Utilizing Mass-Directed SFC in Support of Medicinal Chemistry

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Overview

- Introduction of Technology Enabled Synthesis group (TES) and associated Purification Group
- Highlight deficiencies of reversed-phase, preparative HPLC currently in use
- Why evaluate SFC to overcome these deficiencies
- How SFC platform has been incorporated in high-throughput area
- Summarize results

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Limitations of Current Reversed-Phase HPLC Platform

- Approaching optimal cycle time of prep LC/MS while retaining respectable resolution.
  - 25 mL/min on a 2 cm i.d. column
- Drying aqueous based HPLC fractions is very slow.
  - At least 8 hours in high speed rotary vacuum evaporation system.

THE ANSWER: Mass-Directed SFC?

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Why SFC???

- More efficient separation per unit time compared to HPLC (3-4 times)
- Complimentary separation technique providing varied selectivity
- Very limited modifier use for reduced salt forms compared to HPLC
- A “Green Technology”
- Dramatically reduce dry down time compared to aqueous based HPLC fractions (30mL aqueous to 3mL MeOH)

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Analytical SFC/MS

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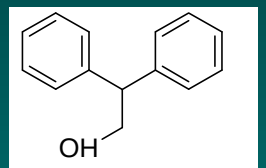
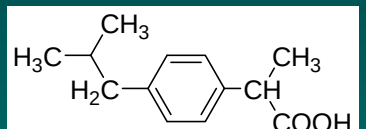
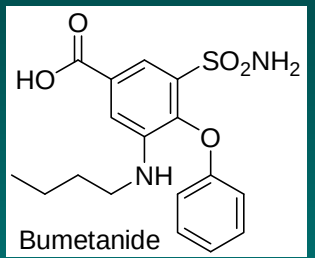
Structures Of Test Components



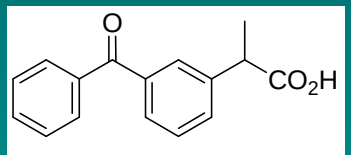
2,2-Diphenylethanol



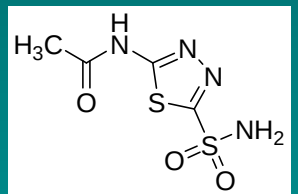
Ibuprofen



Bumetanide



Ketoprofen



Acetazolamide

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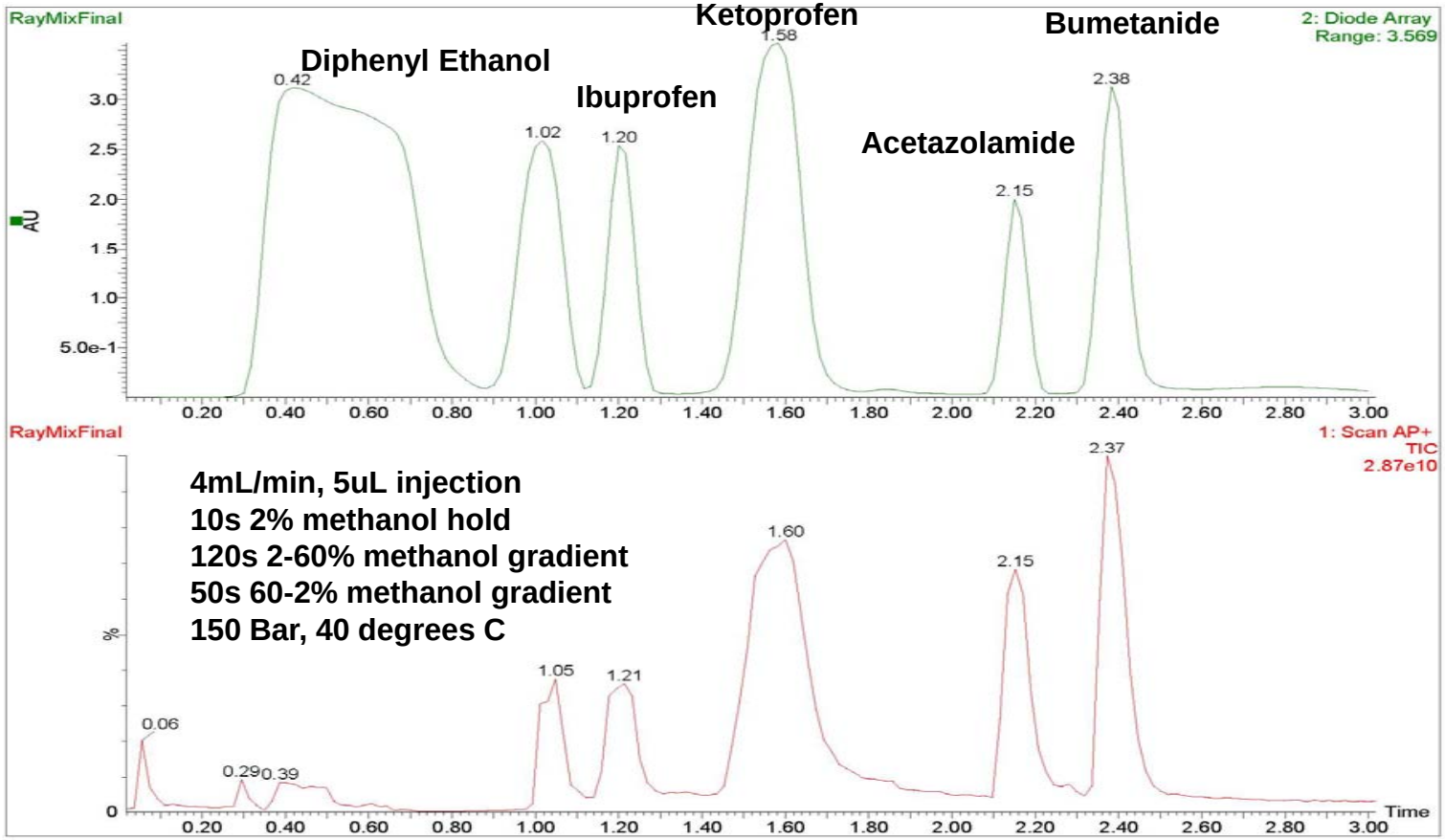
Analytical SFC/MS Screen



SFC MS Prep-30

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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

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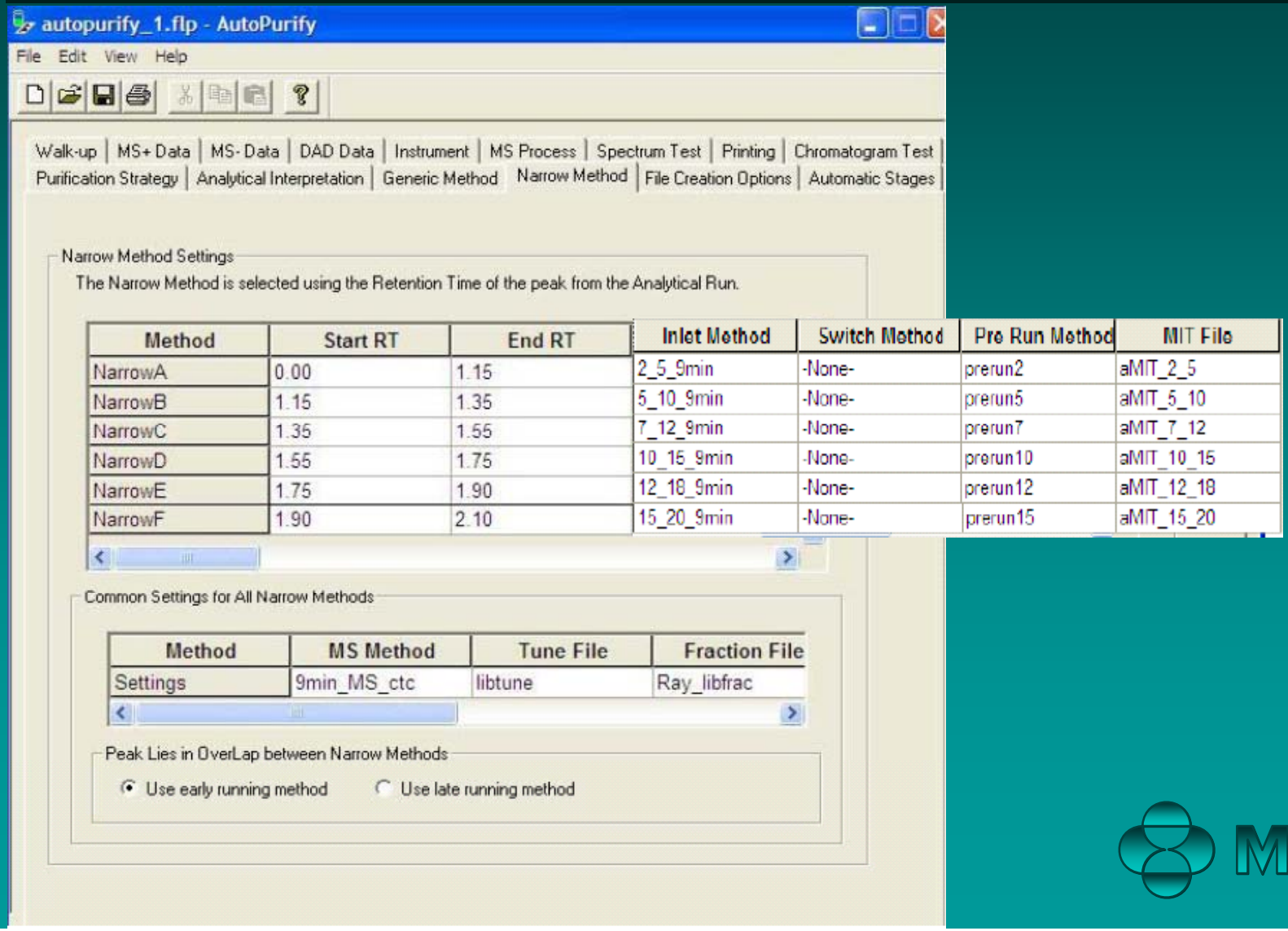
Narrow Gradient Table in FractionLynx



Method	Start RT	End RT	Inlet Method	Switch Method	Pre Run Method	MIT File
NarrowA	0.00	1.15	2_5_9min	-None-	prerun2	aMIT_2_5
NarrowB	1.15	1.35	5_10_9min	-None-	prerun5	aMIT_5_10
NarrowC	1.35	1.55	7_12_9min	-None-	prerun7	aMIT_7_12
NarrowD	1.55	1.75	10_15_9min	-None-	prerun10	aMIT_10_15
NarrowE	1.75	1.90	12_18_9min	-None-	prerun12	aMIT_12_18
NarrowF	1.90	2.10	15_20_9min	-None-	prerun15	aMIT_15_20

Method	MS Method	Tune File	Fraction File
Settings	9min_MS_ctc	libtune	Ray_libfrac

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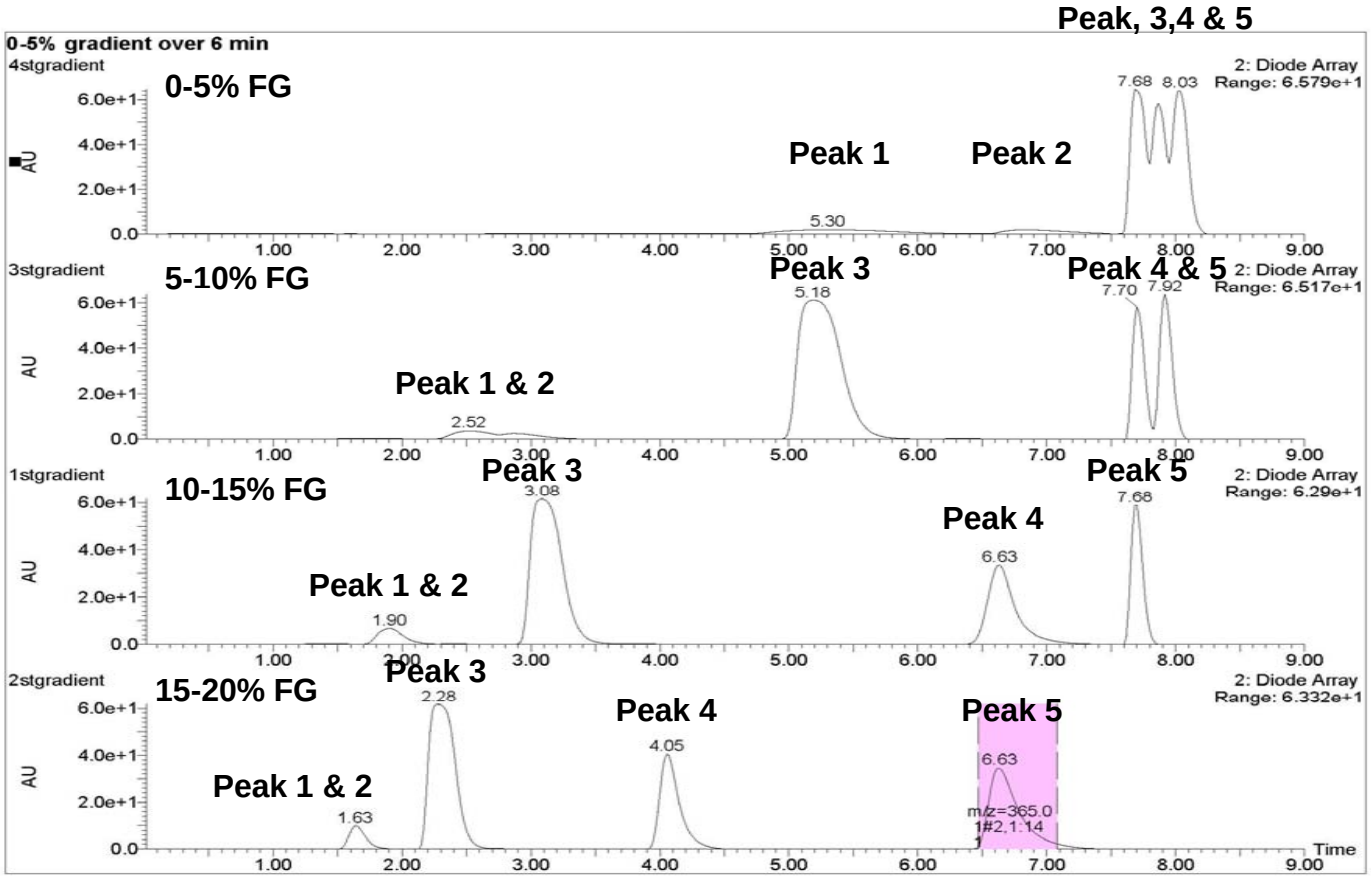
Std Mix on Focused Gradients



1cm i.d. x 10cm, 5um, Ethylpyridine
20mL/min, 120 Bar, 40 degrees C

6 min focused gradient
1 min 60% flush
2 min 60-2% reverse gradient

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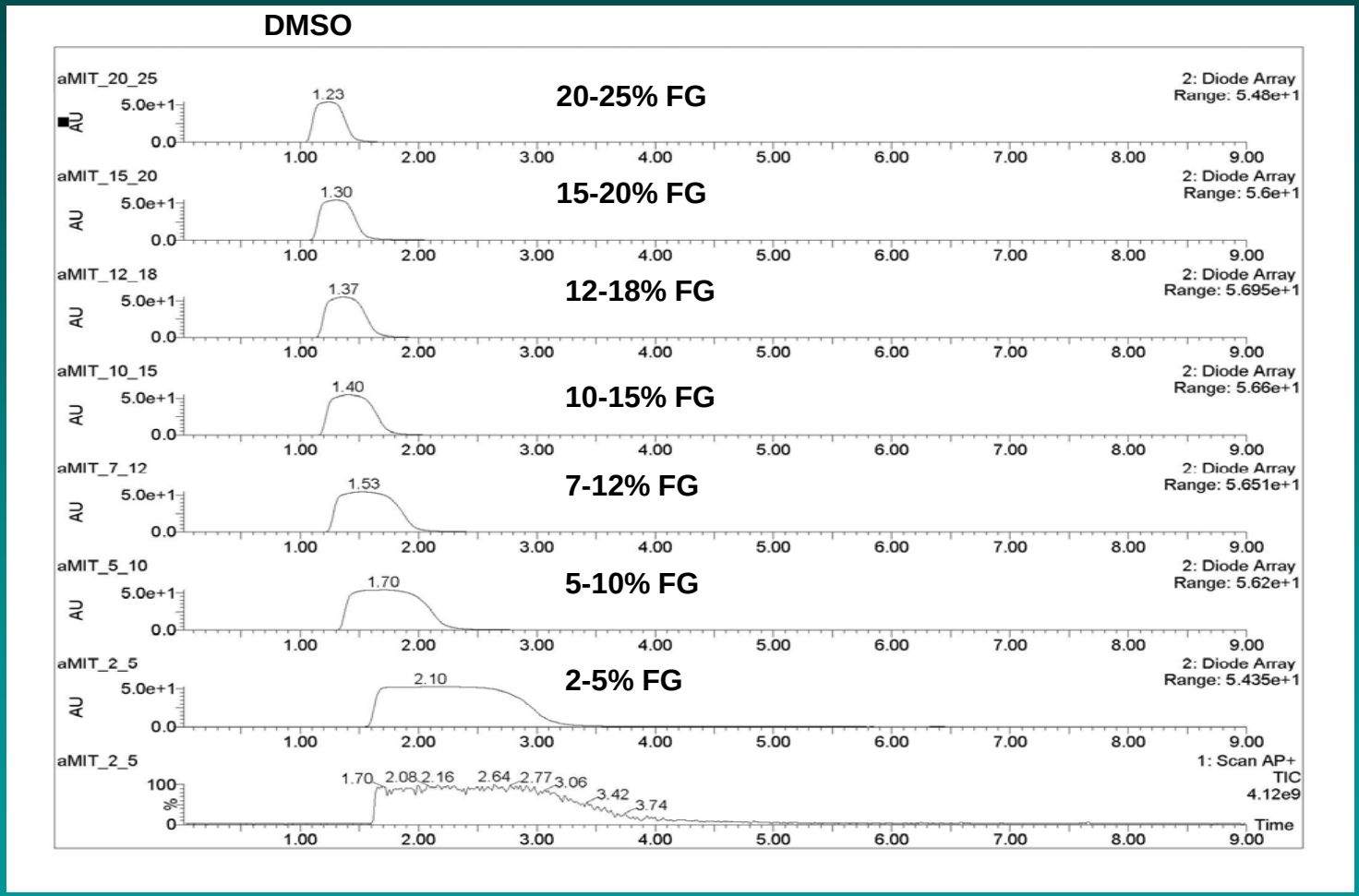
Modifier Stream Injection/Loop Clearance Time



