

## Abstract:

One of the advantages of the covalently bonded Pirkle-Type chiral stationary phases (CSP's) is its compatibility with any solvent system. This can lead to using certain solvents, which may be detrimental to other CSP's, to improve solubility, resolution and therefore production rates when going to large scale separations. In this presentation employing SFC and a Whelk-O®1 CSP, we will use a dual co-solvent system which is comprised of Ethanol/CH<sub>2</sub>Cl<sub>2</sub> and compare this to a single co-solvent system comprised of Ethanol alone. Solubility as well as loading studies will be detailed on both systems to evaluate the final production rate for each of the SFC conditions.

## SFC Method Development

- SFC strategy very similar to LC
- Three variables involved in the method development process:
  - Co-solvent composition
  - Pressure
  - Temperature

## SFC Co-Solvent Systems

Ethanol  
IPA  
Methanol  
Acetonitrile  
THF  
Ethanol/CH<sub>2</sub>Cl<sub>2</sub>\*\*  
Methanol/CH<sub>2</sub>Cl<sub>2</sub>\*\*

## SFC Co-Solvent Modifiers

Acetic Acid--0.1%-0.5%  
TEA-- 0.1%-0.5%  
DEA-- 0.1%-0.5%  
TFA-- 0.1%-0.5%  
Ammonium Acetate--0.01M-0.1M

