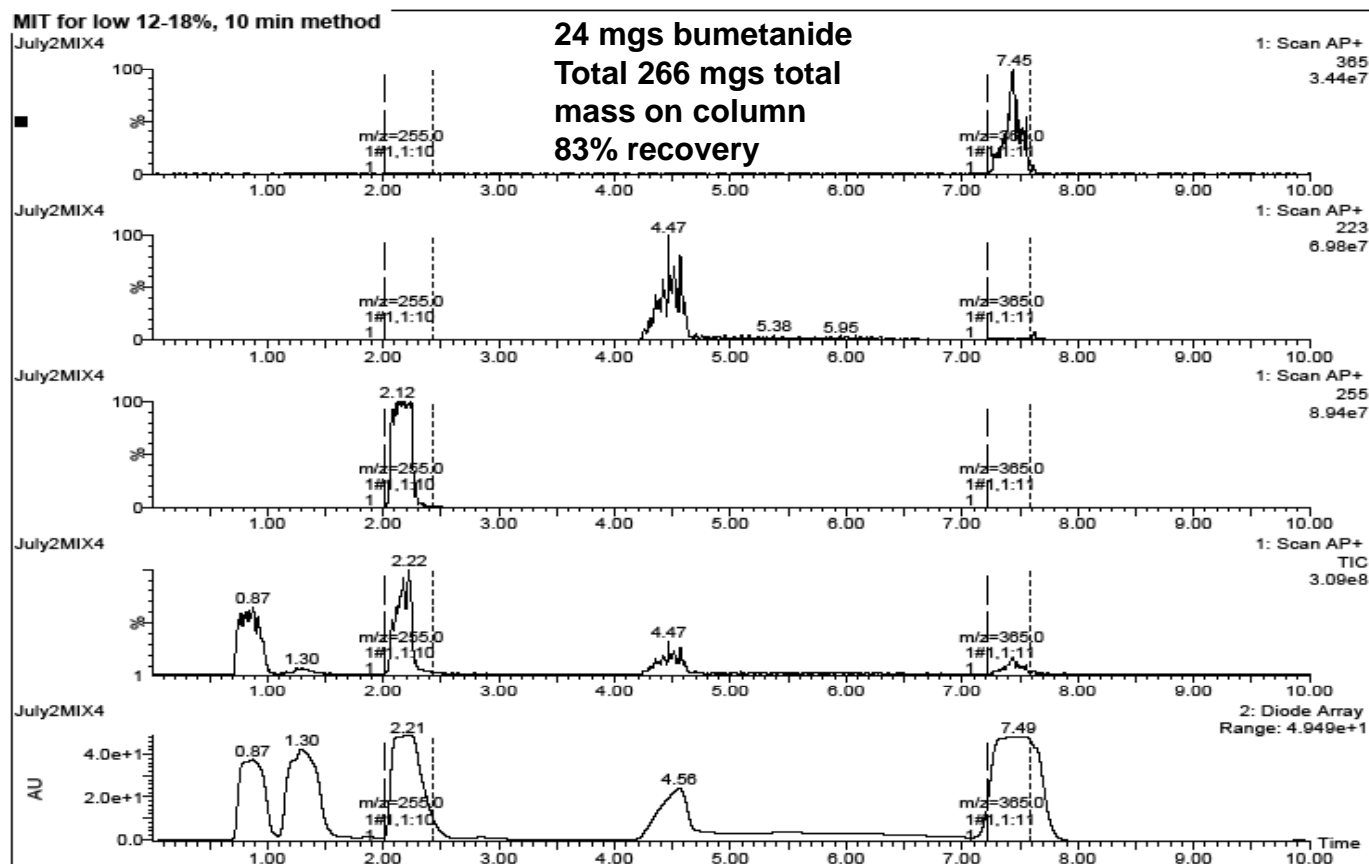
300s 2-40% methanol gradient

90s 60% methanol hold

30s 60-2% methanol gradient



# Multiple Peak Fractionation

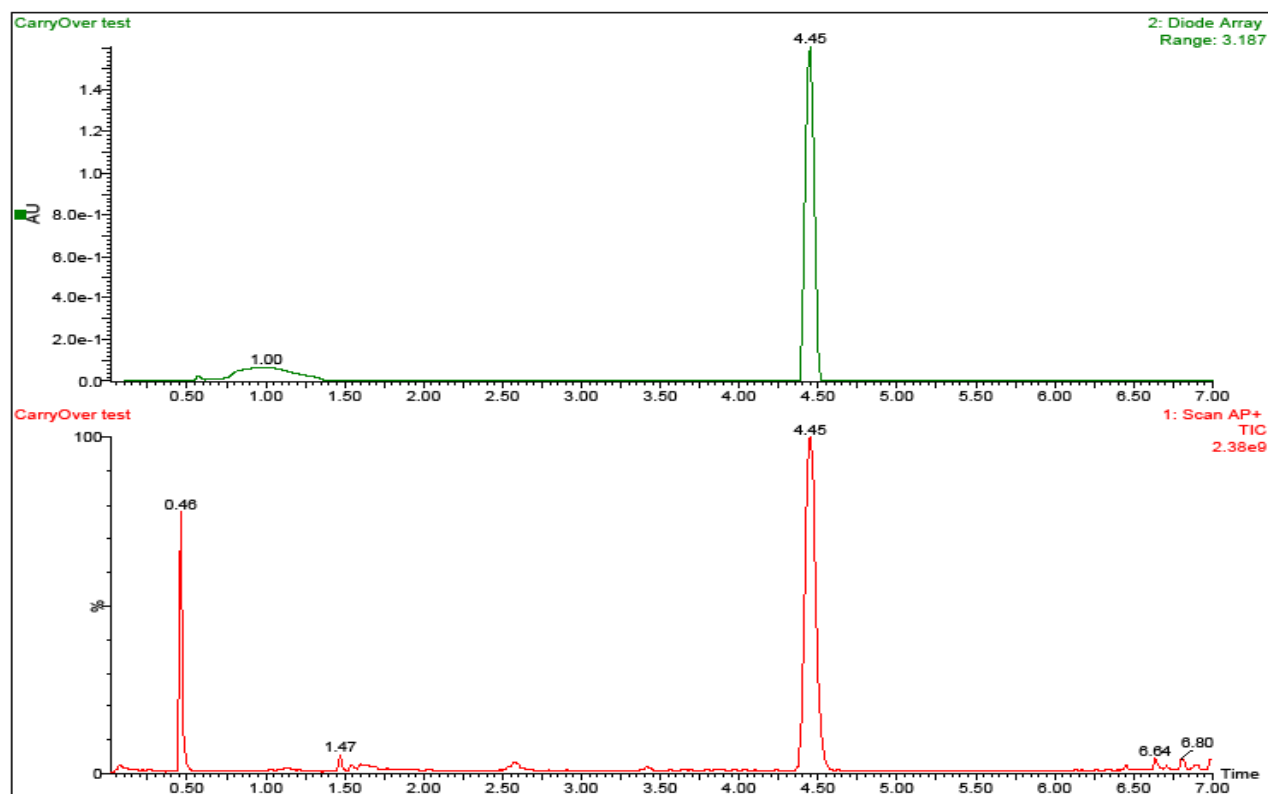


3cm i.d. x 10cm, 5 $\mu$ m, Ethylpyridine  
100mL/min, 120 Bar, 40 degrees C

7 min 12-18% focused gradient  
