SFC and HPLC – two complementary techniques?

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Outline

- Sepmatix SFC screening system
- Screening strategy
- Different interaction mechanism in LC and SFC mode
- Case studies
- Conclusion

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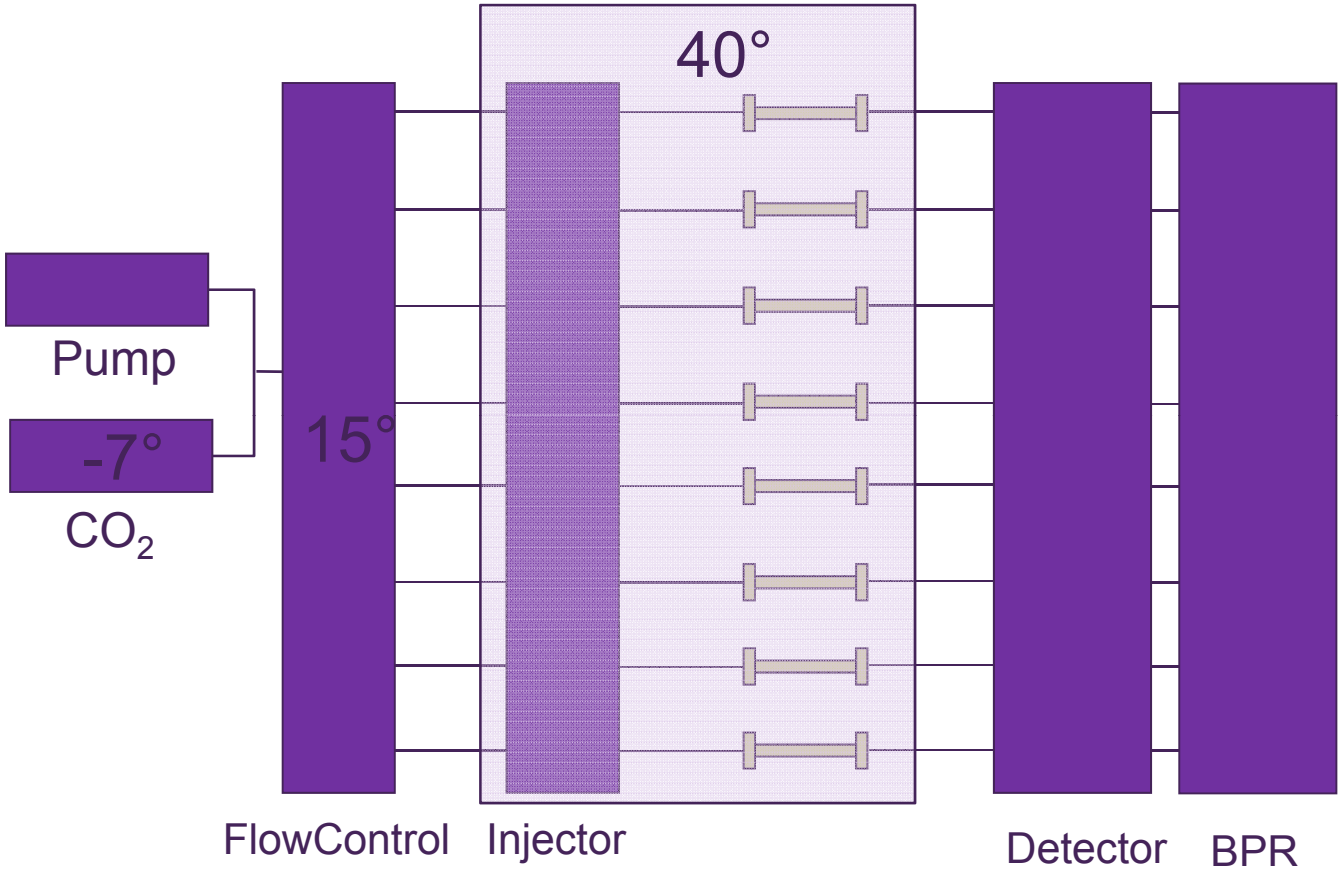
Sepmatix SFC screening system



The flowcontrol can regulate up to 8 parallel channels giving equal flow rates on all columns

Only one pump, injector and detector

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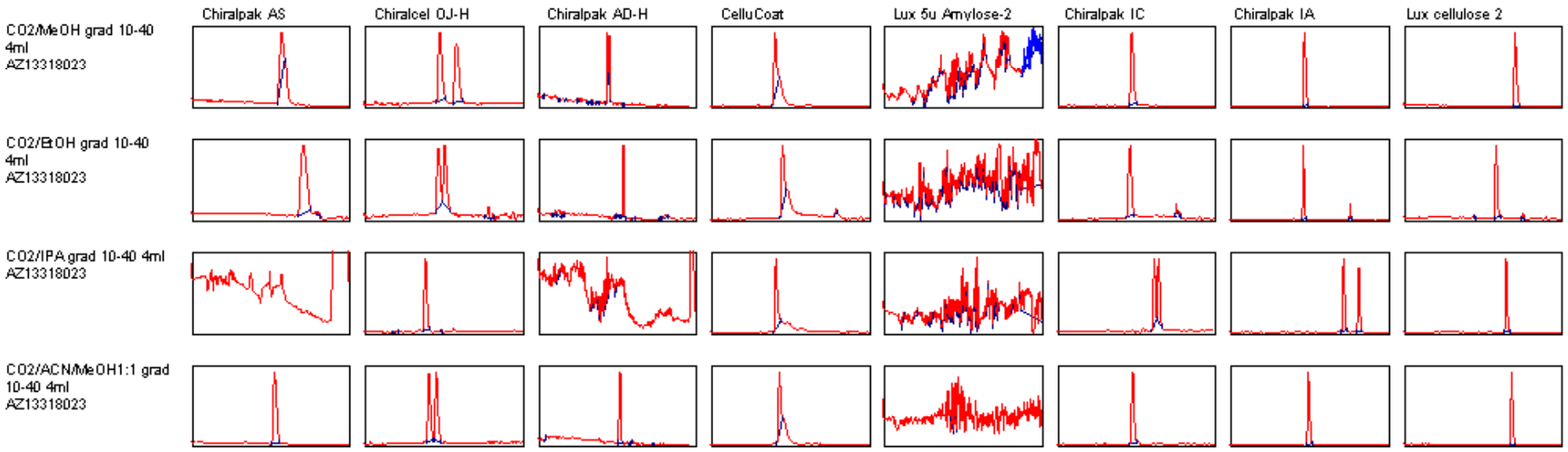
Sepmatix screen overview



- All columns are 250 x 4.6 mm
- Gradient 10 - 40% modifier in 10 min, 100 bar, 4 ml/min, 40 °C
- Total run time (4 modifiers) less than 1 hour giving 32 variations of conditions

