

# Evaluation of Operating Conditions in Analytical and Preparative SFC

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# Agenda

- Introduction
- Preparative SFC Injection Evaluation
- Evaluation of co-solvent for Analytical Chiral Resolution
- Evaluation of co-solvent on Analytical Efficiency

# Introduction

- **SFC History**
  - Initial growth in the '80s (capillary SFC)
  - Resurgence in '90s (packed column)
- **Majority of users are in industry**
  - Mostly application based research
  - Little fundamental research

# Preparative SFC Injection Techniques

- Two approaches currently used
  - Modifier stream injection™ (Berger/Mettler-Toledo)
  - Mixed stream injection
- Berger presented data showing modifier stream injection™ better than mixed stream injection
  - Achiral examples under gradient conditions
  - Do same trends hold under isocratic conditions and for chiral separations?

# Modifier Stream Injection

Berger SFC MultiGram™

Flow Rate: 50 mL/min

Pressure: 100 bar

Temperature: 35 °C

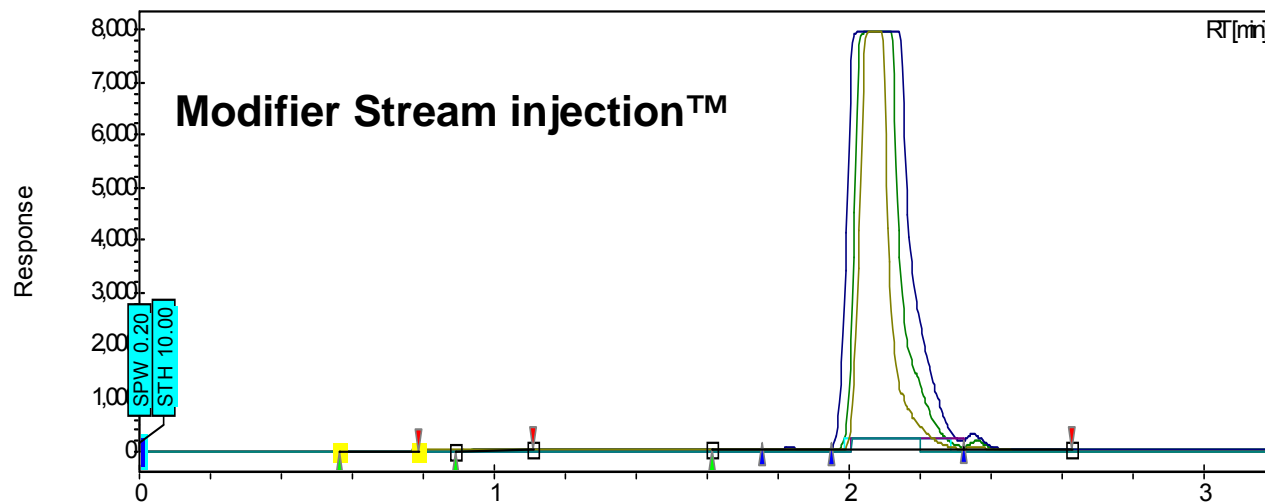
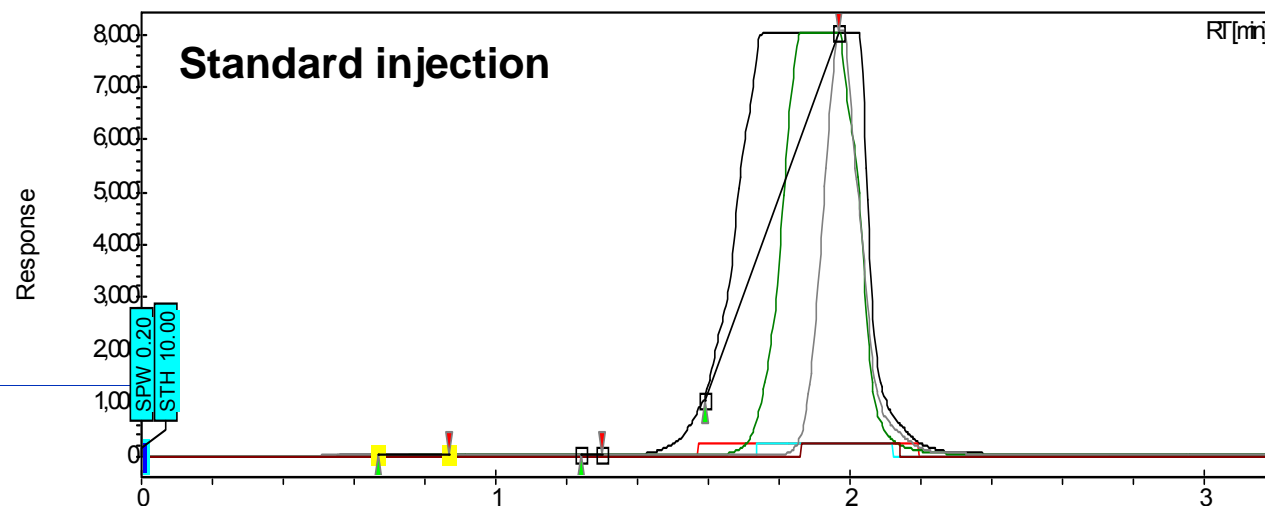
Gradient: 20% to 50% MeOH at  
10%/min

Column: 21.2 x 150 mm,  
Diol, 6 µm, 60 Å

Sample: Sulfanilamide  
conc. 5 mg/mL in MeOH

Injections: 0.5 mL, 1.0 mL and  
1.8 mL

Source: K. Fogelman, et al  
HPLC 2004 Poster  
communication



SFC Conference October 1, 2008

# Injection Schematics

## Mixed stream Injection



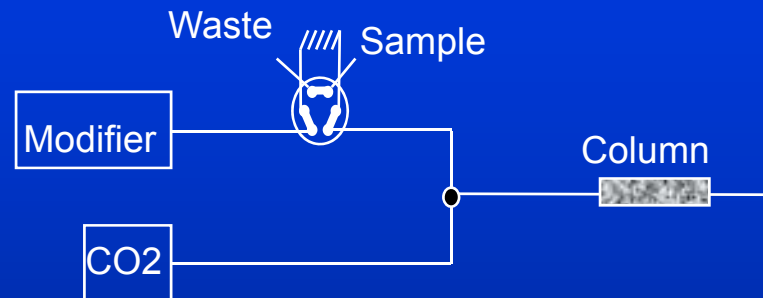
Advantage:

- Injection at head of column (no delay)

Disadvantage:

- Increased risk of sample precipitation (?)

## Modifier stream injection™



Advantage:

- Less risk of sample precipitation (?)

Disadvantage:

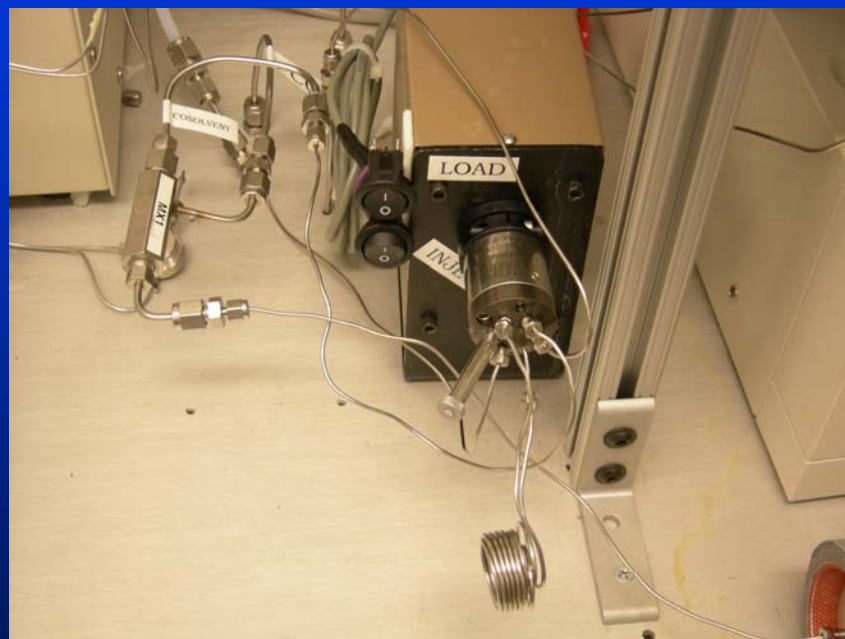
- Delay to sweep sample onto column at low co-solvent percentages

