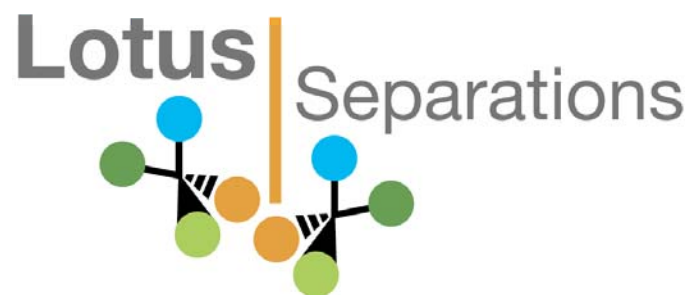


# SFC Applications to Research in Chemistry and Molecular Biology at Princeton University

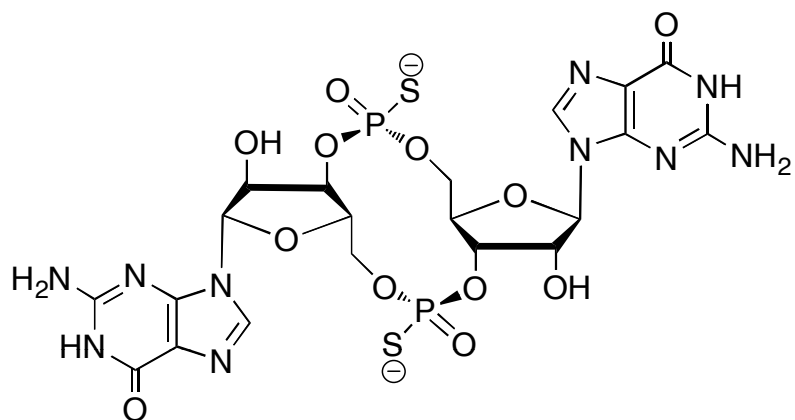


[www.LotusSeparations.com](http://www.LotusSeparations.com)

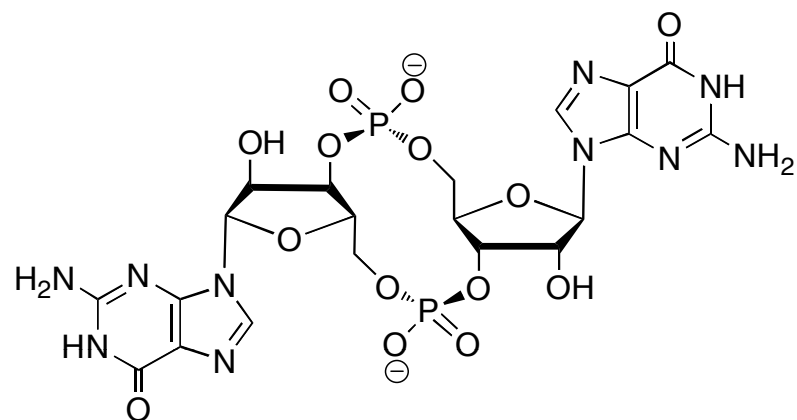
# The Limitations of Supercritical Fluids



# Large Polar Ionic Molecules

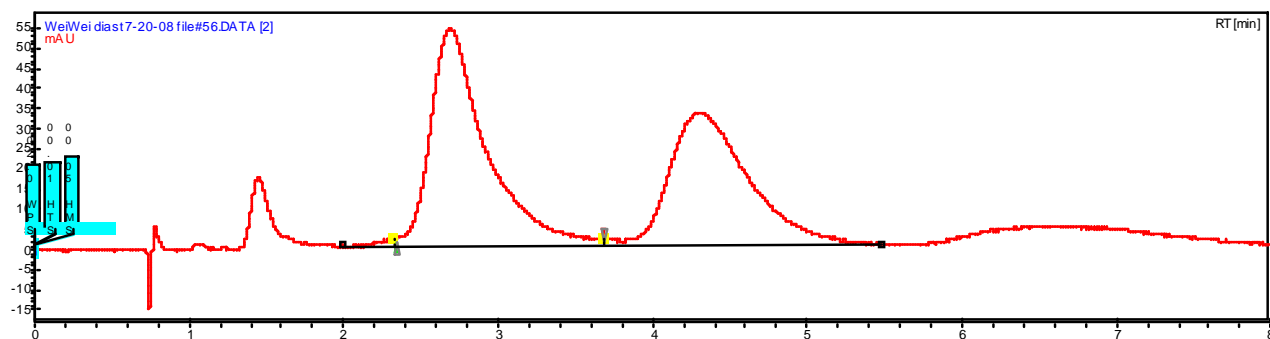


1 (one diastereoisomer)



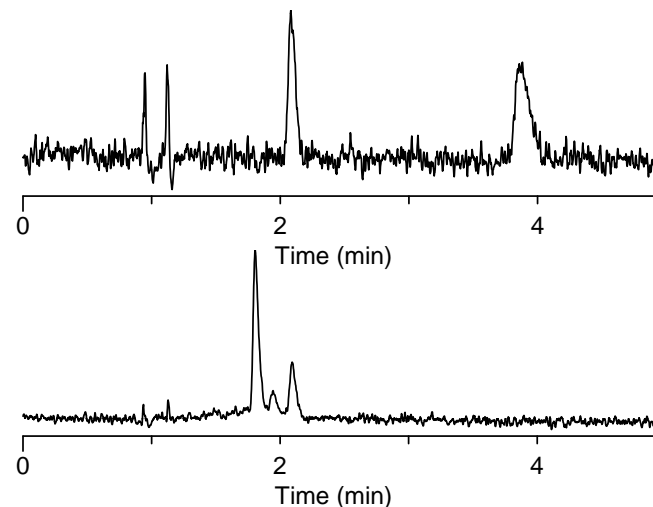
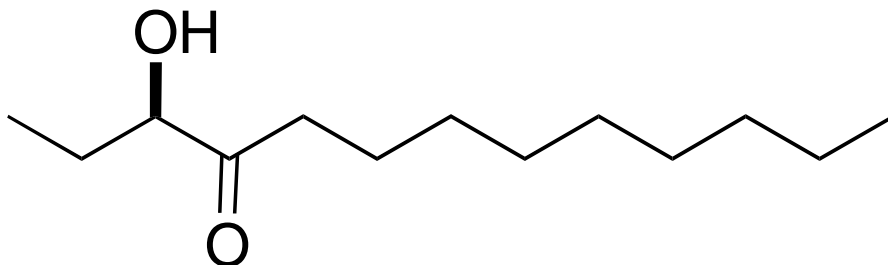
2 (cyclic di-(guanylate monophosphate))

## Cyclic di-GMP and analogs chiral at phosphorous



IC, 40% methanol(0.2% TEA)/CO<sub>2</sub>, 100 bar, 3 m/min.