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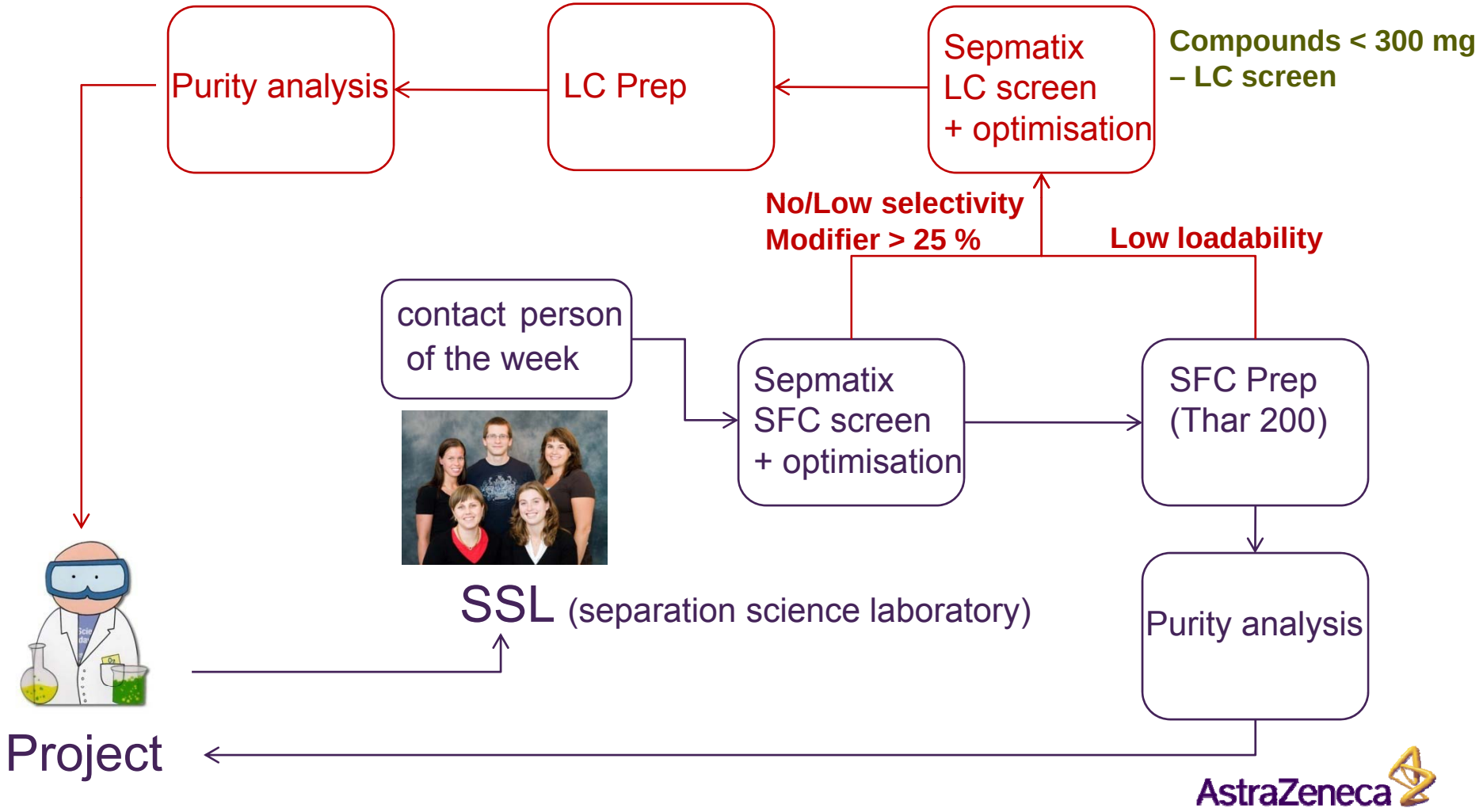
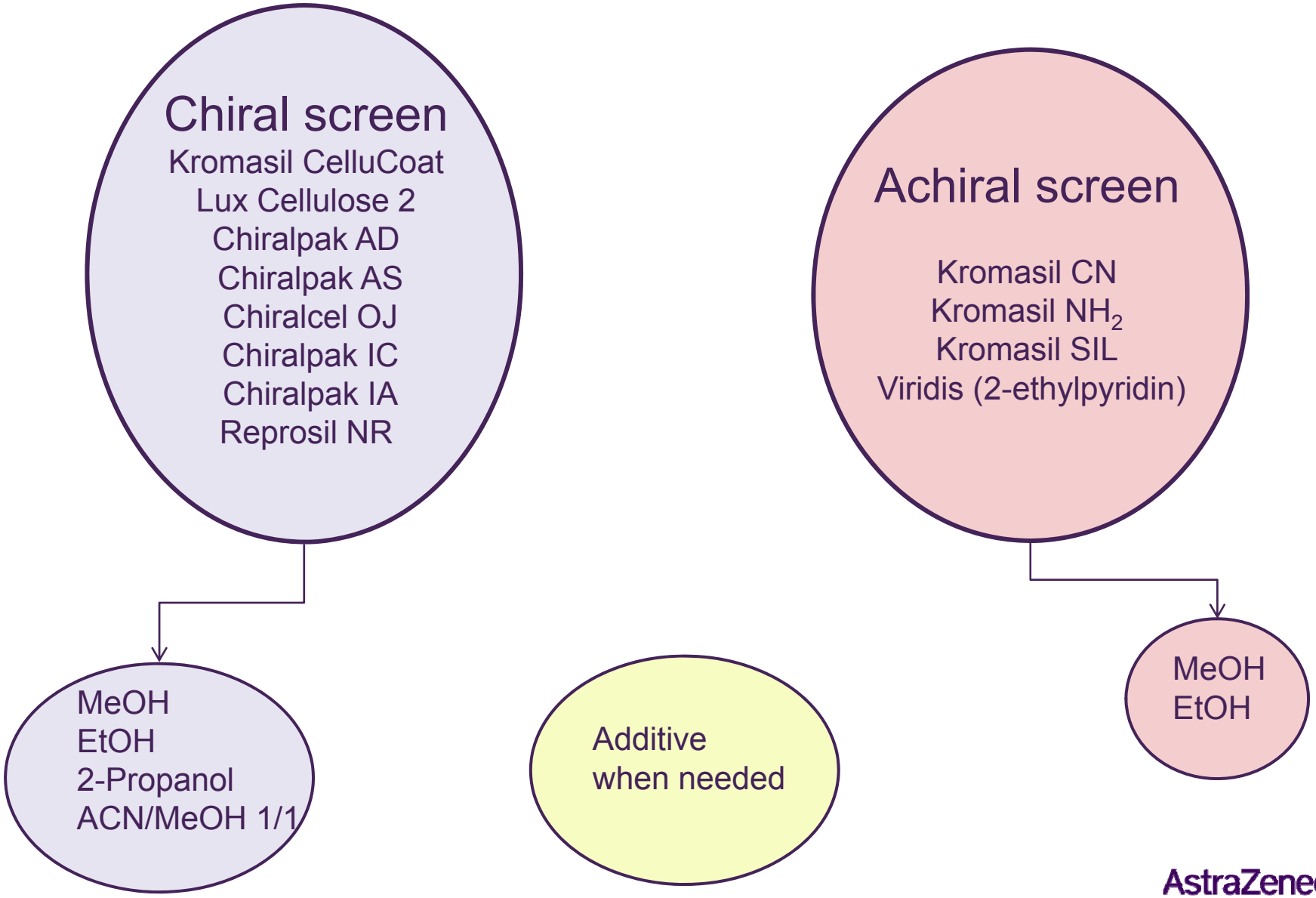
Screening strategy



Screening strategy



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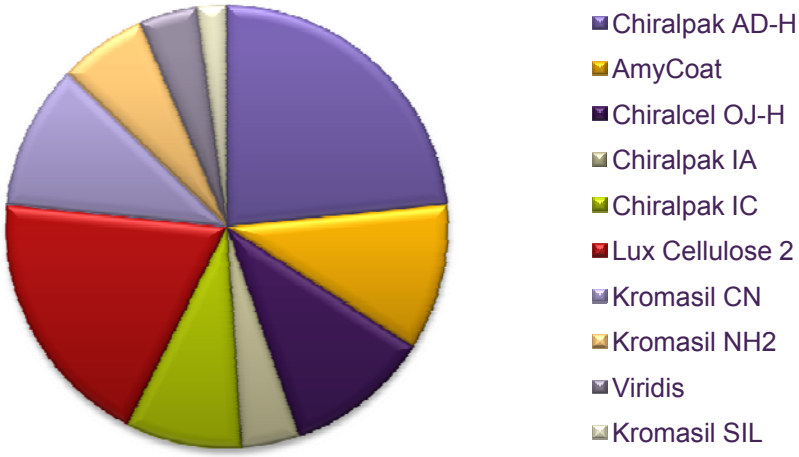
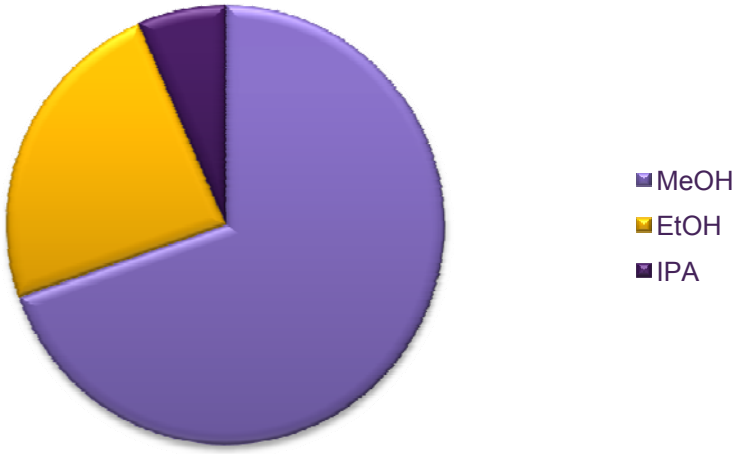
Usage of stationary phases in SFC mode