# Equipment Setup

## Mixed Stream Injection



## Modifier Stream Injection™

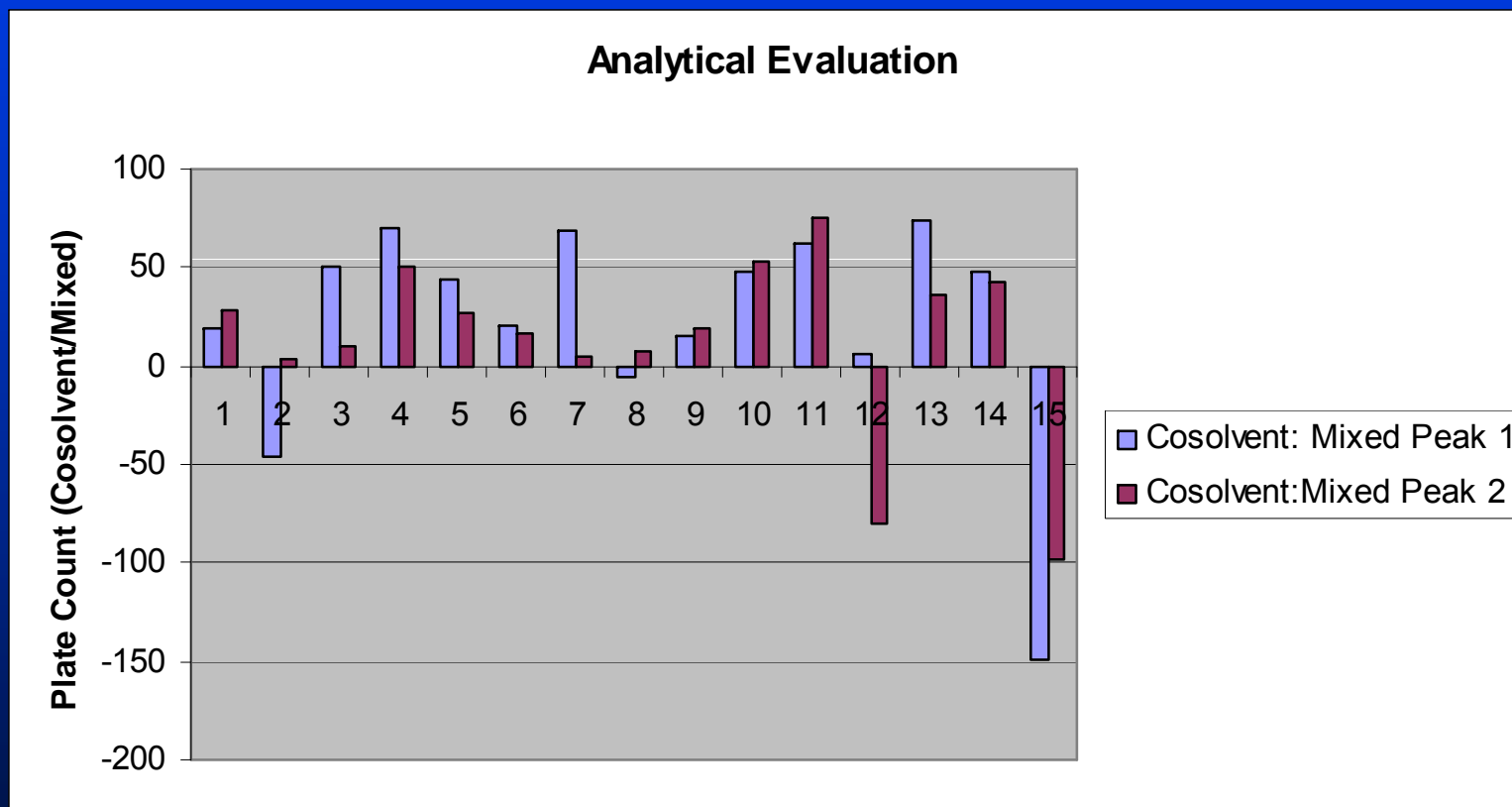


Thar SFC350, equipped with manual injector

# Analytical Evaluation

- Chiralpak AD, 3 x 25 cm, 1 mg injections
- Fifteen racemates, elution at various methanol percentages, plate counts measured
- Results
  - Five racemates showed no difference between injection techniques (<25% difference)
  - Eight racemates were better with modifier stream injection™
  - Two racemates were better with mixed stream injection

# Analytical Evaluation



# Preparative Injection Evaluation

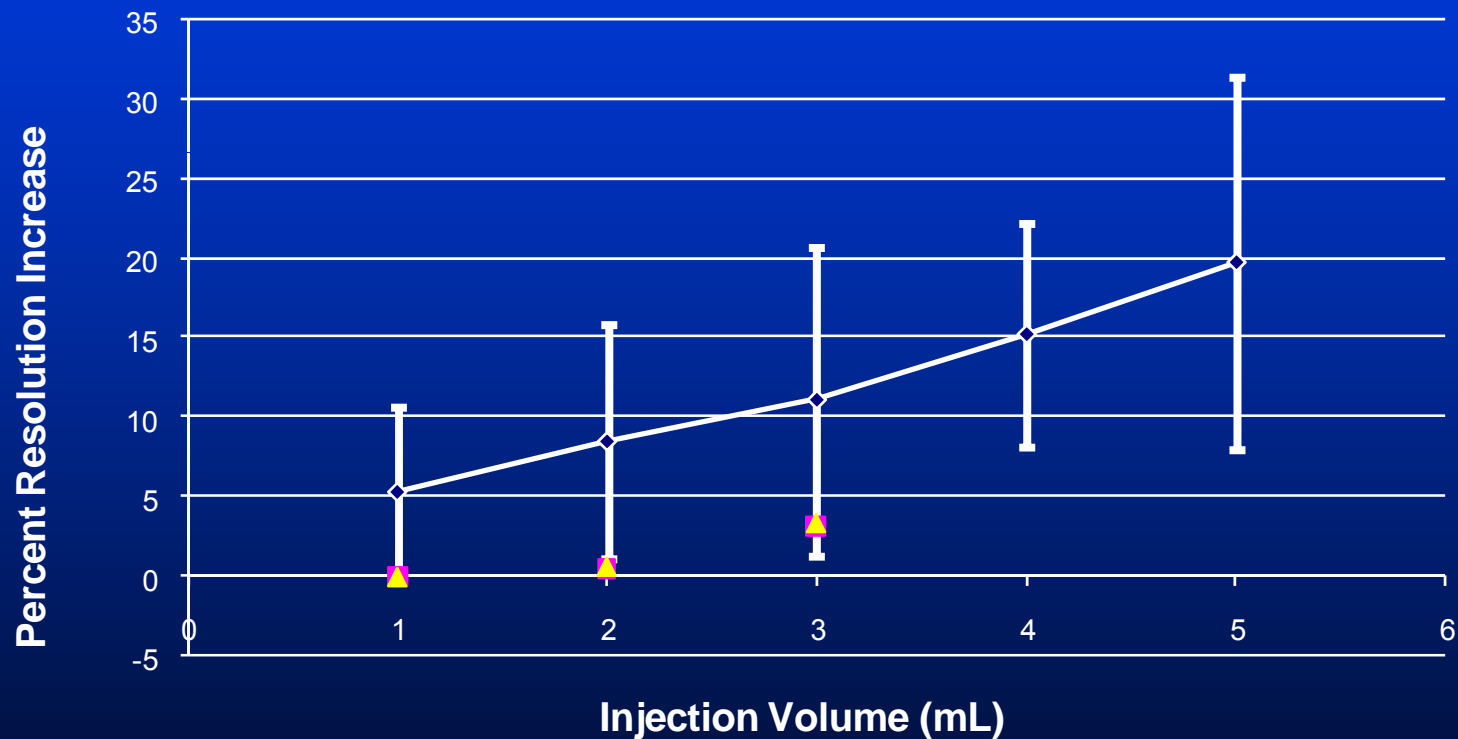
- Chiralpak AD, 3 x 25 cm, 1-5 ml injections of 20 mg/ml solution
- Elution at varied methanol percentages
  - Maintain elution at 1-3 minutes
- Flowrate: 127 ml/min (0.38 cm/sec)
- Resolution measured for each injection

