VRIJE UNIVERSITEIT BRUSSEL

LIMITS OF SEPARATION PERFORMANCE IN SFC

SHIMADZU SFC USER MEETING

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CHEMICAL ENGINEERING DEPARTMENT

ADVANTAGES OF SFC

- Supercritical Fluid Chromatography/SFC (incl. sub-critical conditions) employs a low viscosity mobile phase that contains mainly CO_2
  - ➔ Viscosity η between 0.1-0.25 cP for 10-50% MeOH at 30°C and 150 bar (vs. 0.8 cP for water at 30°C) (1 cP = 1mPa·s)
  - ➔ Fast separations possible: high $D_{mol} \sim 1/\eta$
    - Optimal velocity $u_{opt} \sim D_{mol}$
    - Fast mass transfer: C-term $\sim 1/D_{mol}$
  - ➔ Low column pressure drop since $\Delta P \sim \eta$ (but also $\sim u_{opt}$)
- "Green" technique (low waste/solvent use), unique selectivity and orthogonality, chiral separations, advantages for scale up to prep…

ADVANTAGES OF SFC

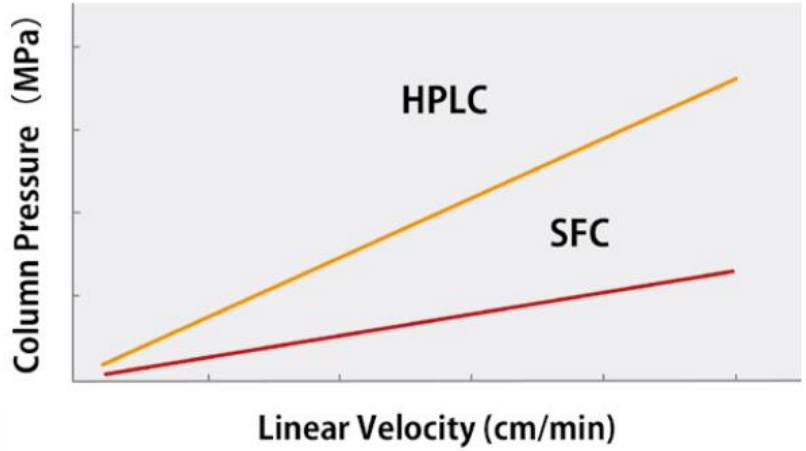
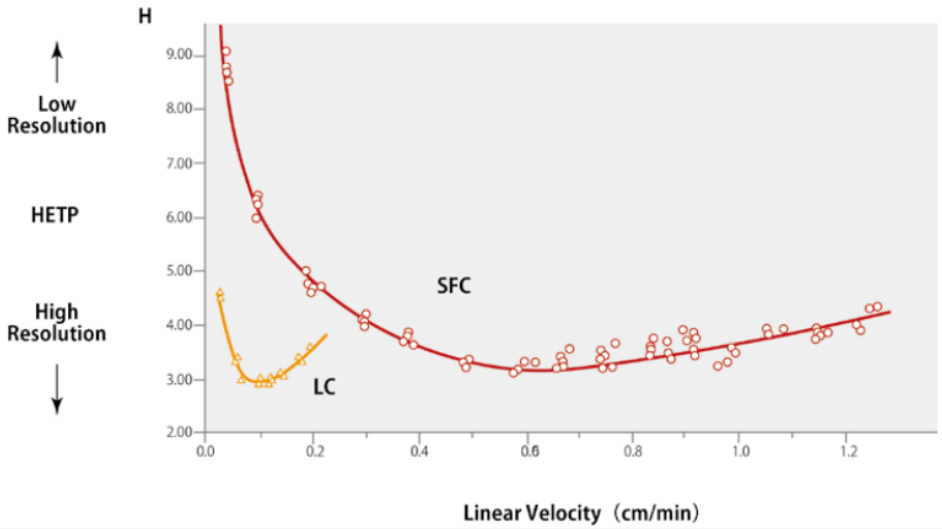


