Improving Productivity in Prep SFC Separations

Jeffrey Kiplinger and Paul Lefebvre
Averica Discovery Services, Worcester MA

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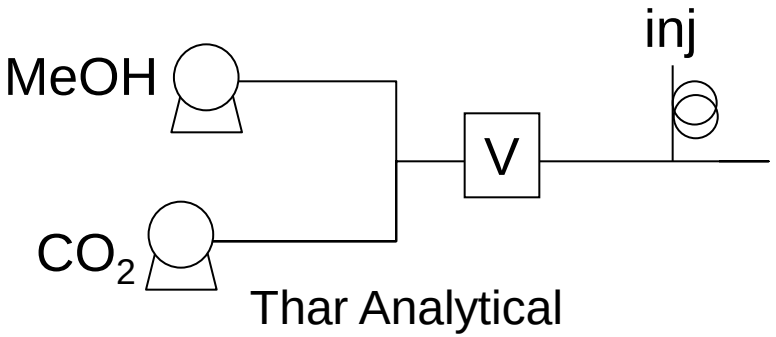
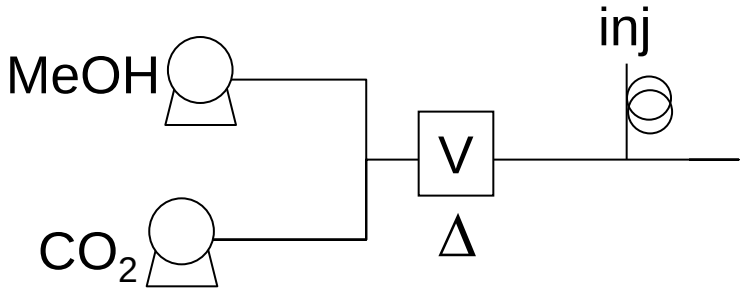
Introduction

- Going faster in isocratic batch separations
  - Increase loading
  - Improve bandshape
  - Enhance resolution
  - Decrease retention
- These are controlled by the balance between the MP density (solubility) and the solute diffusivity in the stagnant solvent layer on the SP
- What characteristics of the SFC system influence efficiency?

Injection Strategies

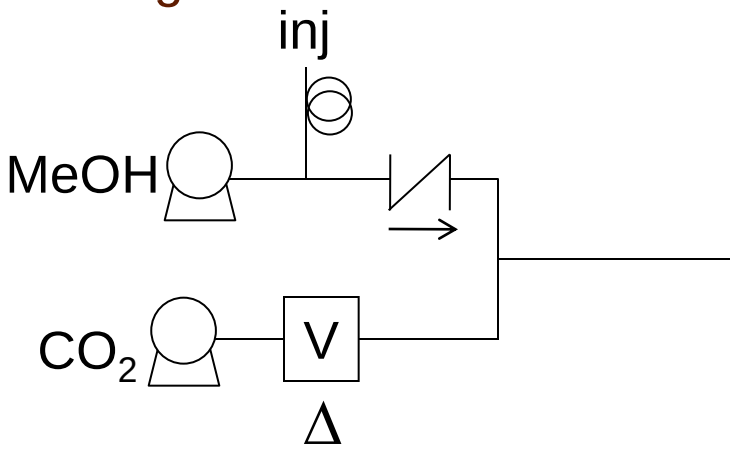


Thar Analytical



Original Thar Prep

- Mixed stream (both left) and Modifier stream configurations
- V = volume, Δ = heated
- volume = empty column or tubing



Modified Thar Prep

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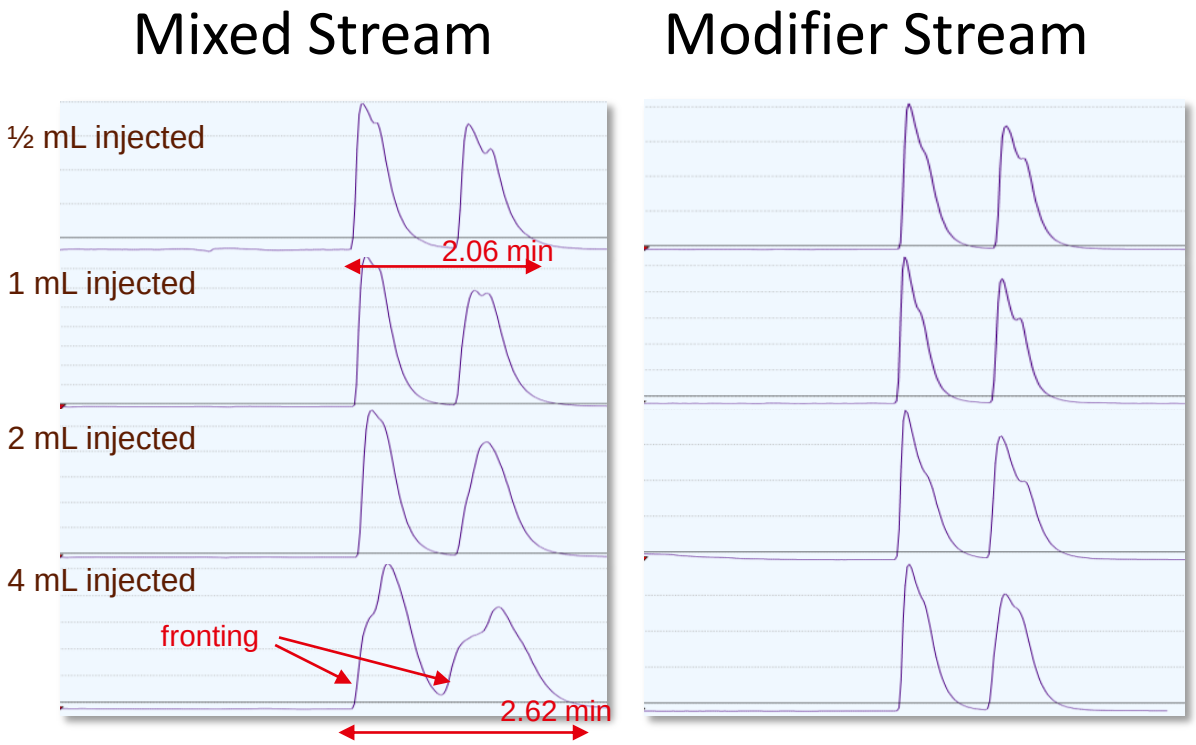
Effects of Injection Type



- Const. mass injections of Tetramisole
- 50% MeOH isocratic
- Mixed stream mode distorted by large ‘plug’ of methanol moving through column

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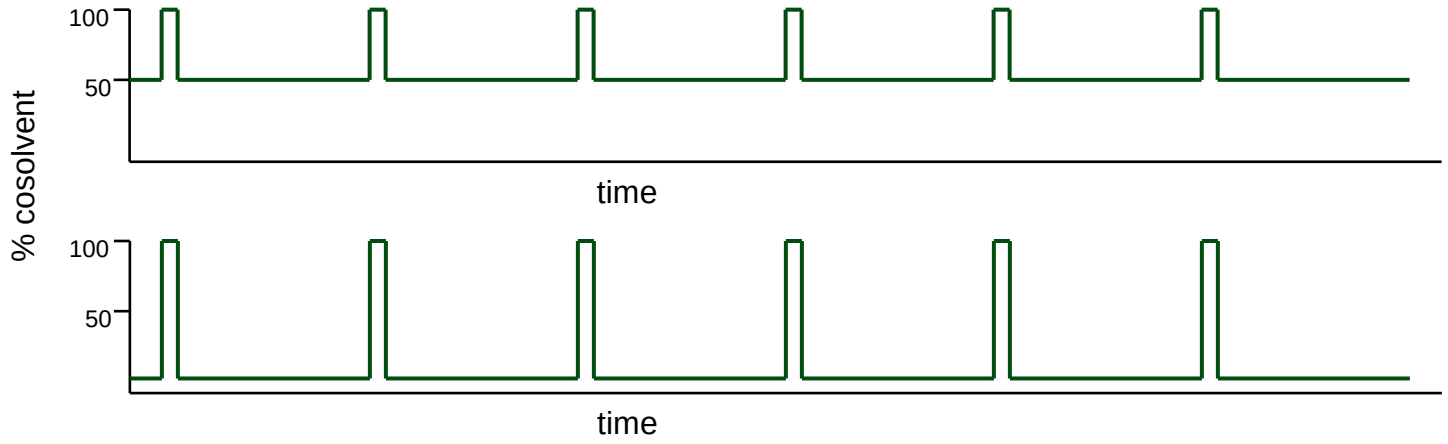
Injection Stacking

- Mixed stream mode sets up a pulsed solvent density gradient, changing analyte solubility (retention)



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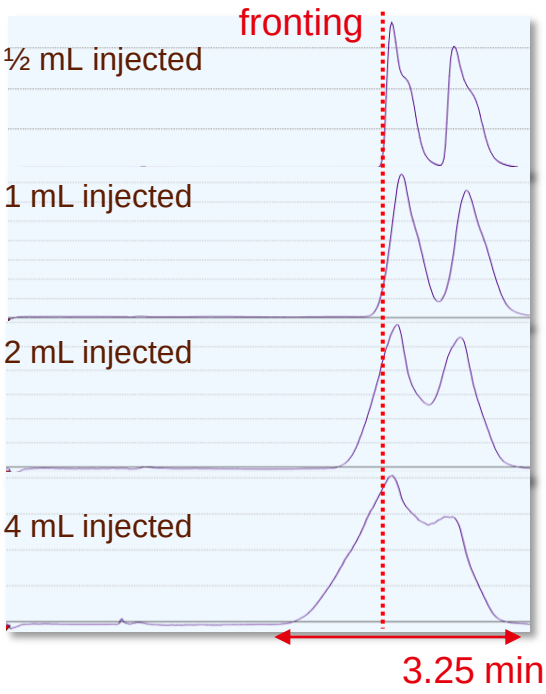
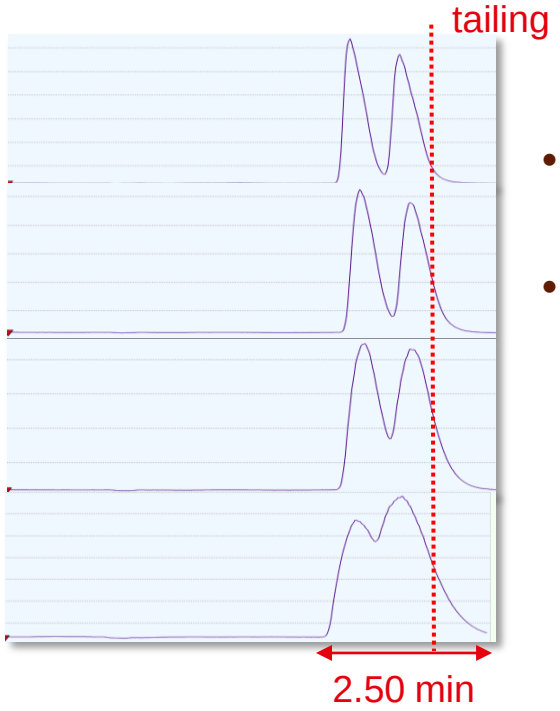
Effects of Injection Type

Mixed Stream



Modifier Stream



- Const. mass injections of Ibuprofen
- 5% MeOH isocratic
- Modifer Stream at low co-solvent clears loop slowly

