REACH
SEPARATIONS

Sustainability considerations for small scale purification workflows - SFC or HPLC?

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Shimadzu SFC User Meeting
October 2025

Reach Separations

- Established over 2 sites
- Experts in **Purification**
- Provide several **analytical** services
- Recently acquired by the **Catsci** Group

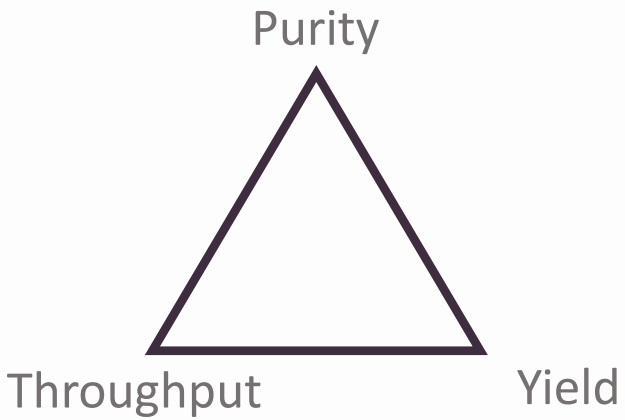
Nottingham

Strasbourg

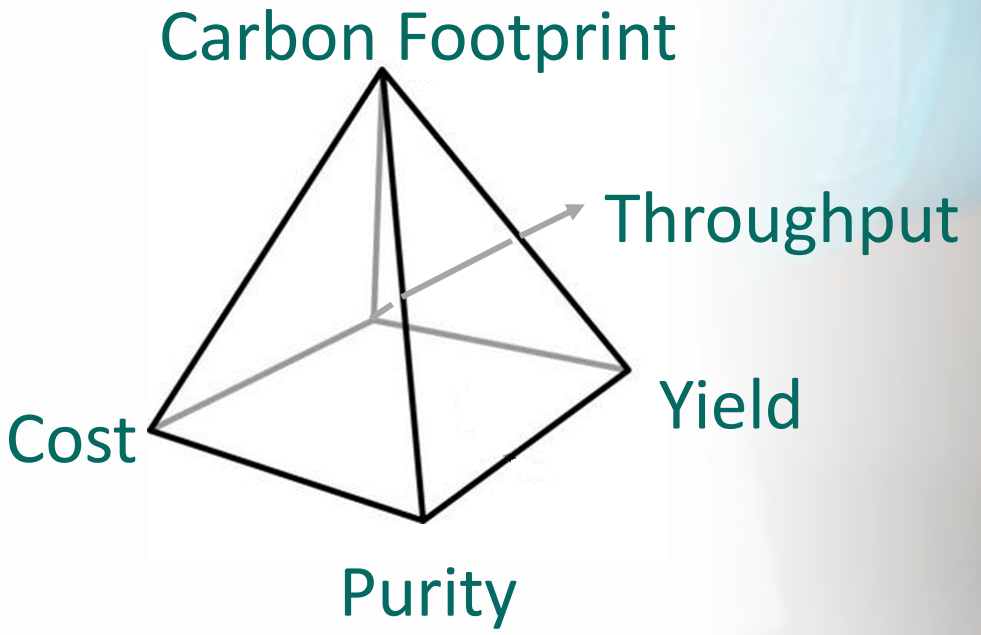
CatSci

REACH SEPARATIONS

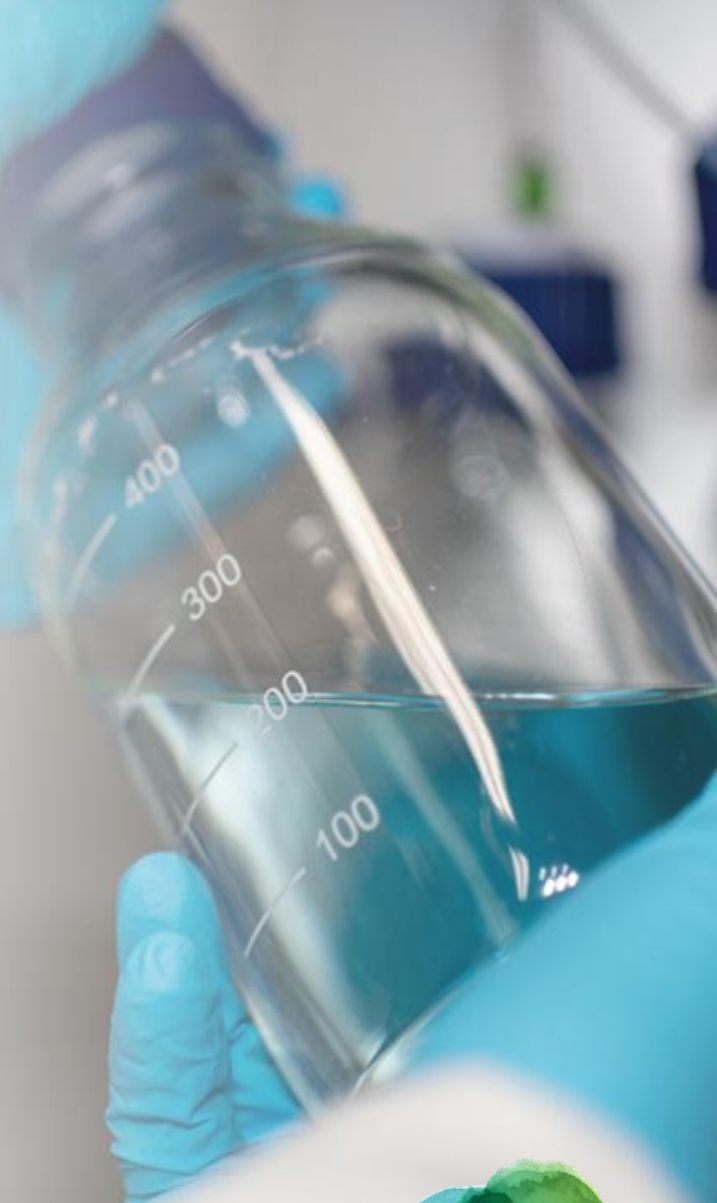
What we do at Reach



Historically for purification



The Reach Paradigm



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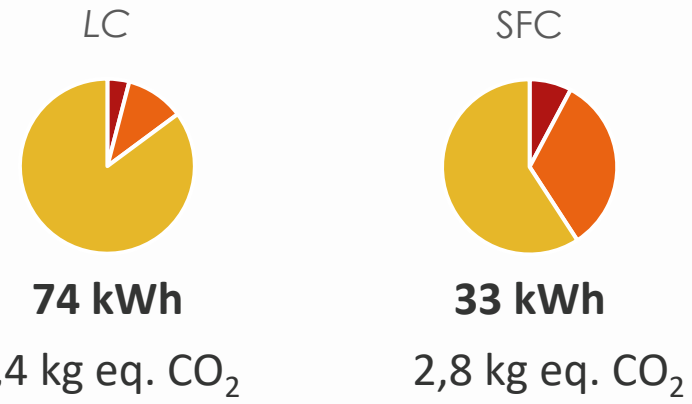
Green Focus – *Energy*

Energy consumption of each purification stage:

- Analytical scale
- Preparative scale
- Dry-Down



74 kWh

6,4 kg eq. CO_2

33 kWh

2,8 kg eq. CO_2

AMGS score

HPLC	SFC
59	58
1575	411

→ The **drying** process = most **energy intensive** stage

→ LC utilises more than **twice the energy** as SFC

Based on manufacturer Data & actual measures in the lab

1 kWh = 87 g eq. CO_2

Average value in France in 2022
(Source: *electricity maps*)

ACS Green Chemistry Institute Pharmaceutical Roundtable

REACH SEPARATIONS

Green Focus - *AGMS*

AGMS score	NP	SFC
Analytical scale	22.8	10.8
Preparative scale	504.4	85.9

→ **Biggest** impact on **preparative** scale

→ **SFC** significantly improves **green score**

ACS Green Chemistry Institute Pharmaceutical Roundtable

Link to AGMS calculator: https://www.acsgcipr.org/amgs/

REACH SEPARATIONS

Chiral Prep example

AMGS calculator comparison									
Nb of injections	Nb of analytes of interest	Diluent	Column	Mobile phase A	Flowrate	Run-time	% cosolvent	Cosolvent	AMGS score
10	2	MeOH	30x250mm, 5µm	CO2	150mL/min	5 min	20	MeOH	330.56
10	2	MeOH	30x250mm, 5µm	CO2	150mL/min	5 min	20	EtOH	735.27
10	2	MeOH	30x250mm, 5µm	CO2	150mL/min	5 min	20	iPOH	512.68
10	2	MeOH	30x250mm, 5µm	CO2	150mL/min	5 min	20	MeCN	4110.3
7	2	MeOH	30x250mm, 5µm	Heptane	42mL/min	5min	20	EtOH	910.14

10	2	MeOH	30x250mm, 5µm	CO2	150mL/min	5 min	0	NA	122.91

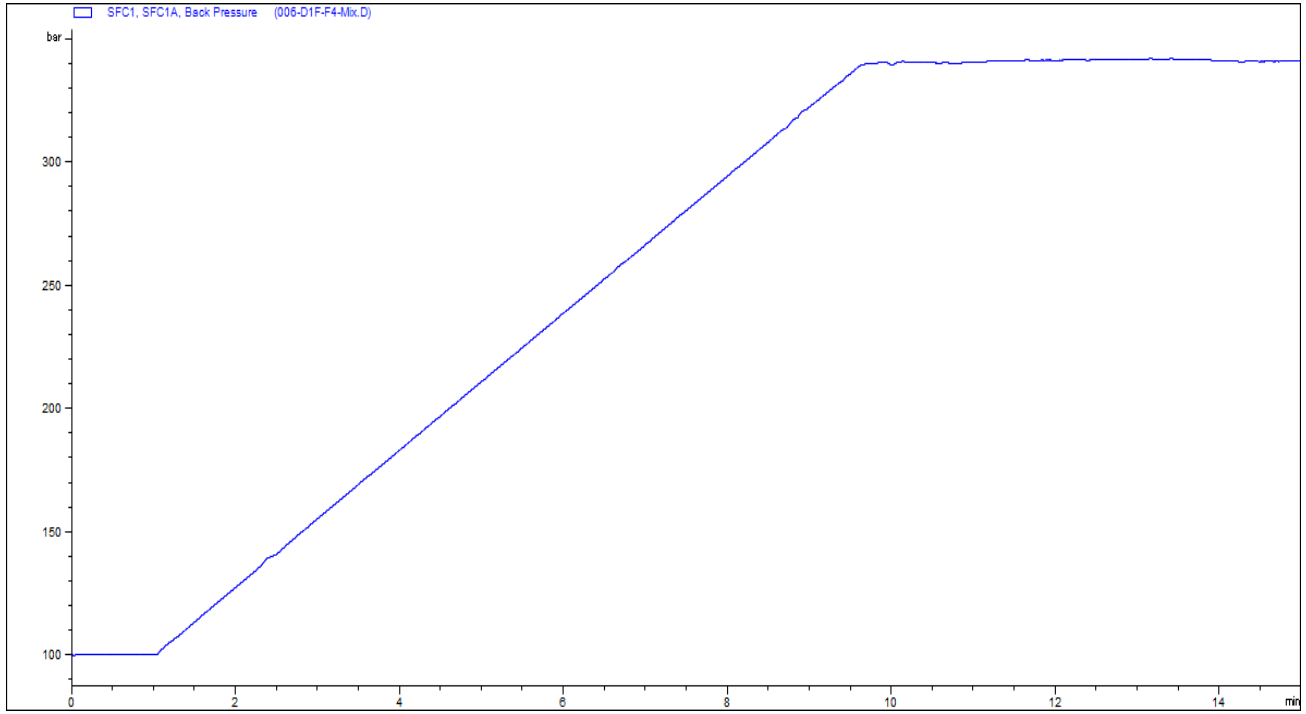
Solvent cost in our lab (1L or 1kg): CO_2 < MeOH < iPOH < EtOH < MeCN