1cm i.d. x 10cm, 5um, Ethylpyridine
20mL/min, 120 Bar, 40 degrees C

6 min focused gradient
1 min 60% flush
2 min 60-2% reverse gradient

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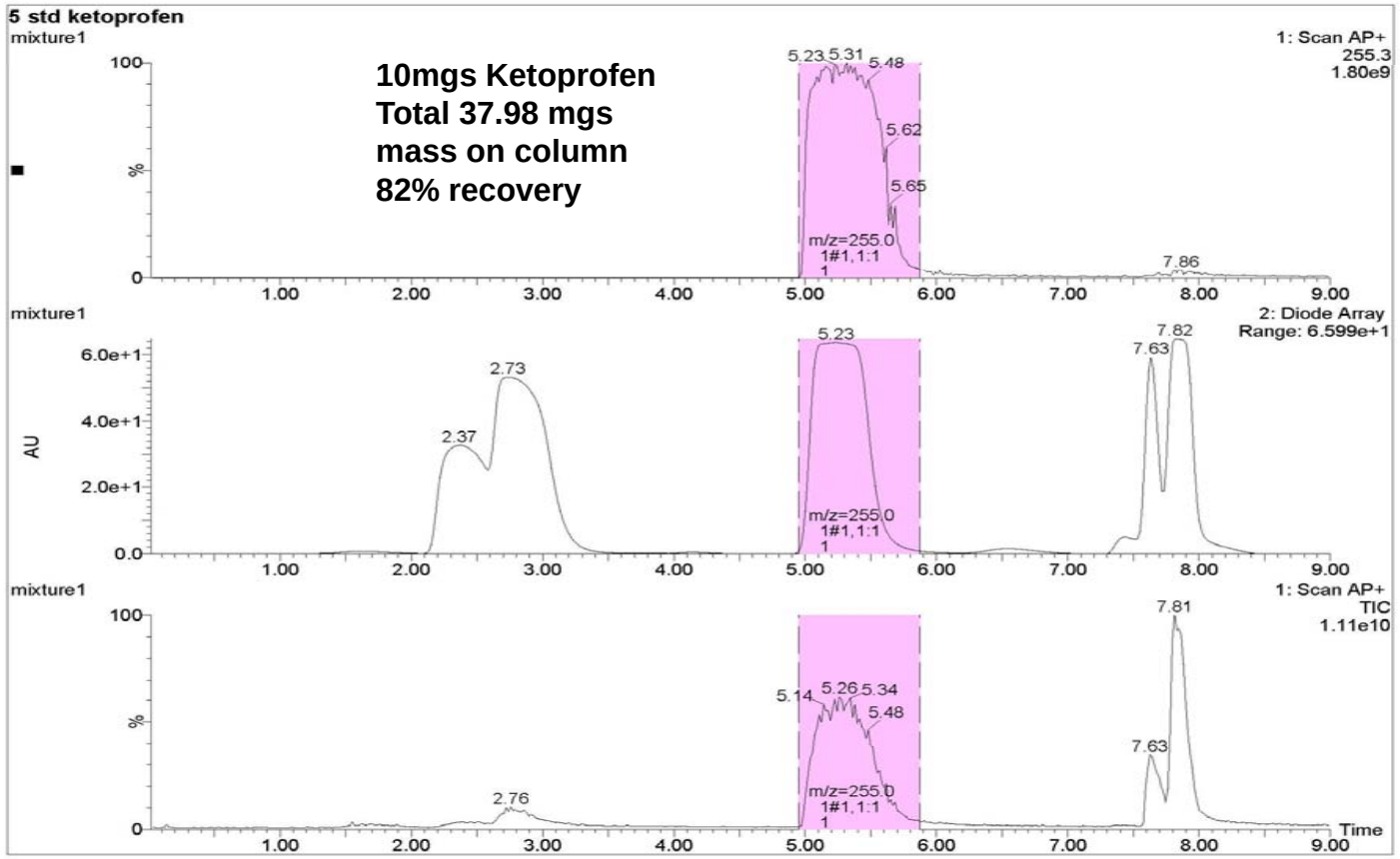
Single Peak Fractionation



1cm i.d. x 10cm, 5um, Ethylpyridine
20mL/min, 120 Bar, 40 degrees C

6 min 5-10% methanol
1 min 60% flush
2 min 60-2% reverse gradient

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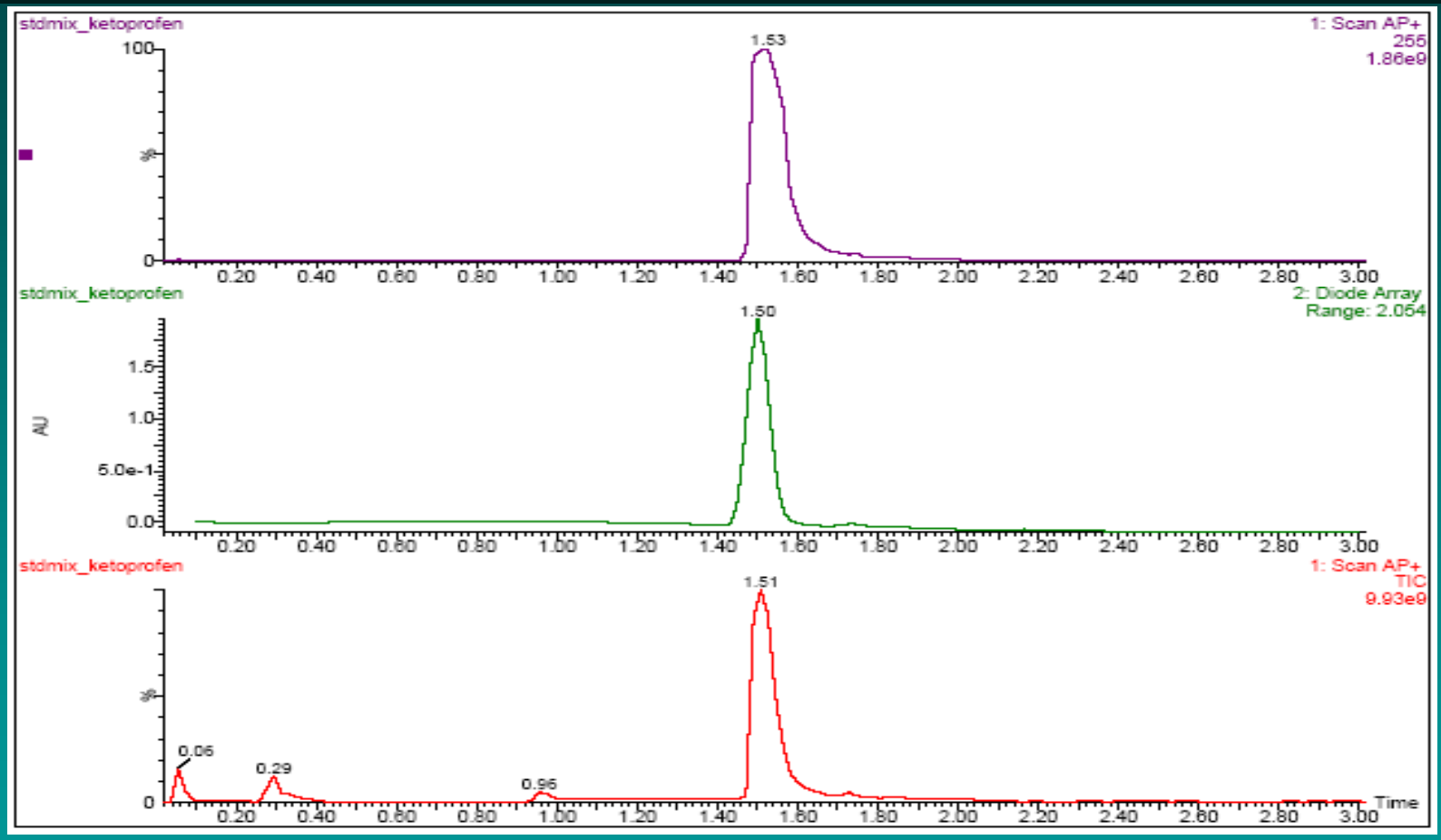
100% Purity of Ketoprofen



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

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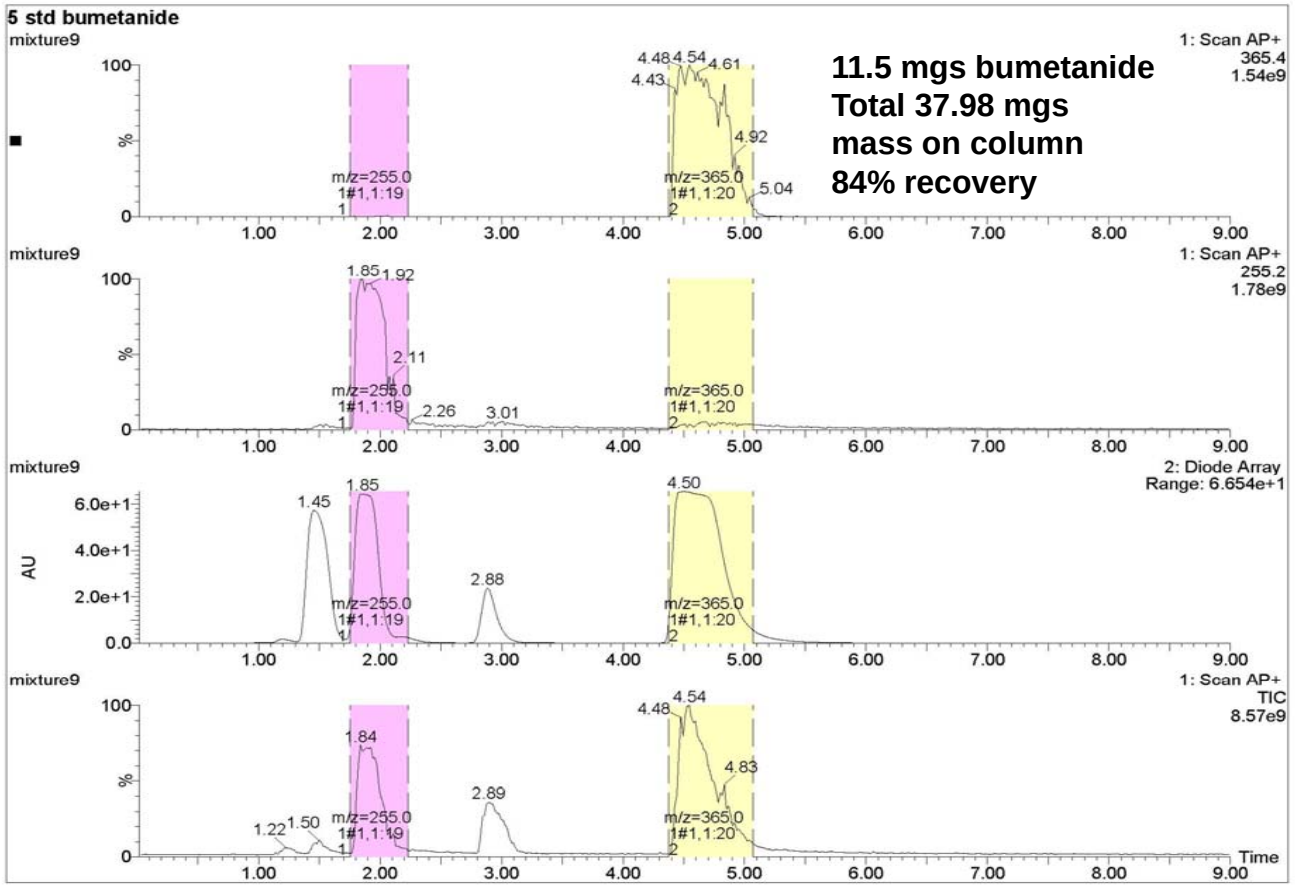
Multiple Peak Fractionation



1cm i.d. x 10cm, 5um, Ethylpyridine
20mL/min, 120 Bar, 40 degrees C

6 min 20-25% methanol
1 min 60% flush
2 min 60-2% reverse gradient

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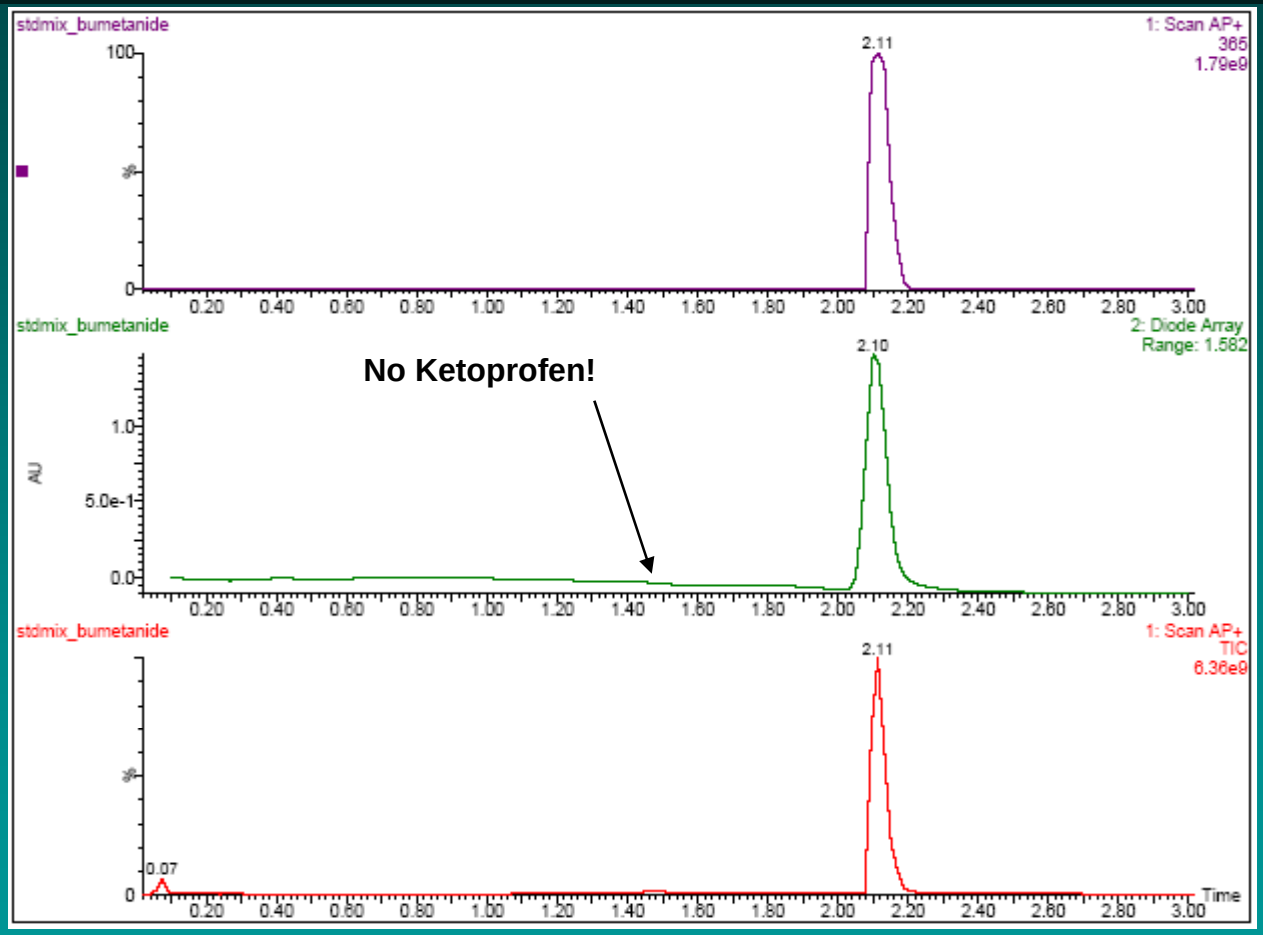
100% Purity, No Carry-Over