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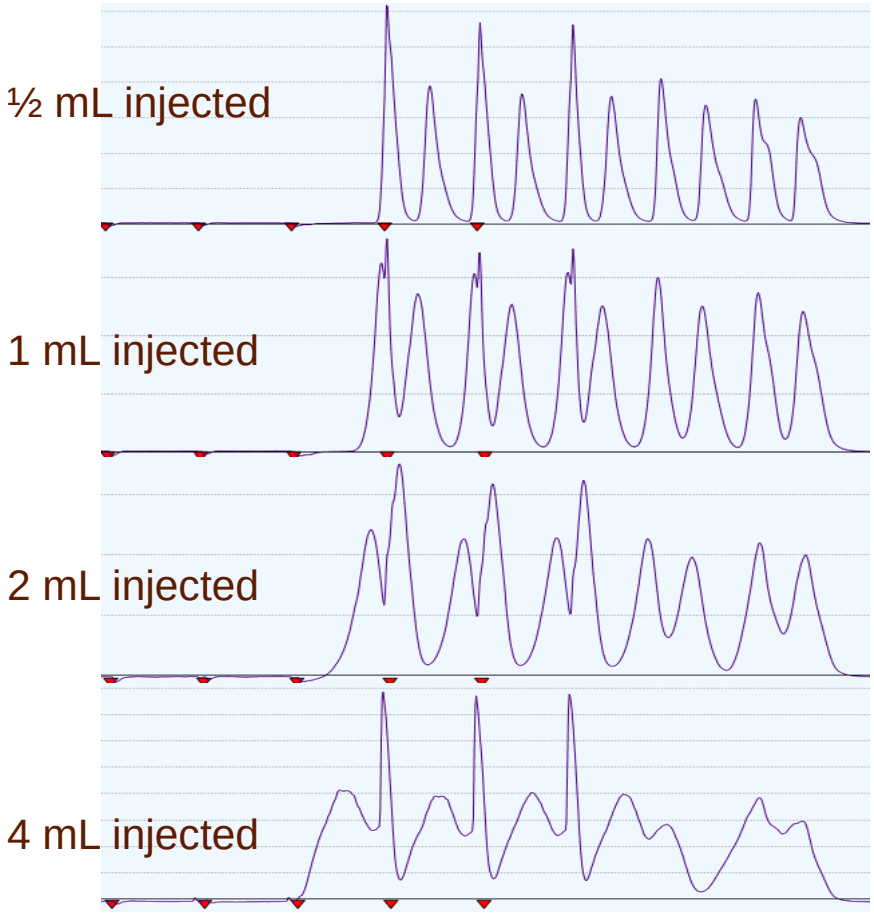
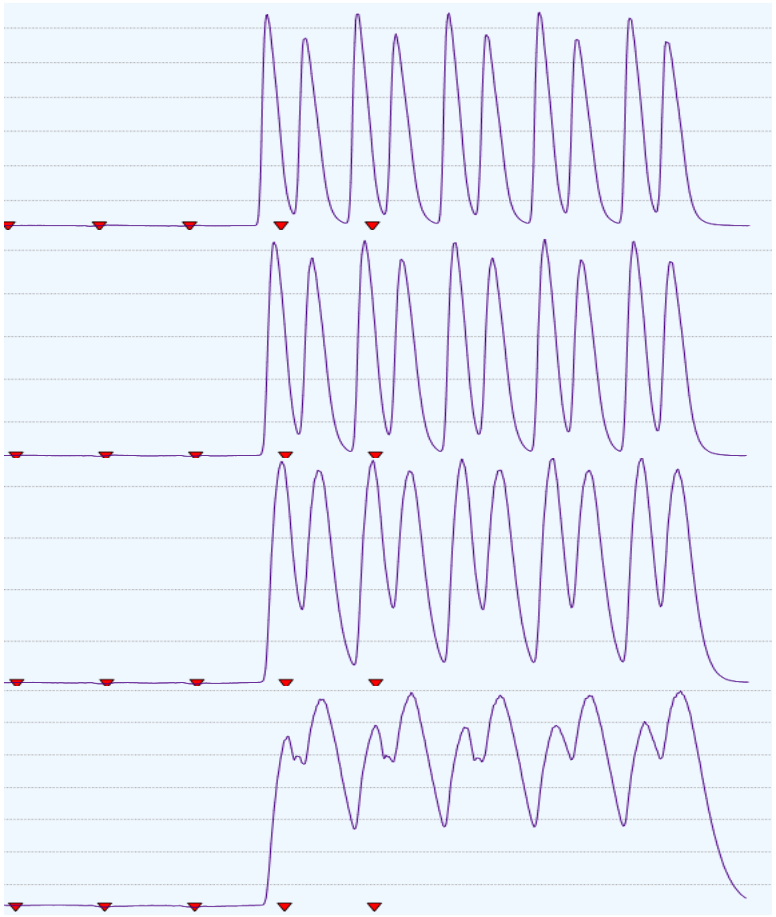
Injection Stacking



Mixed



Modifier

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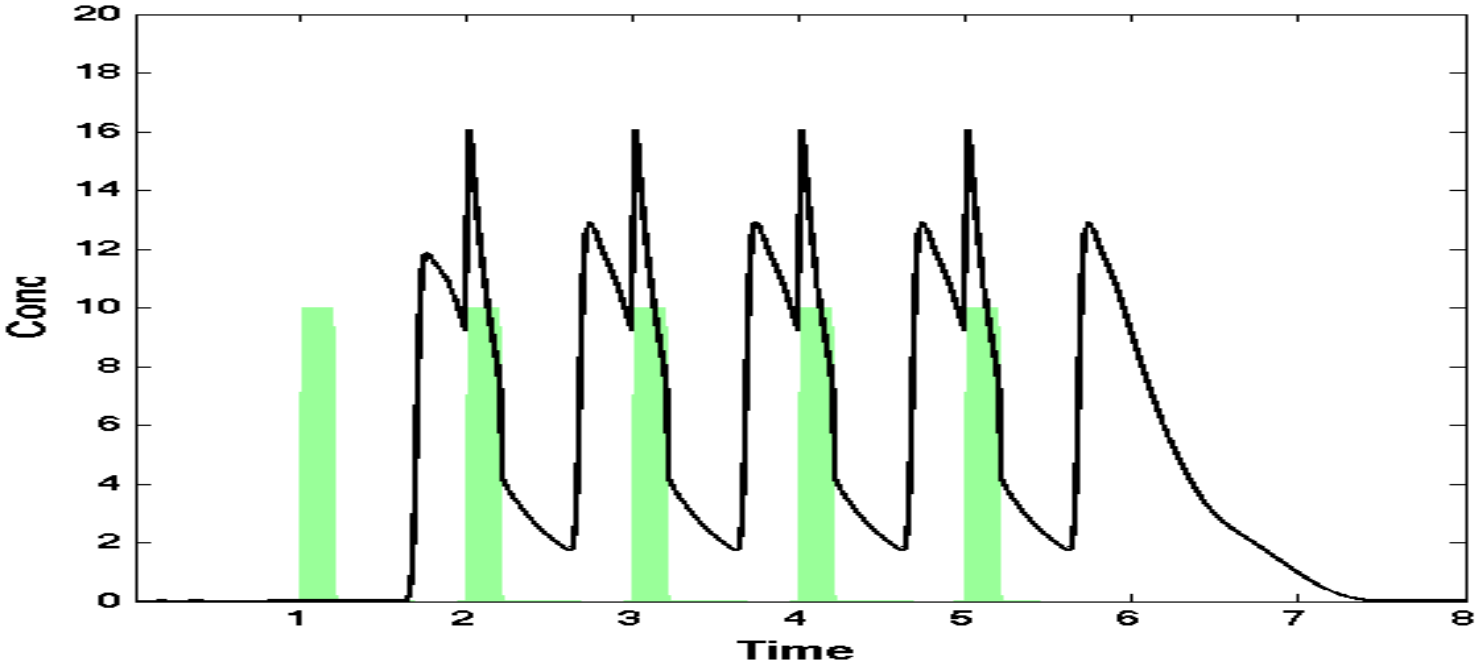
Effect of Solvent Gradient



- *Courtesy Arvind Rajendran, Nanyang Tech. U., Singapore*
- *Wenda et. al., J. Chromatogr. A 1218(2011) 162-70*

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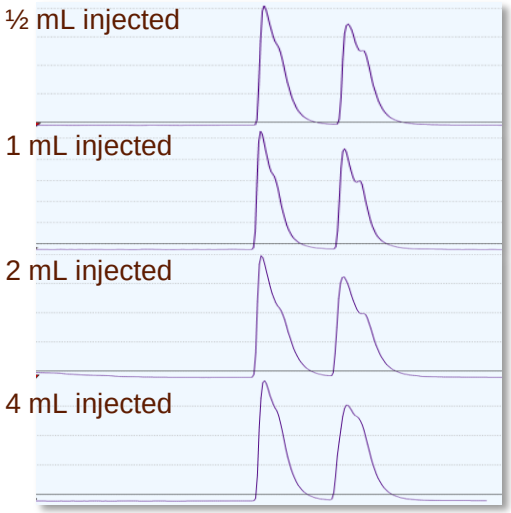
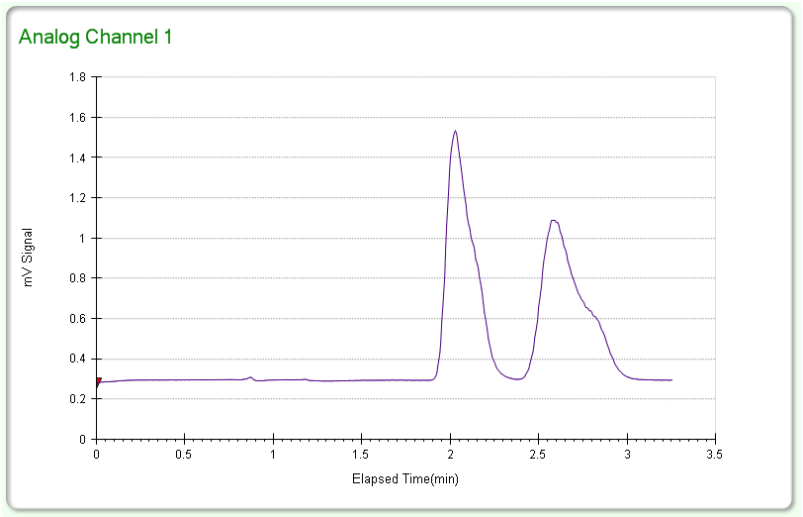
Band Shape Issues



Tetramisole Mod.Str. test,
50% MeOH



TSO, IC, 10% MeOH

- Injection strategy well-characterized, but peaks deformed
- Reproducible on both prep SFC systems
- Eluent is same solvent as diluent
- Same ‘profile’ on both peaks

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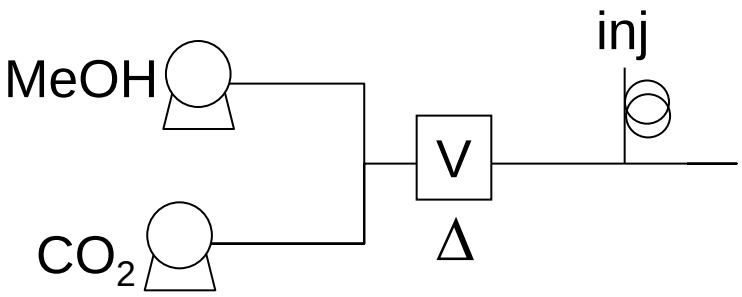
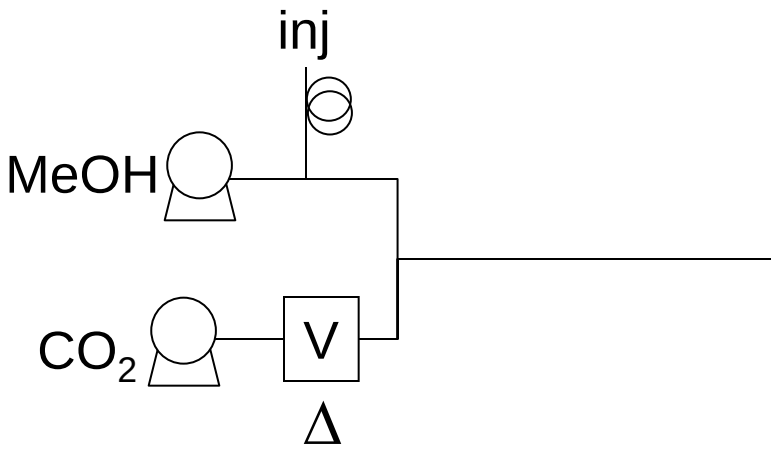
Assisted Mixing

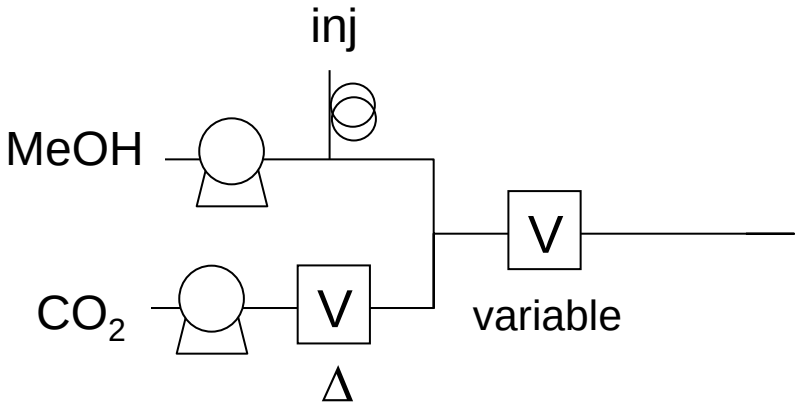


Original Thar Prep



Modified Thar Prep

- Original Thar configuration included a 25 mL heated tubing section post-tee
- If CO_2/MeOH mixing is incomplete it could cause uneven solute deposition