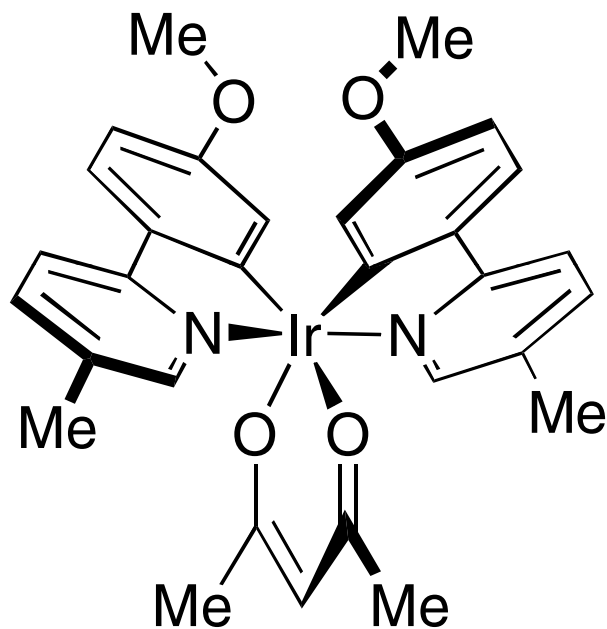
# Low Visibility Molecules



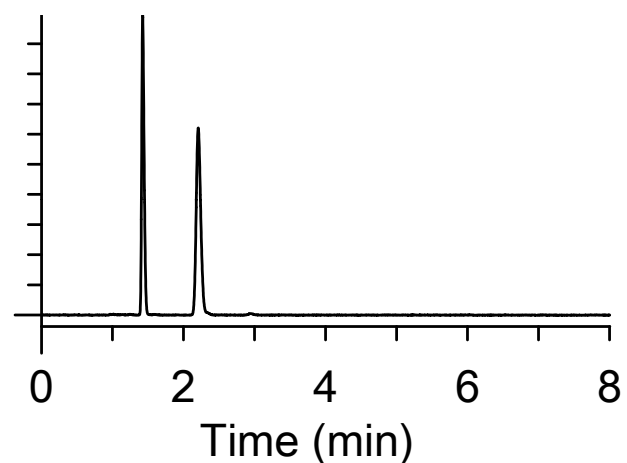
Small molecule which can activate virulence in a bacterial colony

The (S) enantiomer of CAI-1 is the 'quorum sensing' signal isolated from the bacteria *Vibrio cholerae*

# Low Solubility Molecules



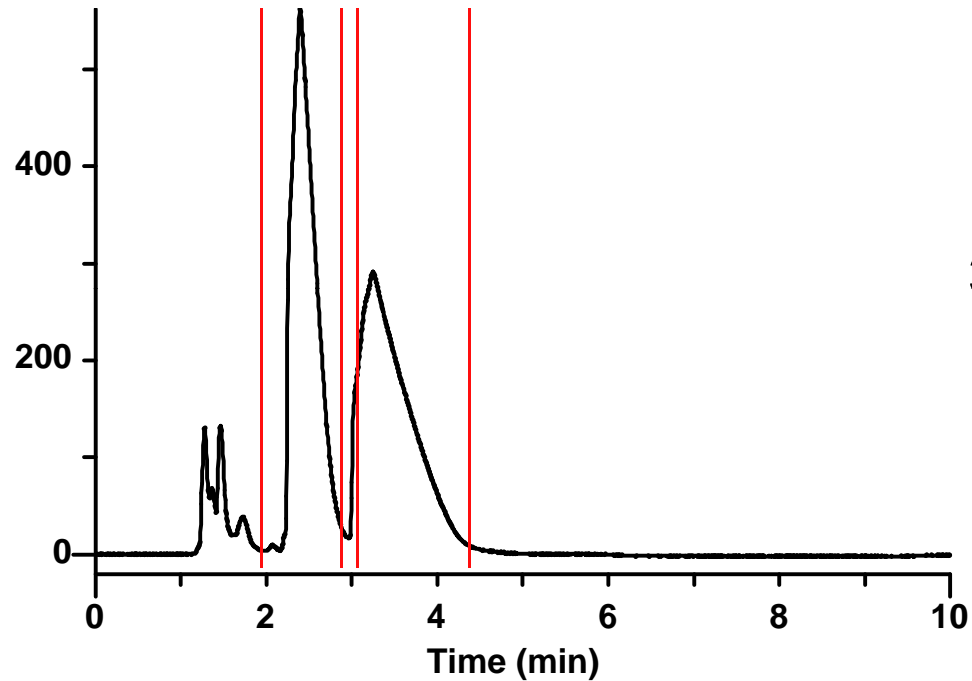
<0.5 mg /4 mL/injection



AD-H, 40% ethanol/CO<sub>2</sub>, 100 bar, 3 m/min.

Iridium(III) luminophores

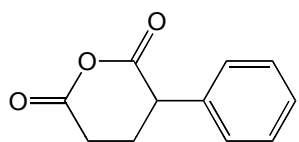
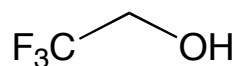
# High Solubility Molecules



3 g/4.5 mL/injection

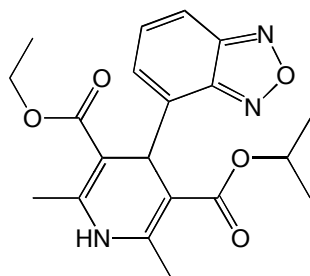
Whelk-01 (R,R) (5 x 25-cm), 43% 2-propanol/CO<sub>2</sub>,  
100 bar, 350 mL/min.

