

AMGEN[®]

Pioneering science delivers vital medicines™



Evaluation of Non-Traditional Co-Solvents for Routine Pharma Discovery Support

Larry Miller, Grace Bi and Wolfgang Goetzinger

Third International Conference Packed-Column SFC 2009

July 22, 2009

Outline

- **Evaluation of non-traditional SFC co-solvents**
 - Analytical evaluation
 - Preparative examples
- **Direct comparison of SFC and RP-HPLC for achiral purification**
- **Conclusions**

Pharma Discovery Support

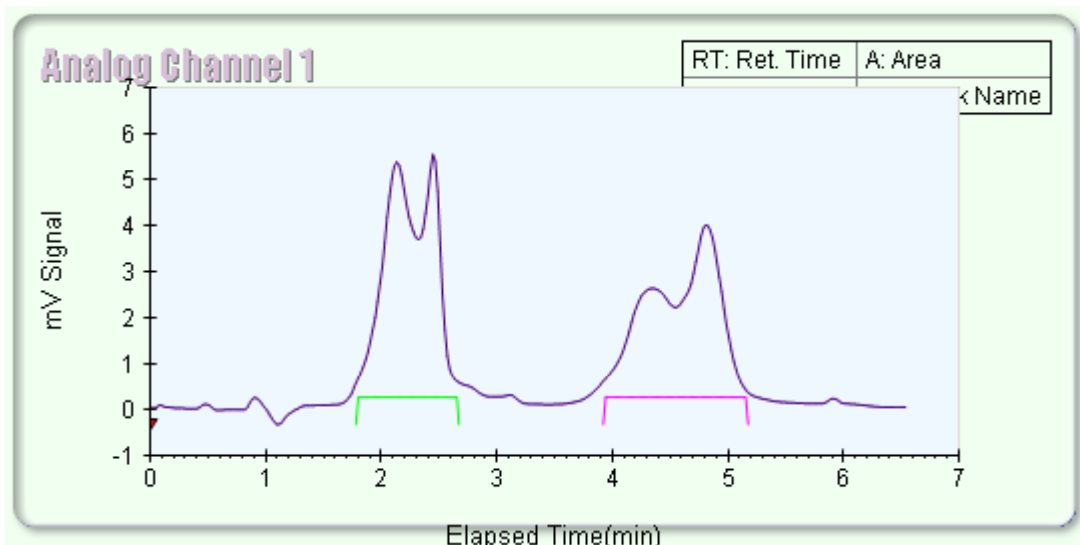
- **50-5000 mg racemate**
 - Often only see each racemate one time
- **Quick turn around required (2-4 days)**
- **Minimal solubility data available**
 - Chemist input
 - Similar racemates previously resolved
- **Standard analytical method development**
 - Rapid gradient screen
- **Preparative method development**
 - Loading only
- **Unexpected difficulties can impact “purification factory”**

Racemate solubility

- **Racemate solubility in mobile phase is critical factor in separation success and purification throughput**
- **Unable to quickly determine racemate solubility in SFC mobile phase**
- **Poor solubility greatly impacts turn around times and productivity of purification laboratory**

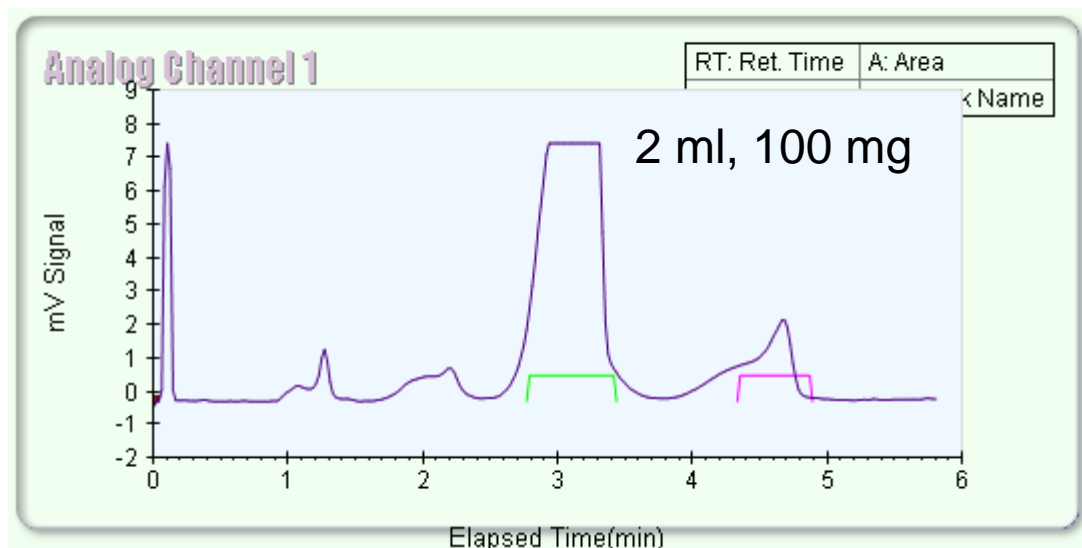
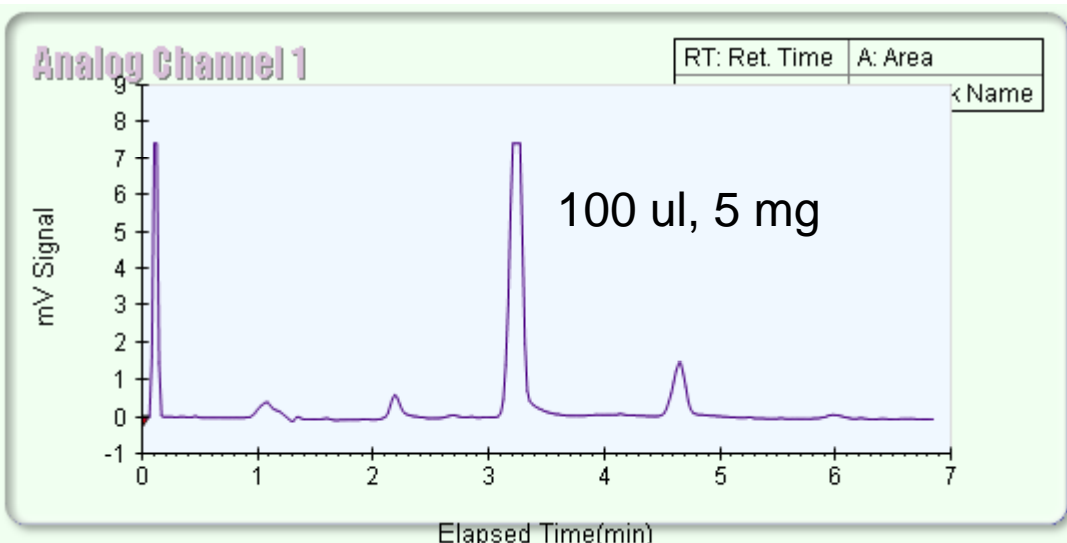
Poor Solubility

- It's been said “if soluble in methanol, can use SFC”
 - True for analytical, but not always true for preparative work
 - At prep loadings, solubility in co-solvent/CO₂ has impact on purification



- Sample dissolved in methanol/DCM
- Poor solubility in methanol/CO₂ results in peak splitting/broadening

Poor Solubility



- **Dissolution: 4:3:1 DCM:THF:DMSO @ 50 mg/ml**
- **Chiralpak IC, 2 x 25 cm**
- **45% methanol (DEA)**
- **80 ml/min**

Poor peak shape, large increase in peak width with increased loading



Due to poor sample solubility in methanol/CO₂???

