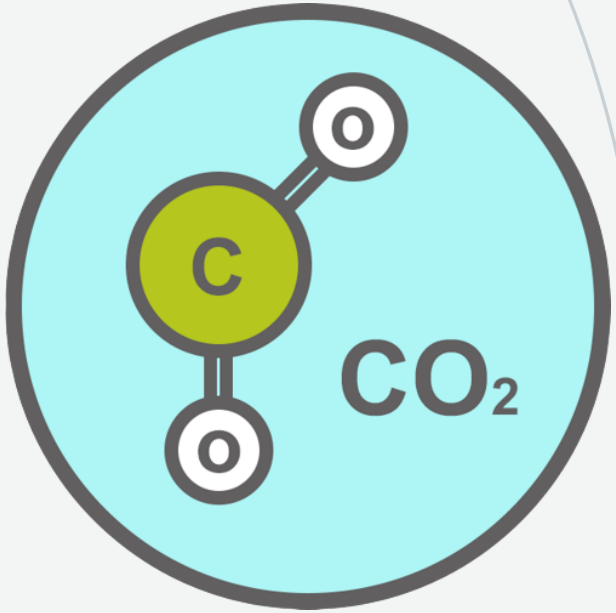


Preparative Supercritical Fluid Chromatography

Principles and applications

Shimadzu SFC User Meeting 2024

Isabelle François, PhD

Chromisa Scientific

Chromisa Scientific

Considerations in favor of SFC purification

All advantages of analytical SFC apply!

Advantages are even more significant at larger scale

Non-toxic
Pure, Stable

Non-flammable

Reclaimed from Industrial plants

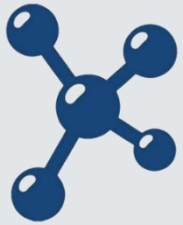
Easy fraction recovery + Limited solvent evaporation

Profitable Total Cost of Ownership

Fast

Recyclable
Minimizes waste

Sustainable
Minimizes energy use



Applicable to many **small molecules**

No liquid solvents
→ **No ATEX**

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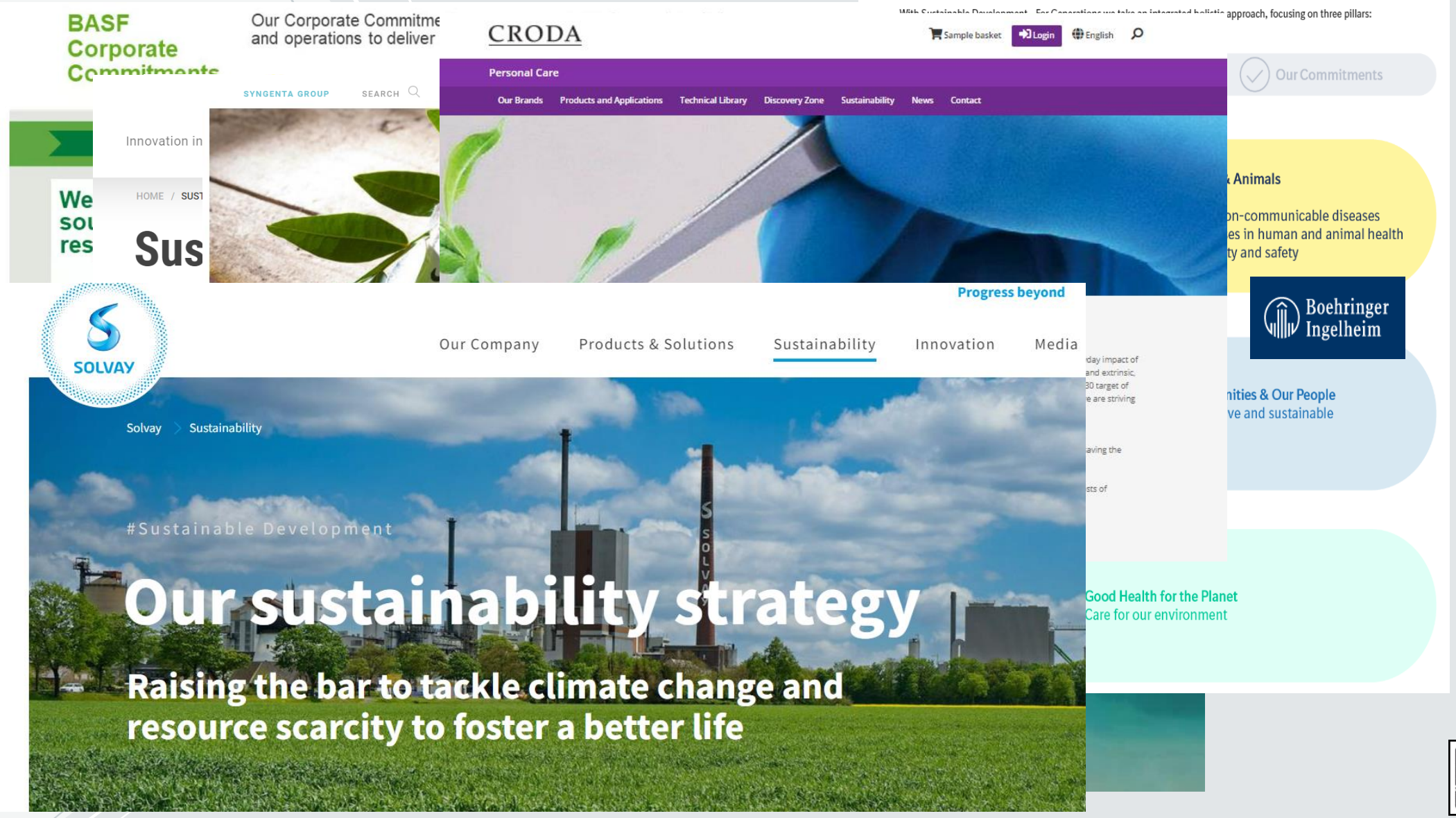
Sustainability and greenness across organisations



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Sustainable chromatography tools

Green Chemistry Solvent Selection:

Preferred	Usable	Undesirable
Water	Cyclohexane	Pentane
Acetone	Heptane	Hexane(s)
Ethanol	Toluene	Di-isopropyl ether
2-Propanol	Methylcyclohexane	Diethyl ether
1-Propanol	Methyl t-butyl ether	Dichloromethane
Ethyl acetate	Isooctane	Dichloroethane
Isopropyl acetate	2-MethylTHF	Chloroform
Methanol	Tetrahydrofuran	Dimethyl formamide
Methyl ethyl ketone	Xylenes	N-Methylpyrrolidinone
1-Butanol	Dimethyl sulfoxide	Pyridine
T-Butanol	Acetic acid	Dimethyl acetate
	Ethylene glycol	Dioxane
		Dimethoxyethane
		Benzene
		Carbon tetrachloride

Greener Solvent Choices

- **SFC**

CO_2, Methanol, 2-Propanol

- **RP HPLC**

Acetonitrile, Water

- **NP HPLC**

DCM, Heptane, Ethyl Acetate

Slide courtesy of Kristina Ohlén, Joanna Raubo

Green chemistry tools to influence a medicinal chemistry and research chemistry based organisation
Kim Alfonsi et al. 2008 **https://doi.org/10.1039/B711717E**

Tools to calculate “Greenness”

Analytical Method Greenness Score (AMGS)

Making the move towards modernized greener separations: introduction of the analytical method greenness score (AMGS) calculator†

Check for updates

Michael B. Hicks, *a William Farrell, b Christine Aurigemma, b Laurent Lehmann, c Lauren Weisel, a Kelly Nadeau, d Heewon Lee, e Carol Moraff, f Mengling Wong, g Yun Huang h and Paul Ferguson i

AMGS Calculator: https://www.acsgcipr.org/amgs

What is taken into account?

- Mass of solvent used and number of injections
- Safety, health and environmental impact of solvent
- Energy used during manufacture and disposal of solvent
- Energy used by instrument

Michael B. Hicks et al., 2019 **https://doi.org/10.1039/C8GC03875A**

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Tools to calculate “Greenness”

Process Mass Intensity (PMI)

$$\text{Process Mass Intensity (PMI)} = \frac{mass\ of\ \text{all}\ reactants}{mass\ of\ products}$$

Each synthesis step increases the PMI with an average of 50 kg/kg

AstraZeneca *Slide courtesy of Kristina Ohlén, Joanna Raubo*

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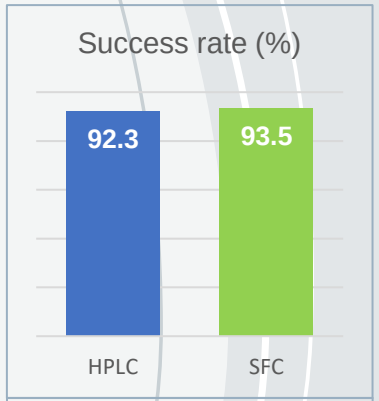
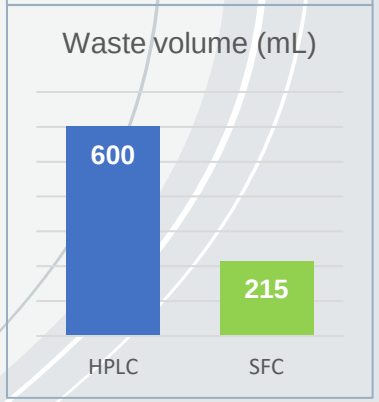
Greenness – SFC vs RPLC

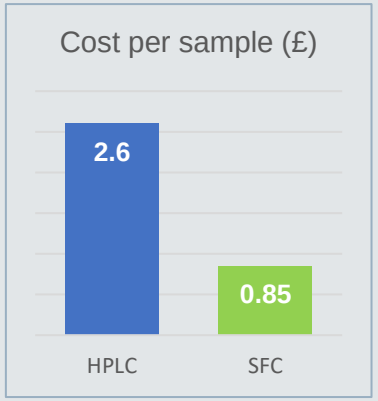
Analytical Method Greenness Score (AMGS)



Equal purification success



64% less waste solvent per sample



67% lower running cost per sample

✓*Lower solvent volumes*

✓*Less waste, lower cost*

HPLC AMGS
1587

SFC AMGS
399

Data generated by Oncology Separation Sciences Team at Astra Zeneca

Calculated using Analytical Method Greenness Score (AMGS)