# Preparative Injection Results

<b>Modifier stream injection better across all loads</b>	<b>5</b>
<b>Initially the same or mixed injection better, but with increased load modifier stream injection™ had higher resolution</b>	<b>5</b>
<b>No difference between injection techniques</b>	<b>6</b>
<b>Initially the same or modifier stream injection™ better, but with increased load mixed injection had higher resolution</b>	<b>2</b>
<b>Mixed injection better across all loads</b>	<b>0</b>

# Preparative Results

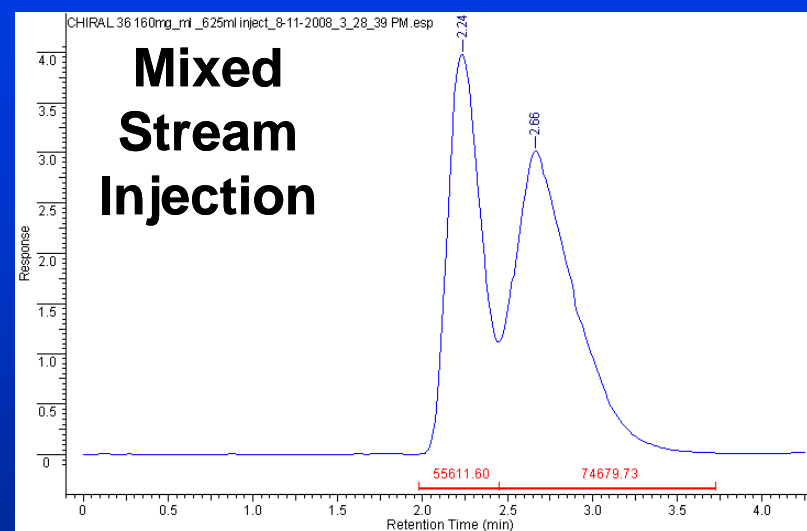
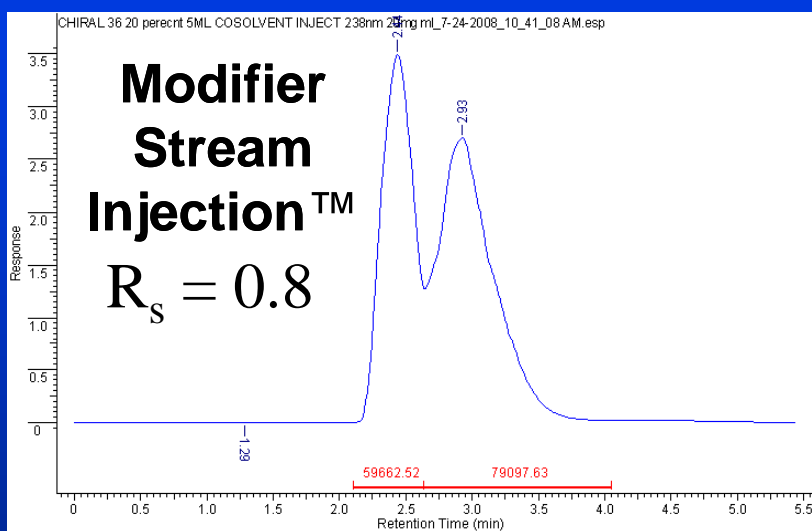
Increase in Resolution: Modifier Stream to  
Mixed Stream Injection™



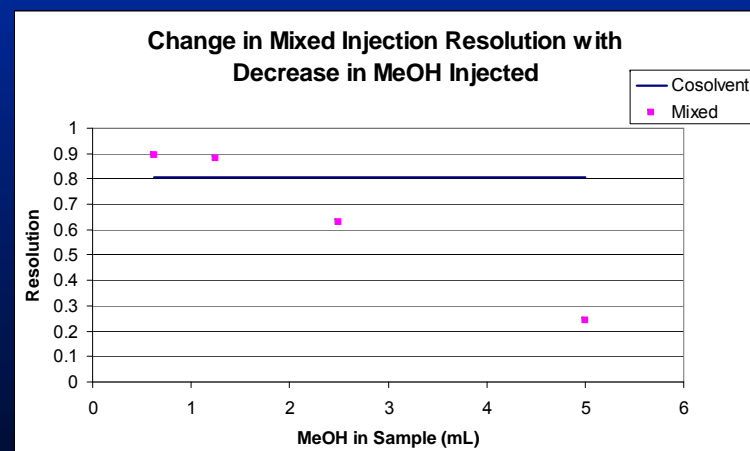
## Preparative Injection Results (cont.)

- With mixed injection technique the temporary, localized increase in methanol due to sample injection could be causing peak distortion
- Injection amount kept constant (100 mg), dissolution volume decreased (5 ml to 0.625 ml)
  - Resolution increased with decreasing injection size
  - Comparable resolution seen for modifier stream injection™ @ higher injection volumes

# Chiral Results: Prep



Injection Volume	Concentration	Resolution
5 mL	20 mg/mL	0.244
2.5 mL	40 mg/mL	0.629
1.25 mL	80 mg/mL	0.882
0.63 mL	160 mg/mL	0.893



# Injection Evaluation Summary

- In majority of cases modifier stream injection™ gave higher resolution vs. mixed stream injection
  - Effect more pronounced as injection volume increases
- No trends seen with regard to percent co-solvent or retention
  - In the range we investigated; may be different at low organic modifier

# Evaluation of Co-Solvent for SFC Chiral Resolution

- **SFC chiral method development screen**
  - Chiralpak AD, AS, Chiralcel OD, OJ
  - Methanol, ethanol, isopropanol, acetonitrile (each with 0.2% diethylamine), gradient
- **Which modifier gives best results**
  - 197 racemates (Amgen and commercial)
  - Sixteen analyses per racemate, 3152 experiments
  - Results evaluated based on difference in retention times of two enantiomers

## Co-solvent Results

Co-Solvent	Percent of Total
Acetonitrile	29.0
Isopropanol	19.5
Methanol	17.3
Ethanol	10.2

Unexpected results: Acetonitrile often used as co-solvent of last resort

# Co-Solvent Correlation

	All CSP	AD	AS	OD	OJ
MeOH to EtOH	0.65	0.42	0.65	0.48	0.68
MeOH to IPA	0.39	0.15	0.59	0.44	0.42
MeOH to ACN	0.20	0.04	0.28	0.25	0.54
EtOH to IPA	0.51	0.26	0.81	0.87	0.42
EtOH to ACN	0.23	0.05	0.25	0.43	0.56
IPA to ACN	0.32	0.10	0.31	0.53	0.56

- Highest correlation seen between methanol and ethanol
- Lowest correlation between methanol and acetonitrile
- Ignores potential for reversal of elution order

## Co-Solvent Summary

- 99% of Amgen racemates resolved
- 90% of commercial racemates resolved
- Commercial racemates more diverse than Amgen racemates

CSP/Co-solvent	% Best $\Delta R_t$
AD	38
AS	19
OD	20
OJ	18
MeOH	24
EtOH	16
IPA	28
ACN	27
None	5

CSP	Co-Solvent	% of Total (all)	% of Total (Amgen)	% of Total (commercial)
AD	MeOH	11.3	13.2	8.4
AD	EtOH	11	11.3	10.6
OD	ACN	10.7	13	7.5
AD	IPA	9.8	12.4	5.9
AS	IPA	8.6	6.7	11.4
OJ	IPA	7.1	7.5	6.6
AD	ACN	5.5	4.1	7.5
OJ	ACN	5.4	0.9	10
AS	ACN	5.1	4.1	6.6
OD	MeOH	4.8	4	6
AS	MeOH	4.2	4.3	4.2
OJ	MeOH	4	4.8	2.9
OD	IPA	2.8	2.9	2.6
OD	EtOH	2.2	2	2.6
AS	EtOH	1.4	0.3	3.1
OJ	EtOH	1.4	1.4	1.6
No sepn	N/A	4.6	0.9	10