CO_2 -> 7tons tank outside the building (food grade)
Solvents -> come in 30L shuttle drum (prep HPLC grade)

Why is MeOH considered greener than EtOH

1. Lower Cumulative Energy Demand
Methanol generally requires less energy to produce and purify compared to ethanol, especially when considering synthetic routes from biomass or CO_2. This lower energy footprint contributes positively to the AMGS score. [About the...– ACSGCIPR]
2. Solvent Health and Safety Profile
While both solvents are flammable and toxic to some extent, methanol is often used in smaller volumes in chromatography and has a well-understood risk profile. Ethanol, although less toxic, may have higher exposure risks due to its volatility and broader use in larger volumes.
3. Environmental Impact
Methanol biodegrades relatively quickly and has a lower potential for bioaccumulation. It also produces fewer harmful byproducts during combustion or disposal. Ethanol, while biodegradable, is often derived from agricultural sources, which can raise concerns about land use and sustainability. [Methanol v...inability?]
4. Waste and Instrument Efficiency
Methanol is compatible with many high-efficiency chromatographic methods (like UHPLC and SFC), which use less solvent and generate less waste. Ethanol, due to its viscosity and polarity, may require longer run times or higher volumes, increasing waste and energy usage. [Analytical...Calculator]
5. Feedstock Versatility
Methanol can be produced from a wide range of feedstocks including biomass, CO_2, and even waste gases, making it more adaptable to green production methods. Ethanol is primarily derived from crops, which can compete with food production and require significant water and land resources

Pressure based method development in SFC



Pressure gradient profile
In this case, 100 to 350 bar (instrument limitation).

Then method optimisation, typically if we can, we go isobaric.

We are working without co-solvent:

- Only the stationnary phase will affect selectivity (large set needed)
- Eluting strength tweaking is done by **adjusting CO_2 density**

Adjusting CO_2 density

There are 2 ways you can affect the density of the fluid in the system:

- Change the pressure in the system:
  - Change the BPR value (100 to 200 bar)
  - Change the flow-rate (2 to 3mL/min)

- Change the temperature

If Pressure  then density  , typically compounds elute faster

If Temperature  then density  , typically compounds elute later

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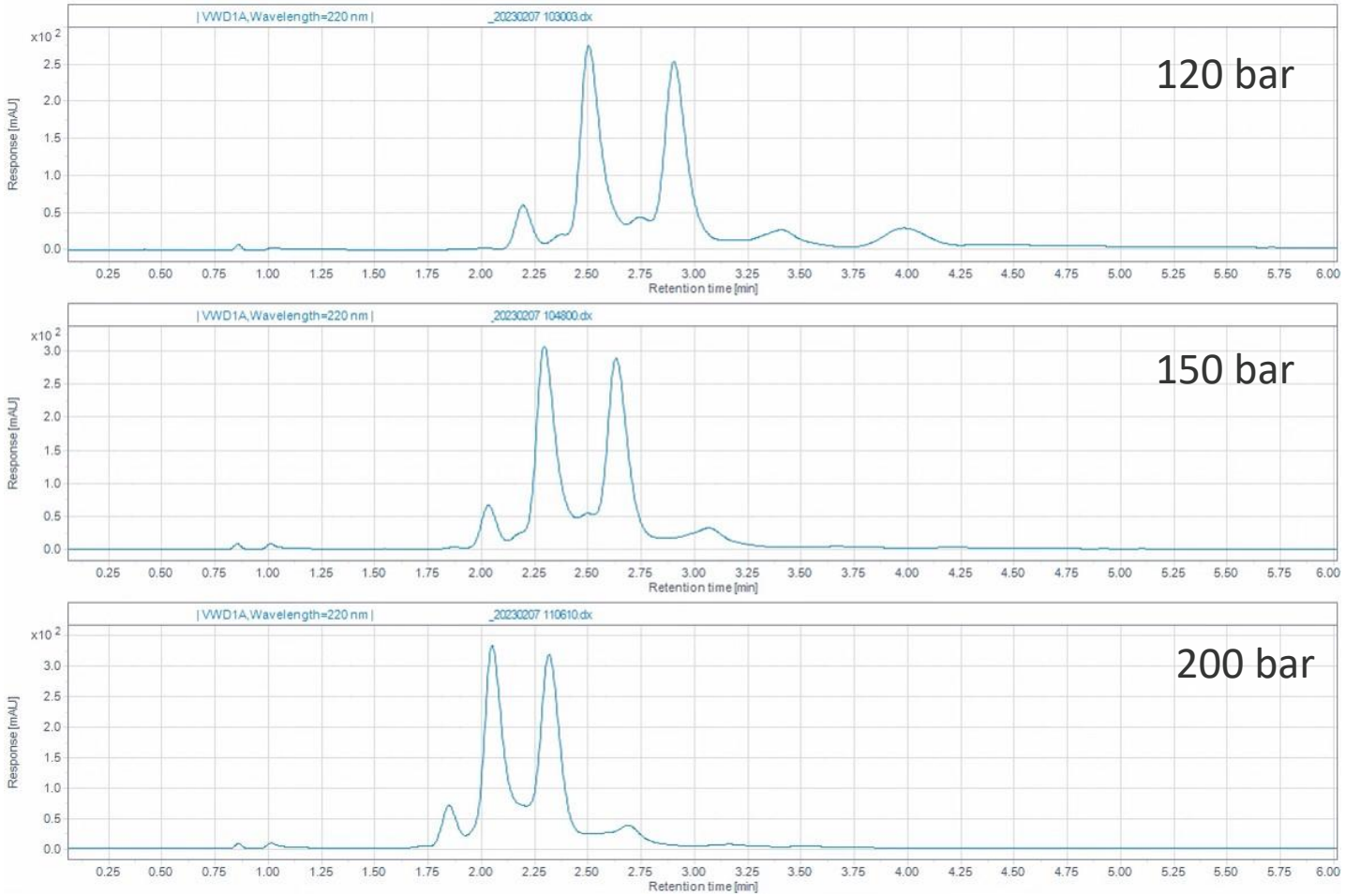
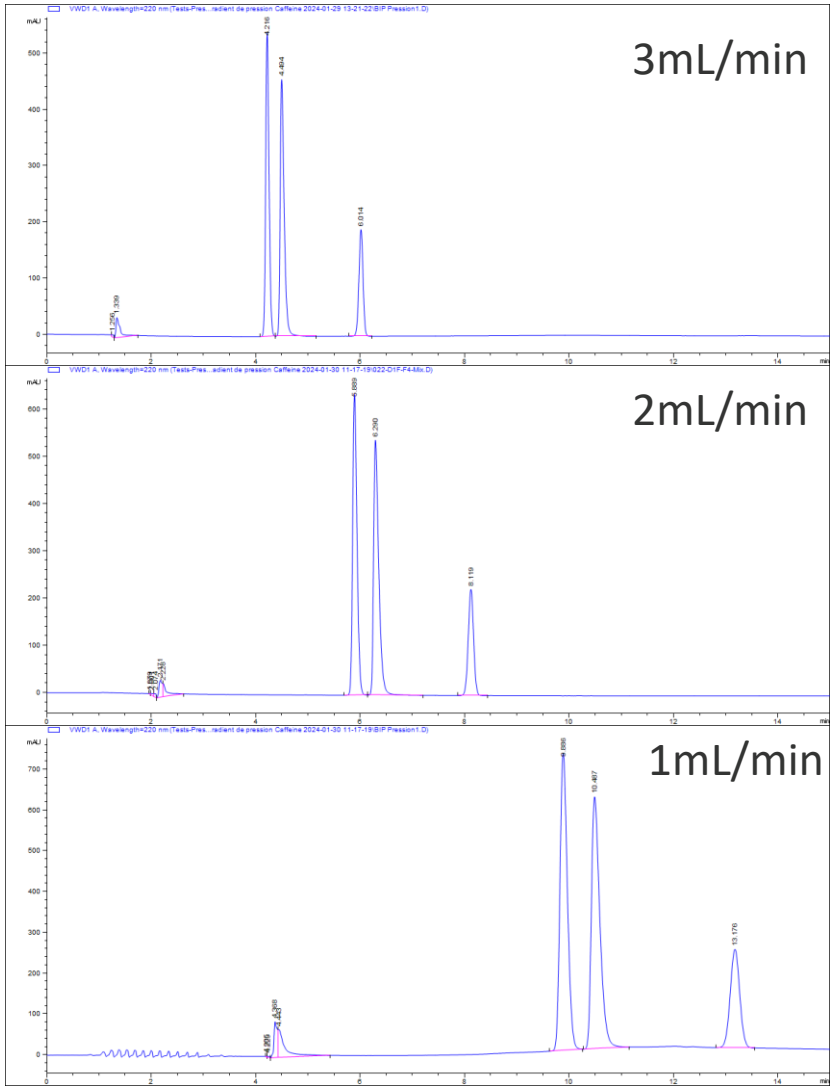
Playing on BPR pressure



Fish oil extract

As pressure increases, compounds are eluting faster.

Playing on flow-rate



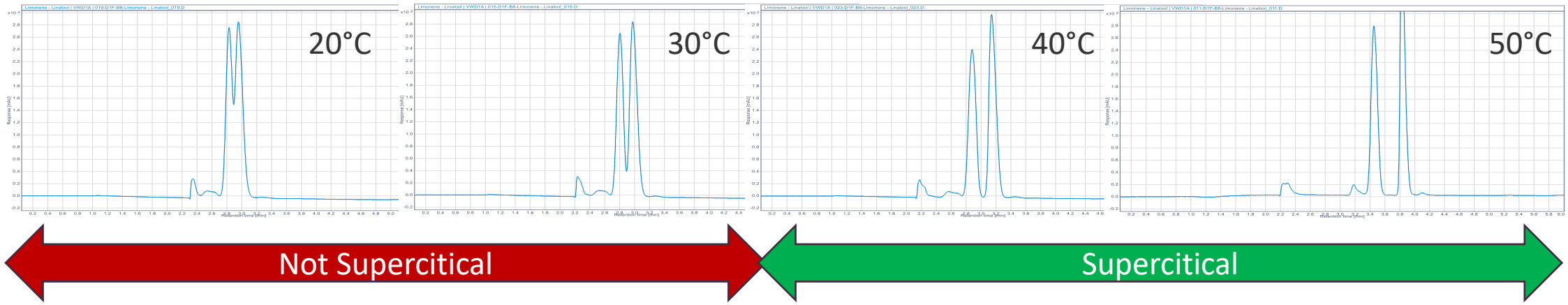
Harder to spot as decreasing flow-rate obviously changes retention time but it will reduce pressure on the head of the column therefore compounds are typically more retained.