AstraZeneca

Slide courtesy of Kristina Ohlén, Joanna Raubo

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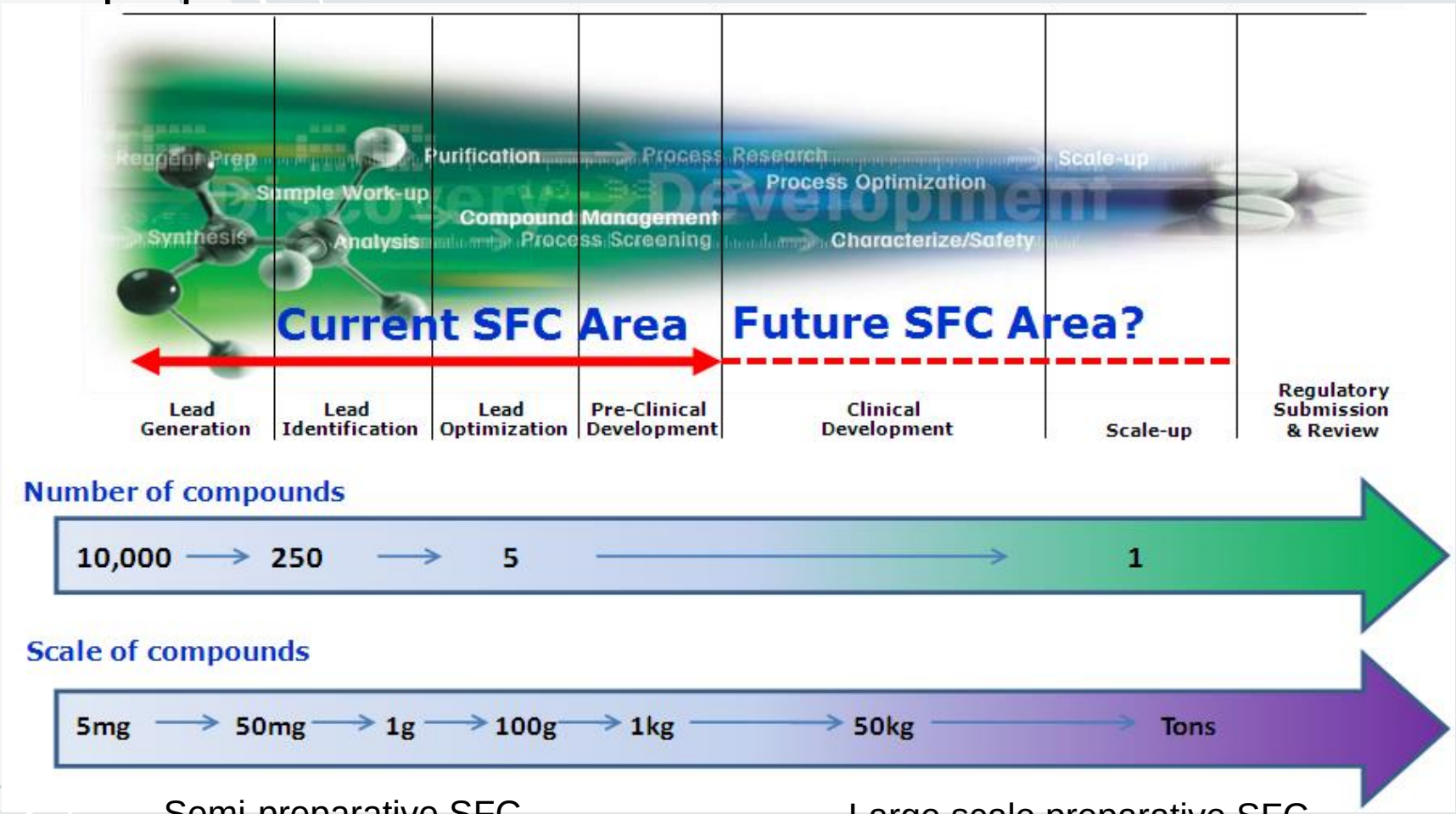
SFC purification in pharmaceutical industry

Pharmaceutical industry has been the main driver for SFC utilization, mainly due to the benefits in prep scale.



Semi-preparative SFC

19, 30, 50 mm ID columns

Large scale preparative SFC

100, 200, 600, 900 mm ID columns

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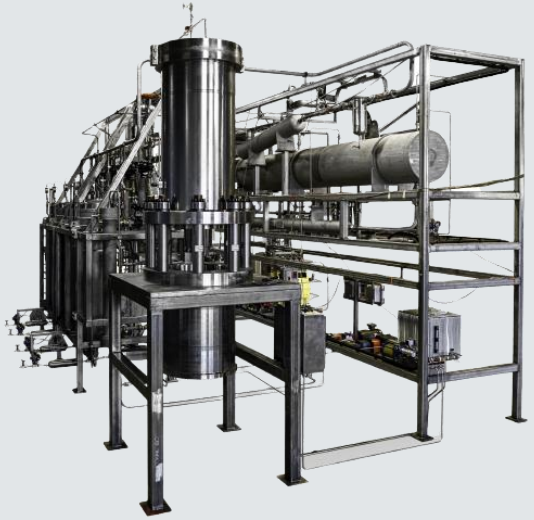
SFC scale – analytical, semi-prep, industrial/large

Waters UPC²

Analytical SFC

Waters SFC Prep100

Semi-preparative SFC



Thar Process Isolator SFC60

Large scale preparative SFC

Chromisa Scientific

SFC scale – analytical, semi-prep, industrial/large

SHIMADZU
Excellence in Science

Nexera Analytical SFC system

Analytical SFC
On-line SFE-SFC optional

Nexera Prep SFC system

Semi-preparative SFC

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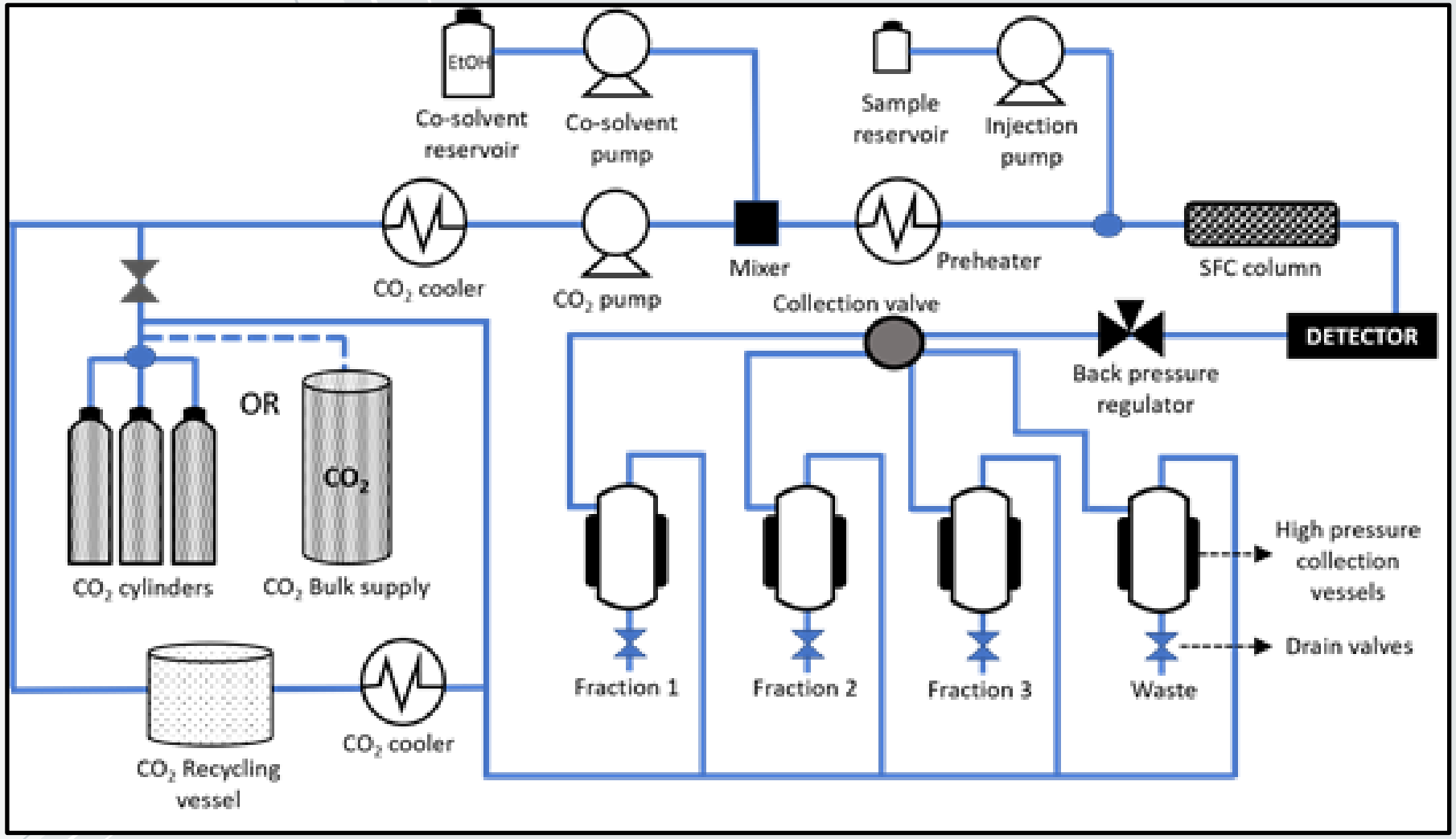
Providers of Prep SFC instrumentation

The providers listed below provide analytical and/or preparative packed-column SFC systems