Columns used



Modifier used



Columns and modifiers used for the last 100 separations

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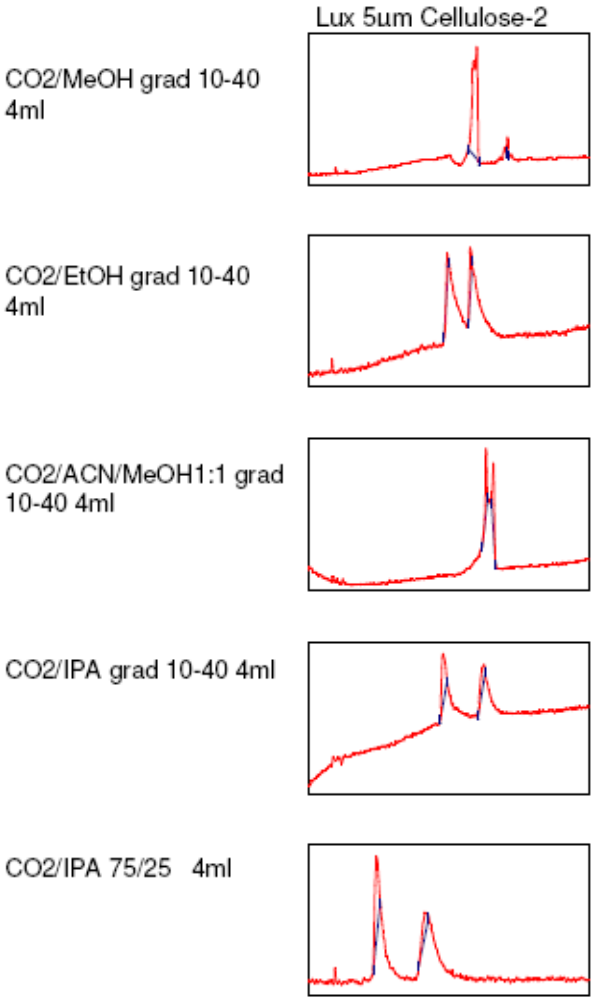
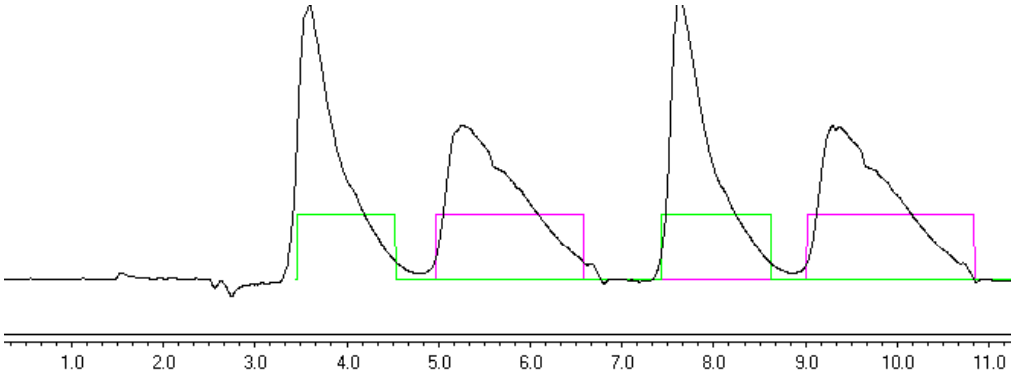
Sepmatix SFC screen - Example





Preparative SFC:

Column: Lux Cellulose-2, 250 x 30 mm, 5 µm
Mobile phase: 25% IPA in CO_2, 110 ml/min,150 bar, 40 °C

Sample: 100 mg/ml in IPA, Injected amount: 350 mg/inj
Total amount: 2 g, Cycle time: 4 min

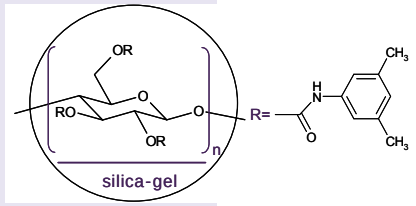
Purity: 99.5 % de, yield: 93 %

Total time for prep: 45 min
Total Cycle time: 2 days

Different interaction mechanism in LC and SFC mode

Outline

- Chiralcel OD-H and Kromasil CelluCoat, same chiral selector
- Acidic, basic and neutral compounds
- Metoprolol and structural analogues
- Heptane/2-propanol or Heptane/EtOH mixtures in LC mode
- MeOH, EtOH, ACN/MeOH 1/1 and 2-propanol in SFC mode



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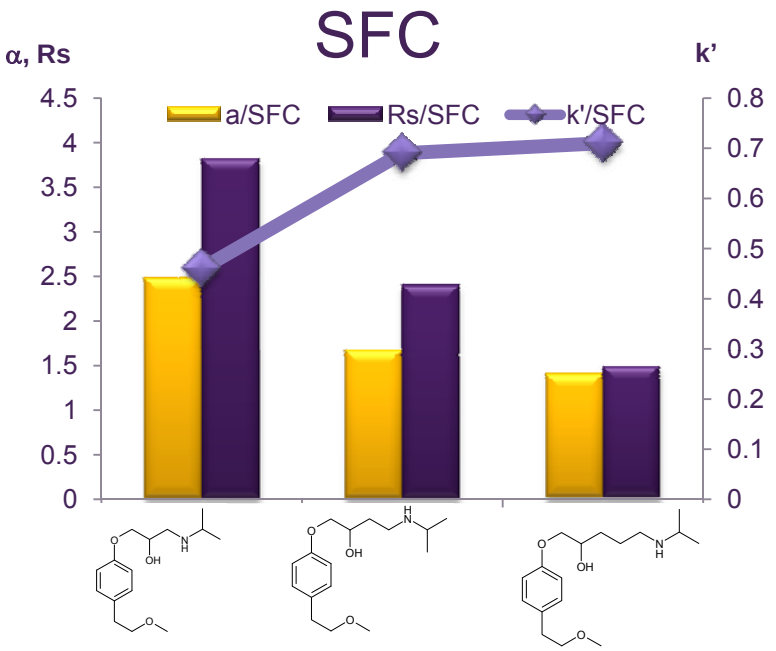
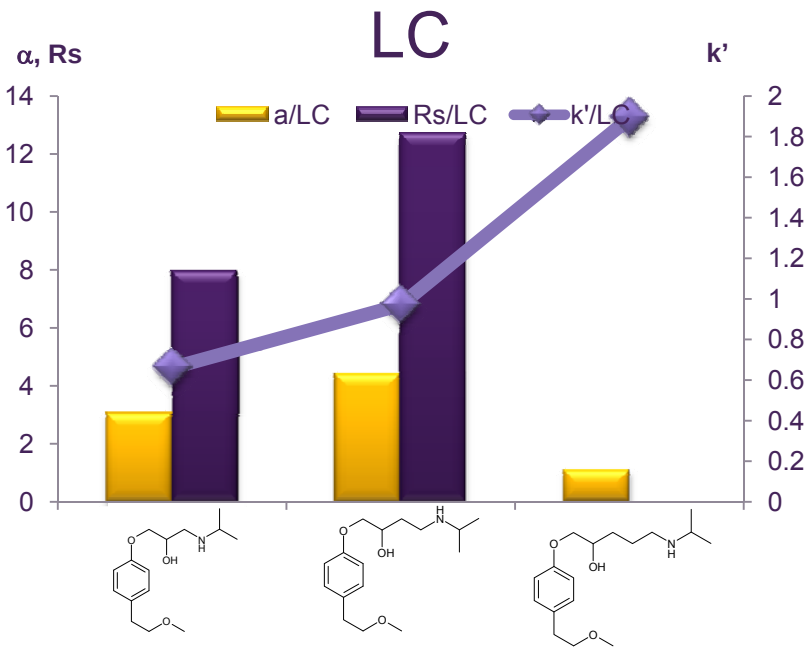
Interactions