# Alcohol-sensitive Molecules



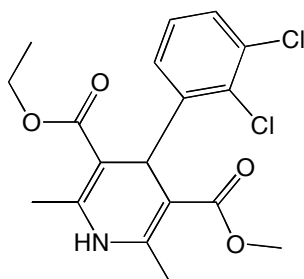
1

2-phenylglutaric anhydride



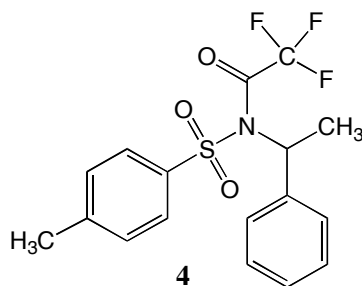
2

isradipine



3

felodipine



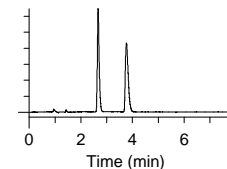
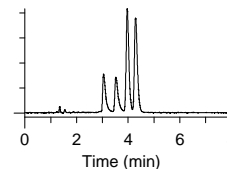
4

2,2,2-trifluoro-*N*-(1-phenylethyl)-*N*-tosylacetamide

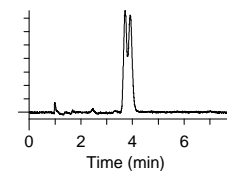
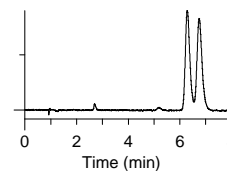
using alcohol modifiers

using TFE

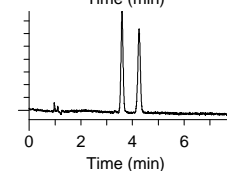
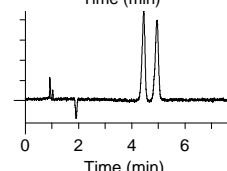
A) 2-phenylglutaric anhydride



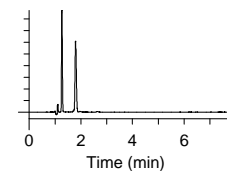
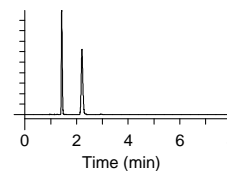
B) Isradipine



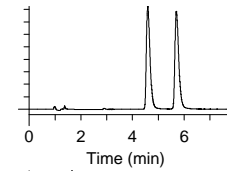
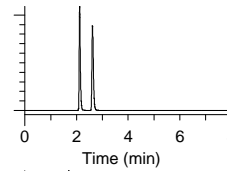
C) Felodipine



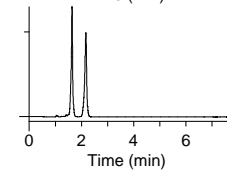
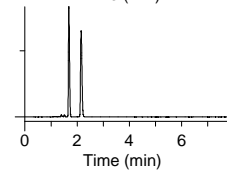
D) 2,2,2-trifluoro-*N*-(1-phenylethyl)-*N*-tosylacetamide



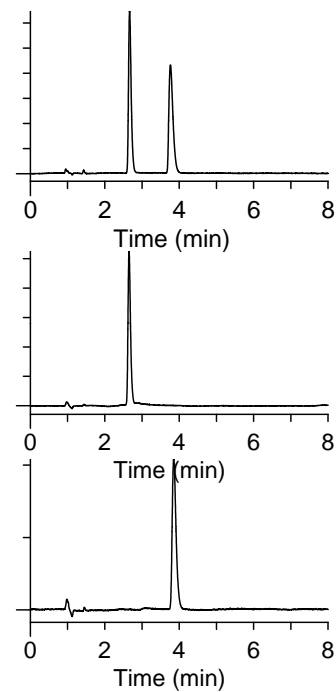
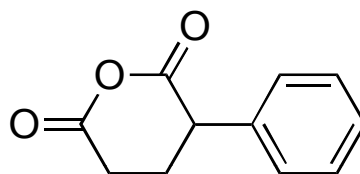
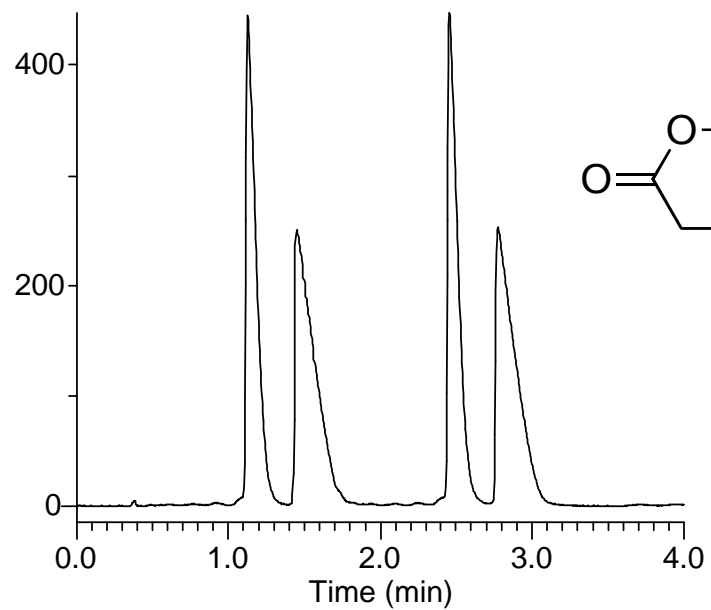
E) Benzoin