When Flowrate ↘ , density ↘

typically compounds elute **later!**

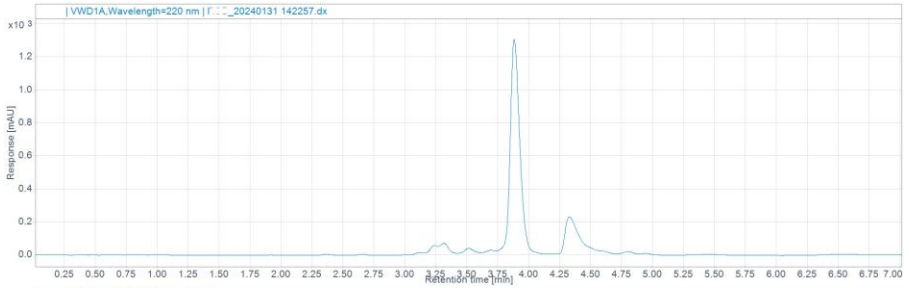
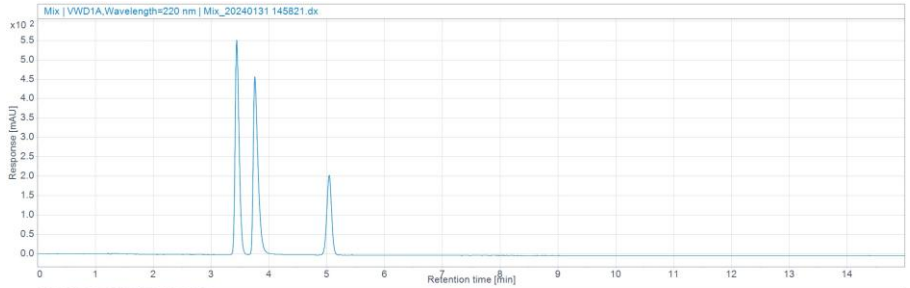
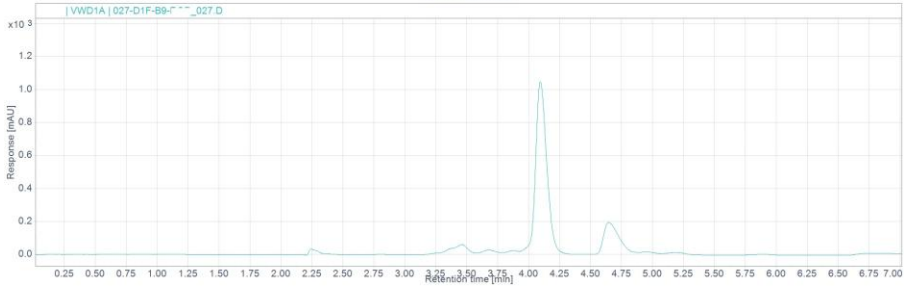
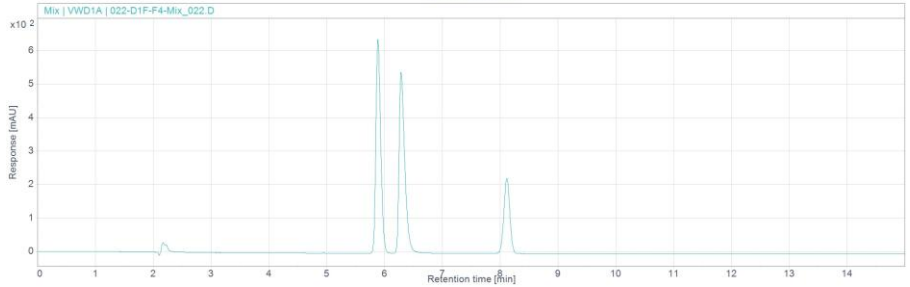
Mix: Amino-Biphenyl, Benzophenone, Dibromobiphenyl

Playing on temperature



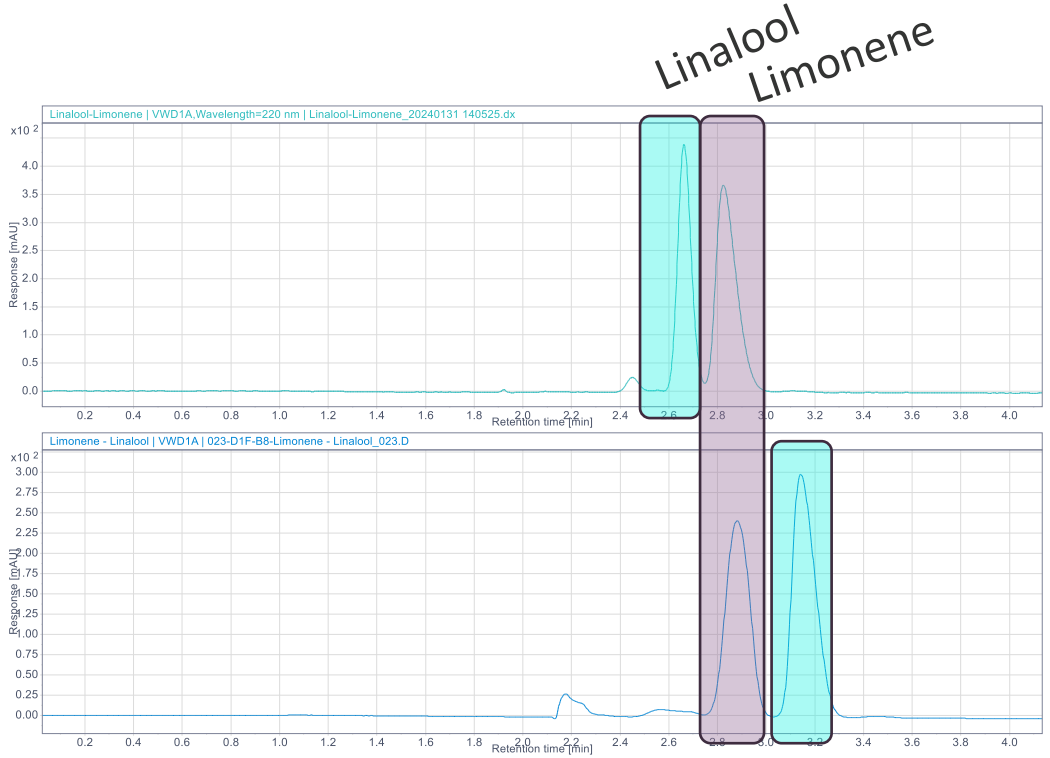
When Temperature ↗ , density ↘ , typically compounds elute **later!**

Transfer from analytical to analytical

	Natural Product Extract	Synthetic Mix[1]
System with lots of valves and narrow tubings		
Simple system, larger tubings		

[1] Mix: Amino-Biphenyl, Benzophenone, Dibromobiphenyl

Transfer from analytical to analytical



Elution order changed!