Metoprolol and structural analogues



Chiralcel OD-H 250 x 4.6 mm, 20% MeOH/DEA 100/0.5 in CO_2, 4 ml/min, 100 bar



Chiralcel OD-H 250 x 4.6mm, Heptane/IPA/DEA 80/20/0.1, 1 ml/min

Increased chain length between the chiral centre and the – N-H group
↑ Binding (k´)
↓ Enantioselectivity (α)
⇒ increased degree of achiral interaction between the enantiomers and the CSP ⇒ lower α

Increased chain length between the chiral centre and the – N-H group
↑ Binding (k´)
Enantioselectivity (α) maximal when -$(CH_2)_2$ between –NH and –OH at the chiral centre

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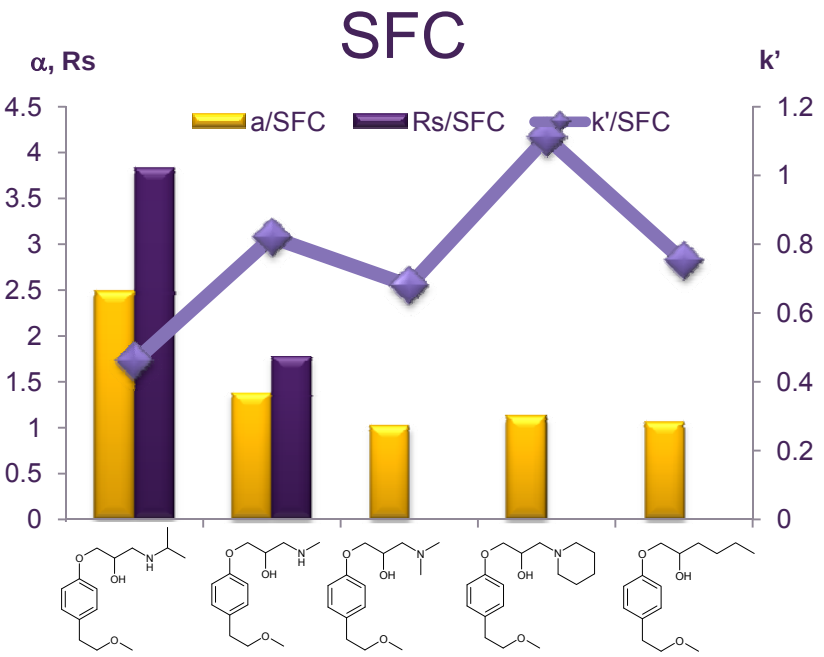
H. Nelander, S. Andersson, K. Öhlén, Evaluation of four commercially available cellulose (3,5-dimethyl-phenylcarbamate) chiral stationary phases by parallel HPLC and SFC, to be submitted.

Interactions

Metoprolol and structural analogues



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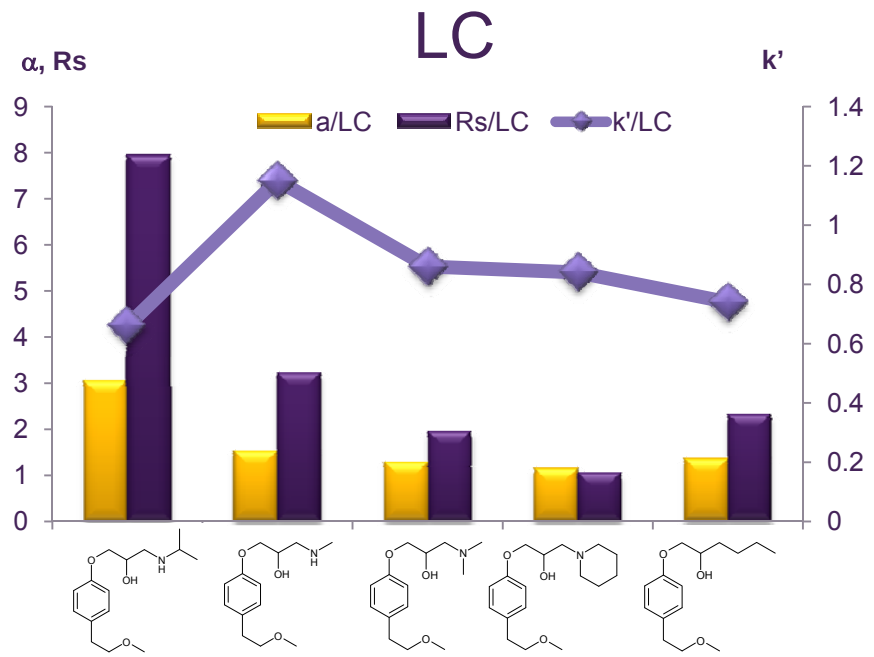

Chiralcel OD-H 250 x 4.6mm, Heptane/IPA/DEA 80/20/0.1, 1 ml/min

In SFC the ability of the CSP to interact with the amino function is much more crucial than in LC
Hydrogen bond (R_2N-H ···CSP) a key interaction for enantioselectivity in SFC mode

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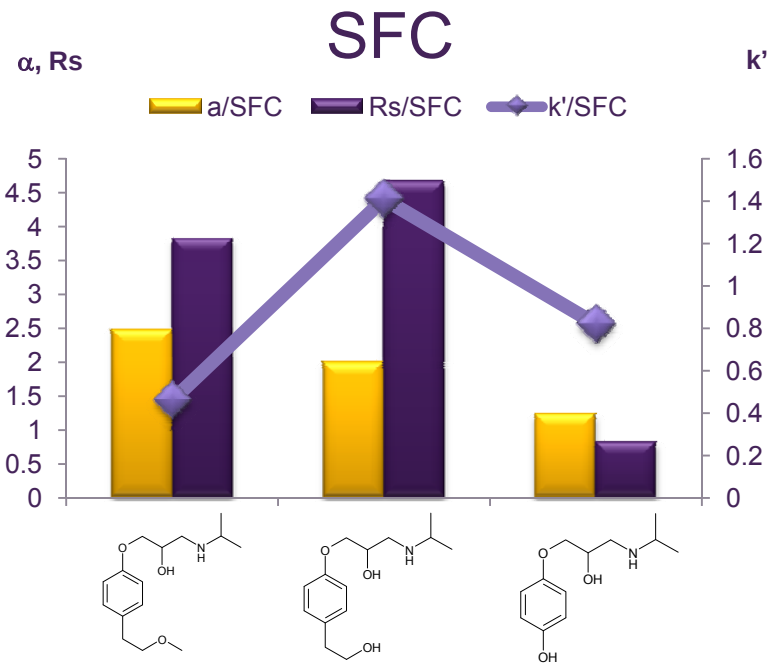
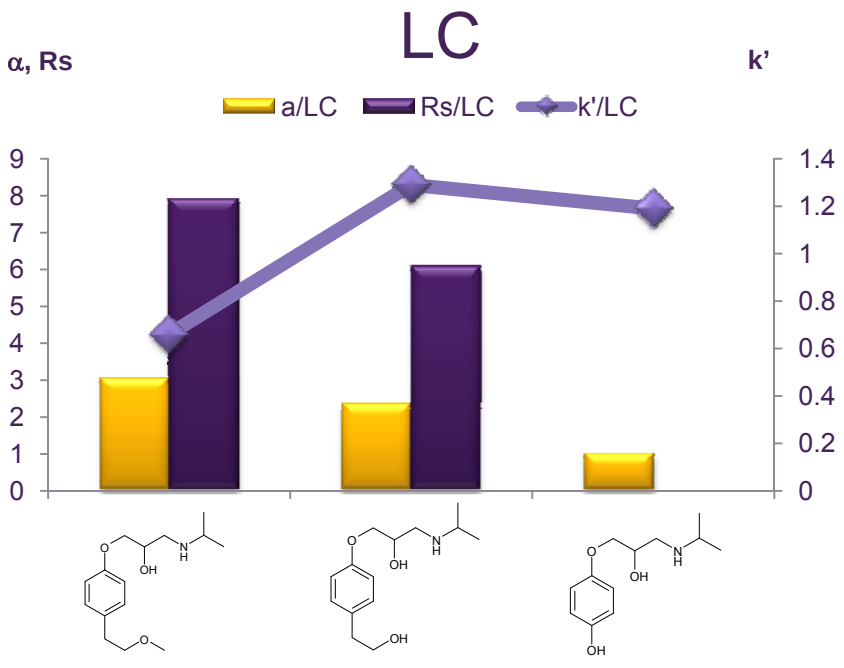
Interactions