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

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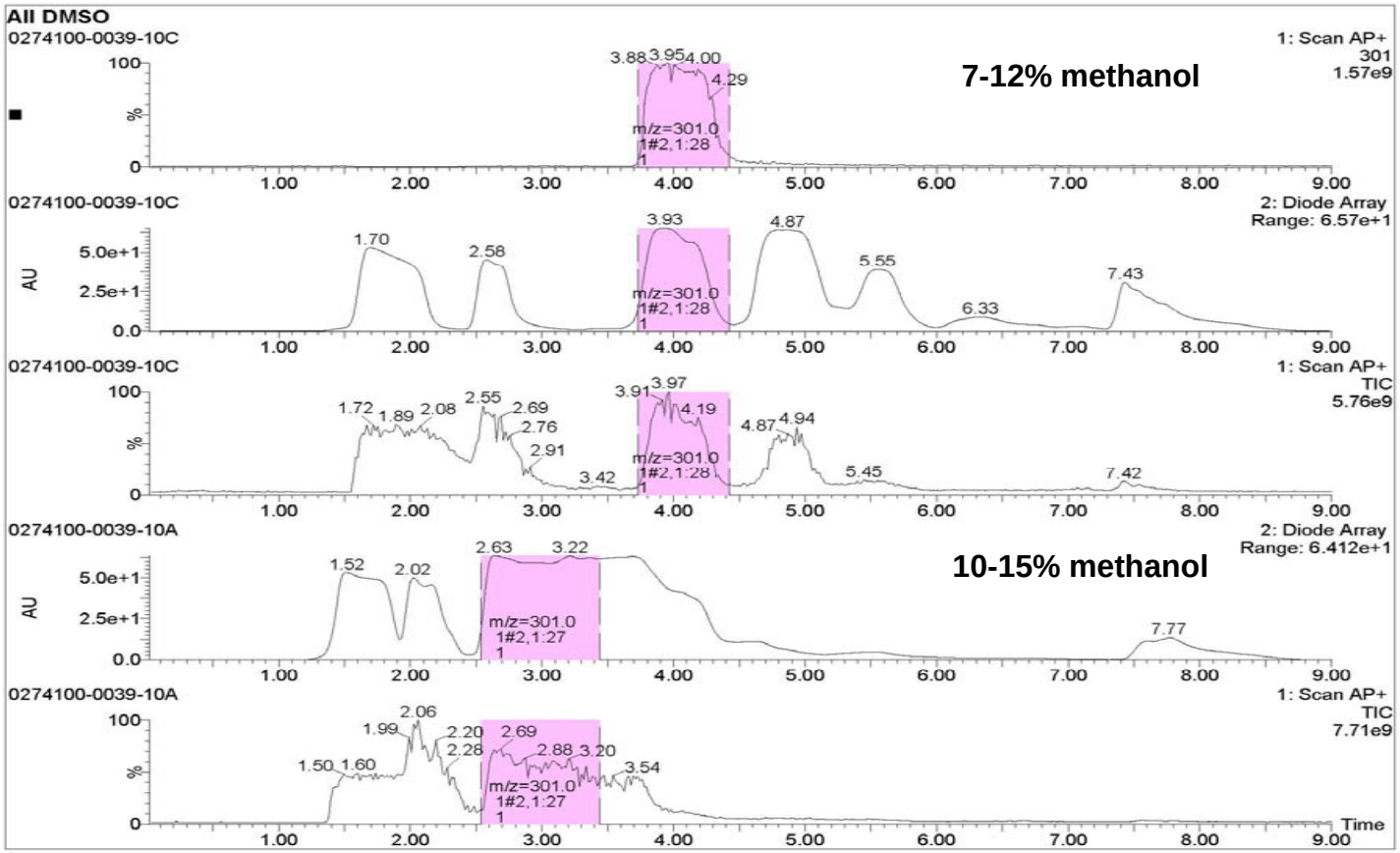
Fine Tuning Focused Gradients



1cm i.d. x 10cm, 5um, Ethylpyridine
20mL/min, 120 Bar, 40 degrees C

6 min focused gradient
1 min 60% flush
2 min 60-2% reverse gradient

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Status Update

- Comparable recovery to RP Prep HPLC, mid eighty percentages
- No carryover between fractions
- Can inject 1/3 of crude product, 333uL, without mass overloading column
- DMSO does not exhibit detrimental strong solvent effect
- Mapping of analytical retention times to focused gradient complete, and automated
- Ready for automated purification!!!!! ROLL THE SAMPLES

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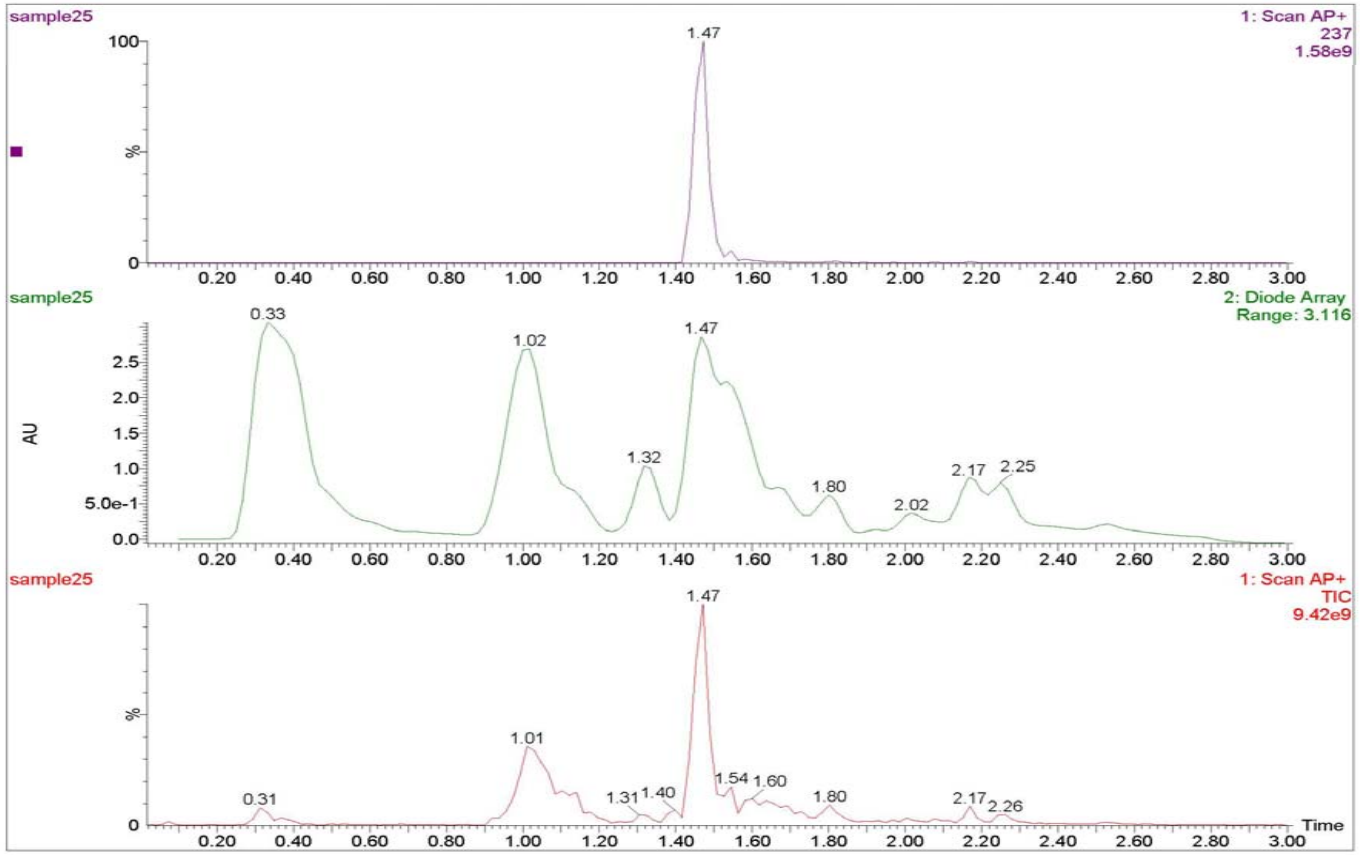
AutoPurify: Analytical Sample One



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

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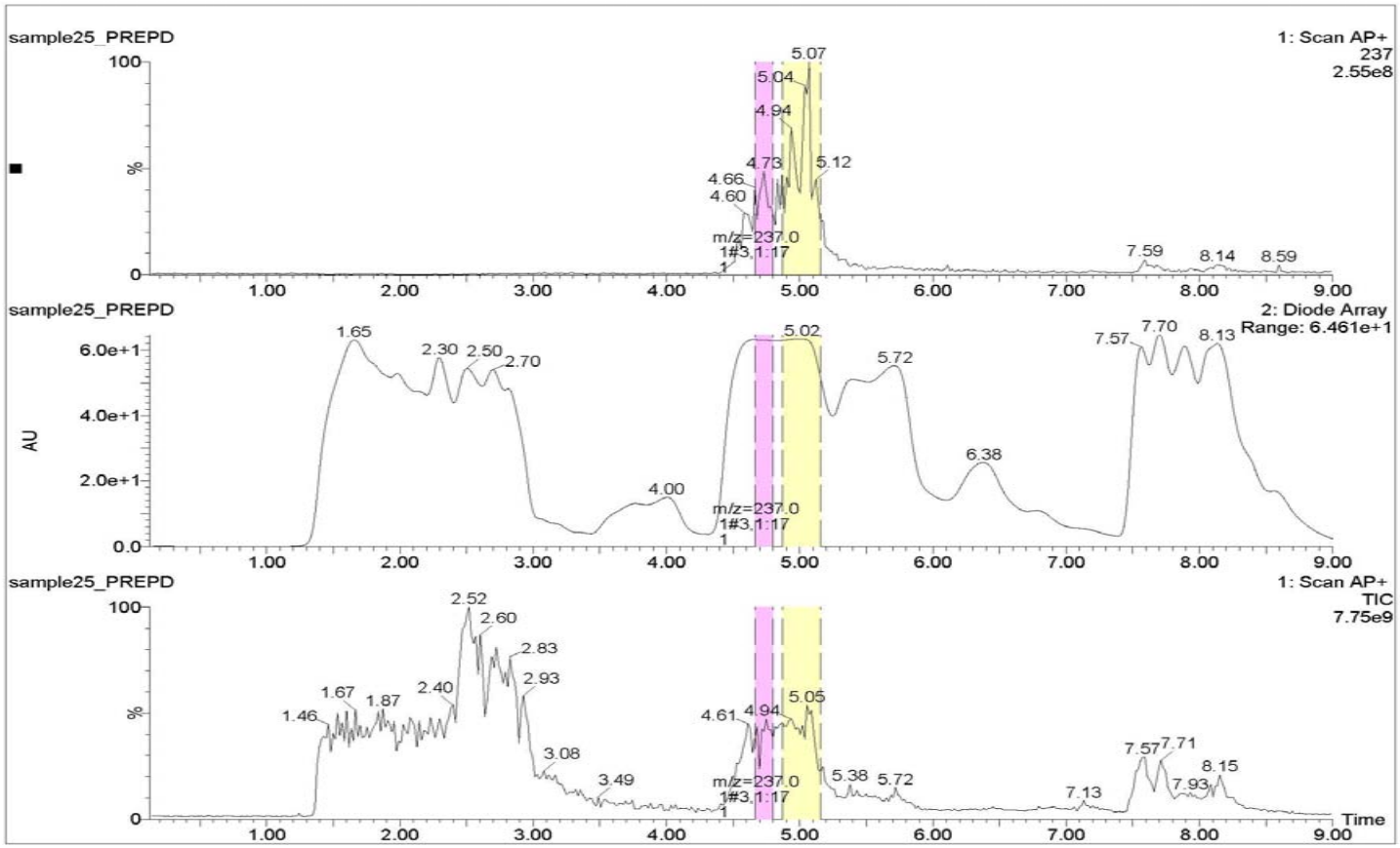
AutoPurify: Prep Sample One



1cm i.d. x 10cm, 5um, Ethylpyridine
20mL/min, 120 Bar, 40 degrees C
333uL injection volume

6 min 7-12% methanol
1 min 60% flush
2 min 60-2% reverse gradient

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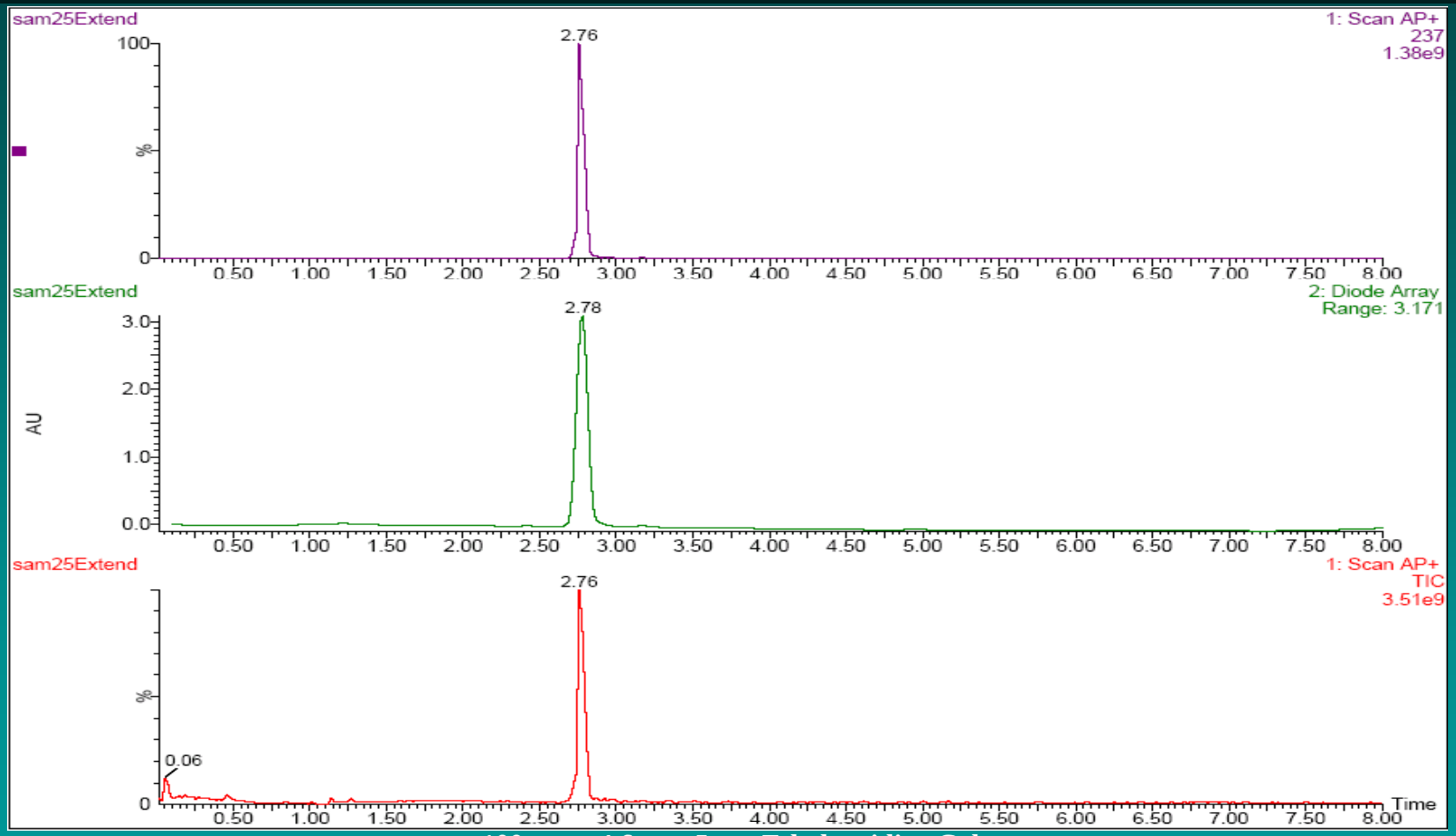
AutoPurify: Pure Sample One



100mm x 4.6mm, 5um, Ethylpyridine Column
4mL/min
360s 2-60% methanol gradient
120s 60-2% methanol gradient

