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Solubility Study in Supercritical Fluids to Support Drug Discovery Purification

-SFE and SFC Purification Method Development

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Overview of Presentation

- **Introduction:** Characteristics of our discovery laboratory
  - Chiral purification: > 95% samples purified by SFC
    - **(10 mg to several kg)**
  - Not much is known about compounds and their components
  - Fast turn around time
  - Two major problems:
    - ***System shuts down***
    - ***Scale-up failure even with a successful analytical method***

- **Experiment**
  - Solubility trends: Several organic compounds
  - Low throughput vs automated multiple chamber solubility measurement
  - SFE purification by leveraging solubility difference

- **Application to real samples: two cases**
  - SFE clean up to remove an achiral impurity
  - Chiral purification throughput increase with consideration of solubility

Introduction: Problem (1) Purification System Down

- **Sample or sample components may precipitate.**
- **Pre-column filters**

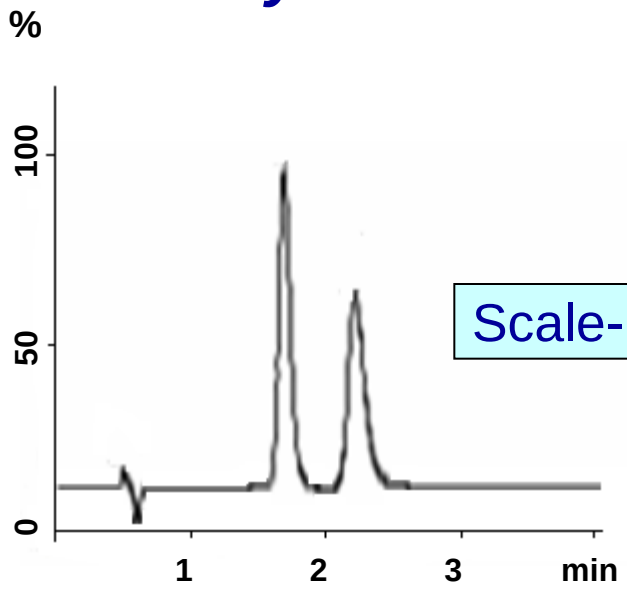
Before use

After SFC system shuts down

SFC system may eventually shut down due to the increased system pressure caused by the accumulation of precipitated sample components.

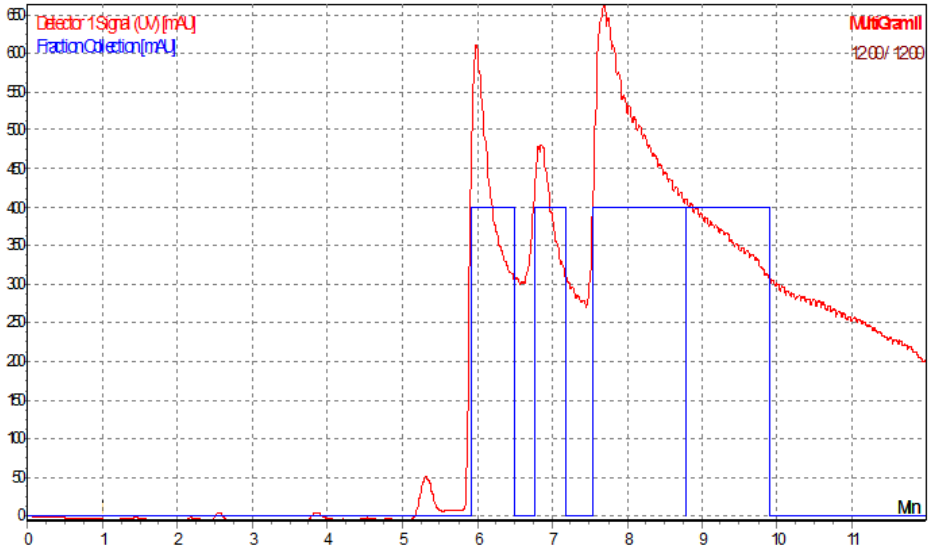
Introduction: Problem (2)
Purification may not be scaled-up

Analytical SFC



Scale-up

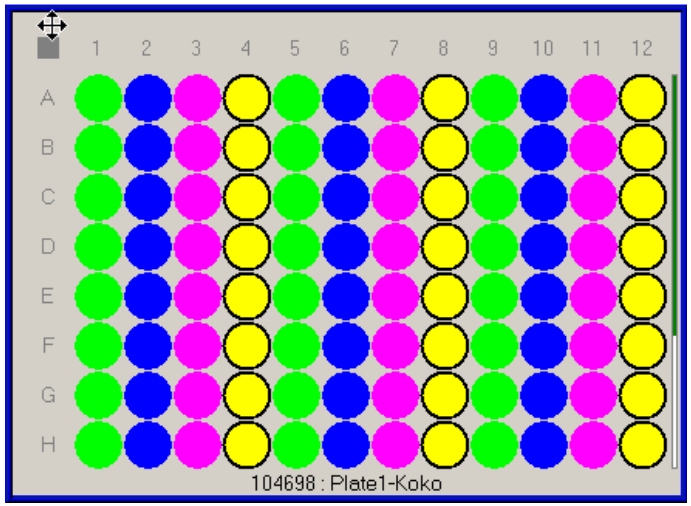
Preparative SFC



- **Solubility in SFC condition is different from the corresponding organic solvent**

Preparative SFC is more affected by solubility than analytical SFC.

Organic Solubility Screening-Gravimetric Method



MeOH
EtOH
ACN
IPOH

- 96-well plate, 24 compounds/plate
- compound ~50 mg/well
- Solvent ~500 µl/well

Equilibrated → phase-separated → 100 µl of supernatant → evaporation → residual solid weighing → back calculation of supernatant concentration

Automatic weighing
High throughput, multiple compounds on the same plate
Eliminates dilution step and reduces error
Symyx solubility station

Tan H, Reed M, Gahm KH, King I, Seran MD, Bostick T, Luu V, Semin D, Cheetham J, Larsen R, Martinelli M, Reider P. Organic Process Research & Development 12 (2008) 58-65

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Image of Compound State in Organic Solution

Plate 1

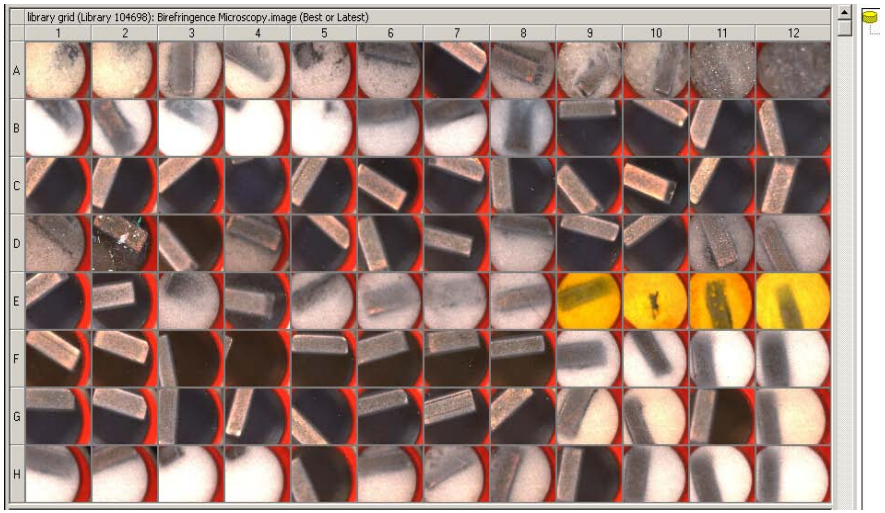
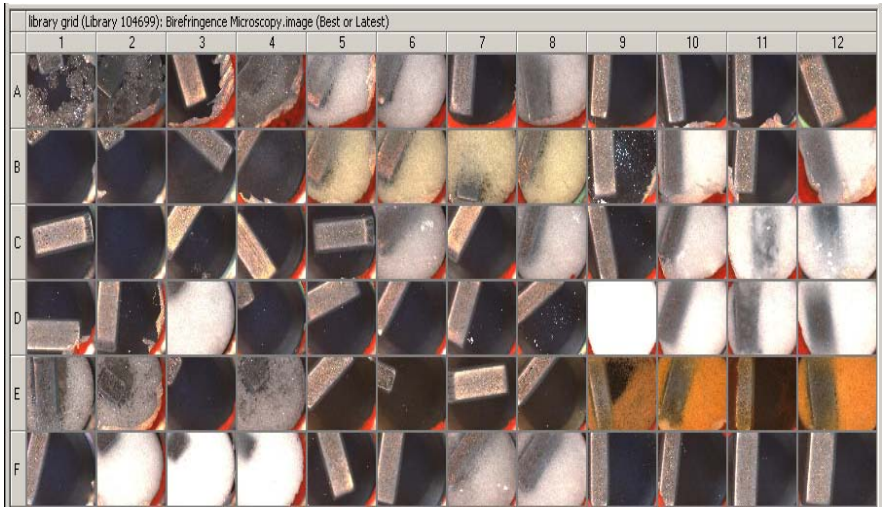


Plate 2



Compound saturation state determined and recorded by camera images after equilibration

High throughput organic solubility measurement has been well automated.

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Solubility in Organic Solvents and Supercritical Fluids (SCFs)

	Solubility (mg/ml)	
Solvent	**Organic**	**SCF**
DME	≥ 100	4
MeCN	12	5
IPOH:DME (50/50 v/v)	54	4
EtOH:MeCN (50/50 v/v)	38	7
IPOH	0.3	3
EtOH	0.8	14
MeOH	10	73

* SCF solubility was measured at organic solvent/CO_2 (50/50 v/v)

It is hard to predict solubilities in SCFs from those in organic solvents.

Gahm KH, Tan H, Liu J, Barnhart W, Eschelbach J, Notari S, Thomas S, Semin D, Cheetham J. Journal of Pharmaceutical and Biomedical Analysis 46 (2008) 831–838.

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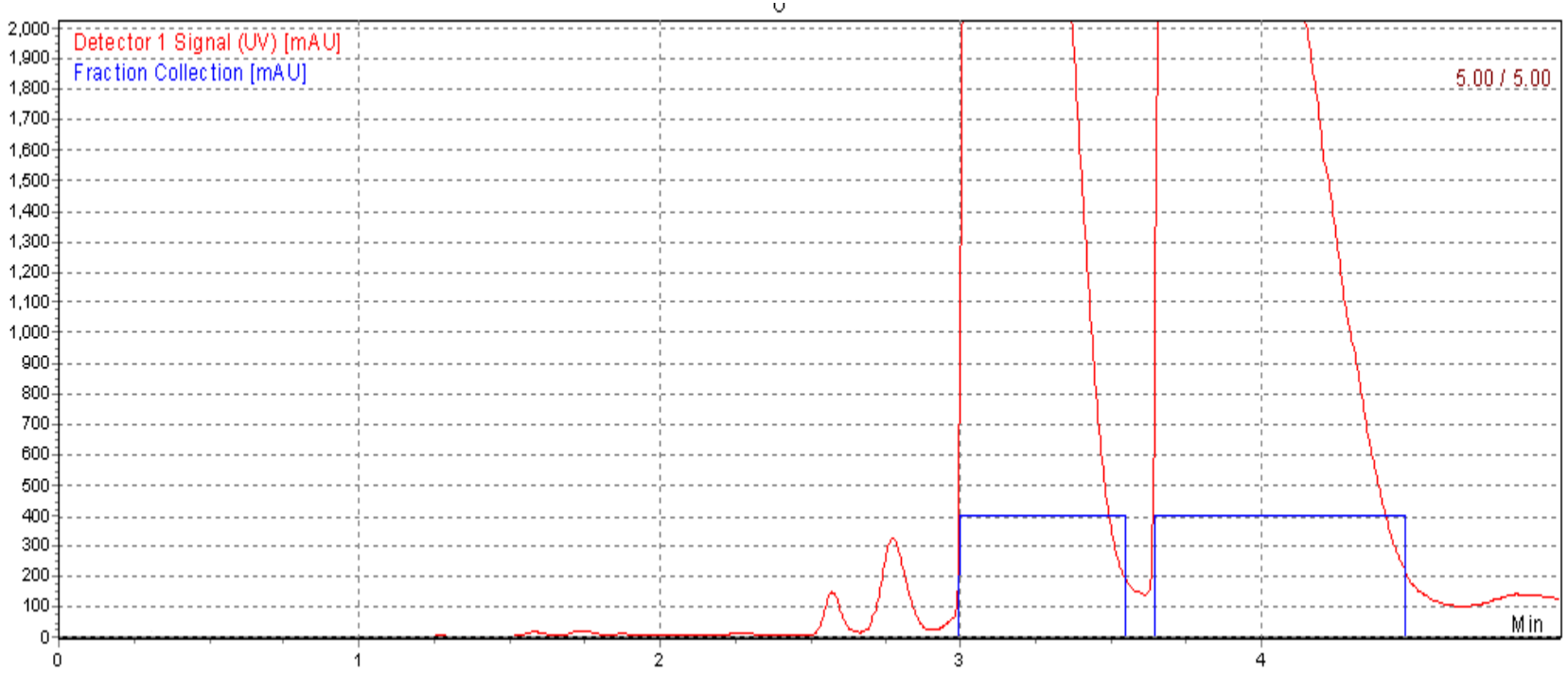
SFC Purification: Solubility Consideration



MP: 40% Ethanol/Acetonitrile (1/1), 60% CO_2, F=55 ml/min, p=193 bar
AD-H, 21 mm x 25 cm, T=40C, 50 mg per injection.

