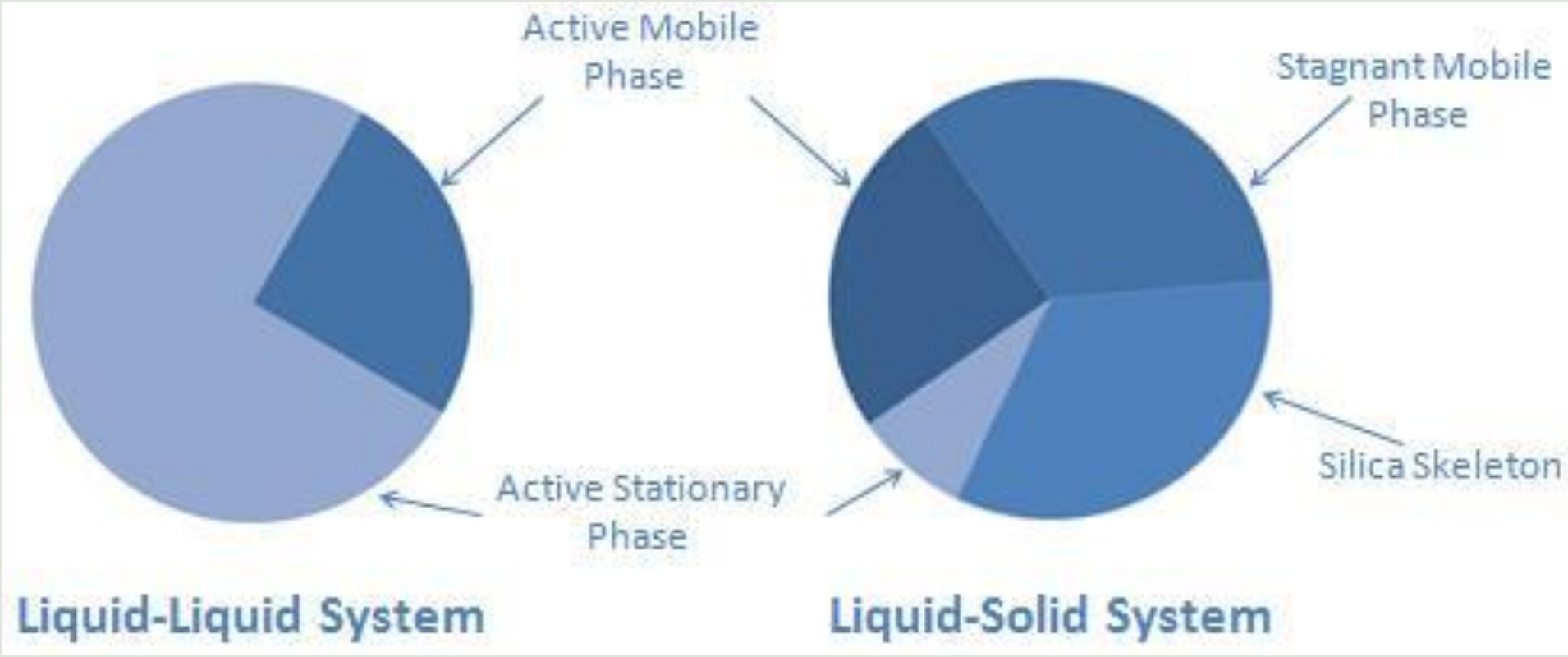
Making liquid stationary phases available for high purity chromatography purification at all scales

Dynamic extractions

Providing separation solutions that make a difference

The difference is the amount of active stationary phase



Dynamic extractions

Providing separation solutions that make a difference

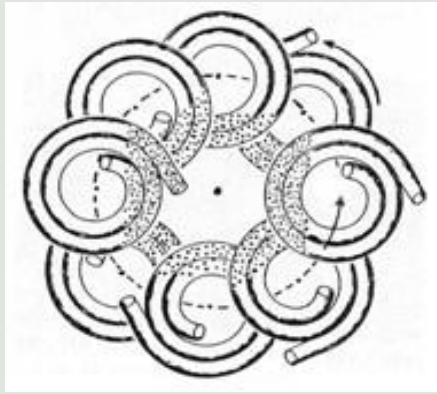
Key benefits of liquid stationary phases

- **High mass and volume injection loadings**
- **Improved handling of sample solubility issues**
- **Ease and cost of scale-up**
- **Extremely low solvent usage**
- **Total sample recovery**
- **Reduced sample preparation**
- **New elution strategies**

What is HPCCC?

High Performance Countercurrent Chromatography

- Crude material is partitioned between two immiscible layers of solvent phases
- Centrifugal rotation around 2 axis creates a planetary motion that causes rapid mixing and the separation of phases **every** revolution
- The stationary phase (SP) is retained by hydrodynamic force field effect
- The mobile phase (MP) is pumped through the column
- Many successive liquid-liquid extractions occur enabling purification of the crude material to occur



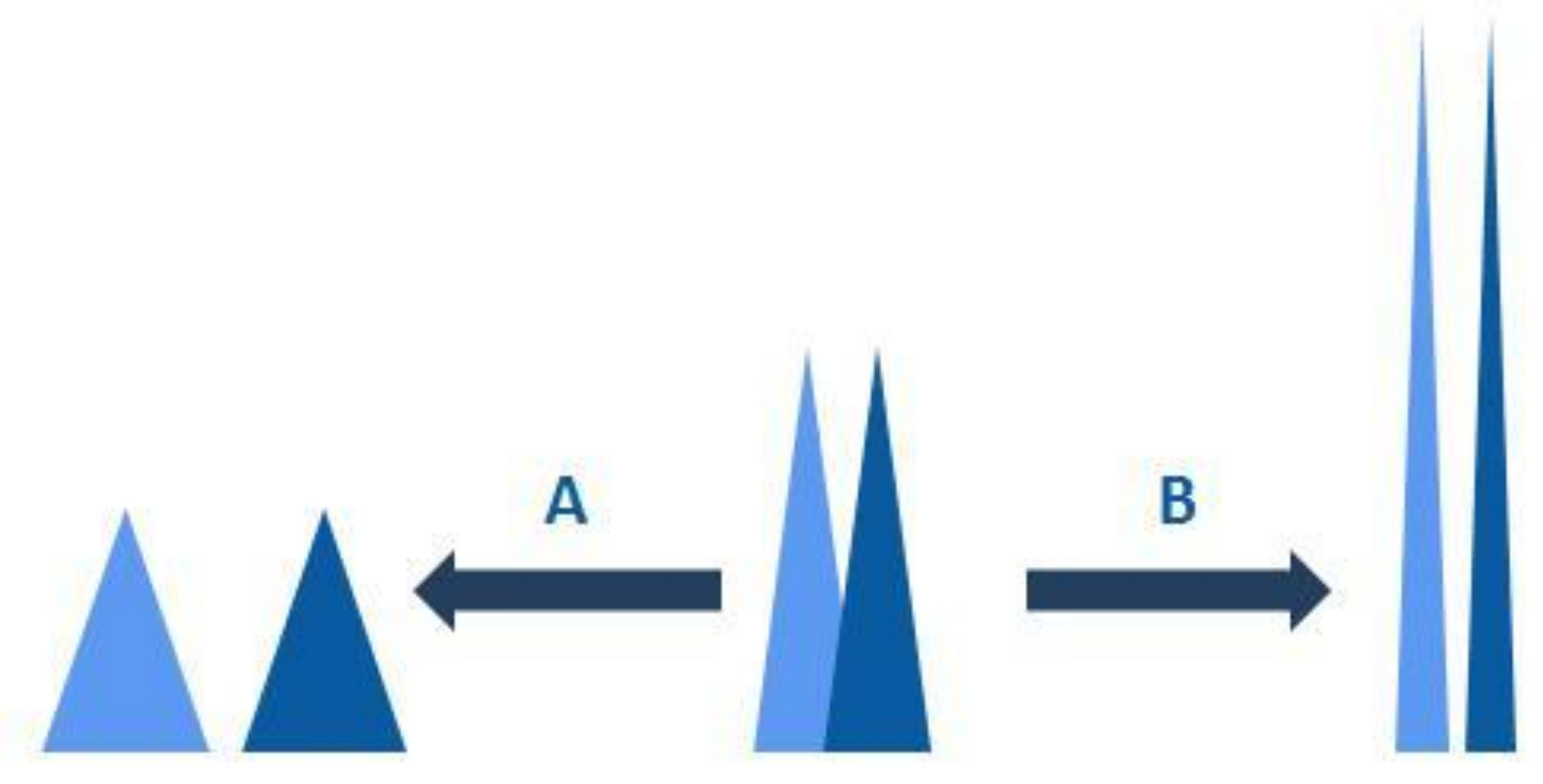
Dynamic extractions

Providing separation solutions that make a difference

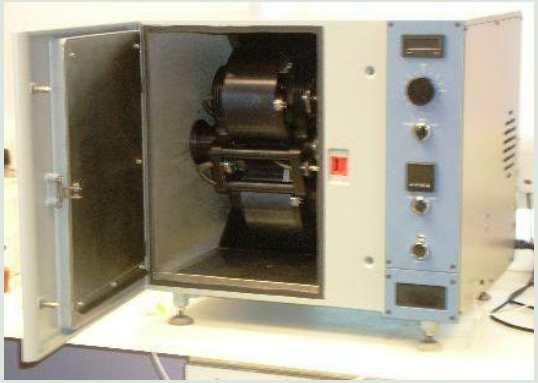
A complimentary and orthogonal liquid chromatography technique