Column Head Pressure: 180Bar

ΔP=80 bar

BPR Pressure: 100Bar

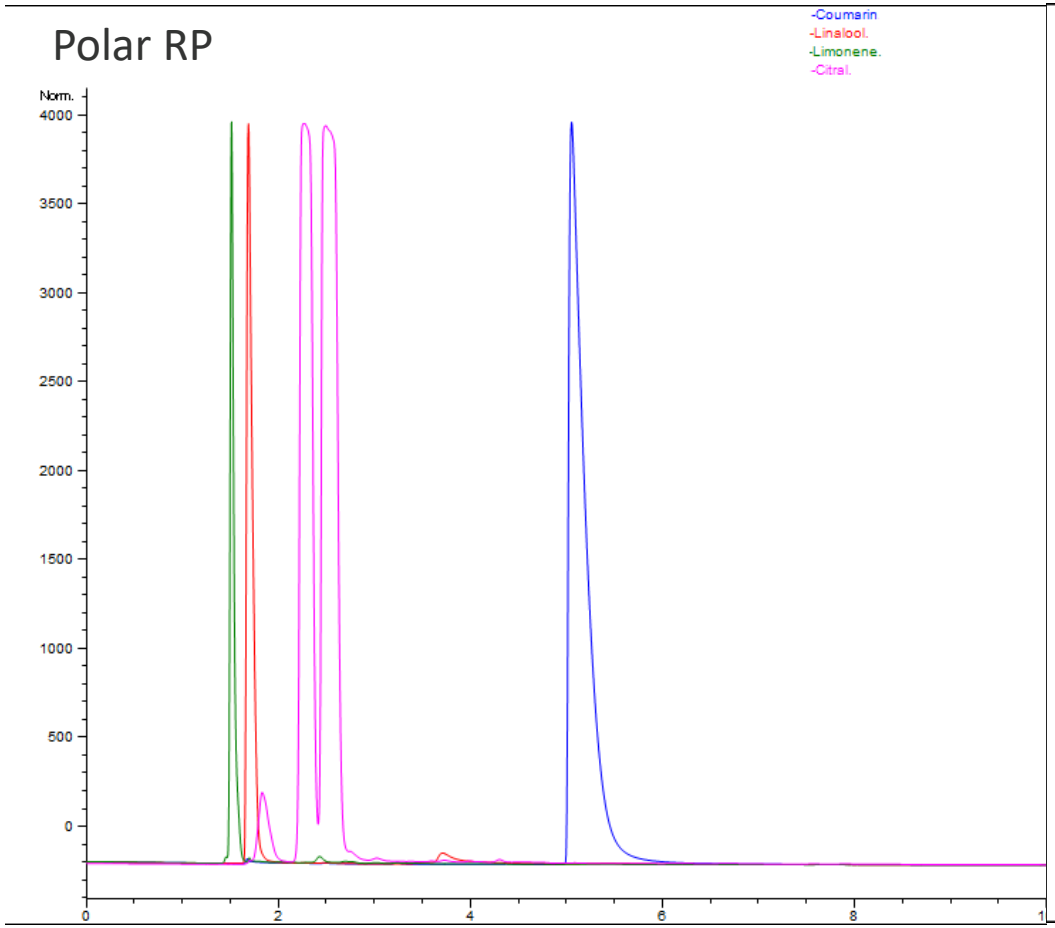
Column Head Pressure: 120Bar

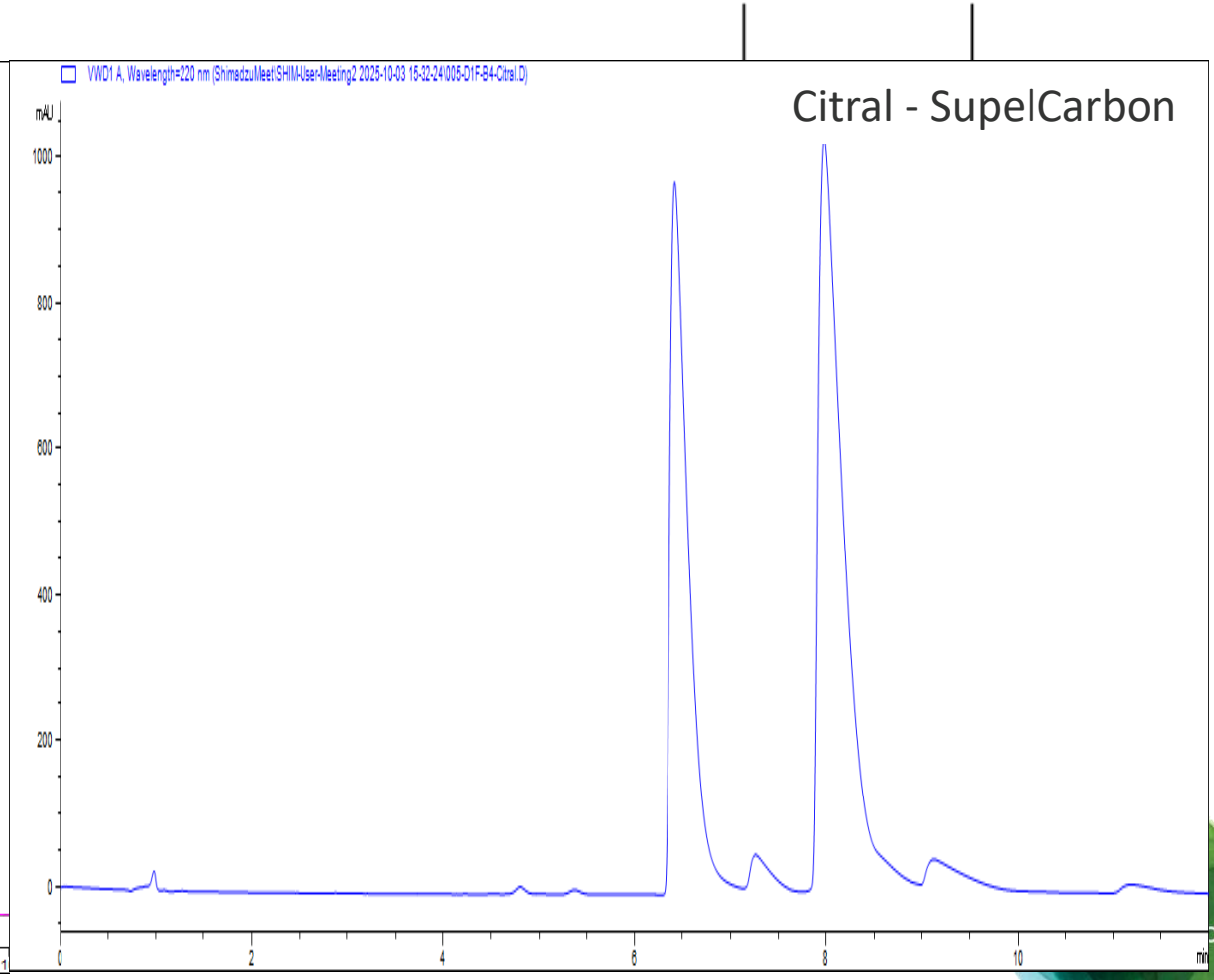
ΔP=20 bar

BPR Pressure: 100Bar

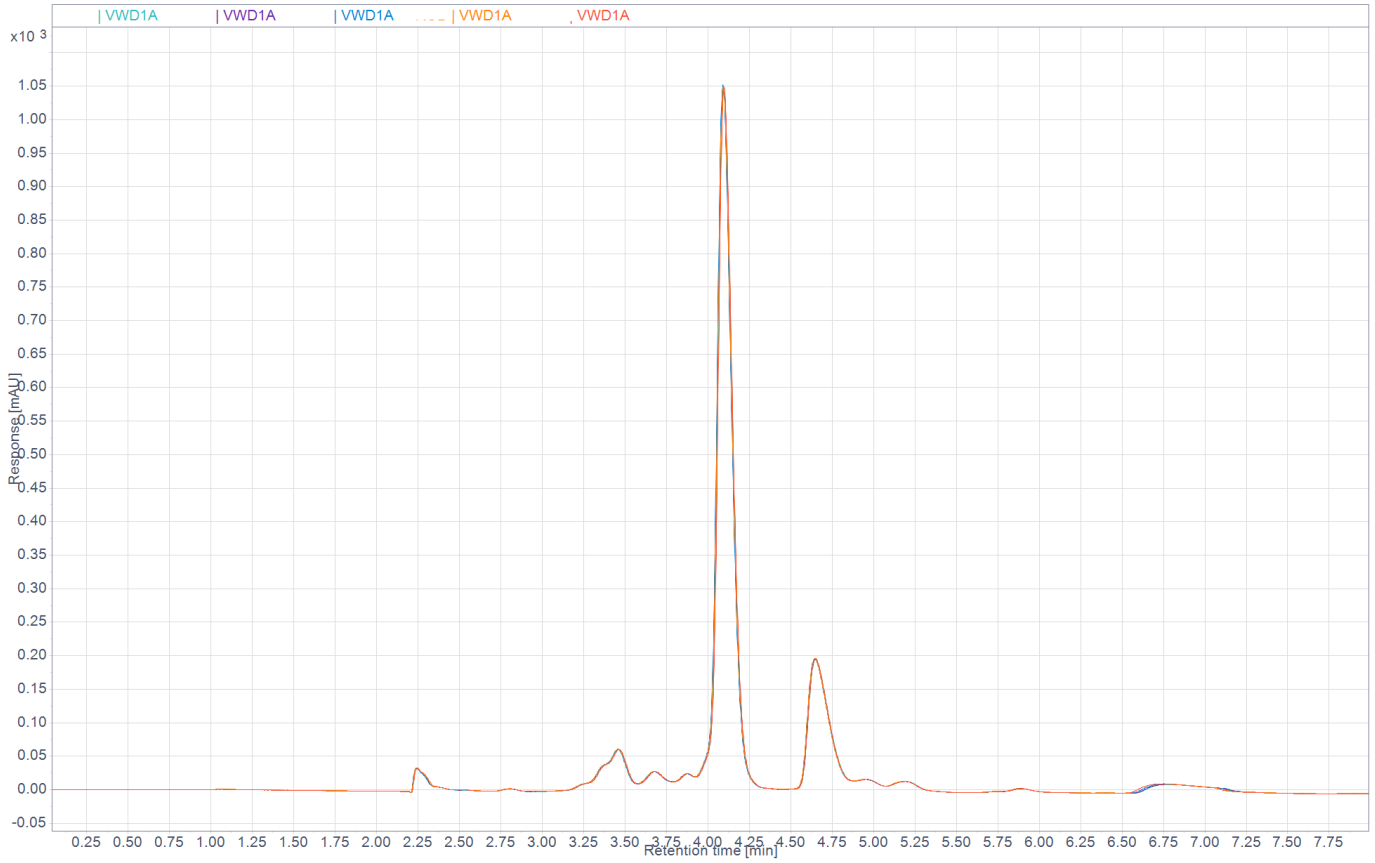
The BPR pressure value is important but so is pressure drop!

Fragrance compounds





Method « repeatability »



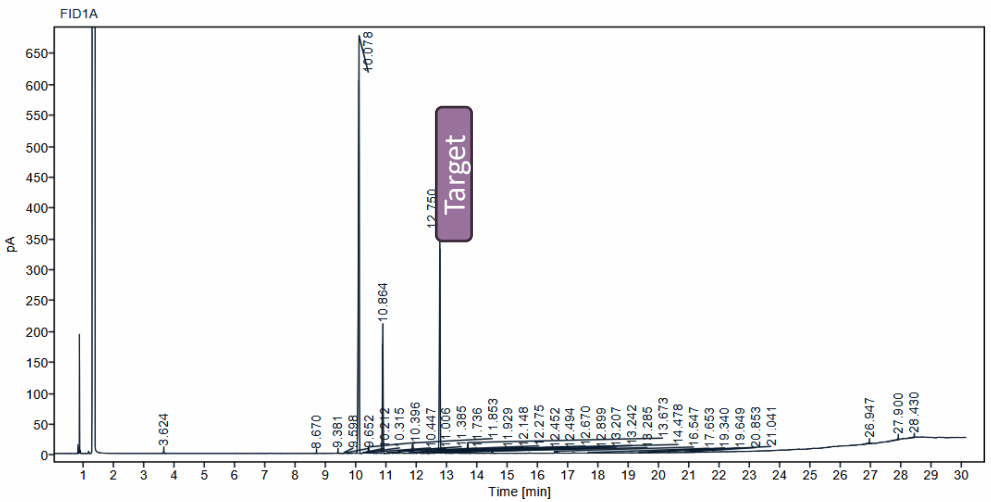
Natural product extract

Overlay of 5 injections in pressure gradient mode

Semi volatile target in plant extract

Semi volatile target from a plant extract

Product is an oil and contains about 24% of the taret molecule (by GC-FID)
This is for indsutrial scale SFC (tons/years)

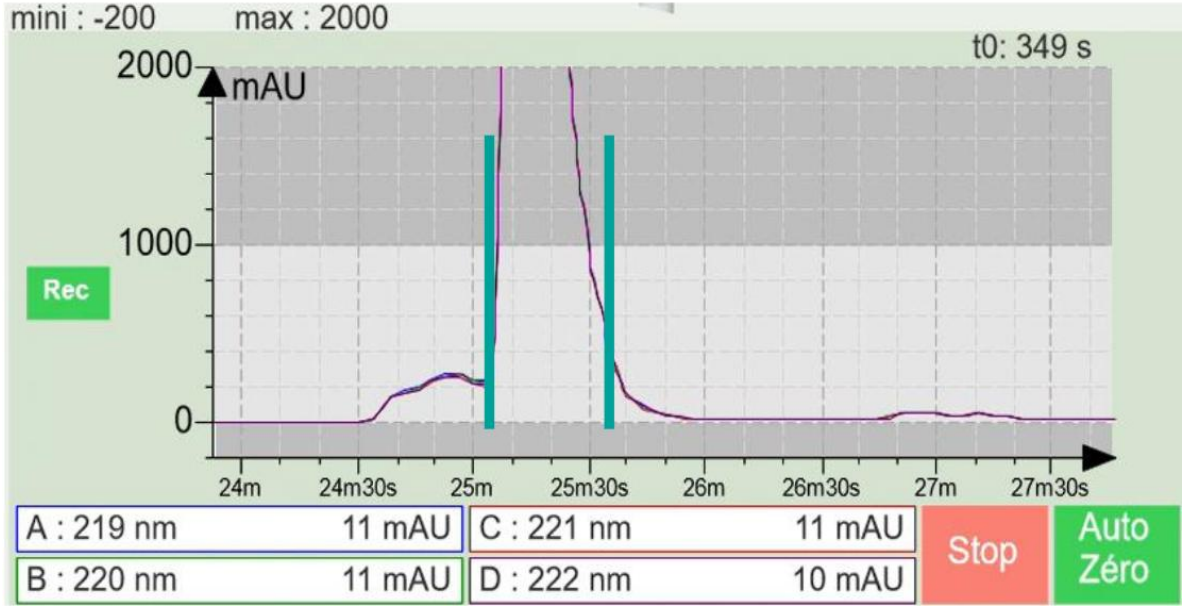


Signal: FID1A

RT [min]	Type	Area	Area%	Name
10.078	BB	1482.1	52.3	
10.864	VB	380.5	13.4	
11.853	VV	36.5	1.3	
12.750	VB	693.8	24.5	Target

Current process is based on many successive distillations to go from 24% to 95% purity (GC-FID)

Semi volatile target from a plant extract



Full screening of columns/conditions
Optimised method: CO_2/EtOH 97/3 on a BiP column.