The higher organic percent in an SFC mobile phase usually helps to solve solubility problems in scale-up.

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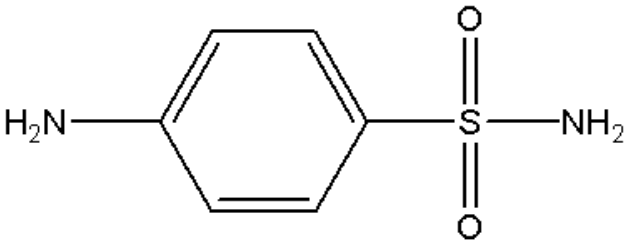
Introduction: Visualization of sample precipitation in SFC column

sulfanilamide



Precipitated compound not silica gel

Flow rate : 50 ml/min
M.P. : CO_2 100%
P = 100 bars
T = 35 ºC

- The above figure demonstrates precipitation of a compound (sulfanilamide) into an **empty column** hardware after multiple injections.

Finding a supercritical fluid mobile phase with a high solubility is one of the key factors in successful purification!

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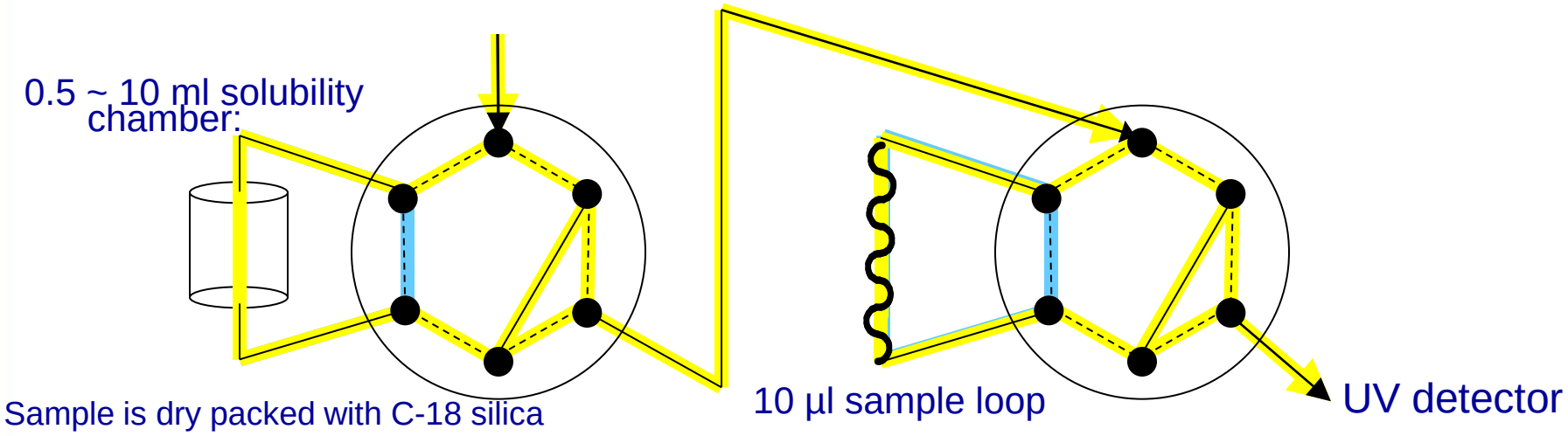
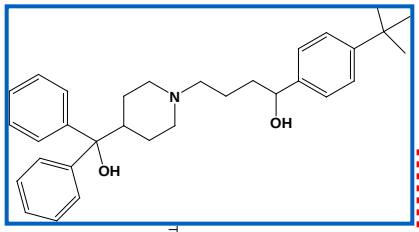
Experiment : Low throughput supercritical solubility measurement

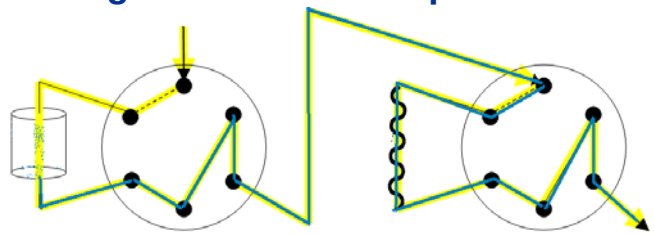
- **Analytical SFC system**
- **2 VICI® 6-port 2-position switching valves**



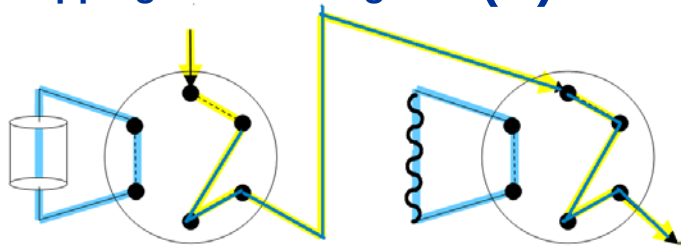
Reproducibility:
Three Repetitive Samplings and Injections of Terfenadine



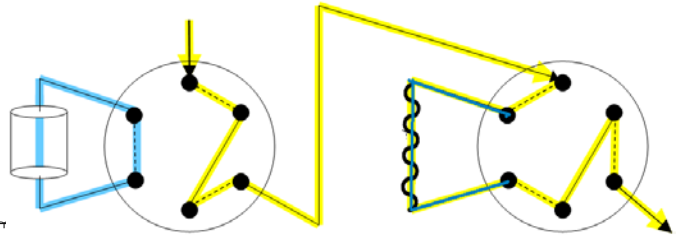
Eluting chamber after equilibration



Trapping and clearing line (A)



Injection of trapped sample (B)



Relative Standard Deviation ~ 3.5 %

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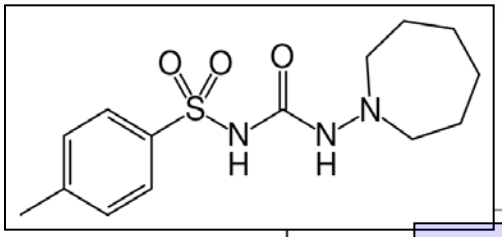
Solubility in organic solvent vs supercritical fluid

- **Is solubility in supercritical fluids easily predictable from the solubility in neat organic solvents?**

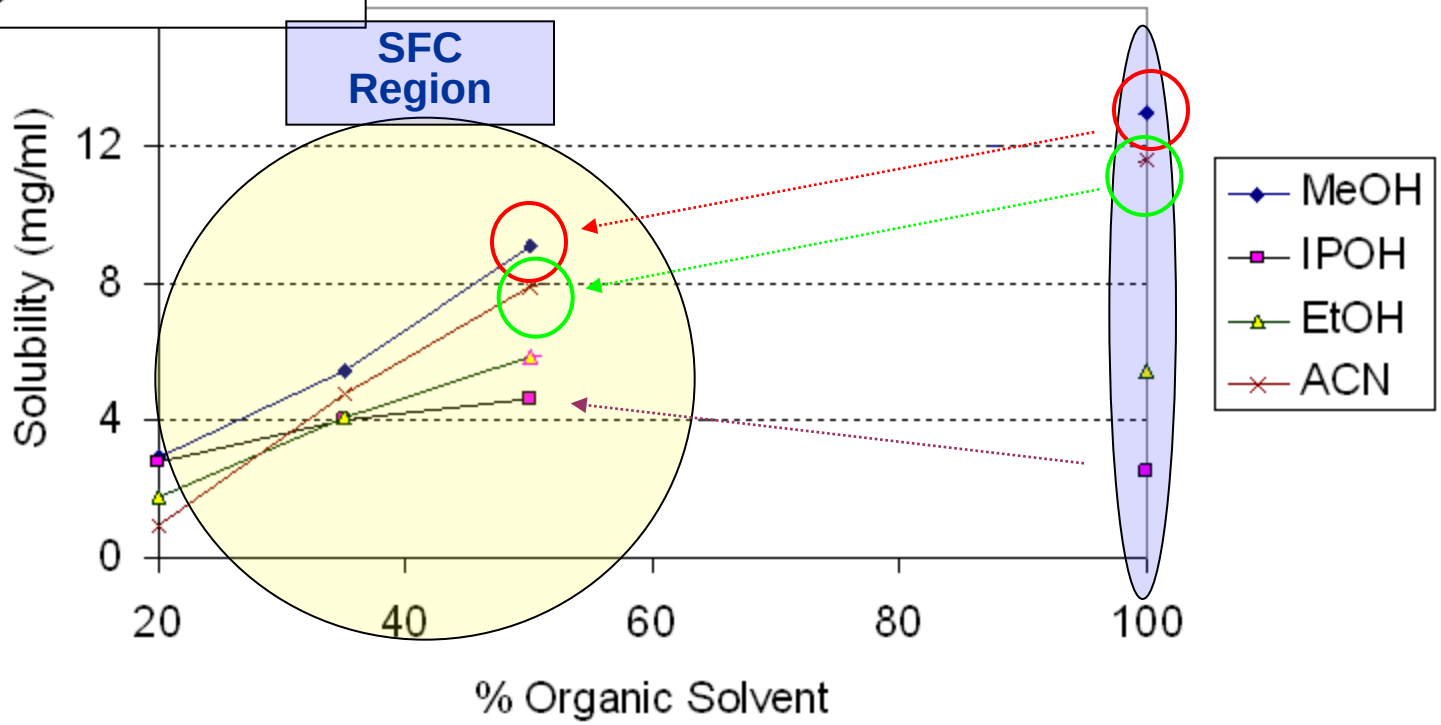
Solubility Trend – Tolazamide (1)



Tolazamide

Neat Organic Solvents

SFC Region



- ***Solubilities of tolazamide are lower both in (50/50 CO_2/organic solvent) than in neat MeOH and MeCN.***

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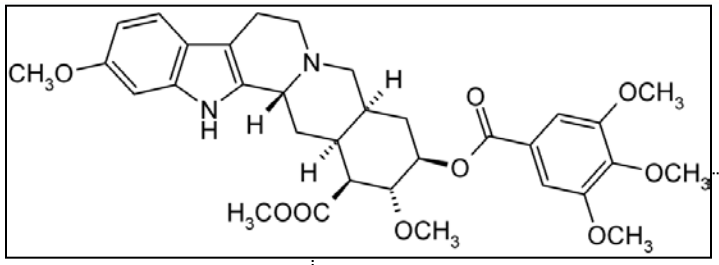
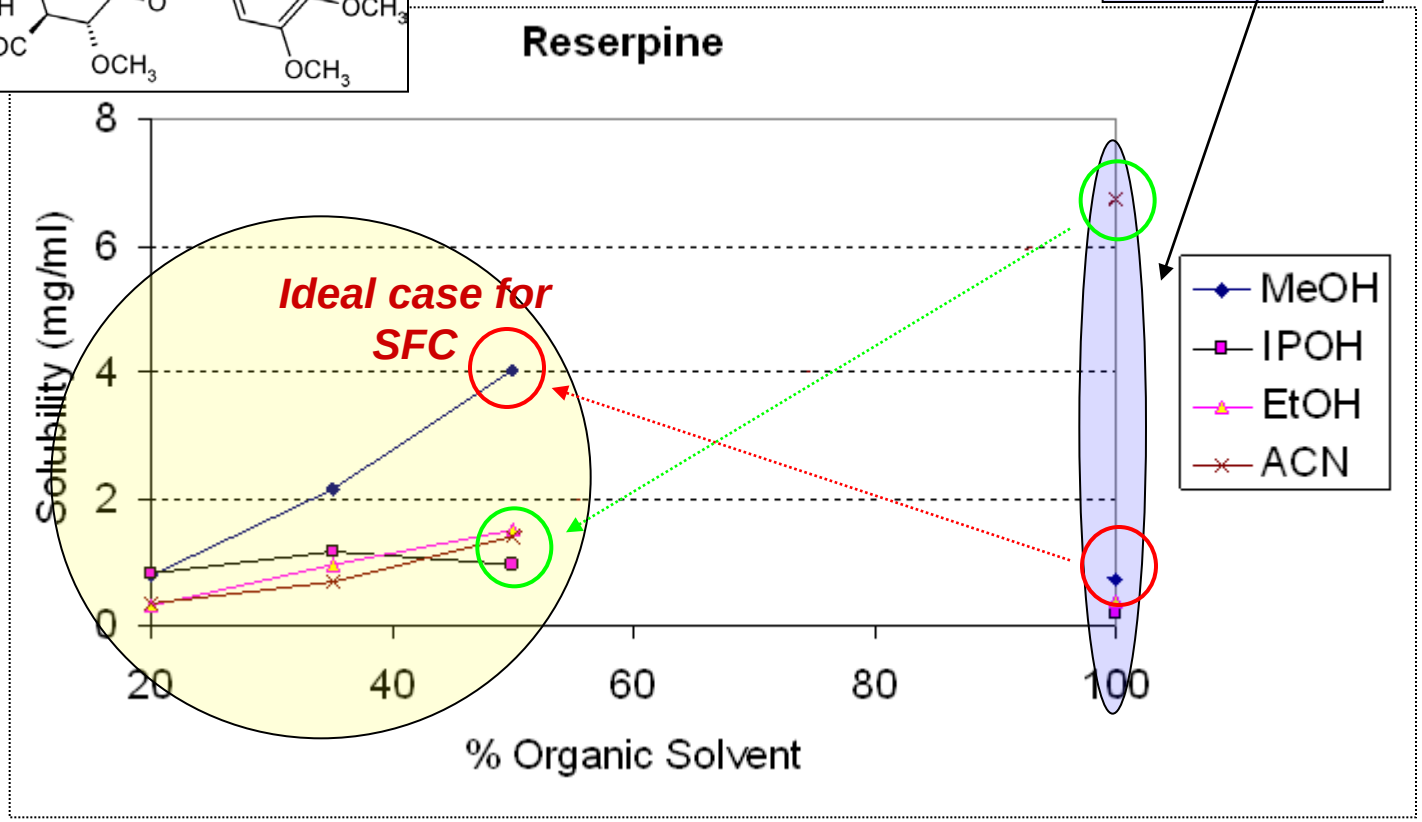
Solubility Trend – Reserpine (2)





Solubility of reserpine is higher in 50/50 CO_2/MeOH than in neat MeOH, but it is lower in 50/50 CO_2/MeCN than in neat MeCN.