2 min 50% flush  
1 min 50-5% reverse gradient



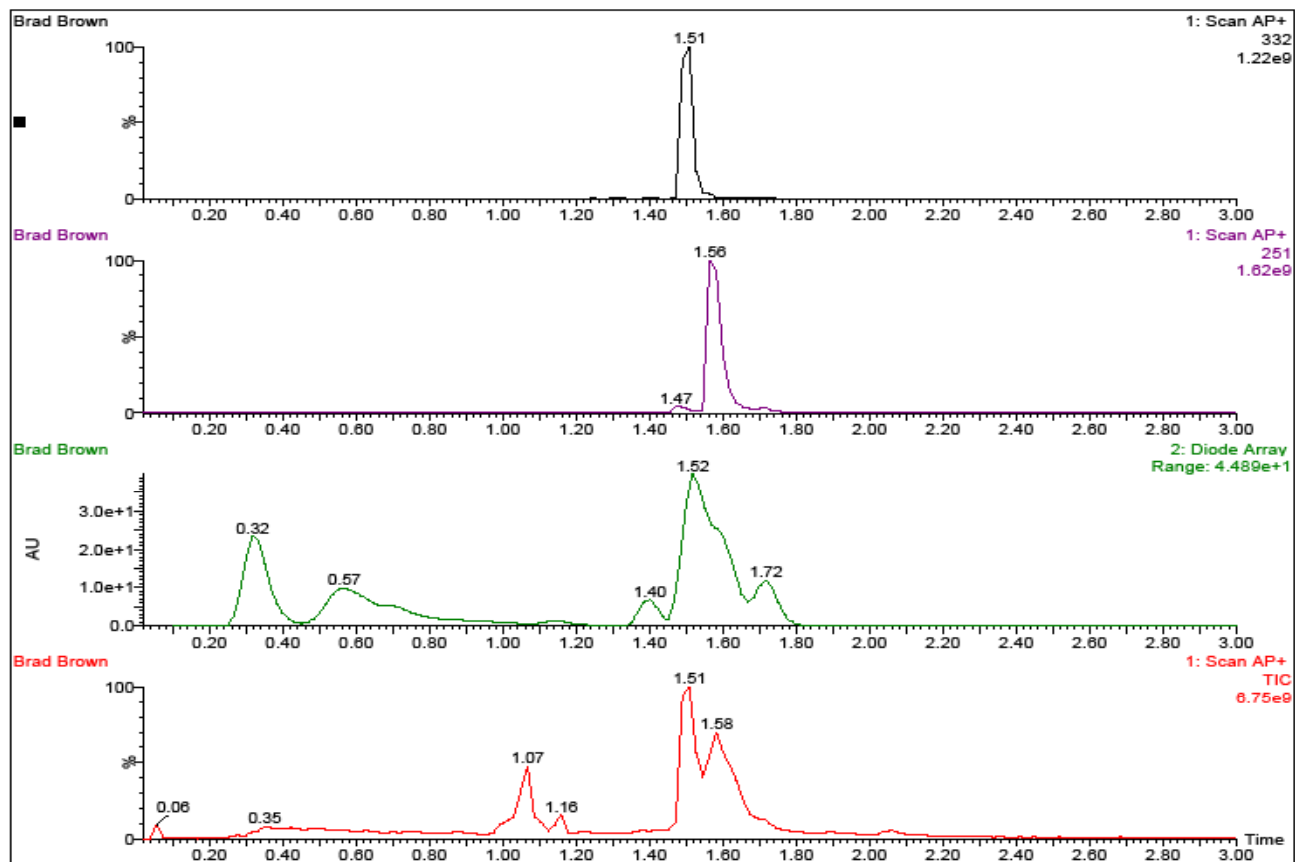
# Carry-Over Evaluation



100mm x 4.6mm, 5 $\mu$ m, Ethylpyridine Column  
4mL/min  
10s 2% methanol hold  
300s 2-40% methanol gradient  
90 60% methanol hold  
50s 60-2% methanol gradient