The method was then scaled to 3cm ID column.
Oil injected neat.

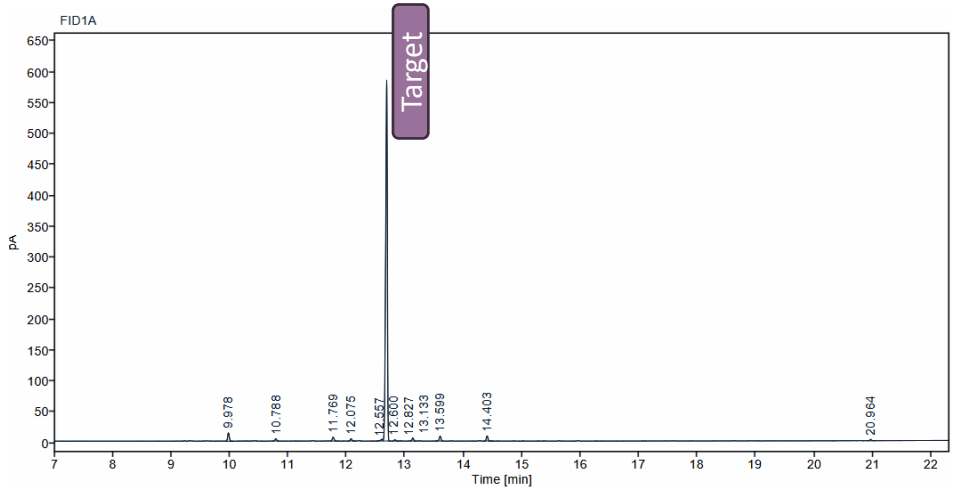
Semi volatile target from a plant extract

Input

Crude (g)	Target content (GC-FID)	Maximum recoverable Target (g)
15,5	24,50%	3,7

Output

Recovered Target (g)	Purity (GC-FID)	Recovery
3,4	91,20%	81%



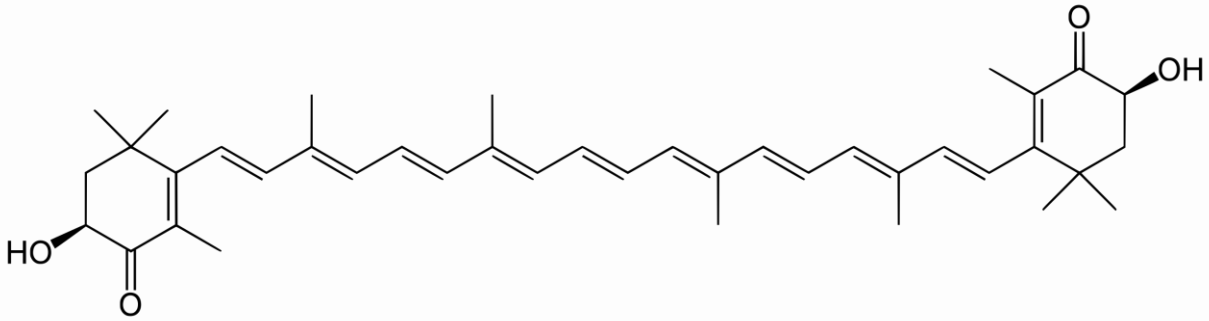
Signal: FID1A

RT [min]	Area	Area%	Name
9.978	25.2	2.0	
10.788	8.1	0.6	
11.769	13.4	1.1	
12.075	7.7	0.6	
12.557	1.7	0.1	
12.600	5.8	0.5	
12.685	1162.9	91.2	Target

Successful project, recovery is good (after dry-down) considering this is a semi-volatile compound! (BP around 110°C)

Sustainability considerations

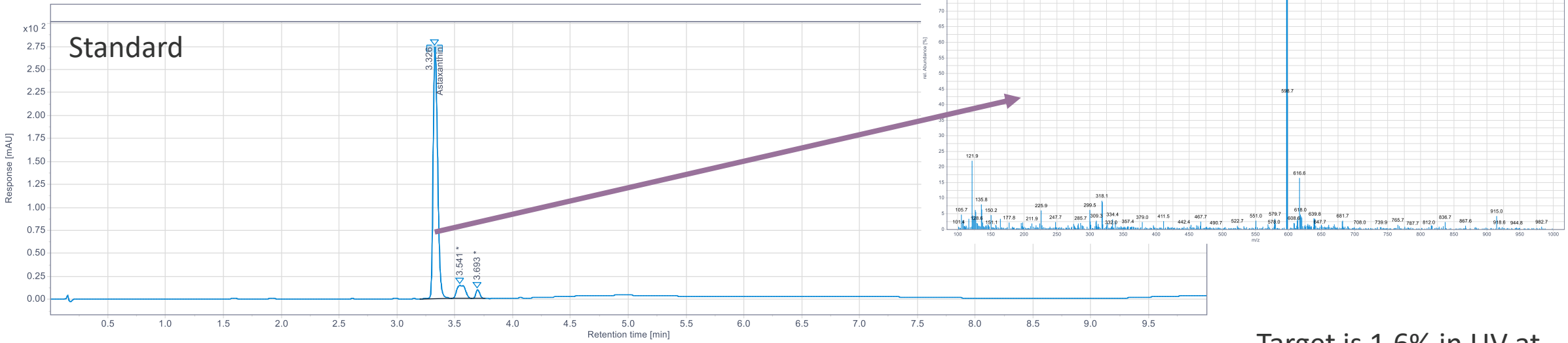
More difficult to compare but this method allows the removal of about 9 distillations steps (customer feedback), so likely it is an overall improvement in terms of sustainability

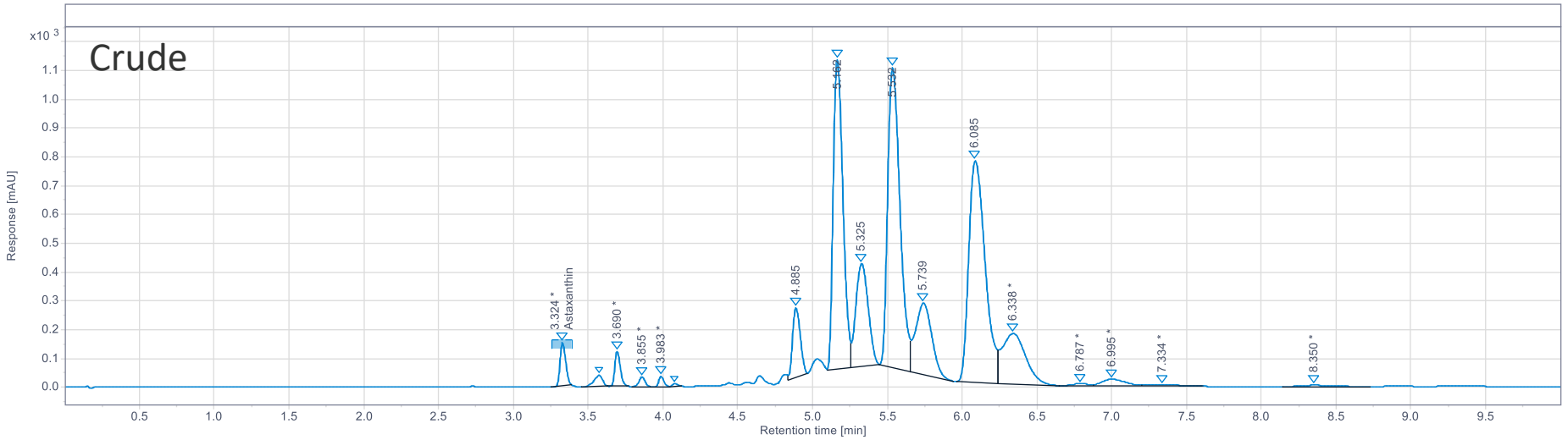


Astaxanthin Purification POC from cyanobacteria extract

REACH SEPARATIONS

RP analytical data





Target is 1.6% in UV at 480nm
Identity was confirmed by RT, MS and UV spectrum.
50-98% MeCN gradient

REACH SEPARATIONS

SFC analytical screening approach

A set of 10 columns were tested to find the best selectivity (all in 4.6x250mm, 10µm).

A pressure gradient approach was tested but did not give enough eluting strength (need for a ***higher-pressure system***!)
A solvent gradient (ethanol -> customer requirement) approach has then been used.

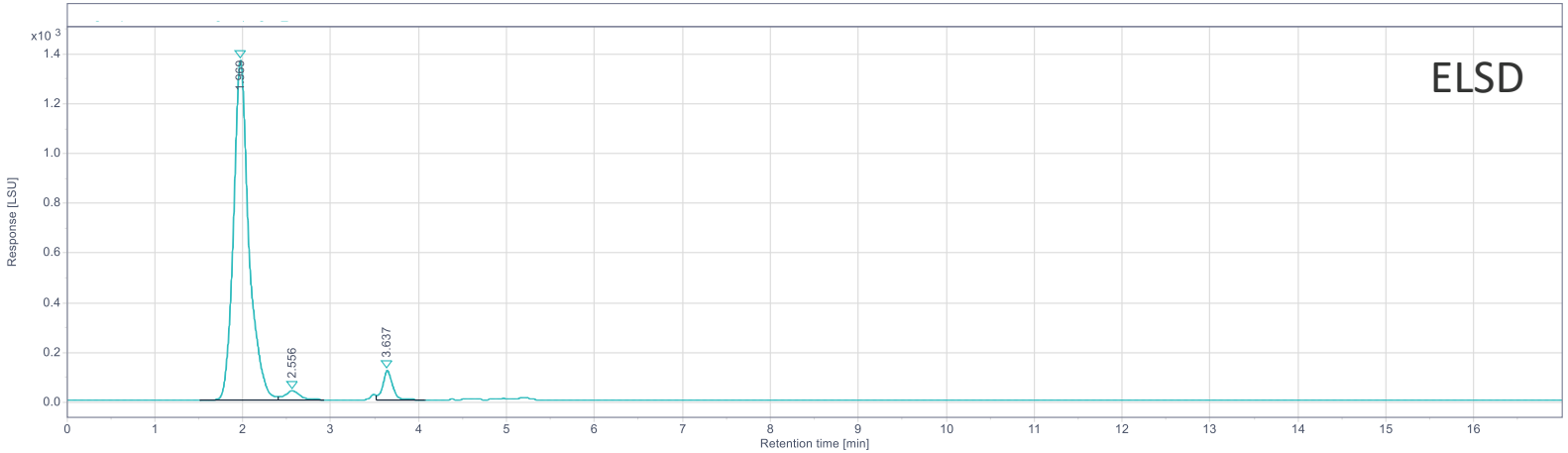
Best column from the screening set was Venusil HILIC.