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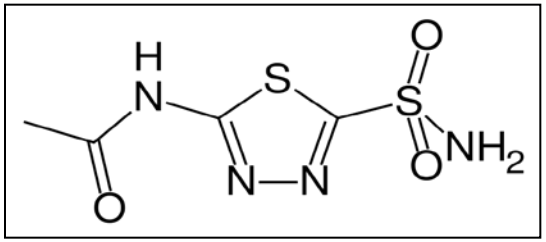
Solubility Trend – Acetazolamide (3)



- ***Solubility of acetazolamide is significantly lower in supercritical fluid condition (50/50 CO_2/MeOH) than in neat methanol.***

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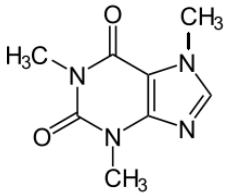
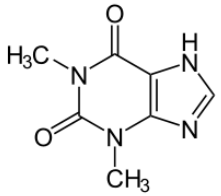
Solubility Trend – Caffeine and Theophylline (4)

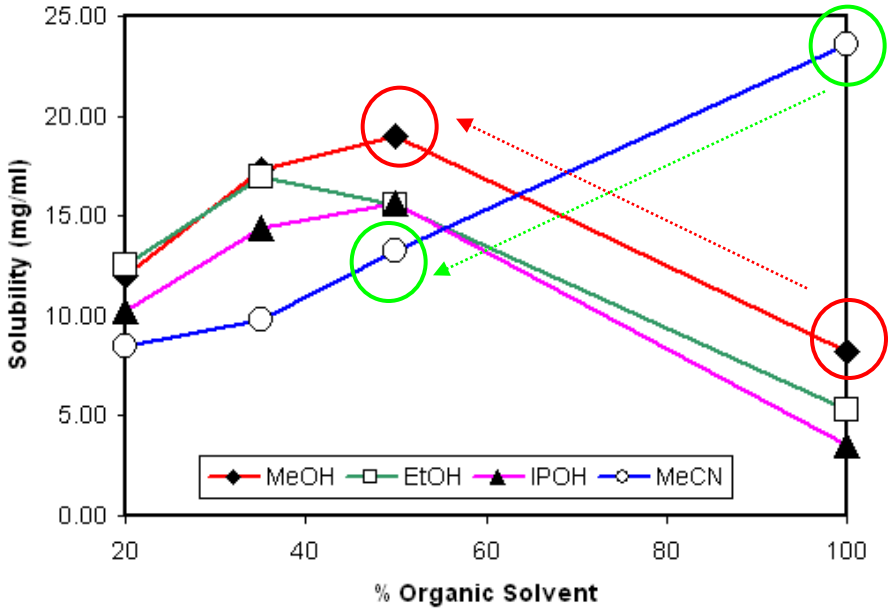
Caffeine

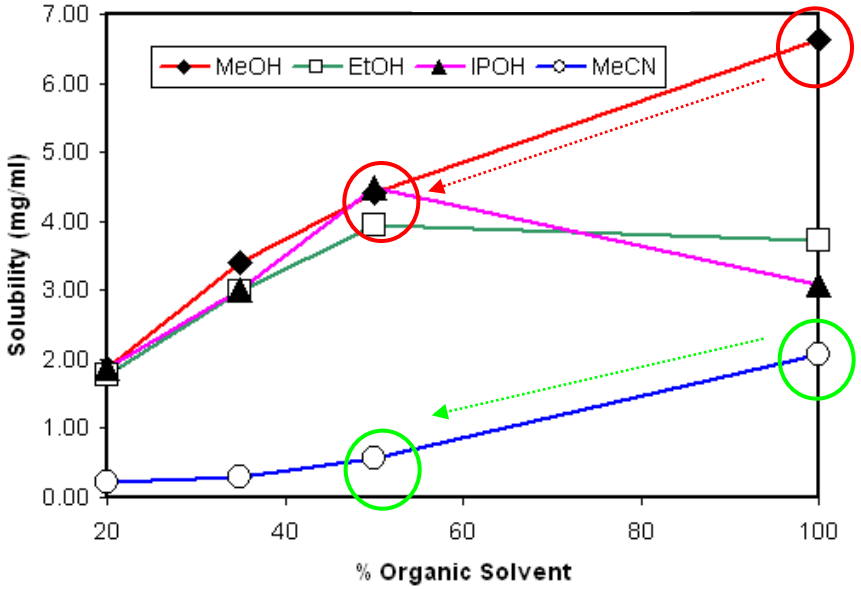


Theophylline



Neat Organic





- ***Solubility of caff. is significantly higher in 50/50 CO_2/MeOH than in neat MeOH. However, solubility of theoph. is lower in the same supercritical fluid condition.***
- ***Solubility of both caff. and theoph. is lower in 50/50 CO_2/ACN than in pure MeCN.***

Solubility trends in neat solvent and supercritical fluids are not easy to predict.

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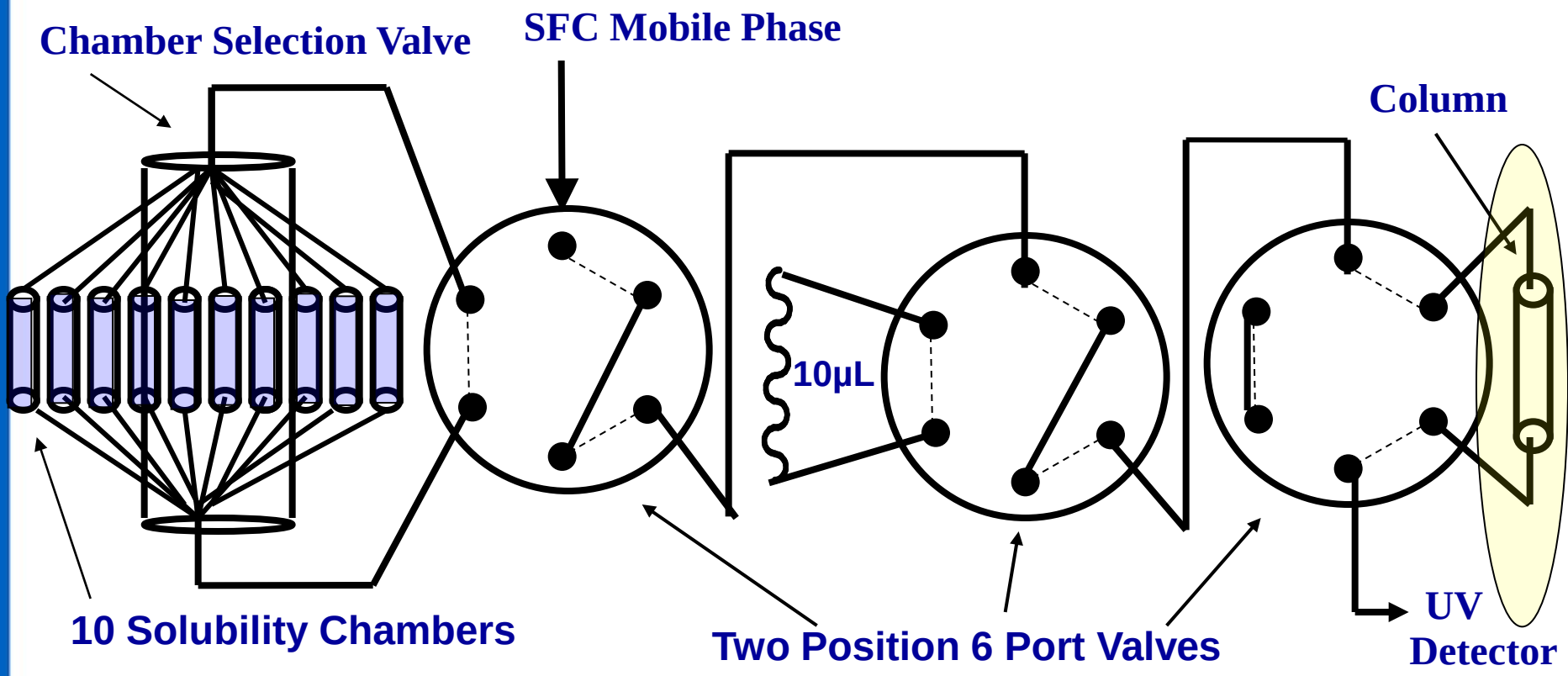
Take home message (1)

- **Now, we learned solubility in organic solvent and supercritical fluids modified by the organic solvent are different and it is not easy to predict a trend.**
- **Experimental measurement is necessary.**
- **Low throughput vs automated multiple chamber solubility measurement**

SFE solubility screening with multiple chambers



Stainless steel chamber was packed with analytes coated on 50 µm C-18 Silica

Typical procedure: 30 sec chamber filling, 3~10 min chamber equilibration

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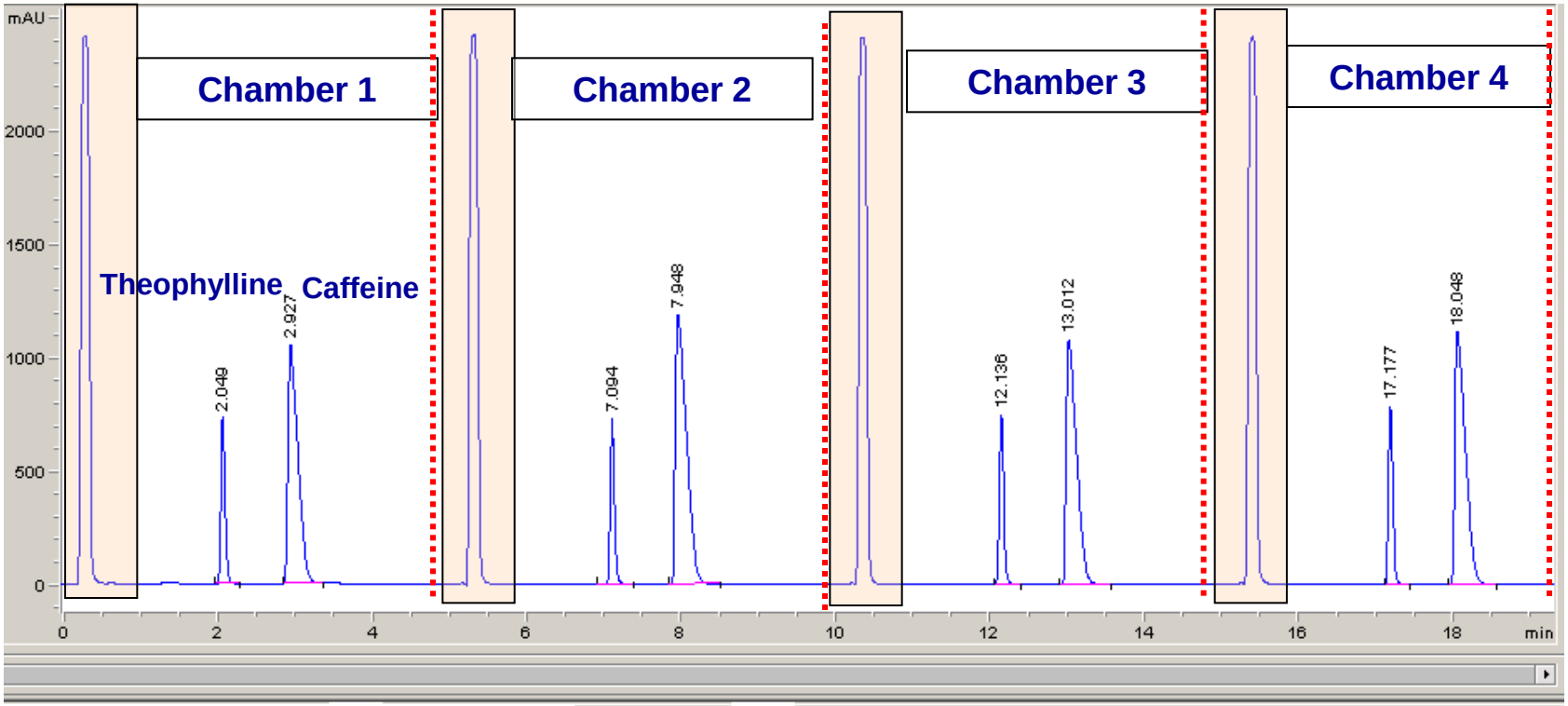
Reproducibility between Chambers