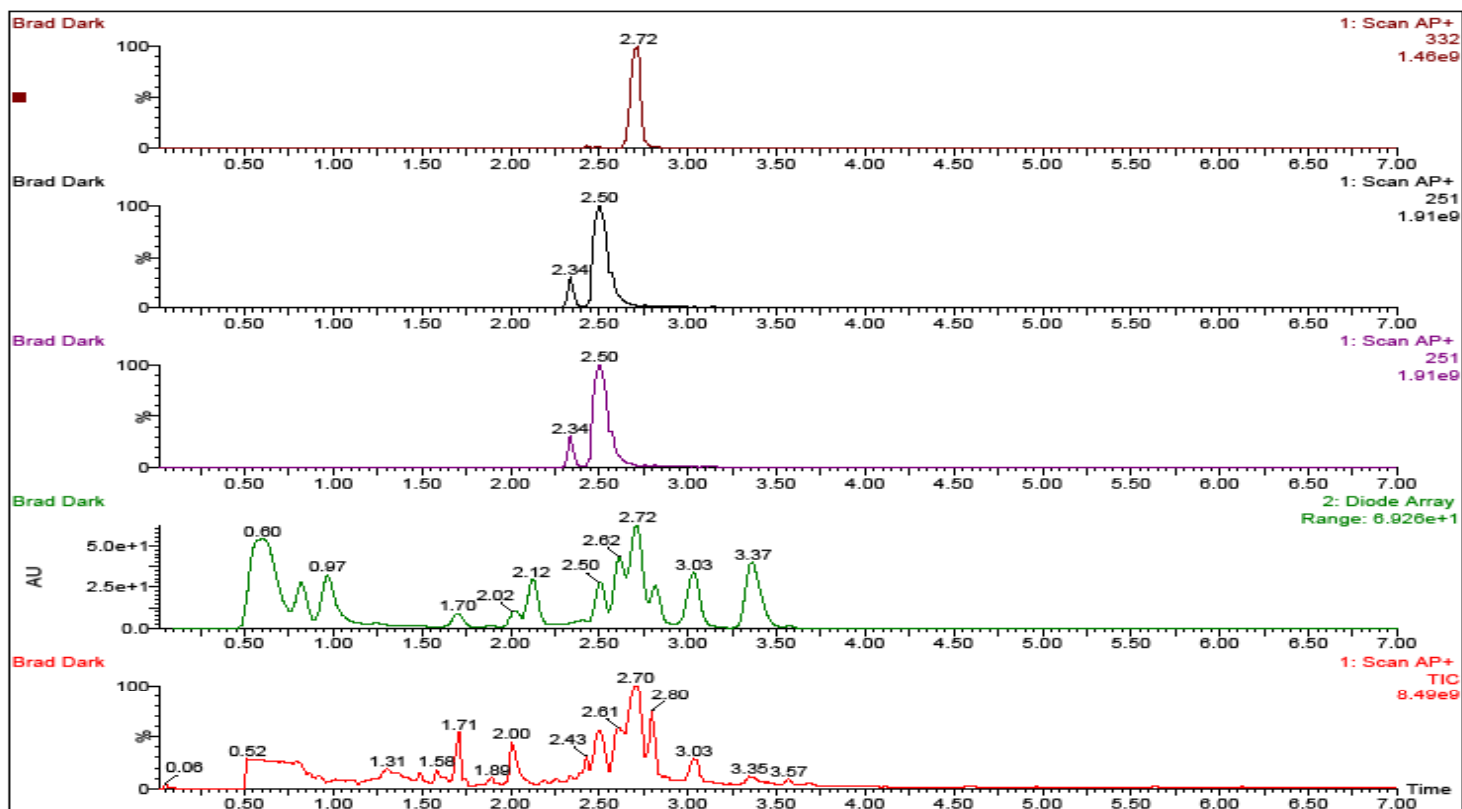
# AutoPurify: Analytical Screen



50mm x 4.6mm, 5µm, Ethylpyridine Column  
4mL/min  
10s 2% methanol hold  
120s 2-60% methanol gradient  
50s 60-2% methanol gradient



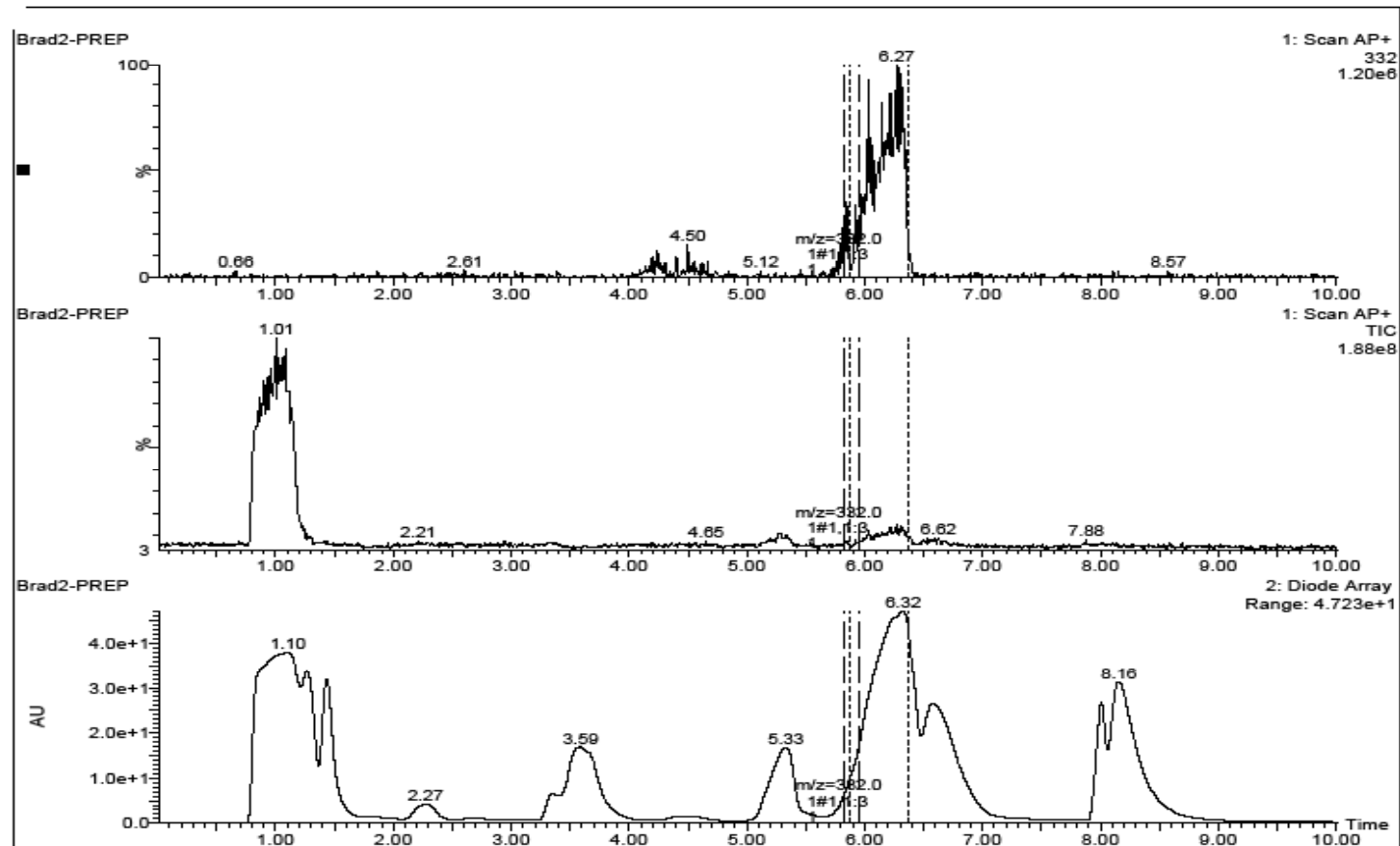
# Screen Performed on Purity Assessment Method To Reveal How Crude It Is!!