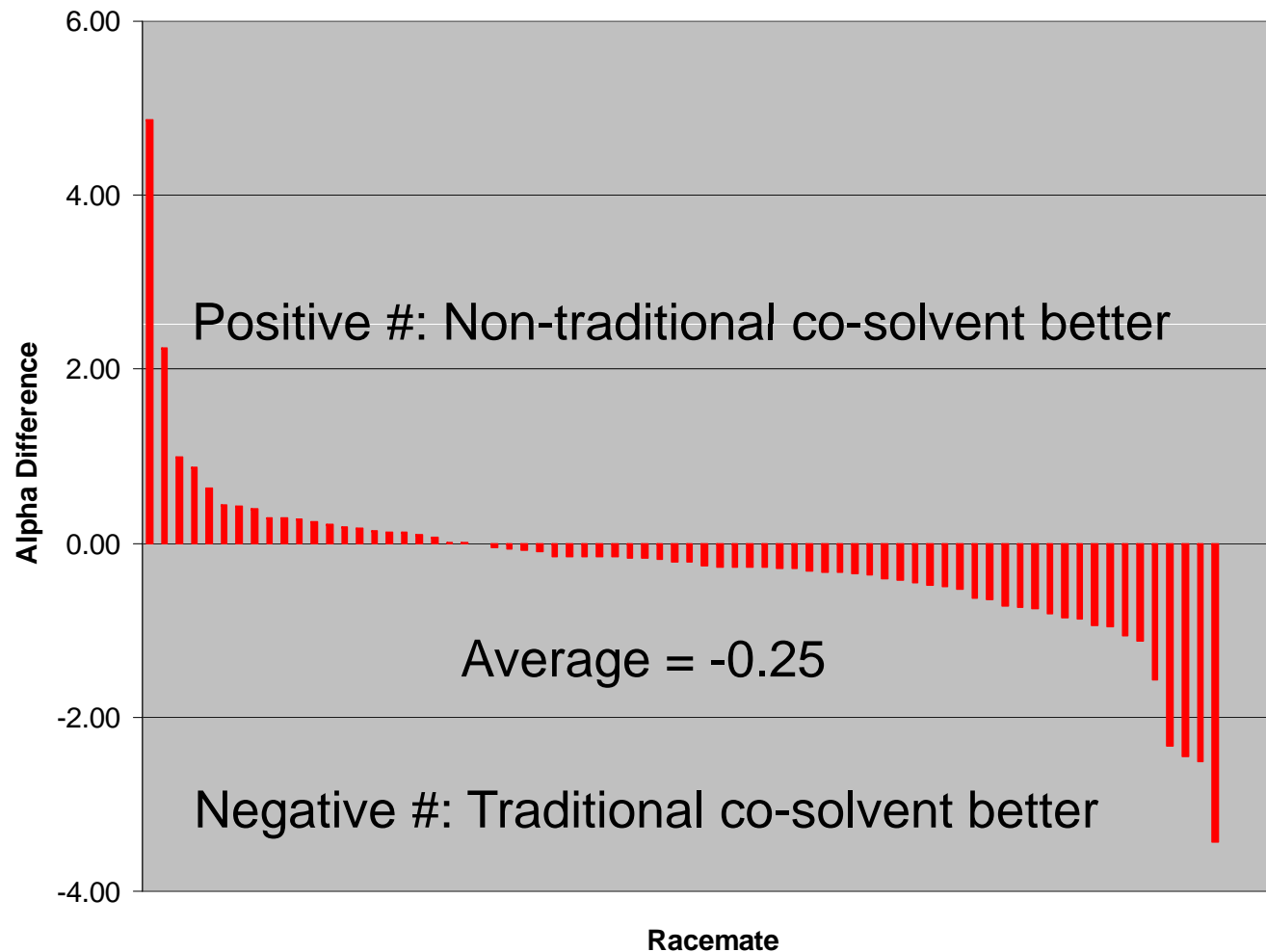
Poor Racemate Solubility In Column

- If low methanol/CO₂ solubility is leading to poor peak shape, could a co-solvent with higher solubilizing power eliminate/reduce this effect, leading to improved preparative peak shape?
- Evaluate dichloromethane (DCM) and THF, each with methanol as SFC co-solvent
 - Use of DCM and THF requires use of immobilized CSP
- Does addition of DCM or THF drastically impact separation compared to traditional co-solvents?
- Can non traditional co-solvents be a replacement for traditional co-solvents?

Experimental

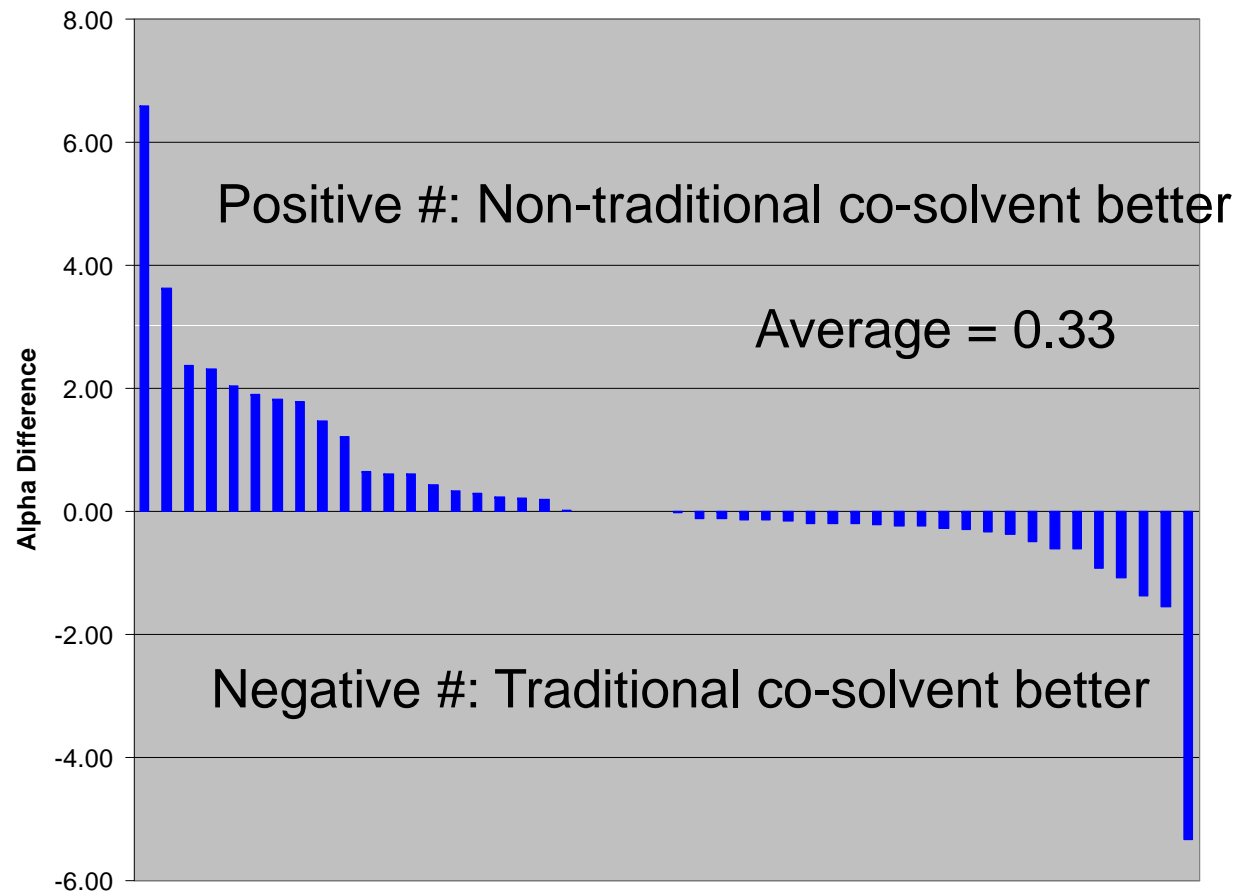
- **72 commercial racemates, 44 Amgen racemates**
- **Develop SFC method using:**
 - Chiralpak AD, AS, Chiralcel OD, OJ
 - Methanol, ethanol or Isopropanol (each w/ 0.2% DEA) co-solvent
- **Develop SFC method using:**
 - Chiralpak IA, IB, IC
 - 20/80 DCM/methanol, 20/80 THF/methanol, 10/10/80 DCM/THF/methanol (each with 0.2% DEA) co-solvent
- **All other conditions (flowrate, column size, etc) constant between two methods**
- **Co-solvent percentages adjusted for 2nd peak to elute under 1.5 minutes**
- **Compare separation obtained using both approaches**