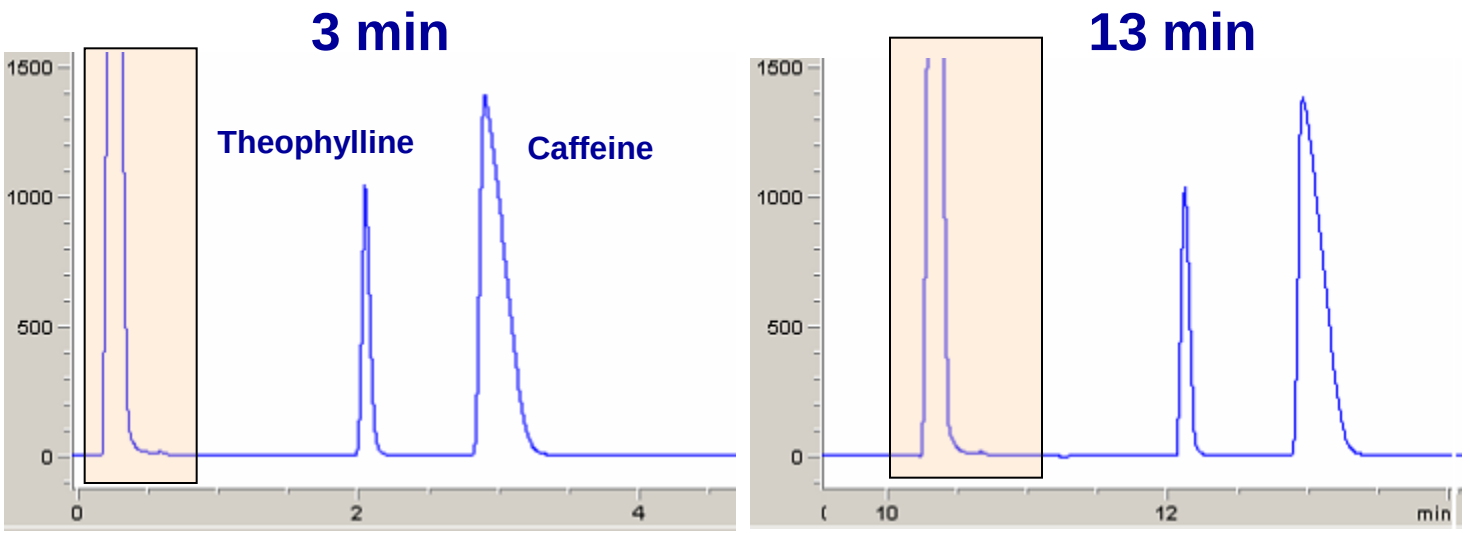
R.S.D: Theophylline: 3.6%
Caffeine: 9.6%

50/50 v/v CO_2/MeOH, p=100 bar

#	Time	Area	Height	Width	Area%	Symmetry
1	2.049	2770.3	732.3	0.0581	5.245	0.755
2	2.927	9426.4	1054.8	0.1403	17.846	0.319
3	7.094	2809.9	731.1	0.0608	5.320	0.76
4	7.948	11713.8	1188.4	0.155	22.176	0.292
5	12.136	2852.5	748.9	0.0584	5.400	0.76
6	13.012	9853.2	1079.6	0.1425	18.653	0.326
7	17.177	3006.2	783.4	0.0588	5.691	0.756
8	18.048	10390.2	1112.5	0.147	19.670	0.316

Chromatographic conditions: AD-H (4.6 mm x 15cm) with 50/50 CO_2/MeOH, 4.0 ml/min

How fast does it reach at equilibrium?



	Theophylline	Caffeine
3 min	4025	15396
13 min	3903	15293
% difference	1.2	1.0

AD-H (4.6 mm x 15cm) with 50/50 CO_2/MeOH, 4.0 ml/min

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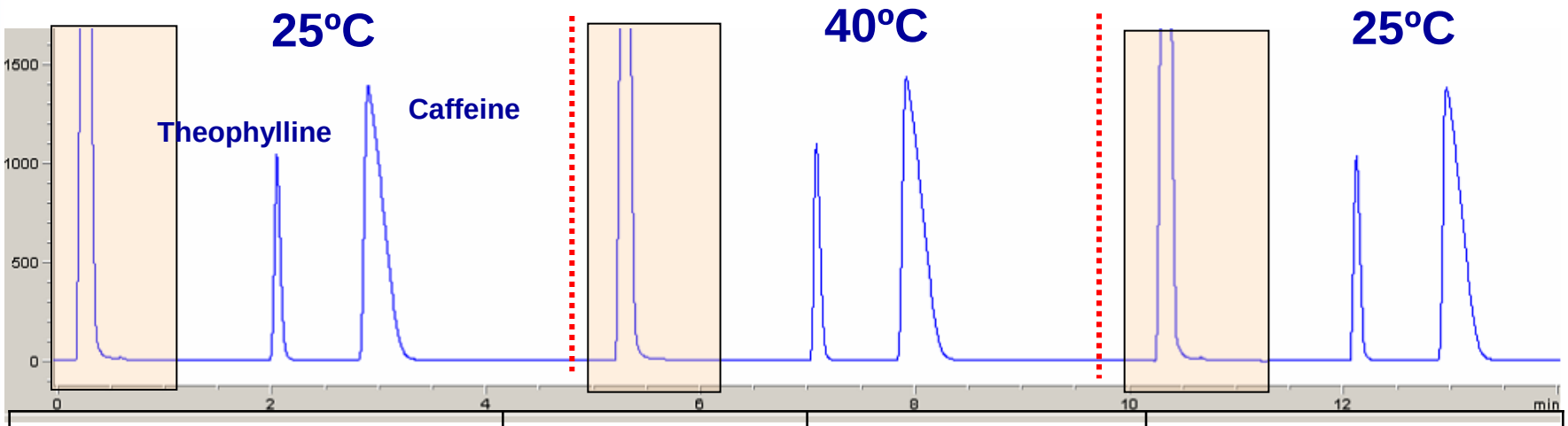
Temperature Study: 50/50 CO_2/MeOH



25ºC	Theophylline	Caffeine	Peak Area Ratio
Ave	4063	15905	1: 4
S.D. (n=4)	126	668	
R.S.D.	3.1	4.2	
40ºC (n=2)	Theophylline	Caffeine	
AVE (% sol. Increase)	4693 (16%)	18830 (18%)	1:4
S.D.	275	1424	
R.S.D.	5.9	7.6	

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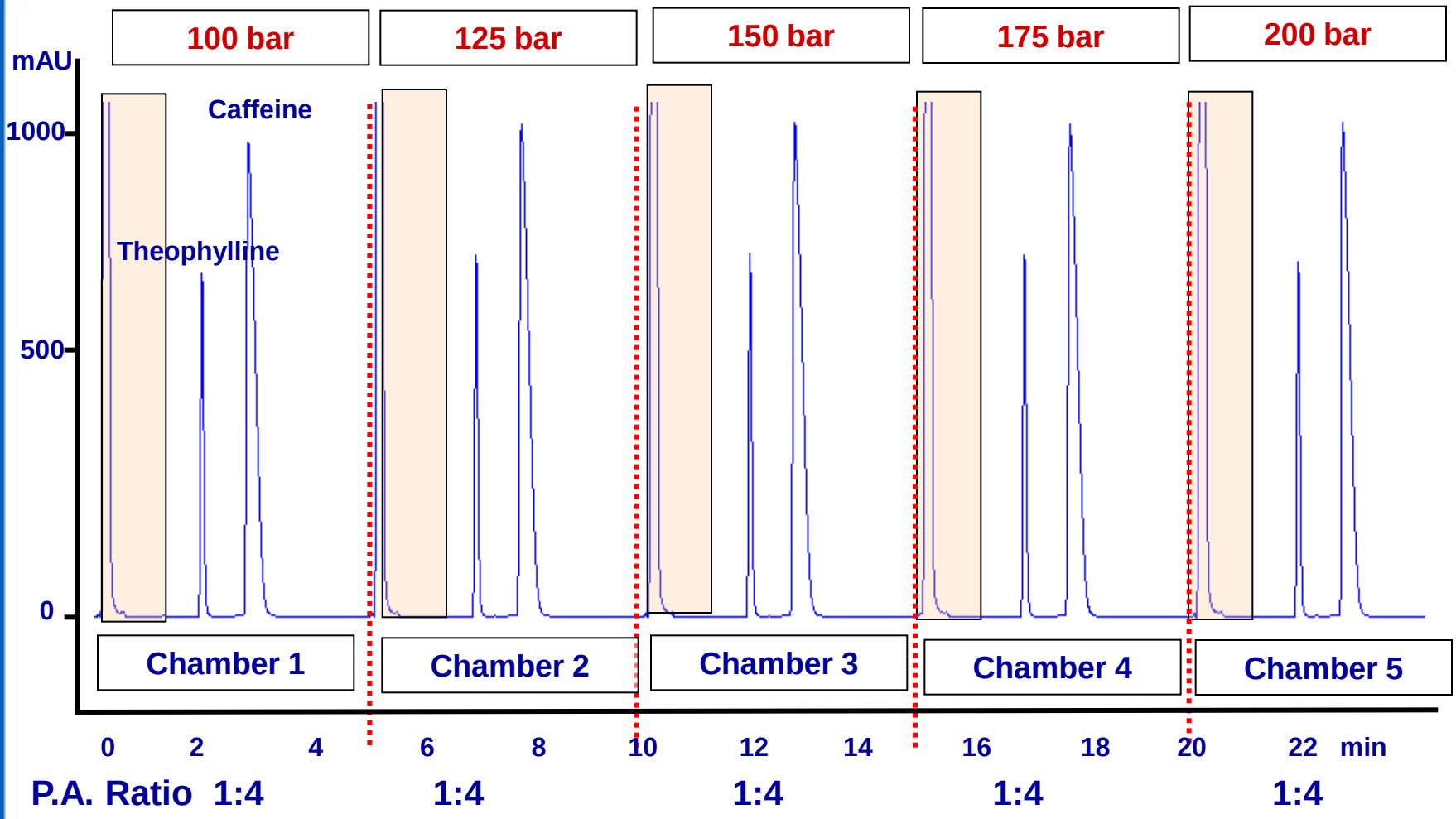
Pressure Study: Theopylline and Caffeine Mixture



AD-H (4.6 mm x 15cm) with 50/50 CO_2/MeCN, 4.0 ml/min

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Take home message (2)

- **Now, we learned solubility can be measured in rapidly using multiple chambers in diverse SCF conditions.**

- **What can we do when we know solubilities?**

  - **Can we maximize solubility difference between sample components?**

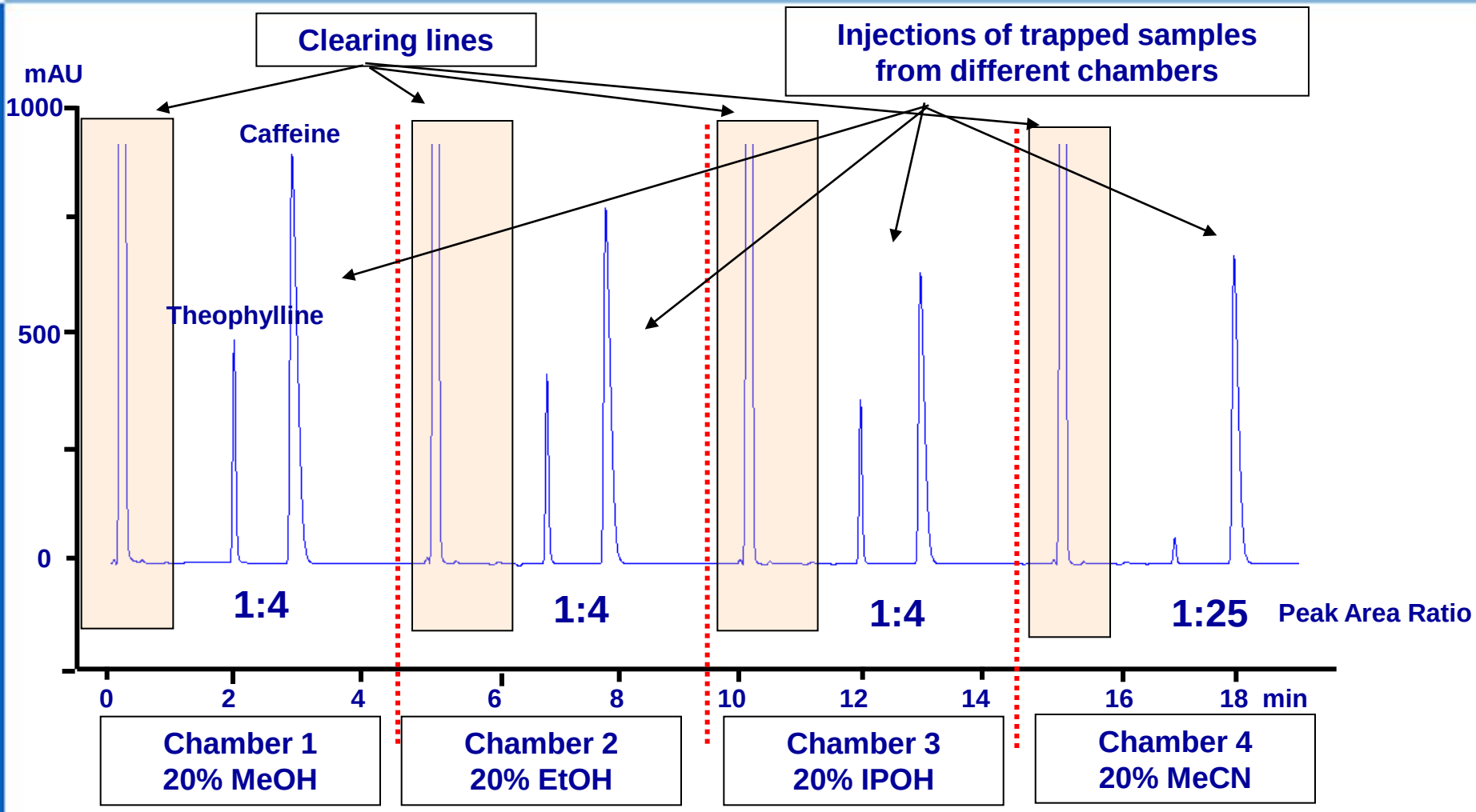
  - **Leveraging large differences in solubility to obtain pure material by *supercritical fluid extraction (SFE).***