F) 1,2,3,4-tetrahydro-2,2,4,7-tetramethylquinoline

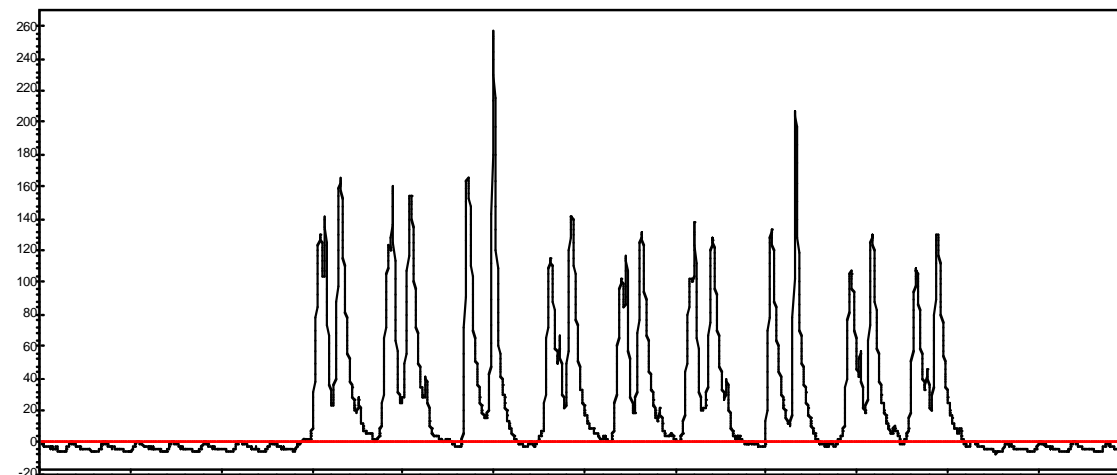


# Alcohol-sensitive Molecules

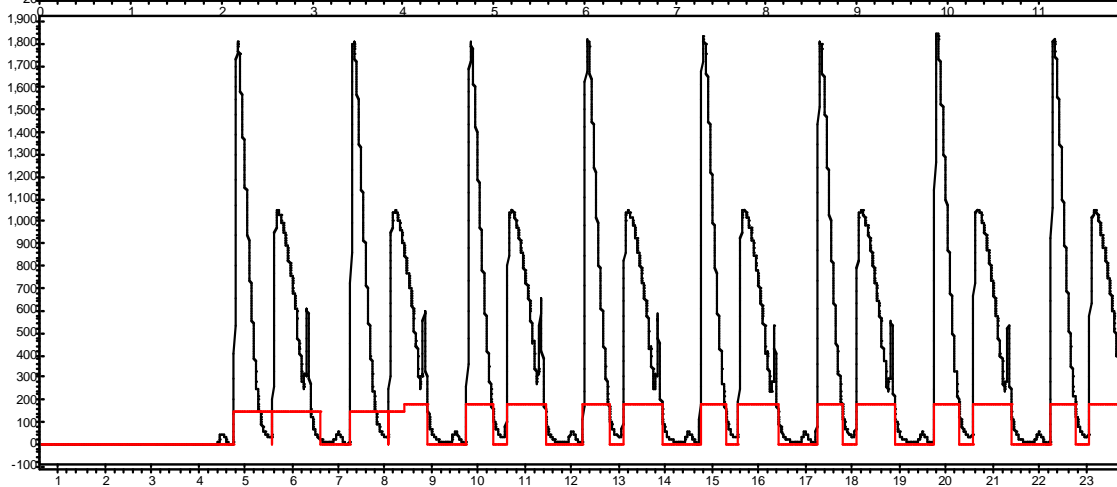


AD-H, 15% TFE/CO<sub>2</sub>, 100 bar, 3 mL/min.

# Non-Retentive Lipophylic Molecules

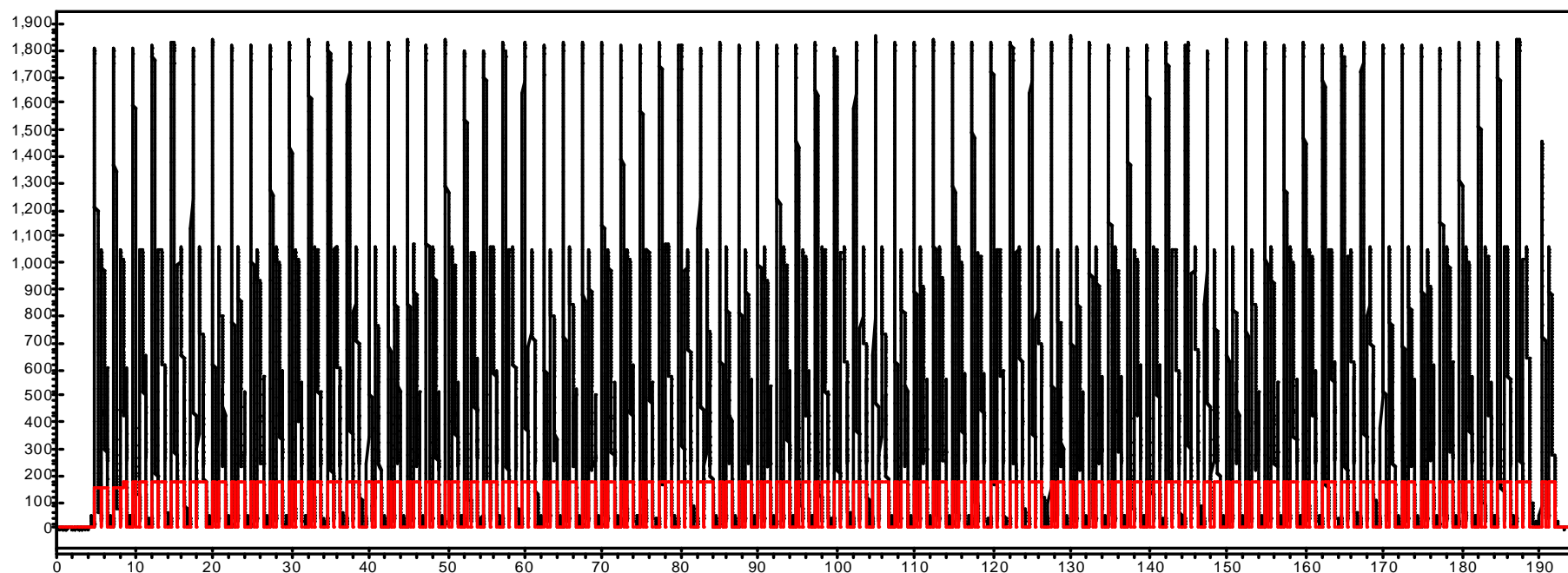


AS-H  
10% isobutanol/ $\text{CO}_2$   
100 bar, 3 m/min.