Commercial Racemates



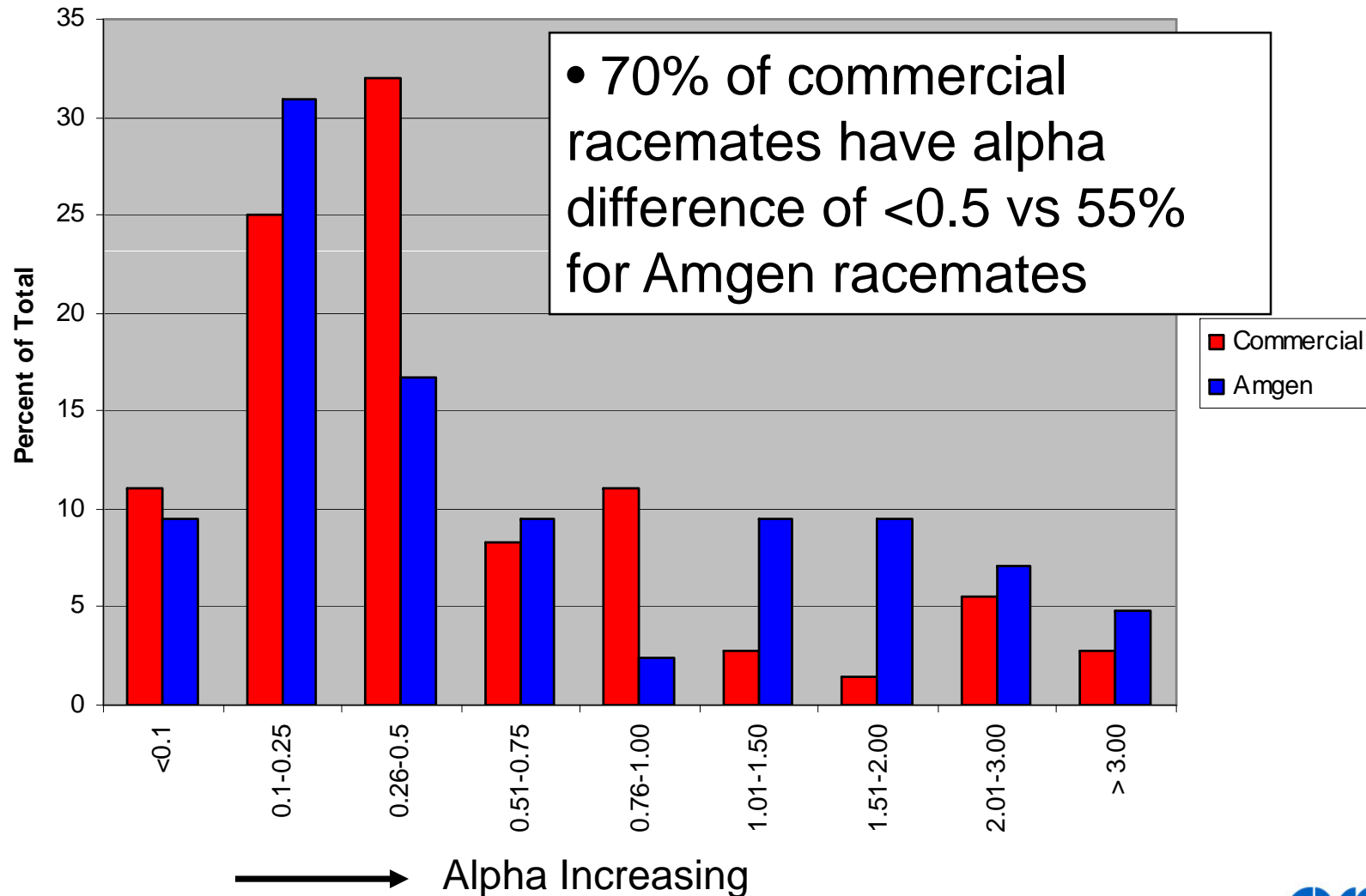
- Traditional co-solvents showed increased separation

Amgen Racemates



- Non-Traditional co-solvents showed increased separation

Alpha Difference Commercial vs. Amgen Racemates



Non Traditional Co-Solvent Summary

	DCM/Methanol	THF/Methanol	DCM/THF/Methanol
Amgen Racemates	26	14	4
Commercial Racemates	32	16	7

- **“Best” co-solvent = largest retention time difference under gradient screening conditions**
- **Racemates with DCM/THF/Methanol as best co-solvent gave minimal (0.02 minutes) improvement over other co-solvents**
 - Evaluation of DCM/THF/Methanol offers no advantage over DCM/Methanol or THF/Methanol