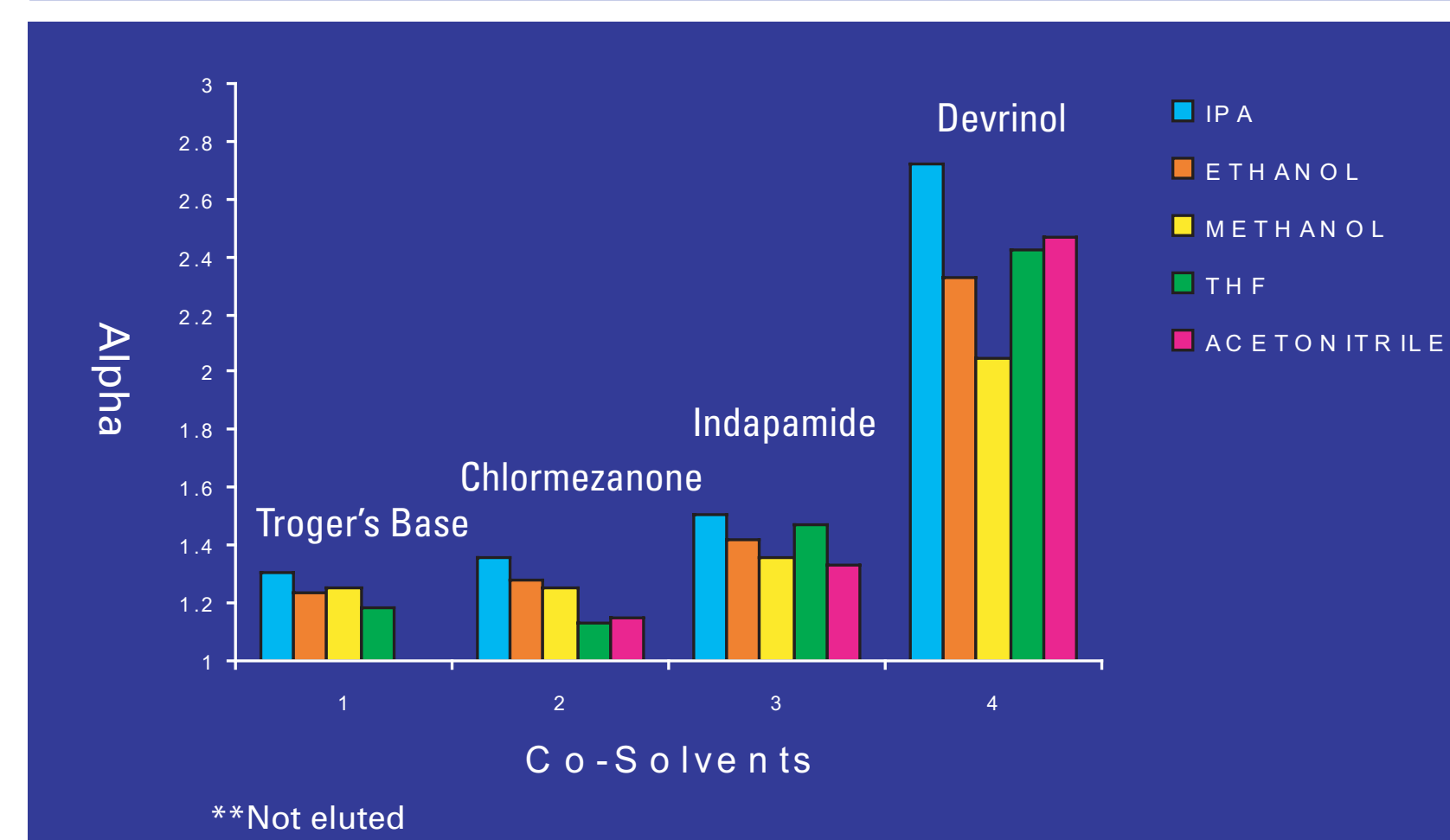
## Effect of Co-Solvent

| Co-Solvent   | Devrinol         |      | Chlormezanone    |      | Indapamide       |      | Troger's Base    |      |
|--------------|------------------|------|------------------|------|------------------|------|------------------|------|
|              | k <sub>i</sub> ' | α    | k <sub>i</sub> ' | α    | k <sub>i</sub> ' | α    | k <sub>i</sub> ' | α    |
| IPA          | 2.50             | 2.72 | 4.23             | 1.36 | 5.95             | 1.51 | 3.82             | 1.31 |
| Ethanol      | 1.66             | 2.33 | 2.75             | 1.28 | 3.47             | 1.42 | 2.85             | 1.24 |
| Methanol     | 1.36             | 2.05 | 2.16             | 1.25 | 2.48             | 1.36 | 2.30             | 1.25 |
| THF          | 1.19             | 2.42 | 5.29             | 1.13 | 4.69             | 1.47 | 4.71             | 1.18 |
| Acetonitrile | 1.70             | 2.47 | 4.12             | 1.15 | 3.33             | 1.33 | N/E*             | ---  |

\*N/E- Not eluted after 40 min  
Column: Whelk-O® 1  
10/100 25 cm x 4.6 mm  
Column Temperature: 40°C  
Back Pressure: 125 bar

\*\*THAR SFC Method Station (analytical scale SFC)

## Graphed Co-Solvent Data



## Conclusions:

Variation in the co-solvent provided an interesting array of results.

Selectivity appeared to be highest using isopropanol for all the compounds, though the trends with the other co-solvents differed among the compounds. Retention showed no uniform trend of co-solvent effect among the compounds. Focusing only on the three alcohols, it appears that increasing polarity correlates with decreasing selectivity and decreasing retention.