Providing separation solutions that make a difference

Using selectivity (A) rather than efficiency (B)



We achieve this by providing High Performance CCC instruments



- These allow high resolution purifications at high mobile phase flow rates
- Provide a range of instruments from milligram to multi-kilo

Dynamic extractions

Providing separation solutions that make a difference

Wide range of application

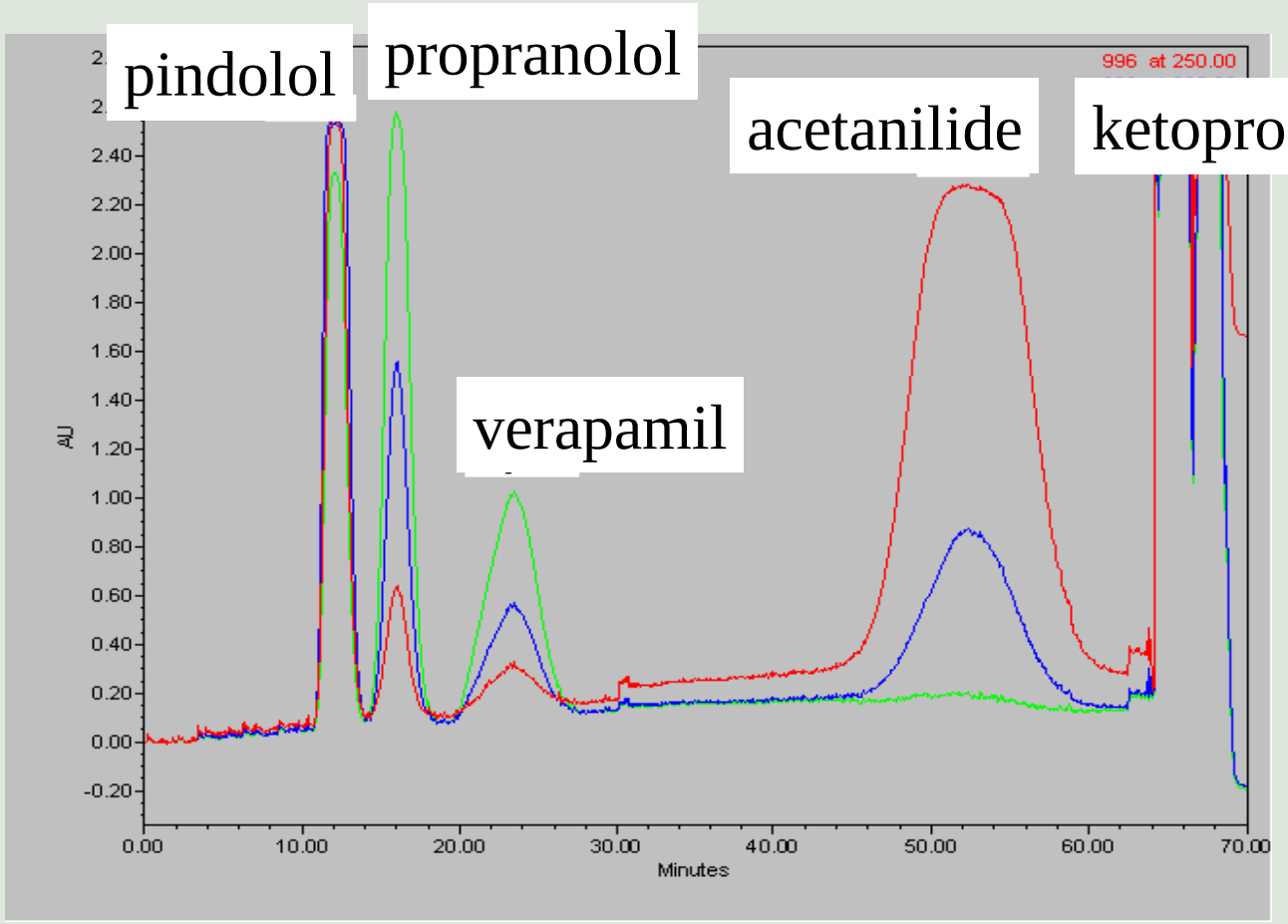
Dynamic extractions

Providing separation solutions that make a difference

Typical HPCCC Applications

- Provides orthogonality to existing separation processes
- Small molecule purification
  - Wide range of targets: synthetics, natural products, peptides
- Preparative separations
  - Highly predicatable scale up, high throughput, low solvent use
- “Difficult” samples
  - Crude samples, problematic solubility
- Low concentration component isolation
  - Impurities or natural extract

Mixed Polarity Synthetic Standards



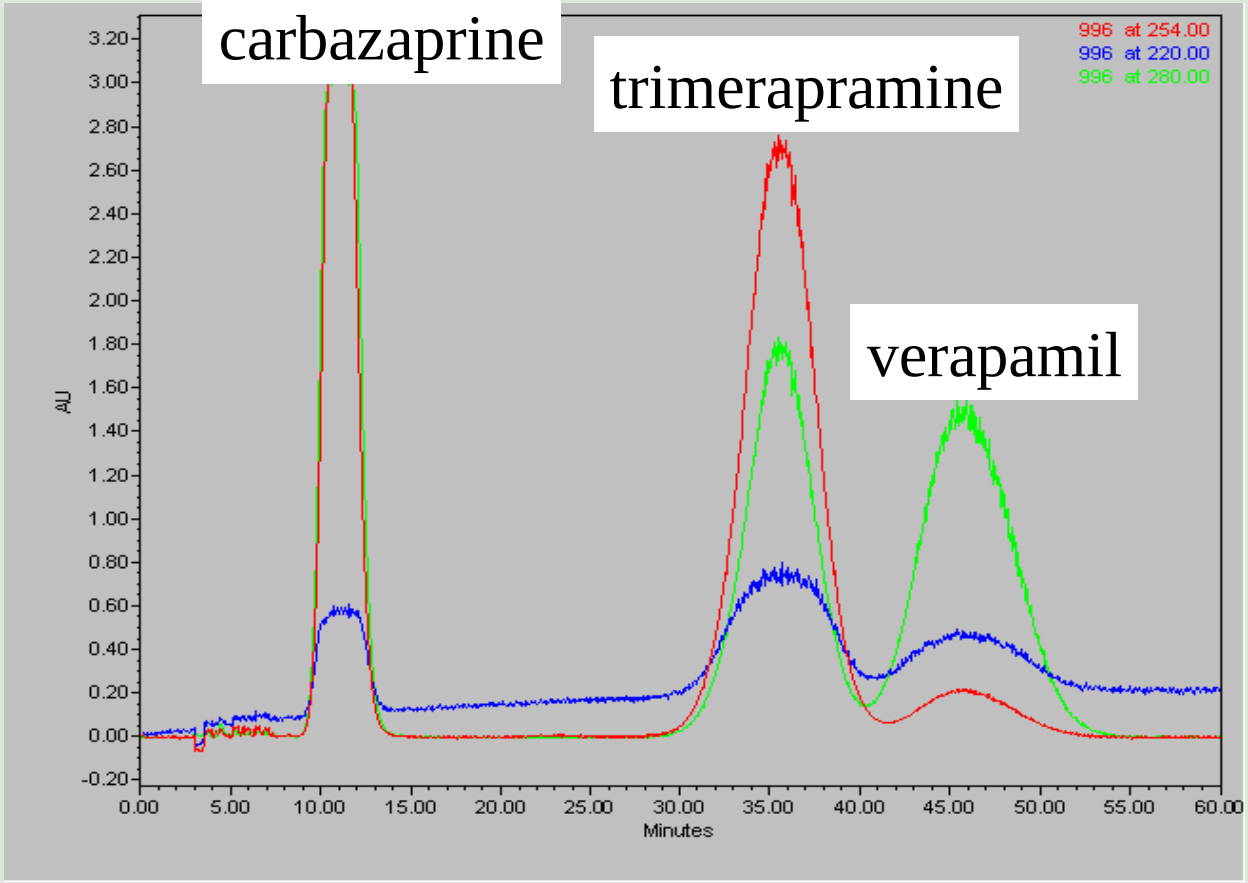
SS11 + 0.1%TFA
RP mode
analytical coil

1mg of each standard
loaded in 1ml
(total 5mg)