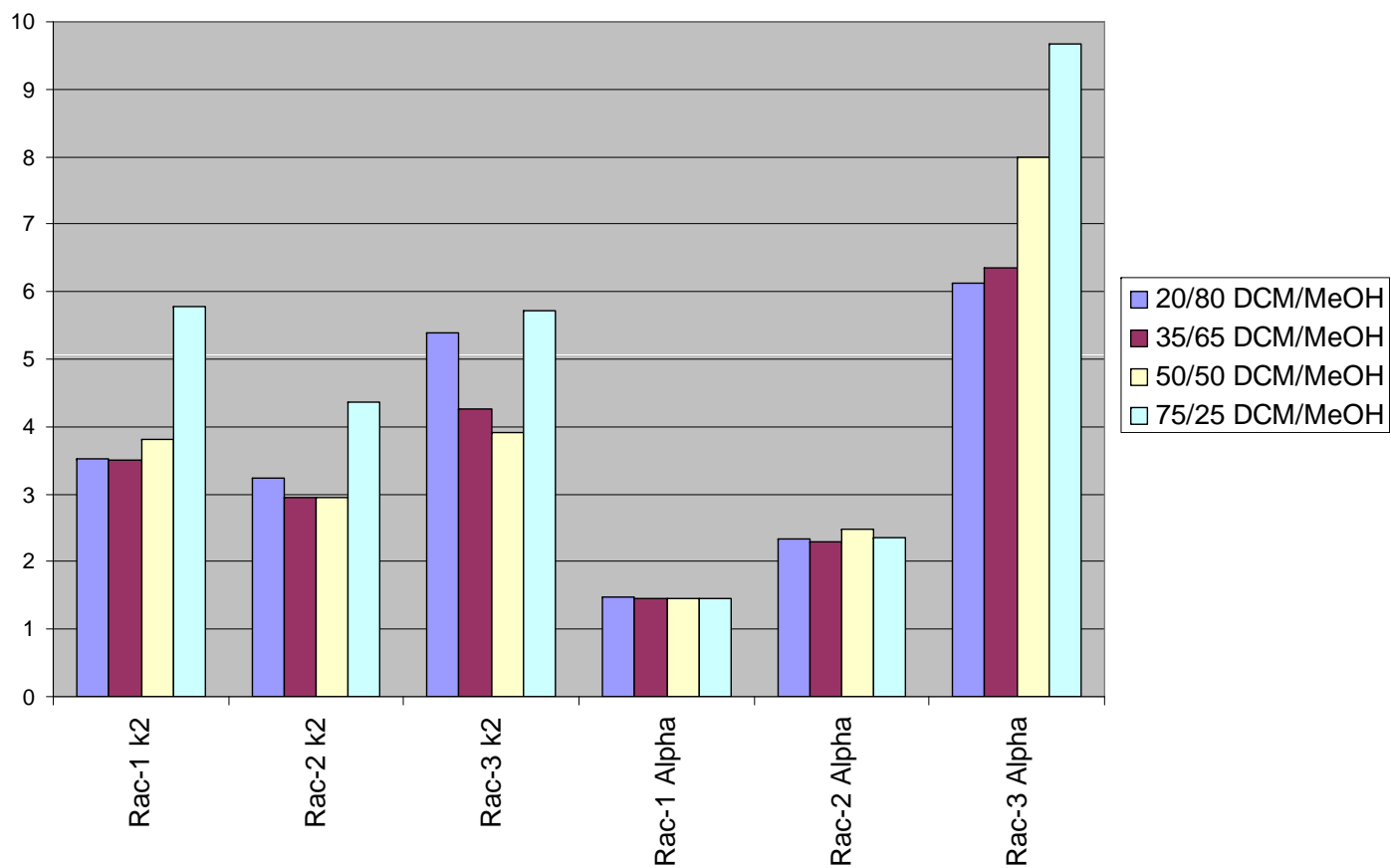
CSP/Co-solvent summary

	Coated CSP				Immobilized CSP		
	AD	AS	OD	OJ	IA	IB	IC
Amgen Racemates	38	0	6	0	12	5	27
Commercial Racemates	30	10	14	11	32	13	10

Best co-solvent for coated CSP		
	Amgen Racemates	Commercial Racemates
MeOH	41	34
EtOH	1	14
IPA	2	17

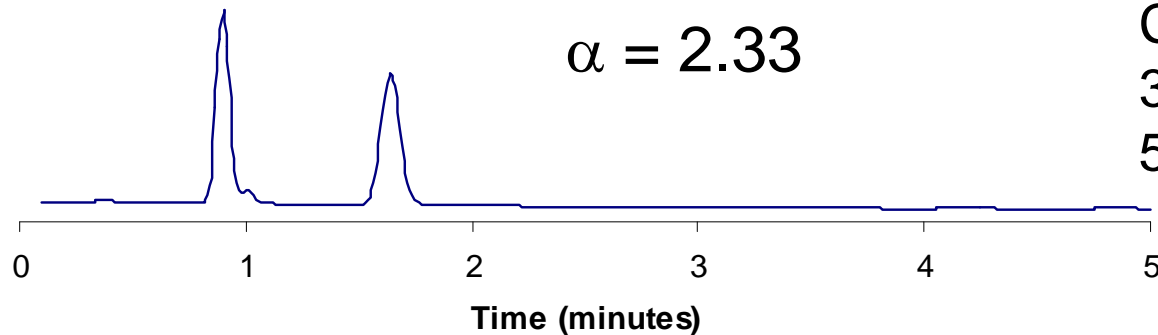
- Amgen racemates not as diverse as commercial racemates

Impact Of Increased DCM Content

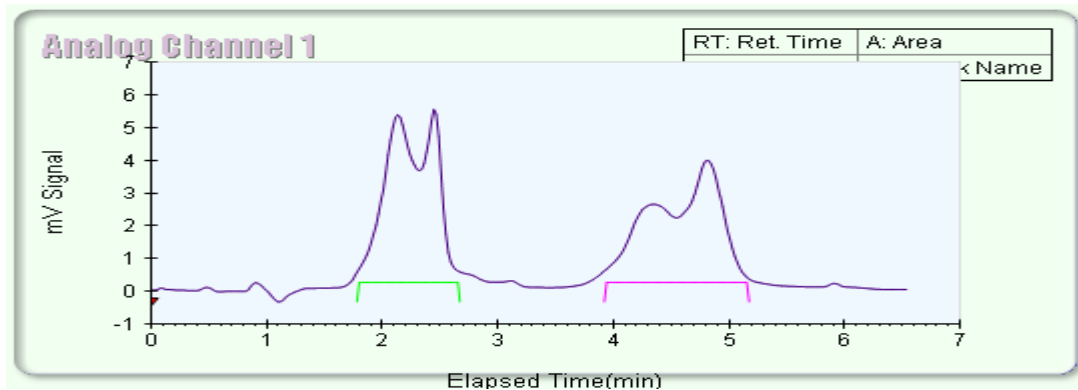


Resolution relatively unchanged with DCM/methanol ratio

Preparative Example



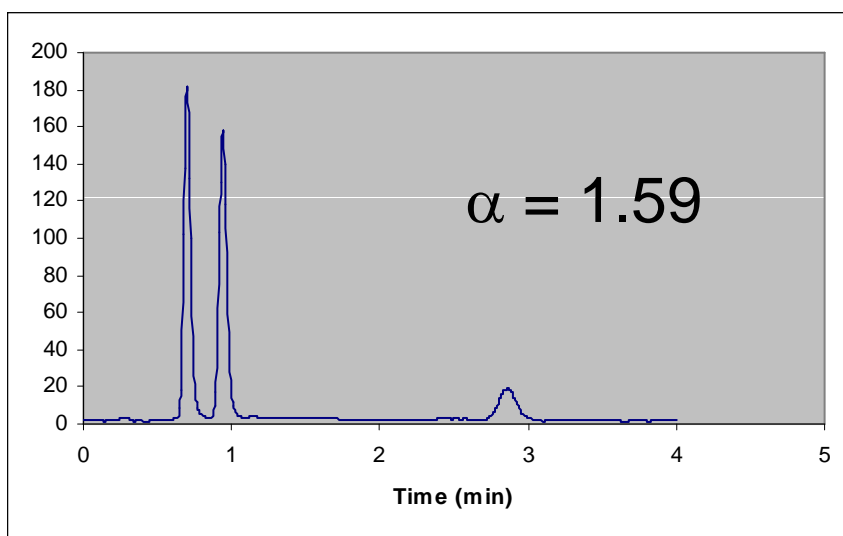
Analytical
Chiralpak AD-H
35% methanol (DEA)
5 ml/min



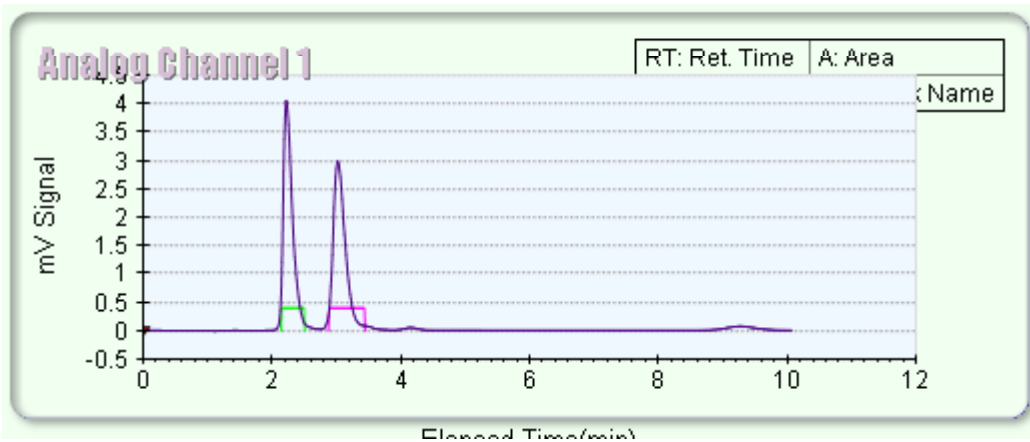
Preparative
Chiralpak AD-H, 2 x 25 cm
35% methanol (DEA)
80 ml/min
15 mg racemate (1 ml of
15 mg/ml in 10:10:1
DCM:MeOH:DMSO