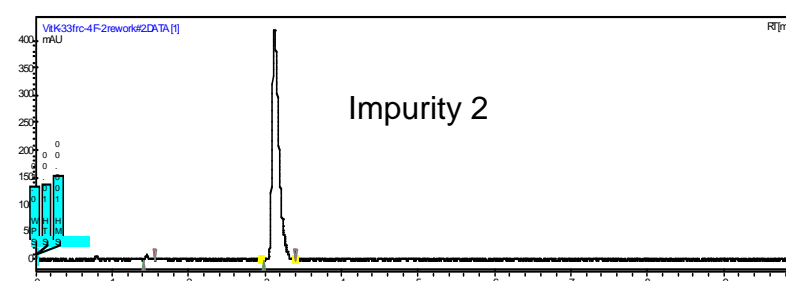
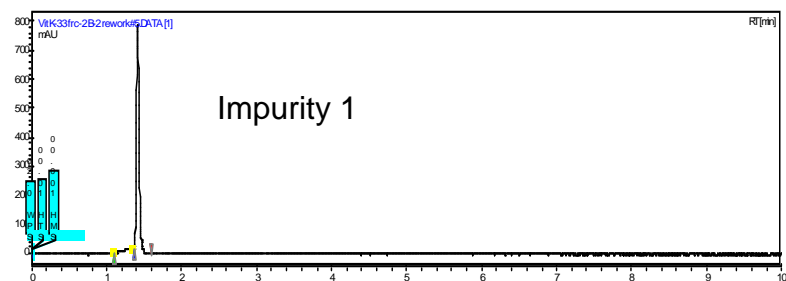
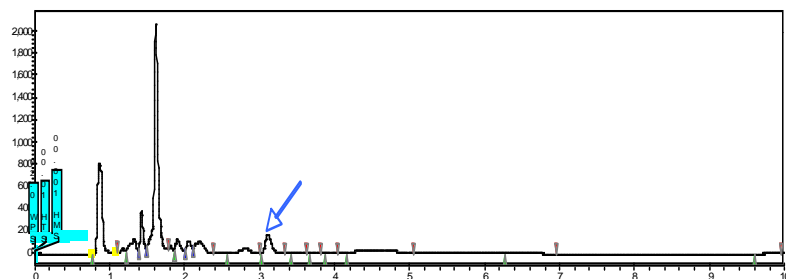
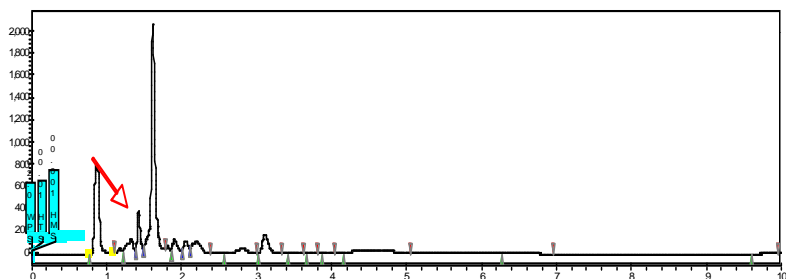
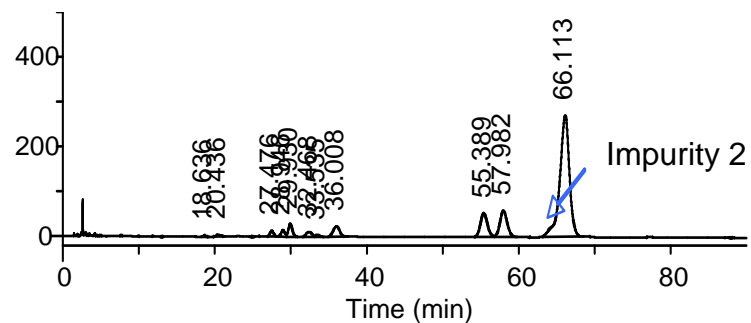
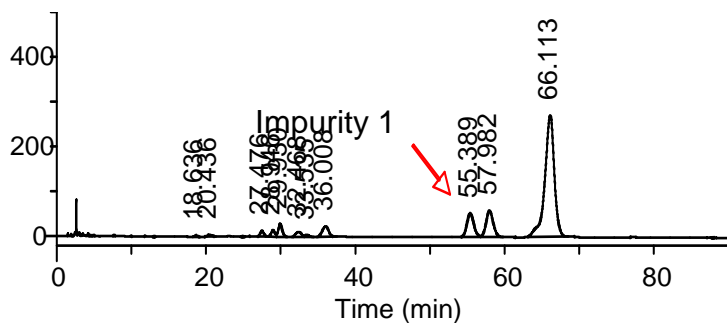
OD-H  
20% 9:1 heptane:isopropanol/ $\text{CO}_2$   
100 bar, 3 m/min.

# Non-Retentive Lipophylic Molecules



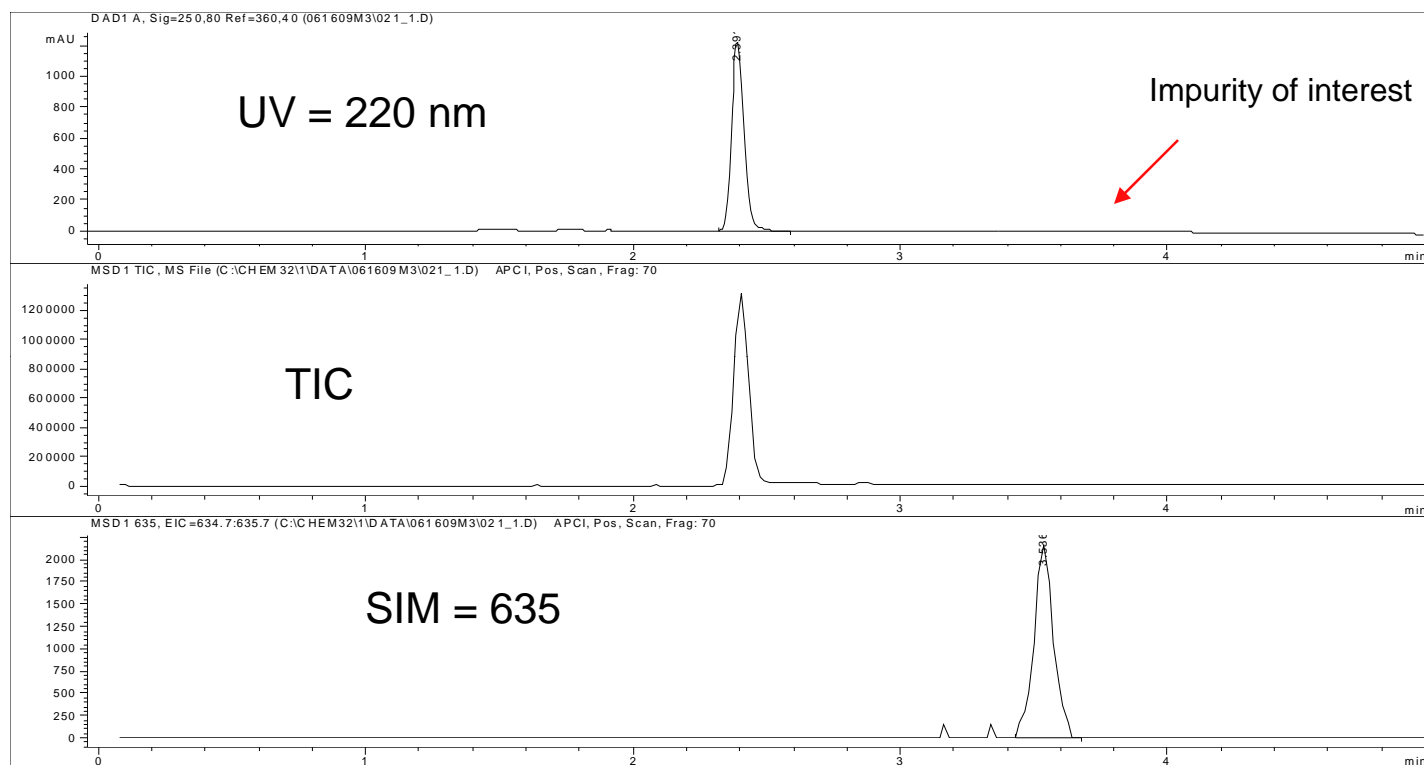
OD-H, 20% 9:1 heptane:isopropanol/CO<sub>2</sub>, 100 bar, 60 mL/min.