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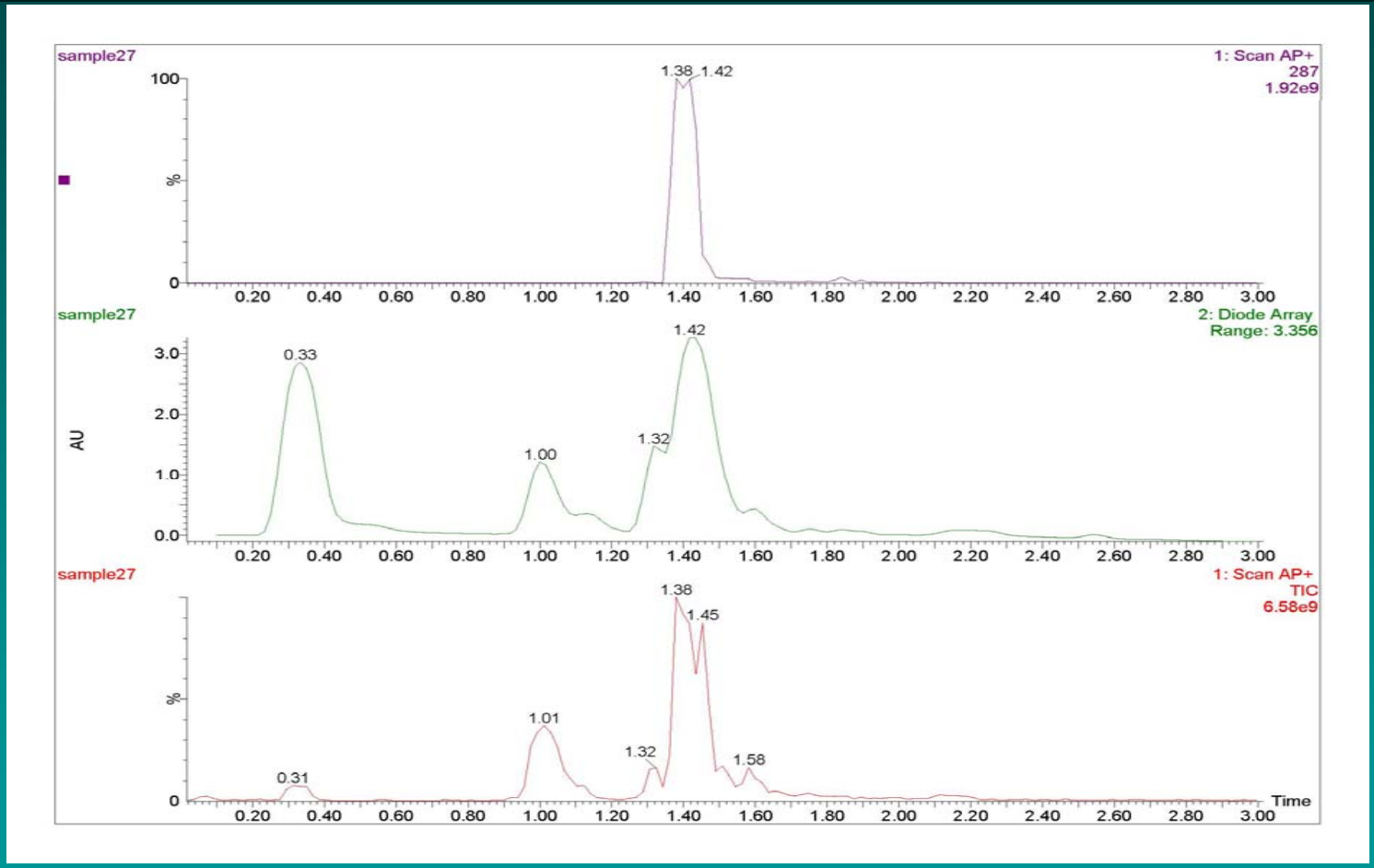
AutoPurify: Analytical Sample Two



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

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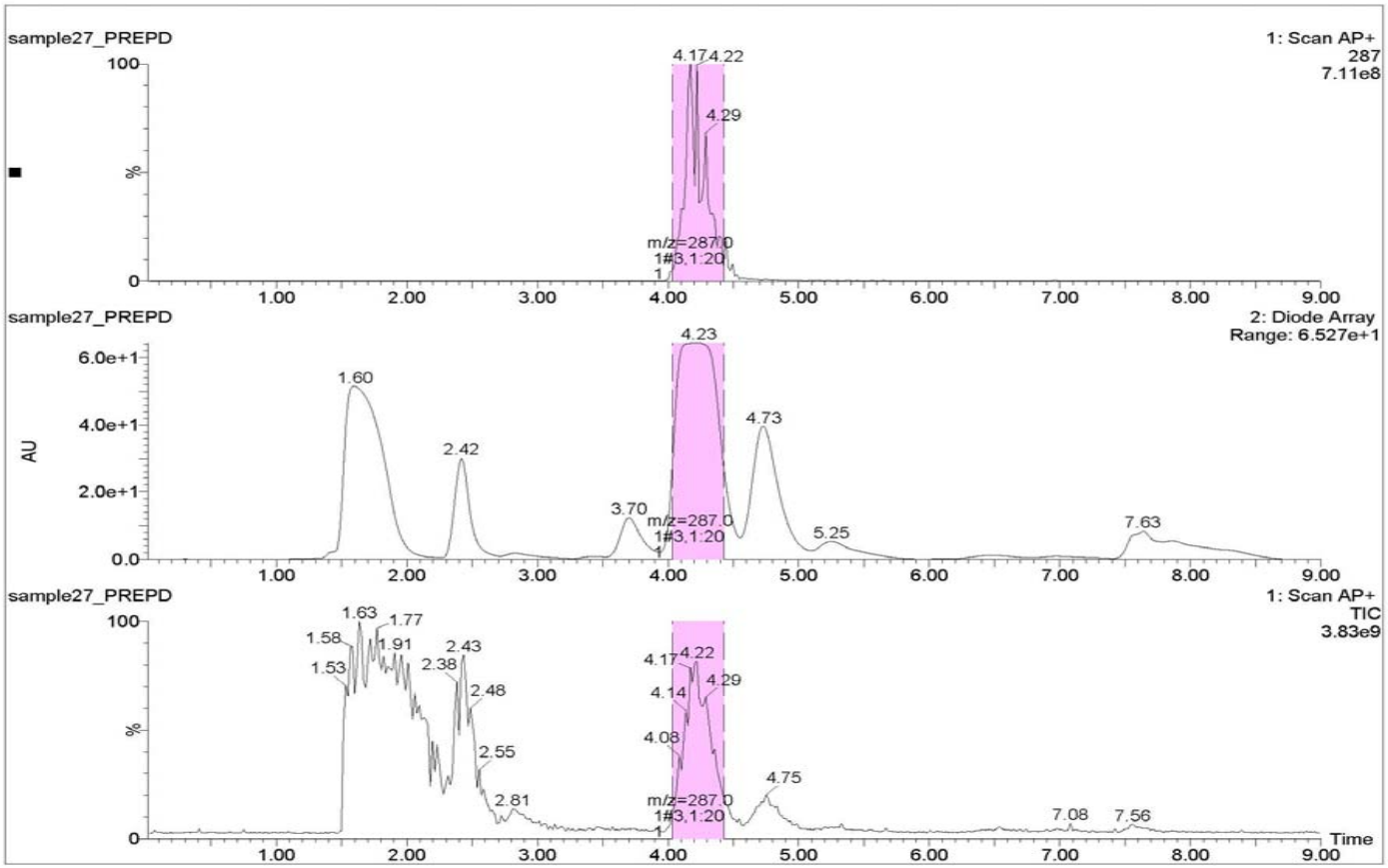
AutoPurify: Prep Sample Two



1cm i.d. x 10cm, 5um, Ethylpyridine
20mL/min, 120 Bar, 40 degrees C
333uL injection volume

6 min 7-12% methanol
1 min 60% flush
2 min 60-2% reverse gradient

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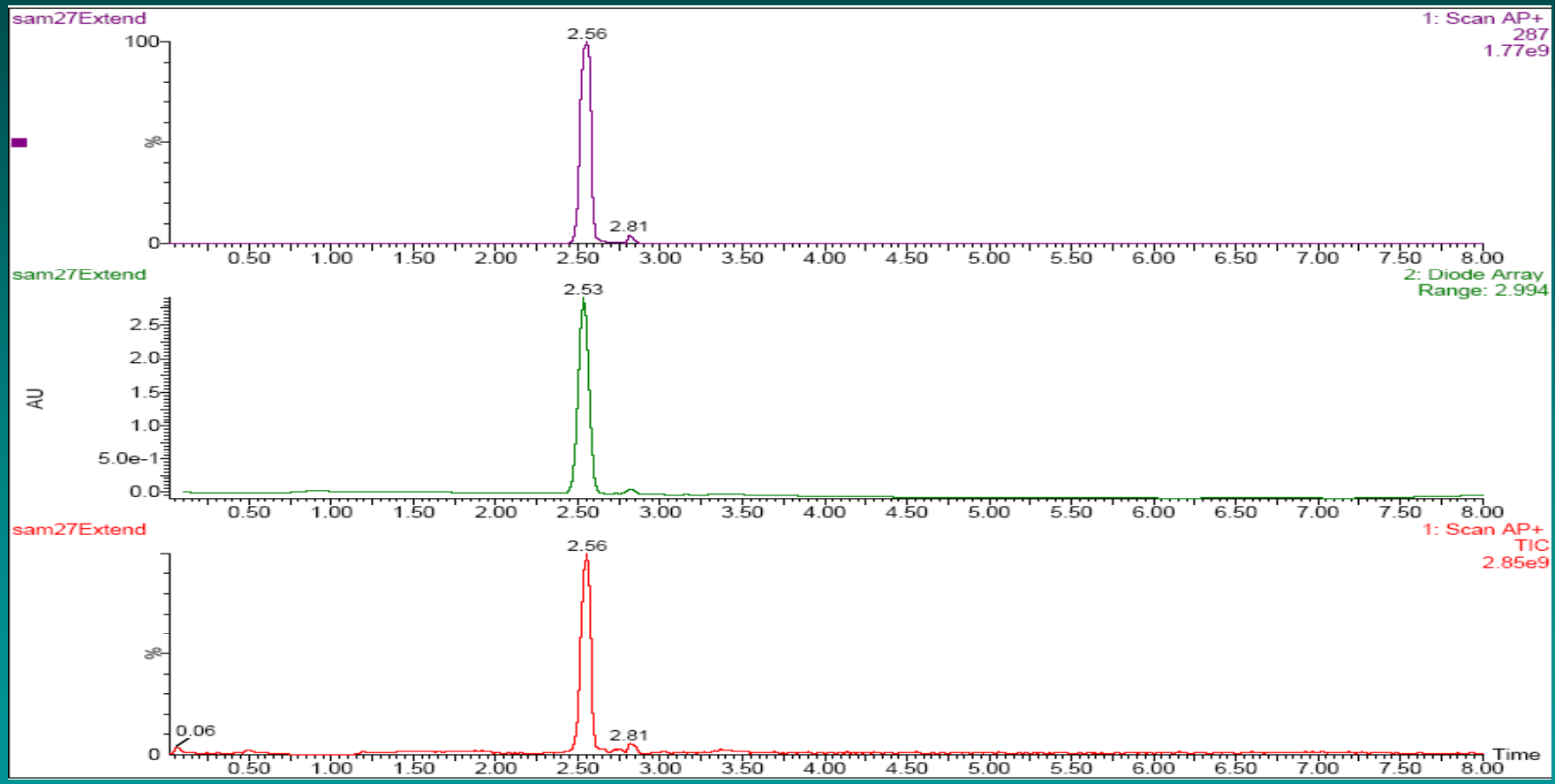
AutoPurify: Pure Sample Two



100mm x 4.6mm, 5um, Ethylpyridine Column
4mL/min
360s 2-60% methanol gradient
120s 60-2% methanol gradient

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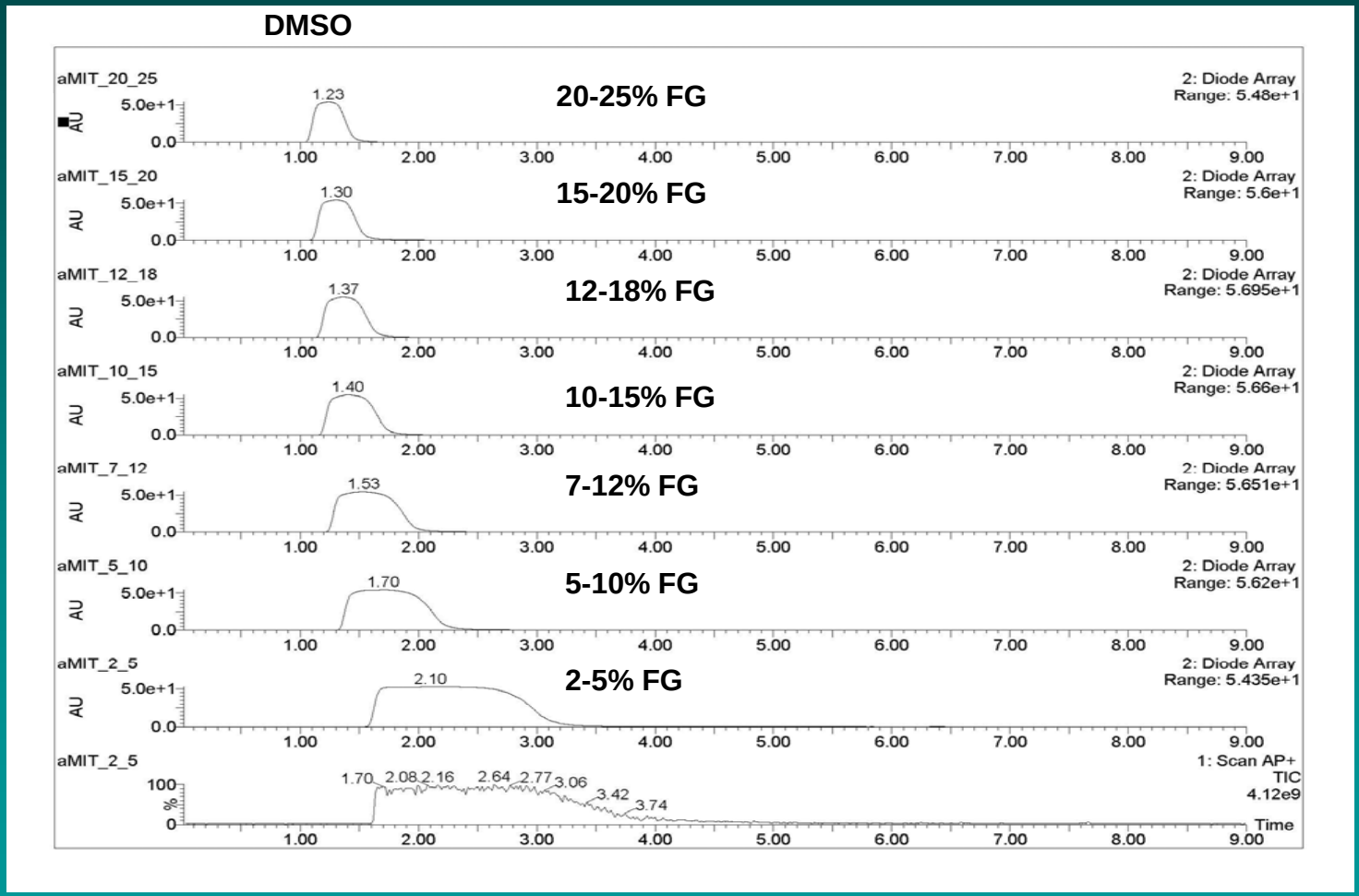
Variable Timing of Modifier Injection



1cm i.d. x 10cm, 5um, Ethylpyridine
20mL/min, 120 Bar, 40 degrees C

6 min focused gradient
1 min 60% flush
2 min 60-2% reverse gradient

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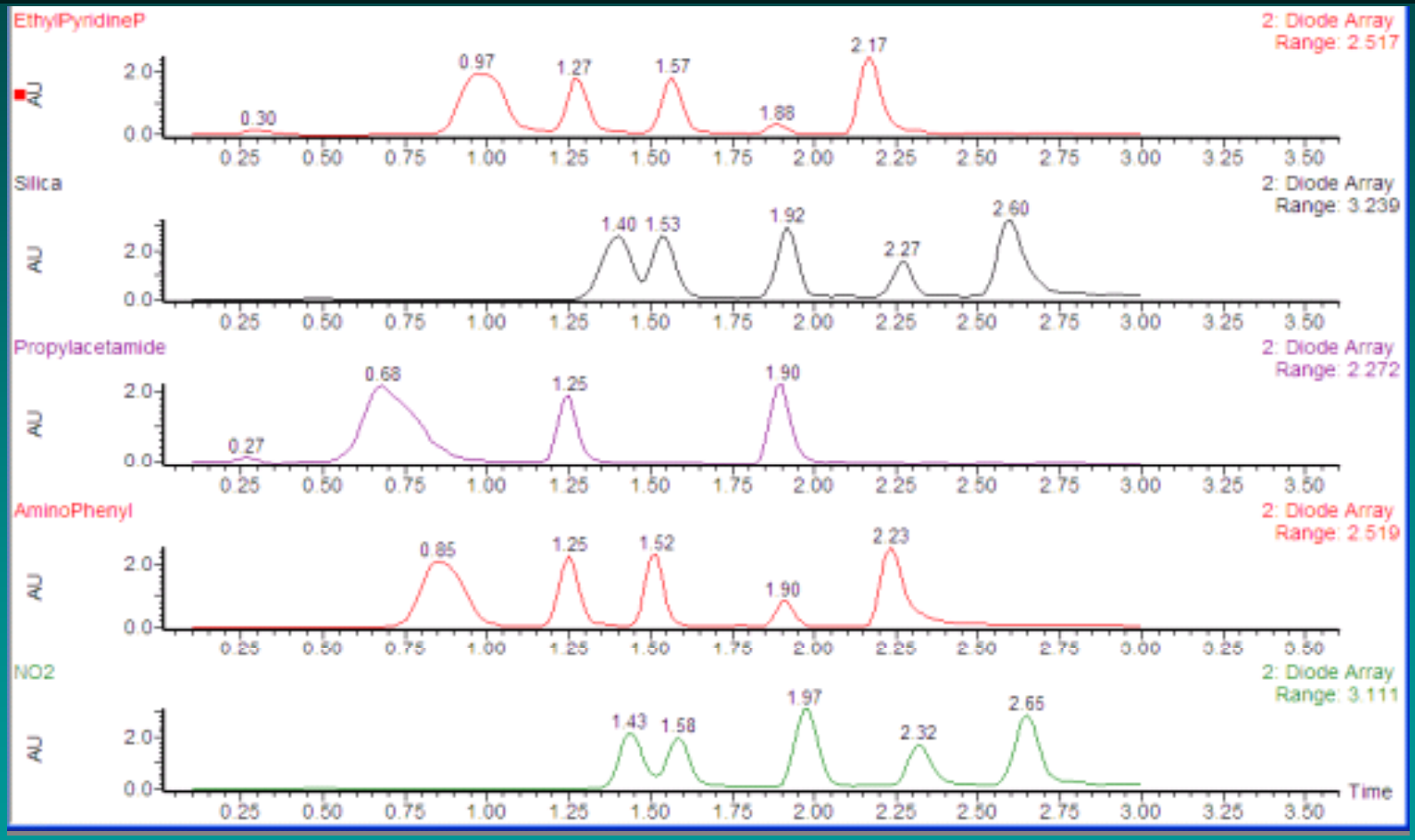
Changing Selectivity / Different Columns