Immobilized CSP

- Investigate DCM as co-solvent to improve peak shape and purification throughput

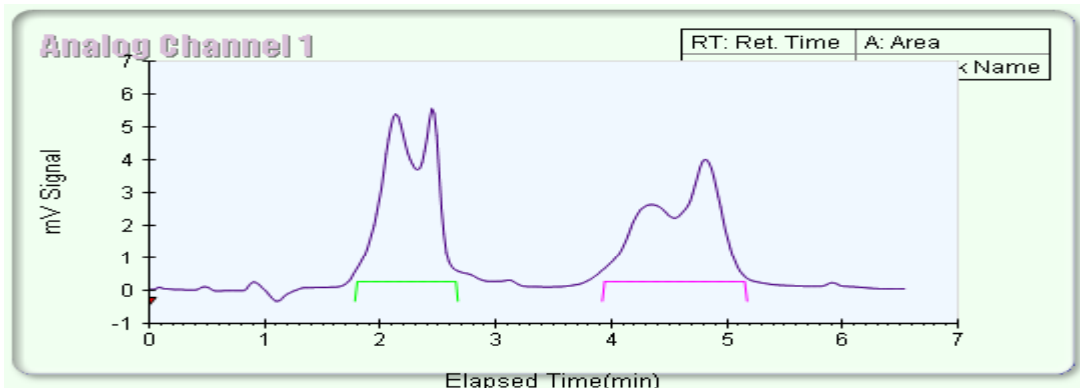


Chiralpak IA, 4.6 x 100 mm
30% of 50:50 MeOH:DCM (DEA)

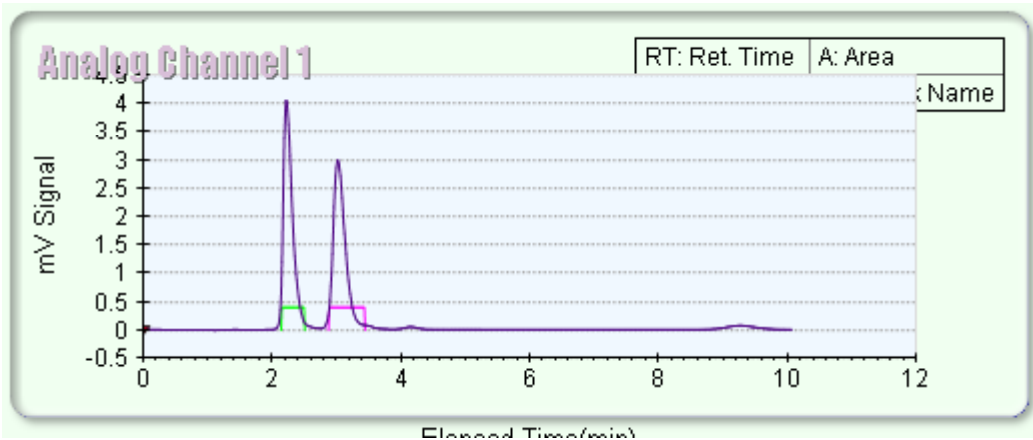


Chiralpak IA, 5 x 25 cm
30% of 50:50 MeOH:DCM (DEA)
160 mg racemate
Dissolution: 40 mg/ml in
10:1 DCM: DMSO

Purification Comparison



- Chiralpak AD, 2 x 25 cm
- 35% methanol (DEA)
- 15 mg racemate
- Productivity: 0.078 kkd
- Solvent Usage: 7.4 L/g racemate



- Chiralpak IA, 5 x 25 cm
- 30% 50:50methanol:DCM (DEA)
- 160 mg racemate
- Productivity: 0.268 kkd (0.42 kkd)
- Solvent Usage: 0.94 L/g racemate

Advantages/Disadvantages

- **Improved chromatography**
- **Solvent reduction**
 - Often lower co-solvent percentage needed for DCM/methanol or THF/methanol vs. traditional co-solvents
- **Increased mobile phase preparation time**
- **Detection issues (215 nm)**
- **Future Plans**
 - Explore additional racemates under preparative conditions
 - Determine universality of approach

MS triggered Purification of Small Molecules by SFC

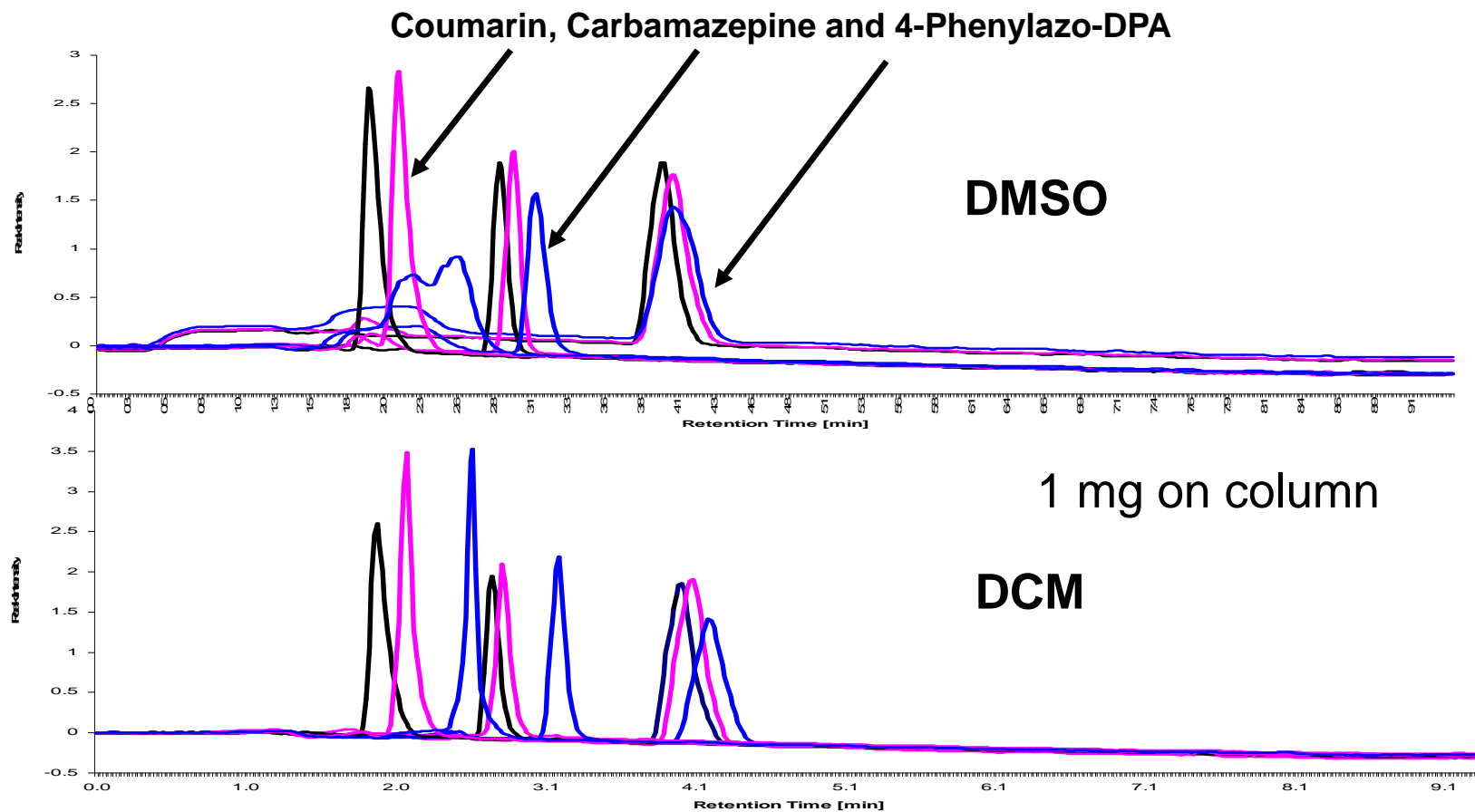
- **The Promise of MS directed SFC purifications:**
 - Faster separations (low viscosity, high diffusivity)
 - Different selectivity compared to RP-HPLC
 - Lower costs of solvents and waste (eliminate Acetonitrile!)
 - Faster evaporation of fractions (reduced volume, methanol)
 - Elimination of stability issues related to common RP modifiers
- **Challenges of MS directed purification**
 - Fraction collection (Cyclones not practical for libraries)
 - Open bed fraction collection now a reality!
 - How does preparative SFC compares to RP-HPLC for achiral purifications in Drug Discovery
 - Which solvents are compatible with both modes of separation?
 - How much volume and material can be injected?
 - Are the systems sized properly for this application?