Dynamic extractions

Providing separation solutions that make a difference

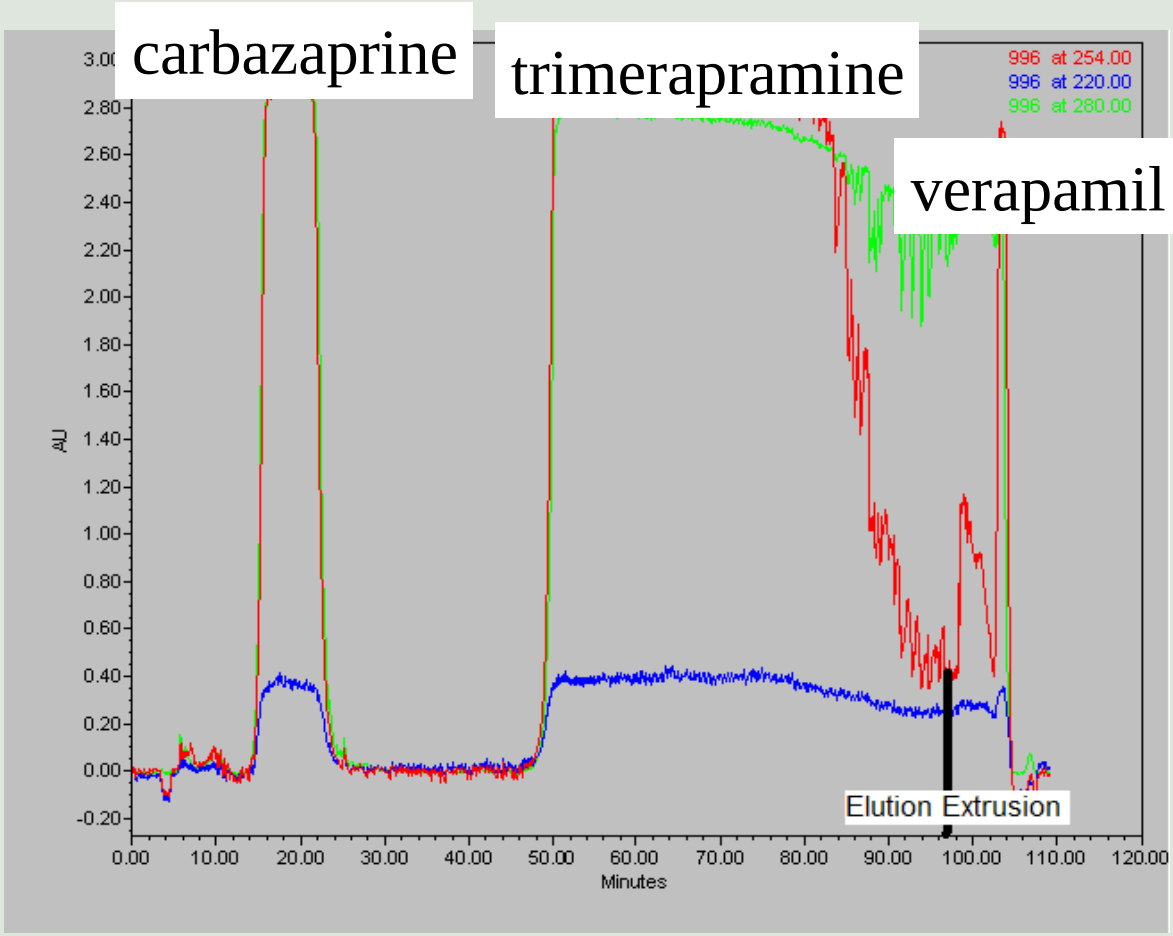
Non-polar Synthetic Standards



SS14
NP mode
analytical coil

1mg of each standard
loaded in 1ml
(3 mg total)

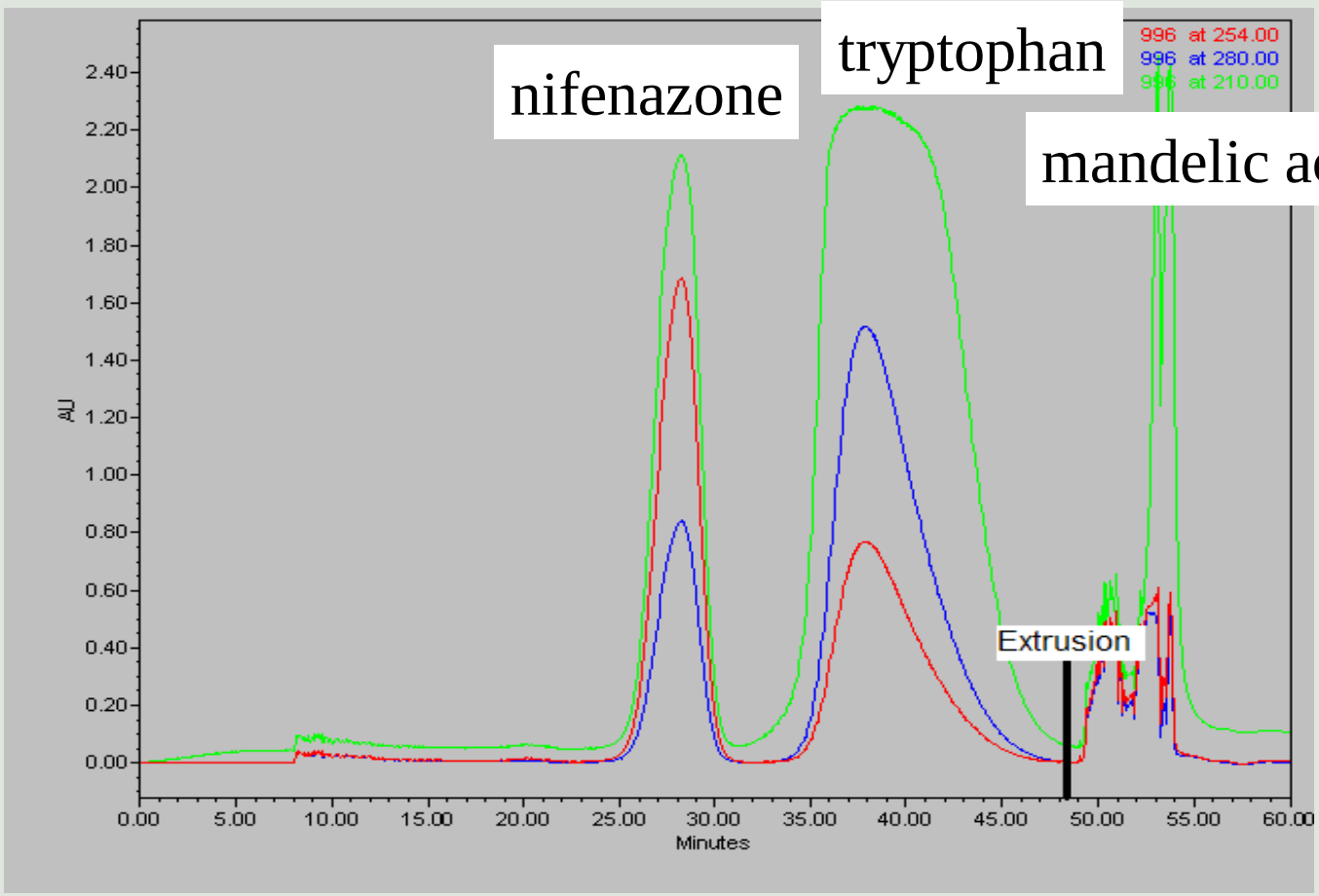
Non-polar Synthetic Standards – 30 times scale-up