Metoprolol and structural analogues



Chiralcel OD-H 250 x 4.6 mm, 20% MeOH/DEA 100/0.5 in CO_2, 4 ml/min, 100 bar



Chiralcel OD-H 250 x 4.6mm, Heptane/IPA/DEA 80/20/0.1, 1 ml/min

The electron density of the aromatic moiety is crucial for chiral discrimination to occur

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Interactions

Metoprolol and structural analogues

	SFC	LC
Optimum length between –OH on the chiral centre and the – N-H group for enantioselectivity	CH_2	$(CH_2)_2$
Hydrogen bonding R2N-H ▪▪▪CSP	Key interaction	Critical for enantioselectivity to occur
Electron density of the aromatic ring	Critical for enantioselectivity to occur	Key interaction

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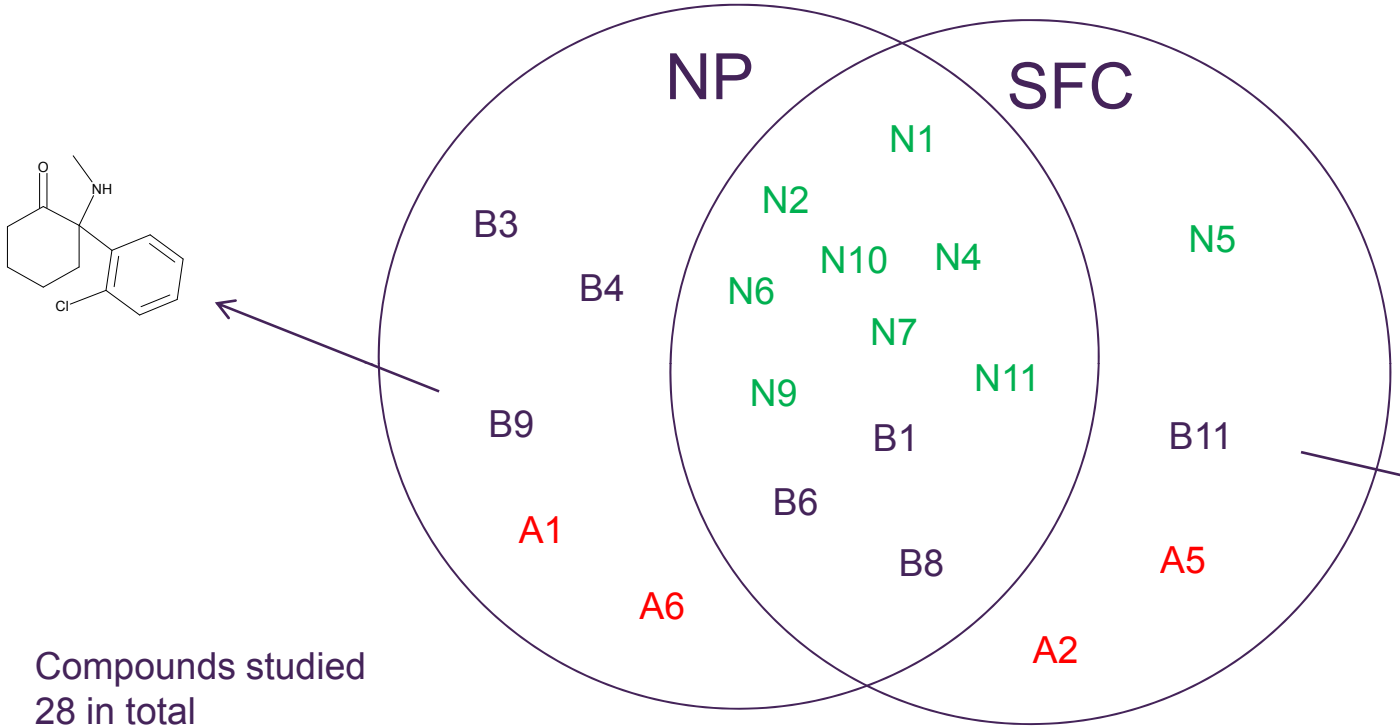
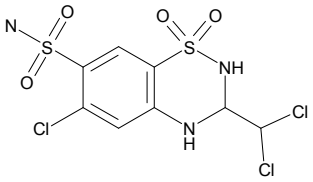
Interactions- binding & selectivity





Compounds studied
28 in total
A = Acid (6)
B = Base (11)
N = Neutral (11)

Number of compounds resolved (α > 1.2) on Kromasil CelluCoat in NP and SFC mode with any of the used mobile phases.

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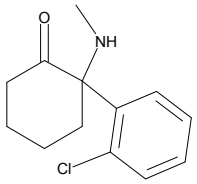
H. Nelander, S. Andersson, K. Öhlén, Evaluation of four commercially available cellulose (3,5-dimethyl-phenylcarbamate) chiral stationary phases by parallel HPLC and SFC, to be submitted.

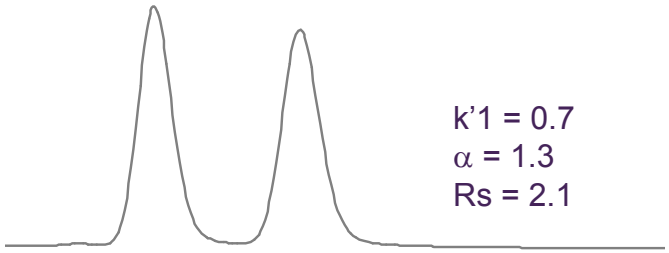
Interactions- binding & selectivity

LC

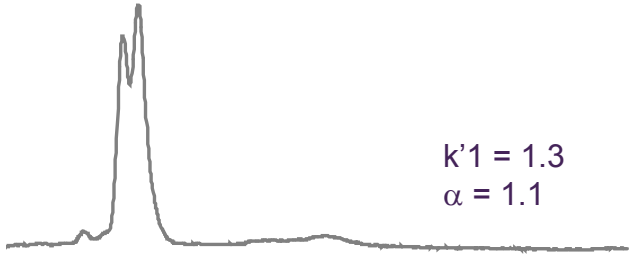
SFC



Ketamin



k'1 = 0.7
α = 1.3
Rs = 2.1



k'1 = 1.3
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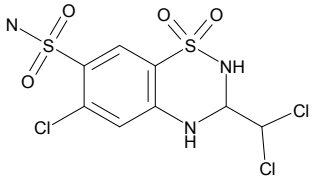

Tri-chlor metiazide

Not eluted after 45 min

k'1 = 2.7
α = 1.2
Rs = 0.7

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Can we use this to obtain purified compounds efficiently?