(Very) large scale SFC systems are sometimes custom-built

Preparative SFC instrumentation



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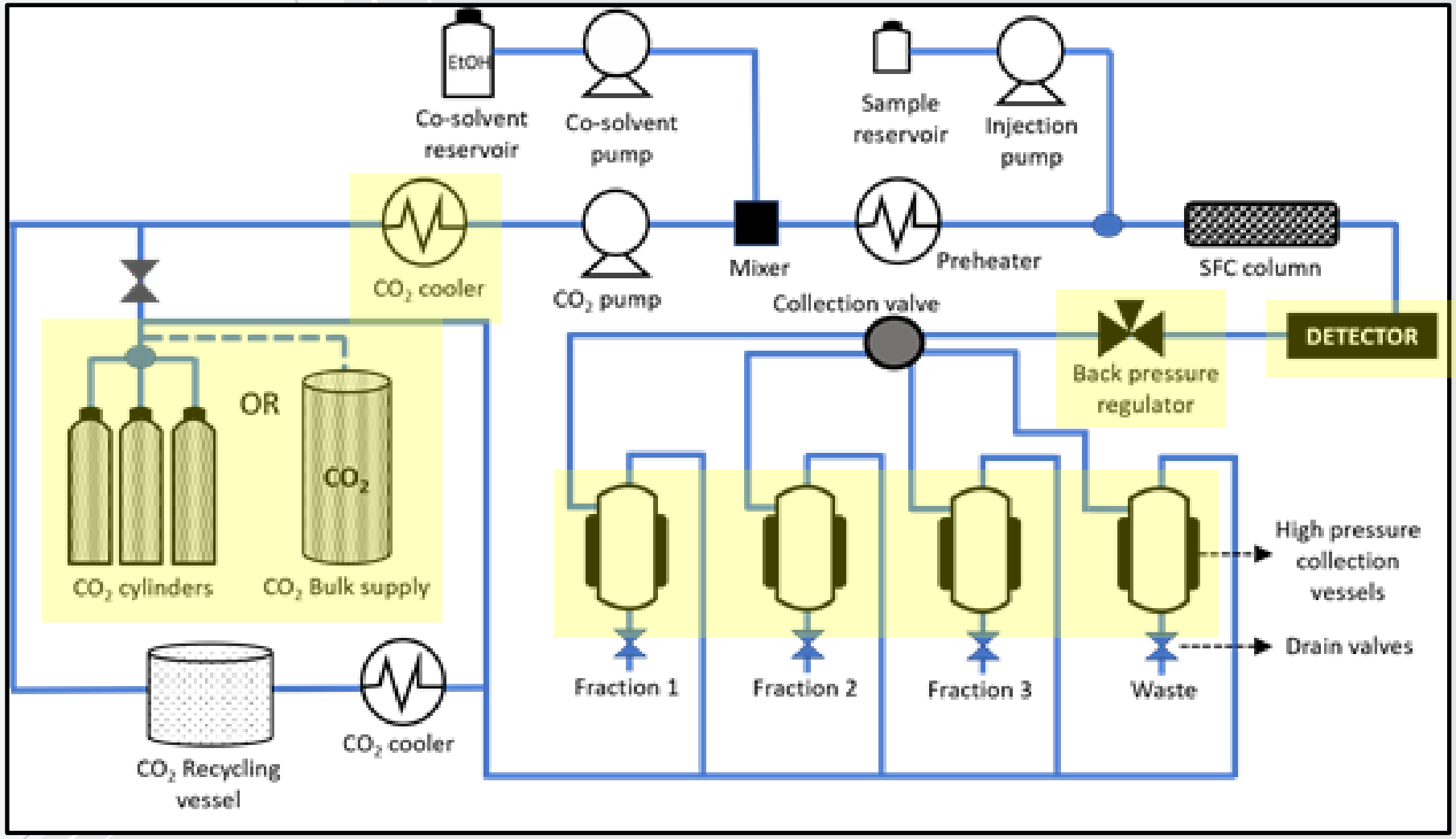
Preparative SFC instrumentation

What is different compared to LC purification?



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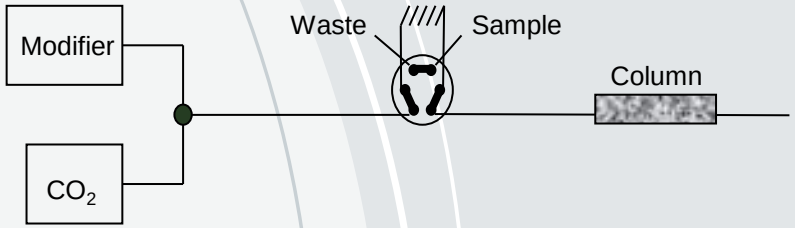
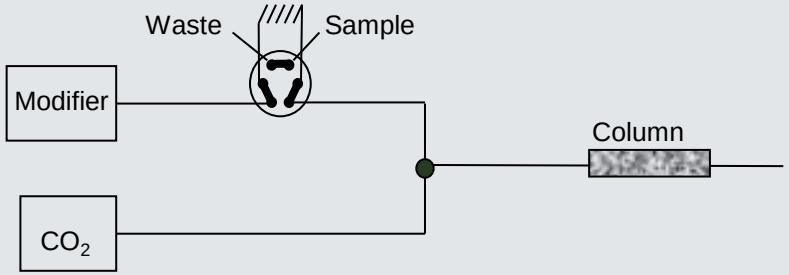
Injection in Preparative SFC

Mixed stream injection



- Sample dissolved in the modifier, or other solvent
- Injection into the mixed mobile phase
- Significant impact of “strength” of injection solvent on peak shape at larger injection volumes
- Sample loop washed for a limited time
- Extensive wash of the injection port required

Modifier stream injection



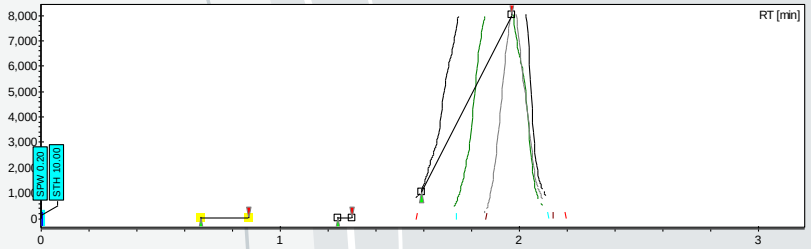
- Principle used for preparative injections
- Sample dissolved in the modifier, or other solvent
- Dilution by CO_2 before the column without precipitation
- Applied at mobile phase strength
- Sample loop washed continuously
- Supporting overlapped injections in isocratic mode
- No sample carry over
- Only functions at sufficient modifier percentages (not below 5%)

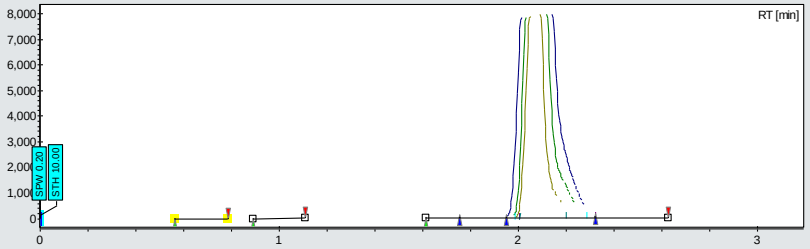
Injection in Preparative SFC

Mixed stream injection

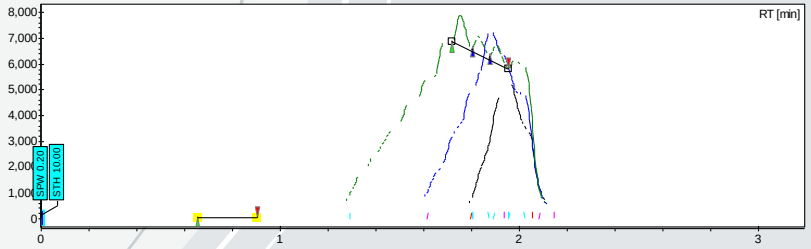
Modifier stream injection

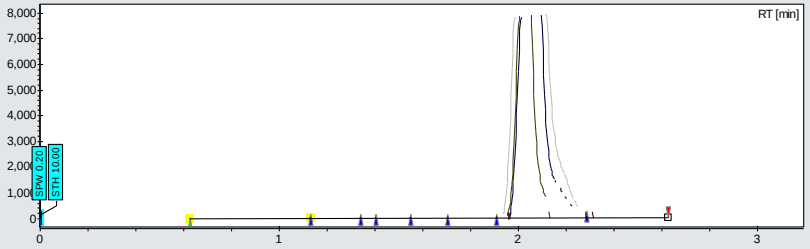
1. Sample dissolved in MeOH





2. Sample dissolved in DMSO





Conditions: Sulfanilamide 5 mg/mL in MeOH or DMSO

Injection volume : 0.5, 1.0, 1.8 mL

Column : Diol 21.2 x 150 mm

Gradient : 20 to 50 % MeOH in CO_2 @ 10 %/min

Flow rate : 50 mL/min

BPR : 100 bar

T : 35°C

Courtesy of Waters

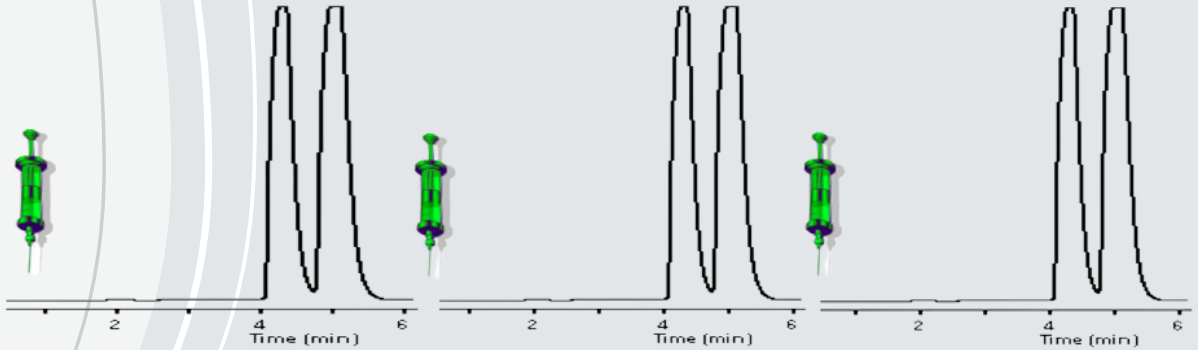
Chromisa Scientific

Injection in Preparative SFC

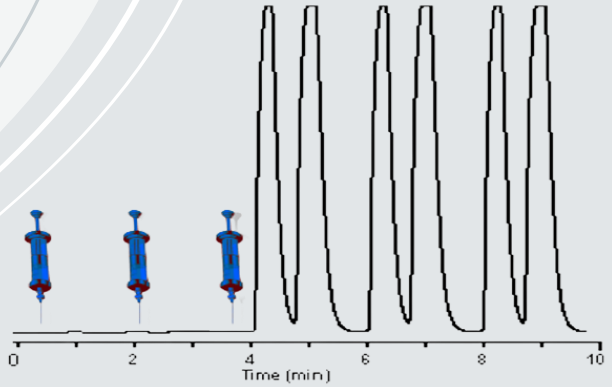
Stacked Injection Mode

- Increased throughput without compromising separation efficiency
- Only works in isocratic mode