One data point per racemate

SFC Conference October 1, 2008

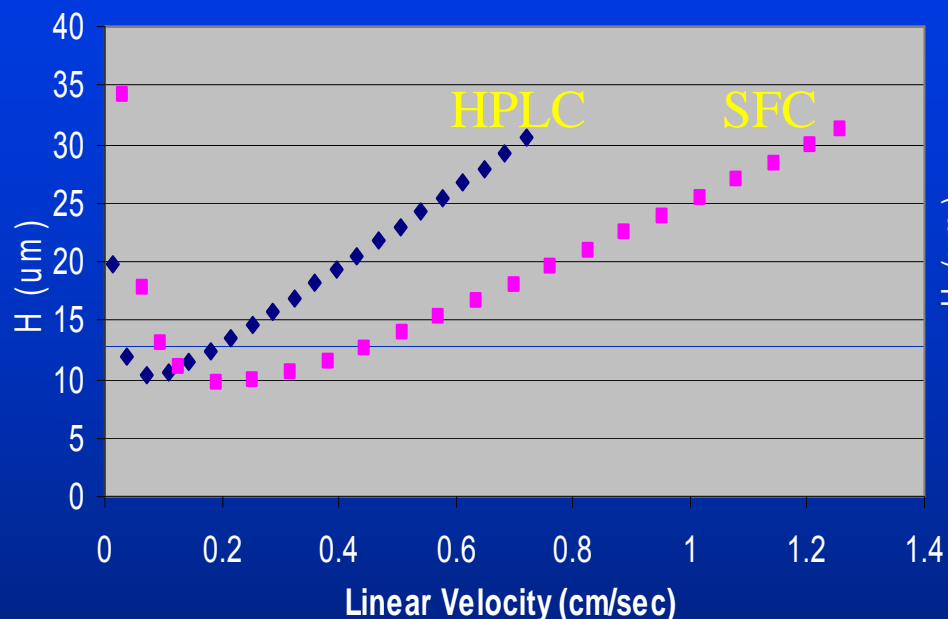


# Evaluation of Co-Solvent on Analytical Efficiency

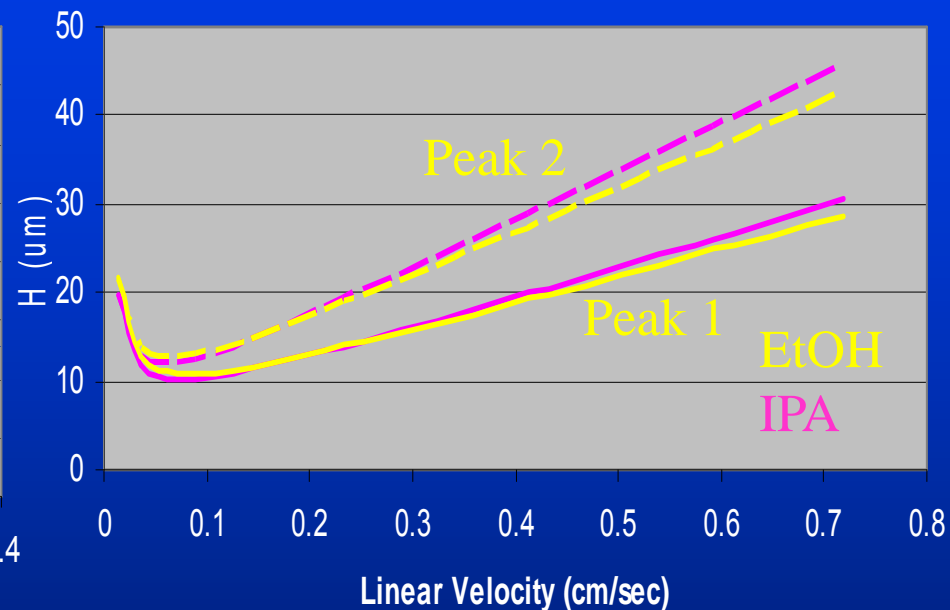
- Co-solvent study showed acetonitrile often gave greatest chiral separation
- When ACN methods scaled to preparative, loadings were lower than expected
- To achieve same retention ACN percentages are often higher than MeOH
  - What impact does this have on analytical efficiency?

# Van Deemter Curves

## Van Deemter HPLC vs. SFC



## Van Deemter-HPLC-IPA-EtOH



- Van Deemter curve shows higher efficiency for SFC at higher flowrates relative to HPLC
- Little difference in Van Deemter curve for IPA/heptane vs. EtOH/heptane (EtOH > IPA)
  - Is same trend seen with SFC co-solvents?

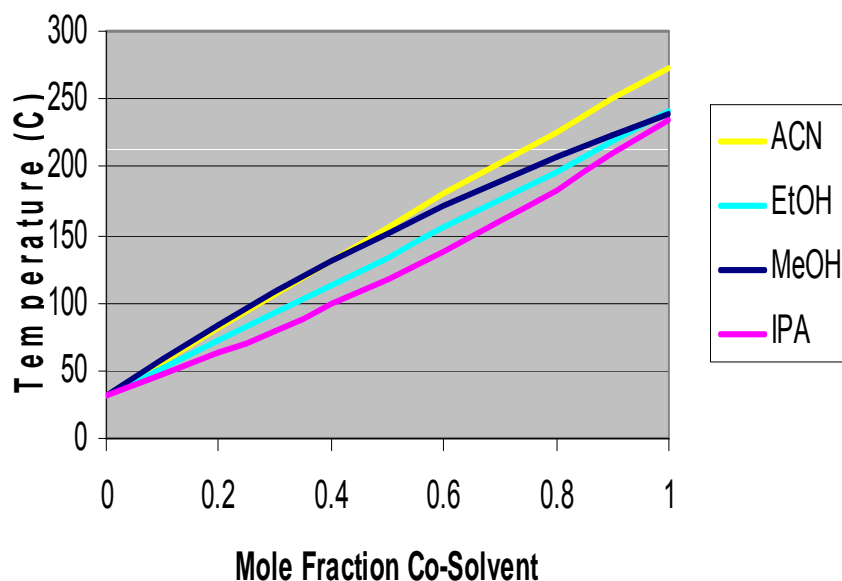
# Critical Points

	Temp (C)	Pressure (bar)
CO <sub>2</sub>	31	74
IPA	236	54
EtOH	241	61
MeOH	239	81
ACN	272	49

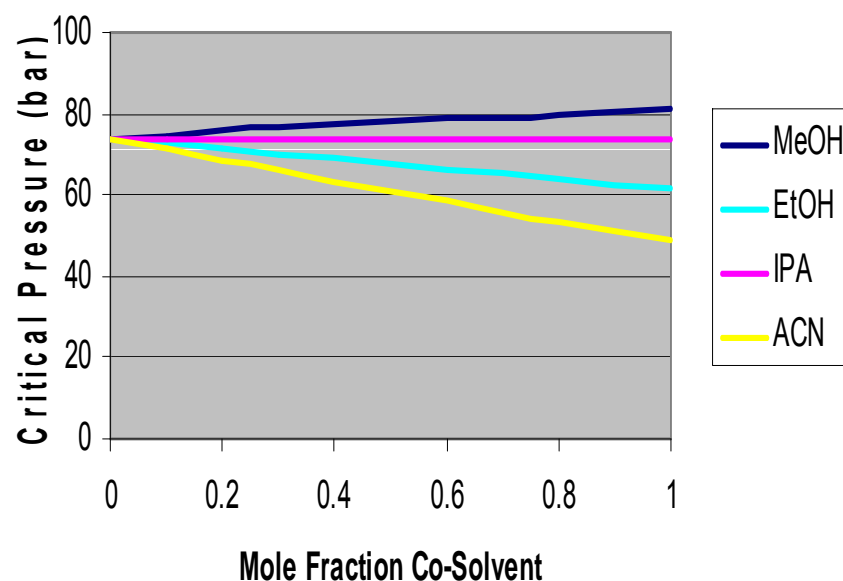
- Usually operate in sub-critical mode
- Each co-solvent has different critical temperature and pressure
- How close to “supercritical” one operates will change with type and percent co-solvent
- What effect does this have on analytical efficiency and preparative resolution?