With added mixing volume

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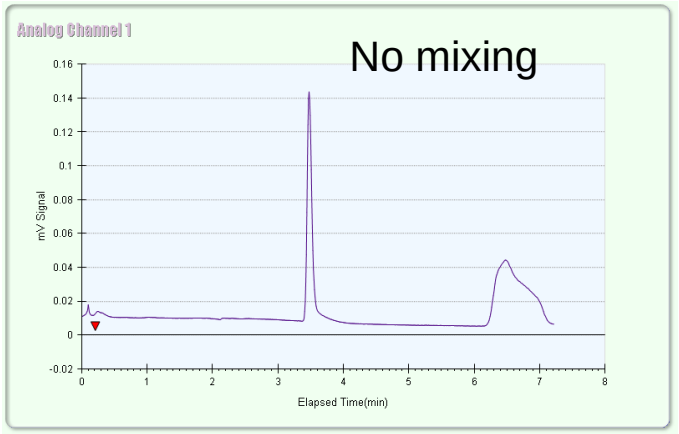
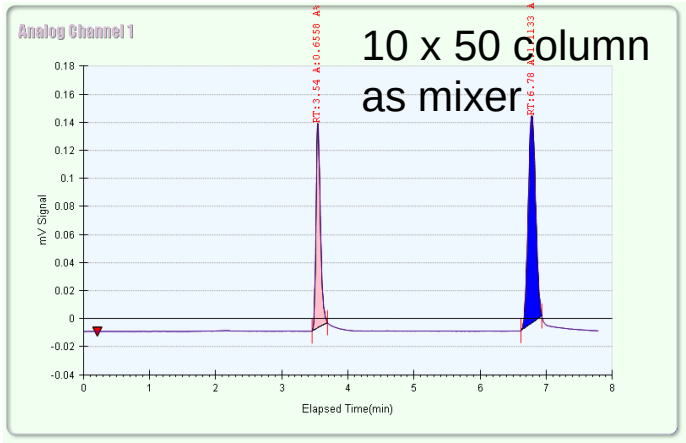
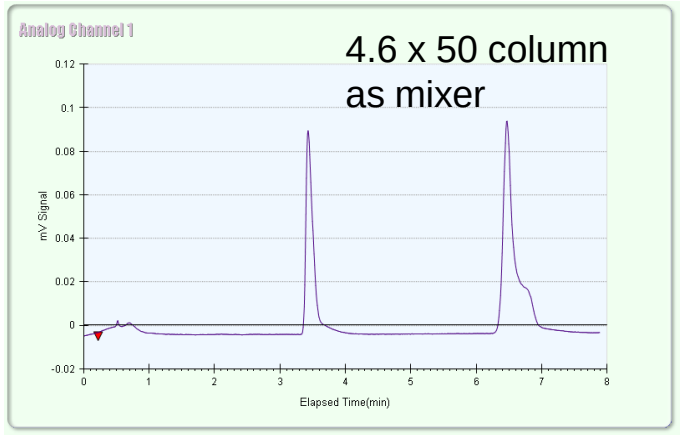
Test sample on 2-EP





N = 20,000



- Test mix of DNB & N-benzylbenzamide
- Reproduces HPLC plate count with 4 mL added extracolumn volume

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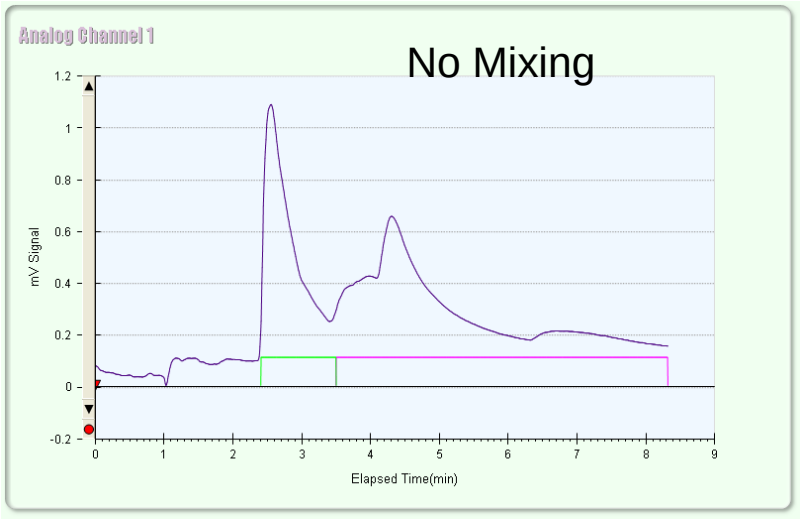
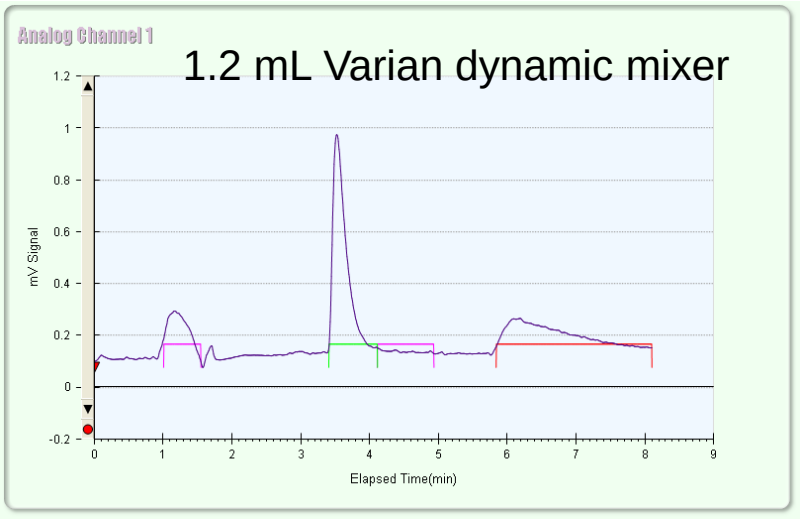
Mixing = Good





- AV010033, RegisCell 21 x 250, 30% Ethanol w/ 1% IPAmine

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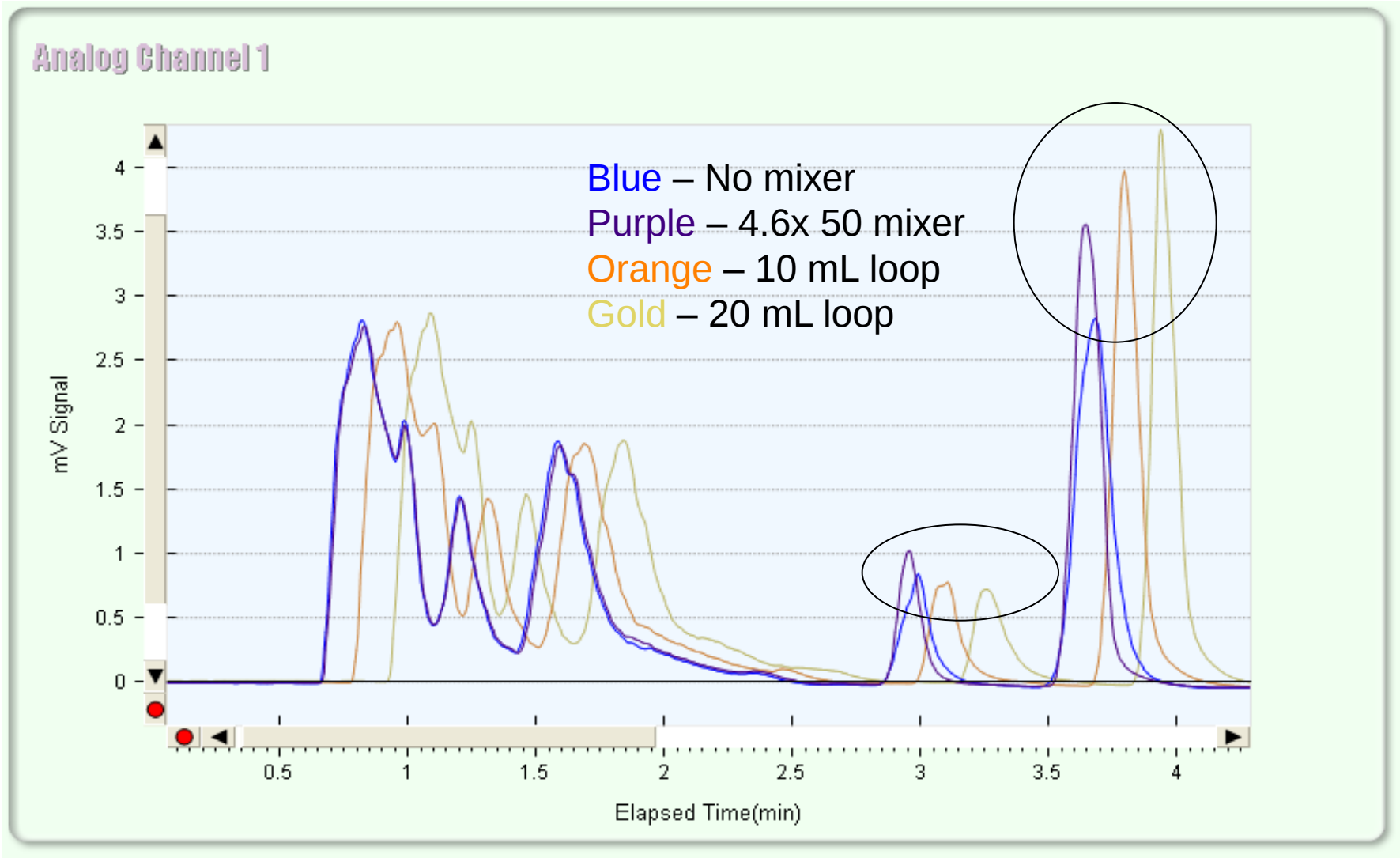

- Test mix of 5 compounds
- Increasing mixing volume has variable effect

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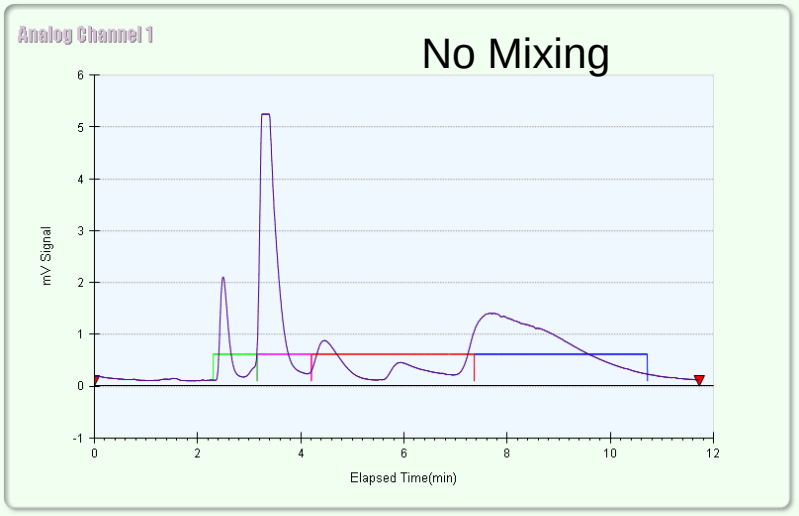
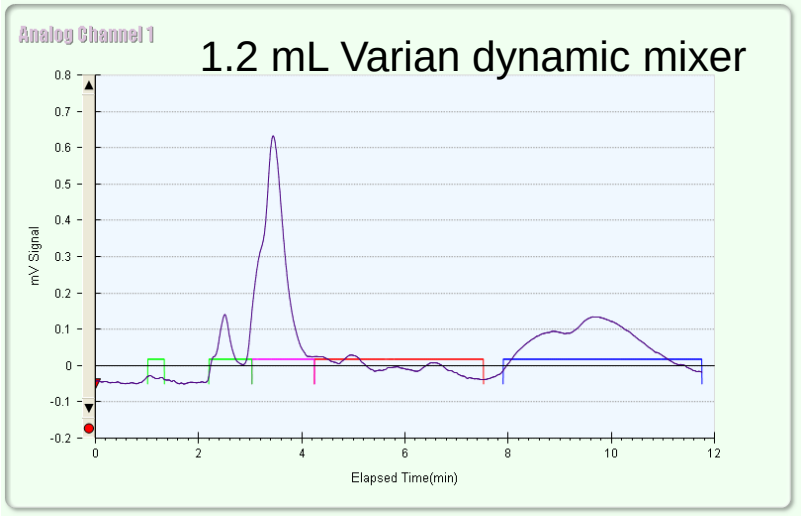
Mixing = Bad