SS14
NP mode
analytical coil

30mg of each standard
loaded in 1ml total

Polar Standards



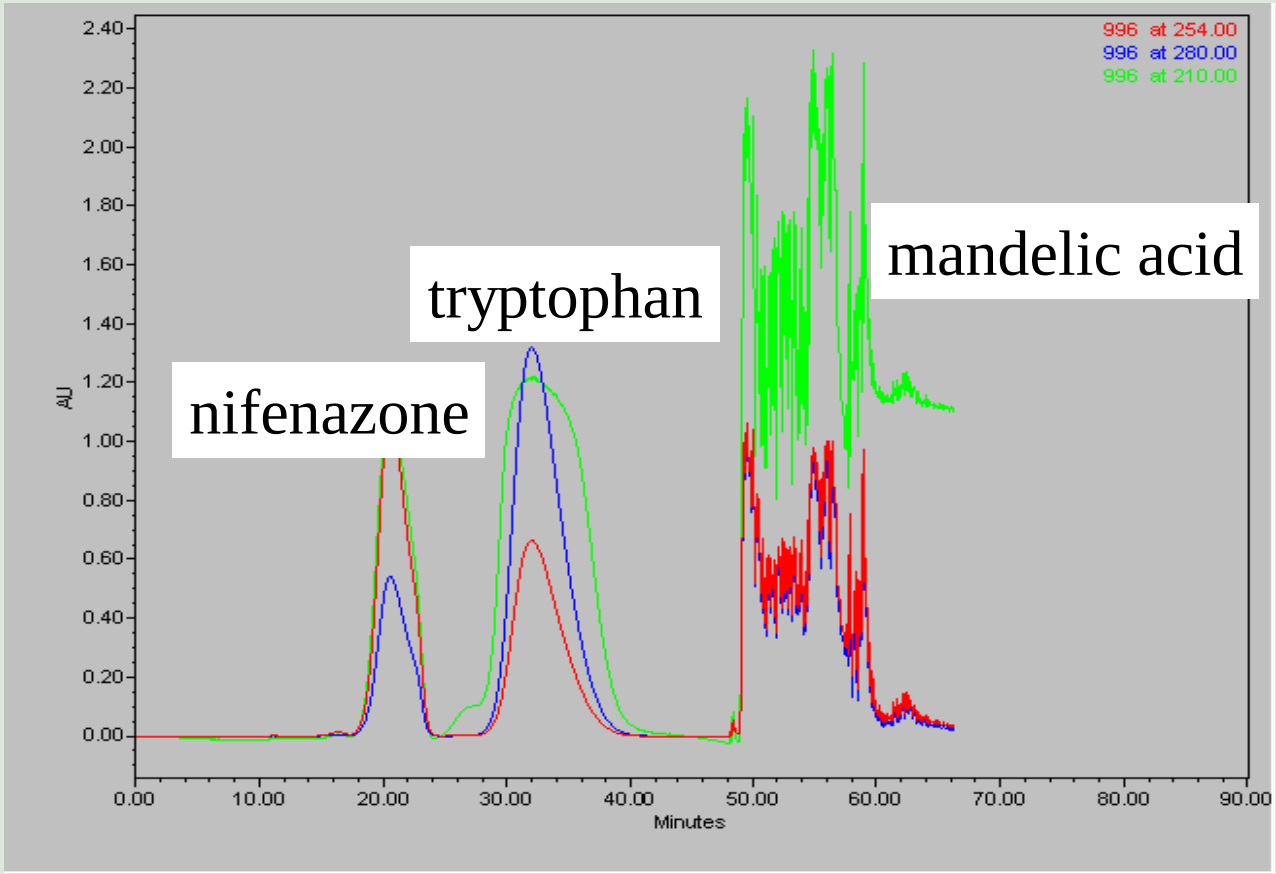
SS01 + TFA
NP mode
analytical coil

1mg of each standard
loaded in 1ml
(3 mg total)

Dynamic extractions

Providing separation solutions that make a difference

Polar Standards – 6 times Scale-up



SS01 + TFA
NP mode
preparative coil

6mg of each standard
loaded in 6ml total

Dynamic extractions

Providing separation solutions that make a difference

Preparative separations

Dynamic extractions

Providing separation solutions that make a difference

Synthetic compound application

- DE Centrifuge: Midi
- Type of separation: Hydrophobic (Non-polar)
- Crude loading per injection: 25 grams
- Target compound isolated per injection: 6 grams (average)
- Purity: > 92%
- Recovery: >95%
- Separation time: 25 minutes
- Total quantity of crude processed: 9 kg
- Total solvent used: 468 litres

Natural product application

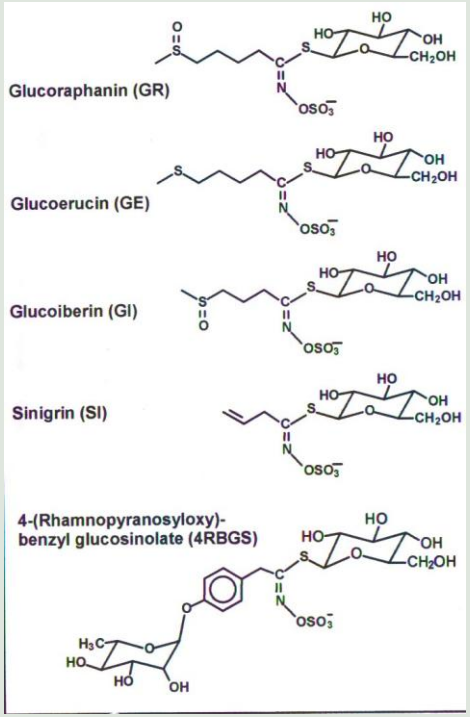
- DE centrifuge: Maxi
- Type of separation: Hydrophilic (polar)
- Crude loading per injection: 160 grams
- Target compound isolated per injection: 23.6 grams
- Purity: >95%
- Recovery: >90%
- Separation time: 25 minutes
- Total quantity of crude processed: 6.7 kg
- Total solvent used: 456 litres

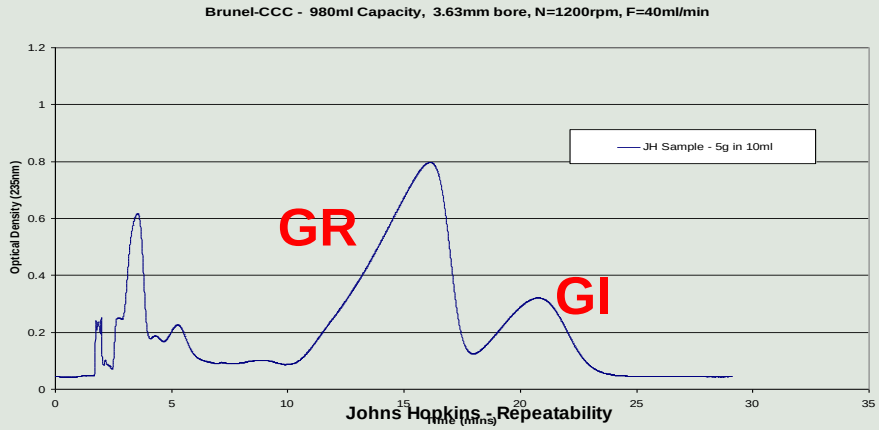
Difficult separations

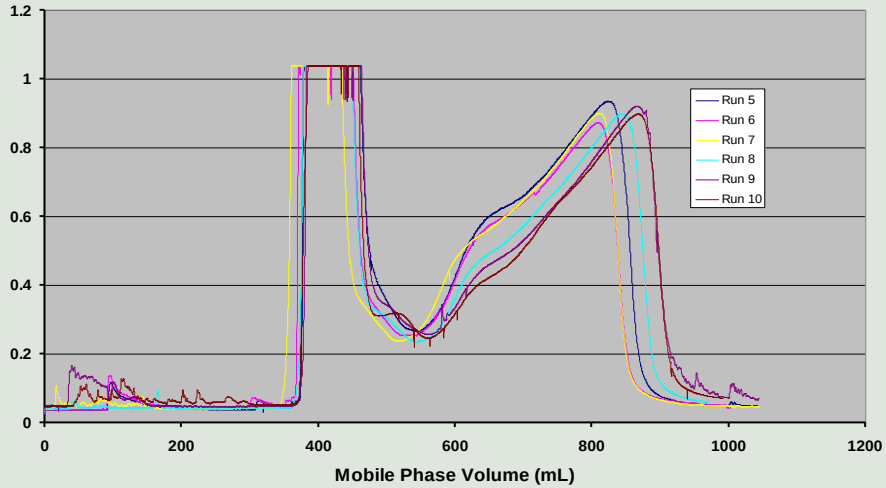
Dynamic extractions

Providing separation solutions that make a difference

Separation of Glucosinolates







- 52.6g from 0.59kg
- 34 runs
- 47%w/w sample conc
- 17g/run sample loading
- 98.5% pure
- 3 days

Dynamic extractions

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Low concentration components

Dynamic extractions

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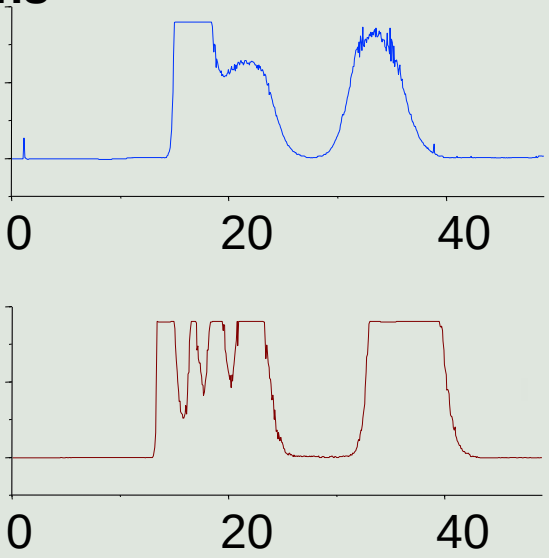
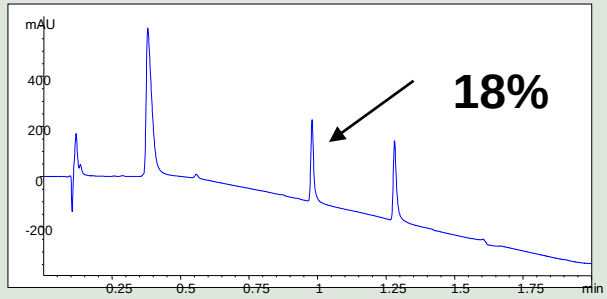
Impurity isolation for identification purposes