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

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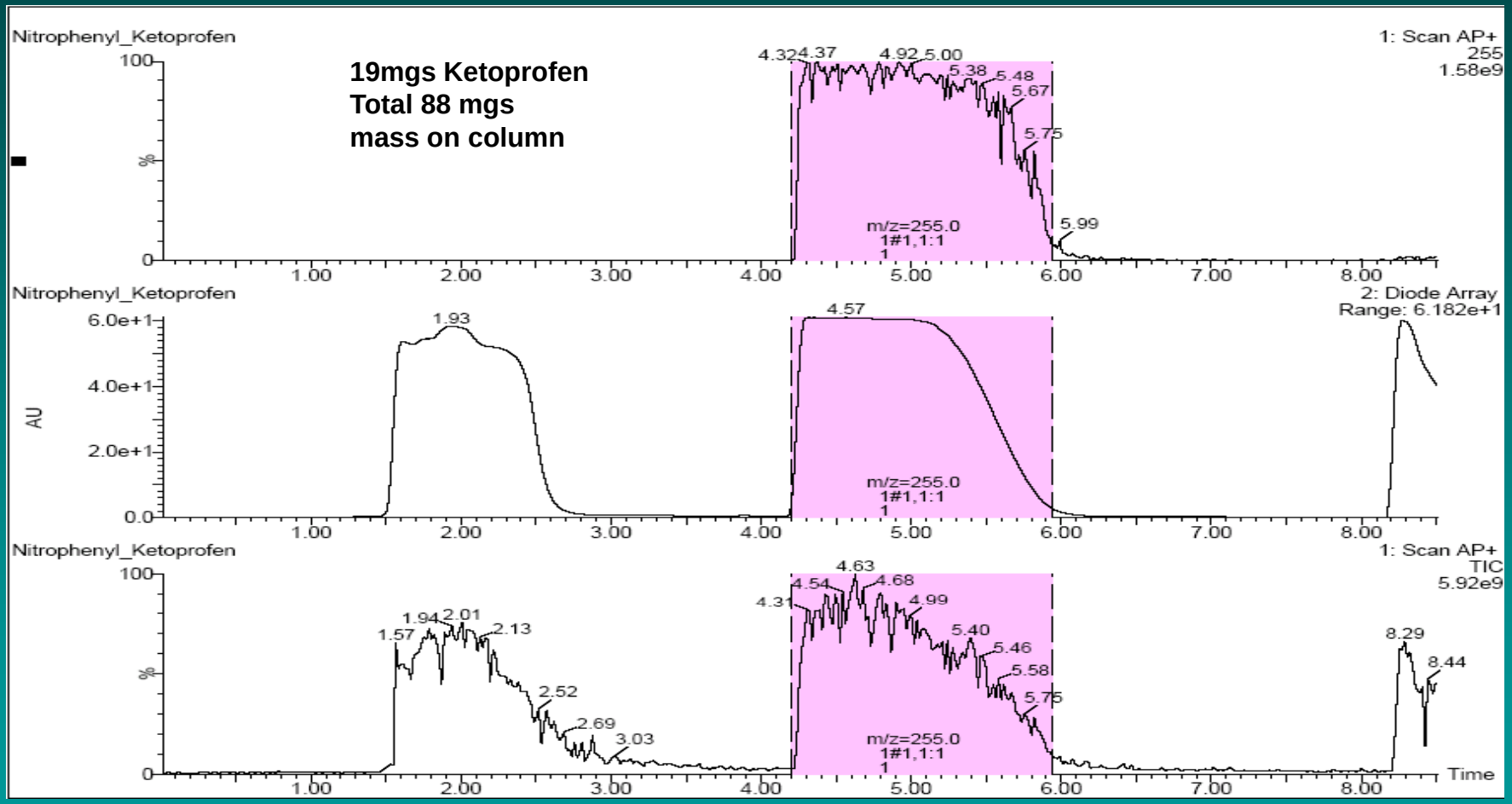
Standard Comparison: NO_2 Versus EP



1cm i.d. x 10cm, 5um, Nitrophenyl
20mL/min, 120 Bar, 40 degrees C
350uL injection volume

6 min 15-20% methanol
1 min 60% flush
2 min 60-2% reverse gradient

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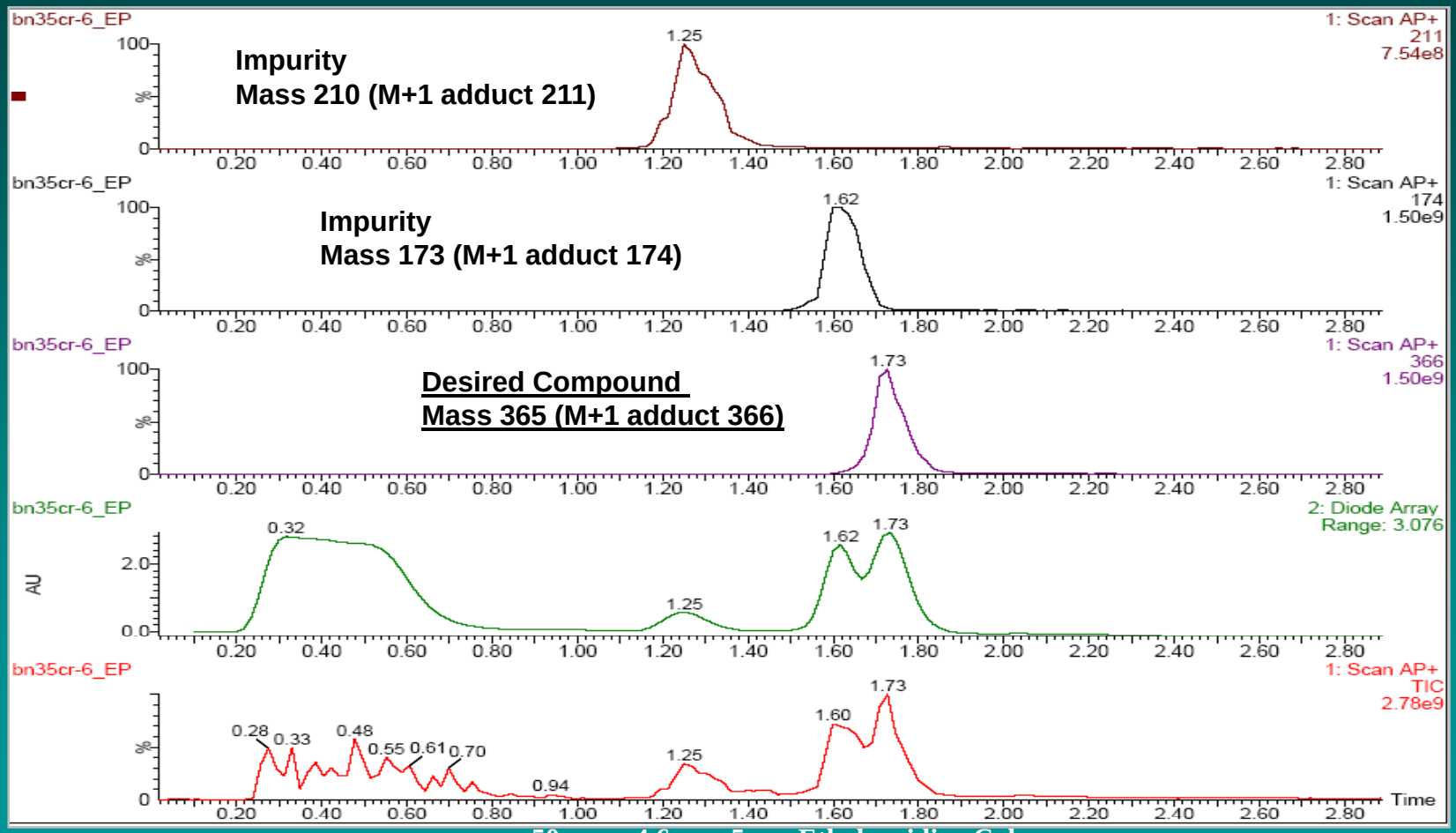
Sample One Comparison: NO2 Versus EP



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

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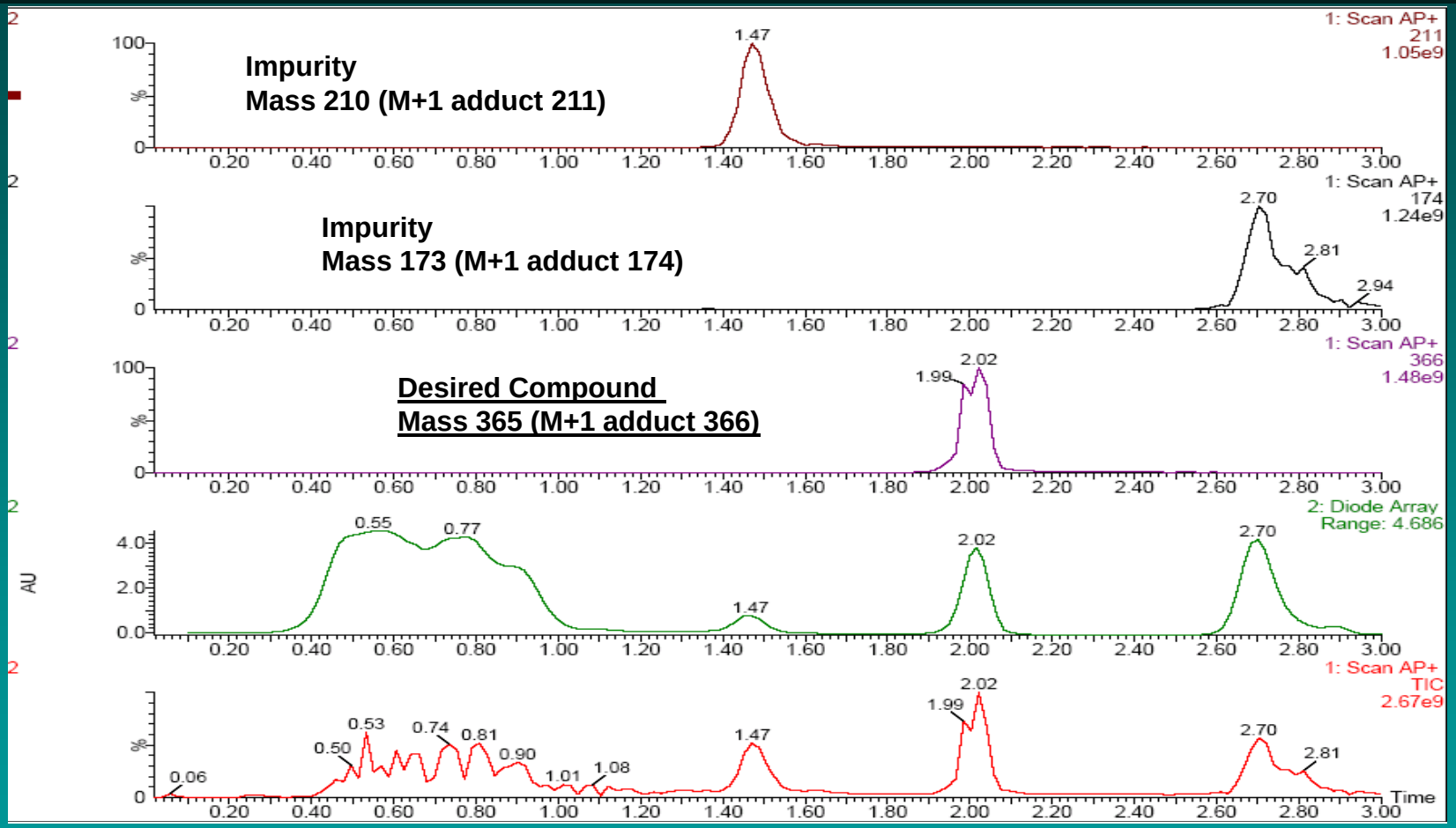
Sample One Comparison: NO2 Versus EP



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

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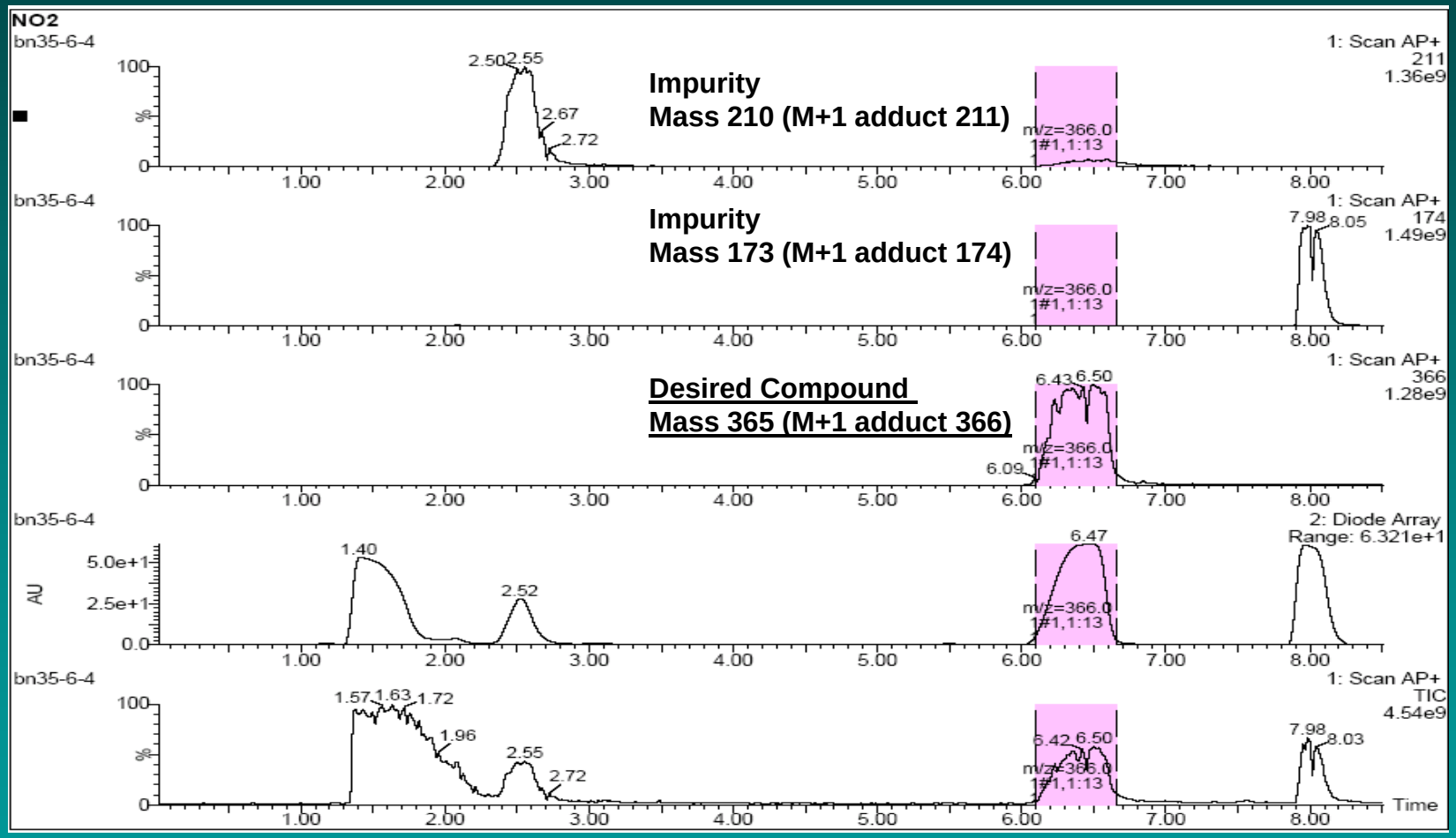
Sample One Comparison: NO2 Versus EP