- AV011082, AD-H 30 x 250, 55% (1:1) MeOH:IPA w/ 1% IPAmine

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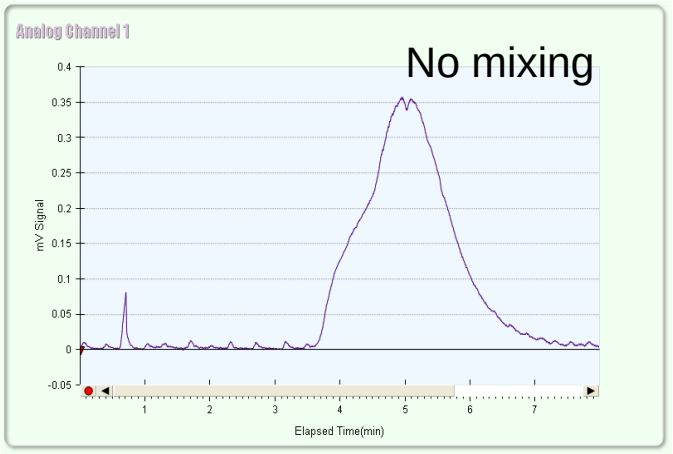
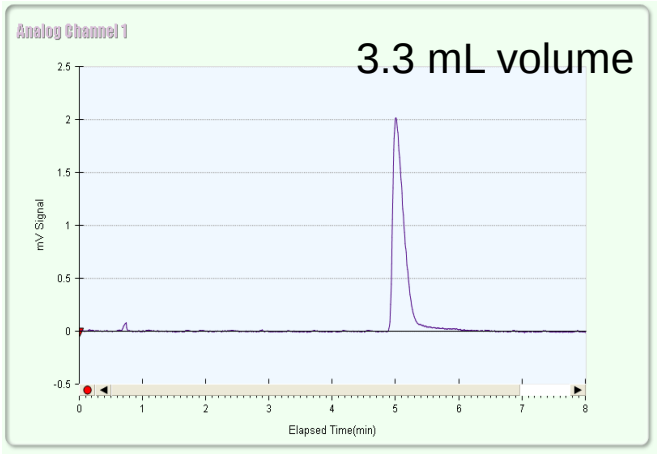
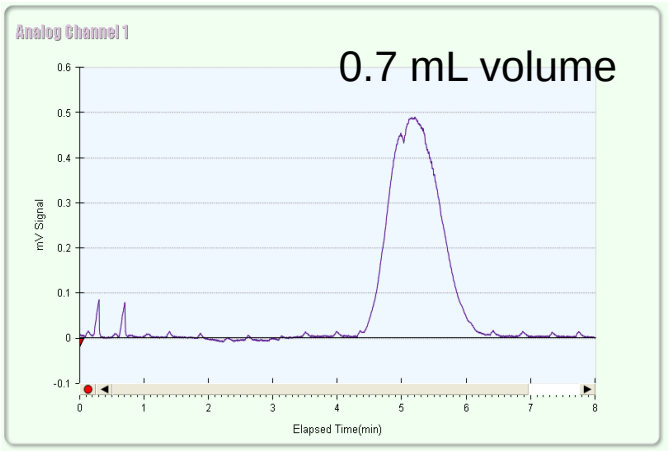
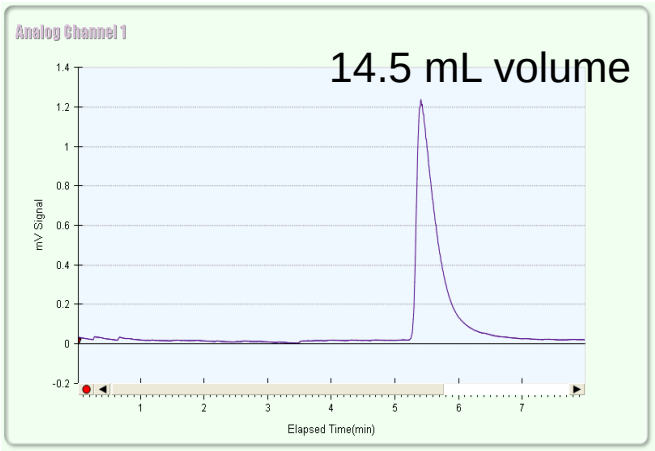
N-benzylbenzamide, LUX Cellulose-2









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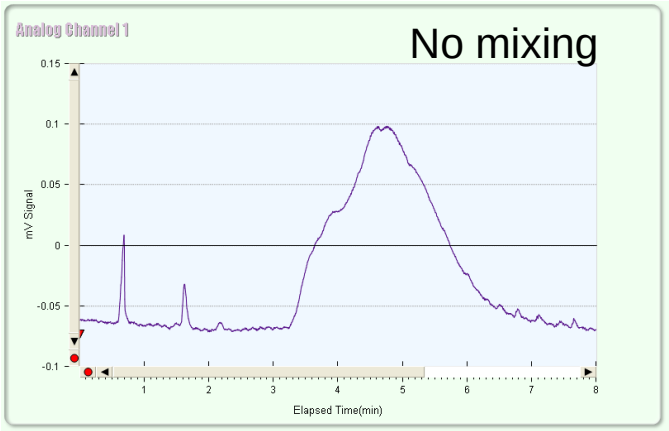
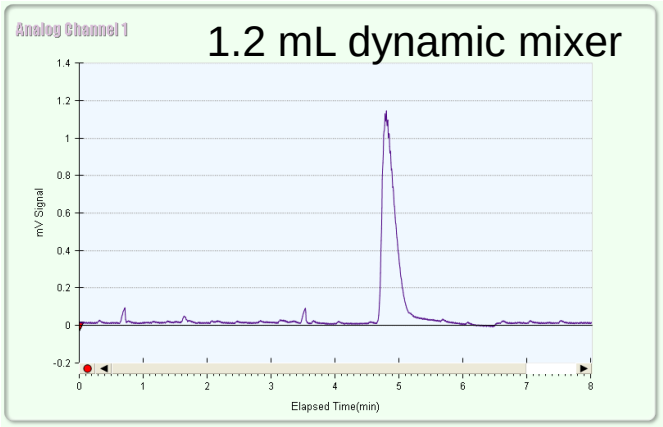
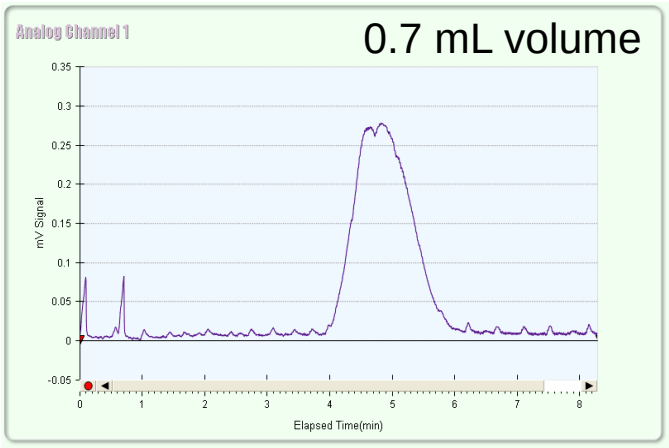
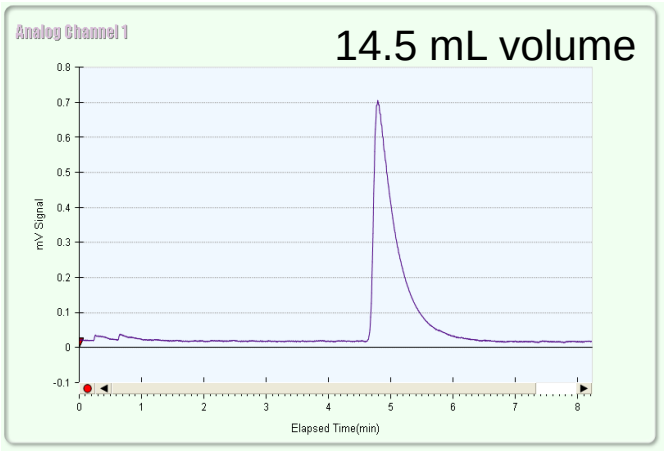
Ketoprofen, PVA-Sil 21 x 250









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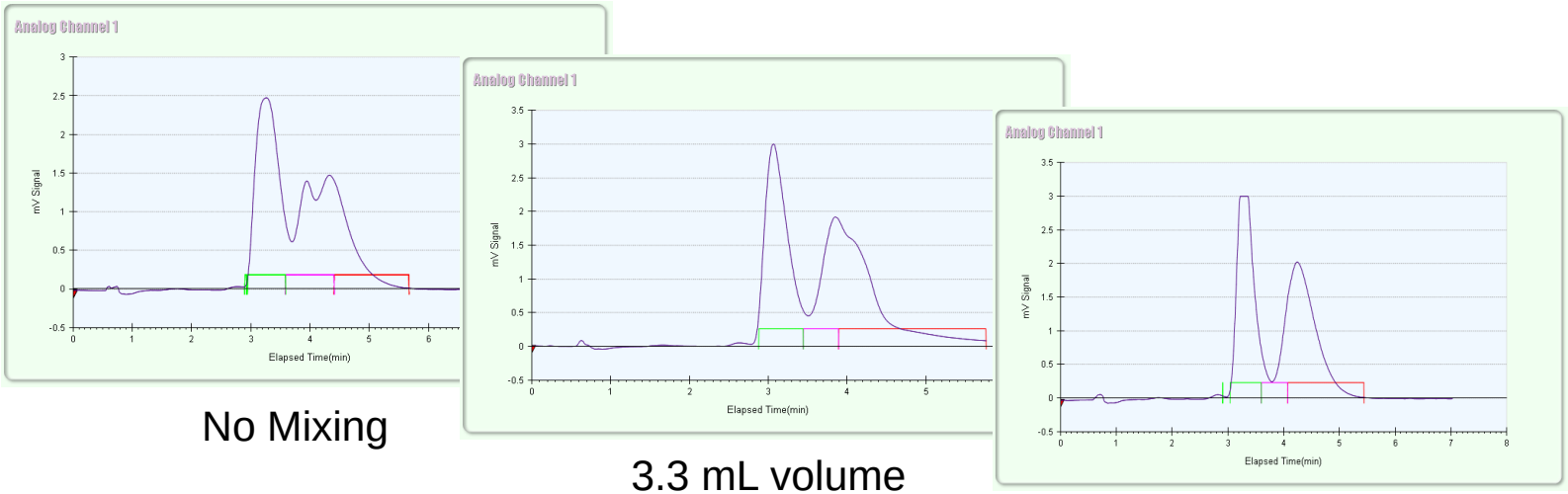
Mixing = Just Right



No Mixing

3.3 mL volume

0.7 mL volume

- AV011021, RegisPack 30 x 250, 25% EtOH

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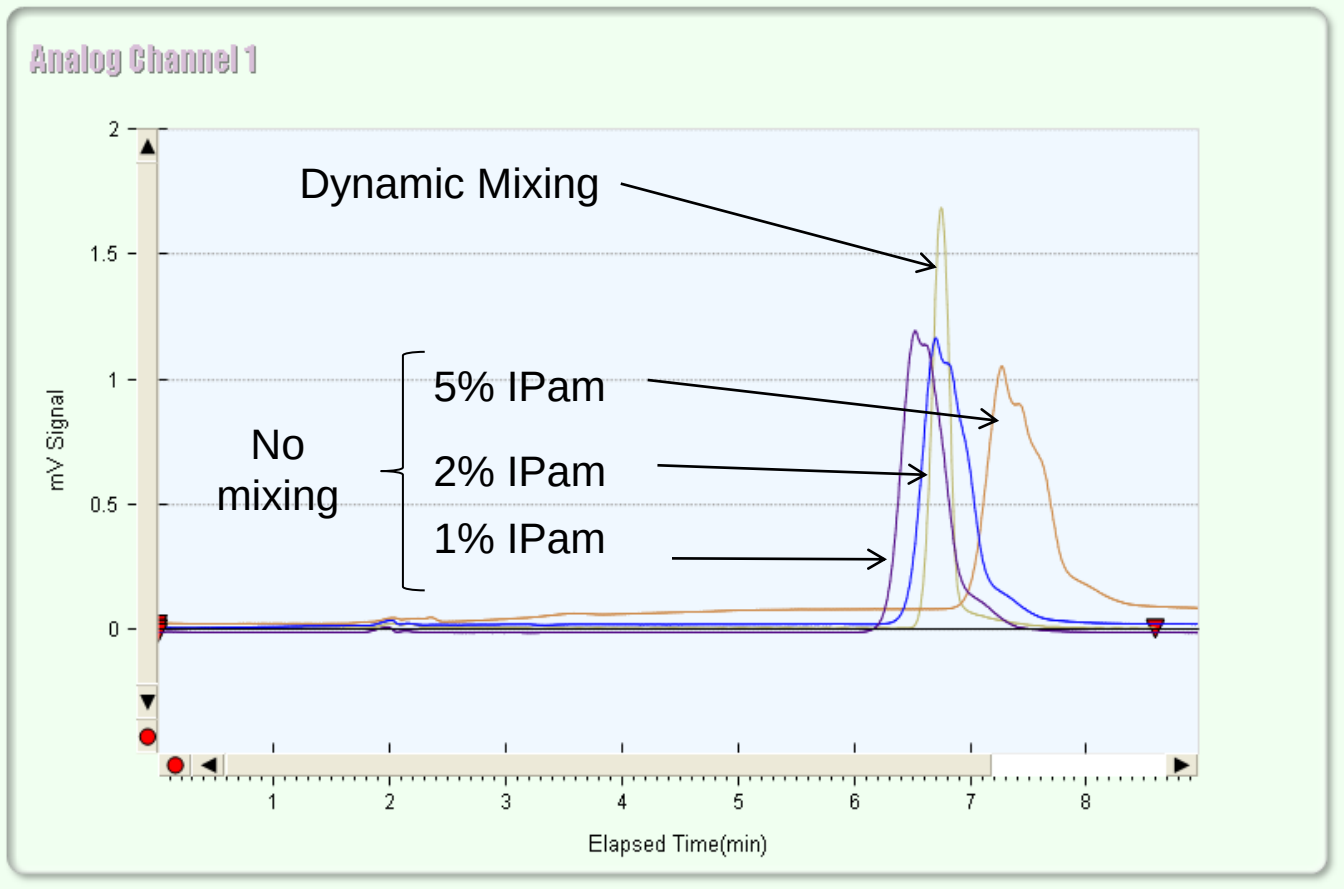
Buffer Effect