Fig. 8 Linear Velocity vs. Column Back Pressure



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Excellence in Science

Supercritical Fluid Chromatograph

SFC Basic Guide

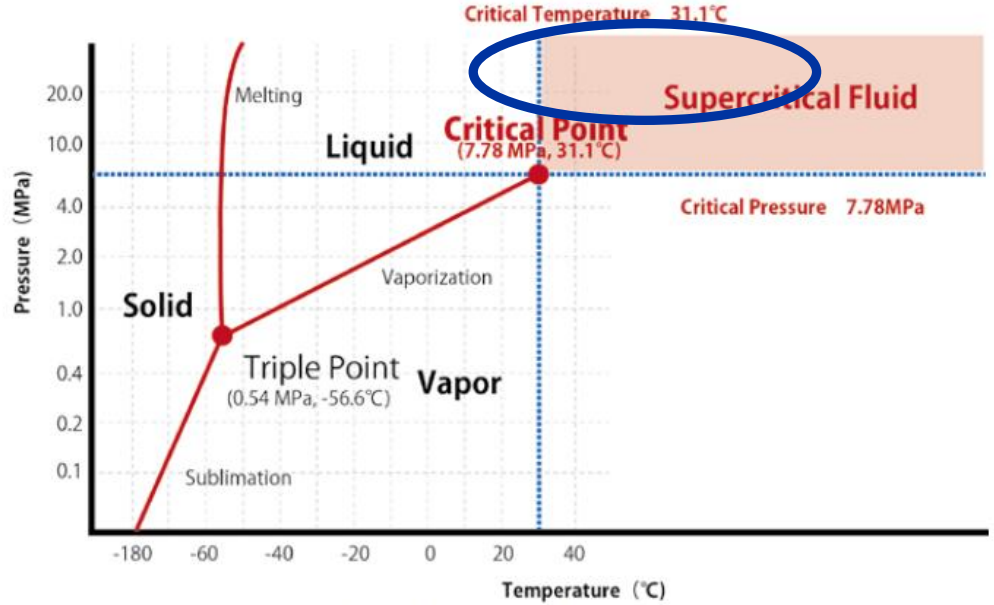


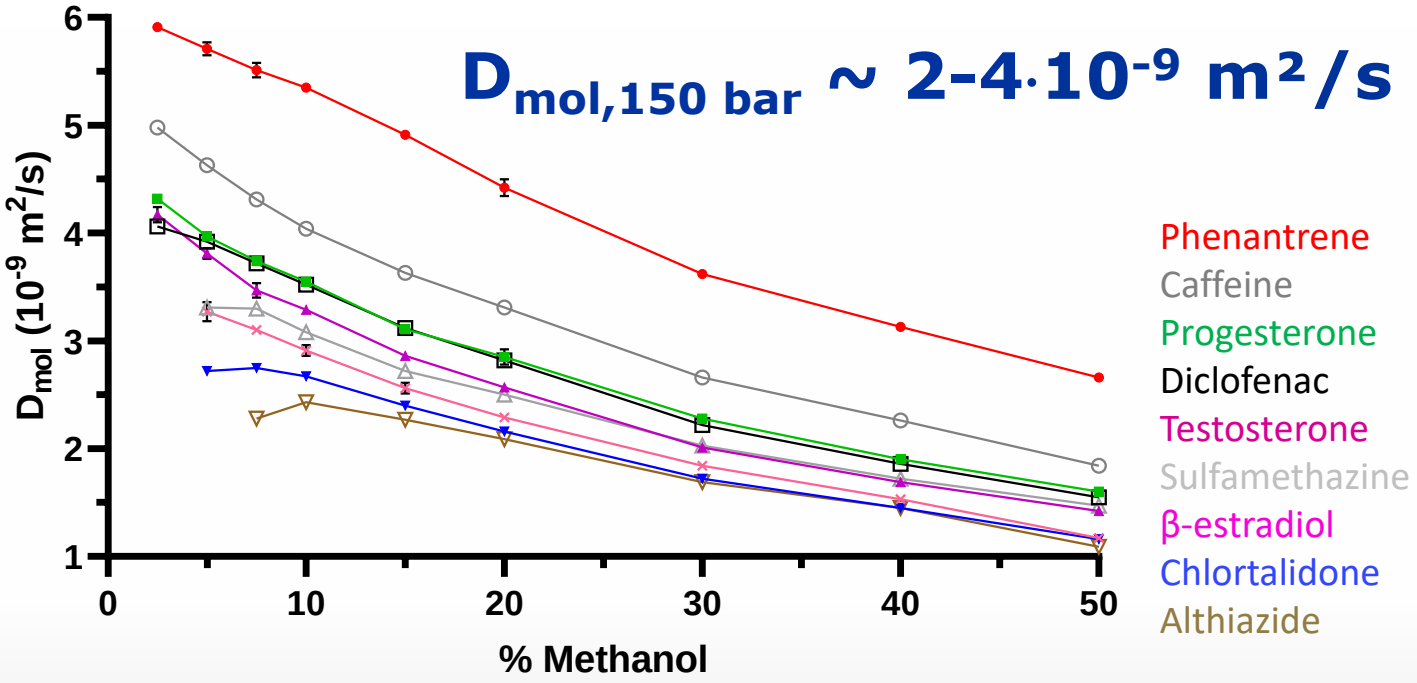
Fig. 1 Phase Diagram for Carbon Dioxide

Table 1 Physical Properties of Supercritical Fluids

	Diffusion Coefficient (m^2/s)	Density (g/cm^3)	Viscosity ($g/cm \cdot s$)
Liquids	10^{-6}	1	10^{-2}
Supercritical Fluids	10^{-3}	0.2-0.8	10^{-3}
Gases	10^{-1}	10^{-3}	10^{-4}

ADVANTAGES OF SFC

- Diffusion coefficients in SFC claimed to be very high (10^{-7}-10^{-8} m²/s) relative to LC (10^{-9} m²/s)
- Range of D_{mol} based on small neutral molecules in pure CO_2 at low backpressure (< 100 bar)
- Addition of minor amounts of co-solvent strongly decrease D_{mol}



T. Januarius et al., J. Chromatogr. A (2022) 463485

ADVANTAGES OF SFC

- Density ρ of SFC mobile phases similar or higher than that of modifier
- Density of neat CO_2 exceeds that of methanol above 160 bar