100mm x 4.6mm, 5 $\mu$ m, Ethylpyridine Column  
4mL/min  
10s 2% methanol hold  
300s 2-60% methanol gradient  
90s 60% methanol hold  
30s 60-2% methanol gradient



# AutoPurify: Prep Sample

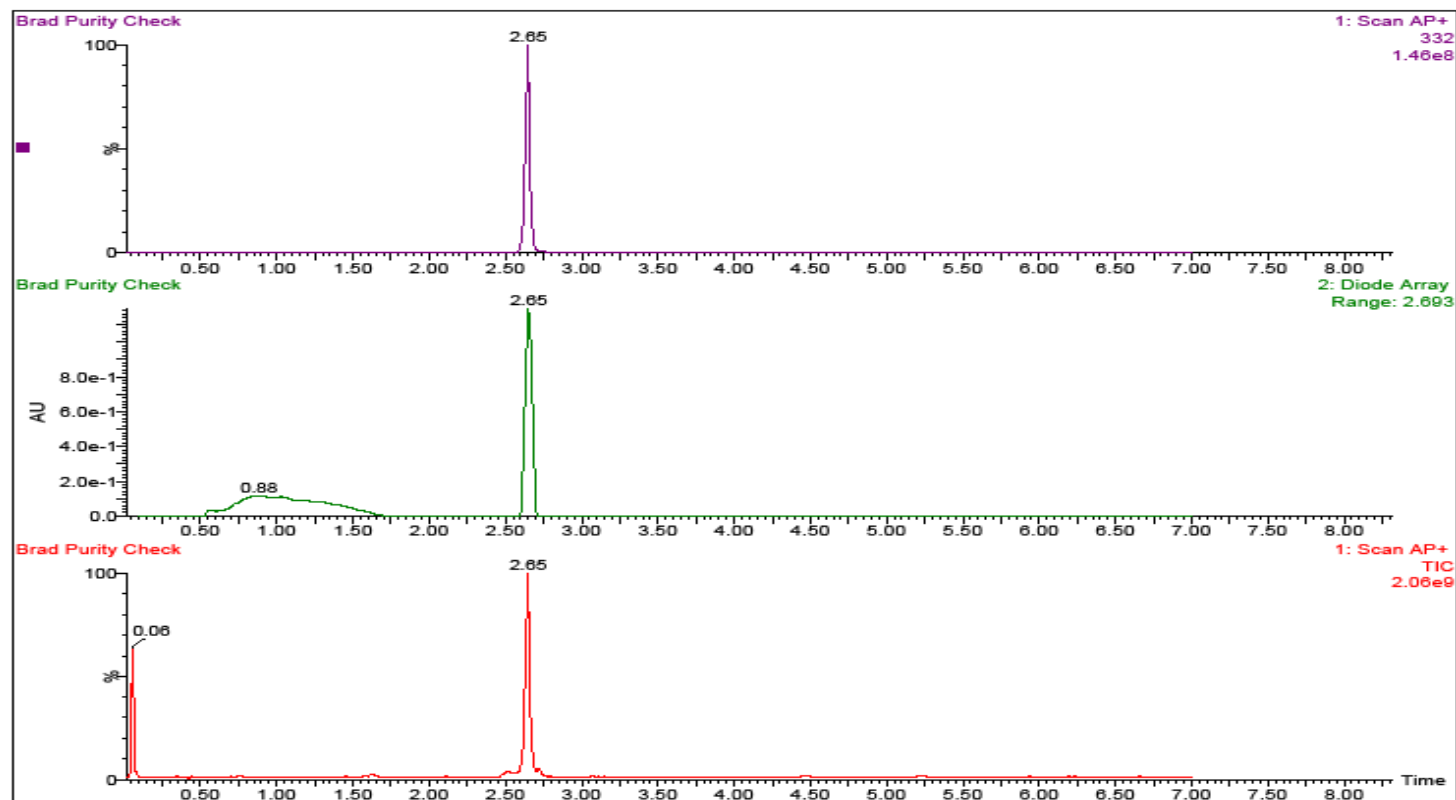


3cm i.d. x 10cm, 5um, Ethylpyridine  
100mL/min, 120 Bar, 40 degrees C

7 min 5-10% methanol  
2 min 50% methanol  
1 min 50-5% reverse gradient



# AutoPurify: Pure Sample One



100mm x 4.6mm, 5 $\mu$ m, Ethylpyridine Column  
4mL/min  
10s 2% methanol hold  
300s 2-60% methanol gradient  
90s 60% methanol hold  
30s 60-2% methanol gradient