Normal injections



Stacked injections



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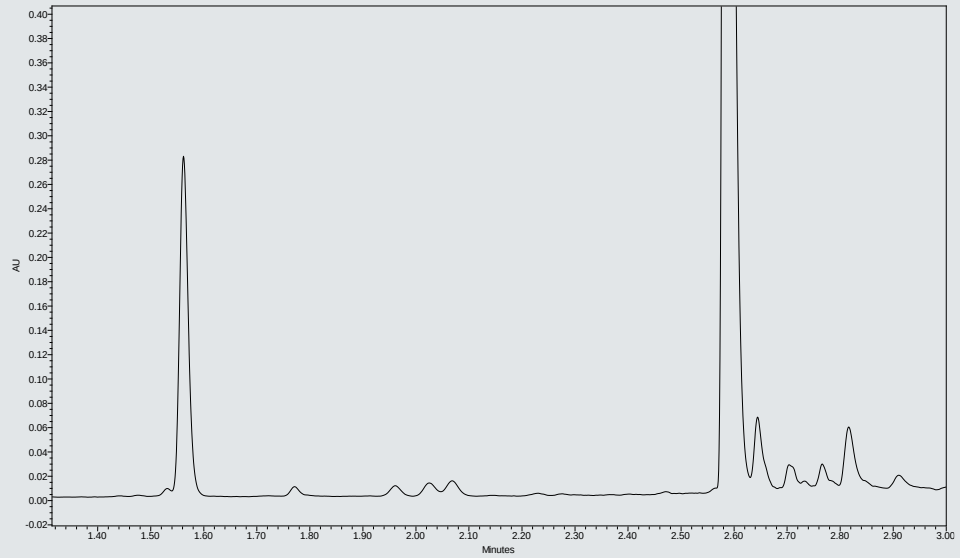
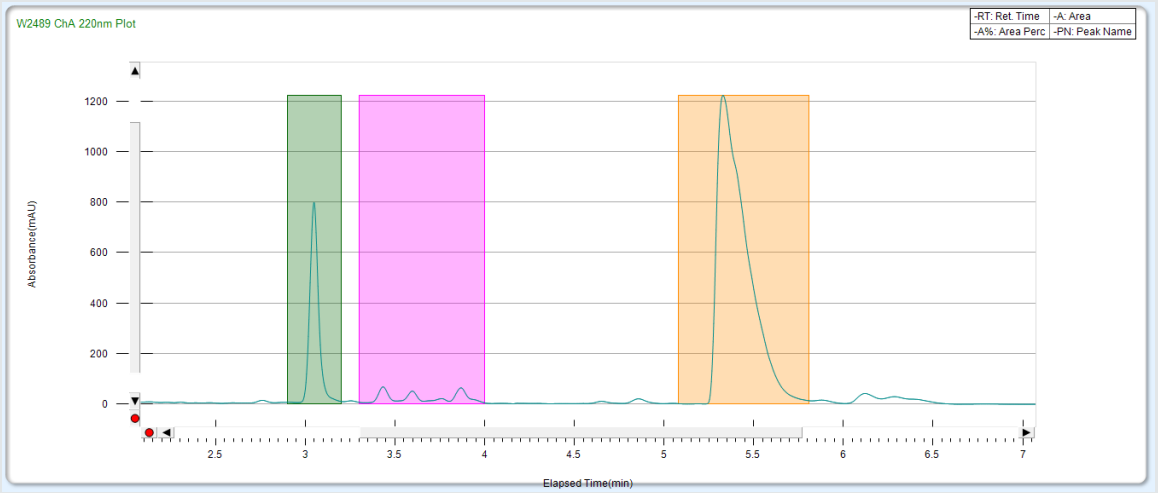
Injection in Preparative SFC

Stacked Injection Mode

- Example : hemp extract
- Analytical analysis was performed on the sample, to determine the appropriate conditions for purification
  - 5-40% gradient
- Method was scaled up
  - Isocratic method was developed @ 15% modifier
  - Three fractions





Courtesy of Chris Hudalla, ProVerde Laboratories, USA

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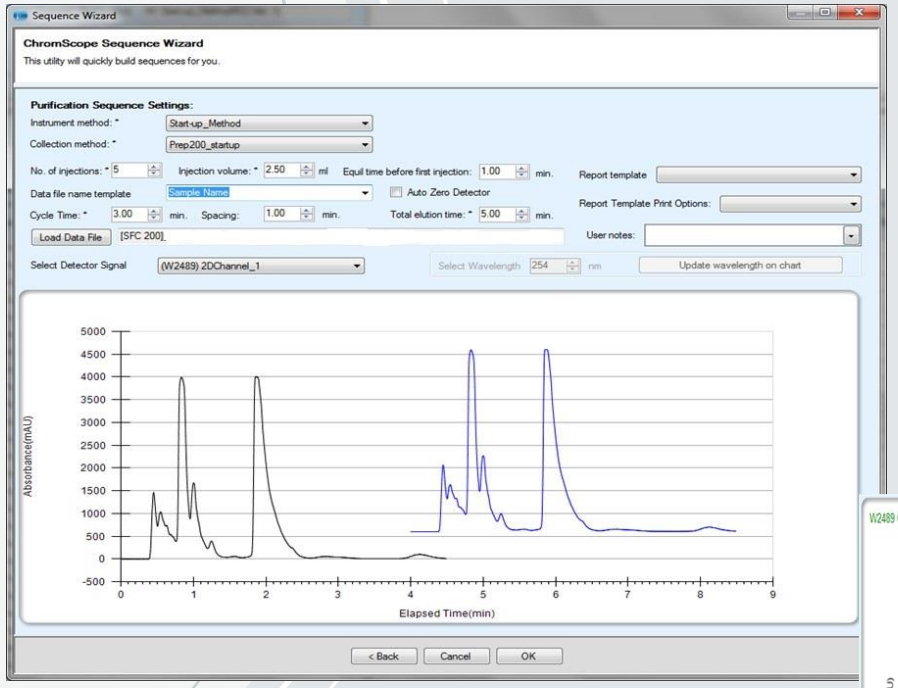
Injection in Preparative SFC

Isocratic analytical method development for conditions to be transferred to SFC 200 for stacking experiments



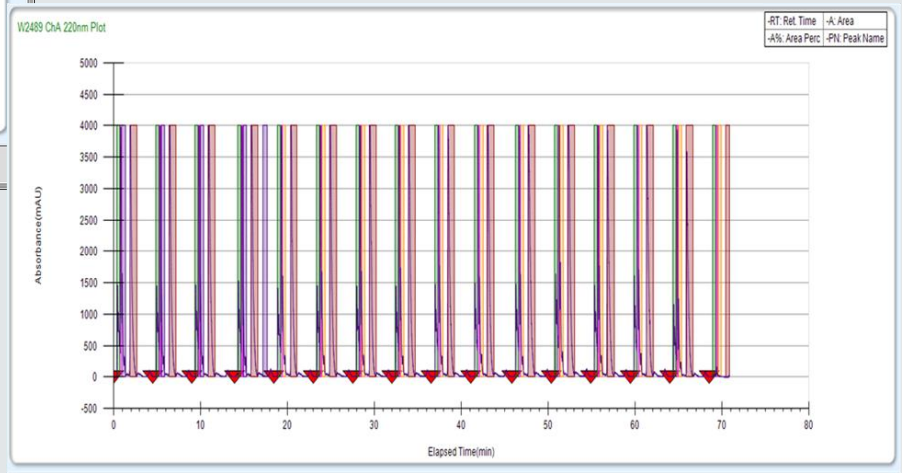
Condition Criteria

Cycle time: 3 min

Spacing: 1 min

Total elution time: 5 min

SFC 200 Isocratic 30x150 mm



Courtesy of Chris Hudalla, ProVerde Laboratories, USA

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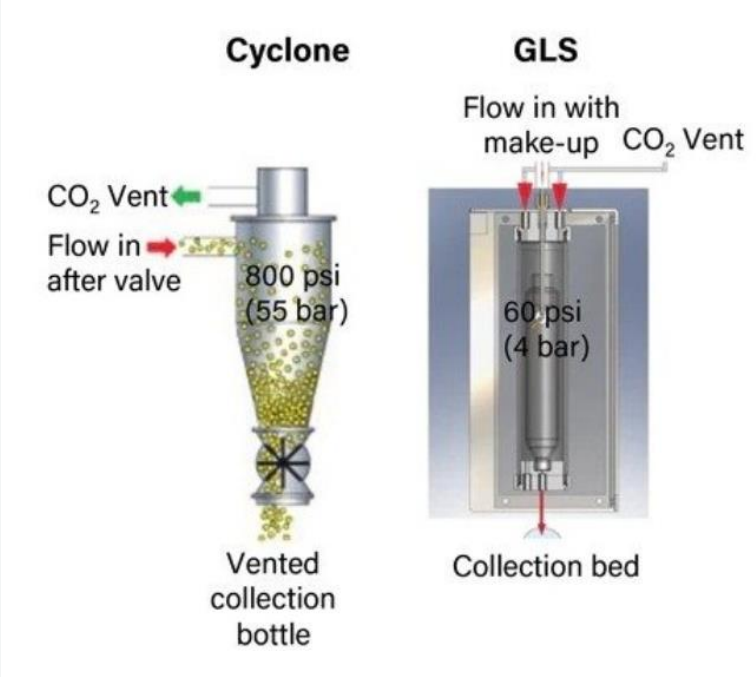
Fraction collection in Preparative SFC

- **CO_2 expansion after BPR makes collection more complicated**
  - Effluent is a biphasic mixture of CO_2 gas and micro-droplets (modifier and compound)
  - Equipment needed to separate gas from liquid without loss of material and without cross contamination
- **Heating is applied after the BPR to mitigate the cooling and associated issues (dry ice / blocked tubing)**
- **In many cases, a make-up solvent is added to avoid cross contamination and keep the purified compounds in solution (avoid blockage and precipitation). This only applies when the modifier percentage is low (below 5%)**