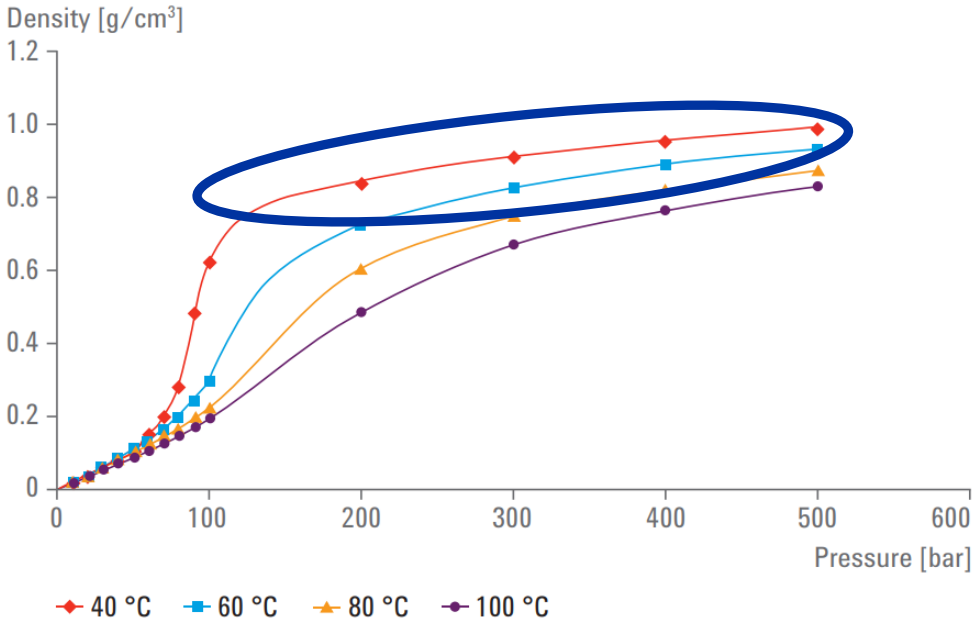
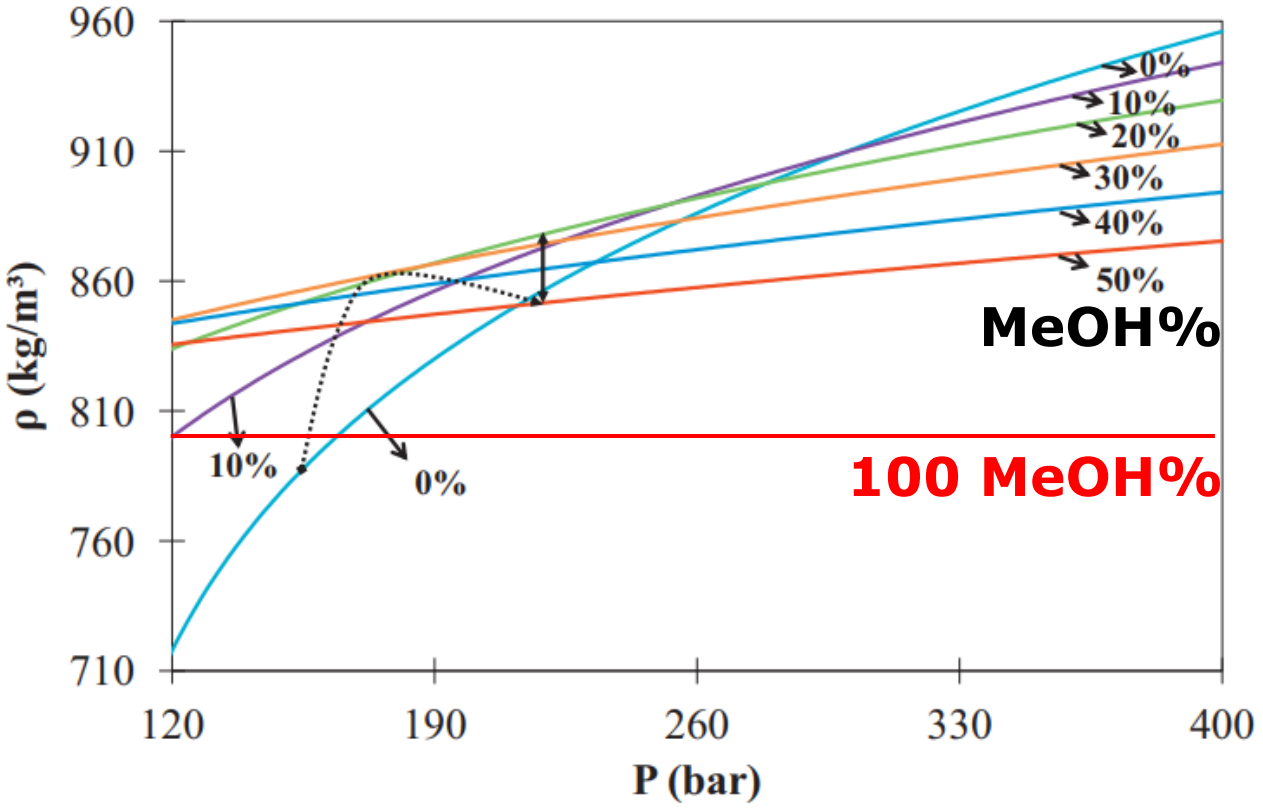


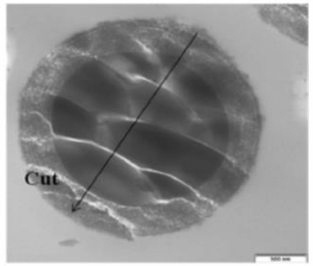
Figure 2.1 Density of pure CO_2 as a function of pressure at four different temperatures. From top to bottom: 40, 60, 80 and 100 °C.