# Critical Point Changes With Co-Solvent Percentage

Critical Temp vs. Mole Fraction Co-Solvent



Critical Pressure vs. Mole Fraction Co-Solvent

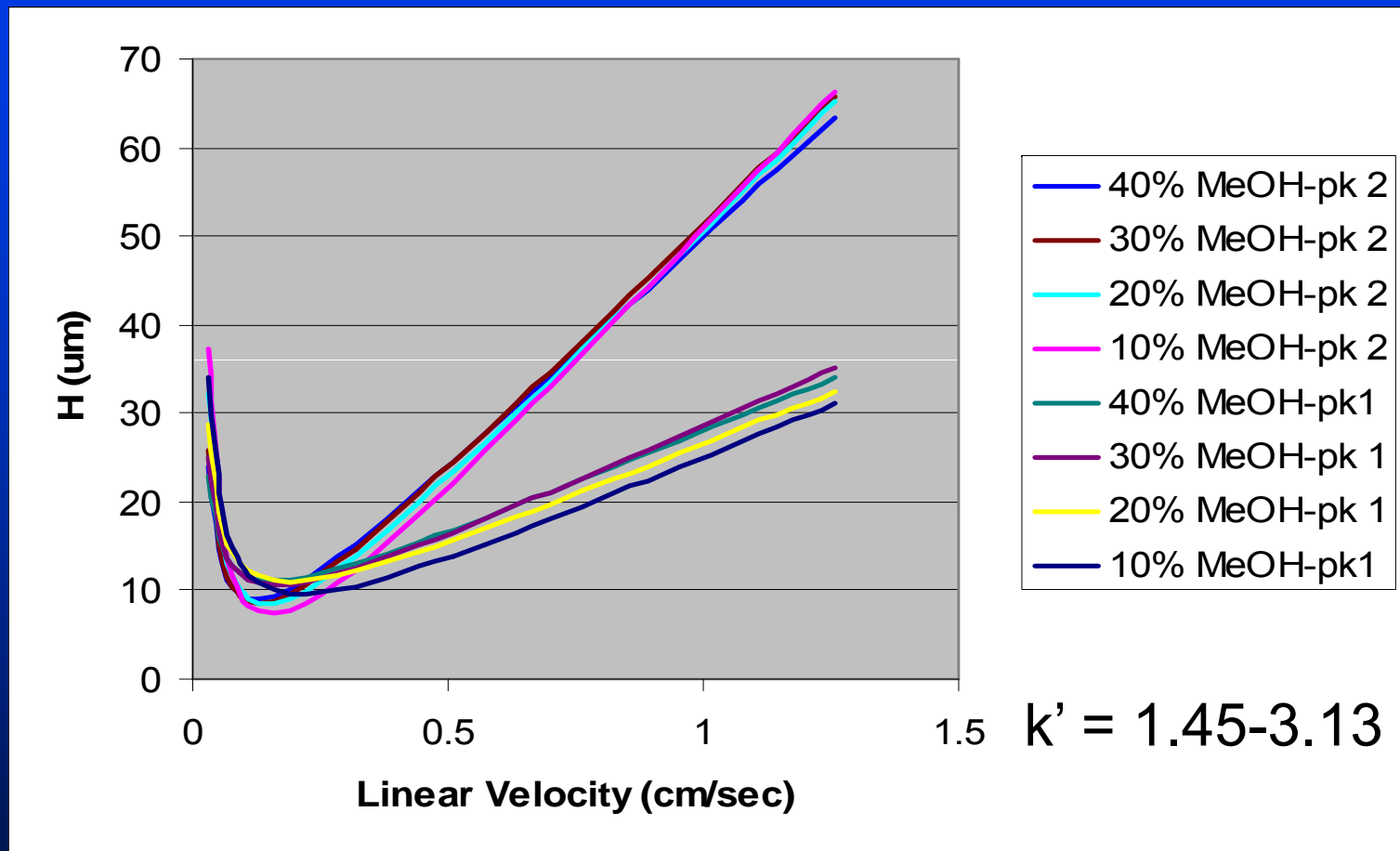


The critical point of a binary mixture can be estimated as the arithmetic mean of the critical temperatures and pressures of the two components

# Experimental

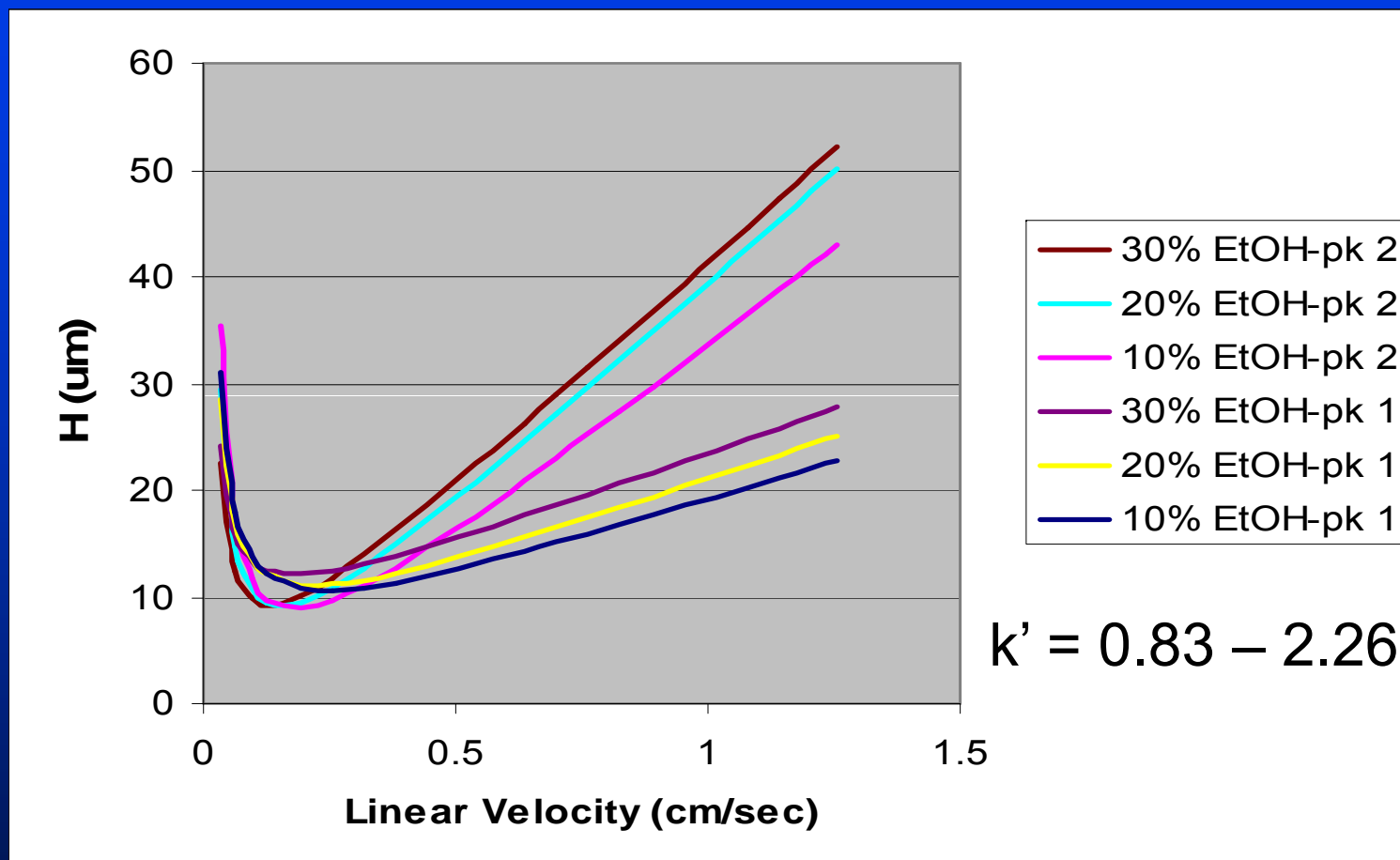
- Chiralpak AD, 5  $\mu$ , 4.6 x 150 mm
- Co-solvents studied
  - Methanol (10, 20, 30, 40%)
  - Ethanol (10, 20, 30%)
  - Isopropanol (10, 20, 30%)
  - Acetonitrile (10, 20, 30%)
- Flowrates from 0.25 to 9.9 ml/min
- Trans stilbene oxide (TSO)