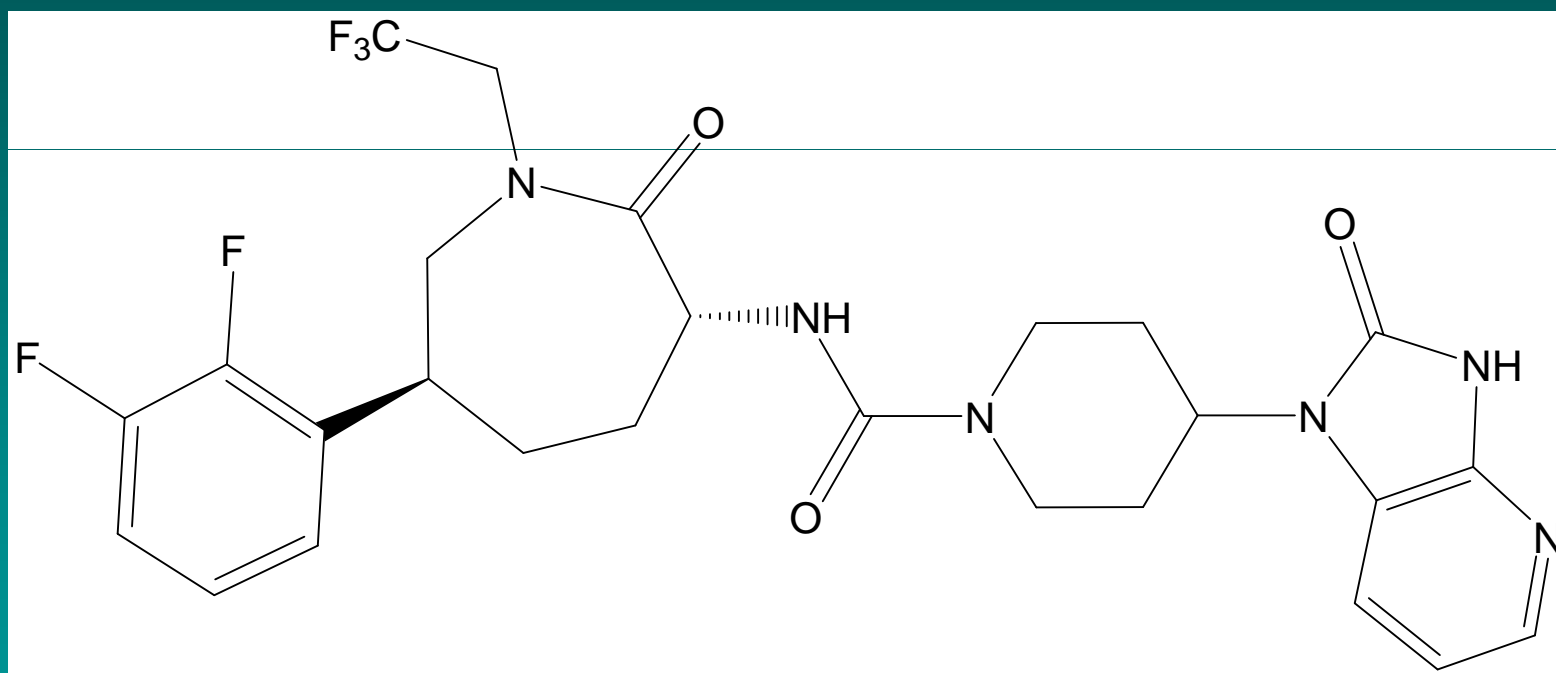
## Case Study: Drug Metabolism Sample

---

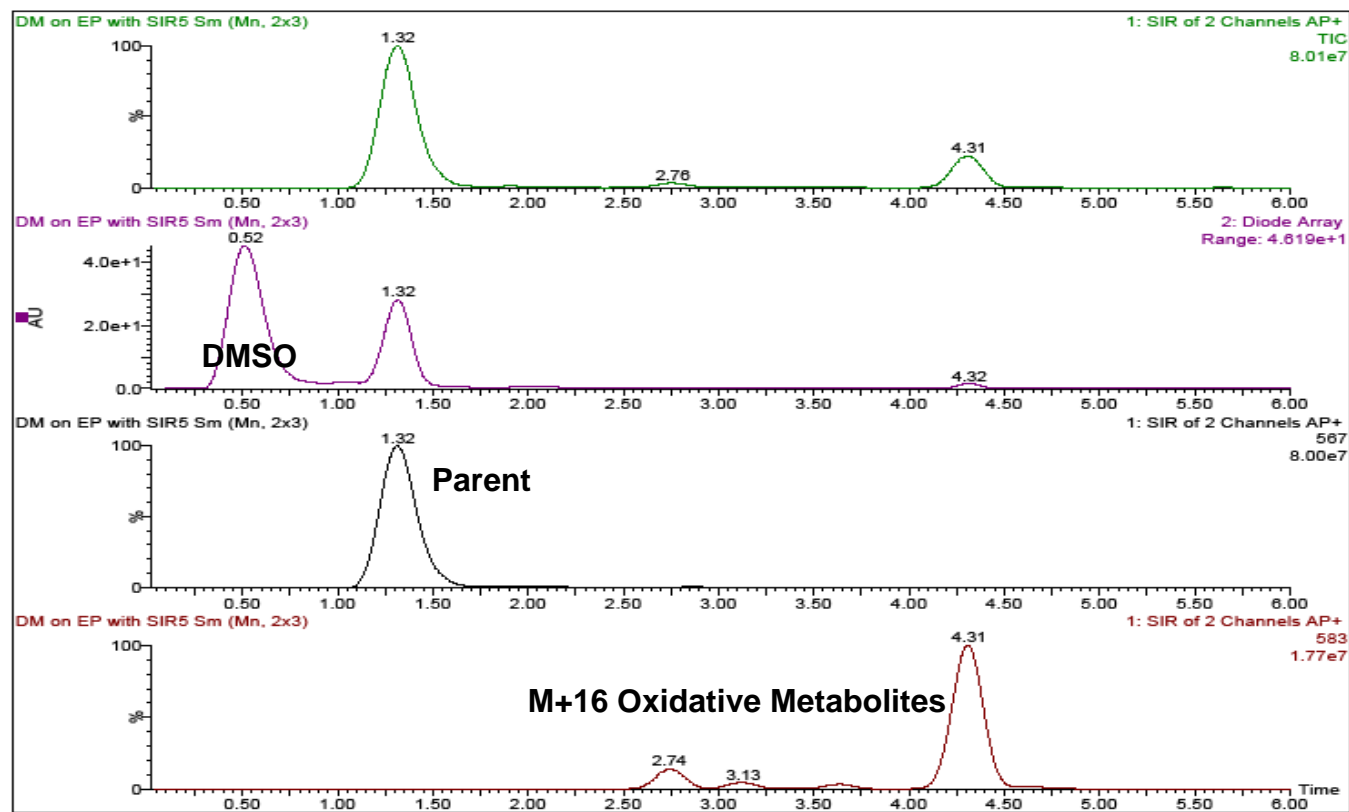
- Purpose: Need to demonstrate metabolites are non-toxic
- Problem: Very expensive to use hepatocytes or synthesize enough material for full characterization and activity studies
- Solution: Isolate desired oxidative metabolites from bioreactors via mass-directed SIR purification.