1cm i.d. x 10cm, 5um, Nitrophenyl
20mL/min, 120 Bar, 40 degrees C

6 min 15-20% methanol
1 min 60% flush
2 min 60-2% reverse gradient

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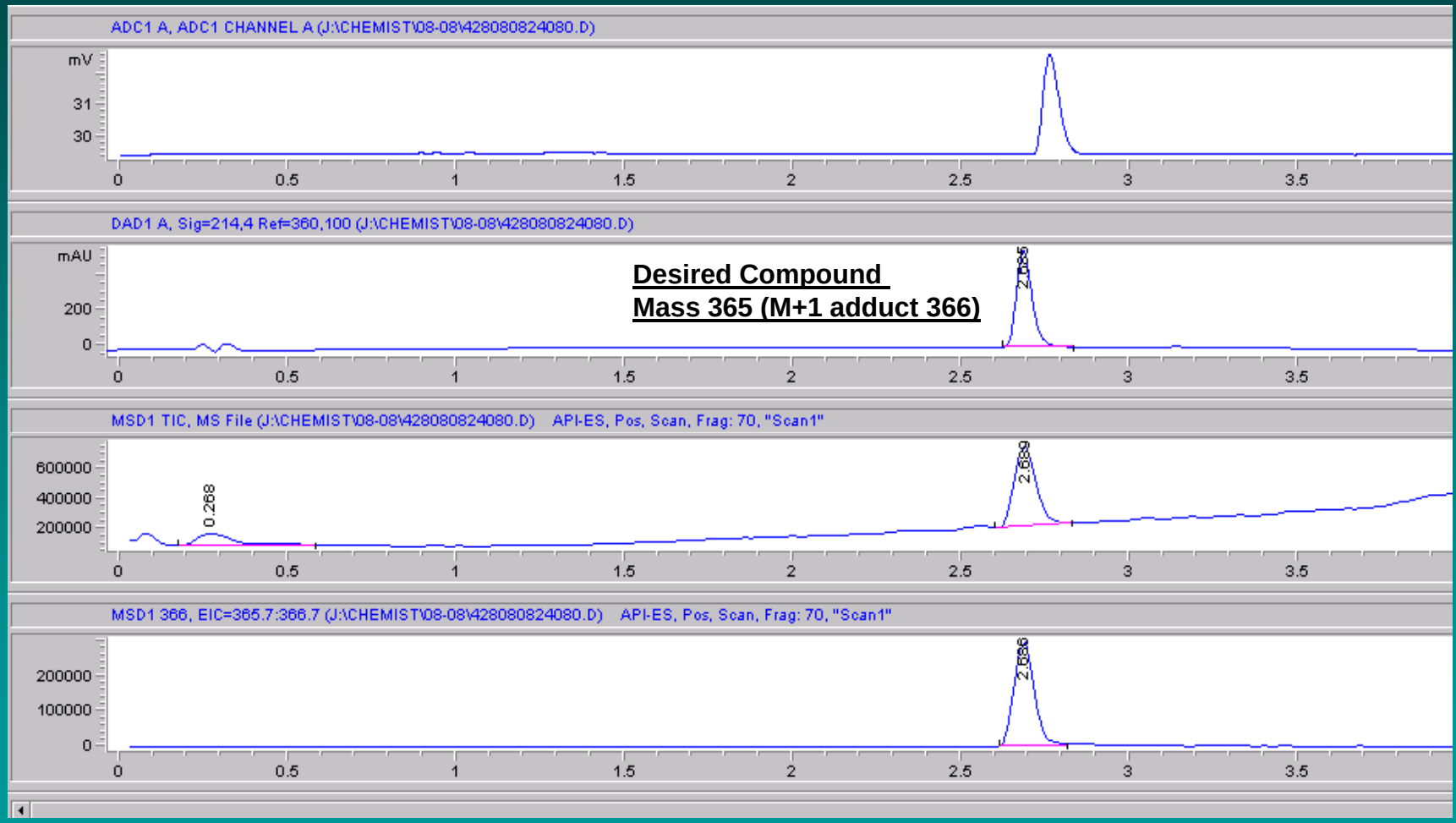
Sample One Comparison: NO2 Versus EP



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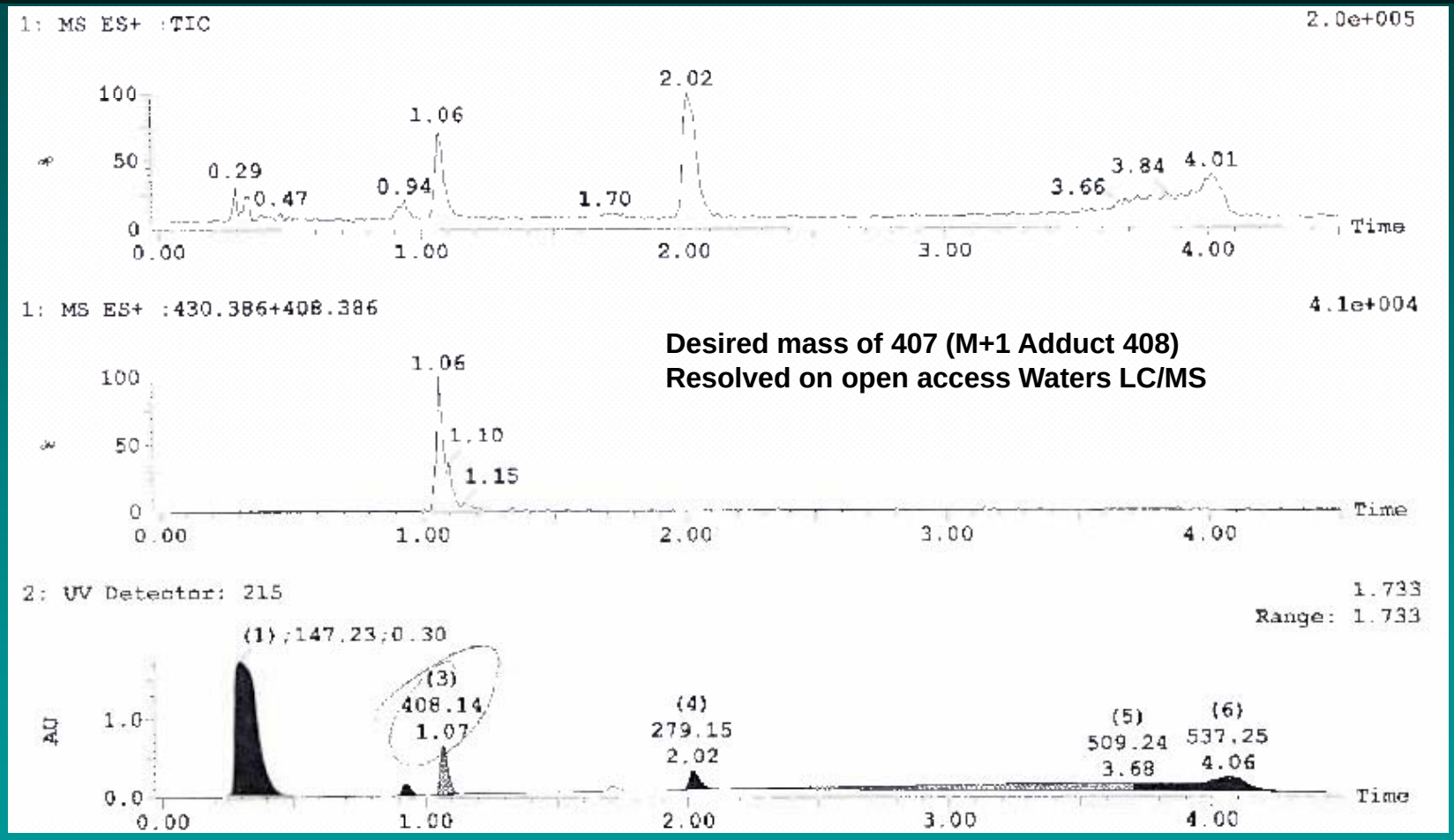
Final Sample



Desired mass of 407 (M+1 Adduct 408)
Resolved on open access Waters LC/MS

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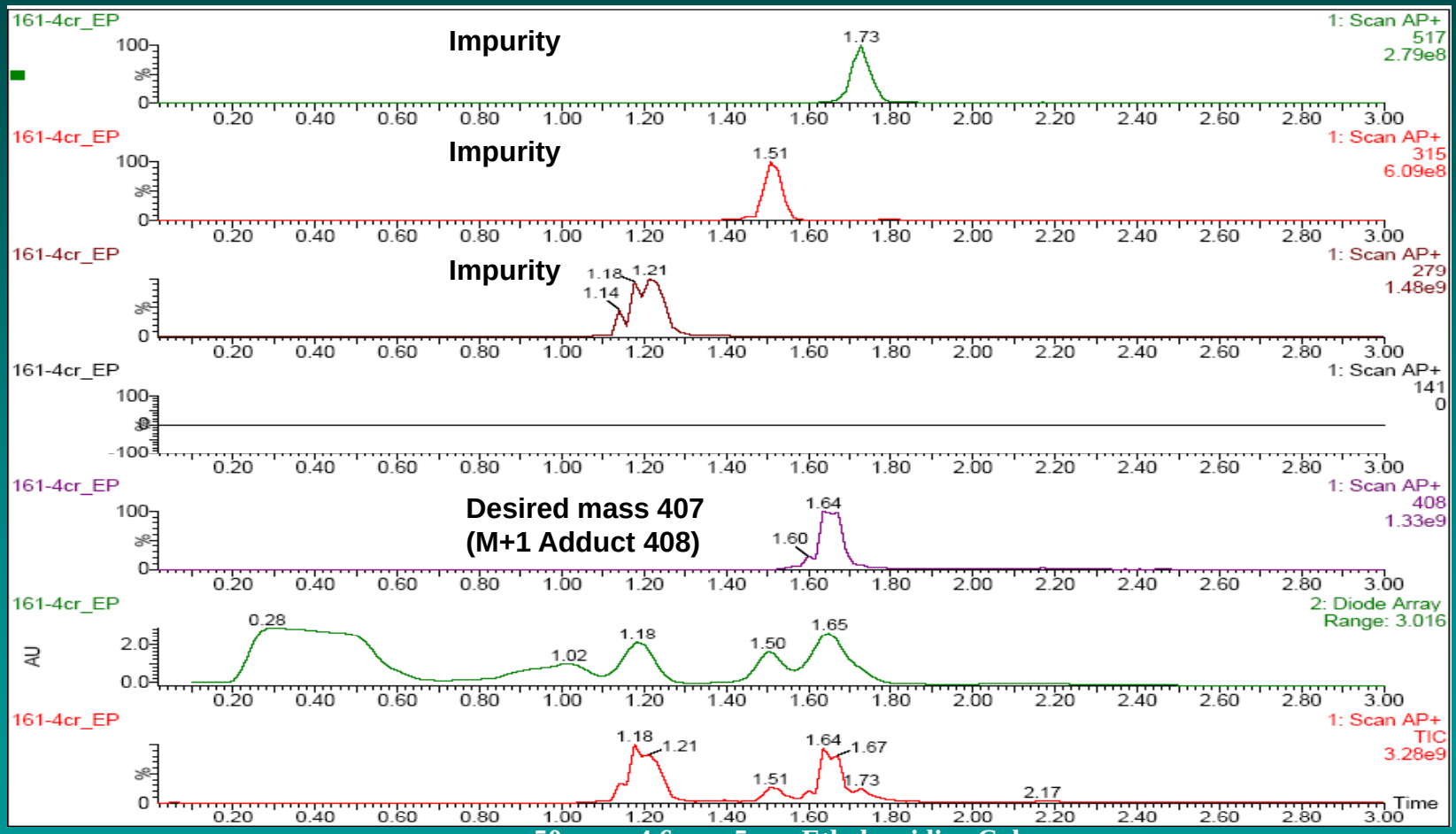
Final Sample



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

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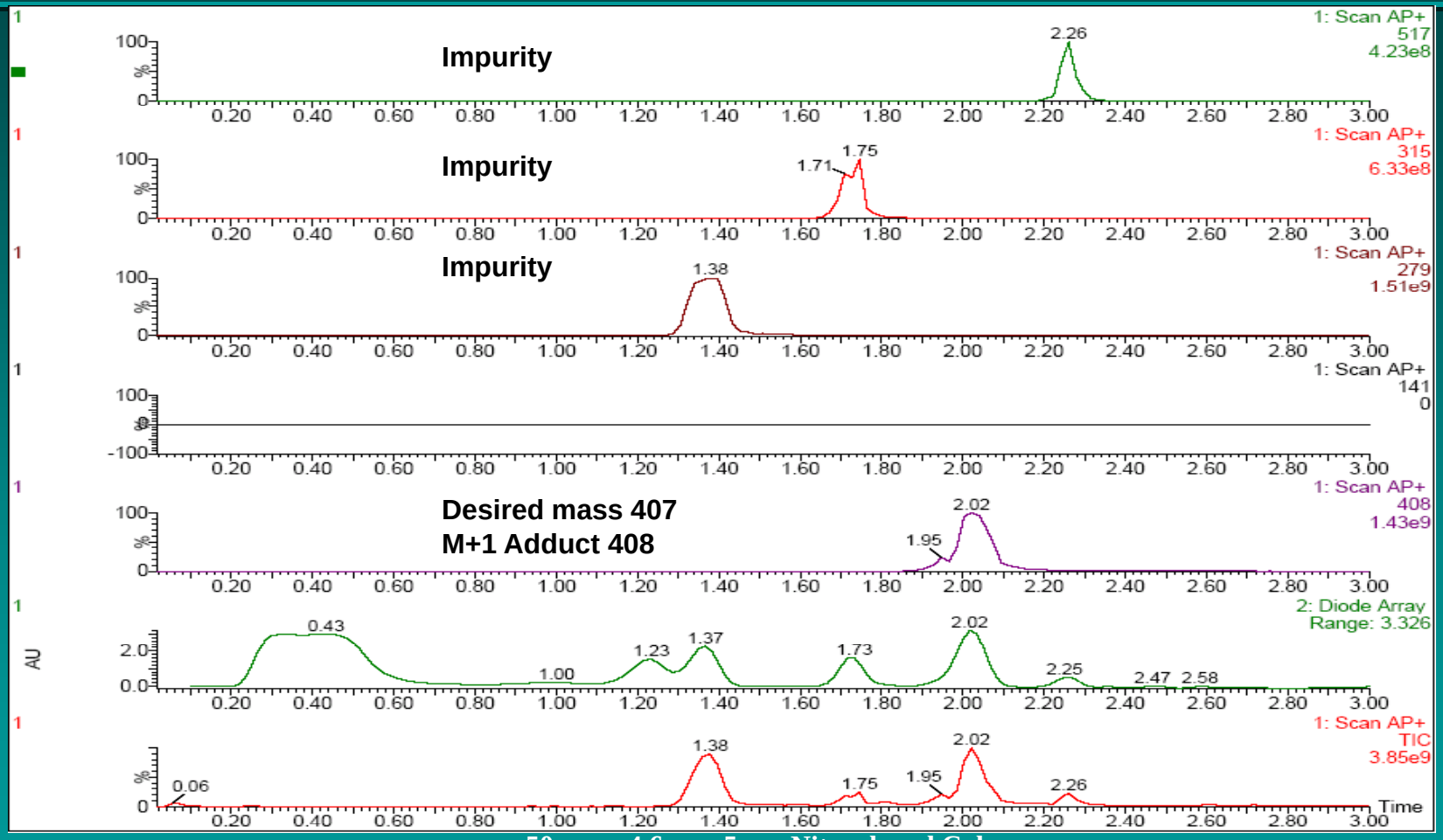
Final Sample