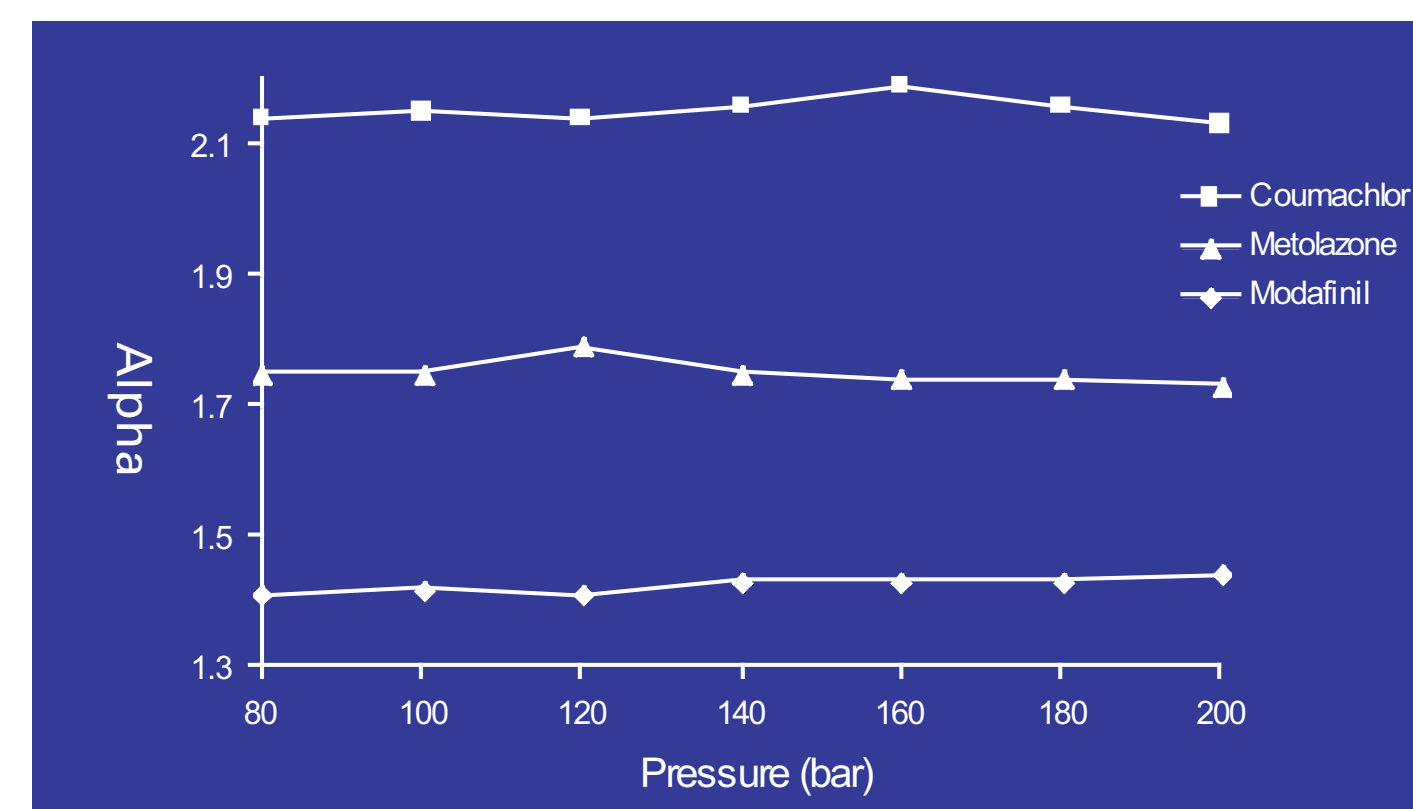
The analyst should consider evaluating more than one co-solvent when developing chiral separations on Whelk-O® 1 columns.

## Effect of System Pressure

| Pressure(bar) | Modafinil        |      | Coumachlor       |      | Metolazone       |      |
|---------------|------------------|------|------------------|------|------------------|------|
|               | k <sub>i</sub> ' | α    | k <sub>i</sub> ' | α    | k <sub>i</sub> ' | α    |
| 80            | 3.30             | 1.41 | 2.07             | 2.14 | 2.29             | 1.75 |
| 100           | 3.09             | 1.42 | 1.95             | 2.15 | 2.15             | 1.75 |
| 120           | 2.92             | 1.41 | 1.88             | 2.14 | 1.99             | 1.79 |
| 140           | 2.73             | 1.43 | 1.79             | 2.16 | 1.98             | 1.75 |
| 160           | 2.62             | 1.43 | 1.70             | 2.19 | 1.93             | 1.74 |
| 180           | 2.49             | 1.43 | 1.66             | 2.16 | 1.86             | 1.74 |
| 200           | 2.41             | 1.44 | 1.65             | 2.13 | 1.80             | 1.73 |

Column: Whelk-O® 1  
10/100 25 cm x 4.6 mm  
Co-Solvent: Methanol  
Column Temperature: 40° C

\*\*THAR SFC Method Station (analytical scale SFC)