Analytical and Preparative runs

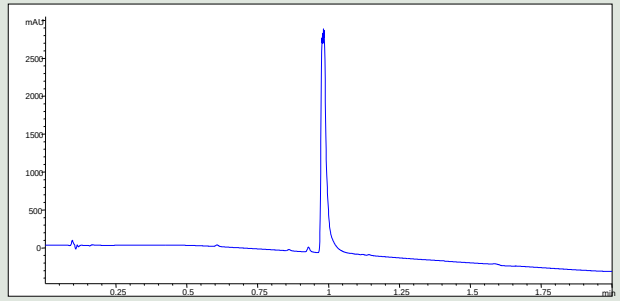


88% yield, 98% purity based on HPLC-UV

Starting material



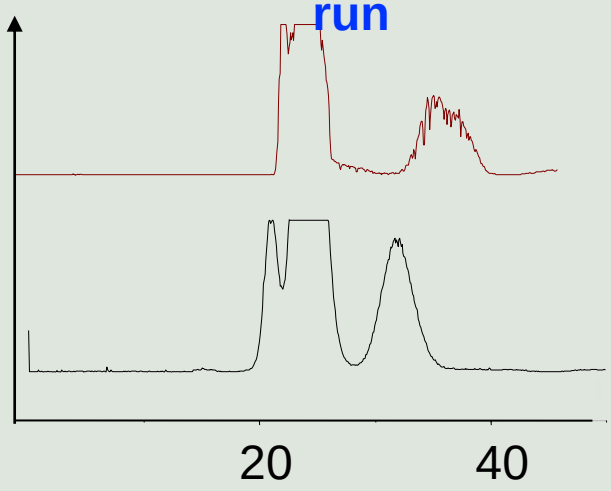
Final sample

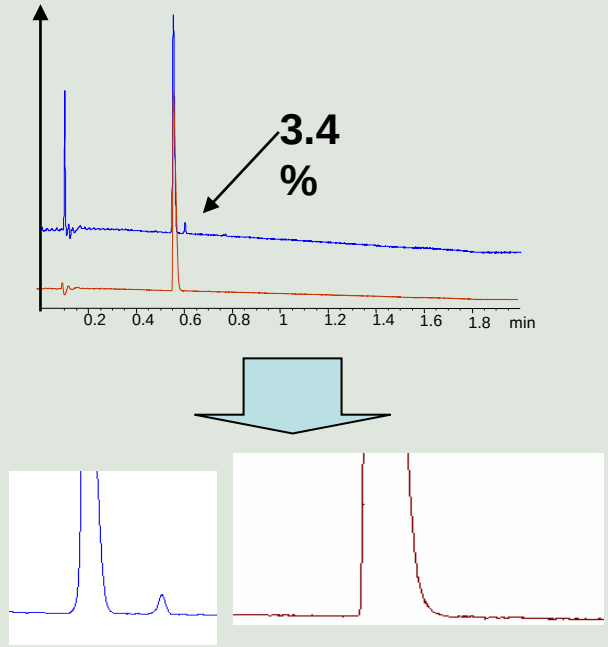


Courtesy of Pfizer UK

Minor impurity removal

Analytical run, Preparative run





92% yield, 99.9% purity on HPLC (impurity not detected)

Courtesy of Pfizer UK

Reduced Sample Preparation

Dynamic extractions

Providing separation solutions that make a difference

Viscous syrup

Dynamic extractions

Providing separation solutions that make a difference

Crude extract including precipitates

Loading capacity up to 50%w/w depending on solubility

Scale up

Dynamic extractions

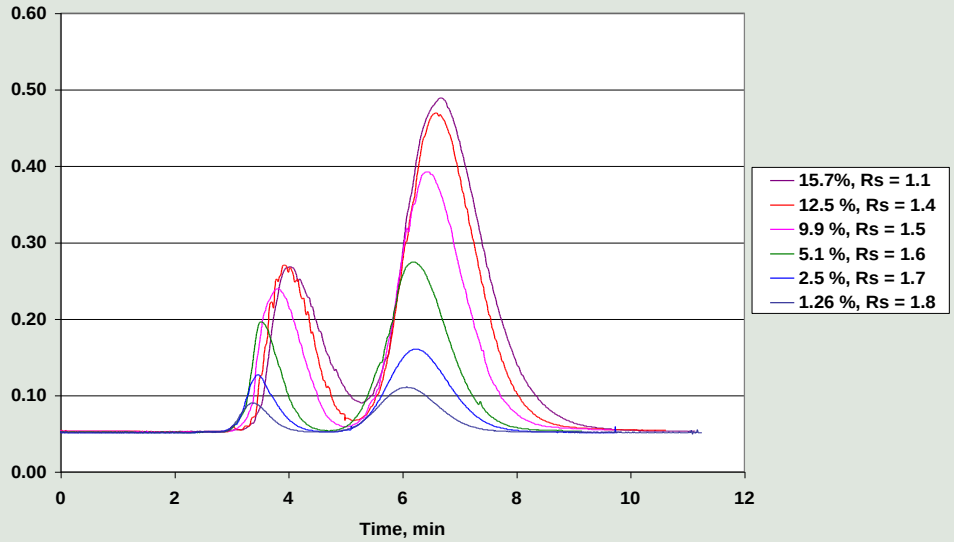
Providing separation solutions that make a difference

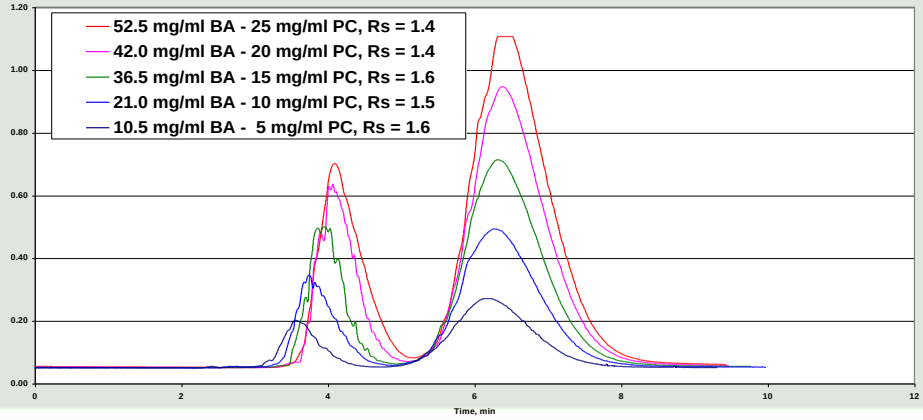
Scale-up is simply volumetric

- You use the ratio of the column volumes that you are scaling between
- For example, 20ml column to 120ml column would be 1:6
- For complete scale-up simply multiply
  1. the sample volume by this ratio
  2. The mobile phase flow rate by this ratio