Fraction collection in Preparative SFC

Typically, cyclones or gas-liquid separators (GLS) are applied



Waters website

	Cyclone	GLS
Pressure & principle	High pressure Closed-bed format Liquid is pushed downwards due to design and gravimetry	Low pressure Open-bed format Spiral flow is created wherein the liquid and compounds are forced towards the wall due to centrifugal force. Spiralling downwards, a vortex is formed. When the diameter is sufficiently small, the clean CO_2 moves upwards through the vortex and is sent to the top of the GLS.
Advantages	No make-up required (CO_2 remains its solvating power through the collection valve)	Cheaper materials can be applied Reduced safety risk Increased number of fractions
Disadvantages	More expensive SS required due to high presssure Increased safety risk Limited number of fractions (dependent on the number of applied cyclones)	Additional make-up solvent required when using low modifier percentages Often fractions need to be combined Not suited for large quantity purification

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Fraction collection in Preparative SFC

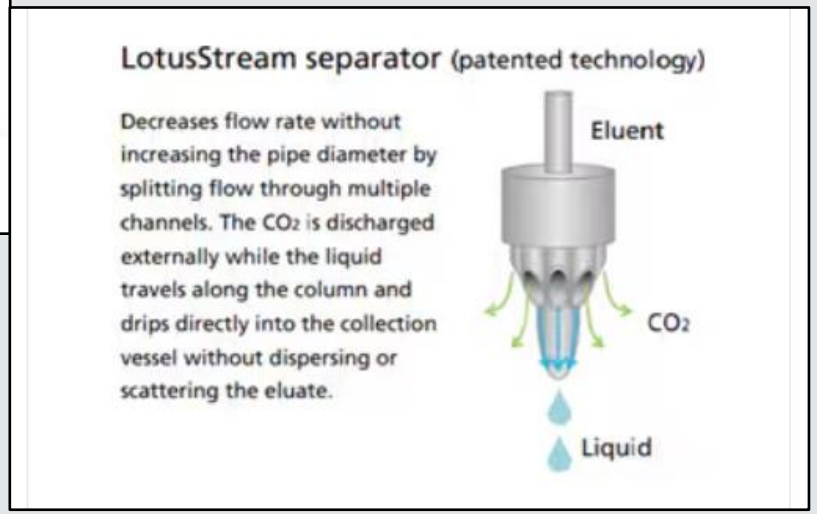
Shimadzu Nexera Prep SFC - LotusStream™

Shimadzu website

SHIMADZU
Excellence in Science

New patented principle for gas-liquid separation :

LotusStream™



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Fraction collection in Preparative SFC

Open bed vs Closed bed collection

Waters Prep SFC100

- **Open-bed**
- **Batch purification**
- **Smaller quantities / smaller campaigns / library purification**
- **Collection in tubes/vials/bottles**
- **1 GLS for all fractions**
  - **Sufficient solvent required (modifier / make-up) to avoid cross-contamination**
- **Detection : UV/PDA/ELSD/MS**

Thar Process Isolator SFC10

- **Closed bed**
- **Bulk purification**
- **Larger quantities / large campaign**
- **Collection in carboys**
- **Cyclone per fraction**
- **Detection : UV/ELSD**

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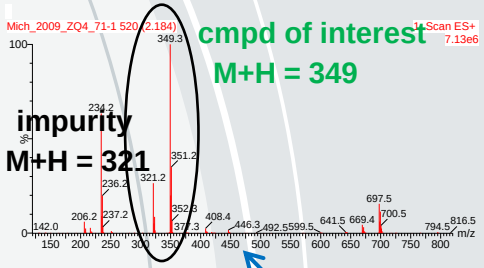
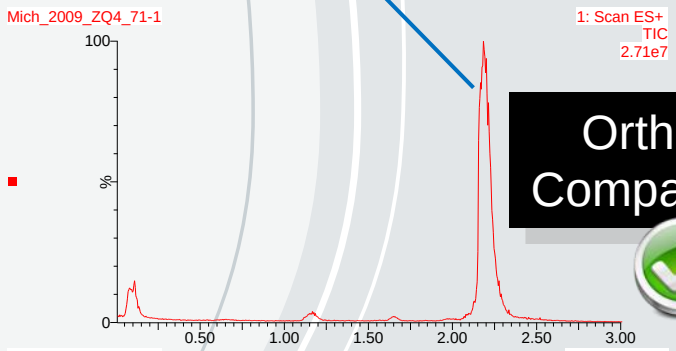
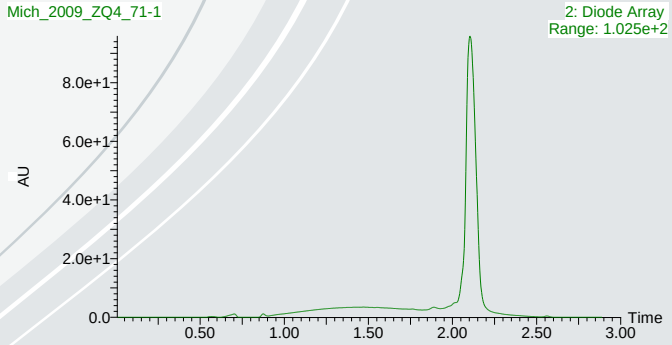
MS & SFC orthogonality advantage

Mass-Directed Prep LC

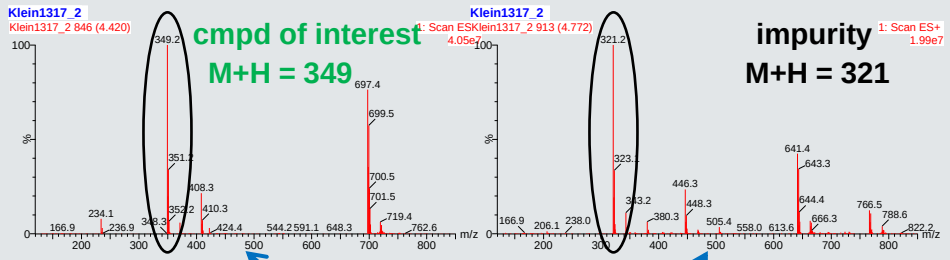


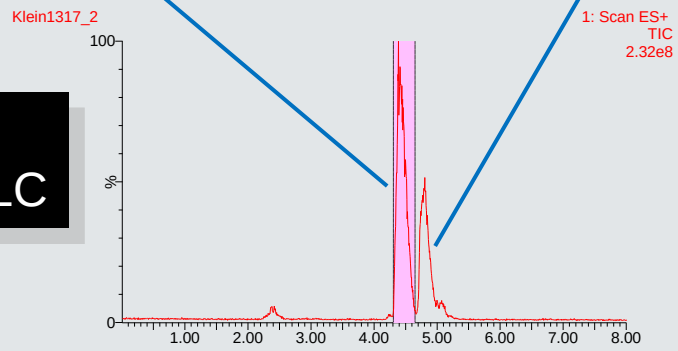


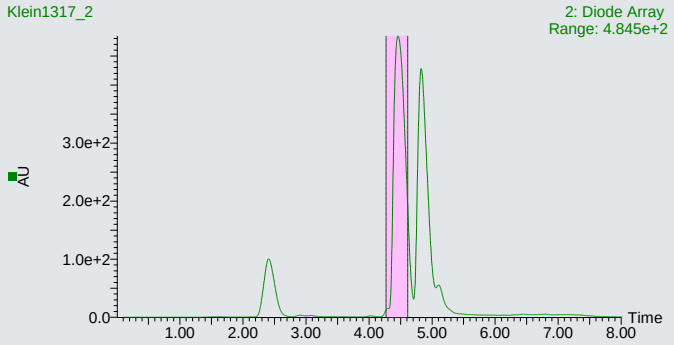


Orthogonal Compared to LC

Mass-Directed Prep SFC







Boehringer Ingelheim

Chromisa Scientific

Columns in Preparative SFC

Pre-packed

Hamilton website

Dynamic Axial Compression (DAC) column

Hanbon website

- DAC : User packs column using a slurry of stationary phase mixed with solvent (MeOH, EtOH)
- Important considerations :
  - Availability and robustness of stationary phase in larger particles
  - Price of stationary phase
    - Eg DAC of 60 cm requires 65 kg stationary phase

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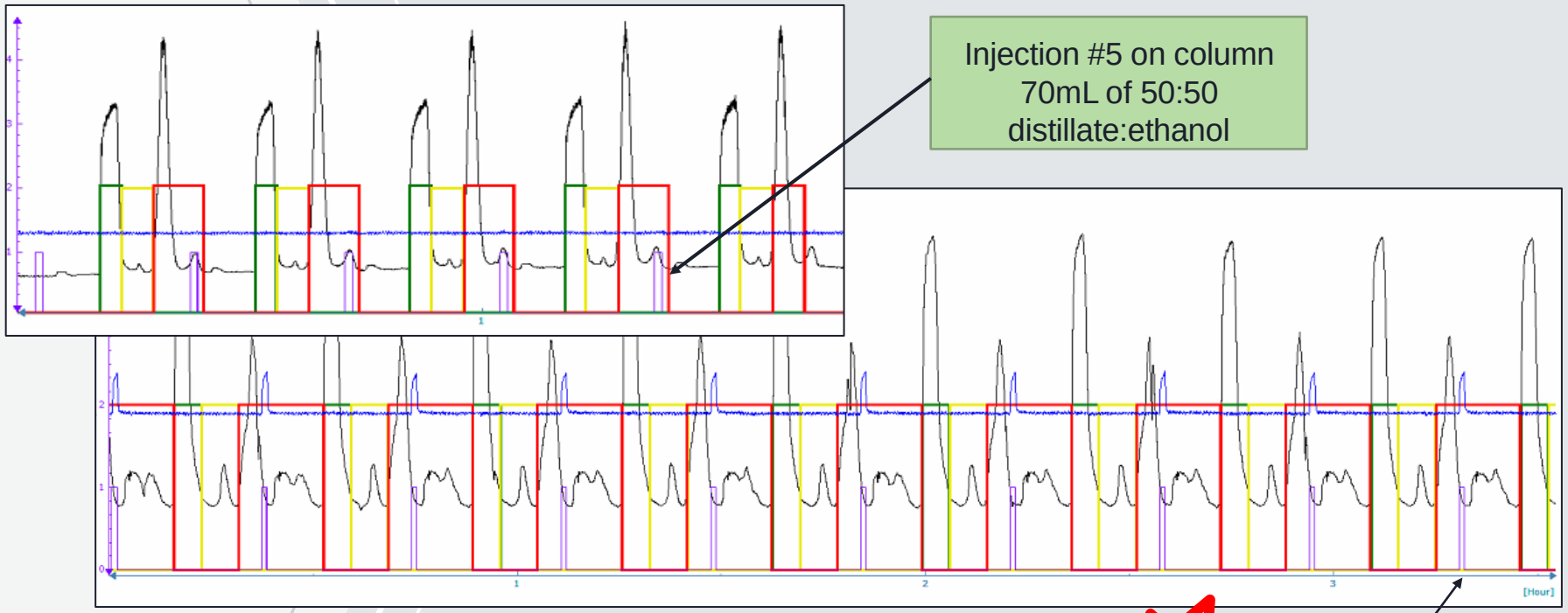
Columns in Preparative SFC - Robustness



Injection #5 on column
70mL of 50:50
distillate:ethanol

Injection #19,684 on column
70mL of 50:50 distillate:ethanol
(10 months since column was packed)

These are hemp-derived products and there is inherent natural variability, but the chromatography shows repeatability and robustness.