Ratio of Caffeine and Theophylline Solubility

%	MeOH			EtOH			IPOH			MeCN		
	Caff	Theo	**ratio**	Caff	Theo	**ratio**	Caff	Theo	**ratio**	Caff	Theo	**ratio**
20	12.01	1.88	**6:1**	12.52	1.78	**7:1**	10.23	1.87	**5:1**	8.45	0.21	**40:1**
35	17.28	3.40	**5:1**	16.99	2.99	**6:1**	14.37	3.01	**5:1**	9.83	0.30	**33:1**
50	18.95	4.40	**4:1**	15.51	3.94	**4:1**	15.62	4.48	**3:1**	13.27	0.56	**24:1**
100	8.24	6.64	**1:1**	5.33	3.73	**1:1**	3.55	3.07	**1:1**	23.63	2.08	**11:1**

- **20% MeCN generated the *highest ratio of Caffeine: Theophylline*.**
- **Large difference in solubility is useful for *"supercritical fluid extraction (SFE)"*.**

Rapid SFE Screening of Theophylline and Caffeine



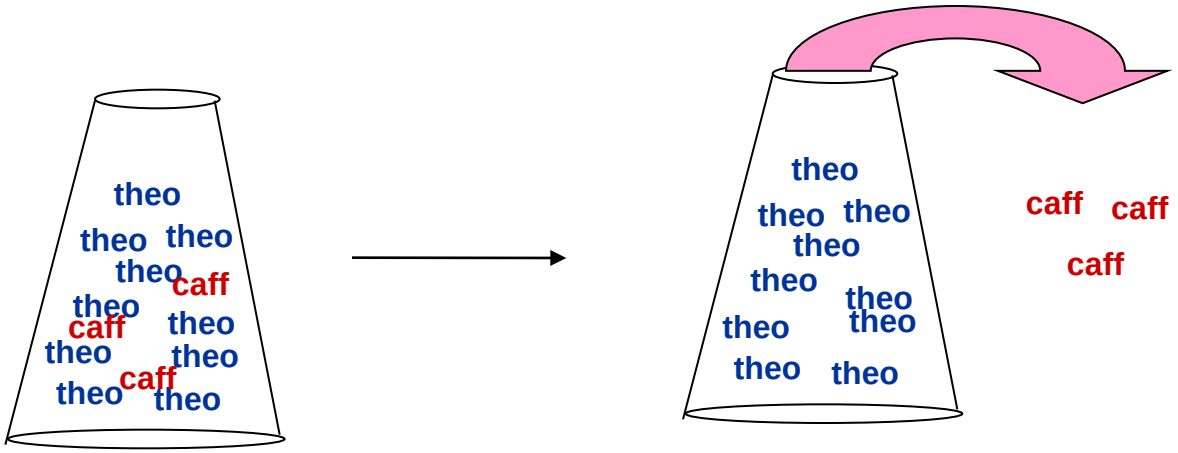
Rapid solubility screening makes it possible to quickly find a potential supercritical mobile phase extraction condition.

Validation of SFE screening for the preparative SFE purification

- **Goal**

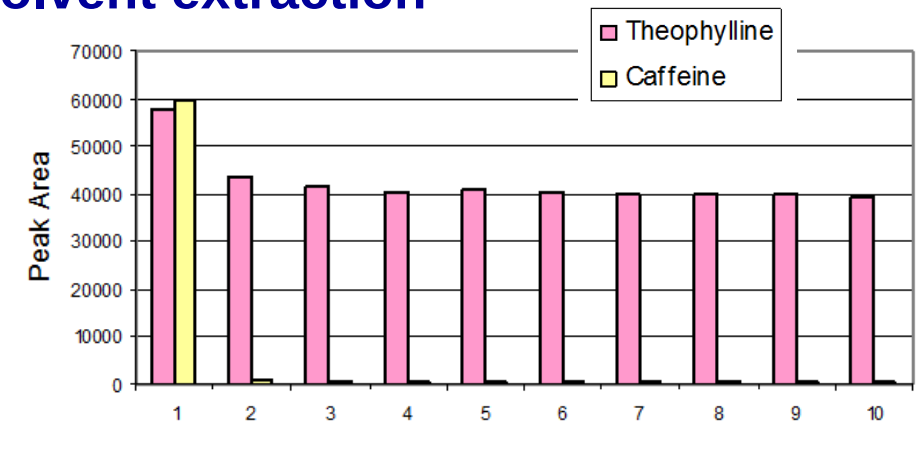
  Highest recovery of theophylline from the mixture of theophylline and caffeine (85/15 wt %) by SFE

  - **Caffeine is an impurity**
  - **Extract out the *minor impurity* *(Reverse purification!)***



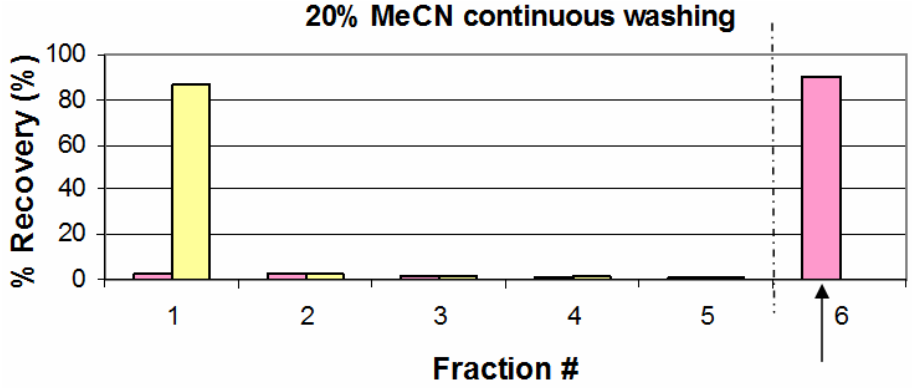
Purification comparison between organic extraction vs SFE

a) Organic solvent extraction



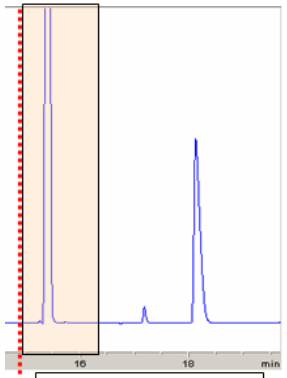
%	MeCN		
	Caff	Theo	ratio
20	8.45	0.21	**40:1**
35	9.83	0.30	**33:1**
50	13.27	0.56	**24:1**
100	23.63	2.08	**11:1**

b) SFE



50% MeOH washing out remaining compounds

SFE Solubility Screening



Chamber 4
20% MeCN

1:26

400mg of theophylline and caffeine (85/15 wt %) mixture packed in column was washed by neat MeCN or CO_2/MeCN (80/20) and fractions were collected.

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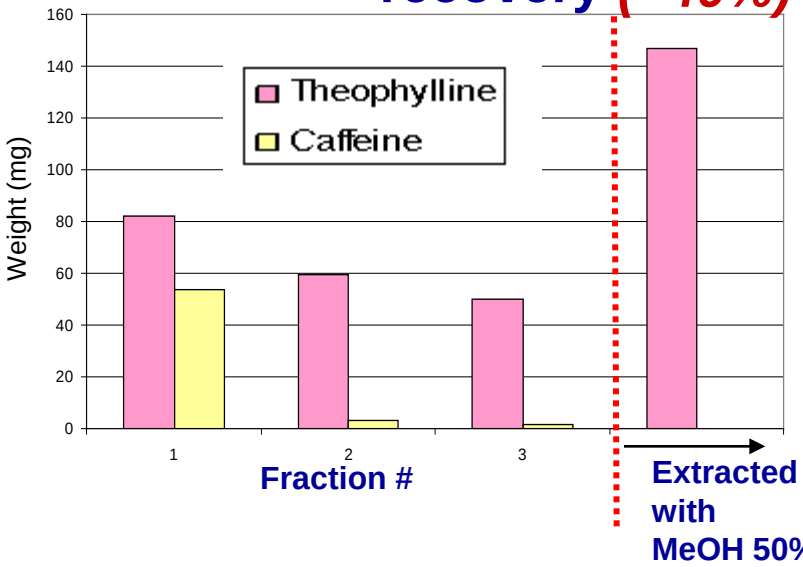
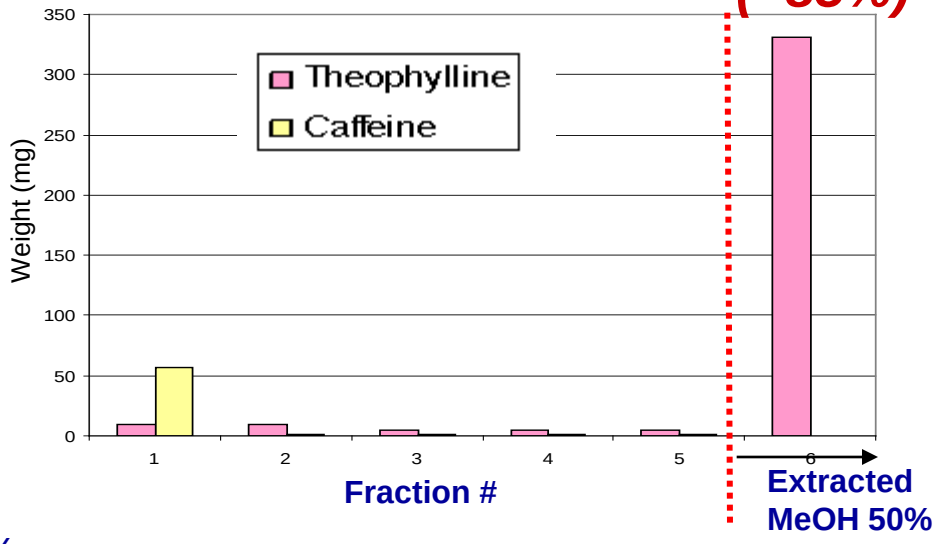
Comparison of Continuous Supercritical Fluid Extraction

- Mass Recovery (mg) in MeOH (20%) and MeCN (20%) SCF

CO_2/MeOH (80/20 v/v) 1:6 recovery *(~40%)*



CO_2/MeCN (80/20 v/v) 1:40 *(~85%)*



- ***CO_2/MeCN (80/20 v/v) is the better eluent to remove an impurity with a better recovery (~85%) than CO_2/MeOH (80/20 v/v) with a recovery (~40%).***