Performed and optimised at analytical scale

DE Mini

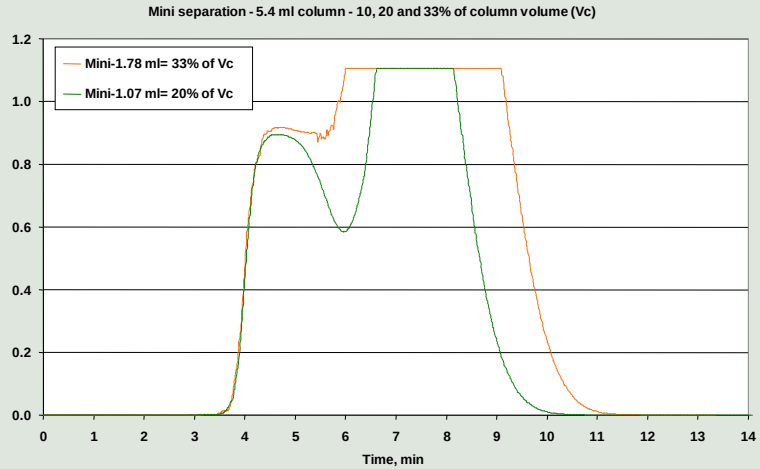


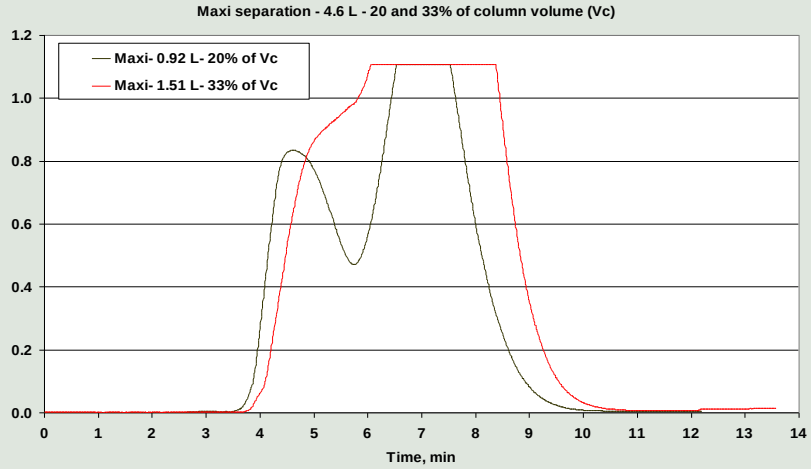


Dynamic extractions

Providing separation solutions that make a difference

Directly transferred to the kilo/pilot scale





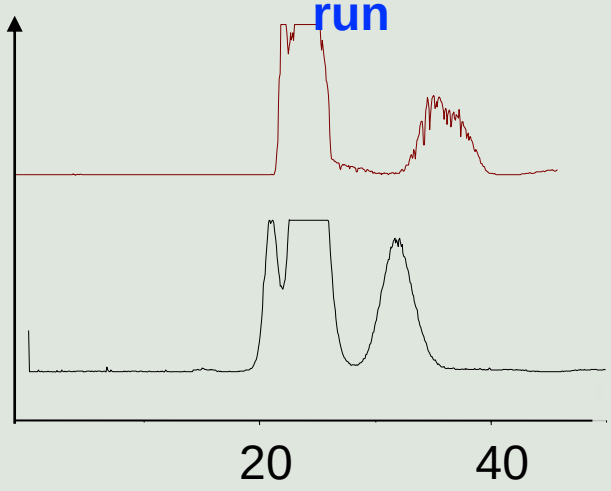
Dynamic extractions

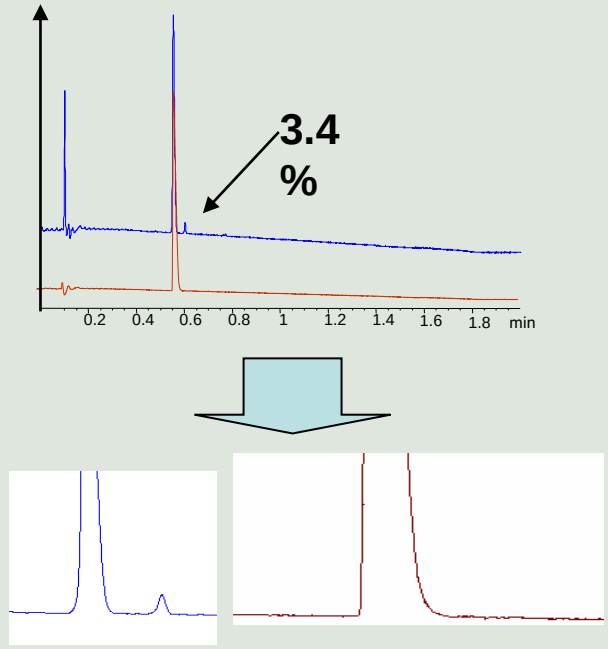
Providing separation solutions that make a difference

Analytical to semi-preparative scale-up

Minor impurity removal to obtain high purity product - Scale-up x26, from 300 mg on a 37 mL coil to 7.8g on a 950 mL coil

Analytical run, Preparative run





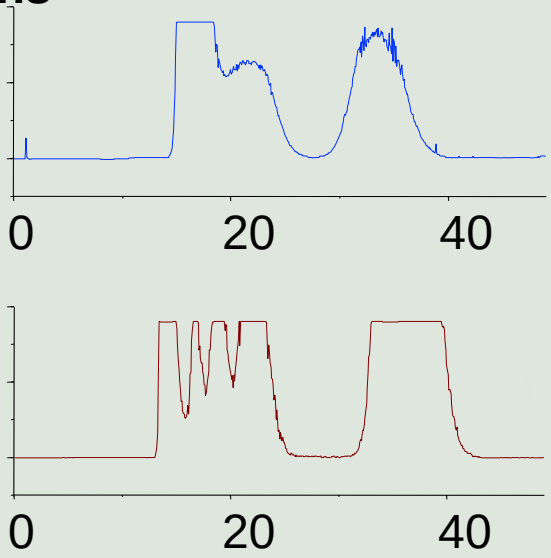
92% yield, 99.9% purity on HPLC (impurity not detected)

Courtesy of Pfizer UK

Analytical to semi-preparative scale-up

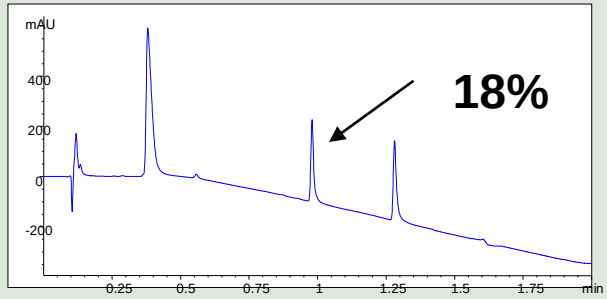
Impurity isolation for identification purposes - Scale-up x26, from 200 mg on a 37 mL coil to 5.2g on a 950 mL coil

Analytical and Preparative runs

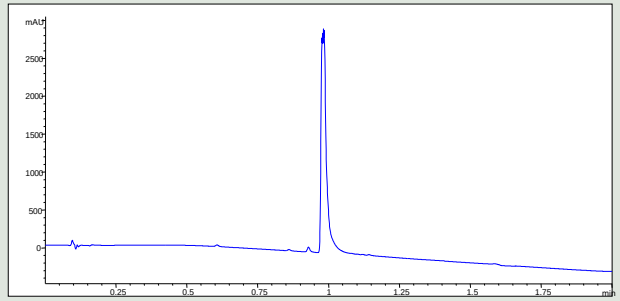


88% yield, 98% purity based on HPLC-UV

Starting material



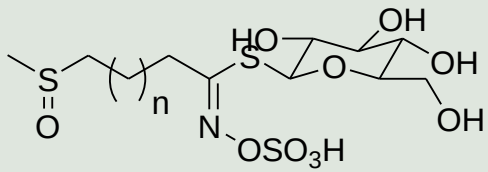
Final sample



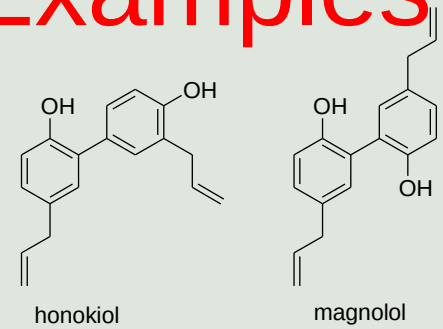
Courtesy of Pfizer UK

Literature Scale-up Examples

DE MAXI



n=2 GR, glucoraphinin
n=1 GI, glucoiberin



honokiol magnolol

	glucoraphinin(GR)	honokiol
Phase System	Propanol:CH_3CN:AS:Water (1.0:0.5:1.2:1.0)	Hep:EtOAc:MeOH:Water (1.0:0.4:1.0:0.4)
Loading/injection	115g crude in 230mL	50g crude in 190mL
Column volume loading	5%	5%
Target Compound/injection	23.6g	20g
Cycle Time	25min	20min
Total crude processed	8kg	150g
Total # runs	42	3
Total Solvent Usage	420L	30L

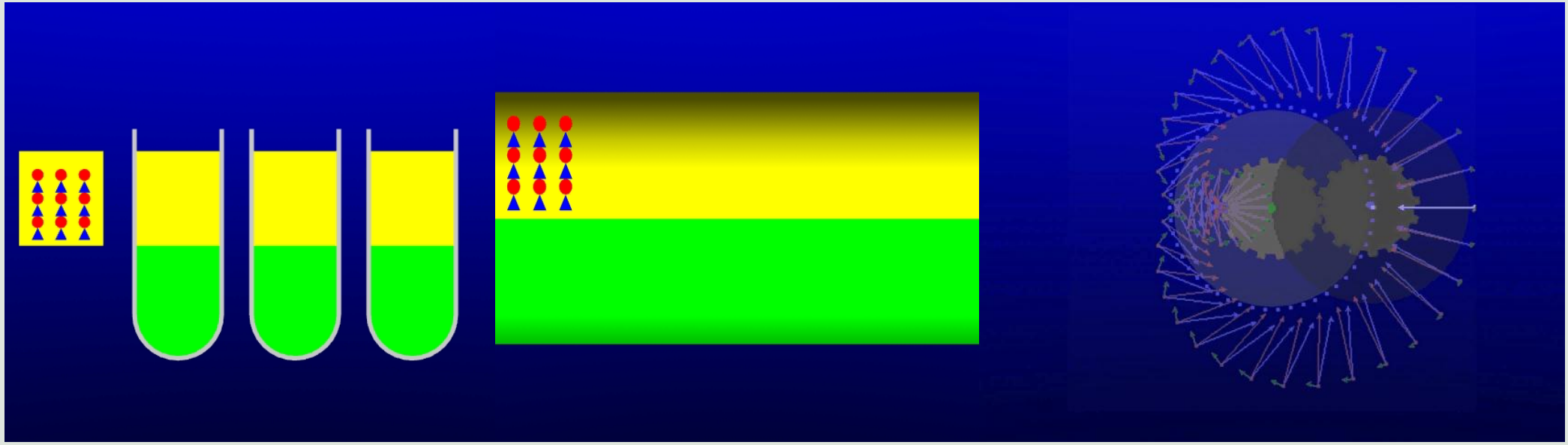
1) Sutherland, I.A., *J. Chrom. A*, **1151** (2007), 6-13;
2) 2) Fisher, D., Garrard, I.J., Heuvel, R. van den, Chou, F.E., Fahey, J.W., *J. Liq. Chrom. Rel. Tech.*, **28** (2005), 1913-1922;
3) 3) Chen, L. et al., *J. Chrom. A*, **1142** (2007), 115-122.