# Van Deemter Curves - Methanol



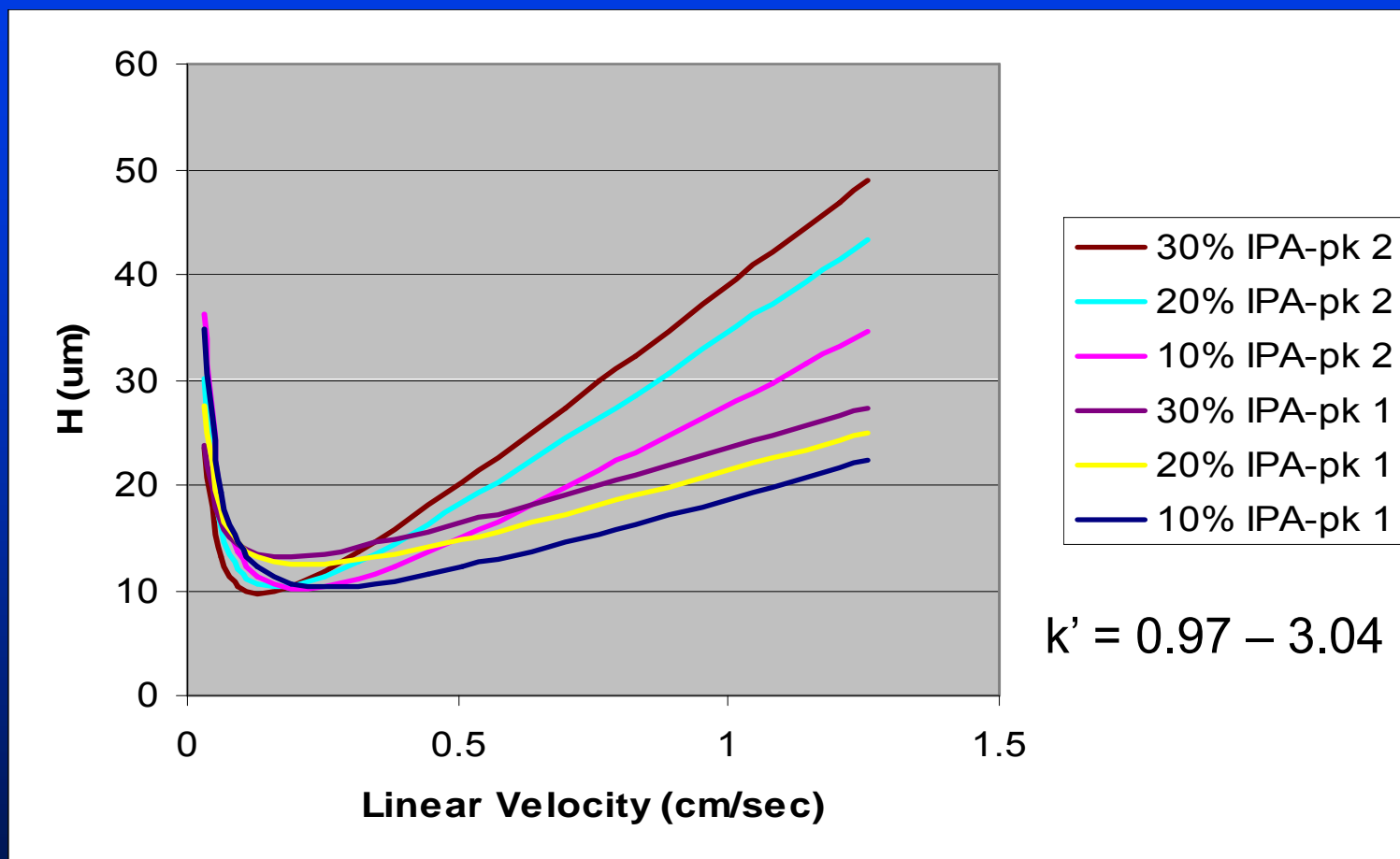
Little impact on efficiency with increasing percent MeOH

# Van Deemter Curve - Ethanol



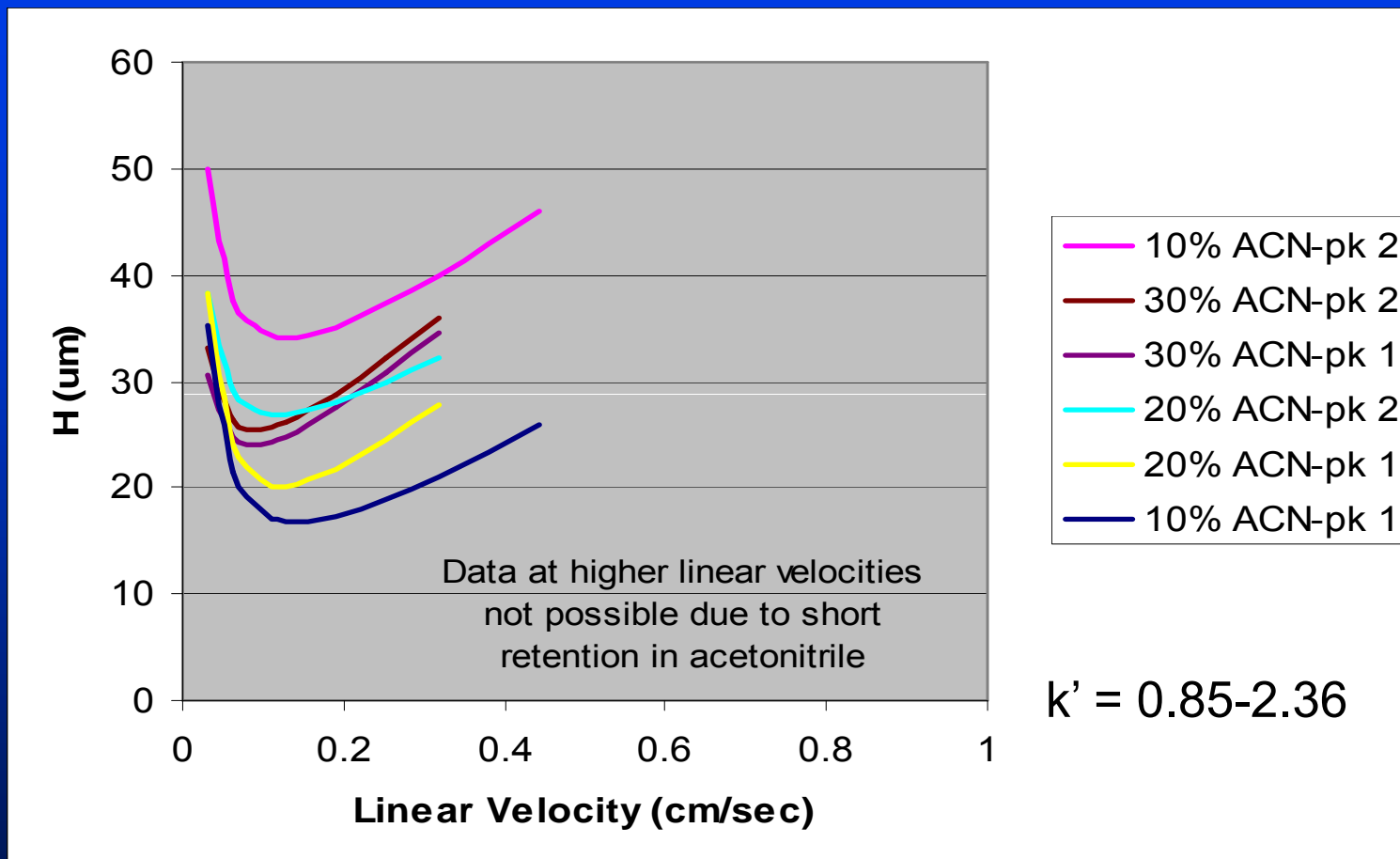
Efficiency decreased with increasing EtOH percentage