Summary of Experimental Parameters

- **Samples: set of 35 “drug-like” commercial compounds**
 - Mw and clogP mostly “rule-of-five” compliant and structurally diverse
 - 6 selected for SFC/LC comparison
- **Dissolution Solvents: DMSO, MeOH, and DCM**
- **Volume loading: 0.2, 0.8 and 3.2 ml (1 mg injections)**
- **Mass loading: 10, 25, 50, 75 and 100 mg (in 2 ml)**
- **Column dimensions: 20 x 100 mm with 5 μ m packing (120A)**
 - **RP-HPLC: Phenomenex C18; SFC: Sepax Pyridine-SFC column**
 - As “similar” as possible
- **Flow-rate: 40 ml/min; Gradient time: 8 minutes**
 - **RP-HPLC: H₂O/Methanol (0.1% Ammonia); gradient from 15-95%B**
 - **SFC: CO₂/Methanol (0.2% DEA); gradient from 5-55%B**
 - As “similar” as possible

Volume loading limitations with SFC

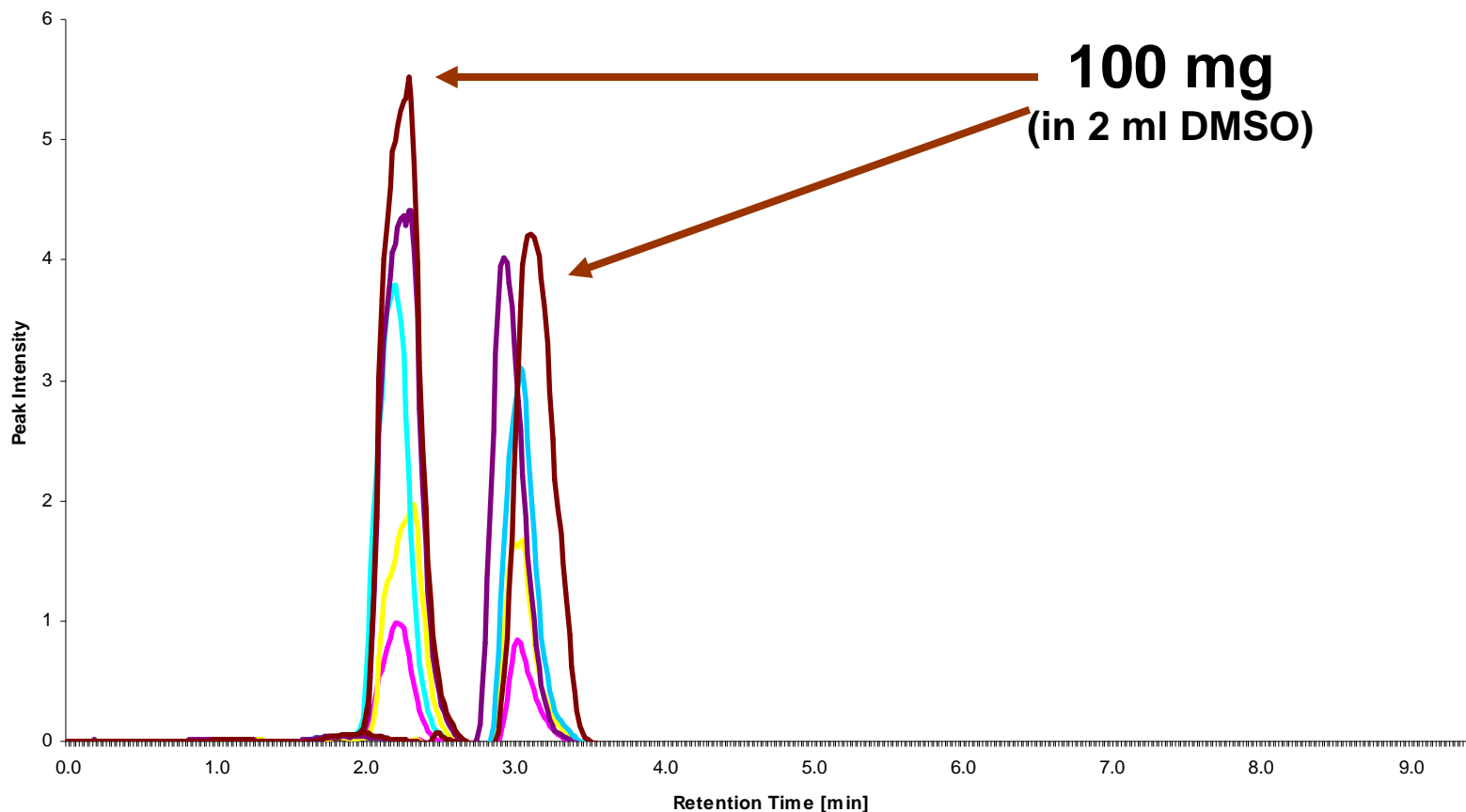
- Modifier stream injection of 0.2, 0.8 and 3.2 ml of samples



- Preferred injection solvent is DCM > DMSO ~ MeOH
 - Issues more pronounced for early eluting/large injection volumes
 - DMSO: watch elution window; DCM: large injection volumes shift elution times