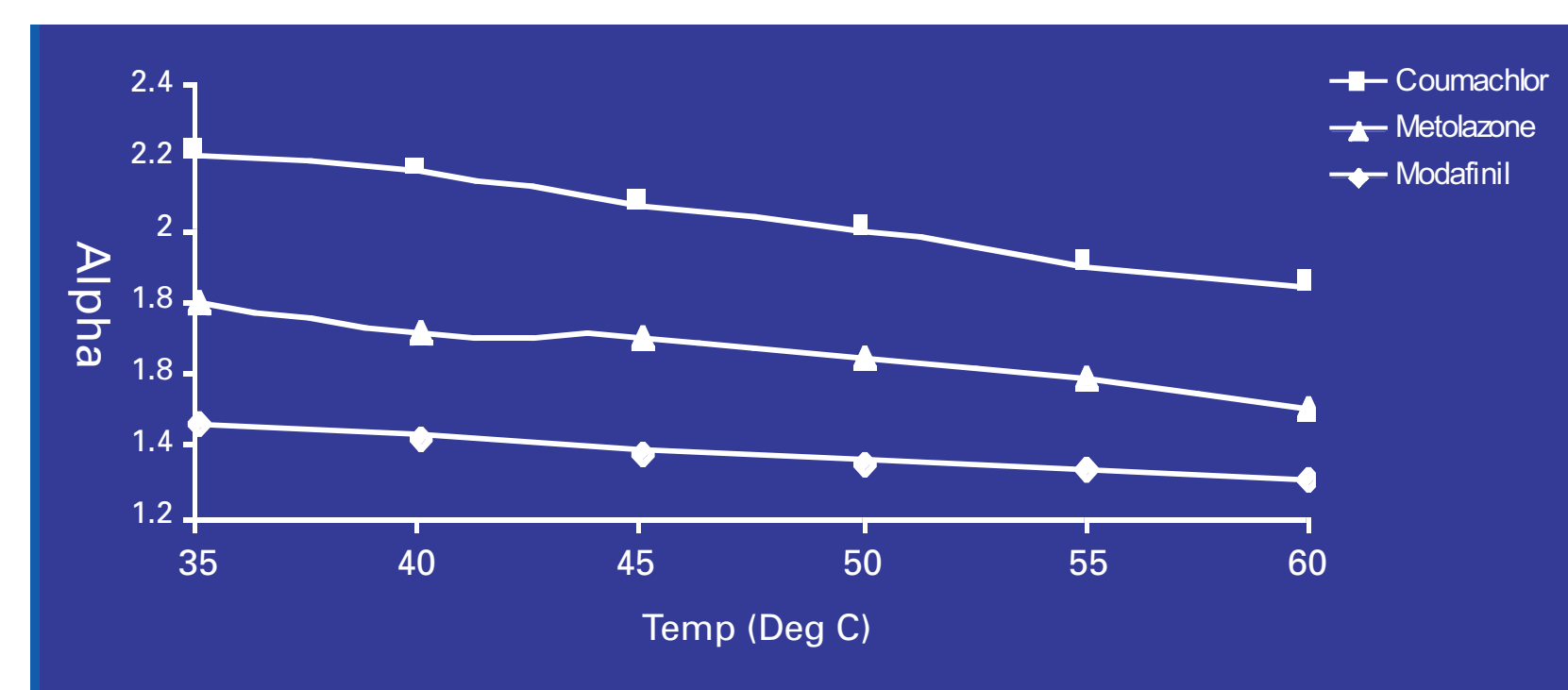


## Effect of Temperature

| Temp (°C) | Modafinil        |      | Coumachlor       |      | Metolazone       |      |
|-----------|------------------|------|------------------|------|------------------|------|
|           | k <sub>i</sub> ' | α    | k <sub>i</sub> ' | α    | k <sub>i</sub> ' | α    |
| 35        | 2.86             | 1.47 | 1.99             | 2.22 | 2.23             | 1.81 |
| 40        | 2.85             | 1.43 | 1.96             | 2.17 | 2.26             | 1.72 |
| 45        | 2.85             | 1.39 | 1.98             | 2.08 | 2.17             | 1.71 |
| 50        | 2.87             | 1.36 | 1.99             | 2.01 | 2.18             | 1.65 |
| 55        | 2.92             | 1.34 | 2.07             | 1.91 | 2.14             | 1.59 |
| 60        | 3.01             | 1.31 | 2.11             | 1.85 | 2.25             | 1.51 |

Column: Whelk-O® 1  
10/100 25 cm x 4.6 mm  
Co-Solvent: Methanol  
Back Pressure: 125 bar

\*\*THAR SFC Method Station (analytical scale SFC)



## Conclusions:

Increasing system temperature caused a modest increase in retention and a decrease in selectivity. Increasing system pressure caused a decrease in retention and no change in selectivity.