# Parent Molecule



# Analytical Screen w/SIR



100mm x 4.6mm, 5um, Ethylpyridine Column

4mL/min

10s 2% methanol hold

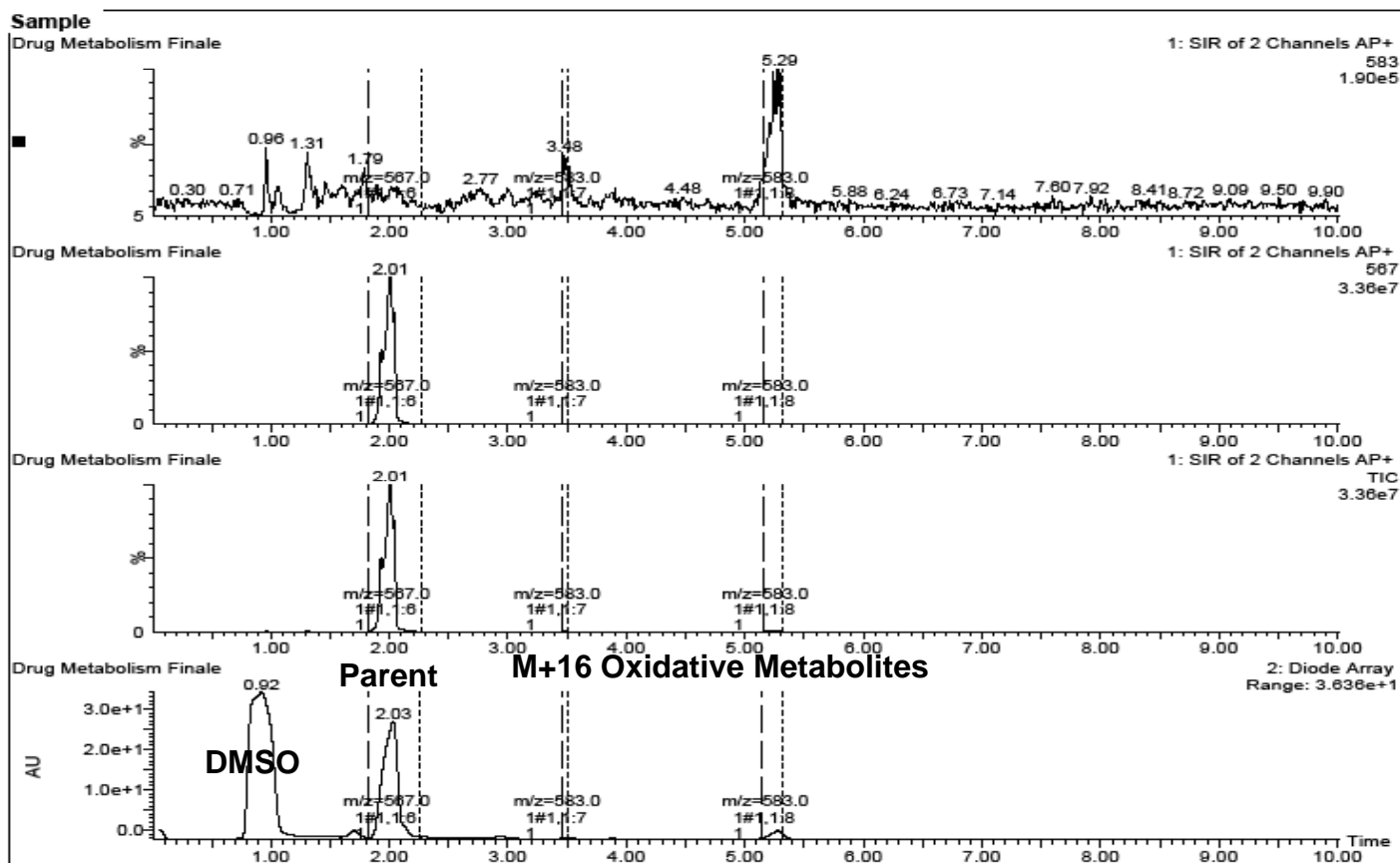
420s 10-20% methanol gradient

60 60% methanol hold

50s 60-10% methanol gradient



# Mass-Directed Purification w/SIR

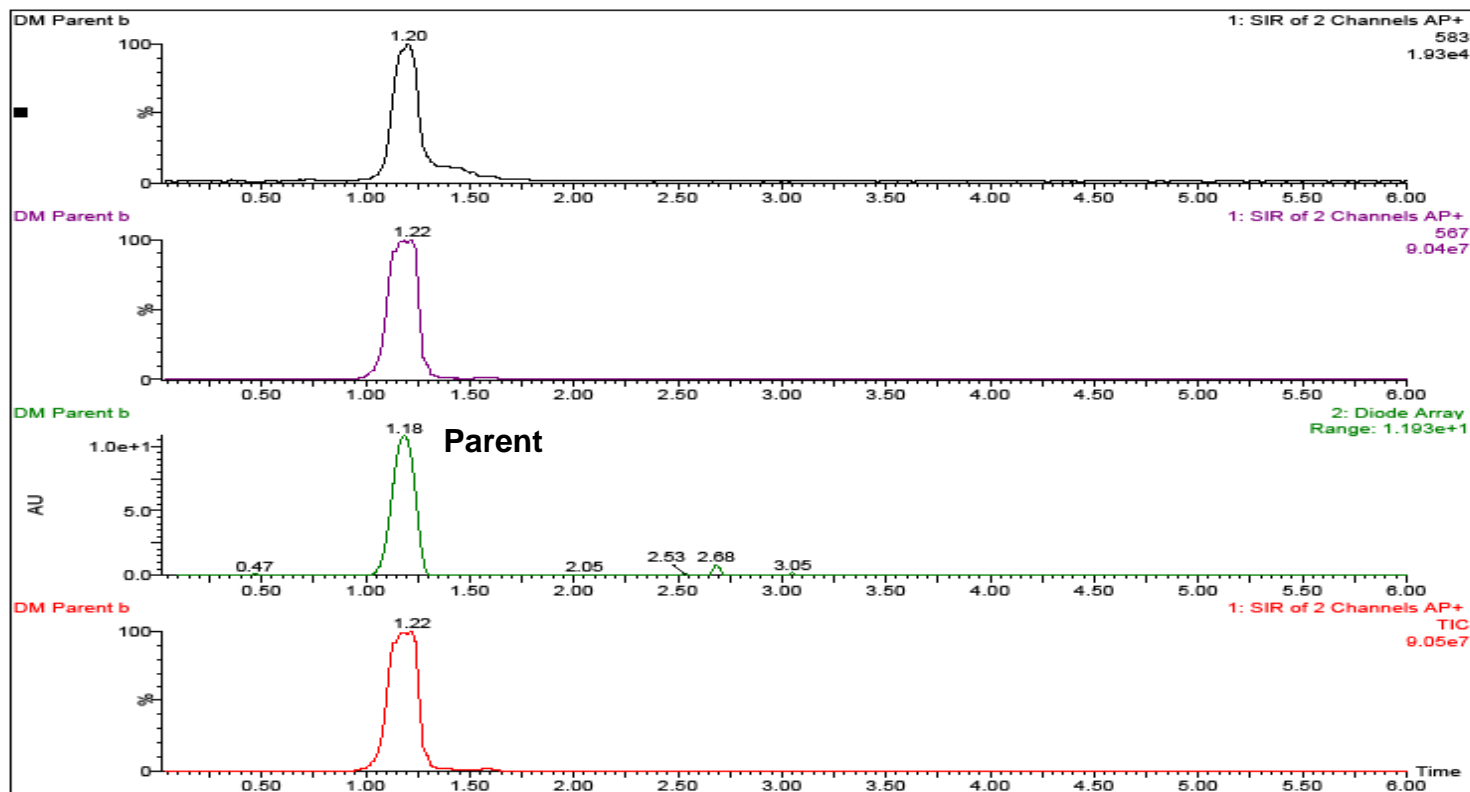


3cm i.d. x 10cm, 5um, Ethylpyridine  
100mL/min, 120 Bar, 40 degrees C

7 min 10-20% methanol  
2 min 50% methanol  
1 min 50-10% reverse gradient



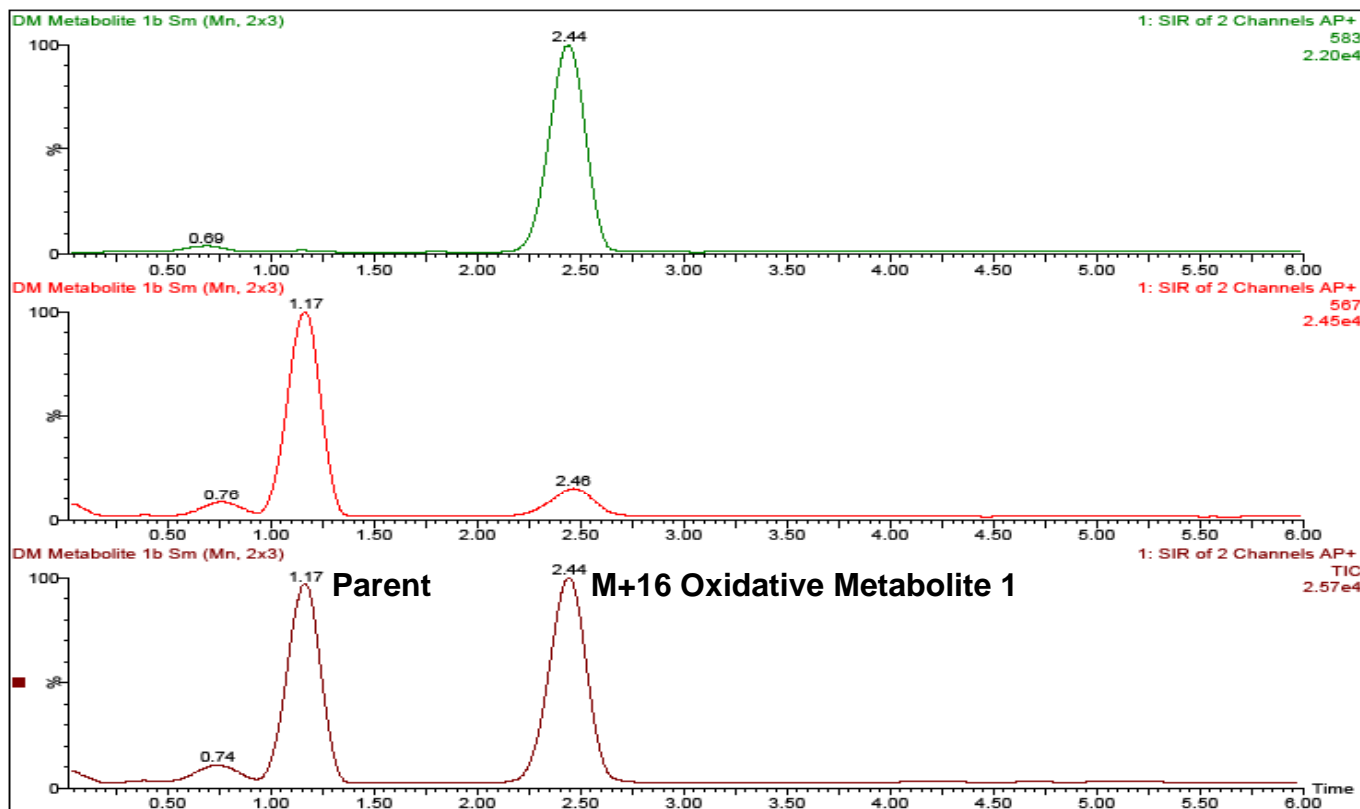
# Purity Assessment of Parent



100mm x 4.6mm, 5um, Ethylpyridine Column  
4mL/min  