Understanding HPCCC

Dynamic extractions

Providing separation solutions that make a difference

CCC mechanism of separation is partitioning



Test tubes

A column

HPCCC
Instrument

Key concepts in HPCCC

Dynamic extractions

Providing separation solutions that make a difference

Mechanism of separation

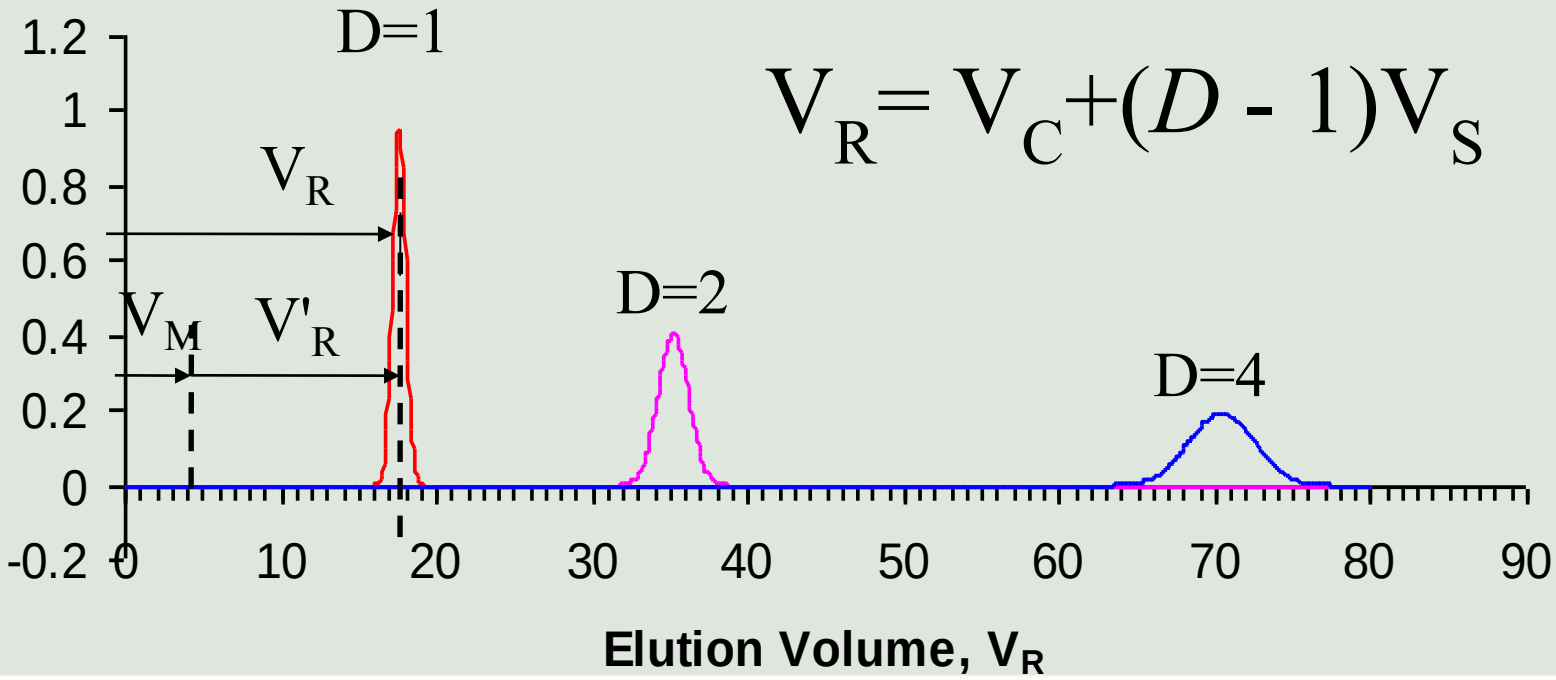
- Separation of compounds in HPCCC is based on liquid-liquid distribution
- Purification occurs because of the different solubility of the components in the liquid mobile and stationary phases
- Compound retention determined by the distribution ratio, D

$$D = \frac{[stationary\ phase]}{[mobile\ phase]}$$

- D can be calculated by partitioning studies

Retention is highly predictable

For, $V_C = 17.6mL$, $V_S = 13.4mL$ ➡ Stationary phase fraction, $S_f = 0.75$



$$V_R = V_C + (D - 1)V_S$$

HPCCC Run Modes

- **Reverse phase (RP)** - Stationary phase (SP) is upper phase (UP)
  - Mobile phase (MP) is lower phase (LP)
  - Advantages: Direct analysis of fractions without need to vac down
- **Normal phase (NP)** - Stationary phase (SP) is lower phase (LP)
  - Mobile phase (MP) is upper phase (UP)
  - Advantages: Easier concentration of fractions
    - NP is a good starting point for method development.
    - If unsuccessful, switching to RP can potentially improve resolution.
    - Advanced run modes: elution-extrusion, pH zone refining

Method Development

Dynamic extractions

Providing separation solutions that make a difference

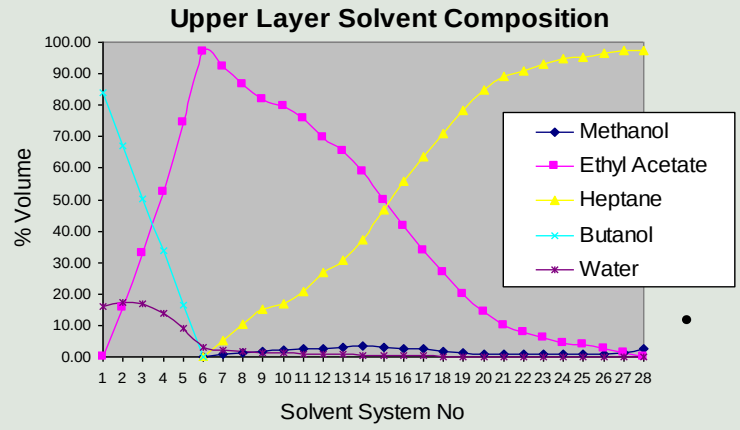
Solvent System Selection

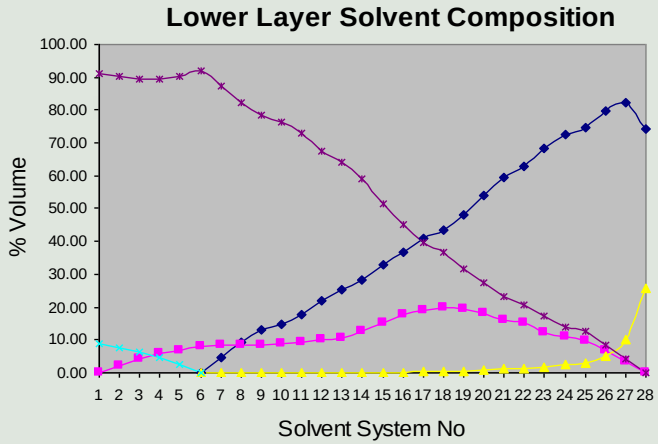
HEMWat solvent systems are suitable for the majority of applications

- 4 components – Heptane(or hexane), ethyl acetate, methanol & water
- Forms 2 immiscible phases
  - Denser lower phase (LP) comprising mostly methanol and water
  - Lighter upper phase (UP) of heptane and ethyl acetate
- Butanol, ethyl acetate and water provides separation in more polar applications
- Additives - Buffers, acids bases expand the selectivity of HEMWat solvent systems
- No pH-column stability issues as inert tubing no solid

HEMWat Solvent System

SS		Heptane	EtOAc	MeOH	Butanol	Water
1		0	0	0	5	5
2		0	1	0	4	5
3		0	2	0	3	5
4		0	3	0	2	5
5		0	4	0	1	5
6		0	1	0	0	1
7	More	1	19	1	0	19
8	Polar	1	9	1	0	9
9		1	6	1	0	6
10		1	5	1	0	5
11		1	4	1	0	4
12		1	3	1	0	3
13		2	5	2	0	5
14		1	2	1	0	2
15		2	3	2	0	3
16		5	6	5	0	6
17		1	1	1	0	1
18		6	5	6	0	5
19		3	2	3	0	2
20		2	1	2	0	1
21		5	2	5	0	2
22		3	1	3	0	1
23		4	1	4	0	1
24		5	1	5	0	1
25	Less	6	1	6	0	1
26	Polar	9	1	9	0	1
27		19	1	19	0	1
28		1	0	1	0	0