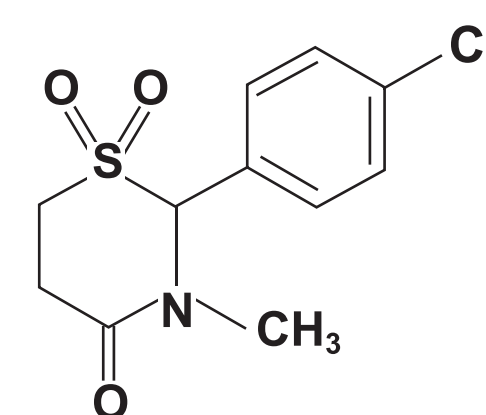
Based on the results from the three compounds evaluated, using The Whelk-O®1 column and methanol co-solvent, there is no compelling reason for employing more aggressive temperatures and pressures in order to improve chiral separation.

Keep in mind that these experiments were performed on an analytical scale. A future study will be conducted to see what effect temperature and pressure will have on solubility and loading in preparative-scale SFC.

## Utilizing Dual Co-Solvent Systems

Equipment: All work was done on a THAR SFC Method Station (analytical scale SFC)

Sample: Chlormezanone



## SFC Conditions

### System 1: Single Co-Solvent

Column: (S,S)-Whelk-O® 1  
25 cm x 4.6 mm  
Co-Solvent: Ethanol  
Co-Solvent Flow: 0.80 mL/min  
CO<sub>2</sub> Flow: 3.20 mL/min  
Column Temp: 40° C  
Back Pressure: 125 bar  
Detection: 254 nm

### System 2: Dual Co-Solvents

Column: (S,S)-Whelk-O® 1  
25 cm x 4.6 mm  
Co-Solvent: (Ethanol/CH<sub>2</sub>Cl<sub>2</sub>) (50/50)  
Co-Solvent Flow: 0.80 mL/min  
CO<sub>2</sub> Flow: 3.20 mL/min  
Column Temp: 40° C  
Back Pressure: 125 bar  
Detection: 254 nm