# Isolation of minor impurities



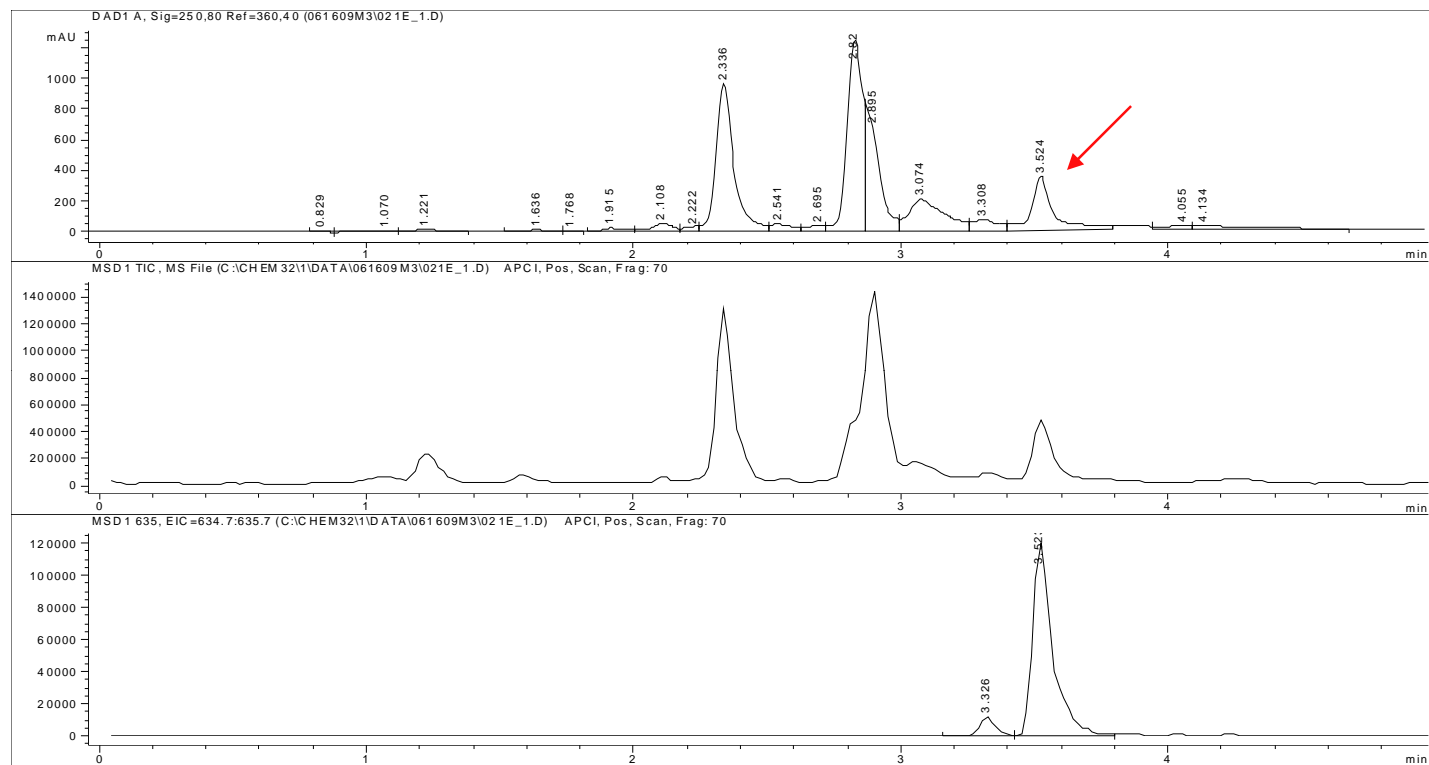
$^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and 2D NMR experiments revealed the structure of E/Z isomers

# Isolation of minor impurities using SFC-MS



Pyridine (25 x 0.46 cm), 10-50% methanol(DEA)/CO<sub>2</sub>  
100 bar, 3 mL/min.

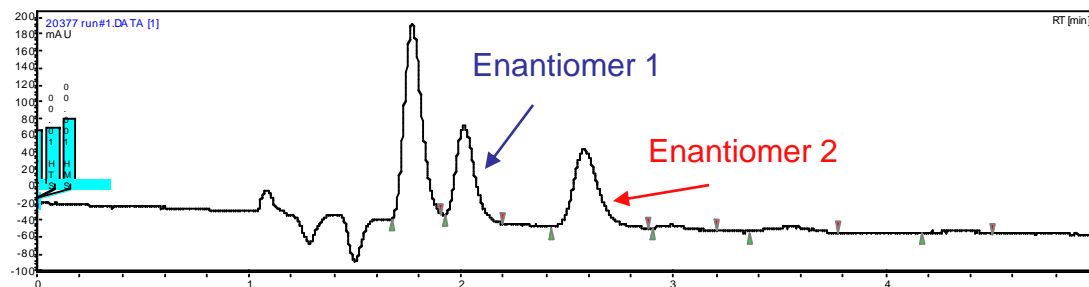
# Isolation of minor impurities using SFC-MS