N-benzylbenzamide, 2-EP, 10% MeOH w/IPam

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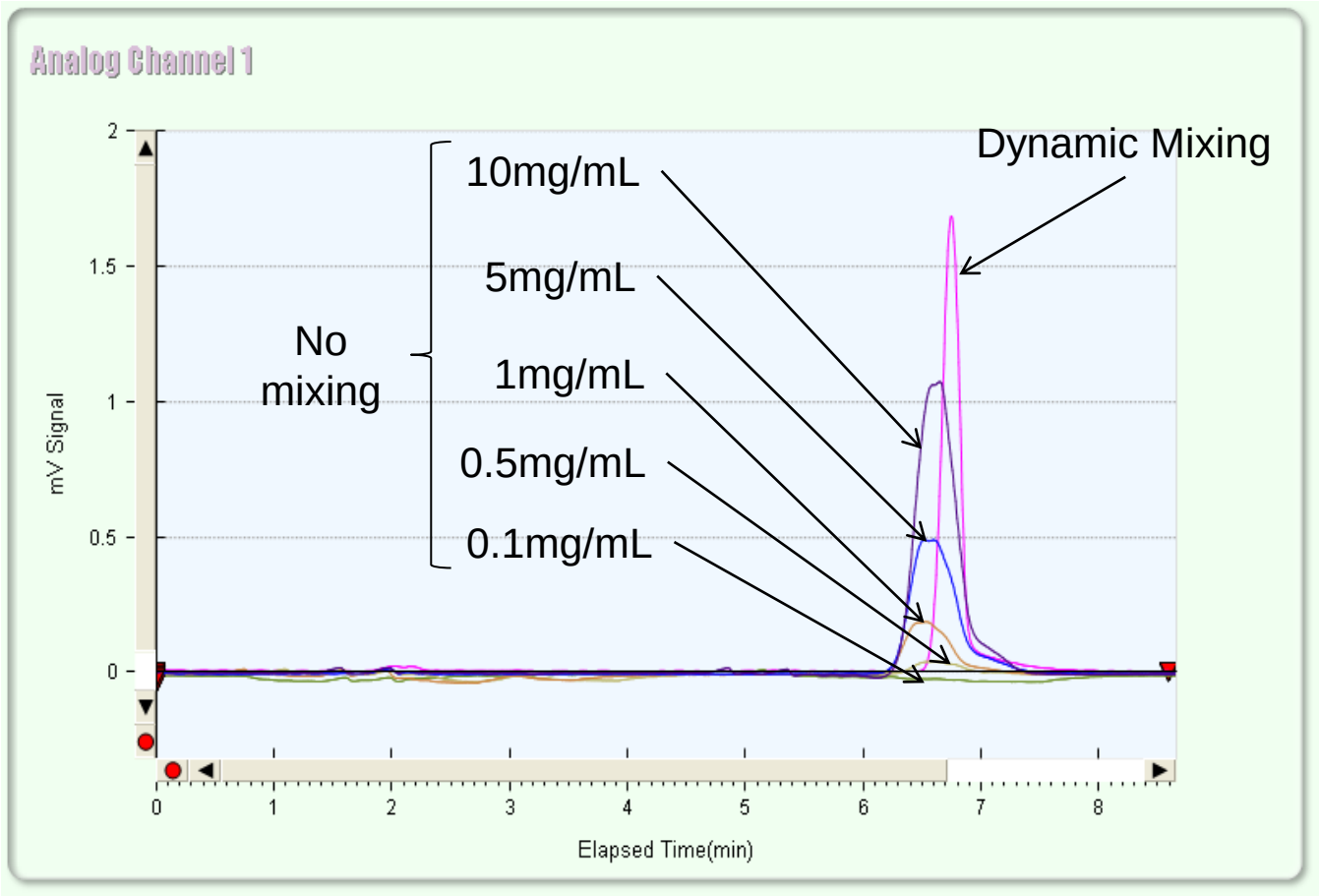
Concentration Effect



N-benzylbenzamide, 2-EP, 10% MeOH w/IPam

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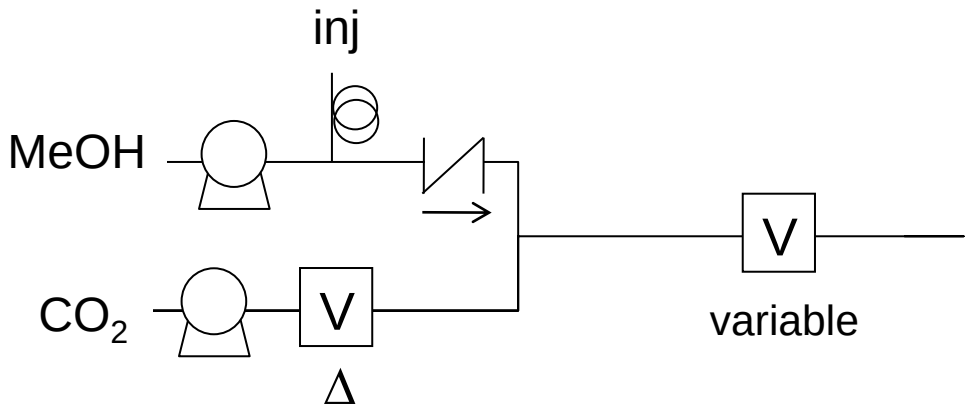
OK, Back Up…

- Band splitting is due to:
  - Change in mobile phase density along flow path

Density Gradients and Band Shape

- In SFC (not HPLC) the MP density, velocity, and viscosity change along the column length (*Tarafder & Guiochon, J. Chromatogr. A 1218 (2011) 4569, 4576*)
- If the $\Delta\rho$ is linear and under const. mass flow, efficiency is unaffected and can be modeled using 'average' condition (*Peaden & Lee, J. Chromatogr. 259 (1983) 1*)
- What if the density gradient is non-linear?



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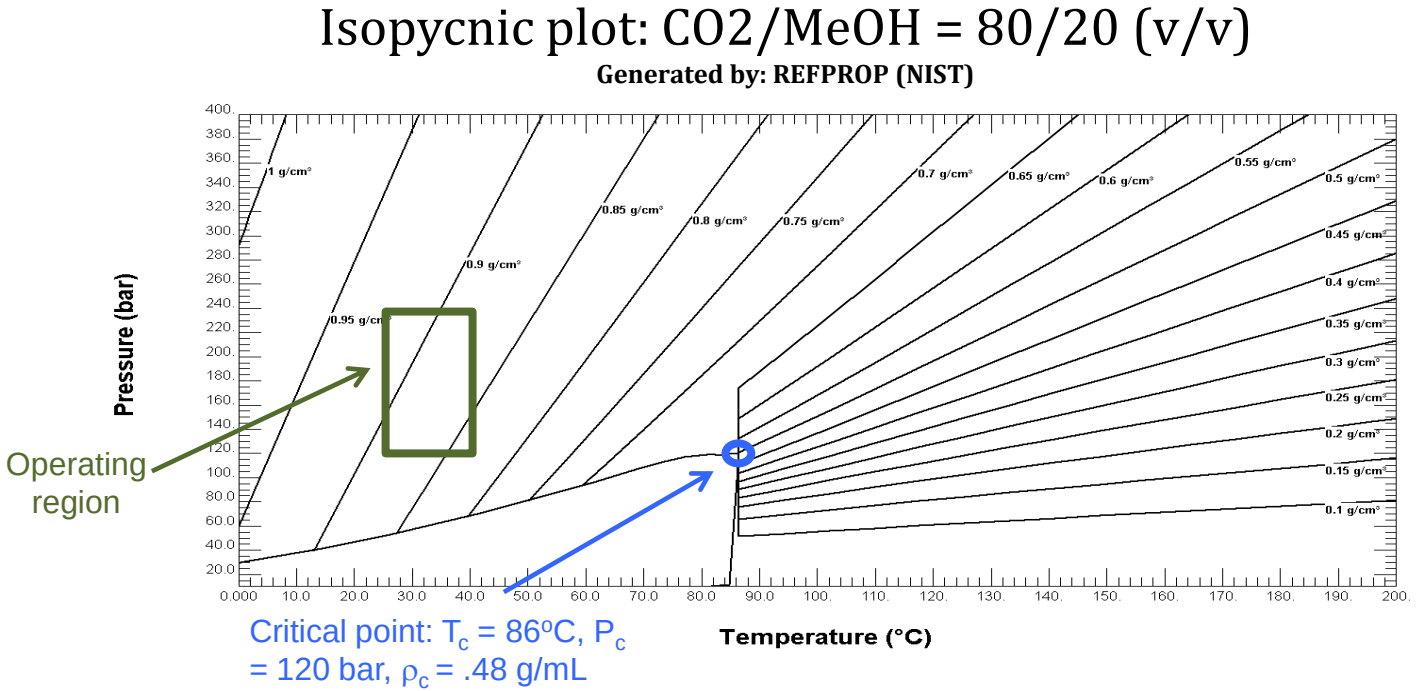
Optimizing Prep Chromatography



- Limited density variation in operating region gives limited control over solubility and k'
- Courtesy Abhijit Tarafder

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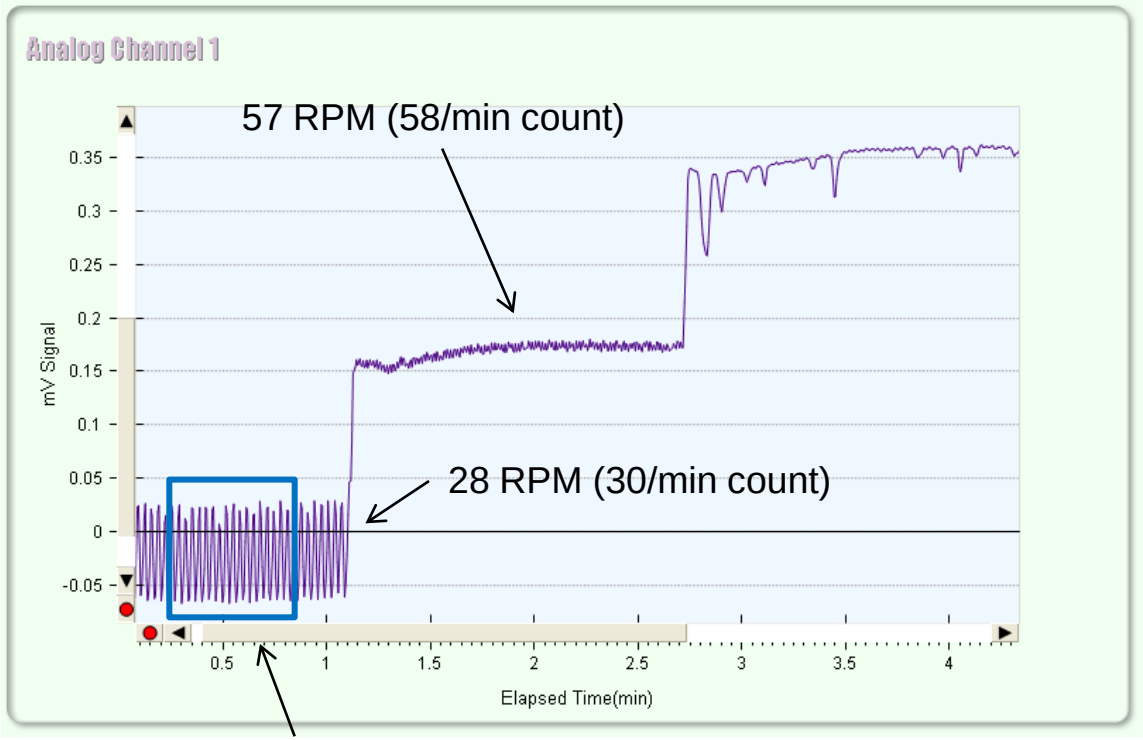
P50 Pump Noise



Detail next slide

- Column removed, 2% EtOAc in MeOH
- Co-solvent at 10, 20, 30%
- Noise can't be seen at higher pump RPM