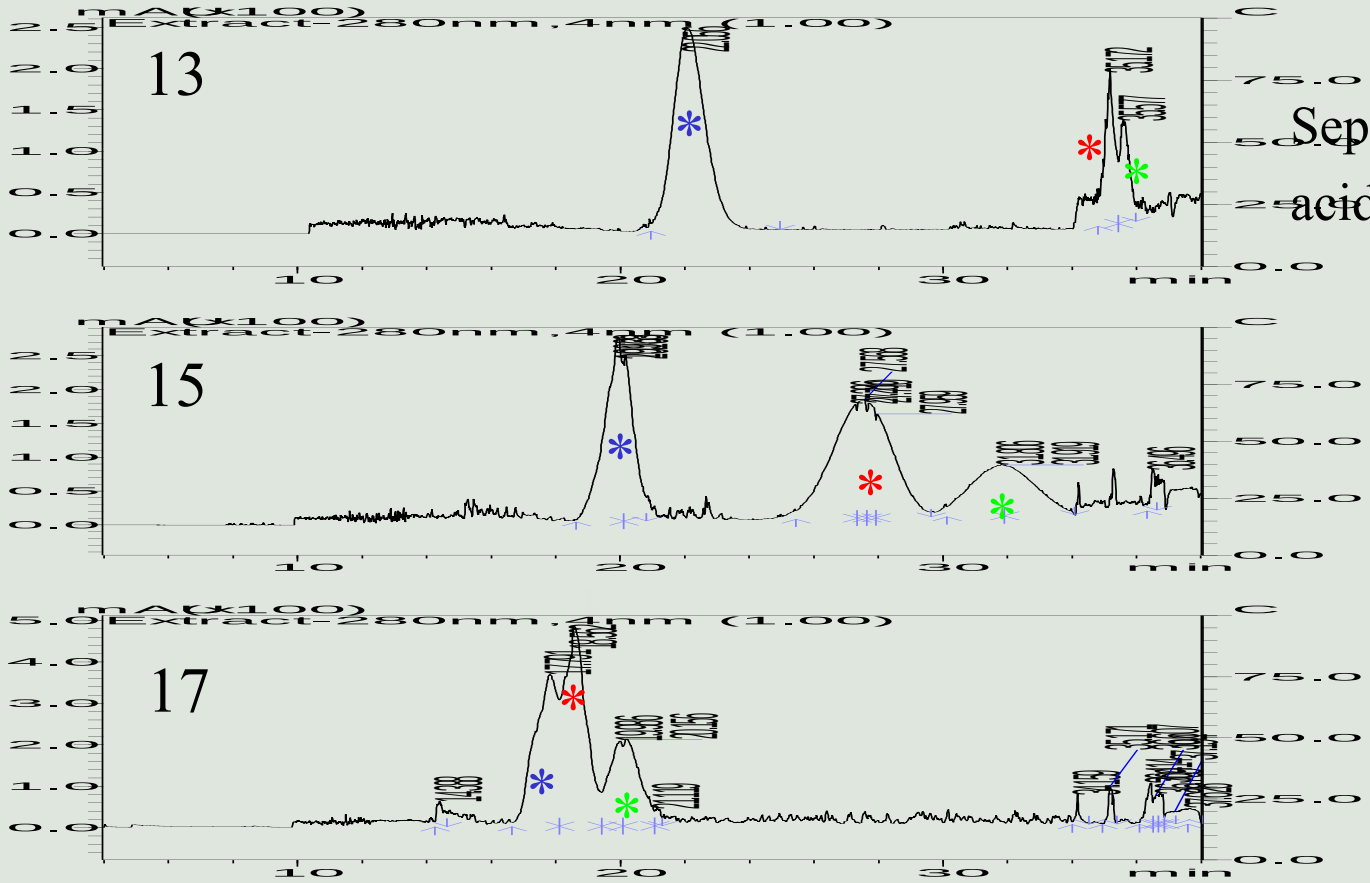




- Provides separation for the majority of compounds
- Good solubility of a wide range of materials

I.J.Garrard. L.Janaway, D.Fisher, *J. Liq. Chrom. Rel. Tech.*, **30** (2007), 151-163

Solvent System Selection



Separation of Caffeine, feruli acid and umbelliferone:

- Analytical coil
- Reverse phase
- 1ml/min flow rate
- 34 min run followed by 4 min extrusion (pump SP @ 8ml/min)

Using the correct SS is key to obtaining an effective and efficient separation

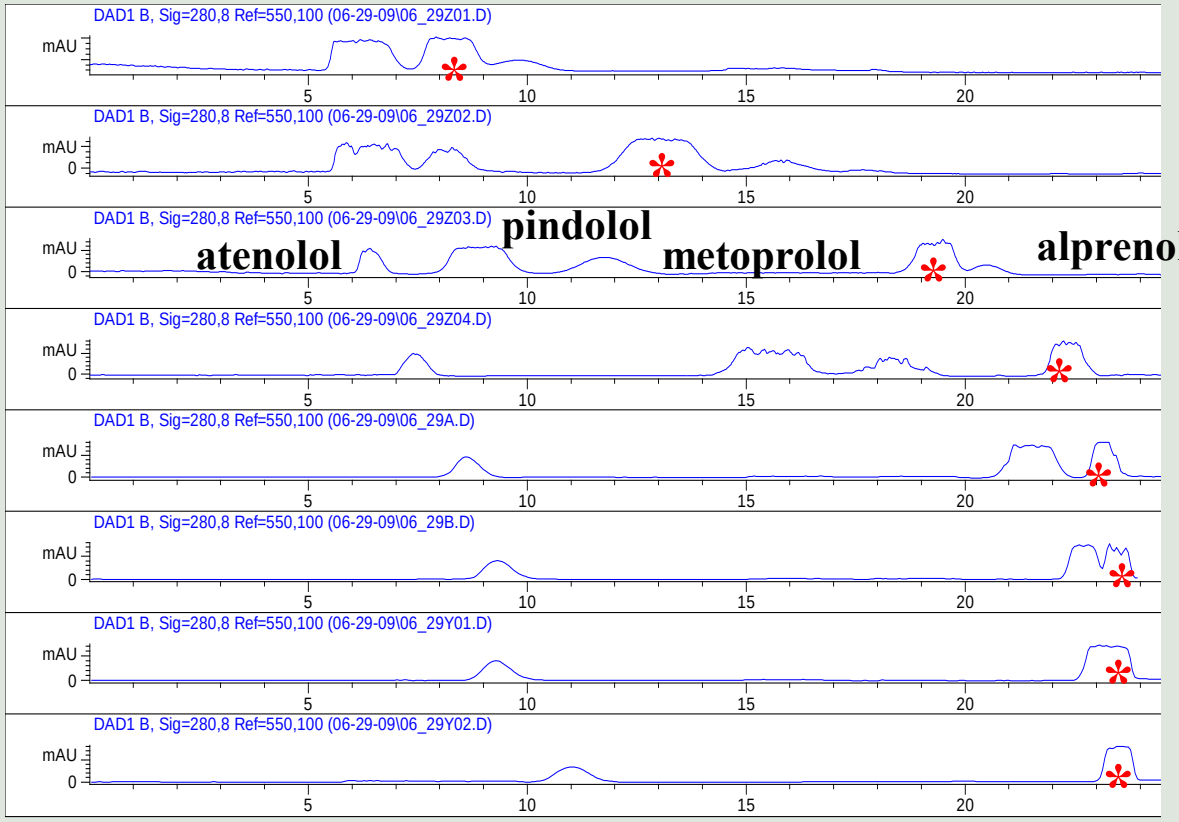
Automated HPCCC Solvent System Screening

- Automated, unattended operation using any standard analytical HPLC systems (Agilent, Shimadzu, etc.)
- On-demand mixing of solvent systems using quaternary HPLC pumps
- 2 to 3 hours/solvent system screen:
  - 5 solvent systems
  - normal and/or reverse phase separation modes
  - any pH can be used

Automated Solvent System Screening Applied to a set of 5 b-Blockers – RP, pH9.5

Solvent System

21
19
17
15
13
11
09
07



* propranolol

Dynamic extractions

Providing separation solutions that make a difference

Range of HPCCC equipment

Dynamic extractions

Providing separation solutions that make a difference

DE HPCCC Instrument Range

	Spectrum	**Midi**	**Maxi**
Loading (grams/run)	up to 2	5 – 40	500-1500
Flow rate (mL/min)	0.5 – 10	30 – 50	500 – 1500
Rotational Speed 240g (RPM)	1600	1400	850
Coil Volume (mL)	20 and 140	38 and 940	4600 or 18000

Integrated solutions

Dynamic extractions

Providing separation solutions that make a difference

DE Solutions & Capabilities

Dynamic extractions

Providing separation solutions that make a difference

Who are we?

- UK manufacturer of High Performance Countercurrent Chromatography instruments
- Instruments are sold and supported internationally from UK and US offices plus a network of specialist international distributors
- Instrument range – Analytical, Preparative and Pilot plant/ Manufacturing scales
- Customer education & training, sample feasibility studies available internationally

Dynamic extractions

Providing separation solutions that make a difference

Products and engineered solutions for end-user use

Dynamic extractions

Providing separation solutions that make a difference

DE Capabilities provided

- Product development and engineering design
- Feasibility studies
- Gram to Kilo separations to GLP
- Training
- Demonstration

Dynamic extractions

Providing separation solutions that make a difference

A diverse range of customers

peakdale MOLECULAR

AstraZeneca UNITED KINGDOM

gsk GlaxoSmithKline

Phytopharm

syngenta UK & IRELAND

Abbott A Promise for Life

NOVARTIS

MERCK

Pfizer

Lilly

四川大學 Sichuan University

National Cancer Institute

BiB

Ecopia BUILDING A PIPELINE THROUGH INNOVATION

Dynamic extractions

Providing separation solutions that make a difference

Key benefits of liquid stationary phases

- **High mass and volume injection loadings**
- **Improved handling of sample solubility issues**
- **Ease and cost of scale-up**
- **Extremely low solvent usage**
- **Total sample recovery**
- **Reduced sample preparation**
- **New elution strategies**

Thank you for your attention

Any questions?

Dynamic extractions

Providing separation solutions that make a difference