- ***Results are in good accordance with the solubility trend.***

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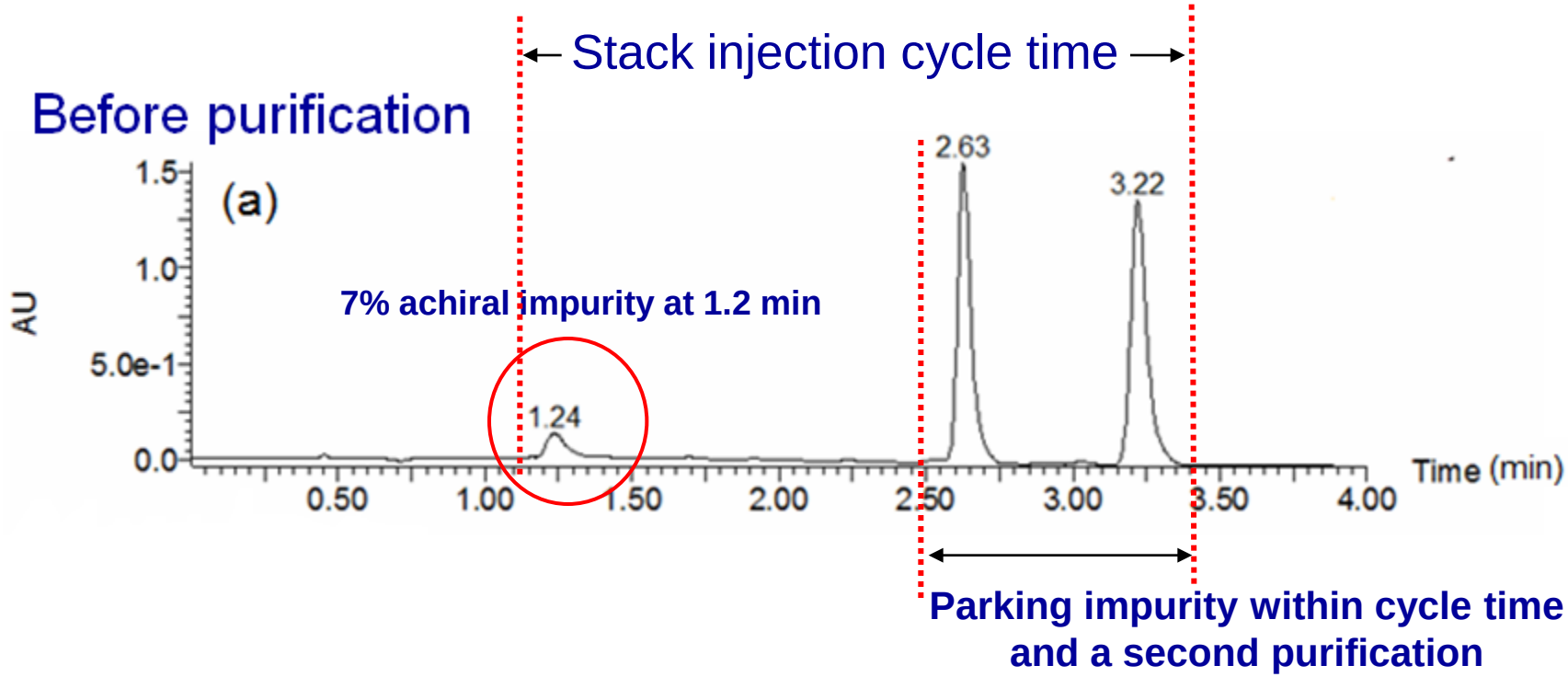
Take home message (3)

- **Rapid multiple chamber solubility screen did help to find a potential SFE condition to leverage large differences in solubility to obtain pure material by *supercritical fluid extraction (SFE).***

- ***Are there examples of solubility work for real samples in the drug discovery environment?***

- **Two case studies of real drug discovery purifications**
  - **SFE on achiral impurity removal**
  - **Increase chiral purification throughput**

Case study (1): Achiral impurity removal by SFE



Other purification methods: flash chromatography, organic solvent extraction, or recrystallization.

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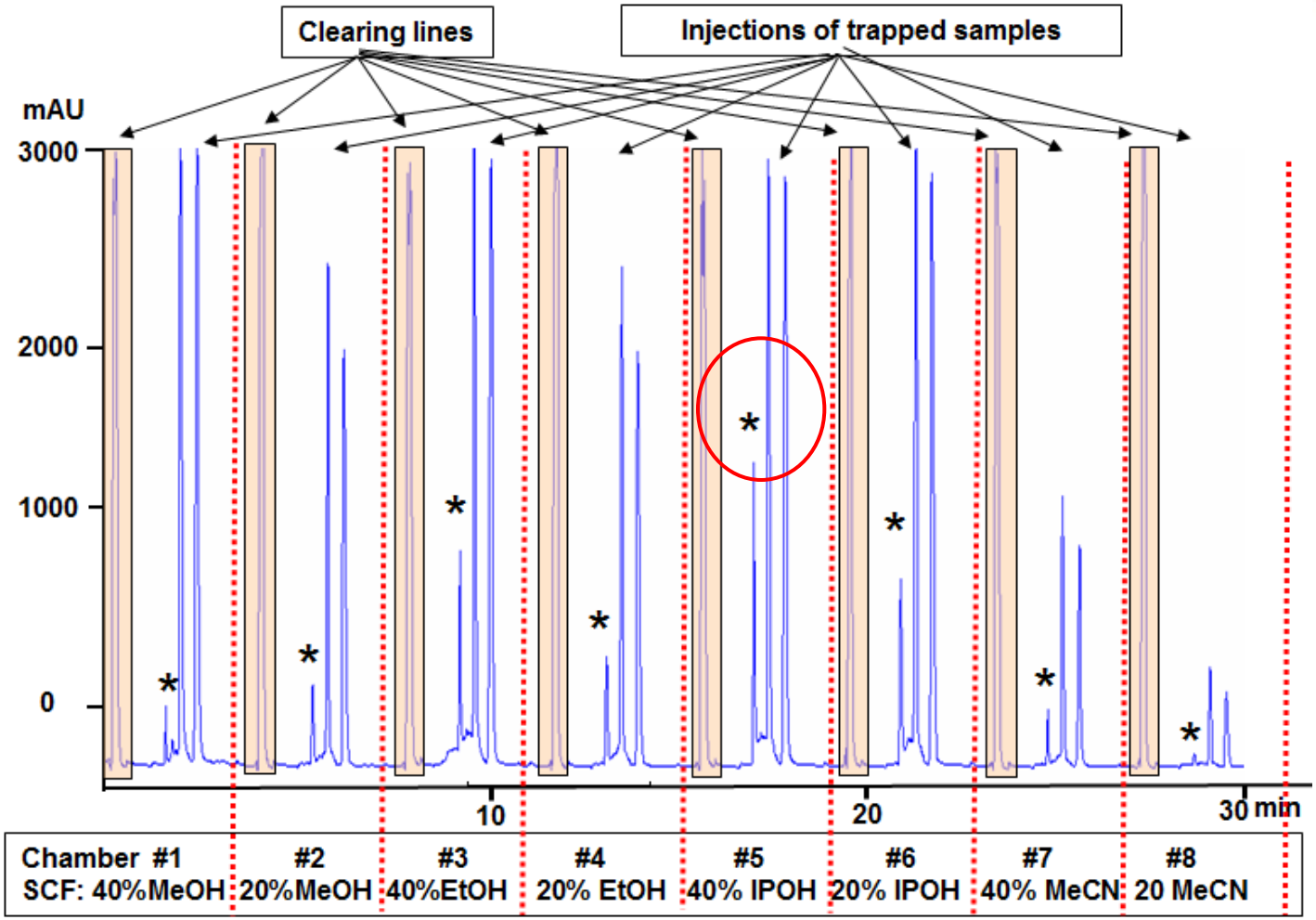
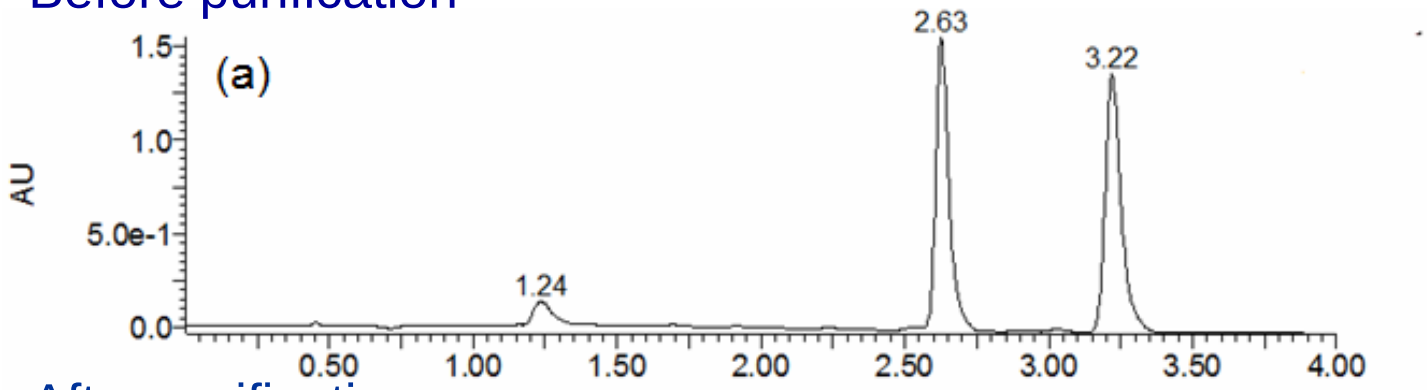
Rapid SFE screening of eight chambers:



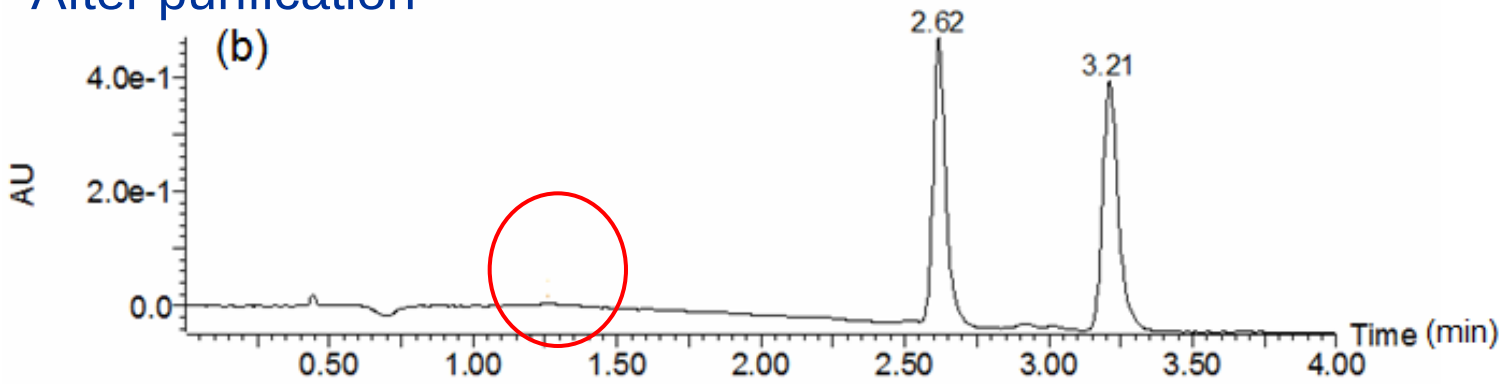
AD-H as an analytical column in m.p. of 50% MeOH (50% CO2).
The major impurity is marked with "*" .

SFE removal of achiral impurities

Before purification



After purification



The recovery was 65%.

(400 mg of sample purified using an analytical SFC system.)

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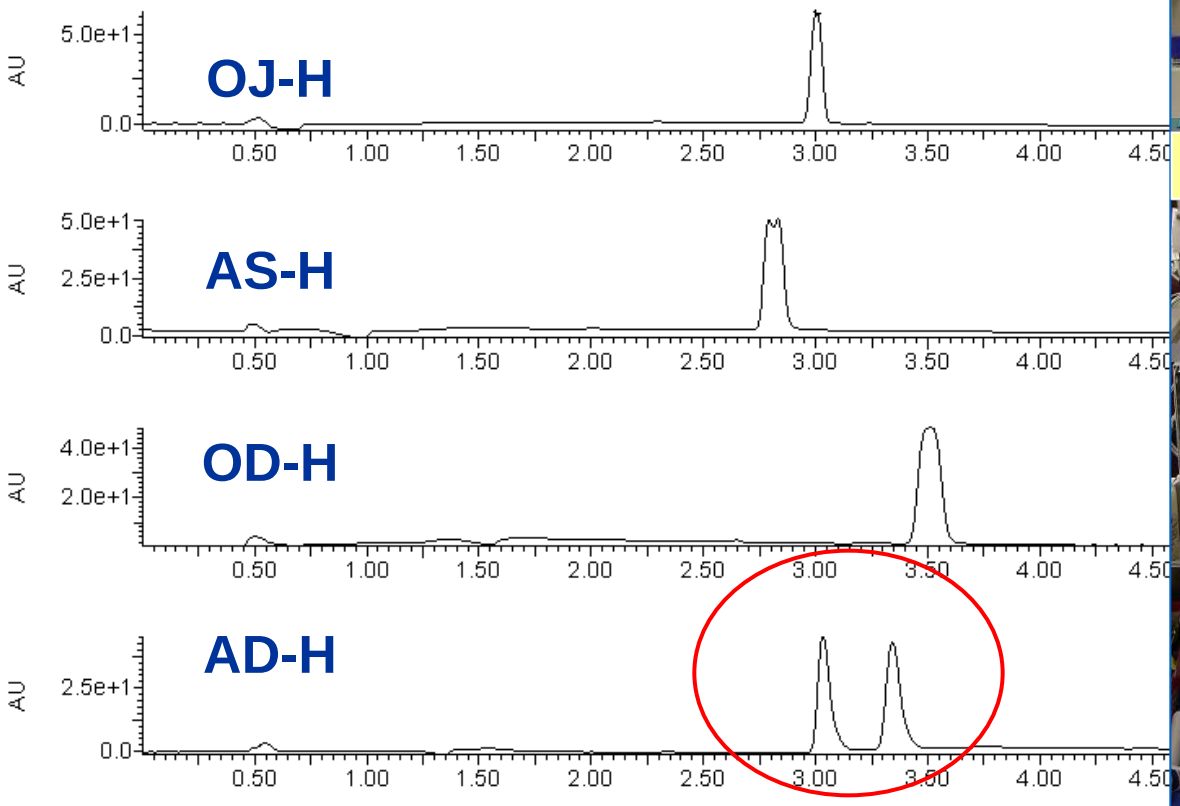
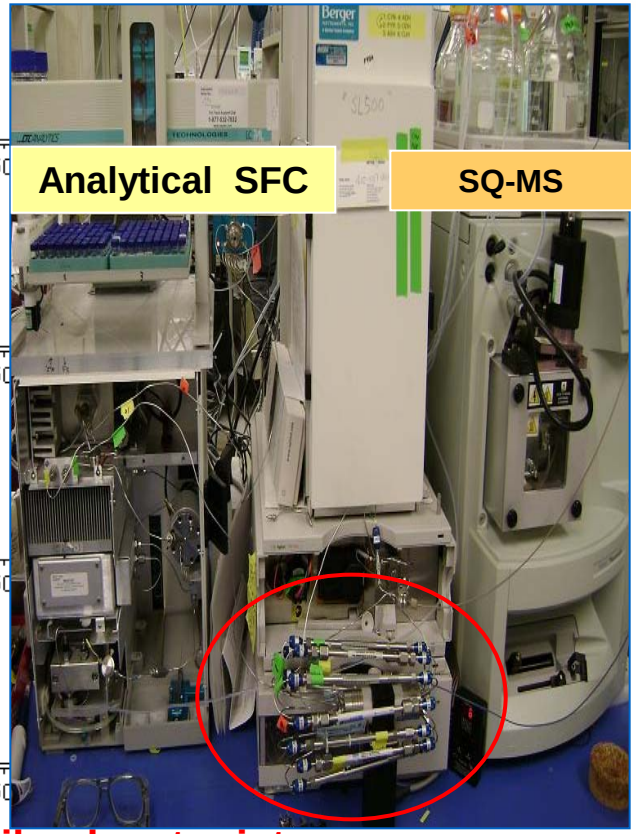
- **Examples of the application of solubility study for the discovery purifications**
  - **SFE on achiral removal**
  - **Increase chiral purification throughput**