Mass loading limitations with SFC

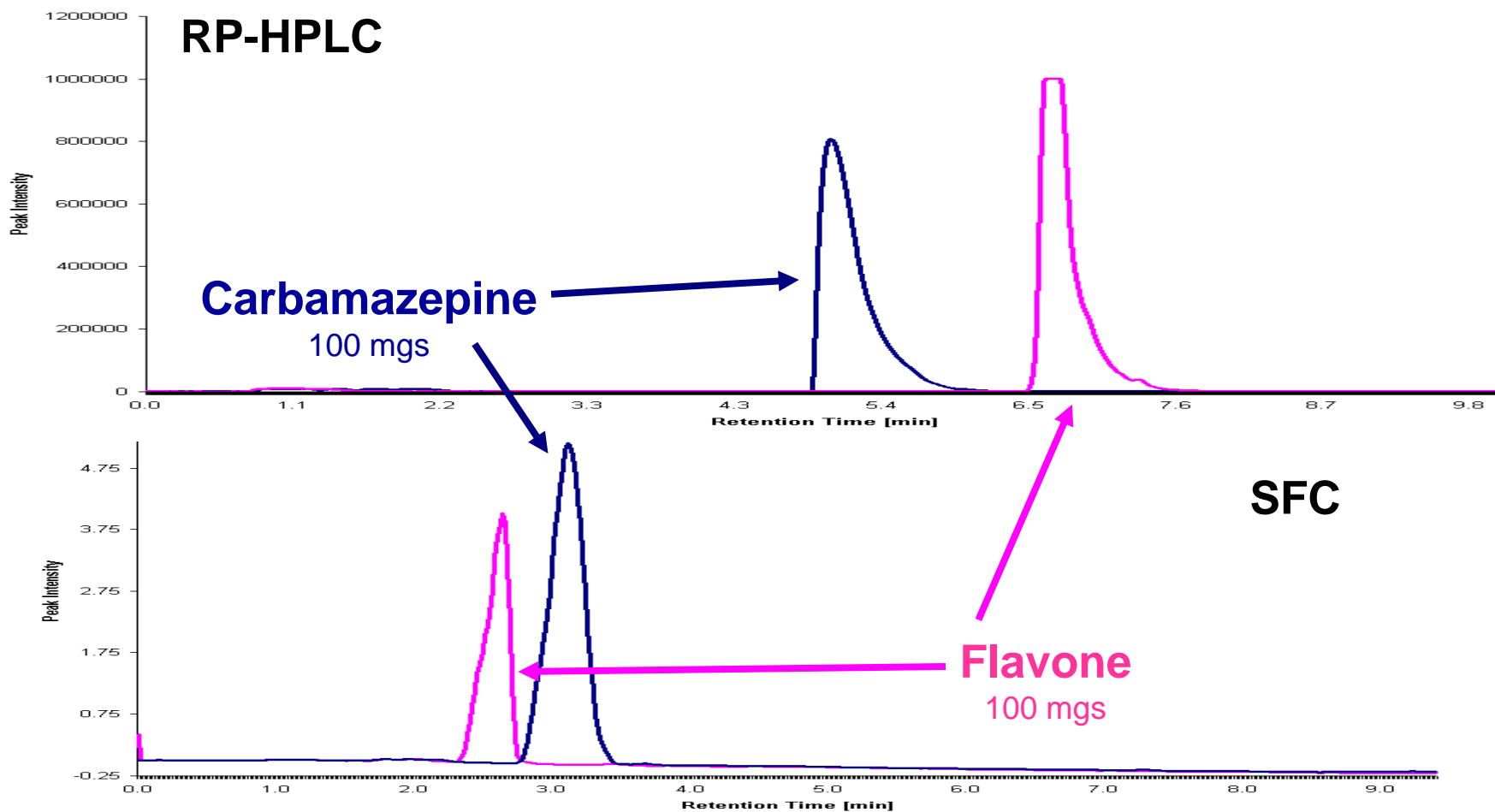
- Good results for individual compounds injected from 2 ml DMSO



- Reasonable mass loading (10-100 mg) with recoveries > 80%
 - Here shown for Coumarin, Diisopyramide

Mass loading comparison RP-HPLC vs. SFC

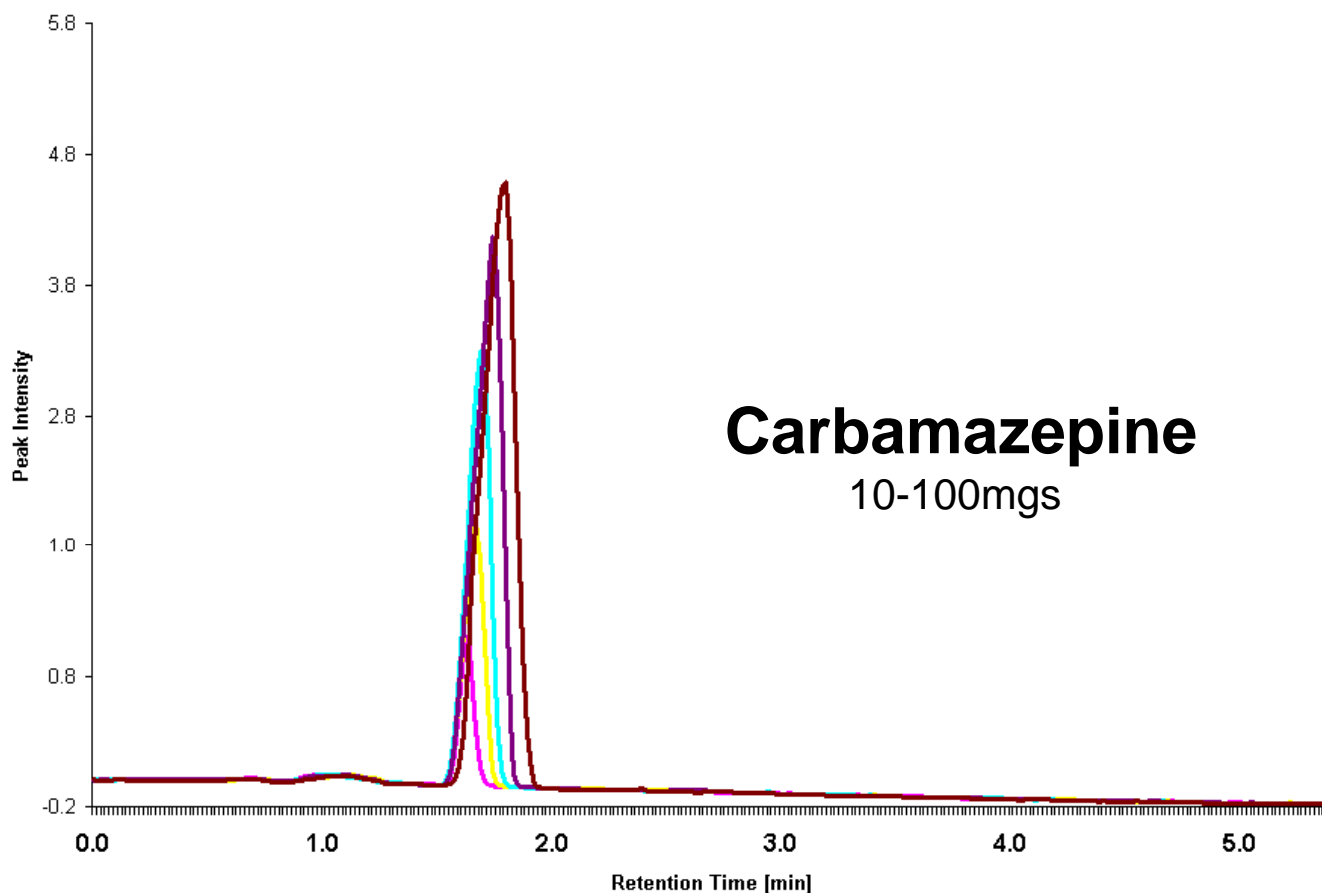
- Direct comparison, all conditions as identical as possible



- For injections from DMSO similar performance for RP and SFC
 - Comparable peak-width, however SFC has smaller final fraction volume!

Fast semi-preparative achiral SFC purifications

- Leveraging the intrinsic advantages of SFC (Flow = 80 ml/min)



- Achiral SFC purifications should be possible in times of 2-3 minutes
 - Flow capabilities of prep. SCF/MS systems may need enhancement

Summary

- **Use of DCM/methanol and THF/methanol co-solvents can improve peak shape and increase productivity for preparative SFC separations**
- **SFC seems promising as a separation technology for achiral purifications directed by MS**
- **Standard 20 mm i.d. columns provide reasonable mass loading capabilities for SFC separations (comparable to RP-HPLC)**
- **Leveraging the intrinsic capabilities of SFC, may require increased flow-rate capabilities**