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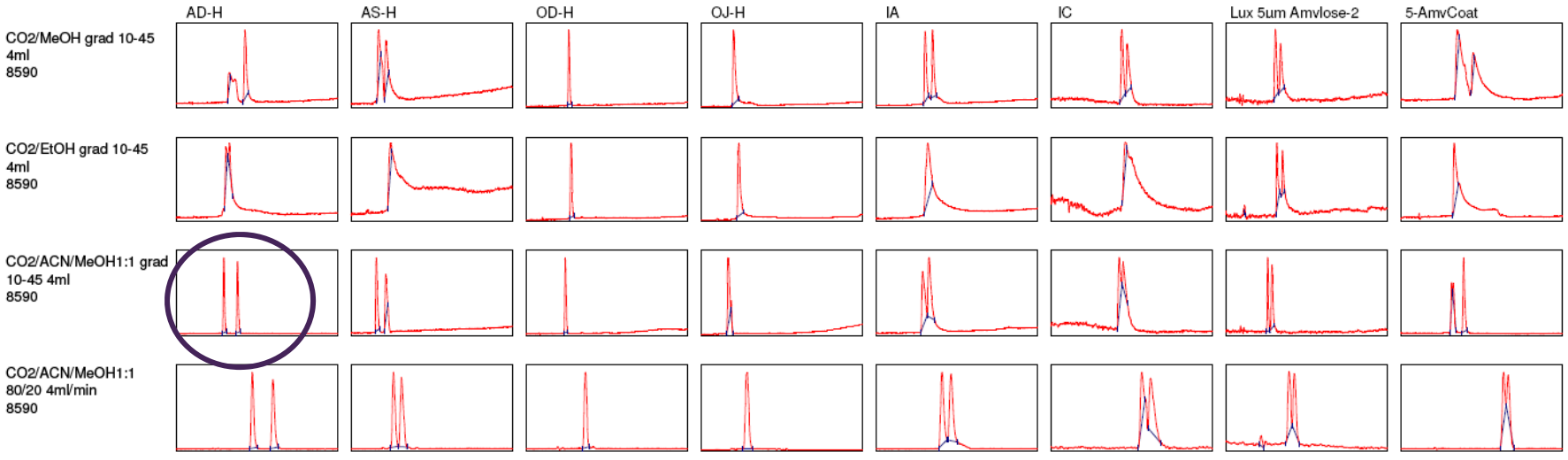
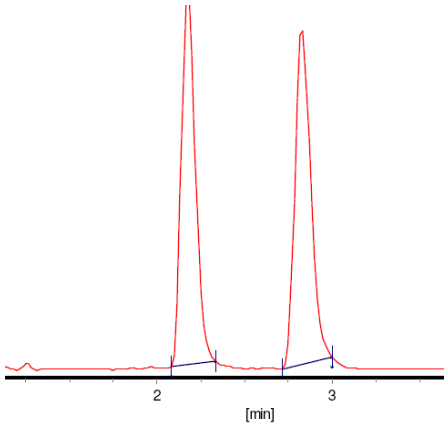
AZ13368590 (amide) – SFC Screen





Chiralpak AD-H 250 x 4.6 mm
20 % ACN/MeOH 1/1 in CO_2, 150 bar, 4 ml/min

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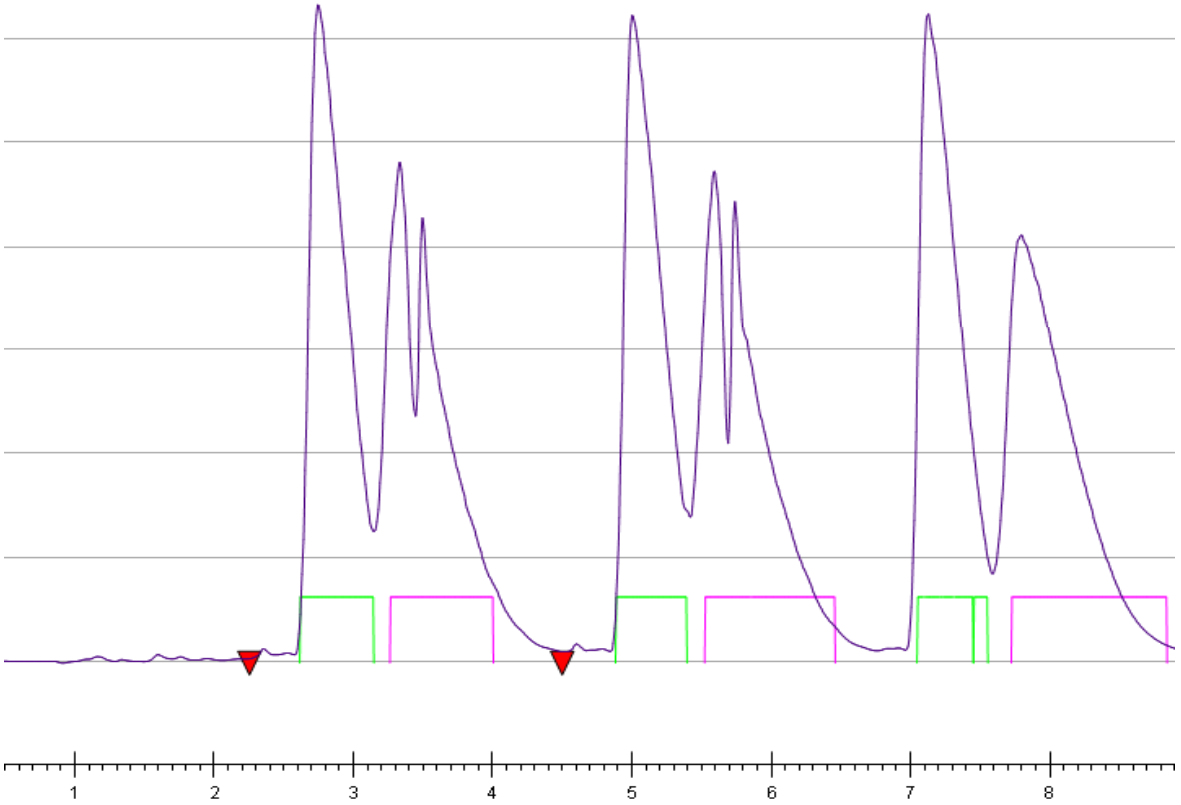
AZ13368590 – SFC Prep



Chiralpak AD-H 250 x 30 mm 10% MeOH/ACN 1/1 in CO2, 150 bar, 80 g/min
100 mg/ml MeOH/ACN 1/1, **inj 8 mg**, tot 5.5 g, **2.3 min** cycle time

690 injections

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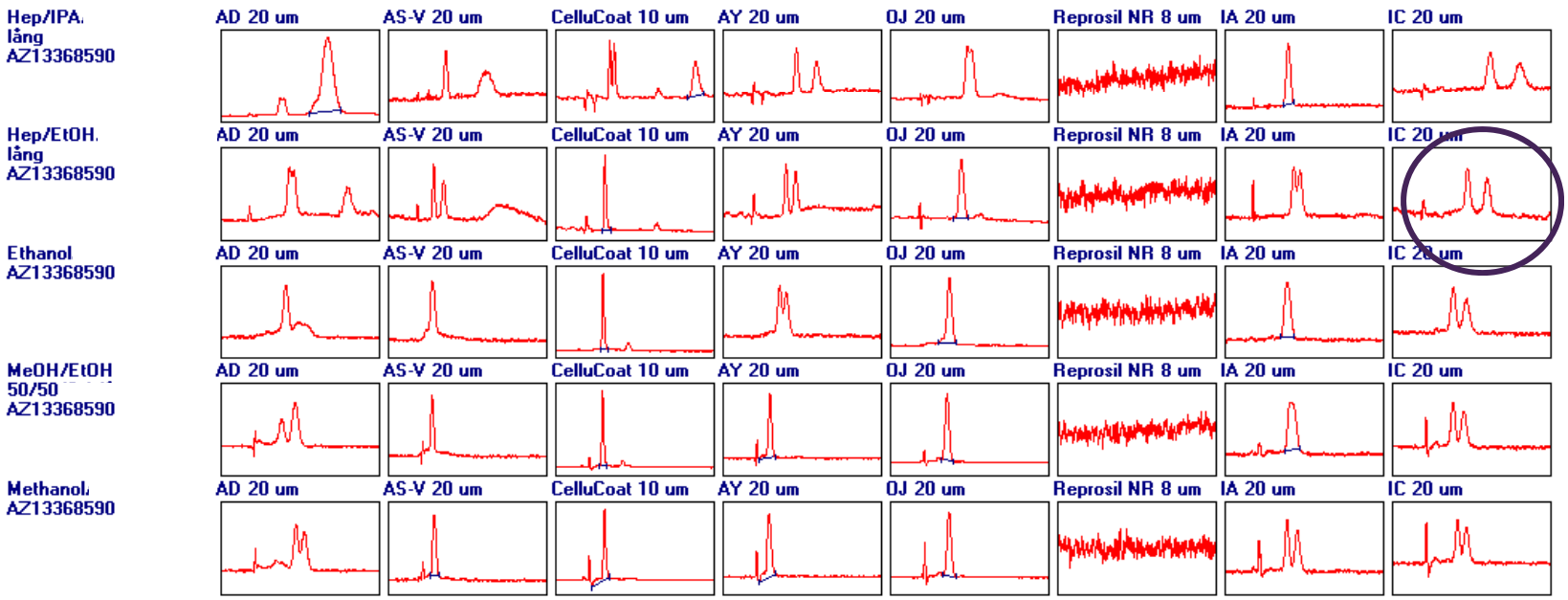
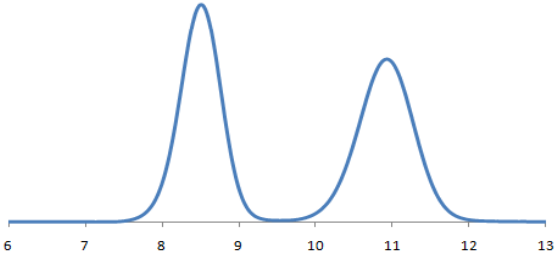
LC - a better option?