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

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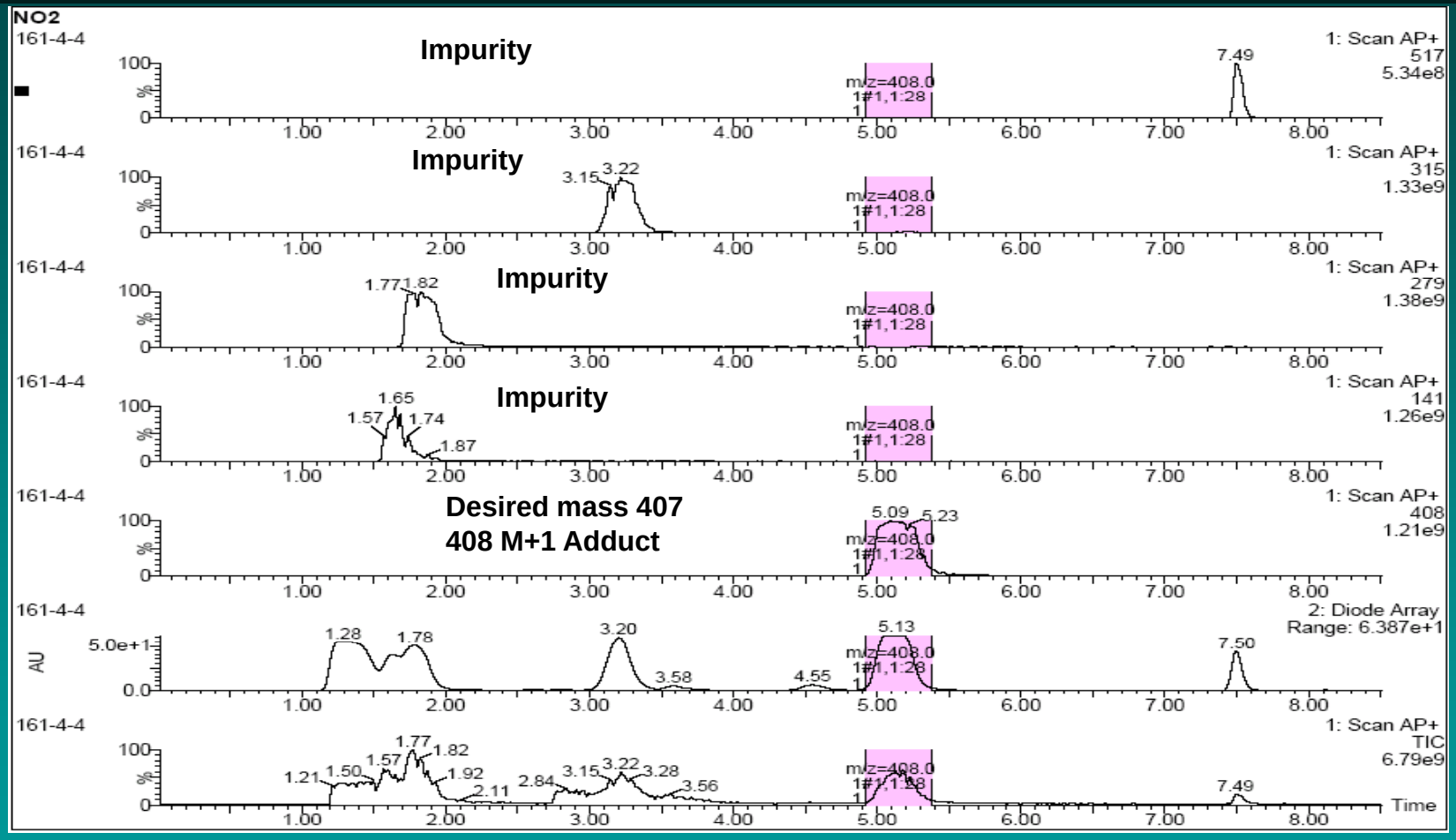
Final Sample



1cm i.d. x 10cm, 5um, Nitrophenyl
20mL/min, 120 Bar, 40 degrees C

6 min 20-25% methanol
1 min 60% flush
2 min 60-2% reverse gradient

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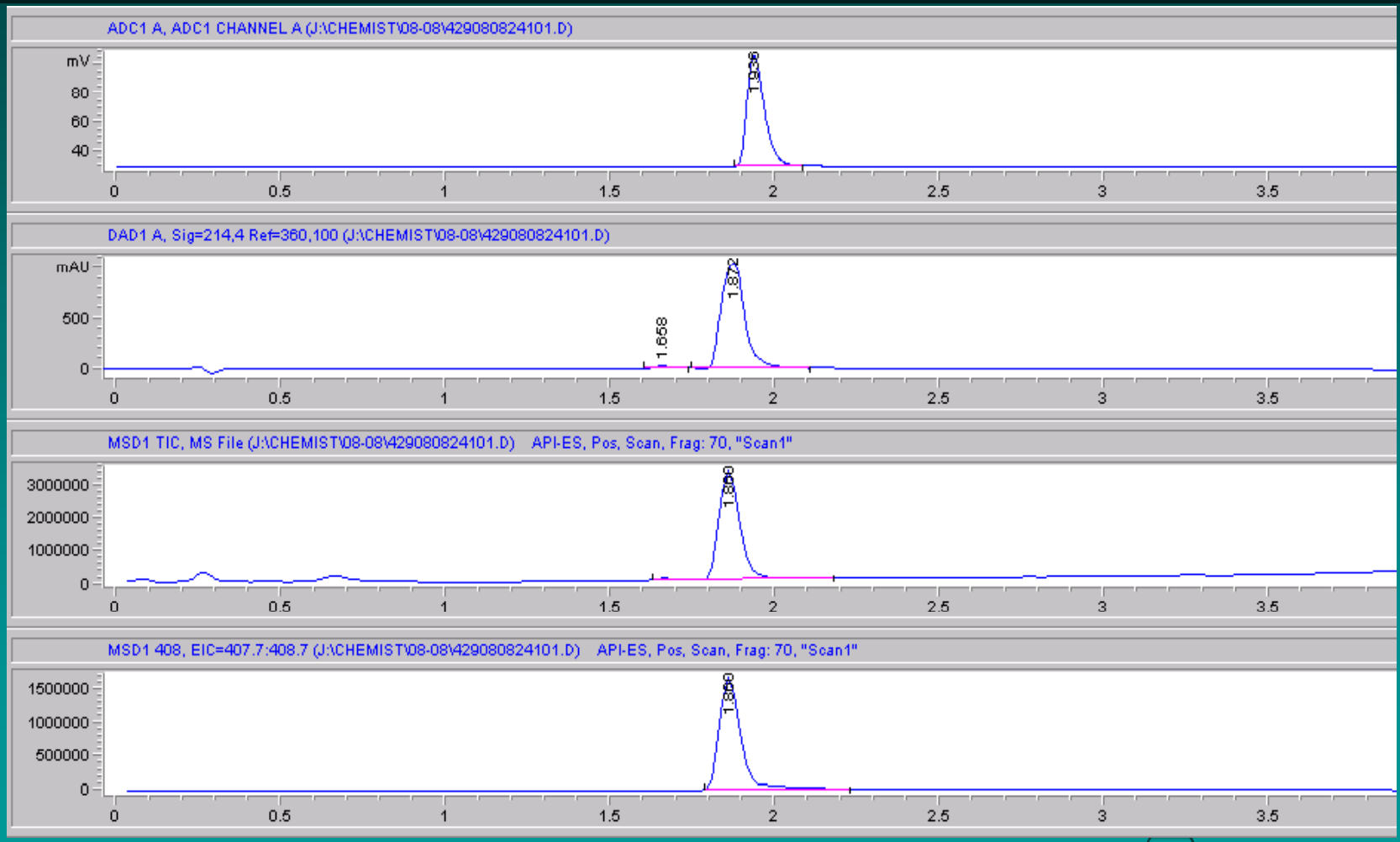
Final Sample



Summary

- Semi-preparative SFC dramatically reduces dry down time of purified compounds compared to the same scale separation on a semi-preparative HPLC system
- Analytical retention times can be mapped to focused gradients on semi-preparative SFC system for automated method assignment
- SFC is a valuable addition to a high-throughput purification lab being able to handle 60% of compounds

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Acknowledgements

- Merck NTRLC
- Chris Welch, Jim Barrow, and George Hartman
- THAR
- Waters
- ES Industries
- Princeton Chromatography
- Bill Wranitz
- Matt Przybyciel
- Denise Heyburn
- And…You

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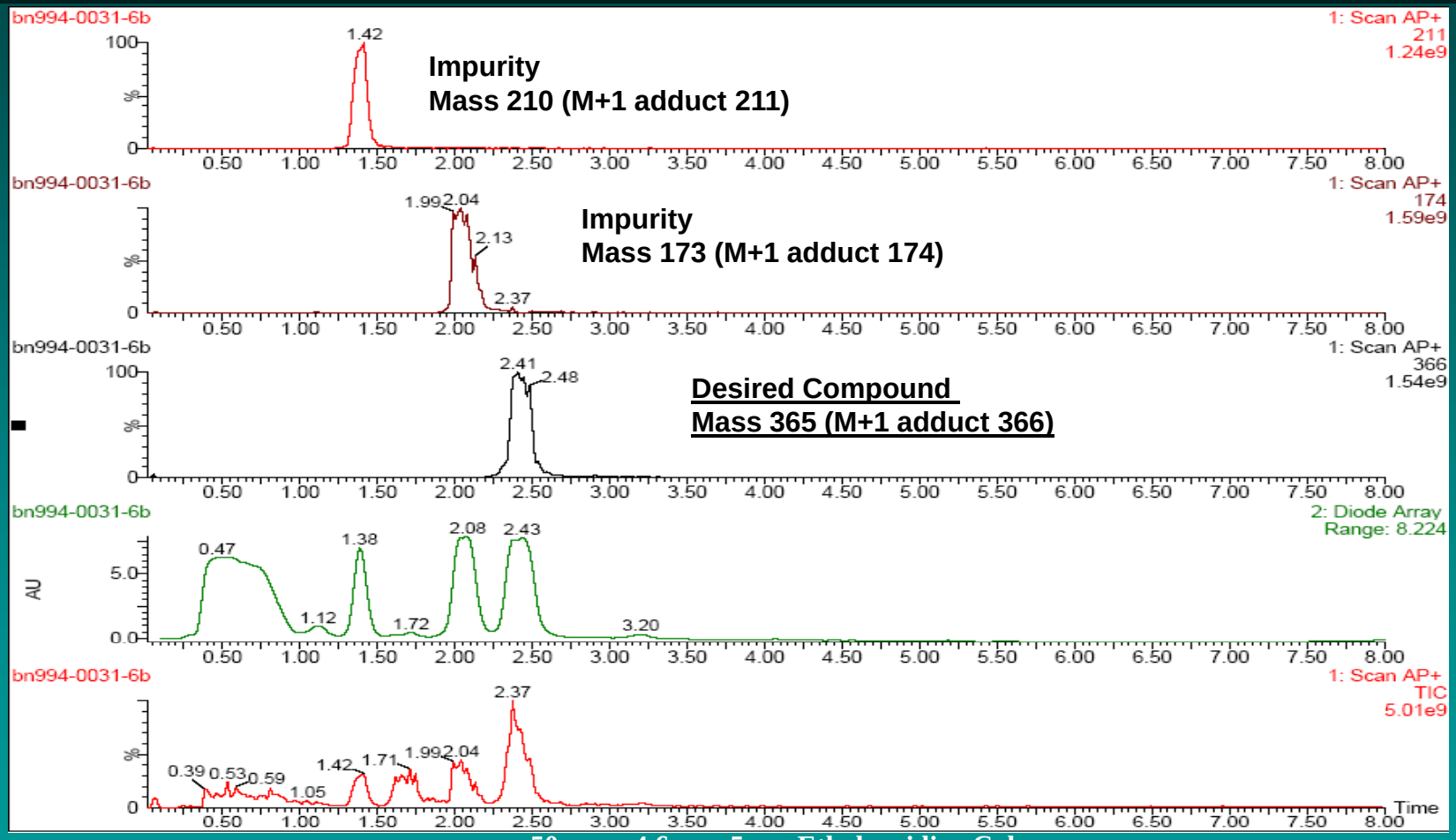
Sample One Comparison: NO2 Versus EP



50mm x 4.6mm, 5um, Ethylpyridine Column
4mL/min
10s 2% methanol hold
360s 2-60% methanol gradient
110s 60-2% methanol gradient

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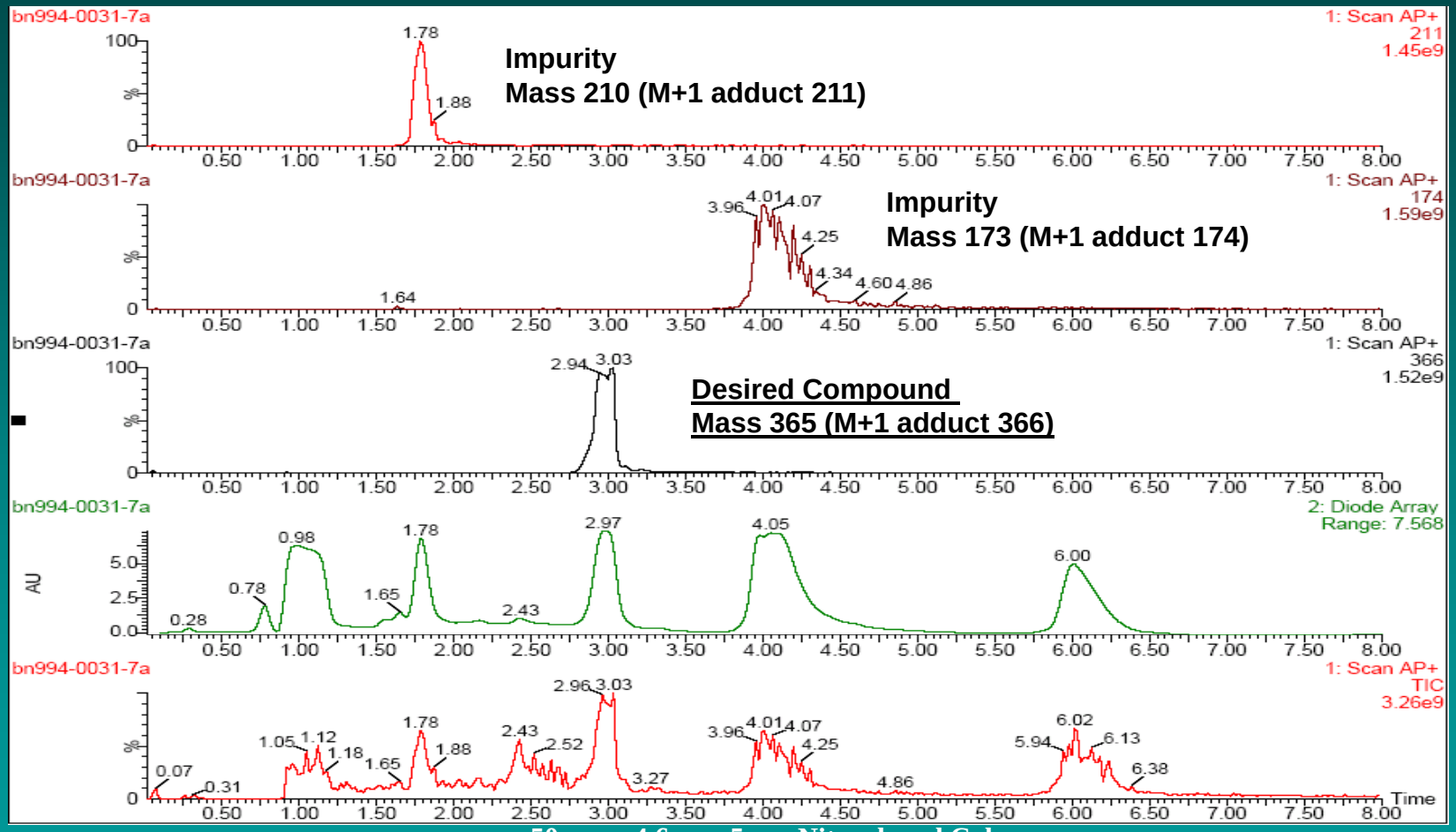
Sample One Comparison: NO2 Versus EP



50mm x 4.6mm, 5um, Nitrophenyl Column
4mL/min
10s 2% methanol hold
360s 2-60% methanol gradient
110s 60-2% methanol gradient

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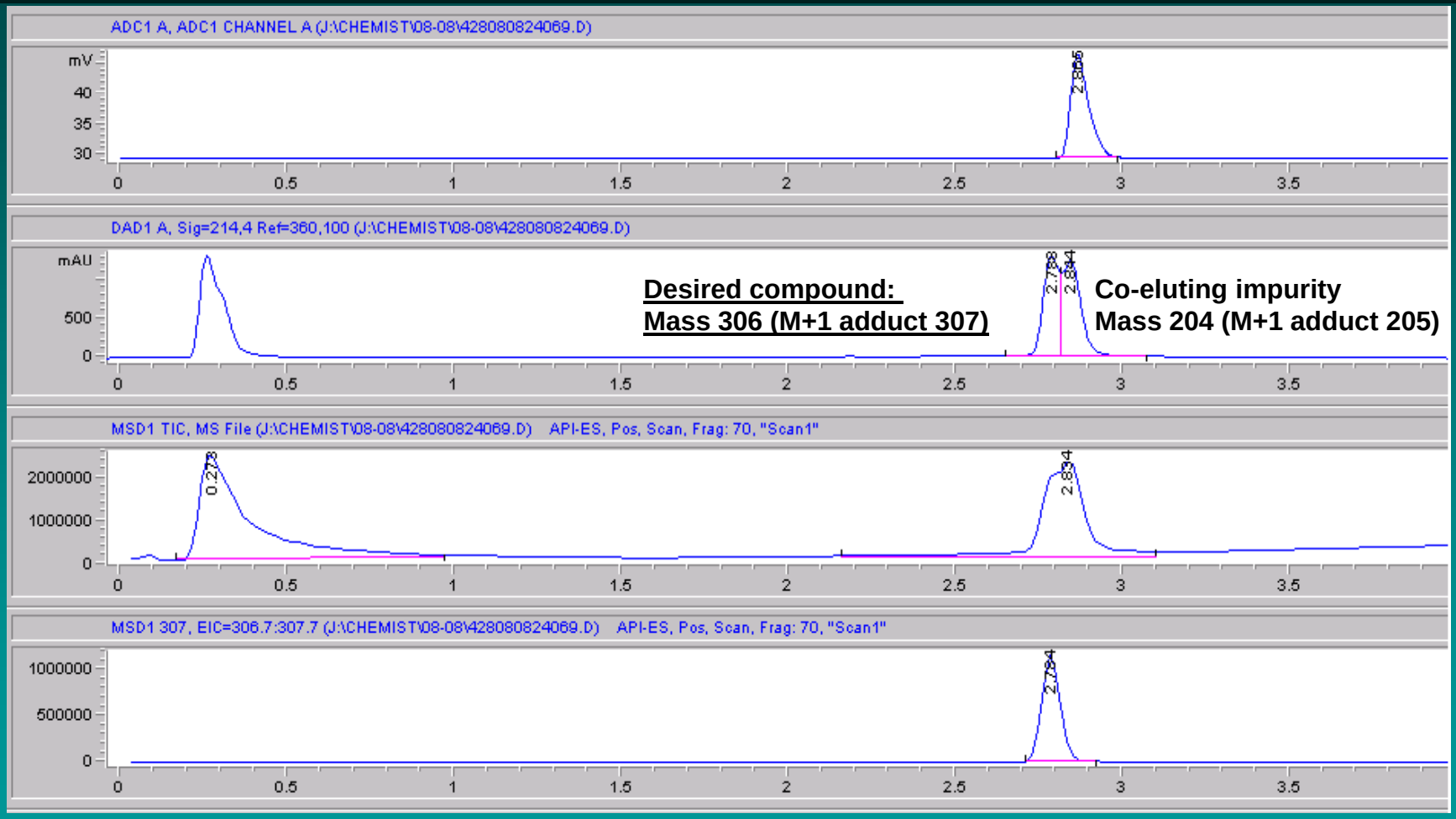
Sample Two Comparison: NO_2 Versus EP