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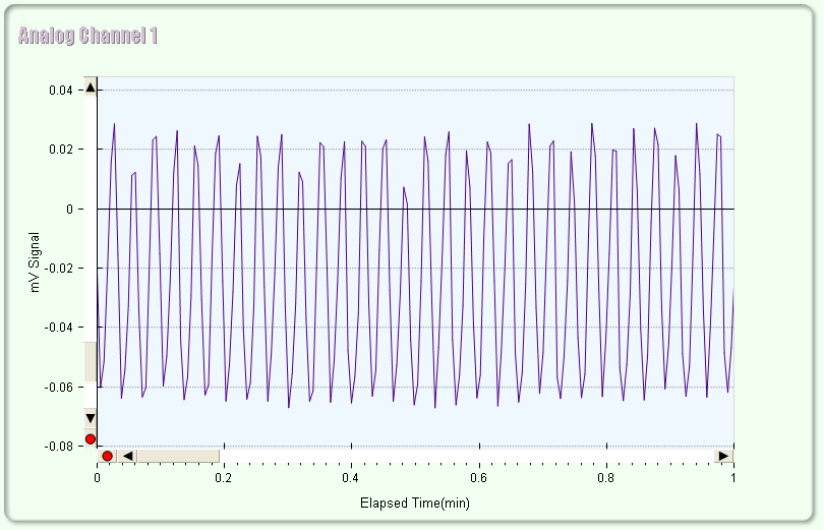
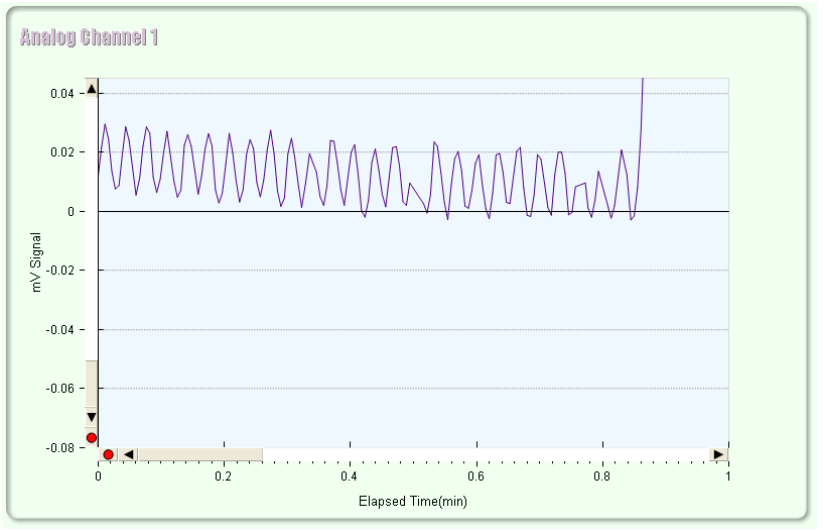
Effect of Mixing on Pump Noise





No Mixing

Varian 1.2 mL Dynamic Mixer

- Note that this is a design feature of the P50 pump, not a service issue

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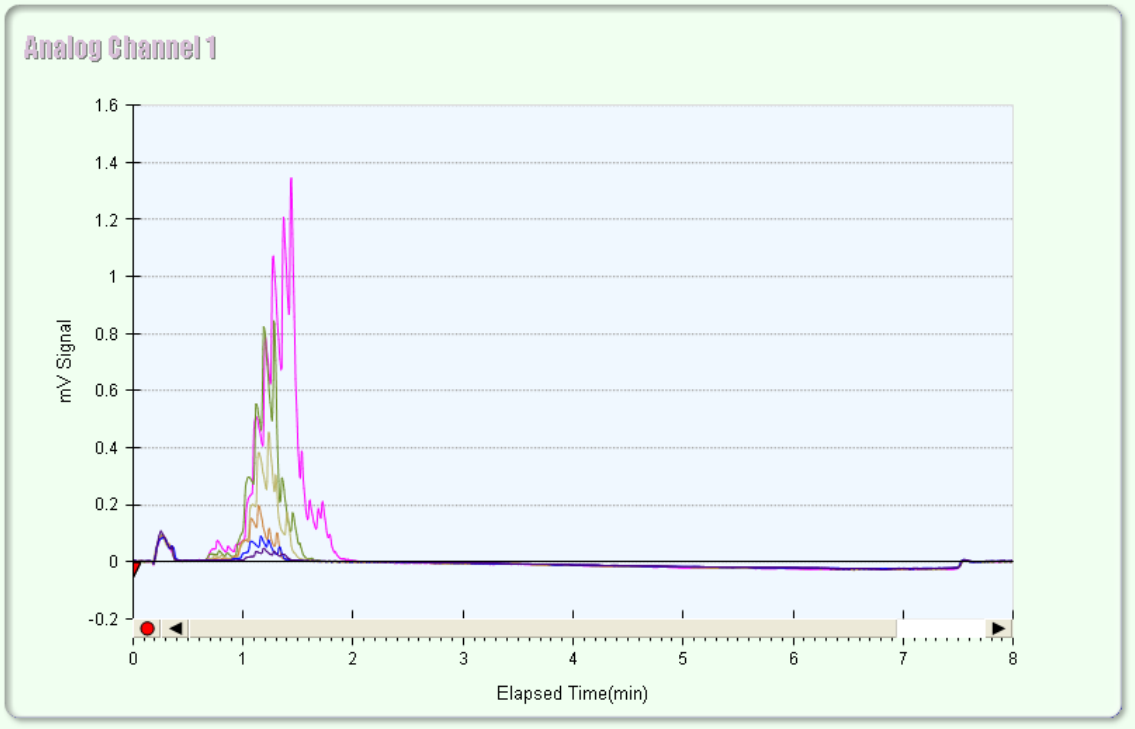
Low Co-solvent Effect



- Flavone, C18 (no retention), 5% MeOH
- Increasing mass at constant volume
- Pump cycle (14 RPM) is close to peak width

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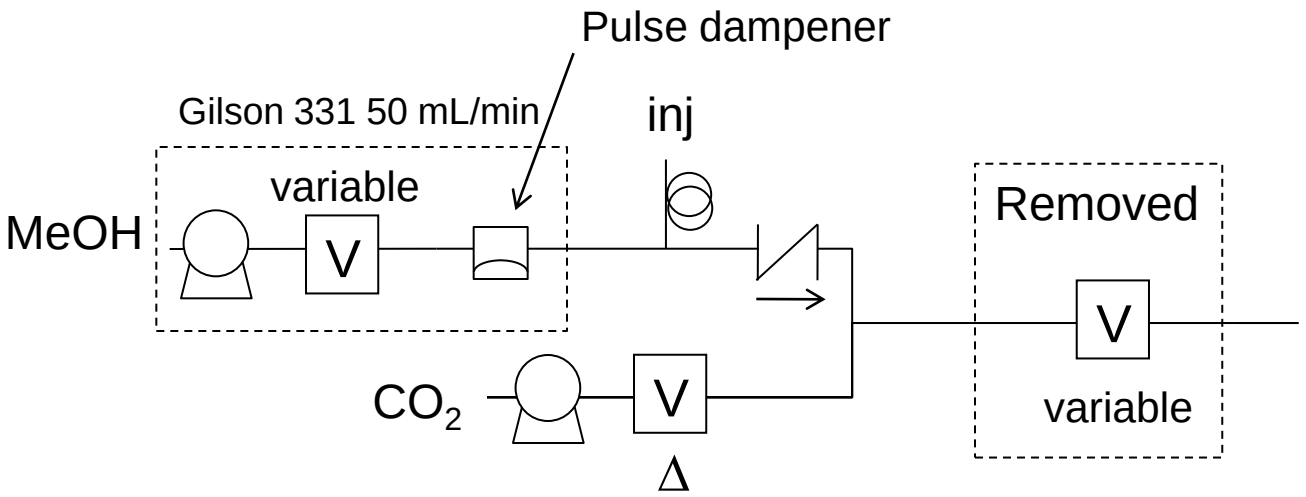
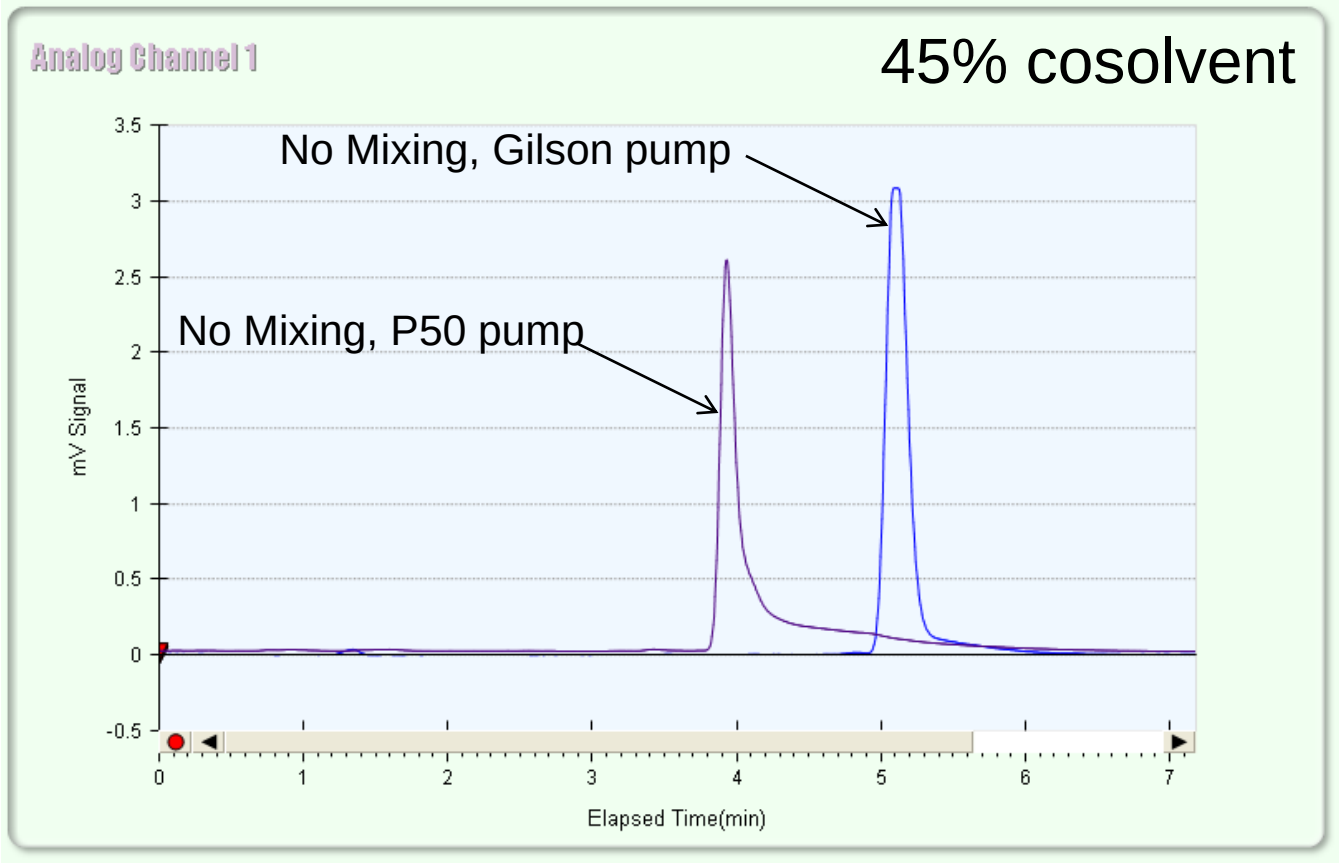
Gilson Co-Solvent Pump

- Gilson 331 pump spec 50 mL/min at 600 bar, low pulsing
- Control via front panel
- Not calibrated against P50: expect some changes in r.t.