Chiralpak IC 250 x 4.6 mm
Heptane/EtOH 80/20, 1 ml/min

Analytical separation identified from Sepmatix LC screen within 2 hours

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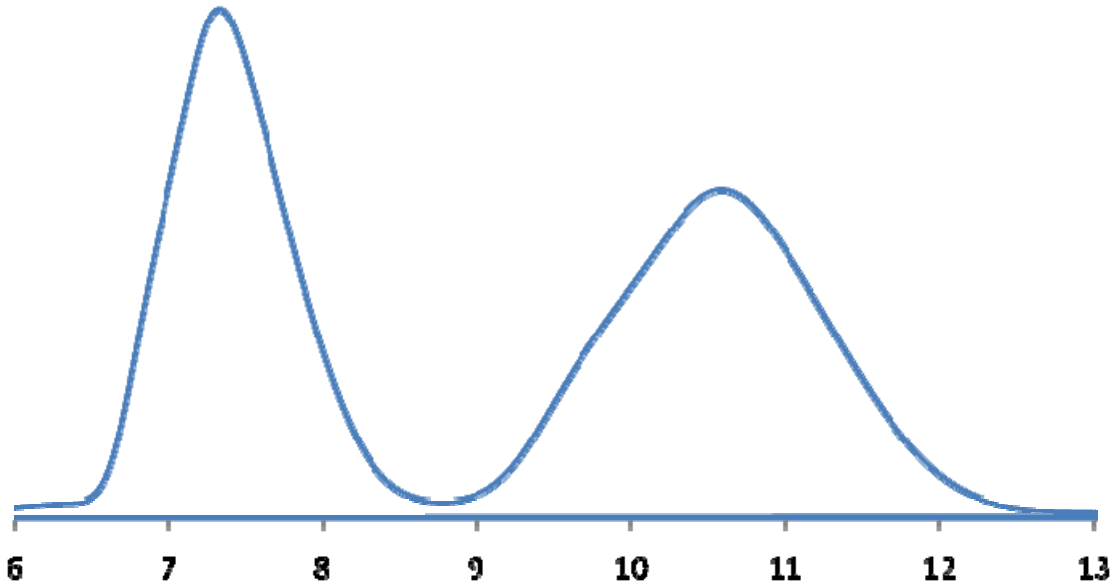
LC is a better option!



LC Prep:
Chiralpak IC 250 x 50 mm Heptane/EtOH 80/20, 120 ml/min
200 mg/ml Heptane/EtOH 1/1, **inj 1.2 g**, tot 5.5 g,
6 min cycle time

5 injections

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Throughput

Preparative SFC

Chiralpak AD-H 250 x 30 mm

Injected amount: 8 mg/inj
Cycle time: 2.3 min
Throughput: 0.04 kg/kg/day
MeOH/ACN: 2.3 L/g

Preparative LC

Chiralpak IC 250 x 50 mm

Injected amount: 1200 mg/inj
Cycle time: 6 min
Throughput: 0.9 kg/kg/day
Hep/EtOH: 0.6 L/g

Yield: 86 %
Purity: > 99.5 % ee

22 times higher throughput and 25 % solvent comsumption!!

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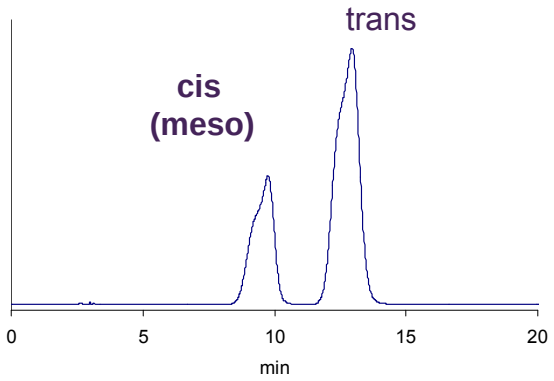
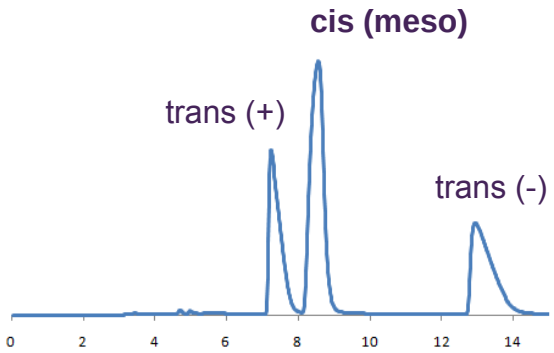
SFC - a better option?



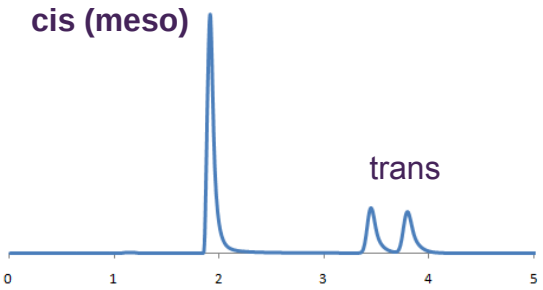
RP – 2 g separated before - now request for 300 g!

Column: XBridge, 250 x 4.6 mm, 10 µm
Mobile phase: 30% MeOH in water, 0.1% TEA
1 ml/min, RT



LC

Column: Chiralpak AD-H, 250 x 4.6 mm
Mobile phase: Heptane/IPA 70/30, 1 ml/min



SFC

Column: Chiralpak AD-H, 250 x 4.6 mm
Mobile phase: 20% MeOH in CO_2, 4 ml/min 120 bar, 40 °C