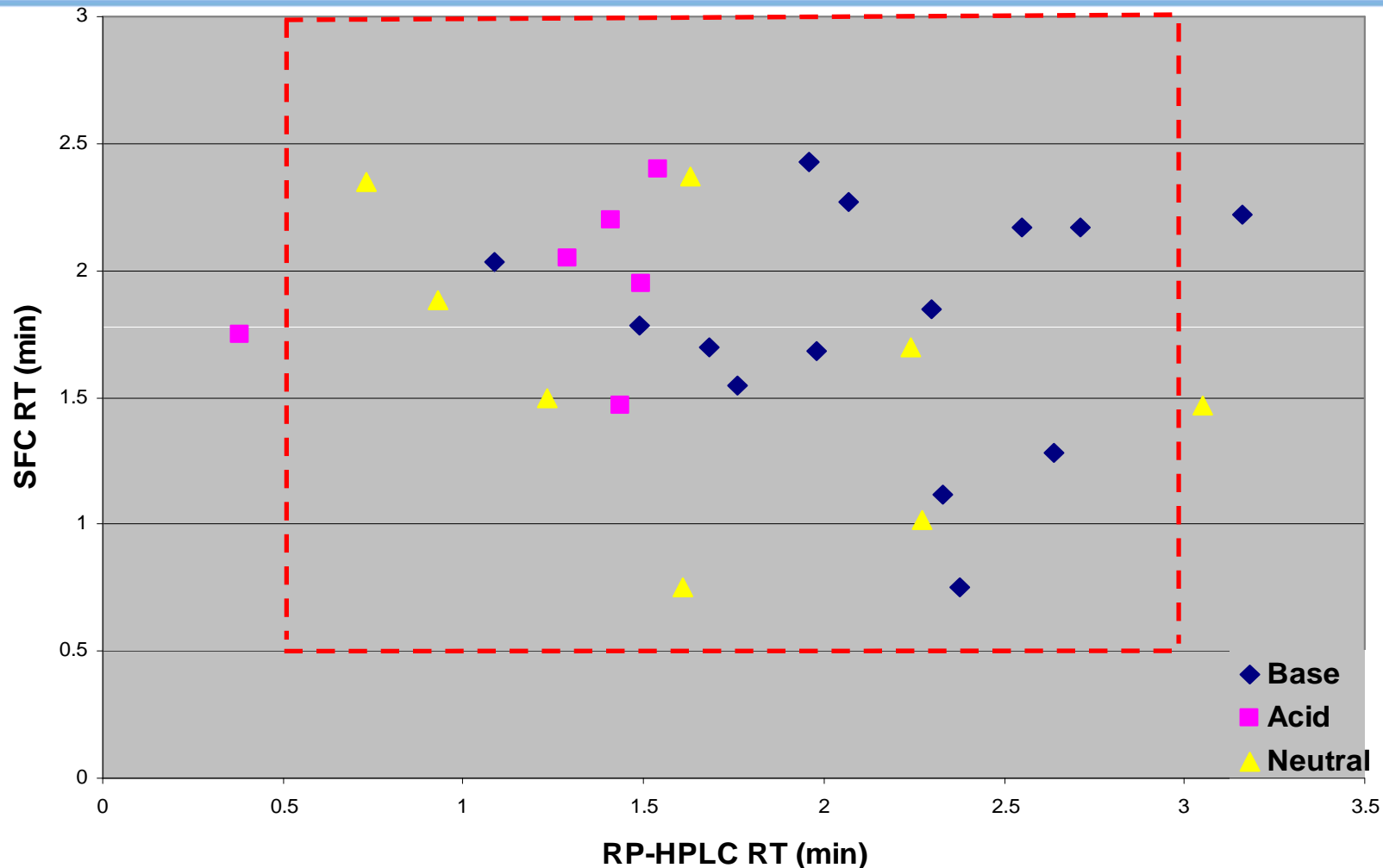
Acknowledgements

- **Jeff Moy (Northeastern University, Co-op student)**
- **Ken Charest, Dan Cinicola (Amgen Research Automation & Technologies)**
- **Discovery Analytical Science Staff (DAS) at Amgen Thousand Oaks and San Francisco**

Extra slides

How comparable are “generic gradients”

- SFC vs. RP-HPLC

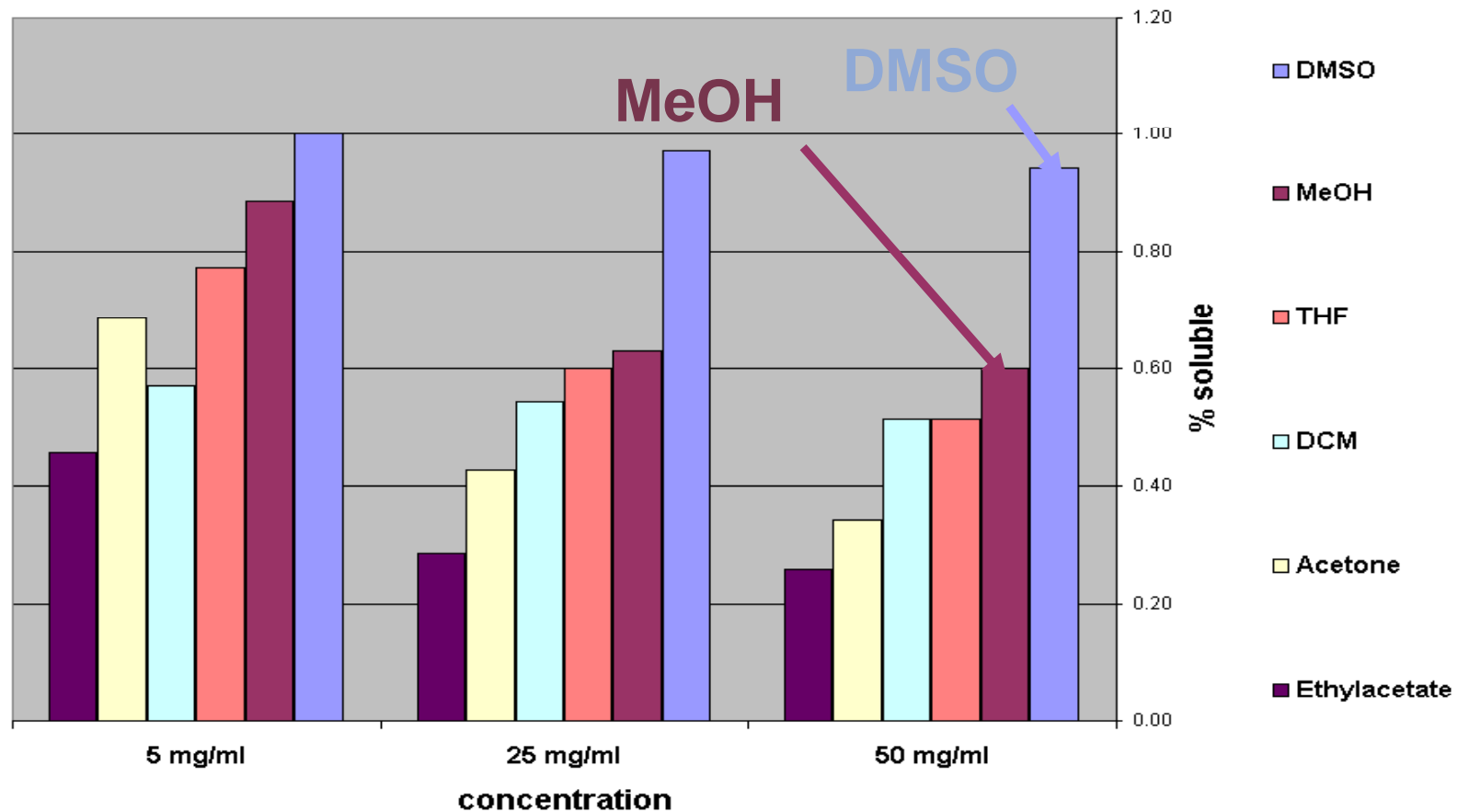


- SFC and RP-HPLC are highly orthogonal
 - Both “generic” gradients provided comparable elution ranges

Solubility of Samples

- Various Solvents and Concentrations

- Solubility is the major limiting factor in preparative chromatography



Commercial and Amgen Racemates