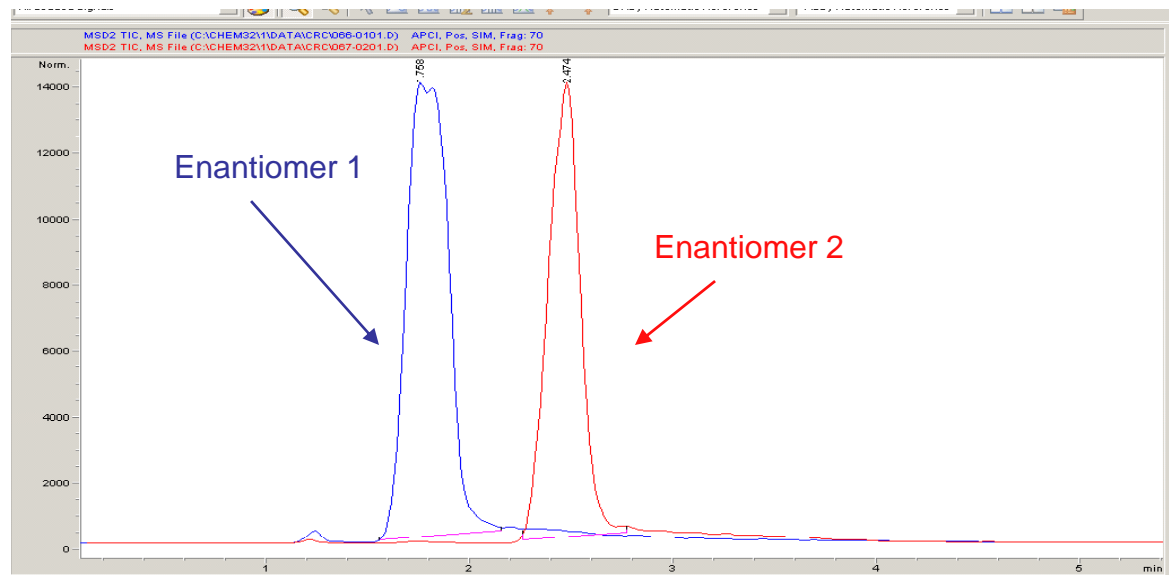
Pyridine (25 x 0.46 cm), 10-50% methanol(DEA)/CO<sub>2</sub>  
100 bar, 3 mL/min.

# SFC-MS analysis of Plasma Extracts

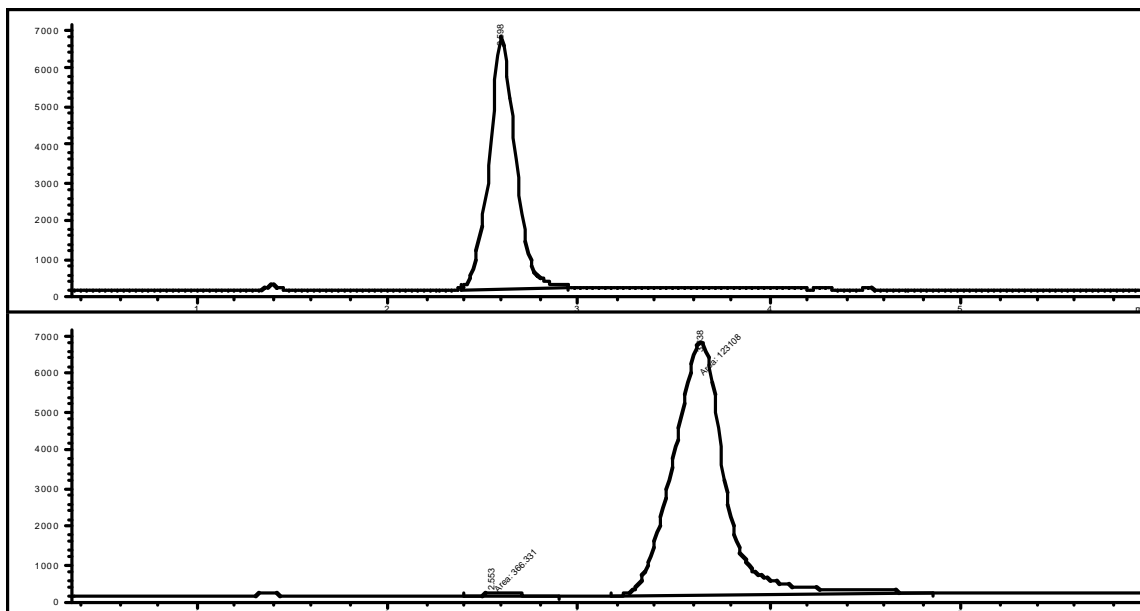
UV = 220 nm



EIC: 500



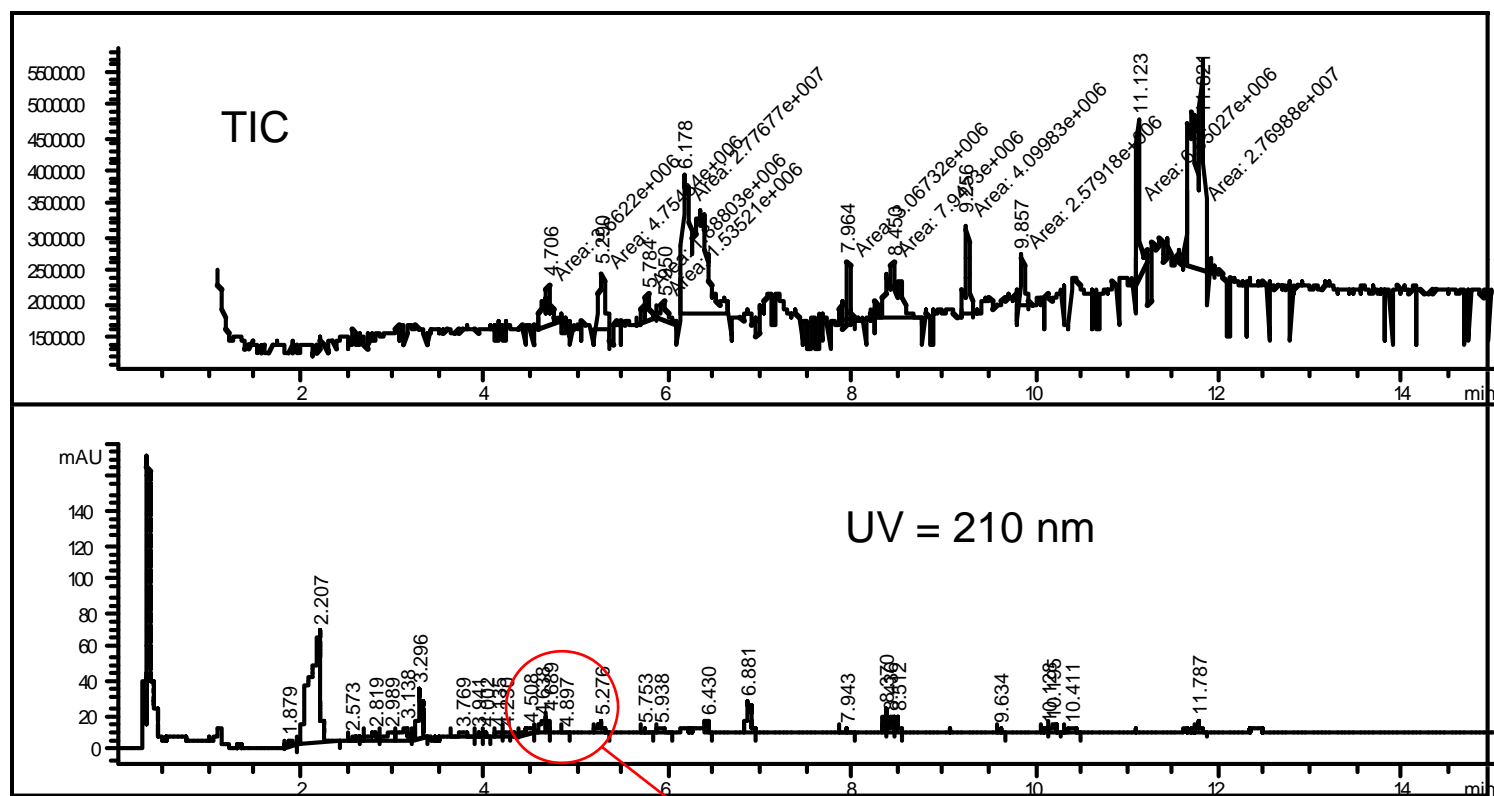
# SFC-MS analysis of Plasma Extracts: ee determination