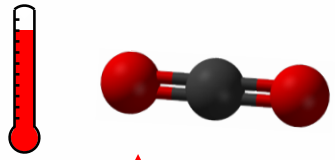
at 40°C



LIMITS OF SEPARATION POWER IN CHROMATOGRAPHY

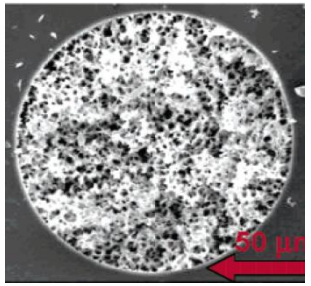
- Time-efficiency trade off is given by the Knox-Saleem equation for a fully optimized system (flow rate, particle size and column length)
- "Money-equation":
  - higher separation efficiency N is valuable/expensive
  - time t = money

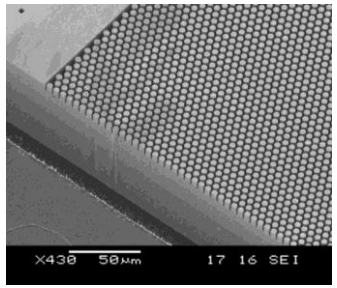


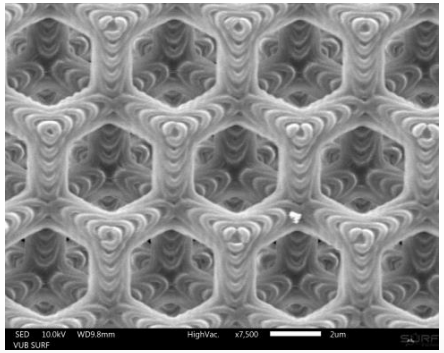


$$t_0 = \frac{h_{min}^{\ 2} \cdot \eta \cdot \phi}{\Delta P} \cdot N^2$$

HPLC → UHPLC







LIMITS OF SEPARATION POWER: LC VS. SFC

- SFC: same columns as in LC (h_{min} and ϕ)
- Viscosity is 3-8x lower in SFC than LC
- What about pressure drop?

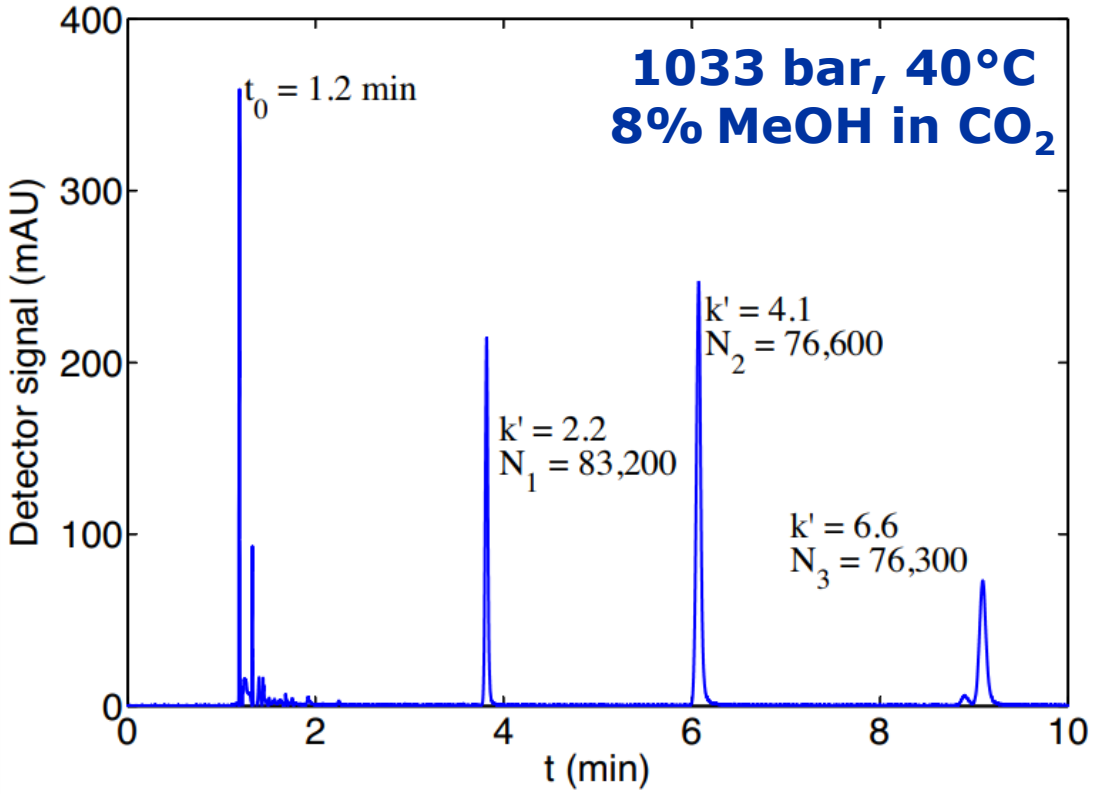
$$t_0 = \frac{h_{min}^2 \cdot \eta \cdot \phi}{\Delta P} \cdot N^2$$