CO_2 as mobile phase is more gentle than liquids for the stationary phase

Courtesy of Thar Process

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Solvent recycling in Preparative SFC

- For larger scale, CO_2 recycling is a must!
  - Improved sustainability and cost effectiveness
- Goal of recycler : liquidify “gaseous” CO_2 coming from collection system

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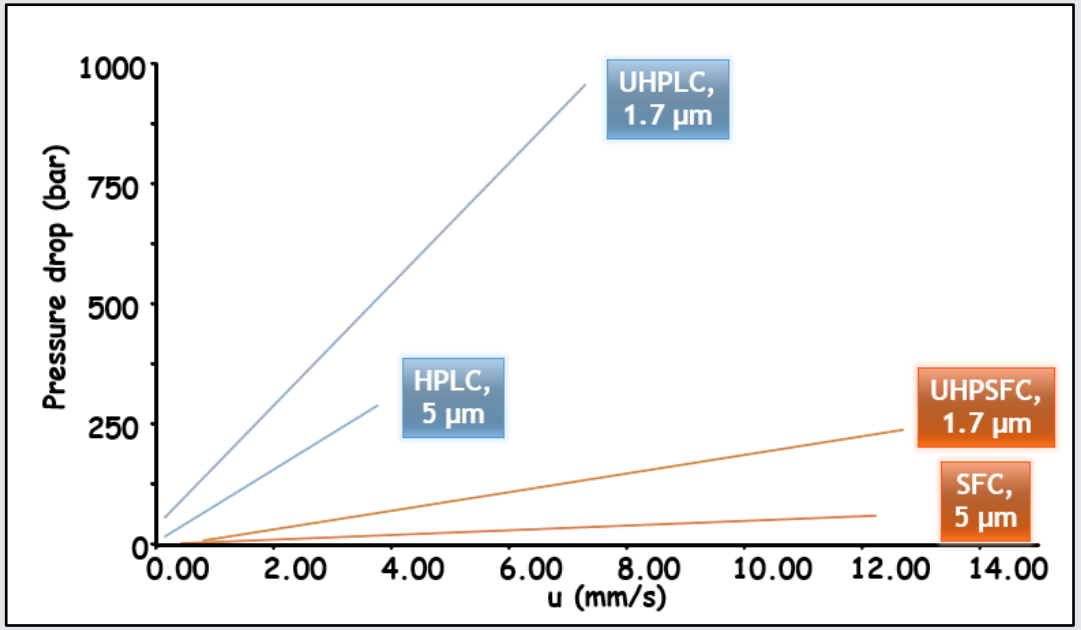
Preparative SFC – Screening and scaling

Requirements for high productivity purification

- High solubility in mobile phase
  - Large injection volumes → reduced resolution
  - Low solubility → poor peak shape (tailing, splitting)
  - Difficult to predict solubility in CO_2
- Short cycle time
- Preference to isocratic methods
- High stationary phase saturation capacity
  - Determines change in retention and peak shape with increasing sample load
- Good availability of stationary phase across particle size / prepacked columns / particles at affordable pricing
- High purity of fractions

Preparative SFC – Screening and scaling

- SFC uses compressible fluid as mobile phase
  - Particle size has significant impact on pressure drop
  - Pressure drop has significant impact on density and mobile phase strength



- When moving from analytical to prep, this pressure drop can be accounted for by adapting the BPR pressure
  - However, this quickly leads to unrealistic settings of BPR pressures and related overall pressures that the prep SFC systems can not achieve due system limitations
- Therefore, particle size is typically maintained from method development/screening to prep (5 or 10 µm)
- Fraction purity assessment can be done using smaller particles

A. Grand-Guillaume Perrenoud et al., J. Chromatogr. A 1266 (2012) 158

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Preparative SFC – Screening and scaling

- Following equations can be used while maintaining identical particle size in both analytical and prep SFC

$$F_{Prep} = F_{Analytical} * D^2_{Prep} / D^2_{Analytical}$$

$$Vol_{Prep} = Vol_{Analytical} * D^2_{Prep} / D^2_{Analytical} * L_{Prep} / L_{Analytical}$$

Example:

Table 2: Strategy used to scale-up the separation when converting the CO_2 flow from mL/min to g/min.

	CO_2 Flow	Co-solvent Flow
Method on UPC²	1.275 mL/min	0.225 mL/min
CO_2 mL to g conversion (density=0.936 g/mL)	1.1934 g/min	0.225 mL/min
Scaled to 19mm (19mm/3mm)² x UPC² Flow	47.87 g/min	9.025 mL/min
Final Prep Method	47.87 + 9.0205 = 56.9 g/min (Total Flow) 9.025/56.9*100=15.9% co-solvent	

Chromatography Today, 2018, August – Runco et al.

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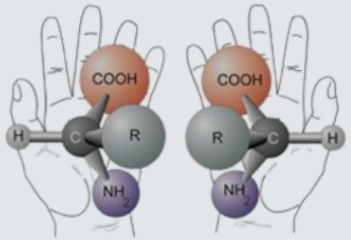
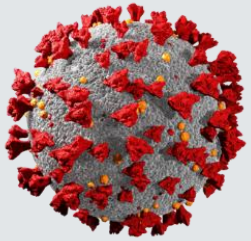
Examples from the industry

Pharma API Purification
(Replacement for NPLC
and Orthogonal to RPLC)



Pharma enantiomer
separation
(chiral)



Synthetic Lipids
Purification

Natural Products
Extraction & Purification

Cannabis
Extraction &
Purification

Fish/algae Oil Omega
3-6 Fatty Acids
(DHA/EPA)

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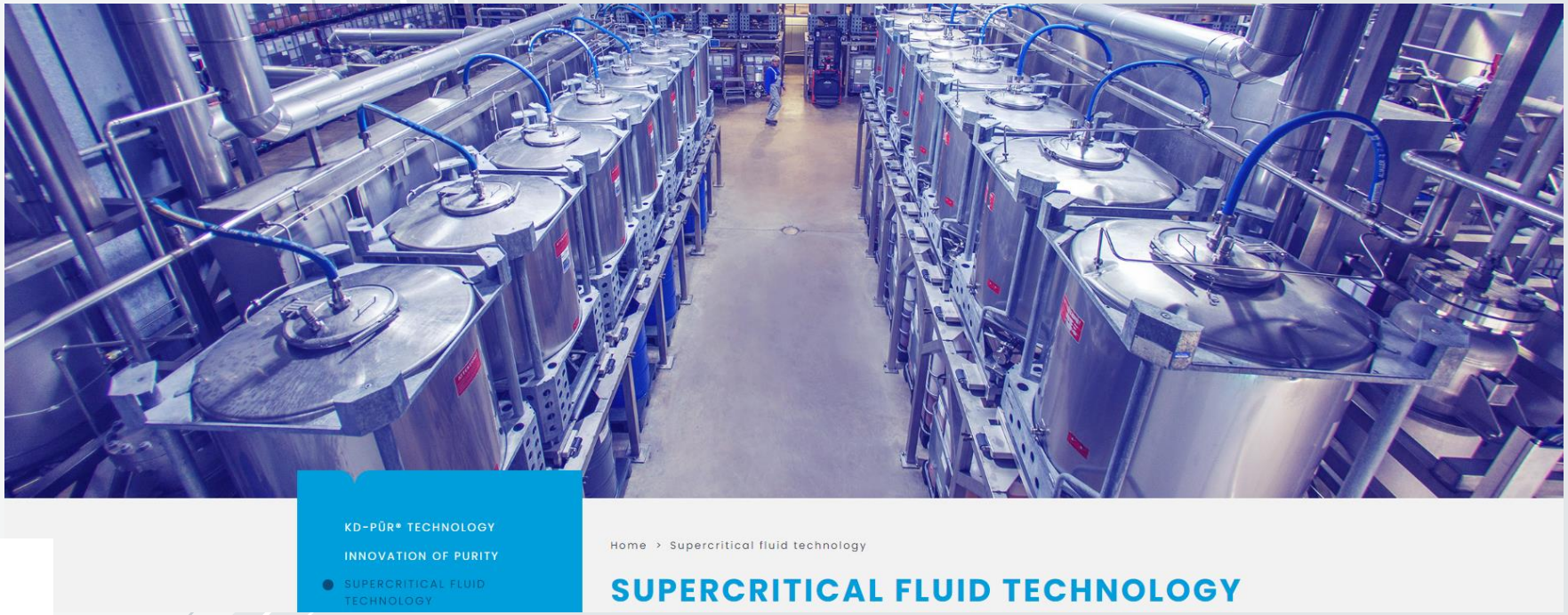
Examples from the industry

Fish/algae Omega 3 DHA/EPA

Multiple custom built large ID SFC systems (>50 cm ID)



KD Pharma Group™
Creating Health Solutions

https://www.kdpharmagroup.com/en/our-difference/kd-pur-technology/supercritical-fluid-technology