k'1 = 0.82, α = 2.8, Rs = 10.5

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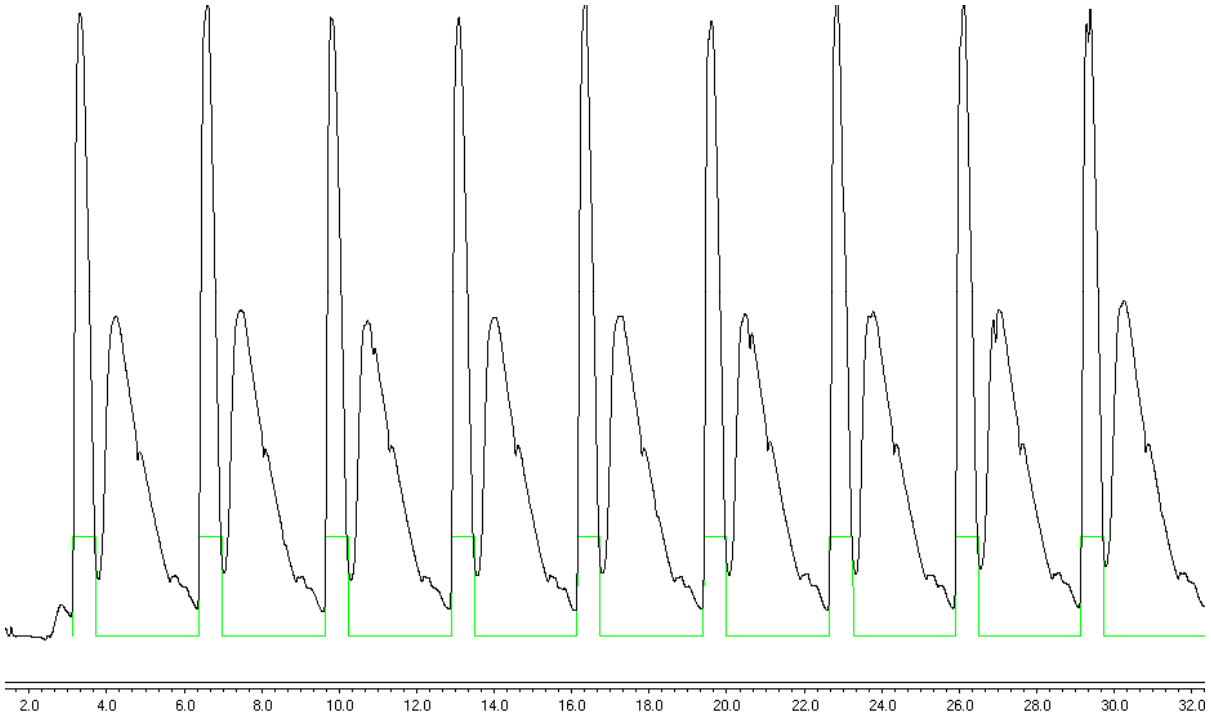
SFC is a better option!



Chiralpak AD-H 250 x 30 mm, 20% MeOH in CO2, 120 bar, 130 g/min
264 mg/ml MeOH, **inj 800 mg**, tot 300 g, **3 min** cycle time

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Throughput

Preparative RP method

XBridge, 250 x 50 mm

Injected amount: 1 g/inj
Cycle time: 14 min
Throughput: 0.3 kg/kg/day
MeOH: 0.5 L/g
H_2O: 1.2 L/g

Yield: 93 %
Purity: 99.2 % de

Preparative SFC method

Chiralpak AD-H, 250 x 30 mm

Injected amount: 0.8 g/inj
Cycle time: 3 min
Throughput: 3.3 kg/kg/day
MeOH: 0.1 L/g

Yield: 85 %
Purity: 98.3 % de

11 times higher throughput and 20 % MeOH comsumption!!

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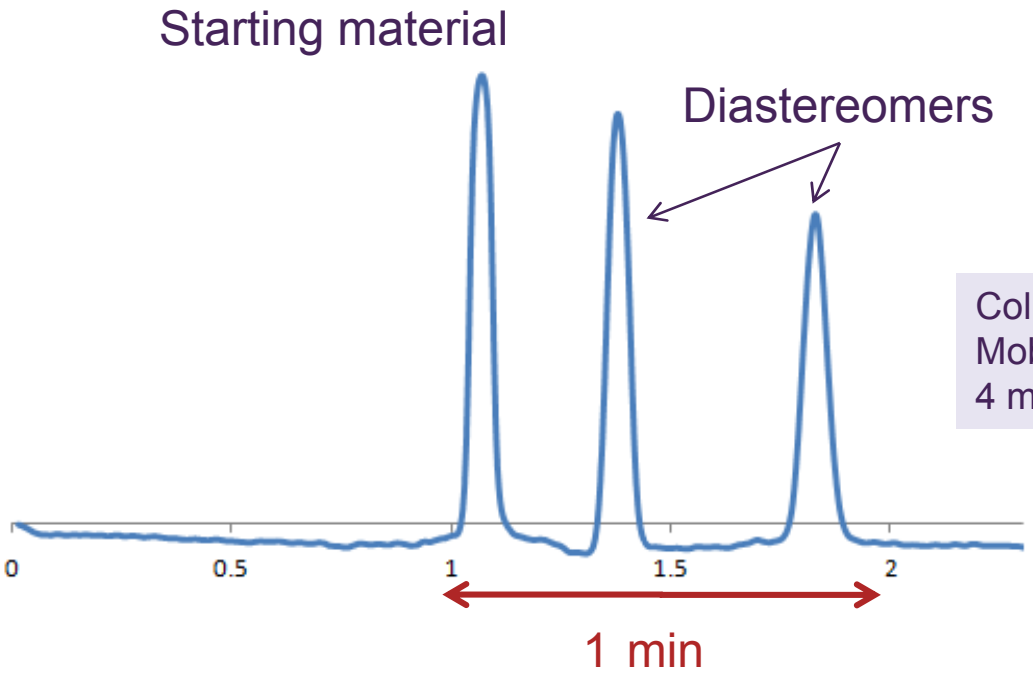
Separation of diastereomers and starting material



Column: Viridis 2-ethylpyridin 250 x 4.6 mm, 5 µm
Mobile phase: 20% MeOH/TEA 100/0.5 in CO_2,
4 ml/min, 120 bar, 40 °C

No good method on any CSP in either SFC or NP mode

The diastereomers can be separated on XBridge

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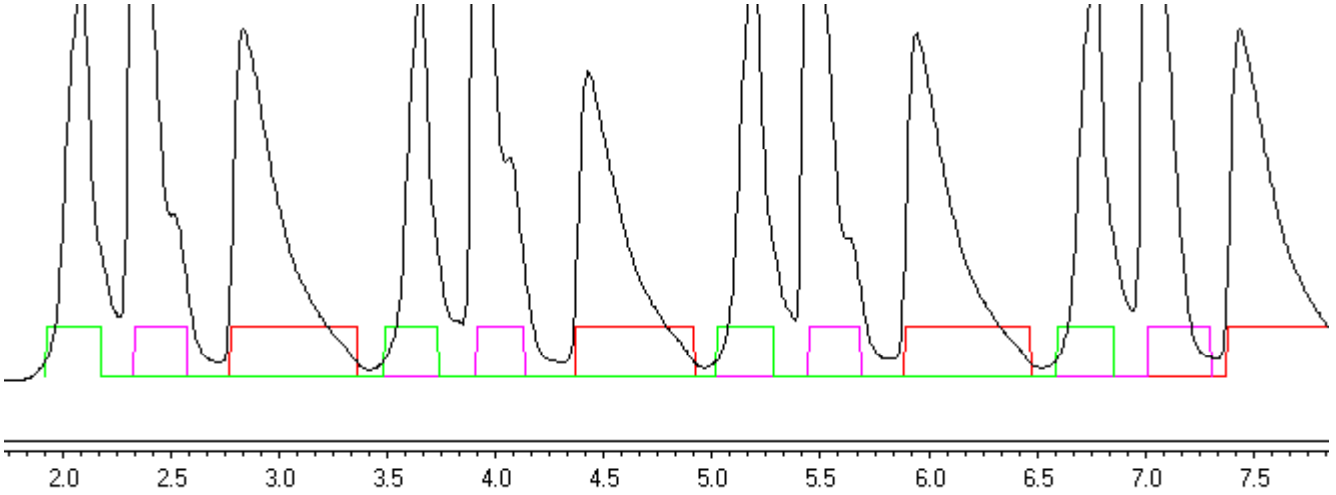
Separation of diastereomers and starting material



Viridis 2-ethylpyridin 250 x 30 mm 20% MeOH/TEA 100/0.5 in CO2, 120 bar, 130 g/min, 200 mg/ml MeOH/DMSO 1/1

Injected amount: 150 mg
Total amount: 4 g, **Cycle time: 1.4 min**

Total time for prep: 60 min

Yield: 80 %
Purity > 99 % de for both isomers

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Conclusion

- Parallel method development is a powerful time saving tool
- Different interaction mechanism and loadablity in SFC and LC can be used to obtain purified compounds in a time and cost efficient manner

SFC is often the fastest technique, consuming less solvent

BUT

In order to have quick turn around times in the drug discovery process you need both LC and SFC and you need to use them for the right compounds!

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Thanks to

Andreas Granfeldt
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Johanna Malm
Göran Nilsson
Shalini Andersson

And to you for your attention!

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