(+/-) Naringenin, 2-EP



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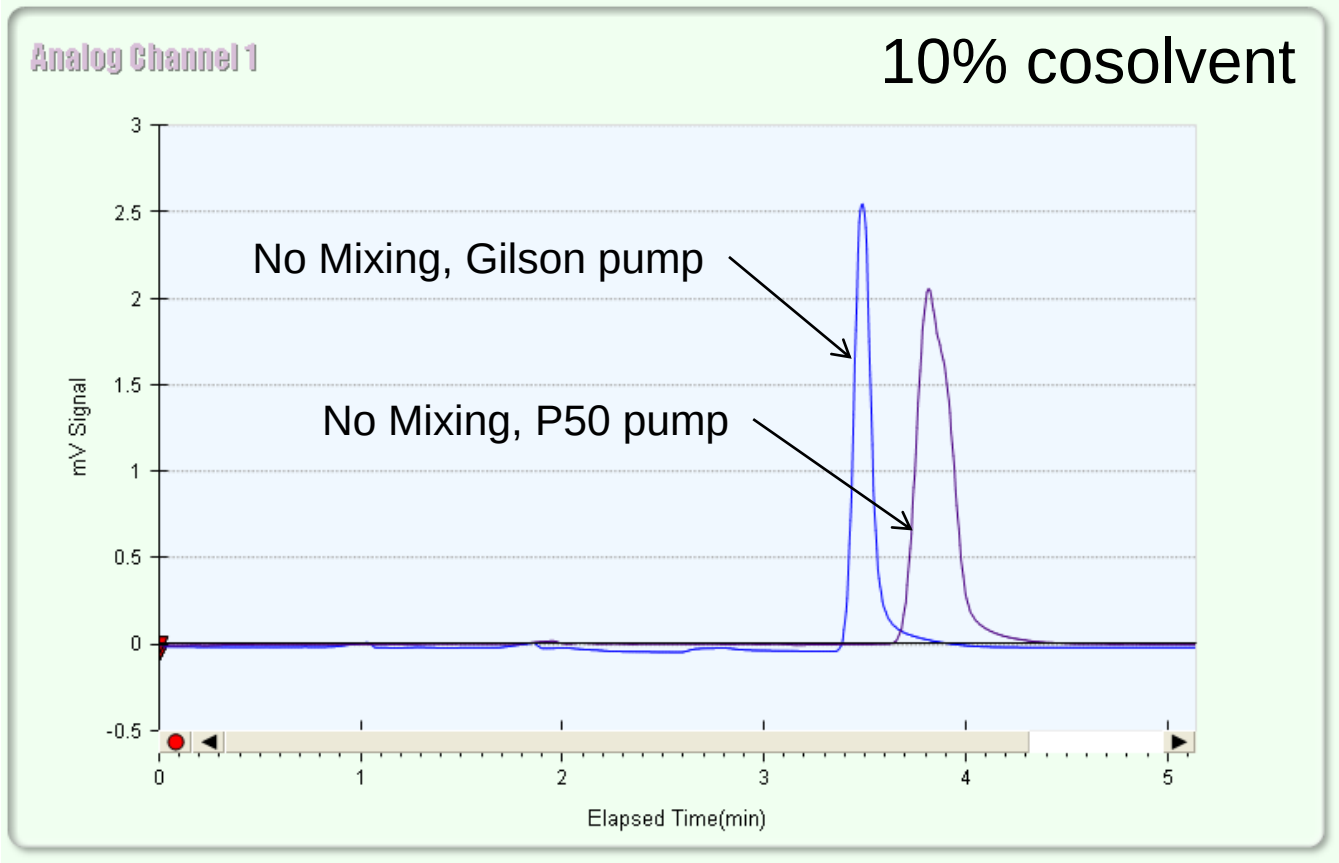
Flavone, 2-EP



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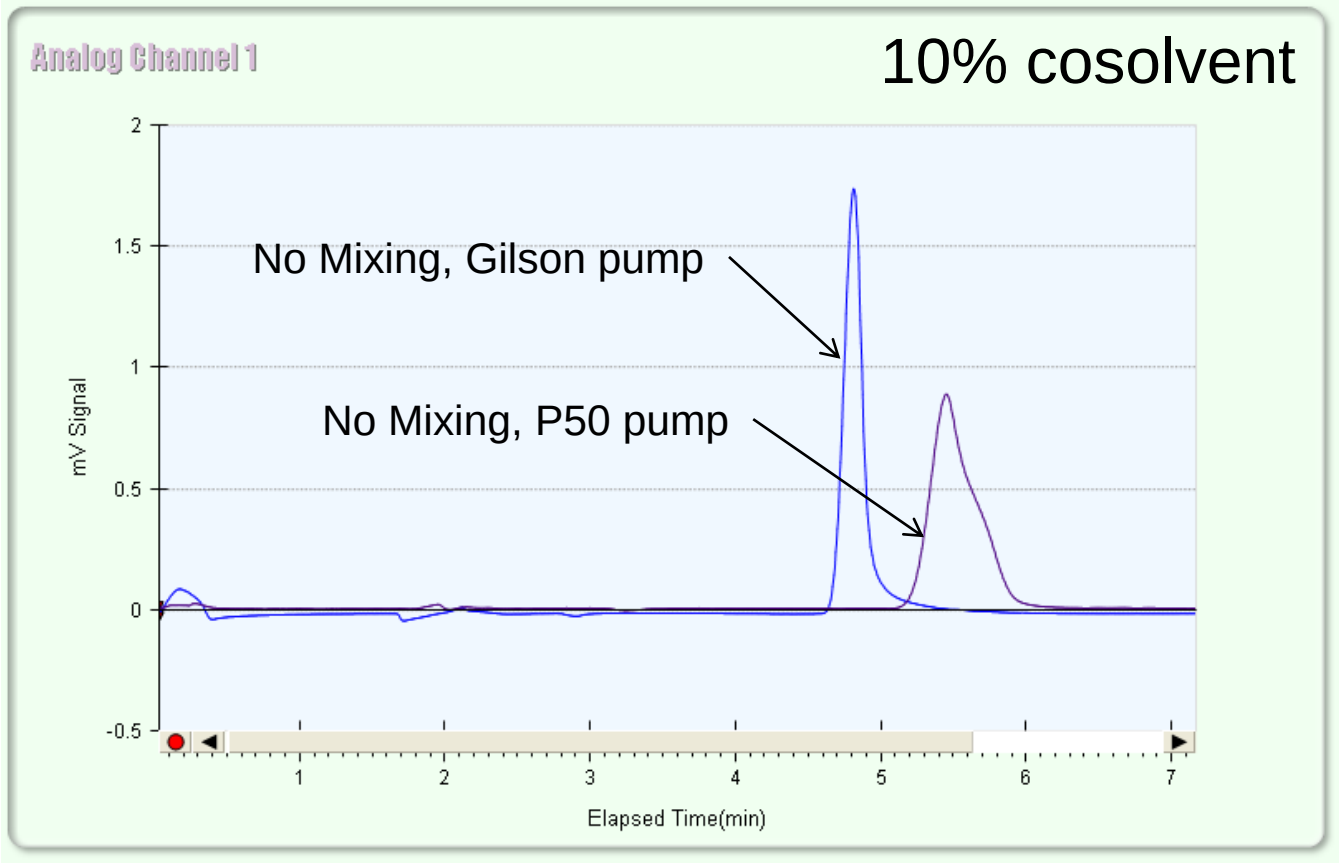
Ibuprofen, 2-EP



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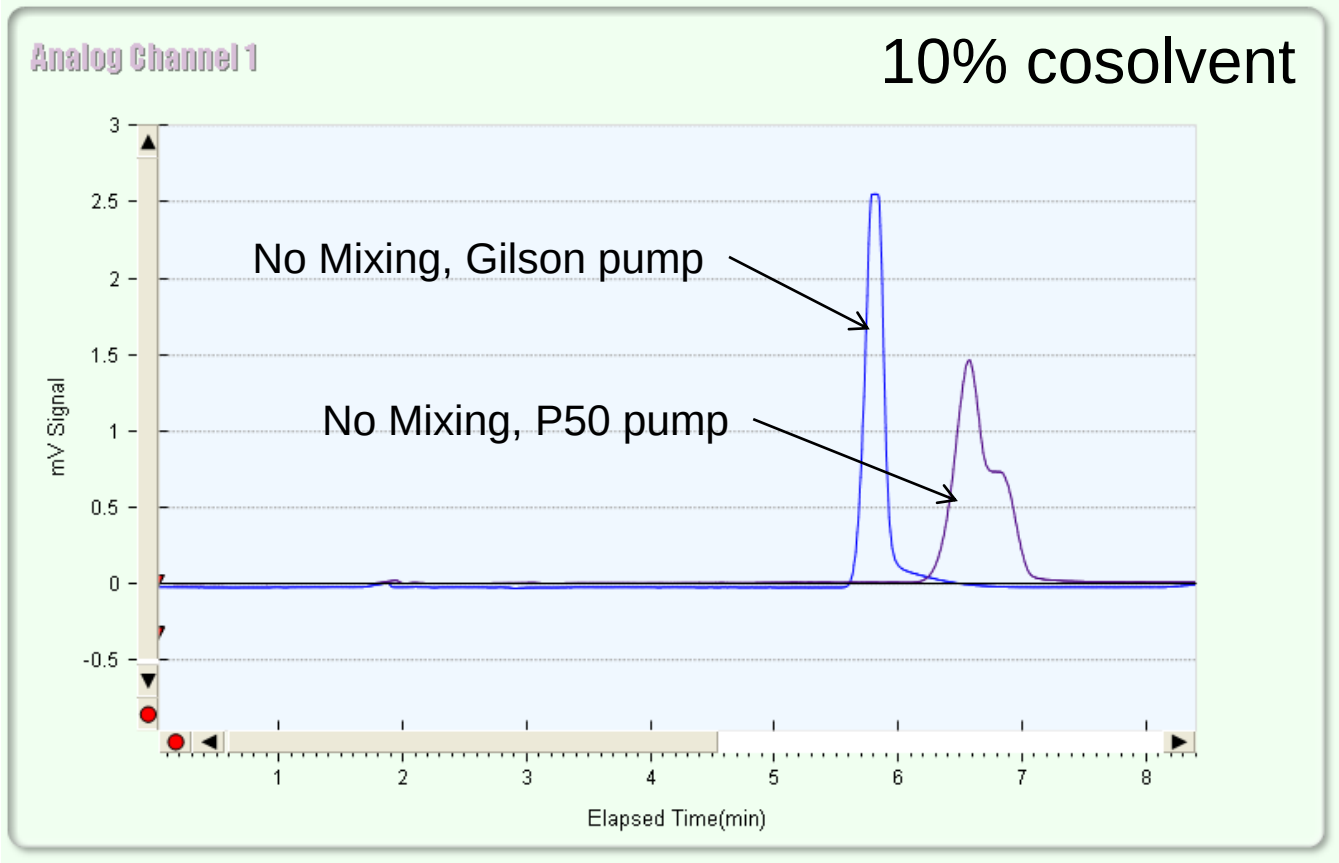
N-benzylbenzamide, 2-EP



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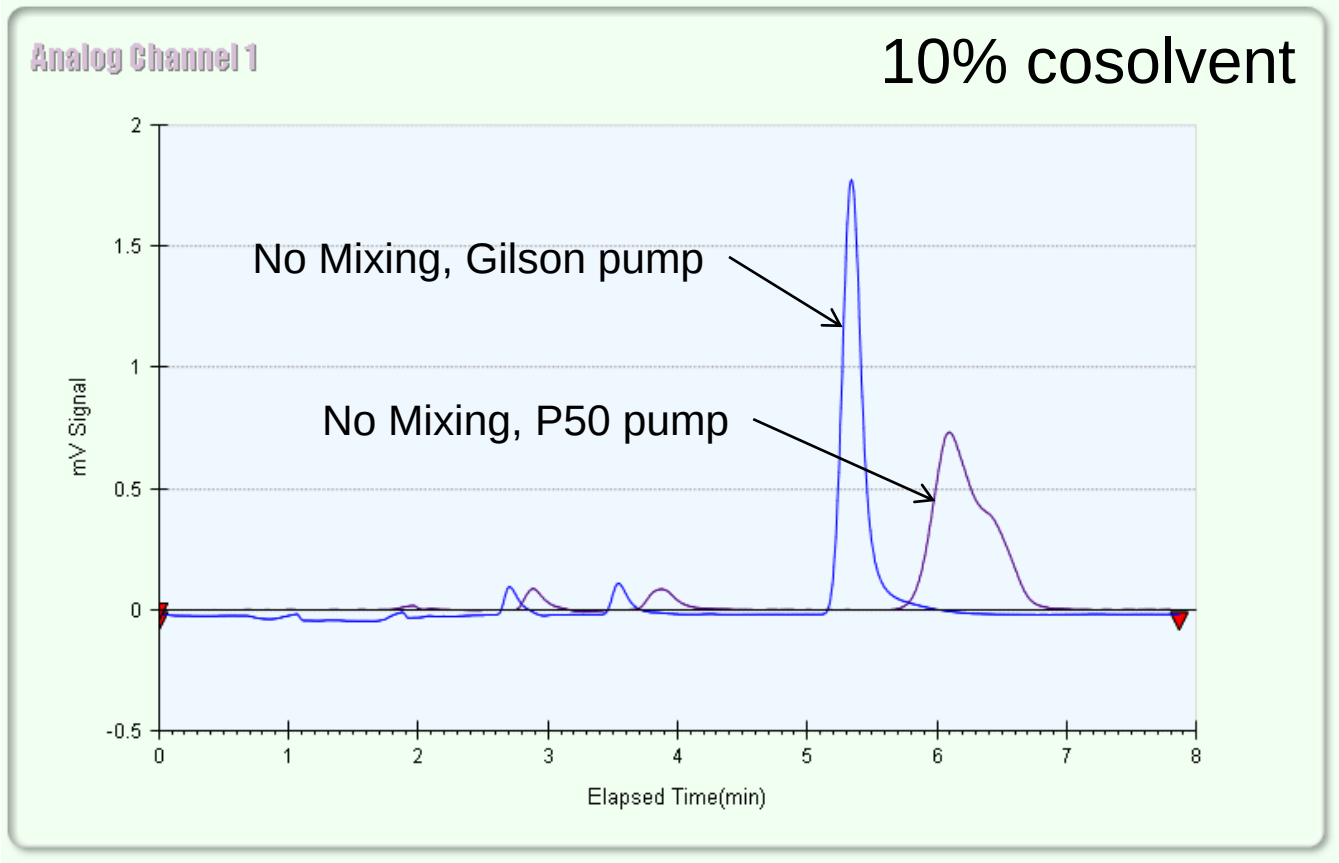
Ornidazole, 2-EP