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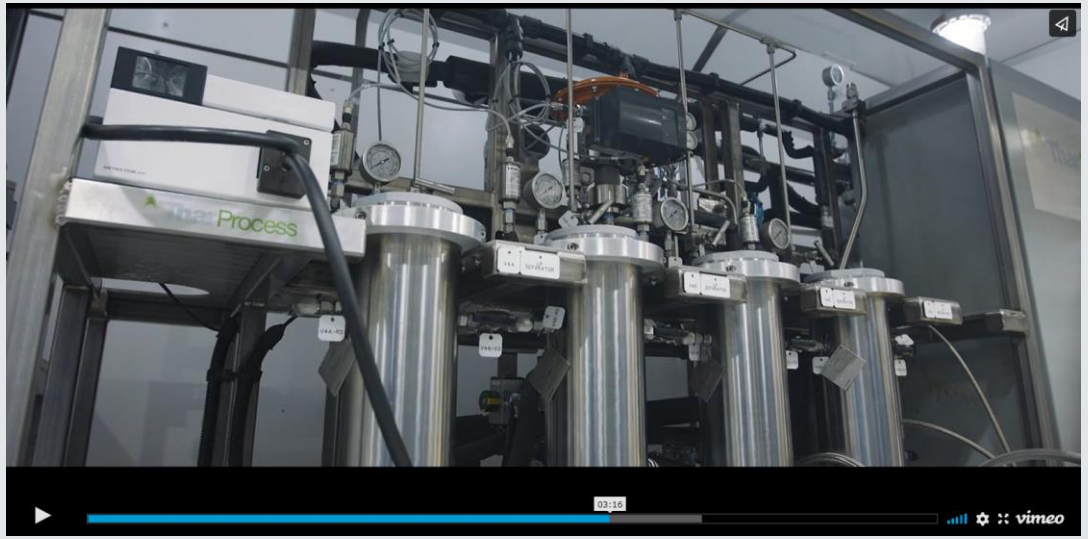
Examples from the industry

Cannabinoids

- THC remediation for hemp derived products
- THC/CBN/CBG isolation



Two Thar Process Isolator SFC60 systems (60 cm ID) + SFC10X as pilot scale system (10 cm ID) to assist method development

https://peakseasonbotanicals.com

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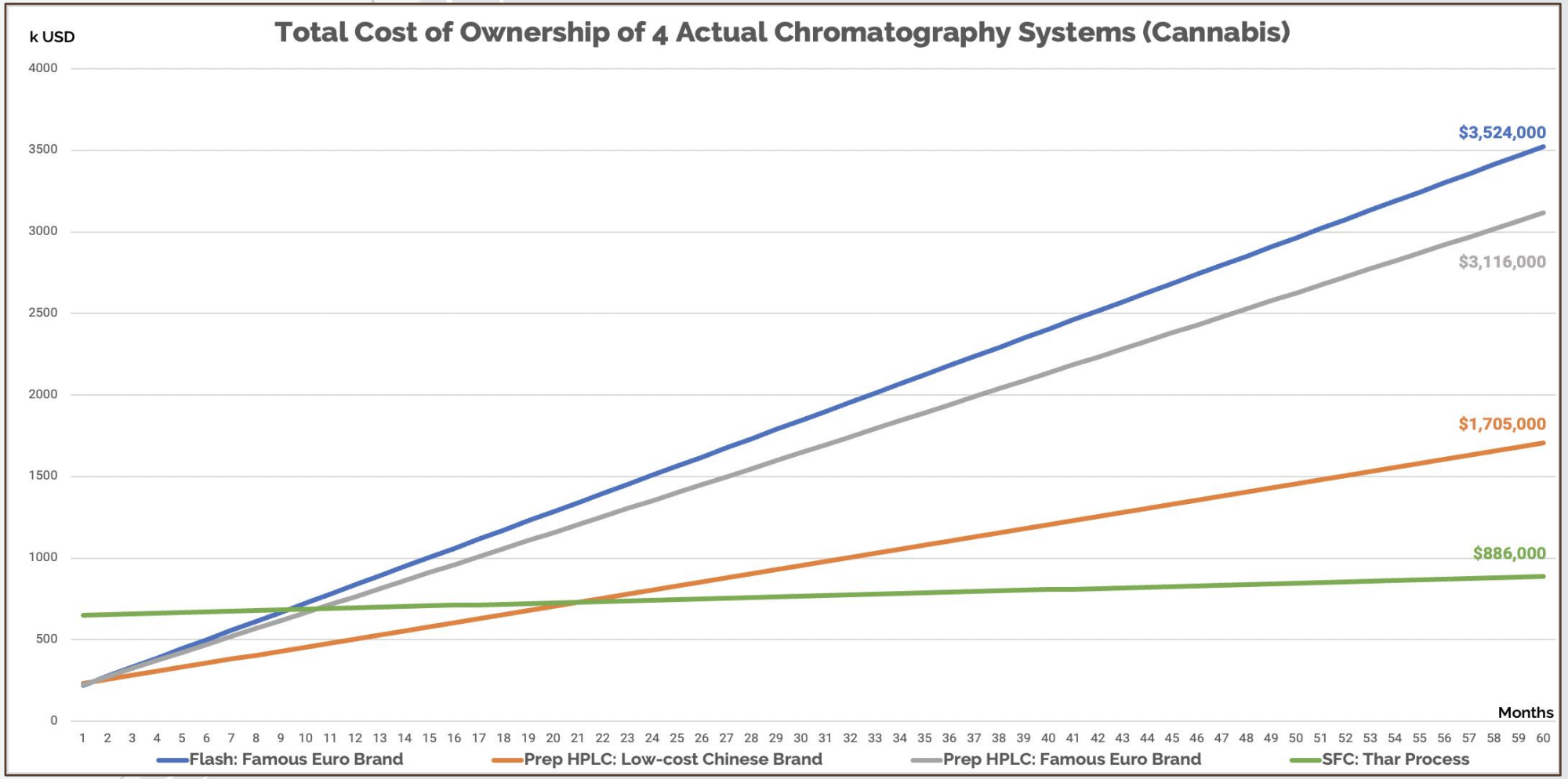
Beneficial Total-Cost-of-Ownership (TCO)

Example for cannabis purification and comparison to other techniques – solvent recycling and reduced evaporation of fractions and mobile phase



Courtesy of Nikos Xynos, Nomad Labs

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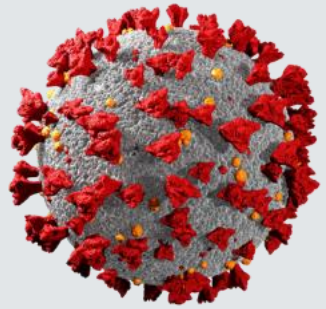
Examples from the industry

Lipid excipients



- Synthetic intermediate for vaccine drug research

Traditional purification method:

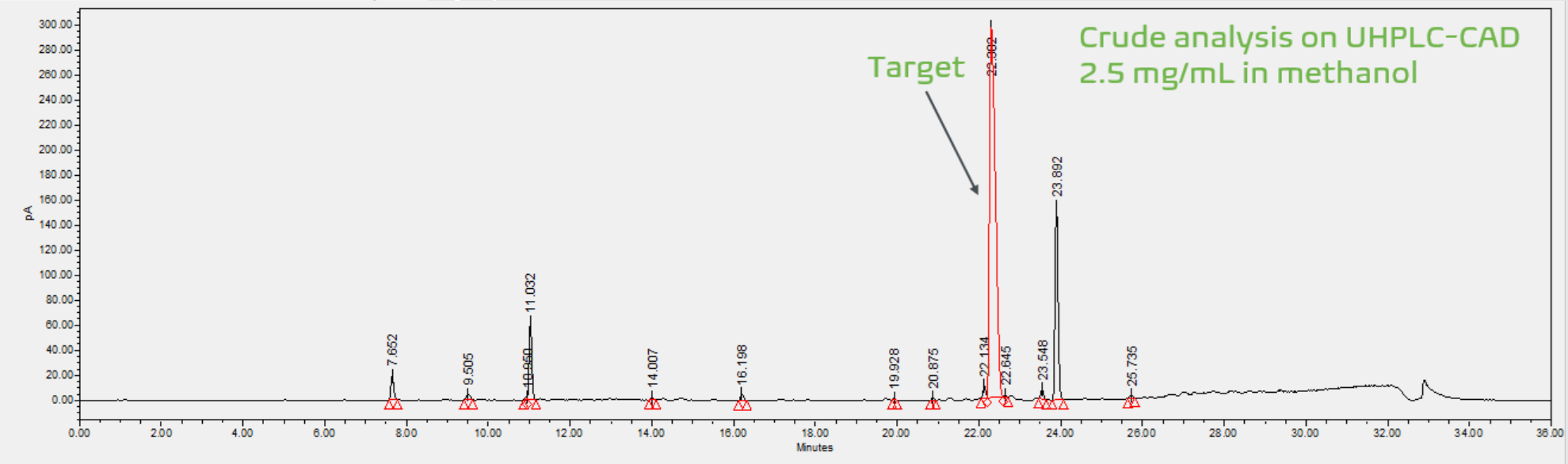
- Normal phase LC purification
- Toxic solvents are used – toluene, heptane, isopropyl acetate
- Large solvent consumption – up to 4,000 - 6,500L for 1 kg of material
- Consists of different steps of column conditioning (pre- and post run), gradient purification and column back-flushing – time consuming process
- Projects are under NDA, so not all details can be shared

Courtesy of Thar Process

Examples from the industry

Lipid excipients

- Crude lipid starting at ~70% purity
- Purity criteria to meet specifications:
  - Total purity ≥ 97%
  - Individual impurities < 0.5%



Courtesy of Thar Process

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Examples from the industry

Lipid excipients

Approach R&D, Feasibility, scale-up

	Phase I	Phase II	Phase III	Phase IV
Goal	Analytical screening & method development	Semi-preparative purification	Repeatability & loading study for semi-preparative stage	Scale-up to preparative fraction collection
Scale	5-50 mg	mg – g		100 g - 10 kg
Column ID	4.6 mm	19 mm		10 cm
System	 *Thermo Vanquish incl CAD detection*  *Waters UPC²*	 *Waters SFC Prep 100 (mass directed)*		 *Thar Process Isolator™ SFC10X*