Case Study (2): Chiral Purification (124 g) - 1st step: Pick a column.

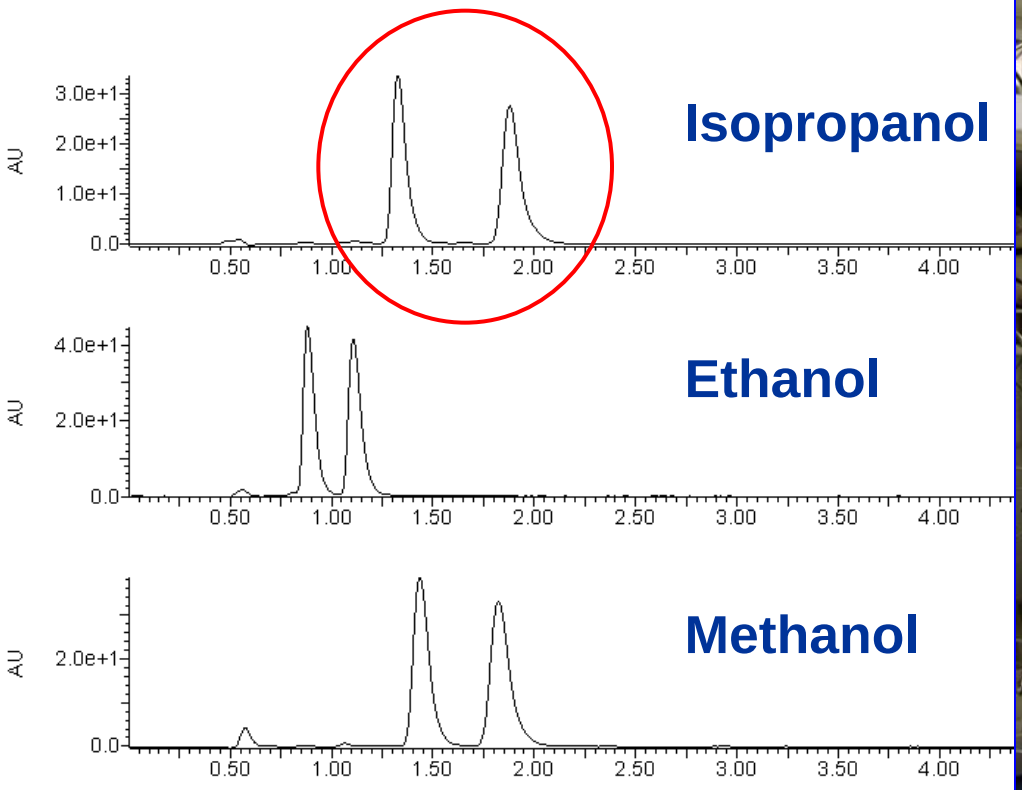


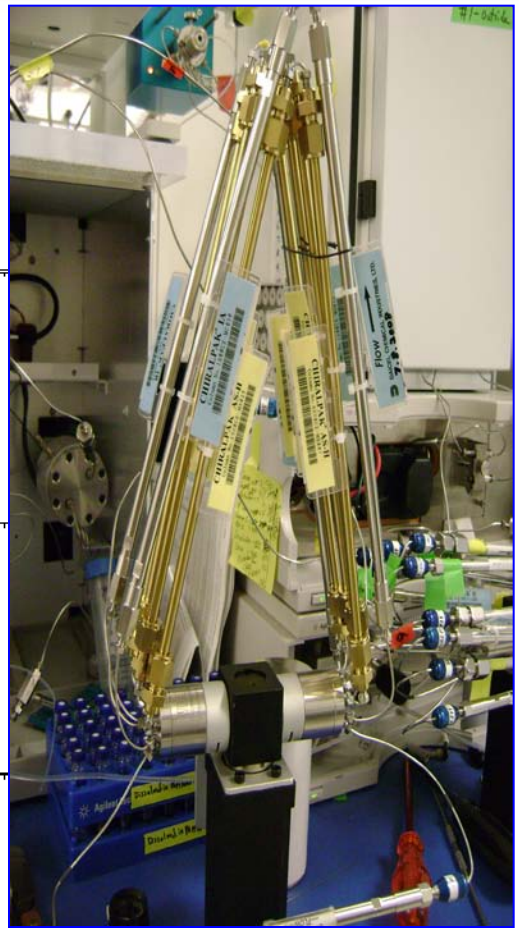


Tier 1: Generic chiral method screening with a cocktail solvent mixture
Mobile Phase A: Liquid CO_2, B: Solvent mixture (0.2% DEA in methanol: ethanol: isopropanol (1:1:1)), Gradient:5% (0.6 min), 15 %/min to 60 % B (1) for AD-H, 50% B (for others), T= 40 C, flow =4.0 ml/min

- ***We try to keep columns outside bundled together not inside a drawer.***

Isocratic Analytical Method Development (AD-H) - 2nd step: Pick a solvent.





Isocratic Method:
Columns: AD-H (150 x 4.6 mm, 5 um), mobile phase:A/B = 60/40, A: Liquid CO_2
B: 0.2% DEA in methanol or ethanol or isopropanol, T= 40 C, flow =4.0 ml/min

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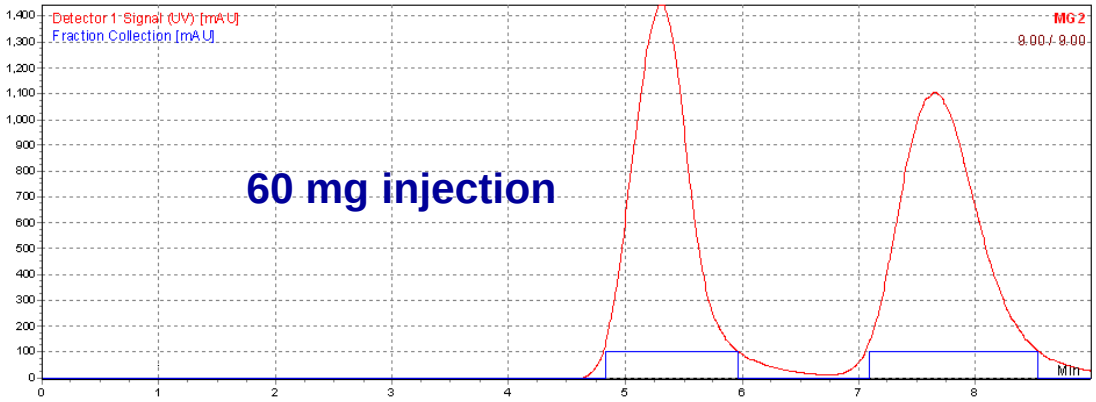
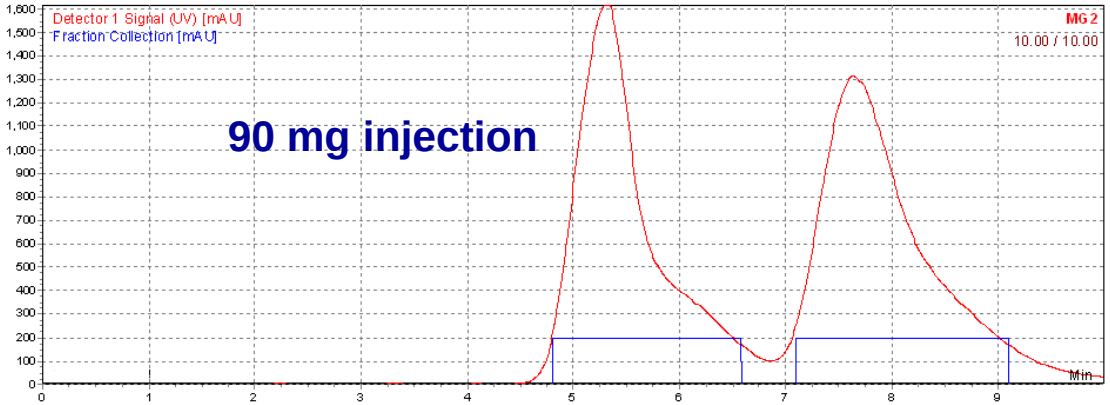
Sale-up Chiral SFC Purification (124 g)

Employ better resolution method: AD-H, IPOH



System pressure increased from 124 to 135 bar after injection and came back down to 124 after a short time.

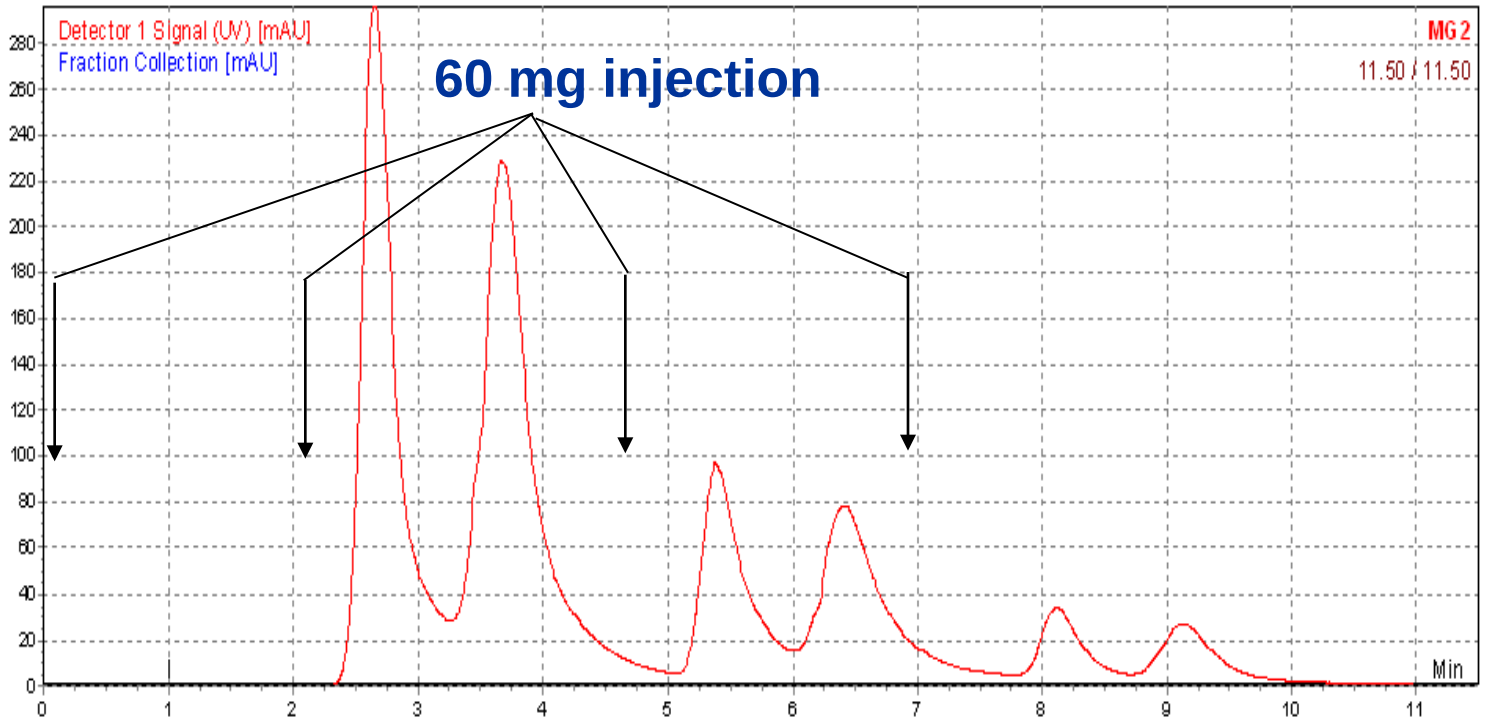


System pressure increased from 124 to 200 bar after injection and came back down to 124 after 1 min.