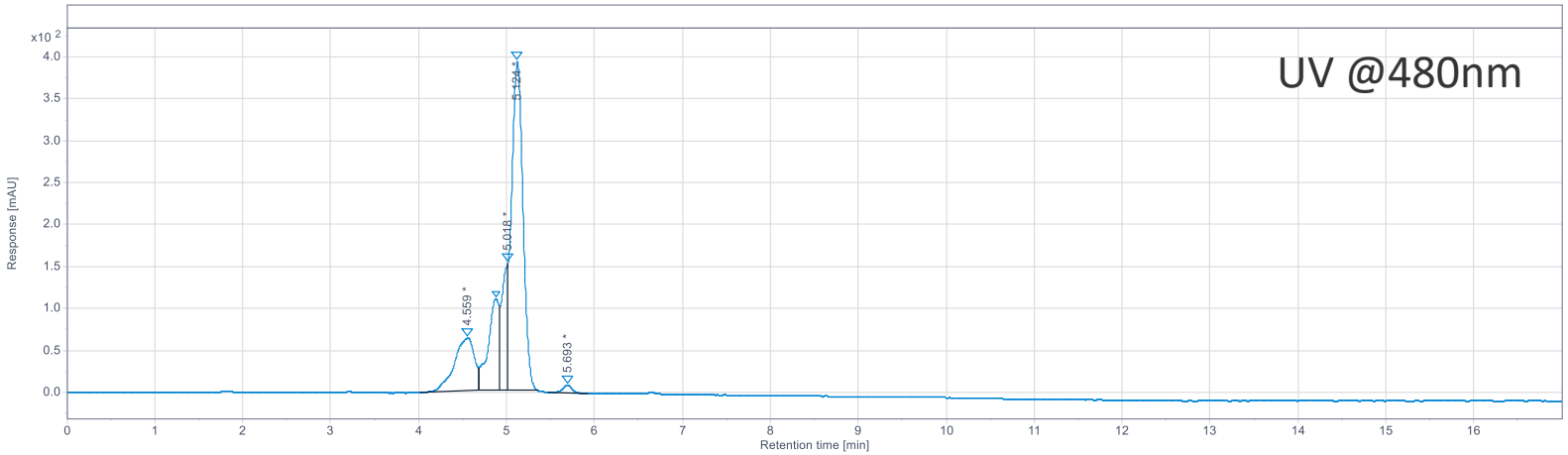
The provided sample was dissolved in DCM (not good but the only solvent that could dissolve everything…).

Analysis was performed with ELSD and UV (480nm) and prep was performed at 480nm.

REACH SEPARATIONS

Promising screening data



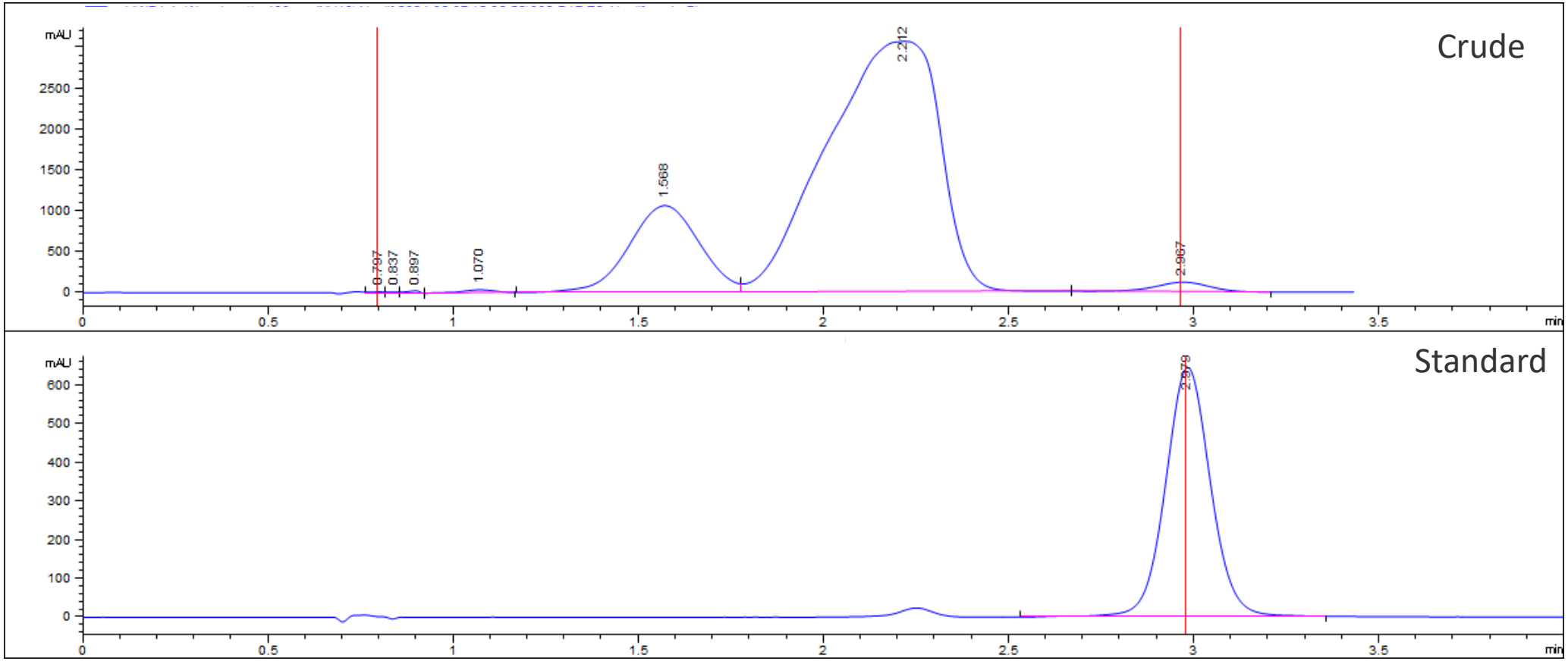


The ELSD allows us to see that the non-UV content of the sample elutes before the UV-visible one.

This combination of column and solvent seems promising.

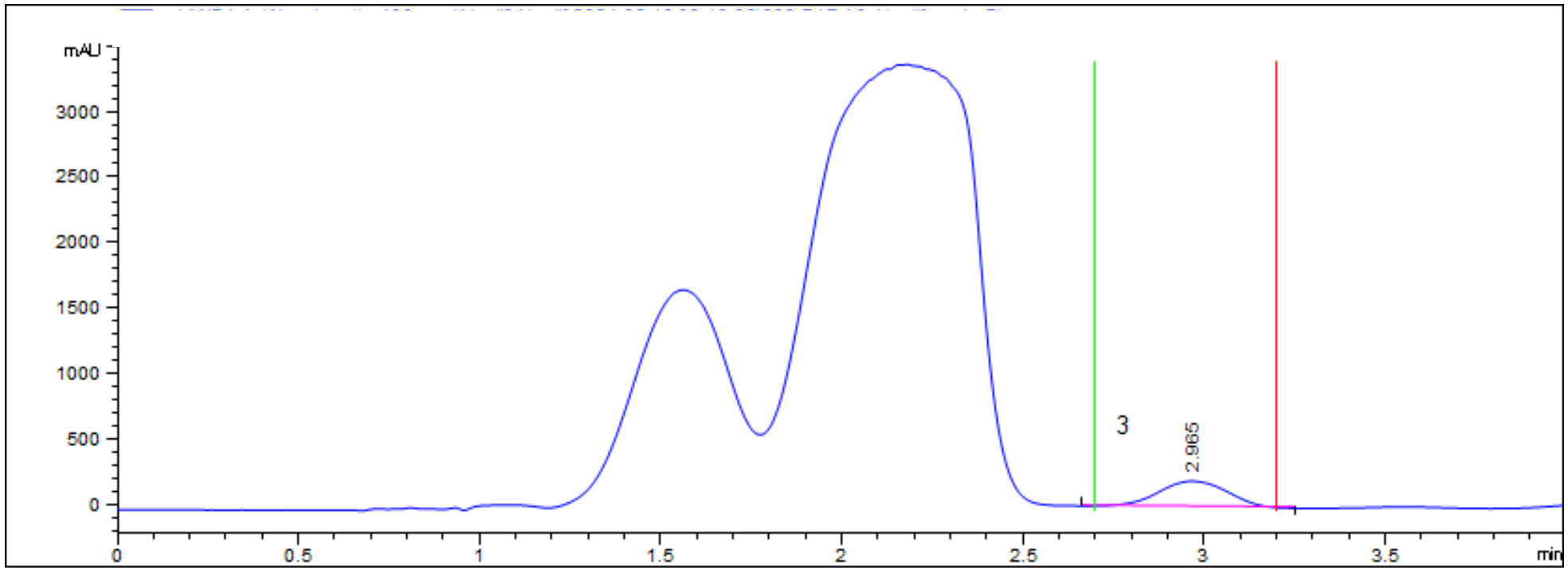
REACH SEPARATIONS

Optimised Isocratic SFC method



REACH SEPARATIONS

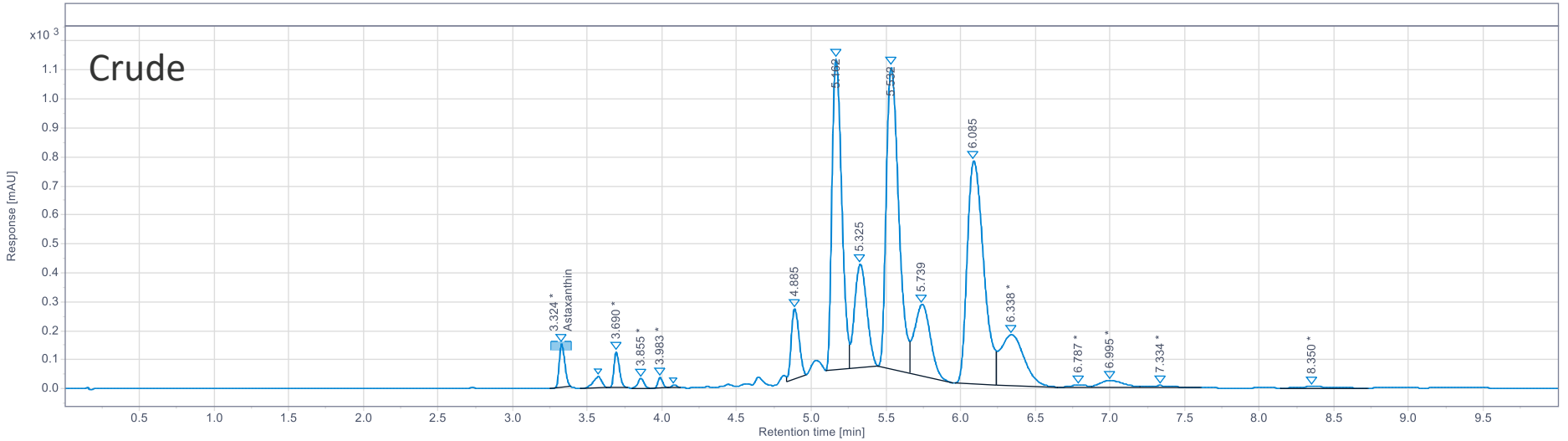
Prep chromatogram example

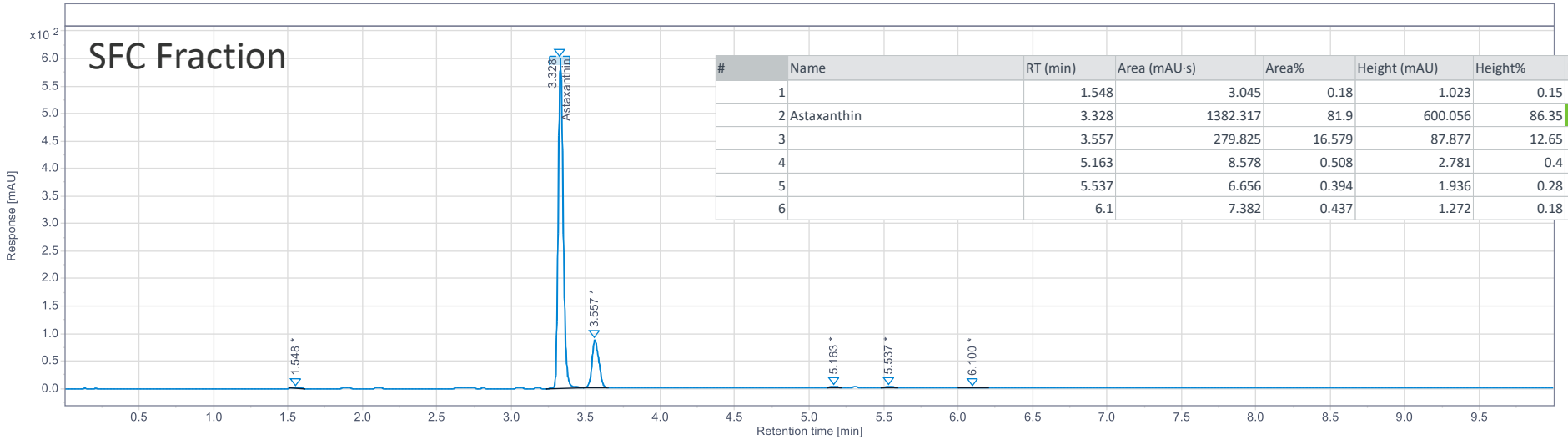


Retention time was very stable over the course of 40 injections.