Courtesy of Thar Process

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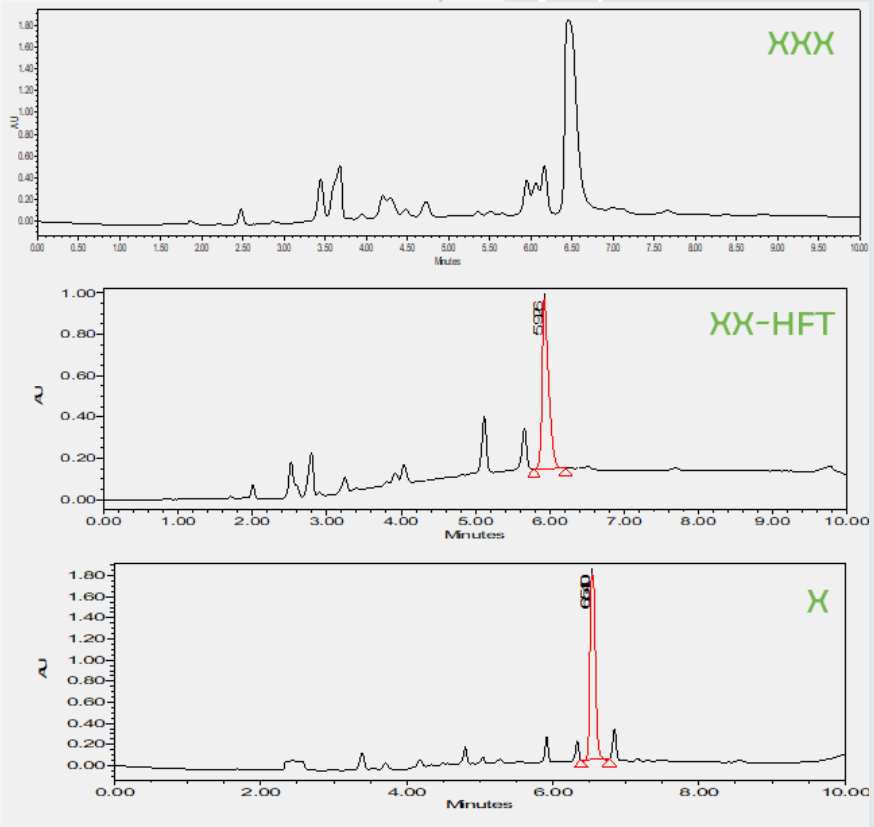
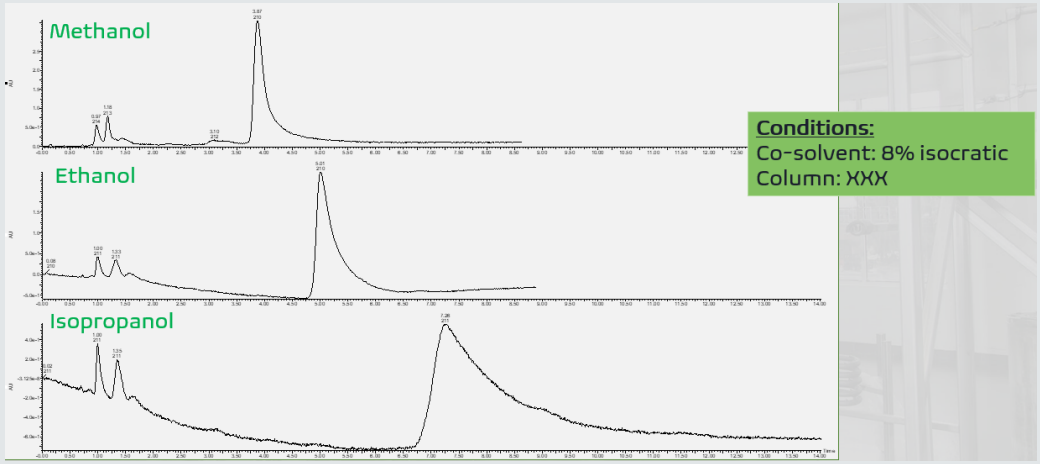
Examples from the industry

Lipid excipients

Column screening





Courtesy of Thar Process

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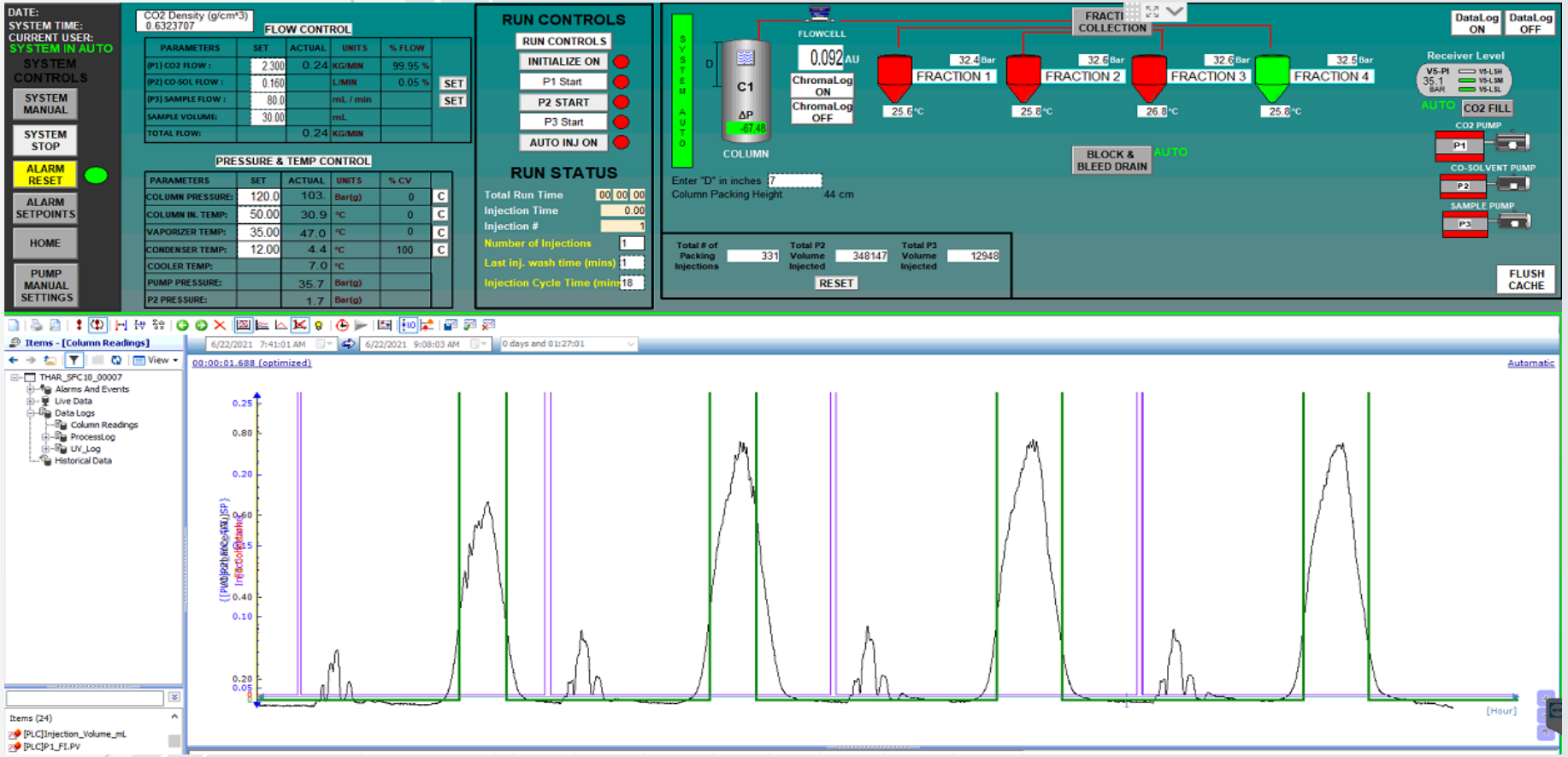
Examples from the industry

Lipid excipients

Isocratic conditions on Isolator SFC10X



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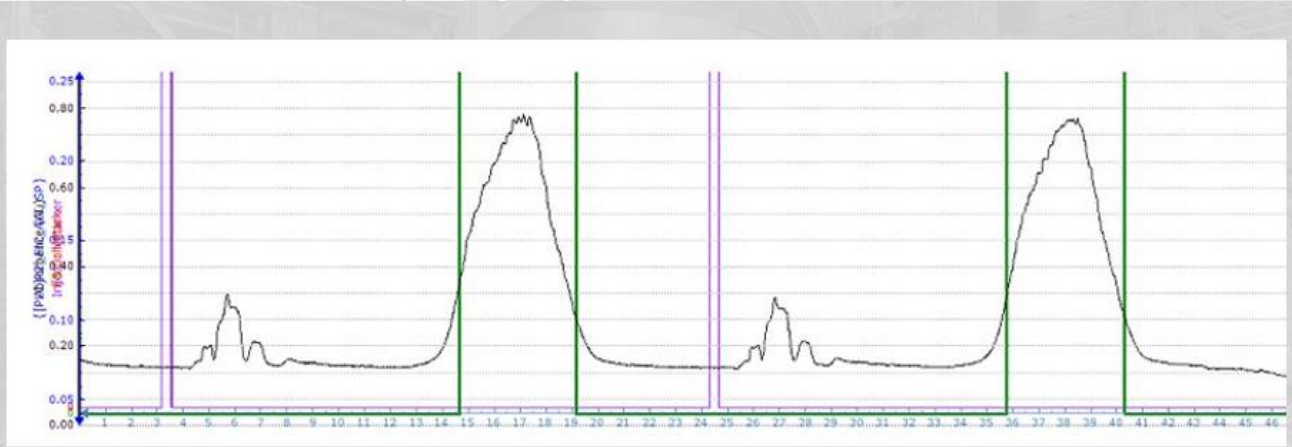
Examples from the industry

Lipid excipients – Ionizable lipid



- Recovery of target > 90%
- Purity = 98.8%
- Highest individual impurity = 0.4%

Conditions:
Co-solvent: 7% methanol
Column: XXX (10 cm)

Cycle time: 21 minutes
~1.9 kg/day

Co-solvent usage : 230 L

Production of 1 kg
→ SFC up to 110 L
→ NPLC up to 6,500 L

Courtesy of Thar Process

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Examples from the industry

Lipid excipients – Ionizable lipid – SFC vs NPLC

1 kg of purified product

SFC
110 L

NPLC
Up to
6,500 L

- Solvent recycling possible (80%)
  → 22 L consumed per 1 kg
- Only one solvent, no additives

- Mixture of solvents
- More challenging recycling and excessive solvent quantities

Courtesy of Thar Process

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Acknowledgements:

- All contributors to this presentation
- AZ Mölndal for facilitation of this meeting
- Shimadzu & Gesa Schad
- You for your attention

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