## Solubility Data of Chlormezanone

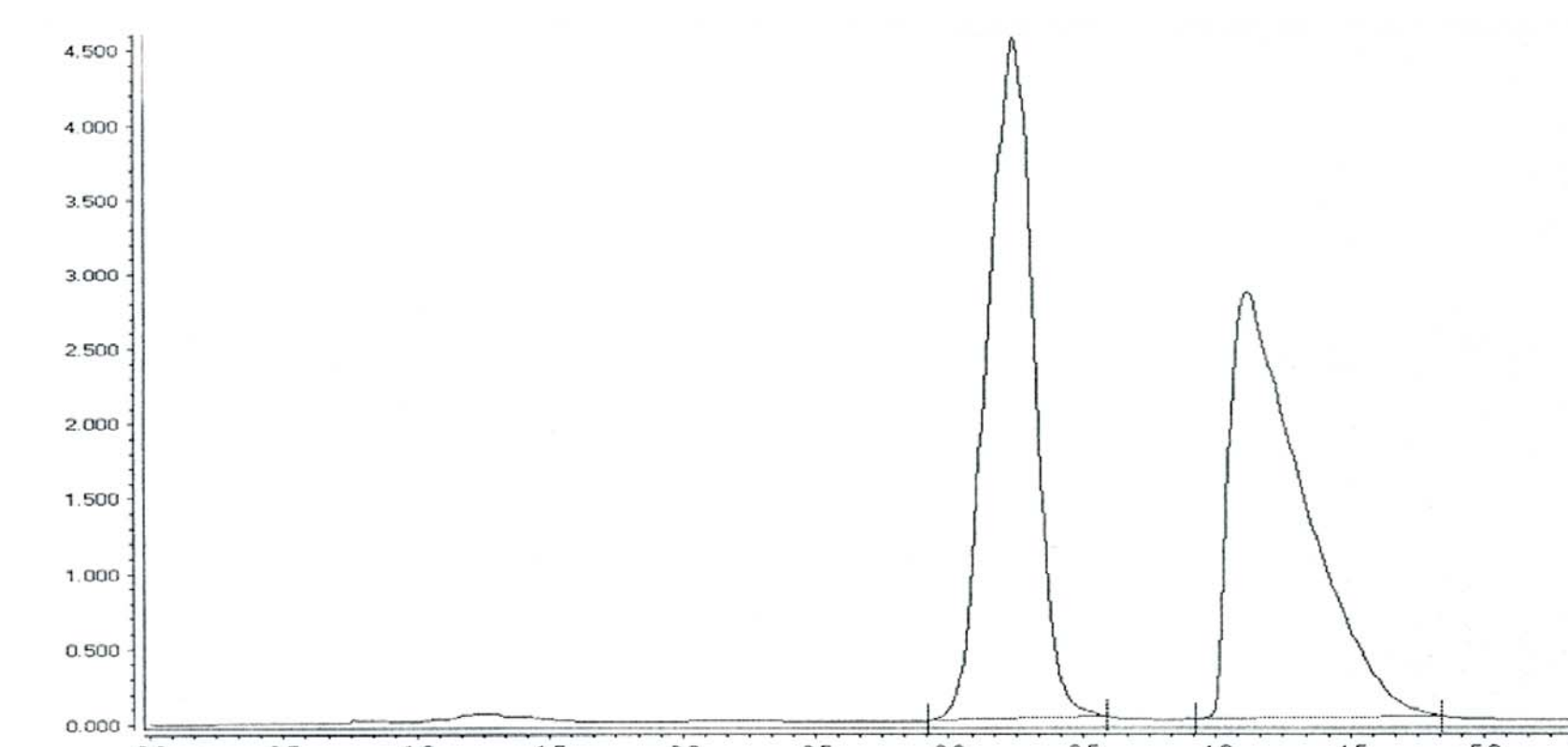
### System 1: Single Co-Solvent

35 mg/mL in Ethanol

### System 2: Dual Co-Solvents

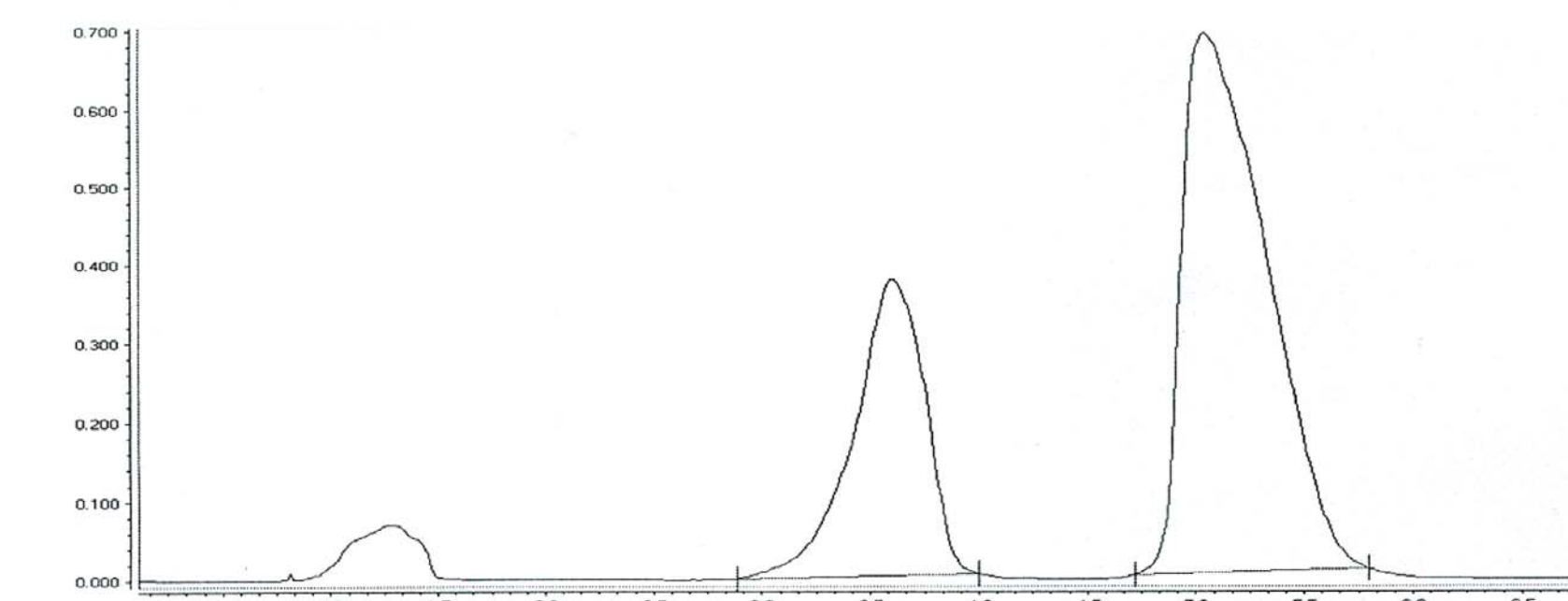
445 mg/mL in (Ethanol/CH<sub>2</sub>Cl<sub>2</sub>) (50/50)