REACH SEPARATIONS

Profile crude/fraction comparison



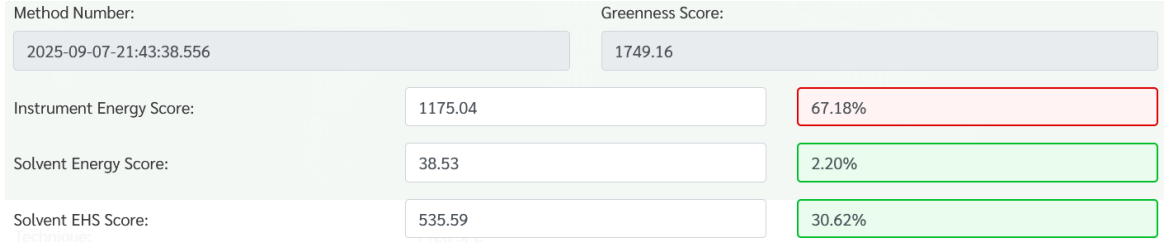


#	Name	RT (min)	Area (mAU·s)	Area%	Height (mAU)	Height%	UV Conf. Match Fac	Symmetry	Width (min)
1		1.548	3.045	0.18	1.023	0.15		0.80973	0.098
2	Astaxanthin	3.328	1382.317	81.9	600.056	86.35	1000	0.81788	0.242
3		3.557	279.825	16.579	87.877	12.65		0.72995	0.169
4		5.163	8.578	0.508	2.781	0.4		0.87974	0.103
5		5.537	6.656	0.394	1.936	0.28		0.94291	0.109
6		6.1	7.382	0.437	1.272	0.18		0.98843	0.203

REACH SEPARATIONS

Outcome

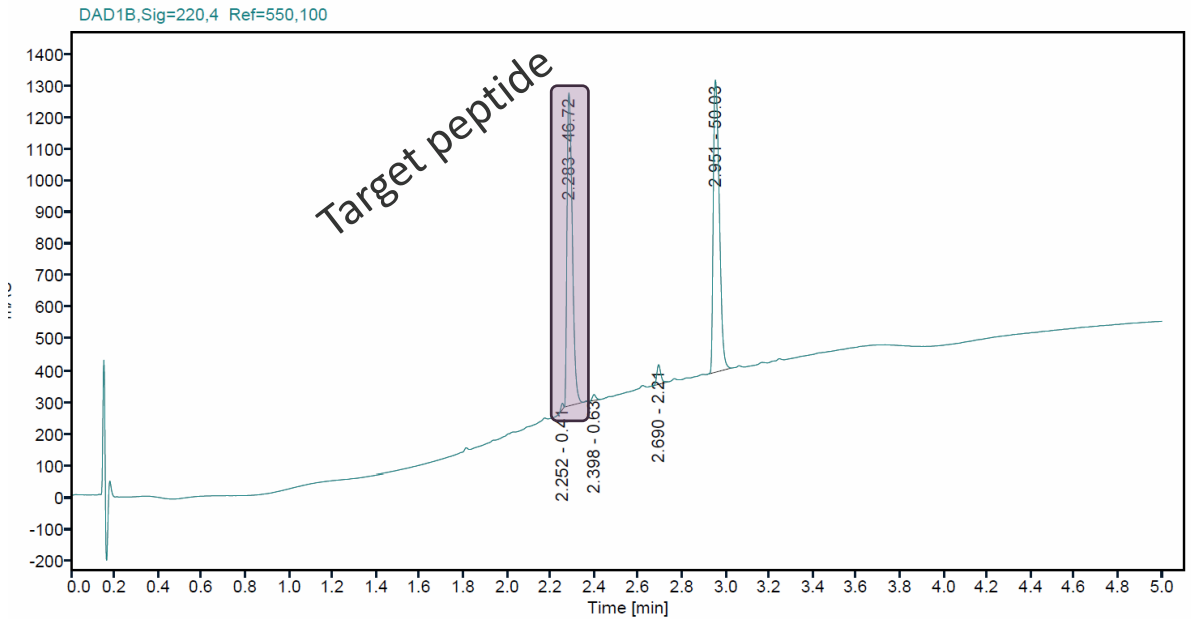
- We have been able to find SFC conditions that seem suitable for astaxanthin purification from the given matrix
- The mobile phase contains only CO2 & ethanol
- The UV purity (@480nm, absorption maximum of astaxanthin and its family) goes from 1.6 to 80% with 1 pass on SFC
- Actual published methods are:
  - Low pressure NP (with DCM & Acetone)
  - High Pressure LC (with high content of ACN)
  - CPC (*n*-hexane–ethanol–water)



REACH SEPARATIONS

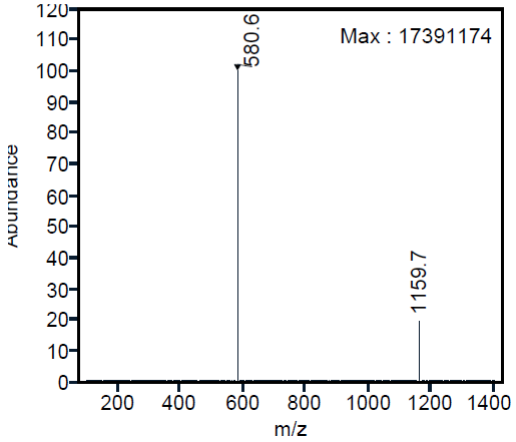
Small Synthetic peptide

Customer sample



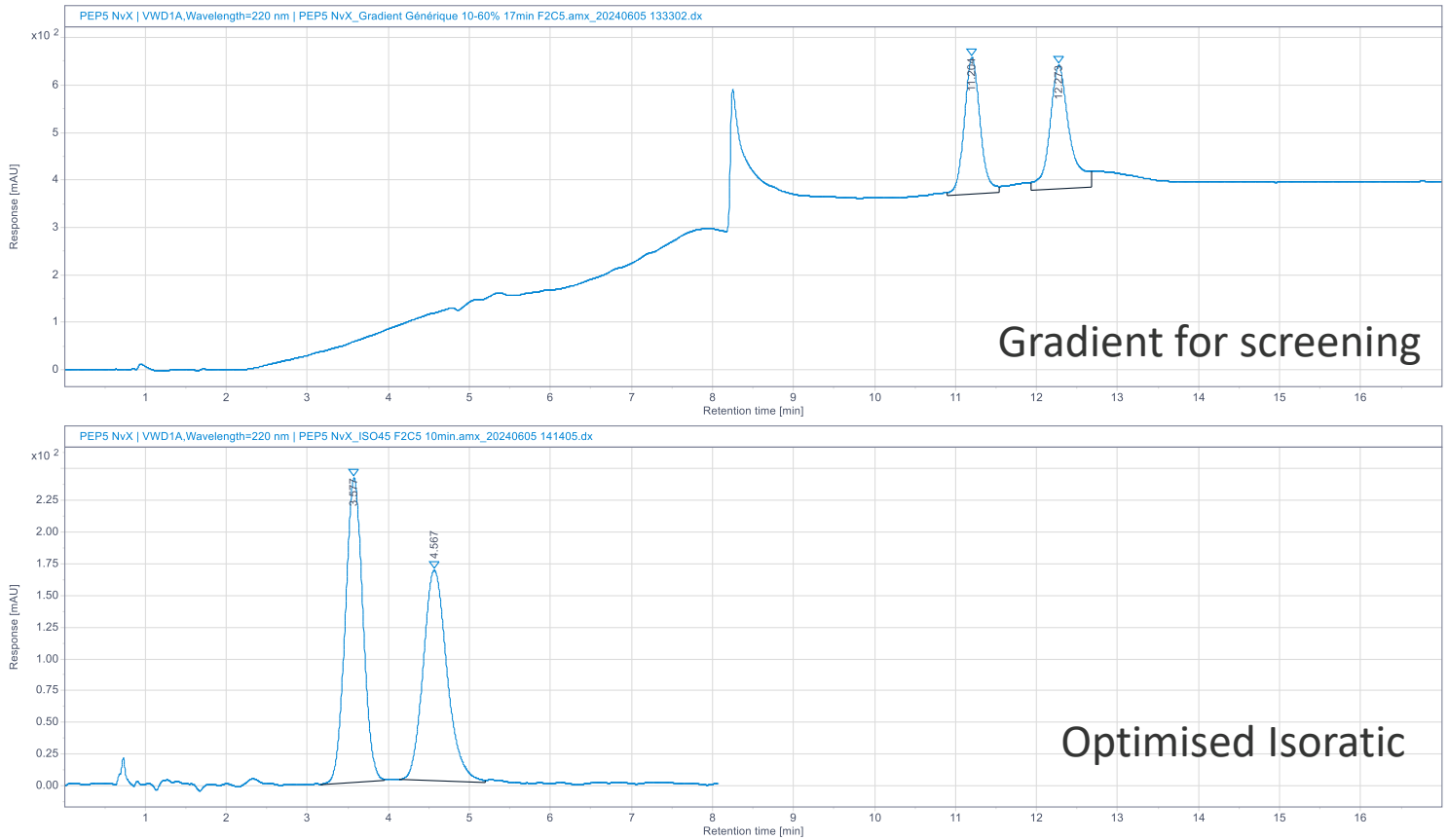
RP analysis with formic acid
PEP5 is a composed of N amino acids to separate from a N-1 amino-acids one.