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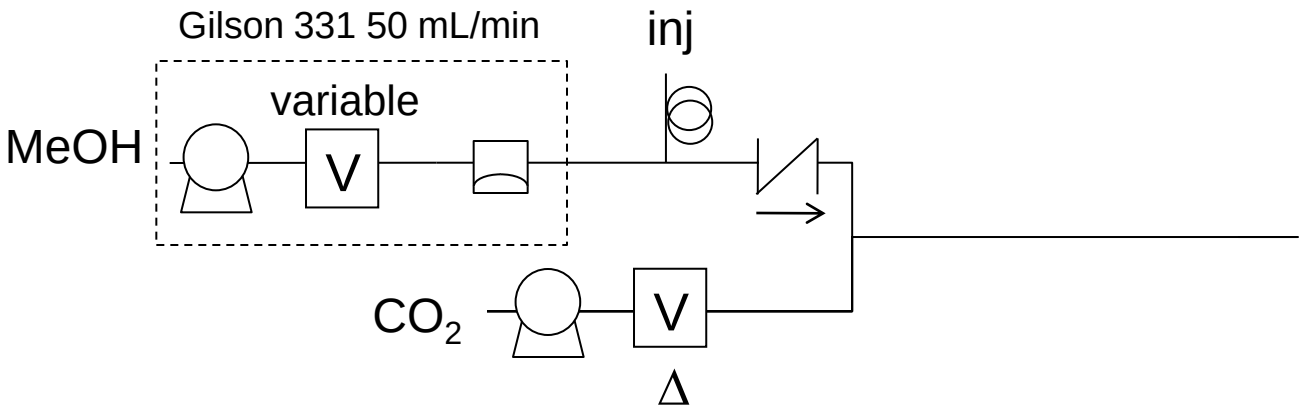
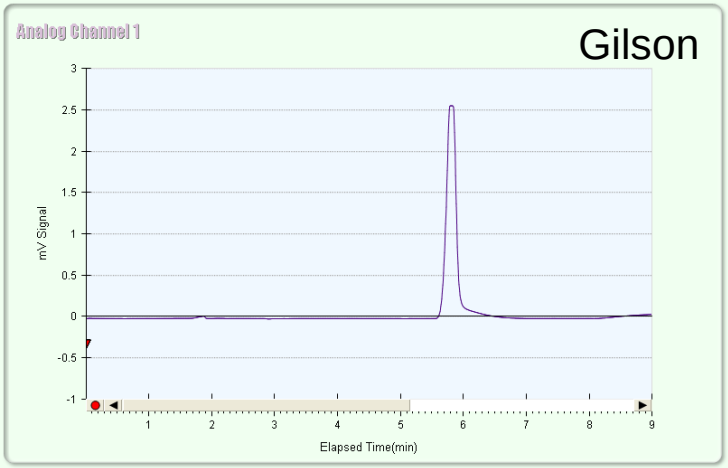
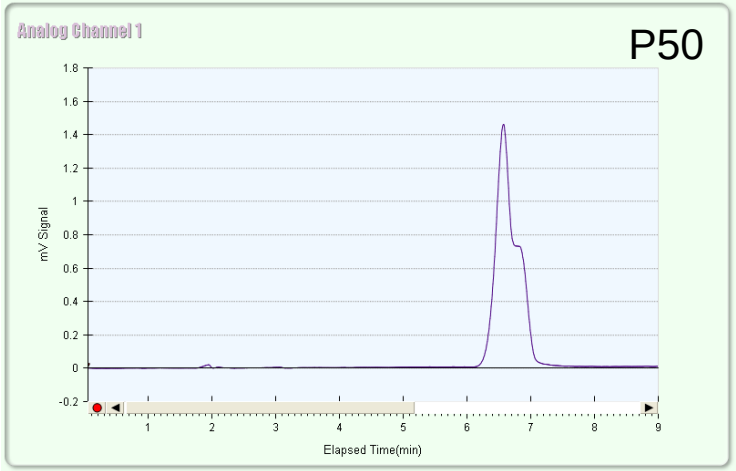
Gilson Co-Solvent Pump

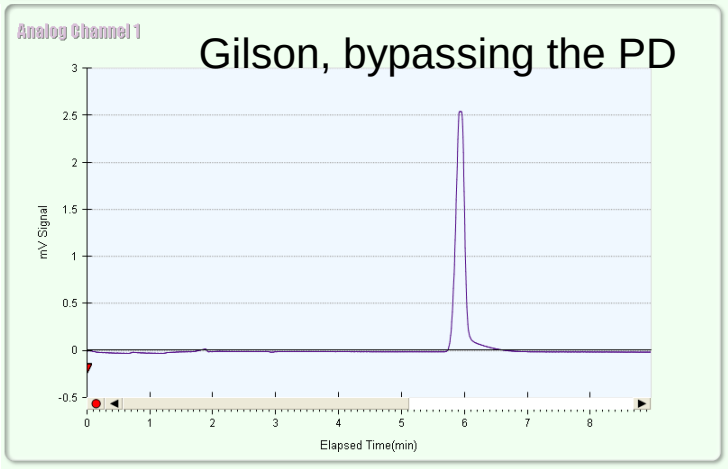
- Gilson variable mixer and pulse dampener are externally plumbed and removable

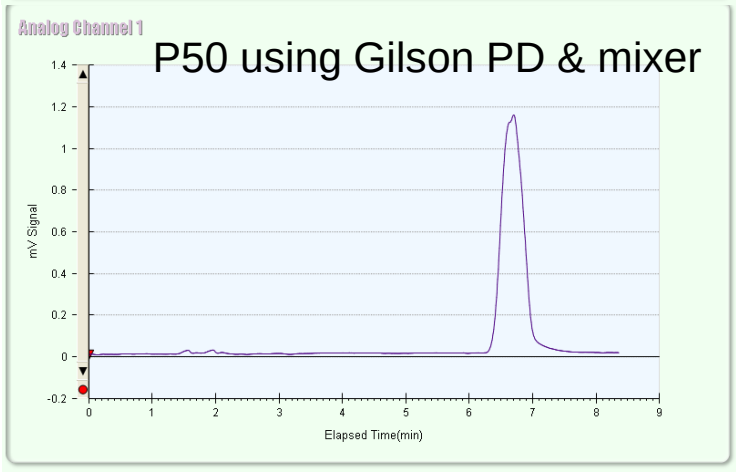


N-benzylbenzamide, 2-EP









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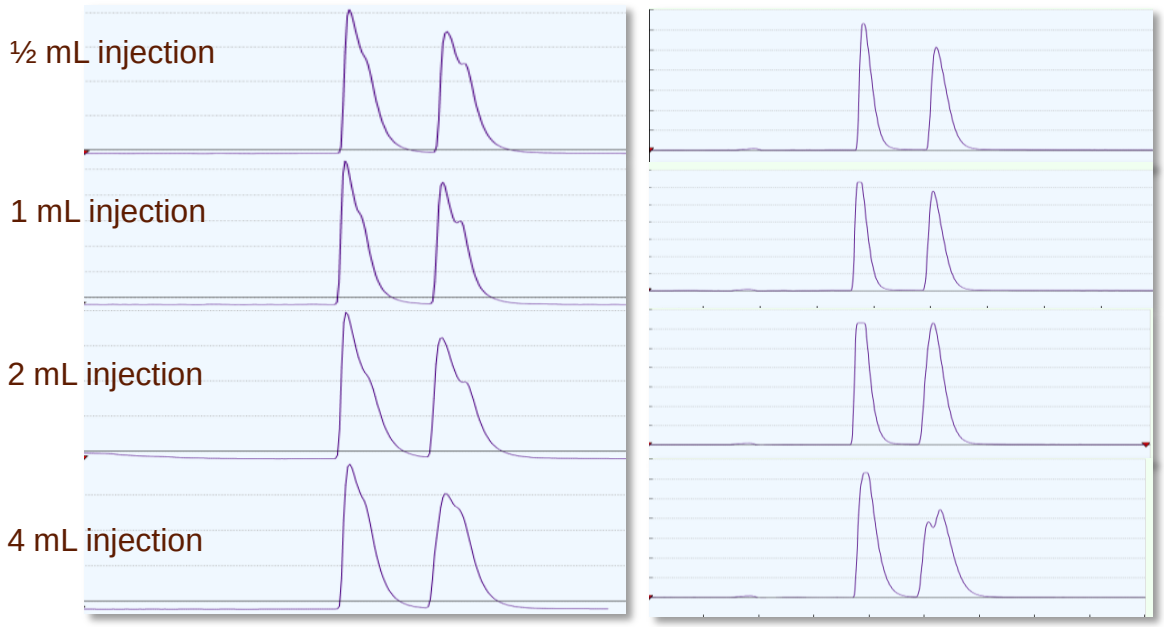
Tetramisole, Modifier Stream



Thar P50

Gilson 331

- Repeat of early modifier stream injection, volume overload experiments

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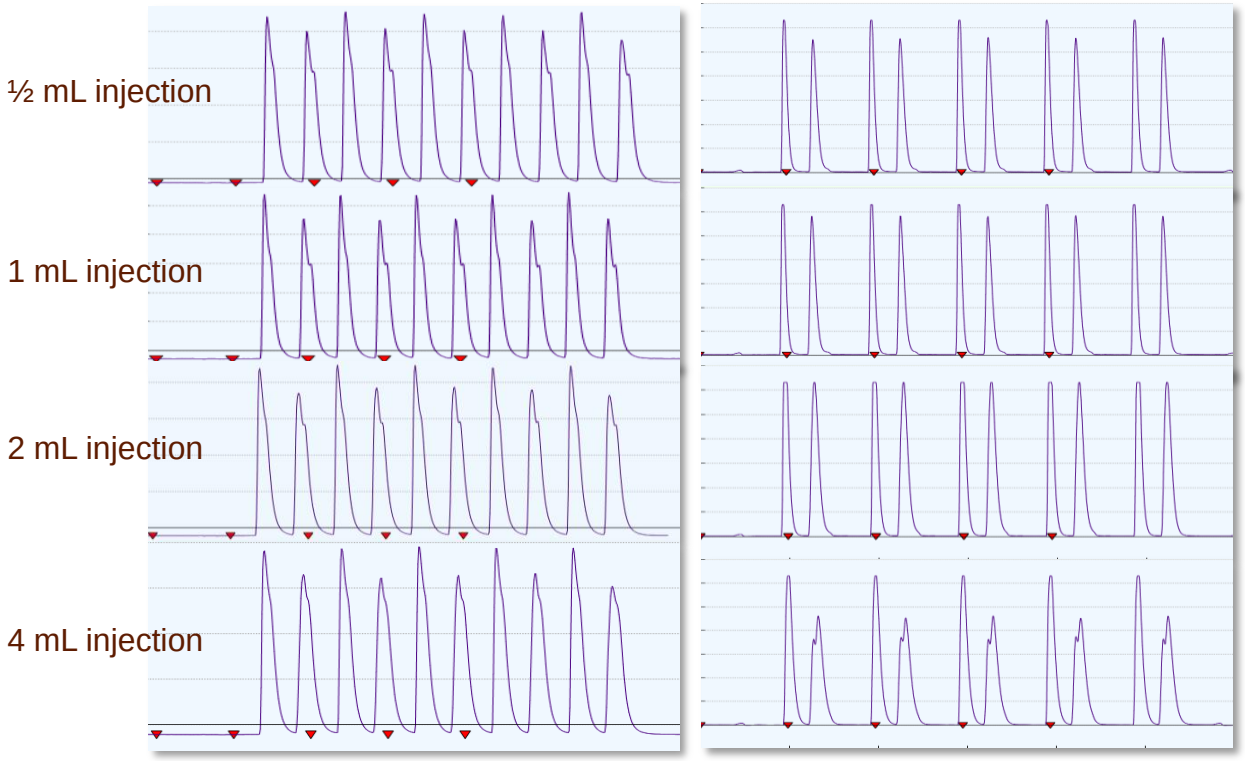
Tetramisole, Modifier Stream



Thar P50

Gilson 331

- Repeat of early modifier stream injection, volume overload experiments

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Conclusions

- Modifier stream injection generates density gradients due to co-solvent pump pulsing
- Pulse dampening may not completely fix P50 pump pulsation
- We do not observe issues derived from CO_2 pump pulsation, presumably due to fluid compressibility
- Using post-tee mixing as a solution is problematic, possibly because changes in co-solvent, diluent, or analyte solubility require different degrees of mixing
- Removal of pump pulsation resolves much of the system-derived problem and should allow further study of solubility phenomena

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