10s 2% methanol hold  
420s 10-20% methanol gradient  
60 60% methanol hold  
50s 60-10% methanol gradient



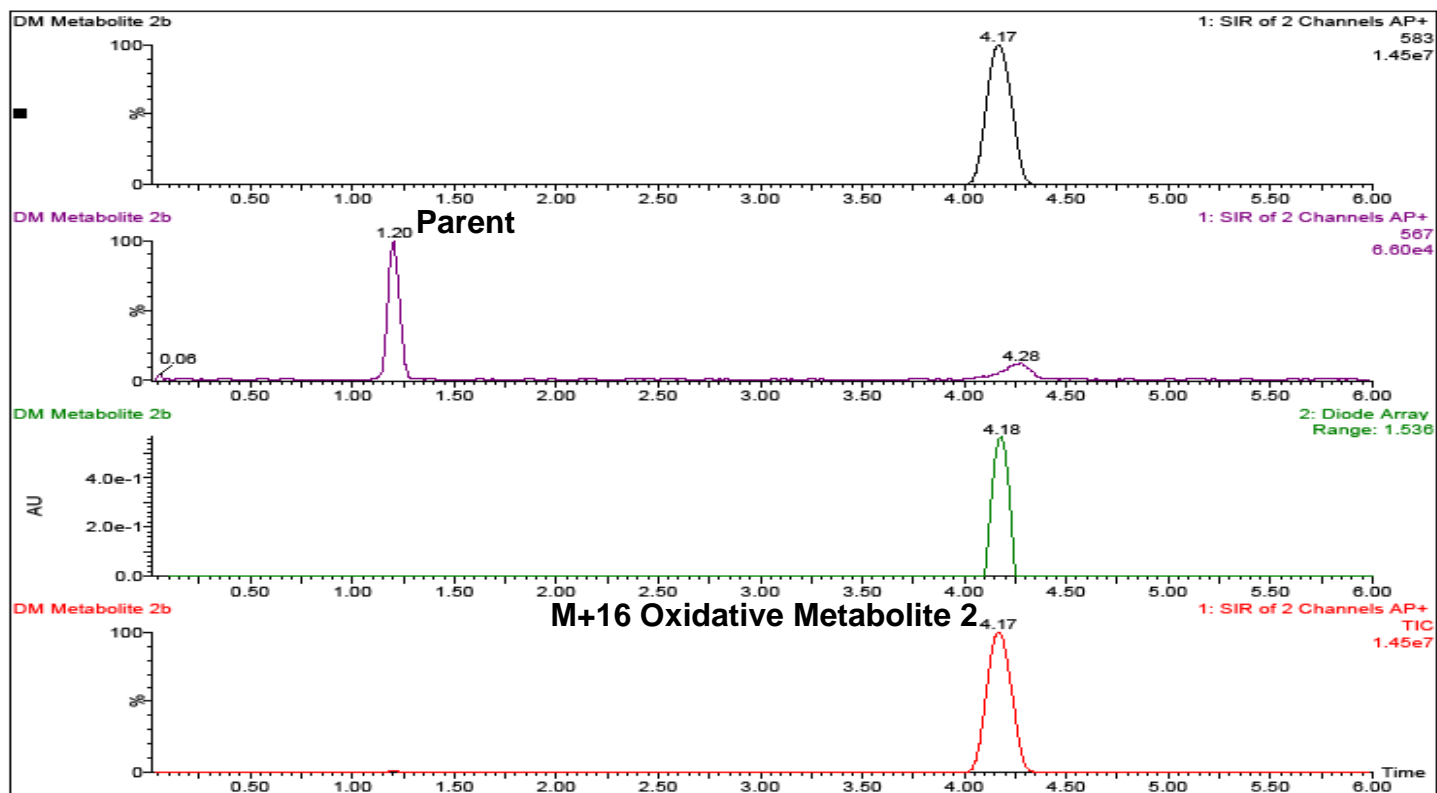
# Mass-Directed Purification w/SIR



100mm x 4.6mm, 5um, Ethylpyridine Column  
4mL/min  
10s 2% methanol hold  
420s 10-20% methanol gradient  
60 60% methanol hold  
50s 60-10% methanol gradient



# Mass-Directed Purification w/SIR



100mm x 4.6mm, 5um, Ethylpyridine Column  
4mL/min  
10s 2% methanol hold  
420s 10-20% methanol gradient  
60 60% methanol hold  
50s 60-10% methanol gradient



# Summary

---

- Analytical retention times can be mapped to focused gradients on semi-preparative SFC system for automated method assignment
- Mass-directed SFC can be used in ways that our traditional RP-LC/MS systems are used such as SIM purification
- Mass-directed SFC is a welcome addition to a high-throughput, purification lab



# Acknowledgements

---

- **Merck:**
  - Chris Welch, Jim Barrow, Jim Small, and NTRLIC
  - Chuck Ross and Vince Van Nostrand
- **THAR:**
  - Bill Wranitz, Pat Amore, and Jen Lefler
- **Waters:**
  - Denise Heyburn
- **ES Industries:**
  - Dave Kohler and Matt Przybyciel
- **And...You**



# Analytical SFC/MS Purity Assessment