# Van Deemter - Isopropanol



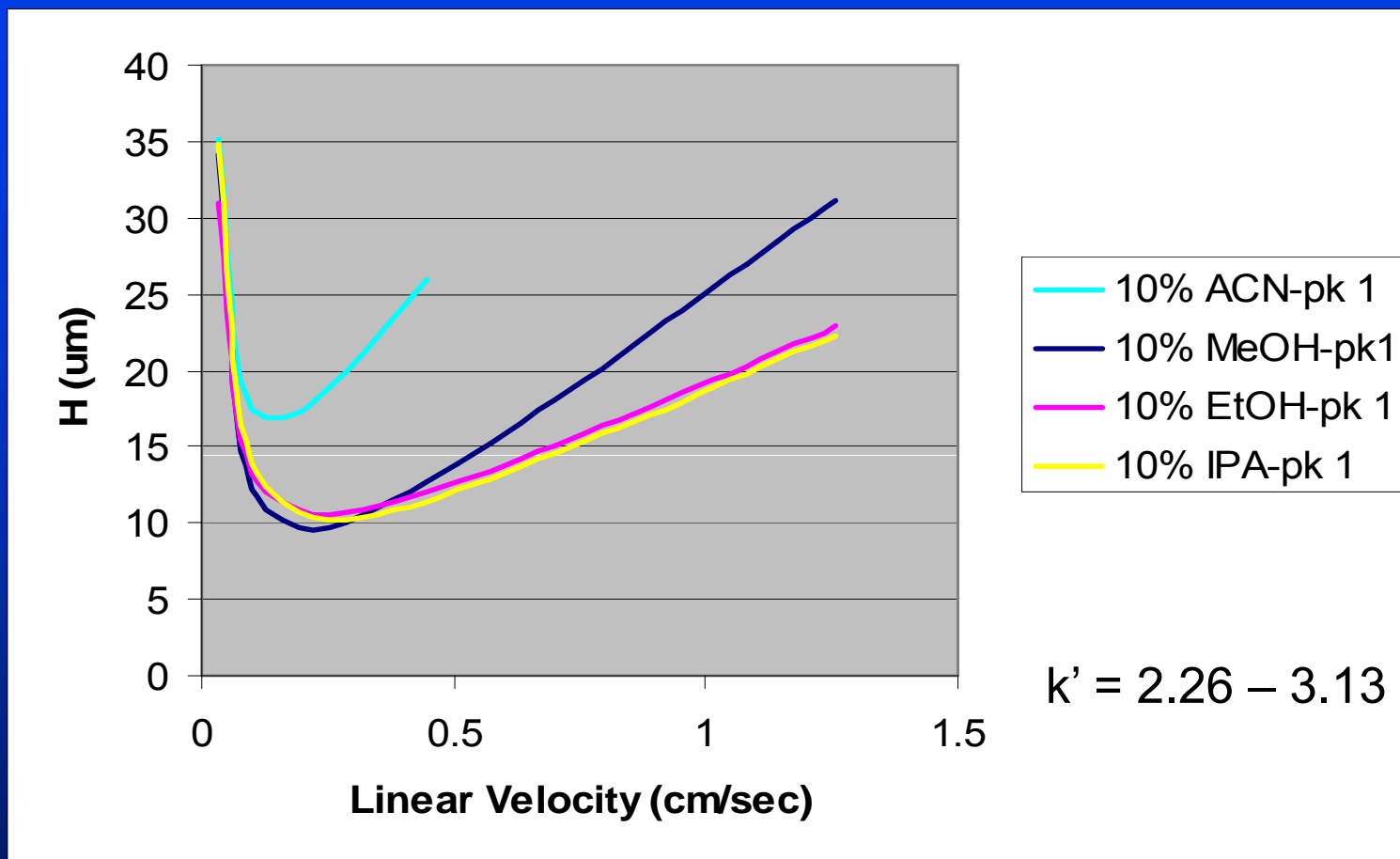
Efficiency decreased with increasing IPA percentage

# Van Deemter - Acetonitrile



Efficiency decreased with increasing ACN percentage  
Poor efficiency for Peak 2 @ 10% ACN

# Van Deemter – 10% Co-Solvent



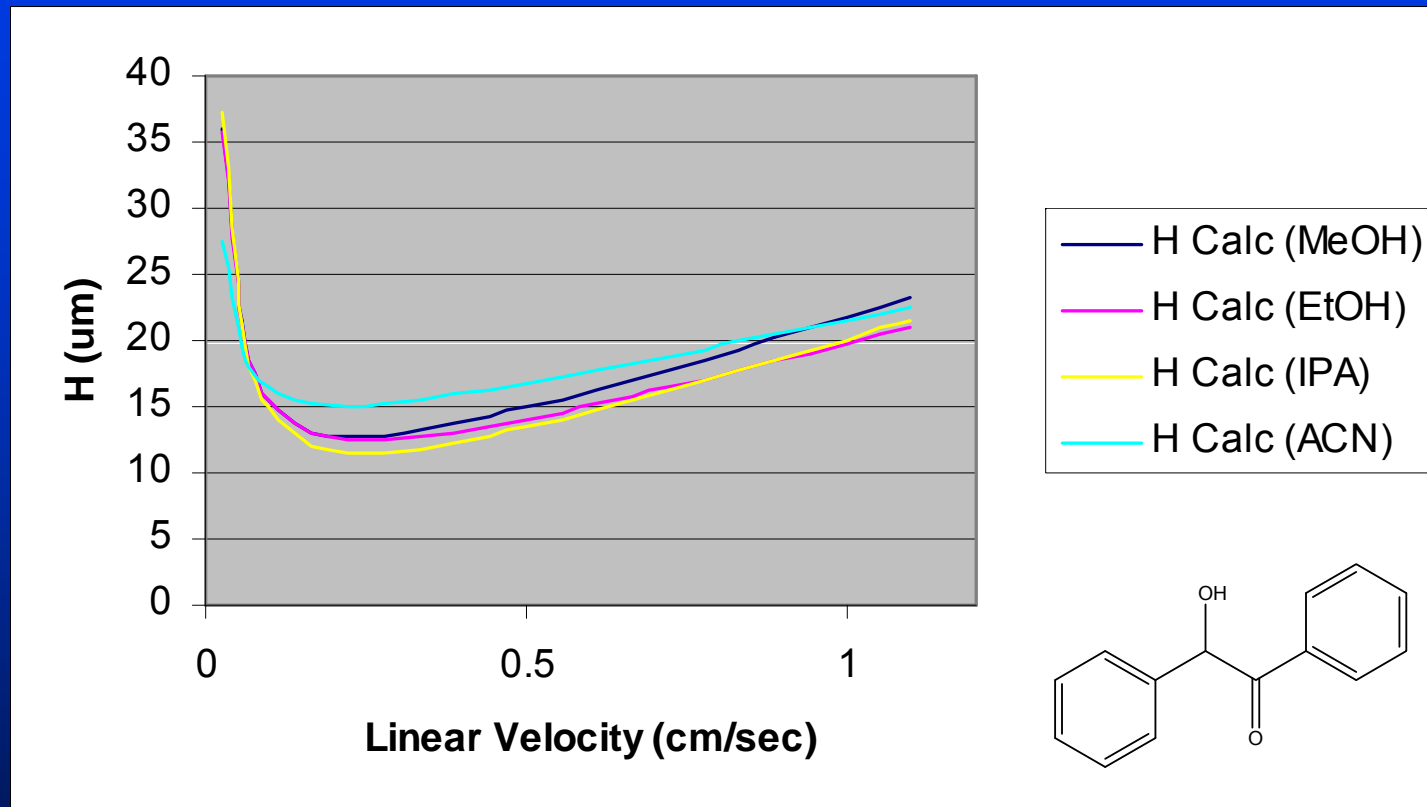
Efficiency approximately the same at lower linear velocity for MeOH, EtOH and IPA