Peak RT: 2.30



Retention Time (min)	Area	Area%
2.25	14.6	0.41
2.28	1655.9	46.72
2.40	22.4	0.63
2.69	78.5	2.21
2.95	1773.3	50.03

SFC conditions screening



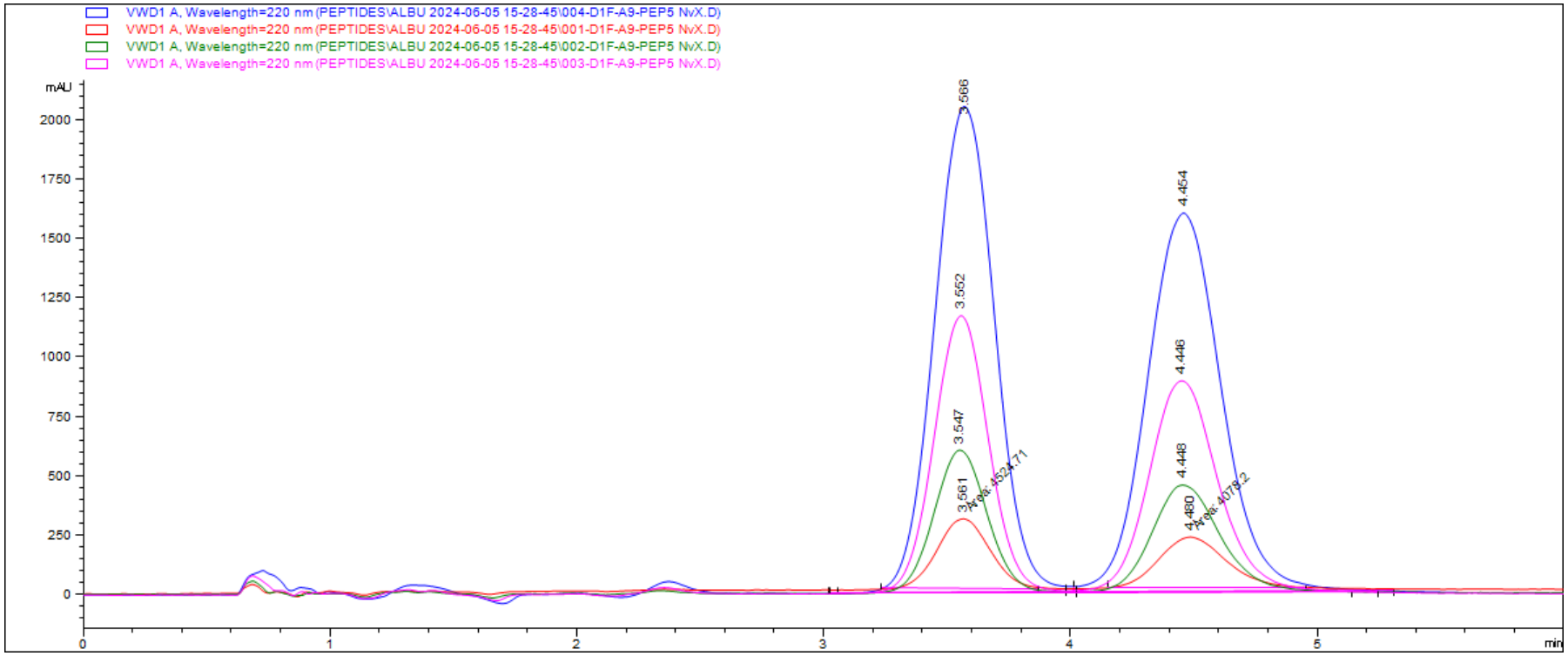
Columns tested:

- Venusil HILIC
- Polar RP
- Gemini C8
- Prep PhenHex
- Luna NH2

4.6x250mm, 10µm

Several mobile phase tested
MeOH/H20 95/5 Amm For 20mM

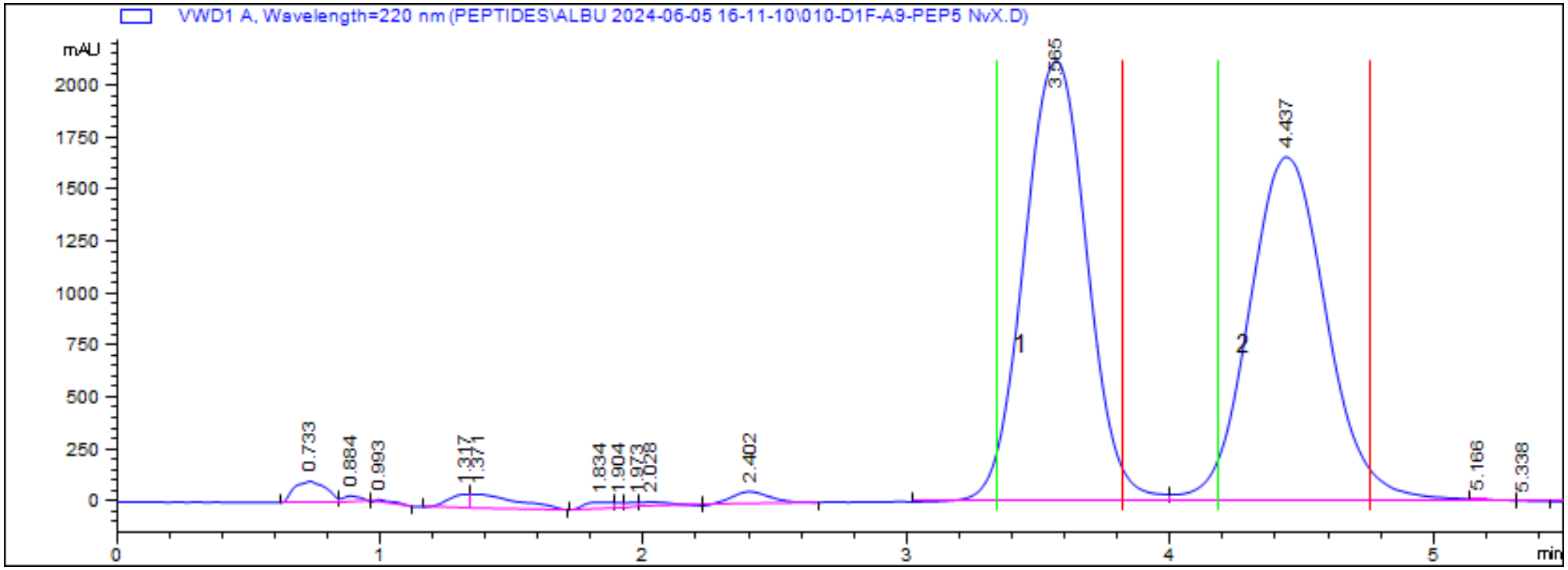
SFC loading study



3mg of crude diluted into 600µL of MeOH (good solubility)
Injection volumes: 5, 10, 20 & 40µL
Resolution still looked ok at 40µL so selected for the prep

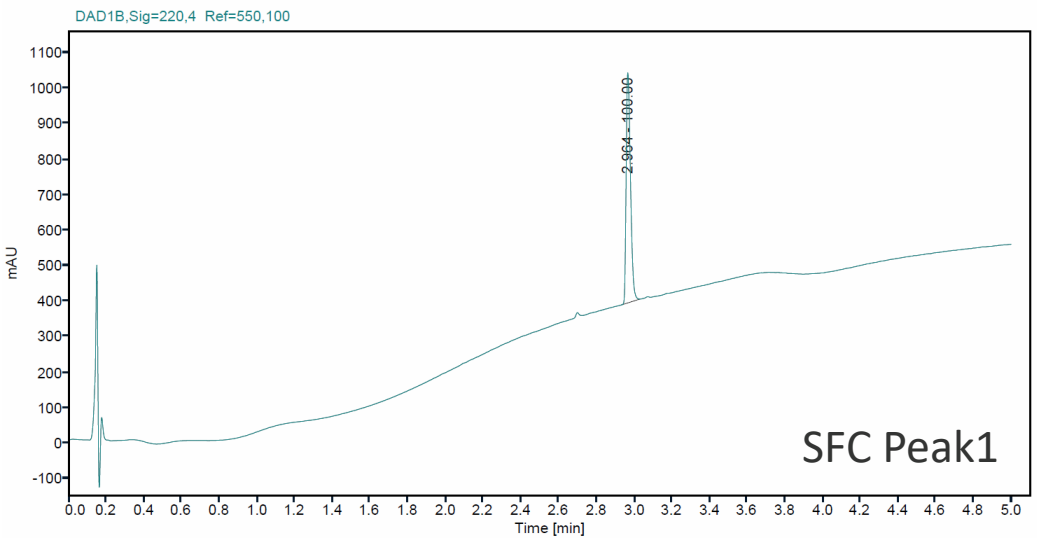
REACH SEPARATIONS

Preparative run example

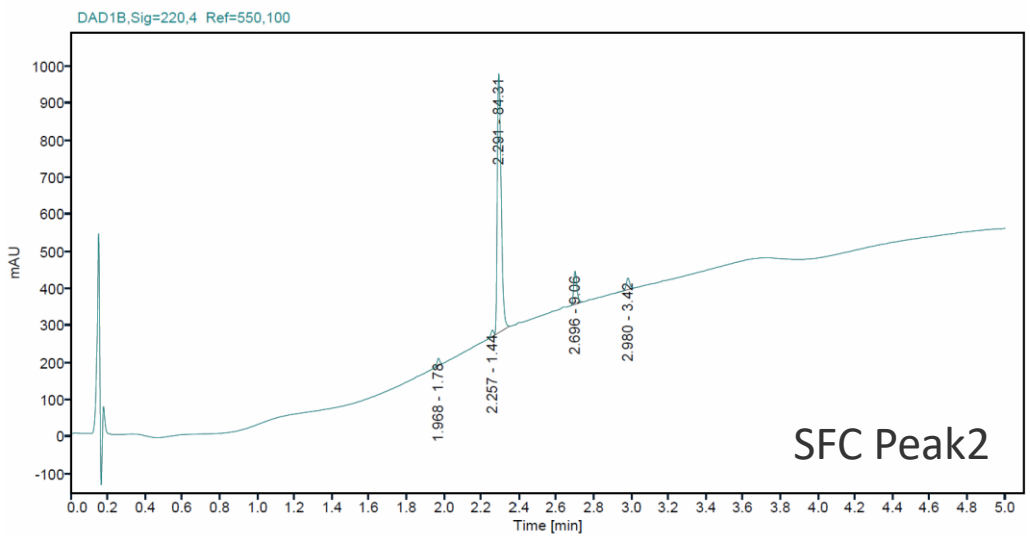


11 injections of 40µL were performed so 2.2mg on column total (200µg/injection)

Results



Retention Time (min)	Area	Area%
2.96	1033.9	100.00



Retention Time (min)	Area	Area%
1.97	21.3	1.78
2.26	17.3	1.44
2.29	1010.3	84.31
2.70	108.5	9.06
2.98	40.9	3.42

	Amount (mg)	UV Purity (%)
PEP5_1	0.9	98
PEP5_2	0.8	84

Elution order is reversed between RP & SFC

AMGS considerations

SFC method score:	50.29
Optimised small scale ***HPLC*** method (not performed)	202.10

Assuming the same number of injections on both techniques

REACH SEPARATIONS

Conclusions

- Not surprisingly, SFC is typically « greener » for prep
- If you can push to go without co-solvent, even better (100% CO_2)
- AMGS calculator is a useful tool to quickly compare different approaches in terms of sustainability

Thanks to the team!

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Thanks for your attention!