Chiralpak AD-H(25 x 0.46 cm), 20% isopropanol(DEA)/CO<sub>2</sub>  
100 bar, 3 mL/min, 220 nm.

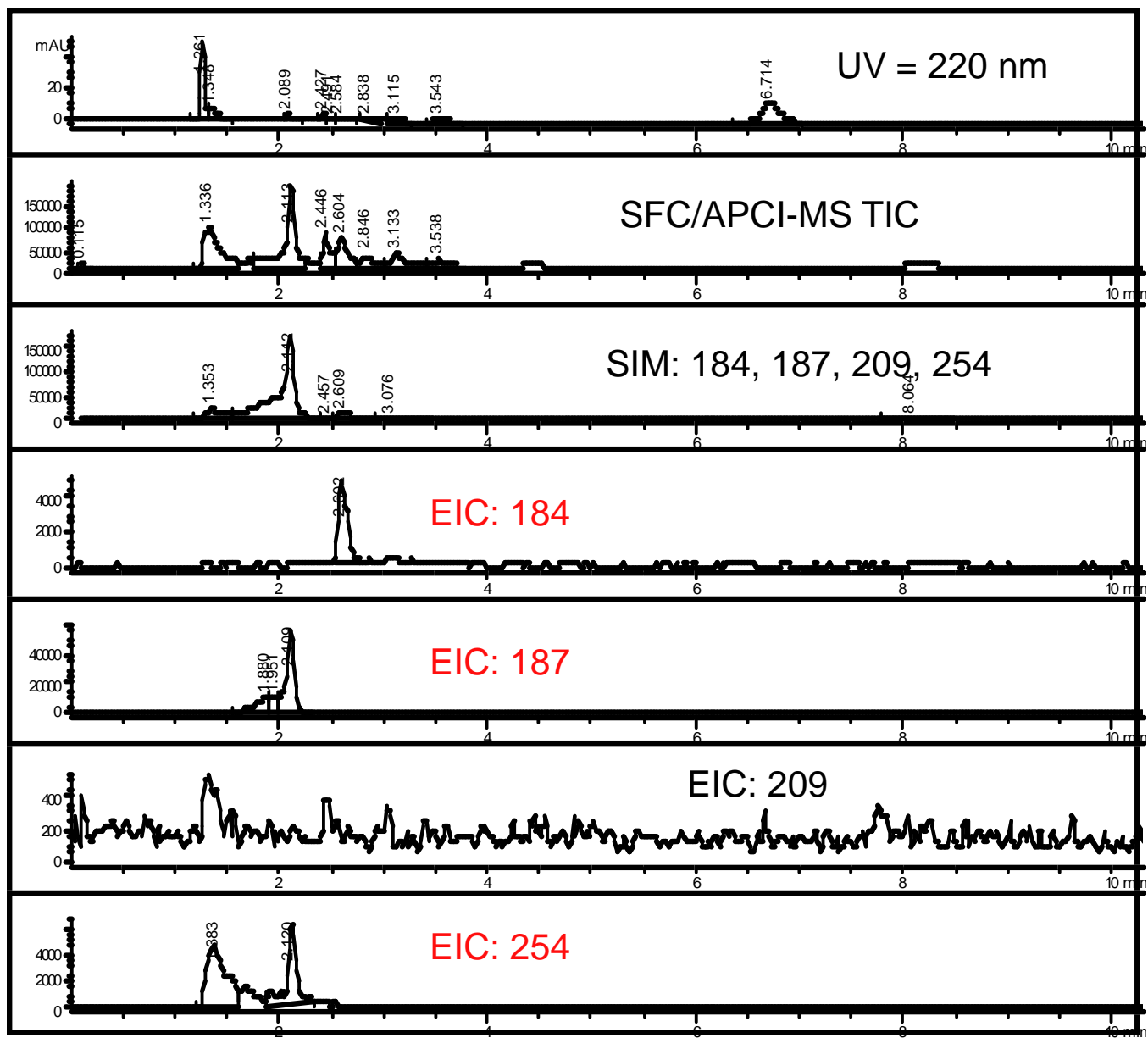
# Translating LC-MS to SFC-MS:

Isolation of a target molecule from *Vibrio harveyi* extract



Active  
Components

# SFC-MS



# Acknowledgements



Daniel Sanner



Neal Byrne

Karin Henigan

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Dr.Yingru Zhang (BMS)

Dr.Paul Reider

Dr.WeiWei Liao

Prof. Bassler

Prof. Sorensen

Prof. Semmelhack

Prof. Groves

Prof. MacMillan

Prof. Doyle

Prof. Bernhard

# We're moving....

QuickTime™ and a  
TIFF (Uncompressed) decompressor  
are needed to see this picture.