Efficiency lowest with acetonitrile co-solvent

# Acetonitrile Results

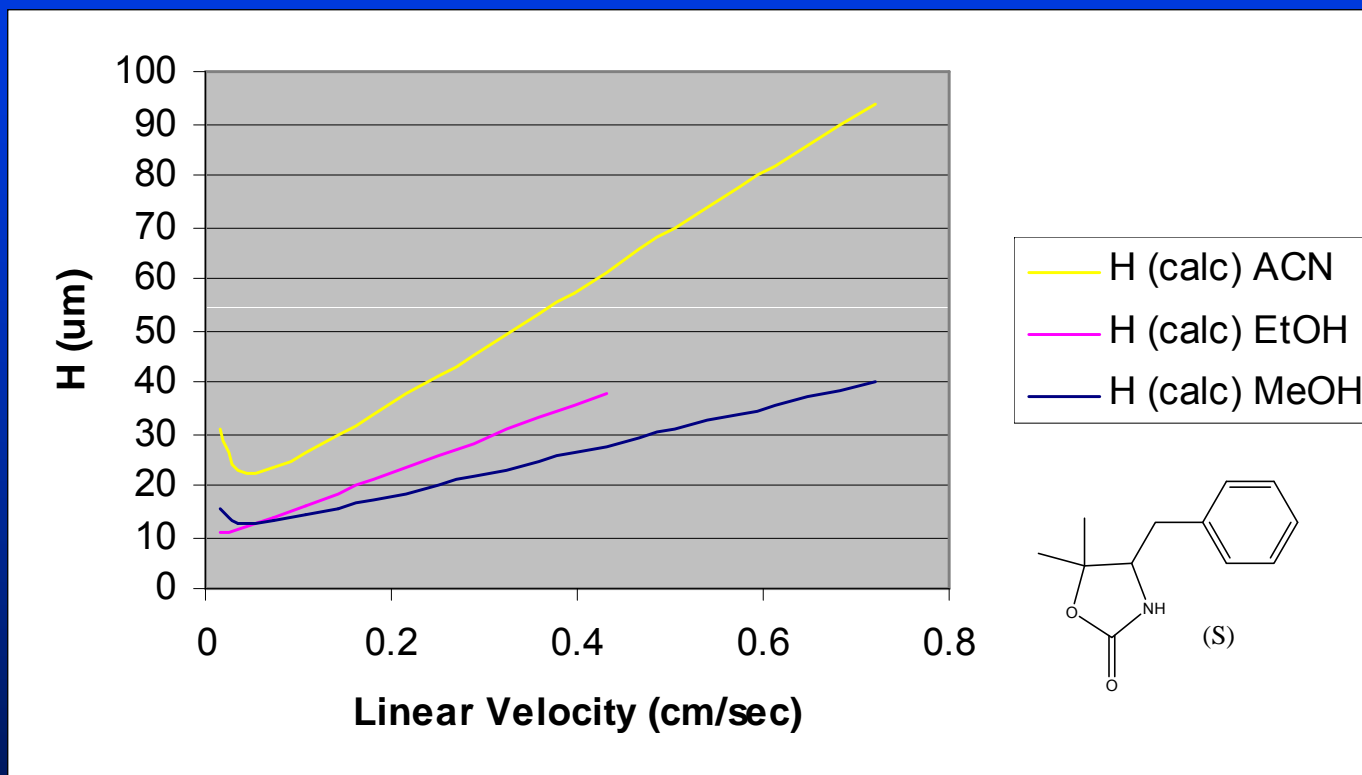
- **Why does acetonitrile give lower efficiencies than other SFC co-solvents for chiral separations?**
  - **SFC effect**
    - Investigate acetonitrile with achiral column
  - **Chiral stationary phase effect?**
    - Investigate acetonitrile with CSP in HPLC (polar organic mode)

# SFC: Acetonitrile/Achiral



Column: 2-EP, 4.6 x 100 mm, 5 micron  
Compound: Benzoin

# HPLC: Polar Organic



Column: Chiralpak AD, 4.6 x 150 mm, 5 micron

Compound: (S) 4-Benzyl-5,5-dimethyl-2-oxazolidinone

# SFC Efficiency Summary

- MeOH, EtOH, IPA showed comparable efficiencies with ACN significantly poorer
- Initial results point to CSP interactions with acetonitrile vs. acetonitrile effect in SFC
- Further studies needed to confirm results
  - Evaluate additional racemates (achiral and chiral)
  - Evaluate increased temperature
  - Compare methanol vs. acetonitrile co-solvents for preparative separations

# Summary

- **Preparative Injection Techniques**
  - Co-solvent injection often better than mixed stream injection, especially for large volume injections
- **Co-solvent for SFC chiral resolution**
  - Acetonitrile often gave best separation of enantiomers ( $\Delta R_t$ )
  - Greatest correlation was seen between methanol and ethanol and between ethanol and isopropanol
  - Ethanol could be dropped from screening approach with minimal impact on success

## Summary (cont.)

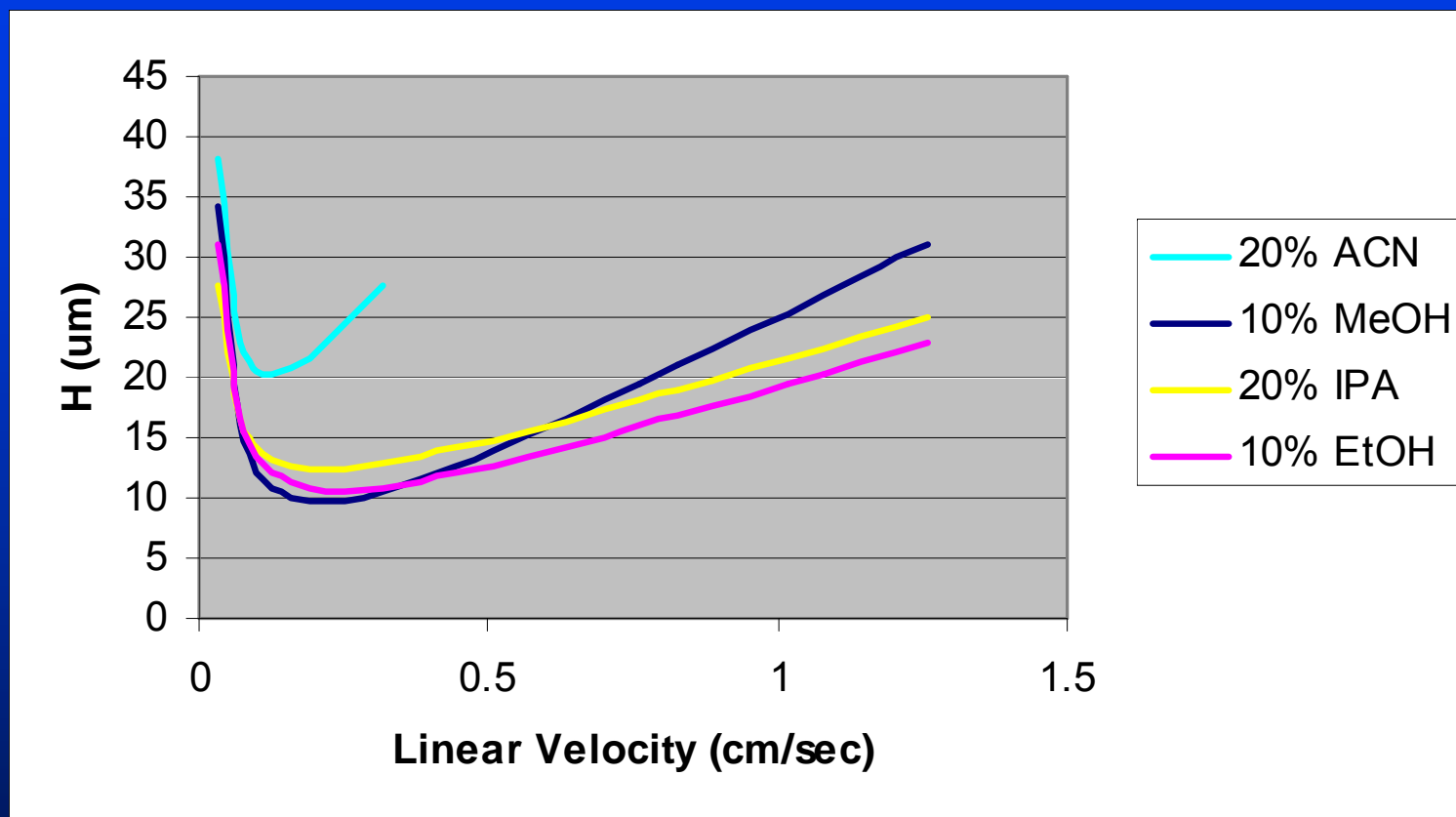
- **Effect of SFC co-solvent on analytical efficiency**
  - Percent methanol has least effect on efficiency
  - Efficiency decreases with increasing percent ethanol, isopropanol and acetonitrile
  - Decreased efficiency seen with acetonitrile relative to other co-solvents
  - Lower acetonitrile efficiencies may be due to CSP interactions

# Acknowledgements

- **Amgen**
  - John Eschelbach
  - Kyung Gahm
  - Wolfgang Goetzinger
  - Manny Ventura

# Extra Slides

# Van Deemter – Co-Solvent @ Same Retention



Negligible difference between methanol, ethanol or IPA  
Efficiency significantly poorer with acetonitrile