## Analytical Chromatogram: System 1



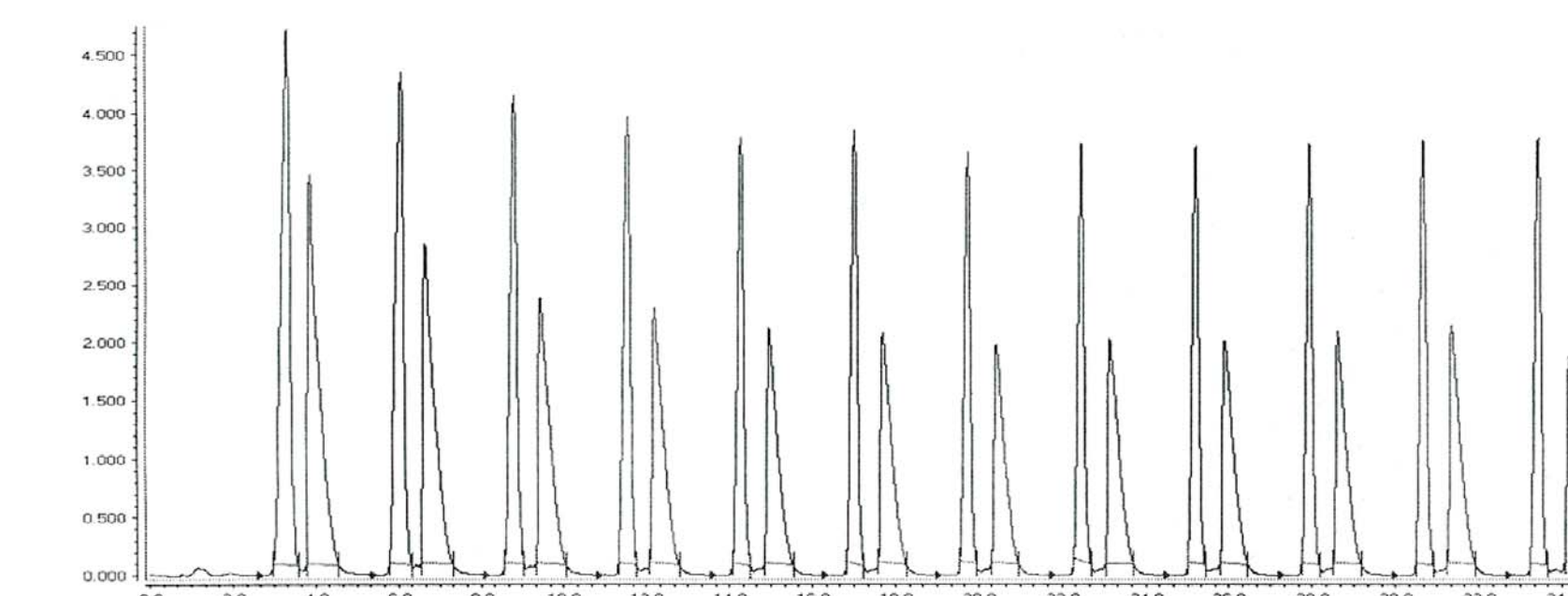
Selectivity 1.36  
Resolution 3.43

## Analytical Chromatogram: System 2



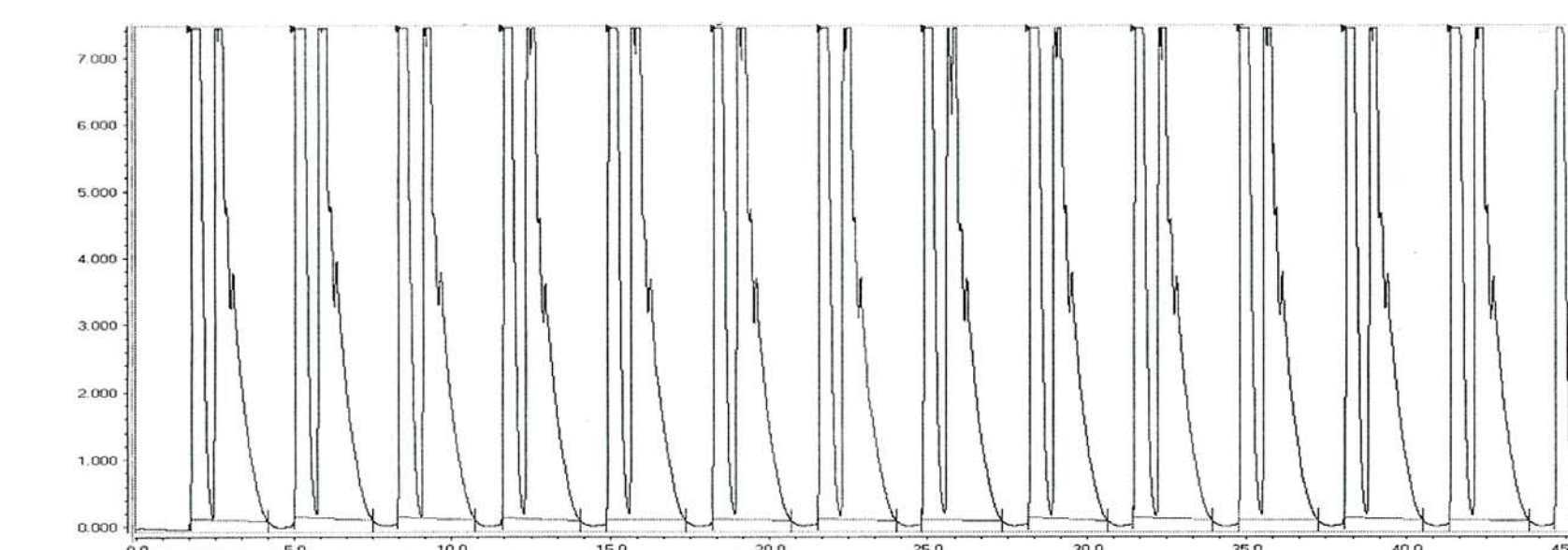
Selectivity 1.51  
Resolution 3.36

## Stacked Injections: System 1



Concentration: 35 mg/mL  
Volume injected: 200 uL every 1.8 min  
Amount injected: 7 mg/injection  
233 mg/hr

## Stacked Injections: System 2



Concentration: 445 mg/mL  
Volume injected: 100 uL every 2.5 min  
Amount injected: 44.5 mg/injection  
1,068 mg/hr

## Production Rate Summaries

### System 1: Single Co-Solvent

Column: (S,S)-Whelk-O® 1  
25 cm x 4.6 mm  
233 mg/hr  
25 cm x 50.0 mm  
27.5 gm/hr  
\*\*1.61 kilos/kilo of SP/day

### System 2: Dual Co-Solvents

Column: (S,S)-Whelk-O® 1  
25 cm x 4.6 mm  
1,068 mg/hr  
25 cm x 50.0 mm  
126.1 gm/hr  
\*\*7.32 kilos/kilo of SP/day

## Conclusions:

Employing a dual co-solvent system in SFC significantly improves solubility and production rates. In this particular study the solubility of Chlormezanone was increased by a factor of ~ 13 and the production rate by a factor of ~ 4.5.