AD (21 mm x 25 cm, 20 um), Mobile phase CO_2/IPOH (75/25 v/v), P= 124 bar, Sample concentration = 150 mg /m l in DCM/IPOH (8/2 v/v)

Problem: Purification could not be scaled-up

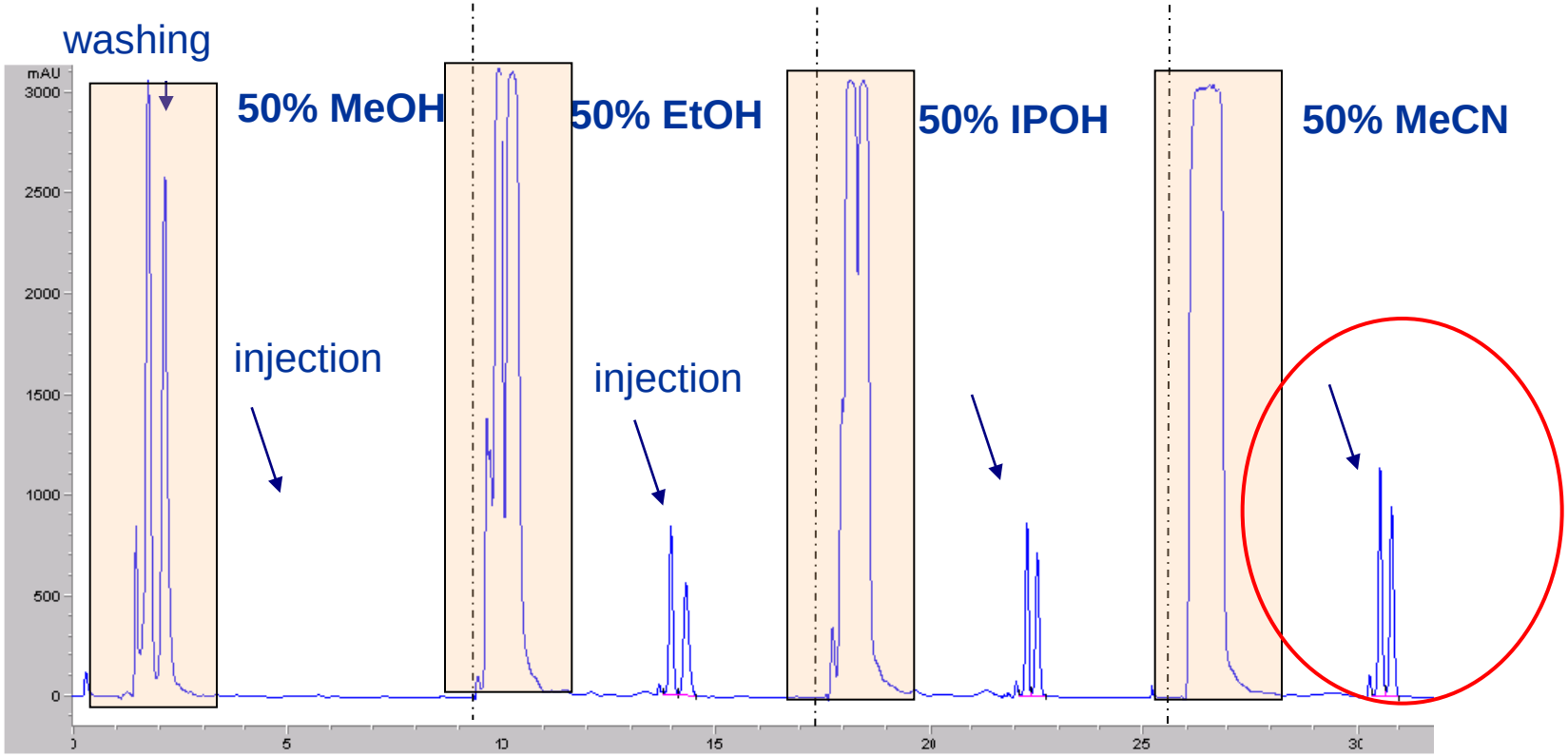
- **Stack injection failed.**



- The SFC system shut down due to the high system pressure.

- ***SFC system was shut down after the four consecutive injections due to the increased system pressure.***

SFC Solubility Screening



- CO_2/MeCN shows the highest solubility for this compound.

SCF solubility screening revealed a potential organic solvent modifier.

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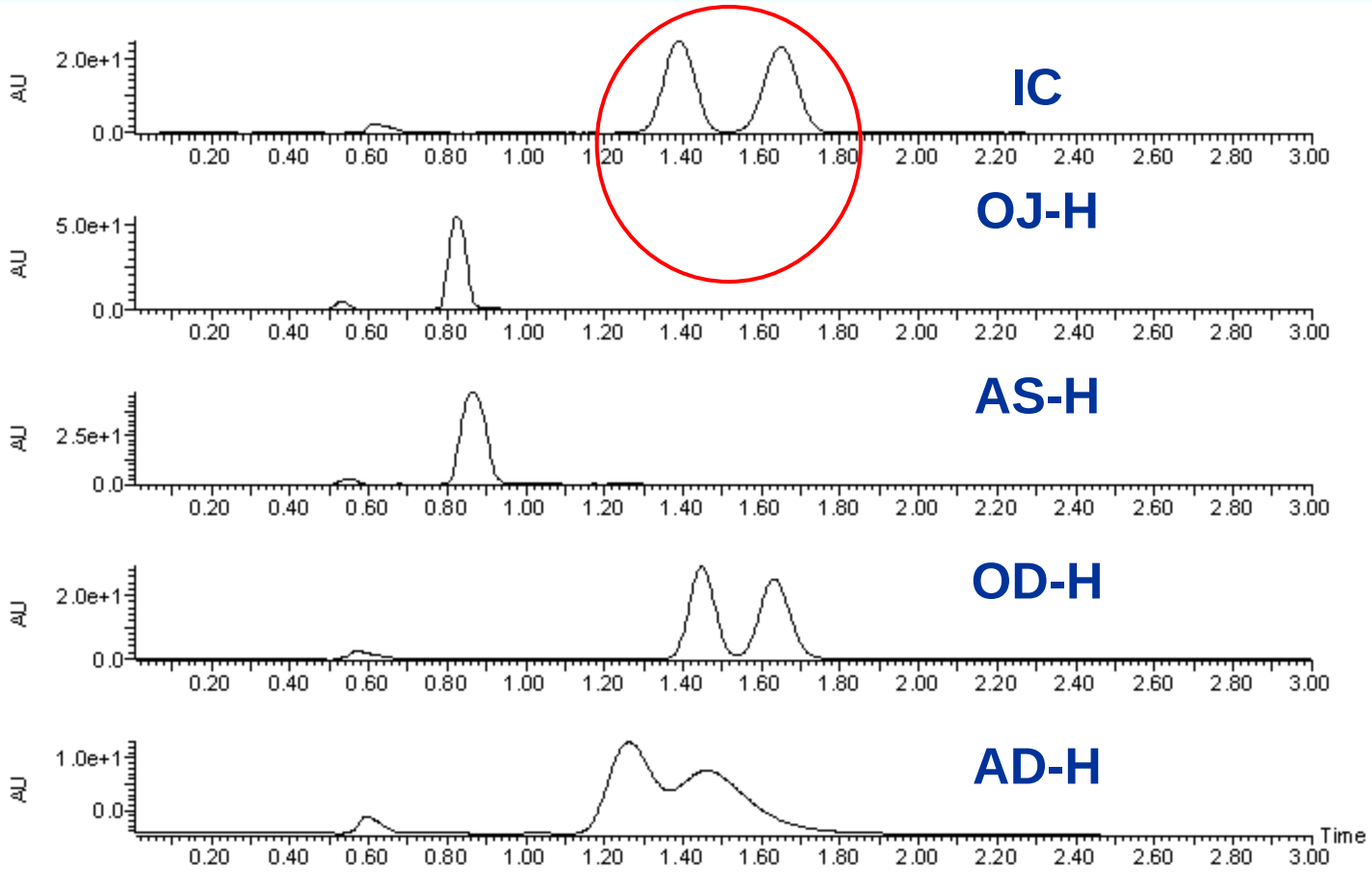
Re-Screening with Acetonitrile



- Column screening with acetonitrile. M.P.: CO_2/acetonitrile (70/30 v/v) flow rate = 4.0 ml/min, IC seems to be the best column of all with acetonitrile.

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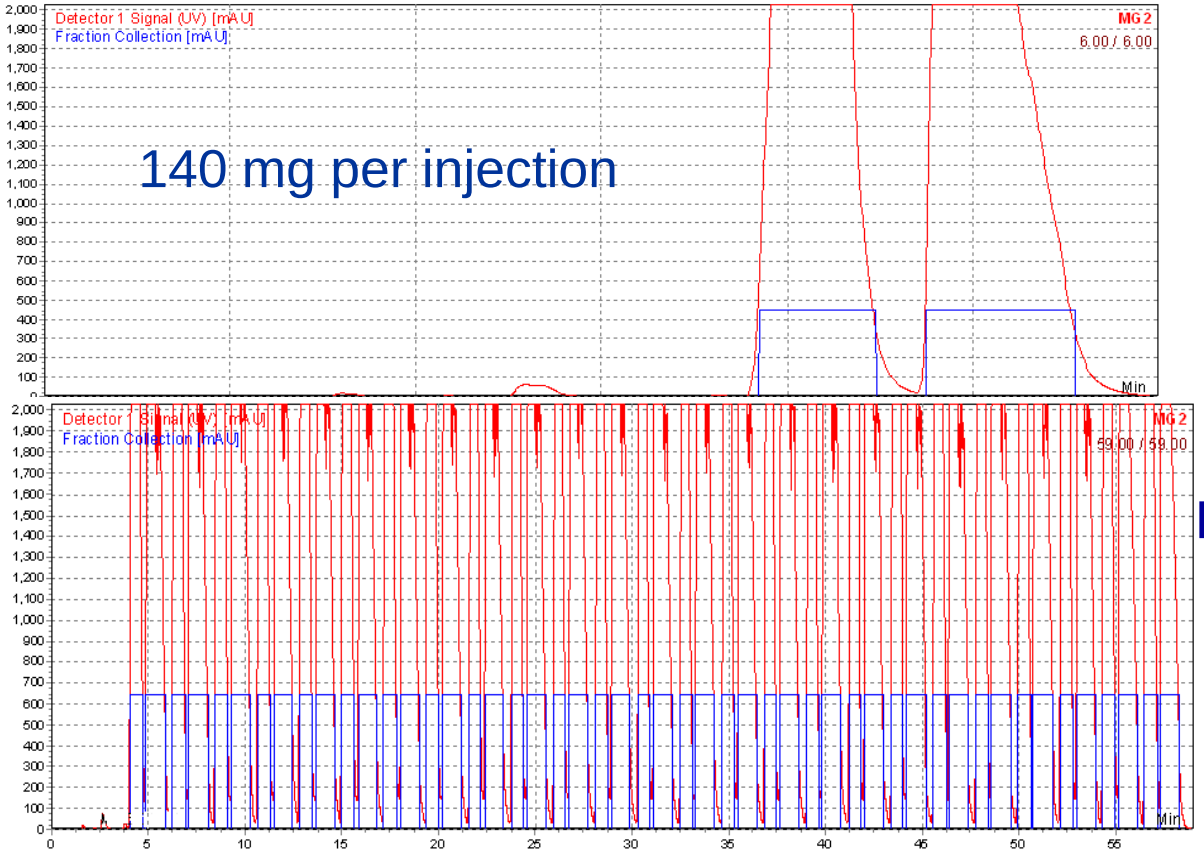
Successful Preparative SFC Chiral Purification (124 g)

- **Employ better resolution method: IC, Acetonitrile**



25 stacked inj.
P=165 ~ 172 bar

IC (2.1cm x 25 cm, 5 um), MeCN 25%, 70ml/min, 140 mg injection.
Purification throughput: 1.9 kkd.

- ***SCF solubility is one of the key factors for a successful purification.***

Conclusions

- Solubility trends are not easy to predict, requiring experimental determination.
- Supercritical fluid solubility can be quickly measured with multiplex chambers.
- Understanding solubility is critical for a successful purification.
- Reversed purification by SFE can be an effective way of getting a pure material by removing an impurity rather than extracting the major component.
- Supercritical fluid solubility knowledge enhances both achiral and chiral purification throughput.

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