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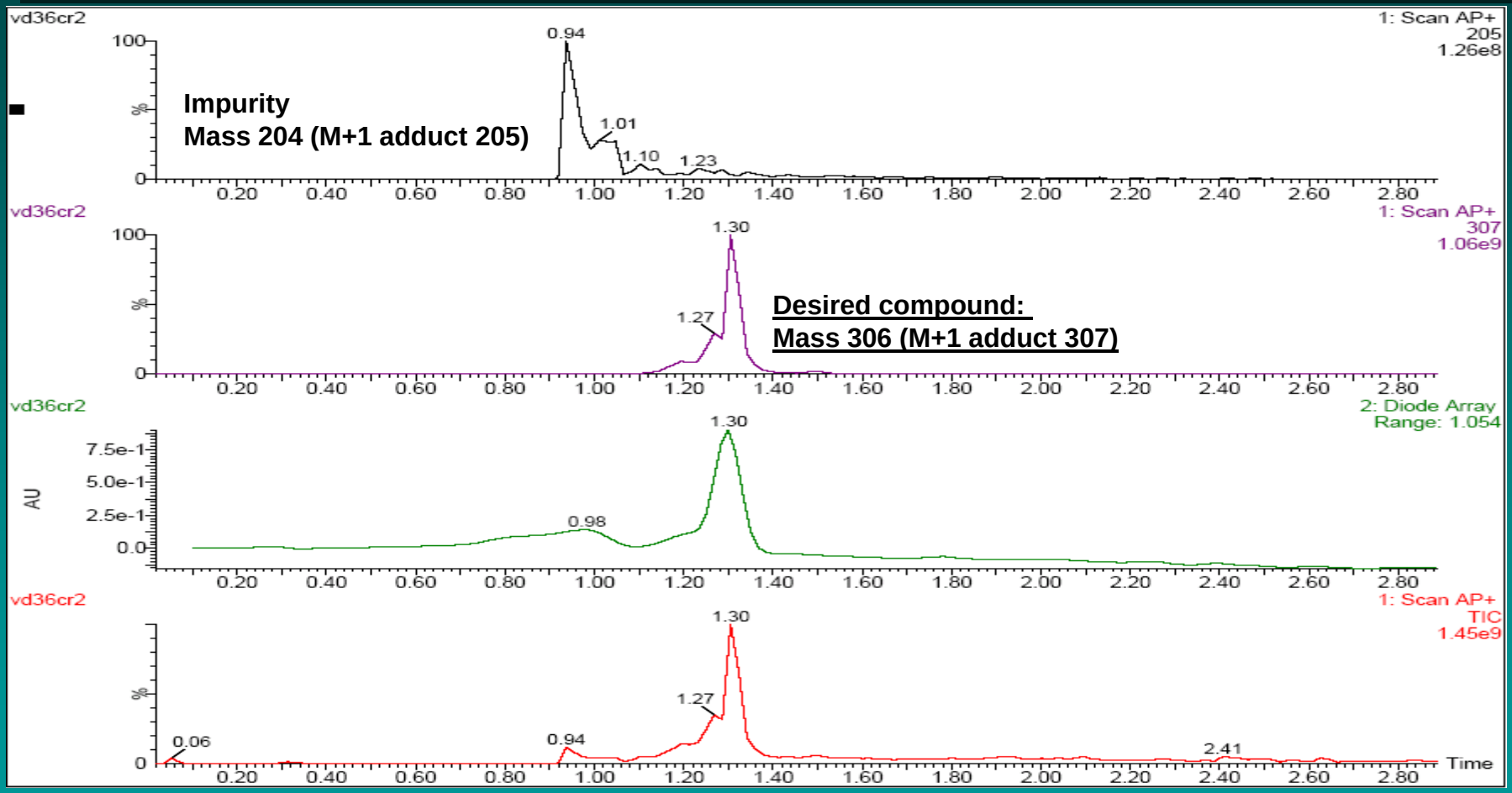
Sample Two Comparison: NO_2 Versus EP



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

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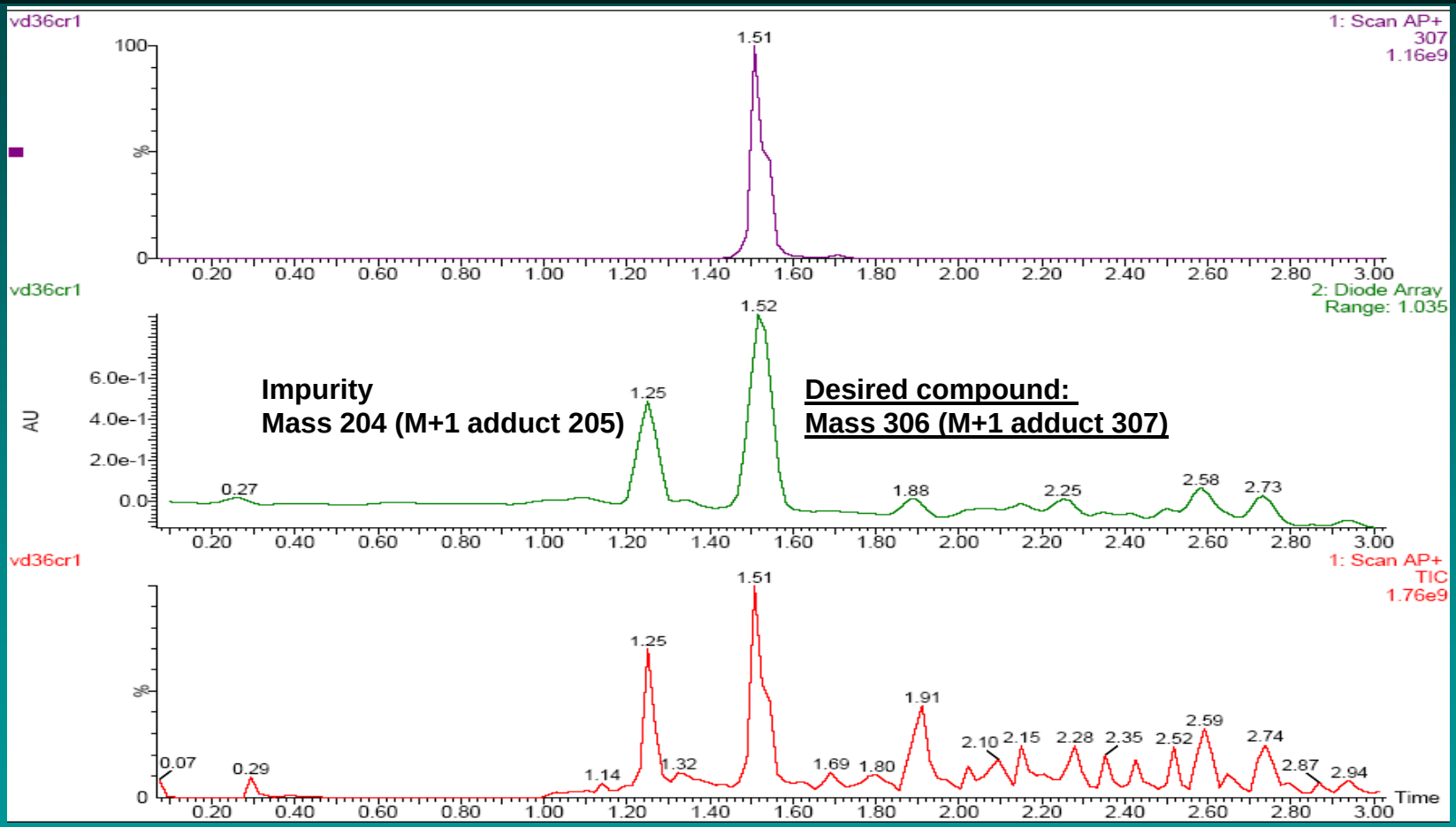
Sample Two Comparison: NO2 Versus EP



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

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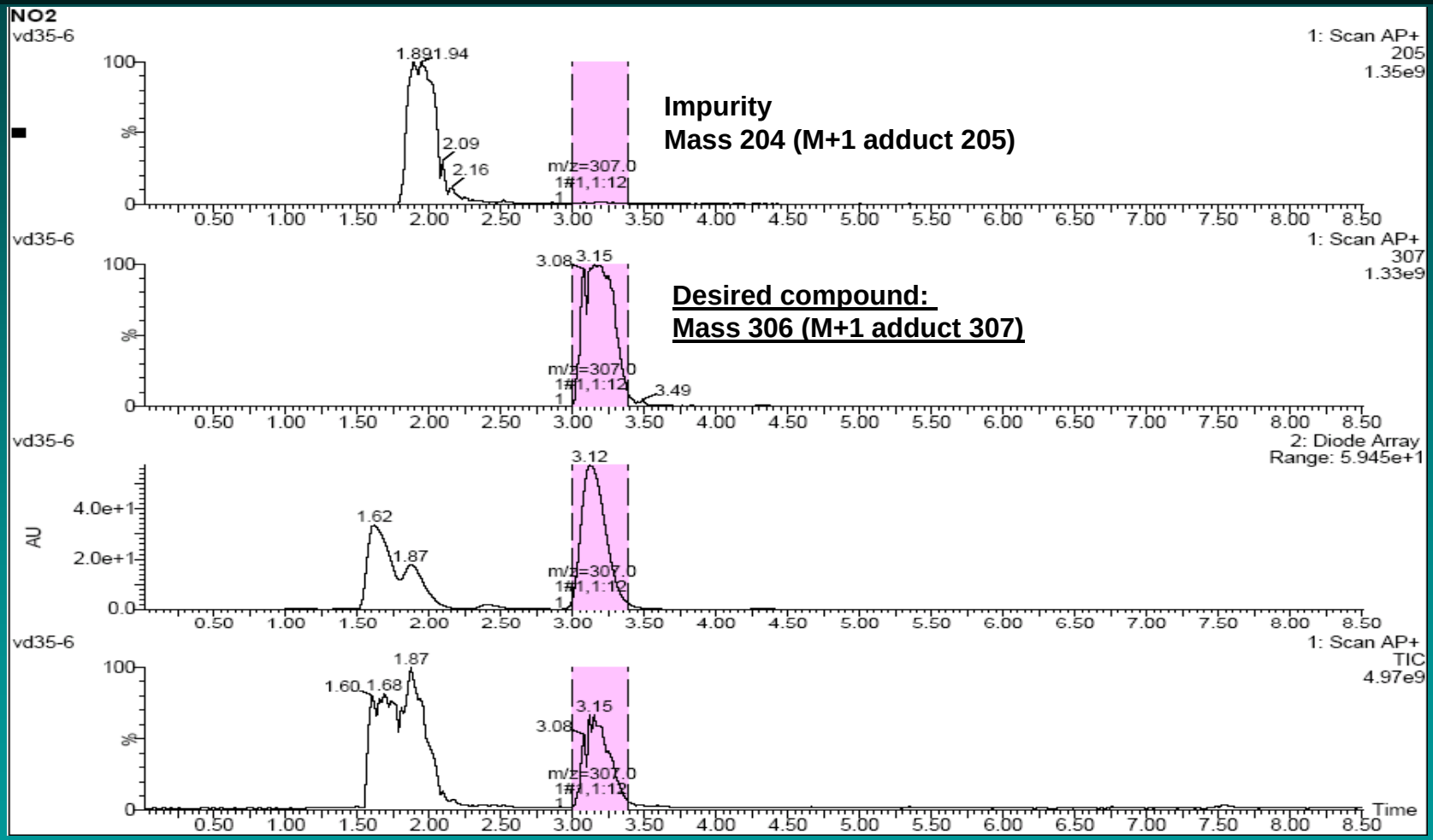

1cm i.d. x 10cm, 5um, Nitrophenyl
20mL/min, 120 Bar, 40 degrees C

6 min 15-20% methanol
1 min 60% flush
2 min 60-2% reverse gradient

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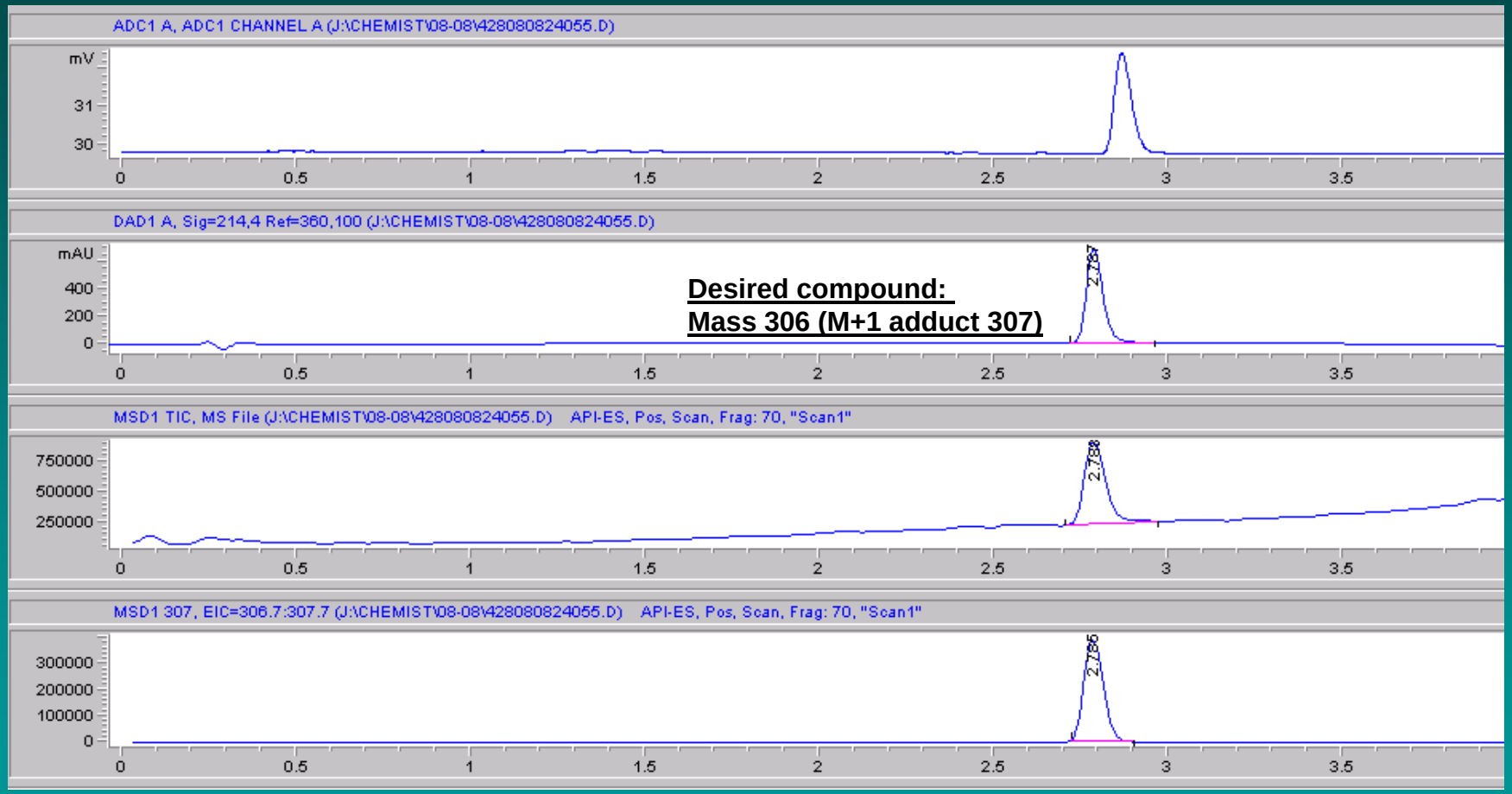
Sample Two Comparison: NO2 Versus EP



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