Mode	P_{max} (bar)	F (ml/min)
LC	1300-1500	2-5
SFC	413-660	3-5

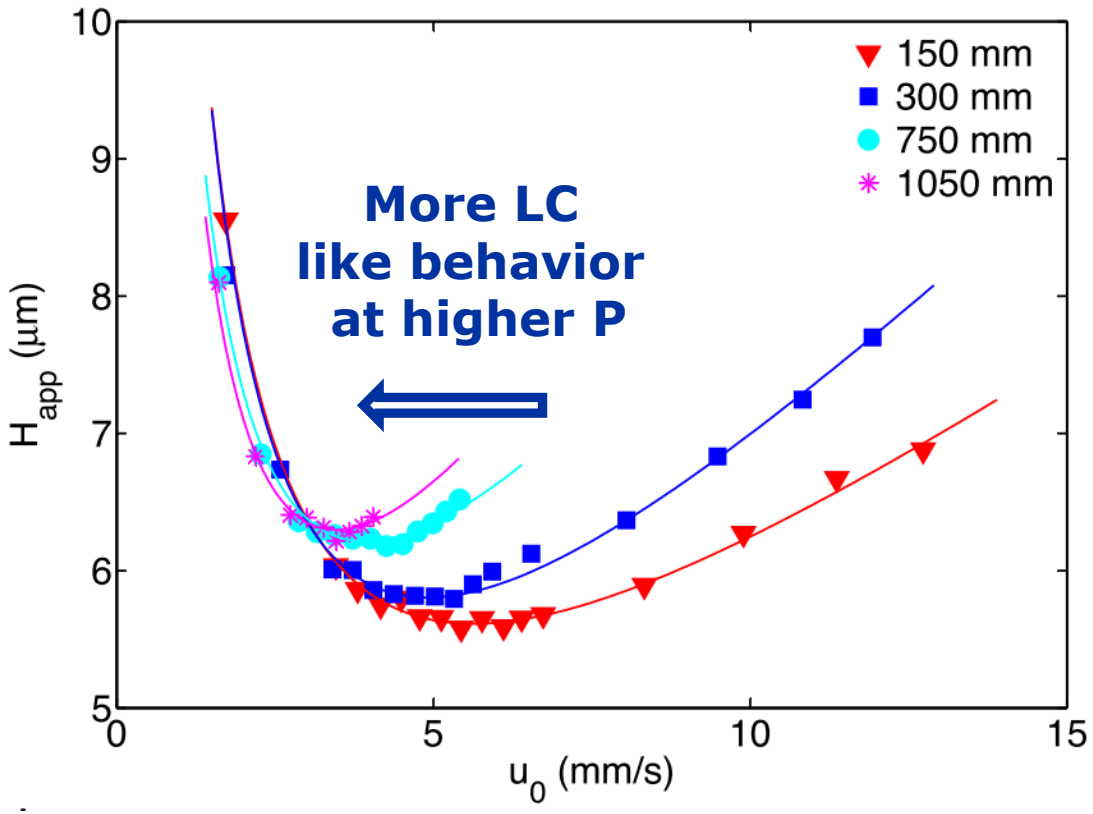
- SFC allows fast separations at low pressure drops, but has similar kinetic performance limits than UHPLC

LIMITS OF SEPARATION POWER FOR SFC

- Is there a fundamental limitation on operating pressure in SFC?



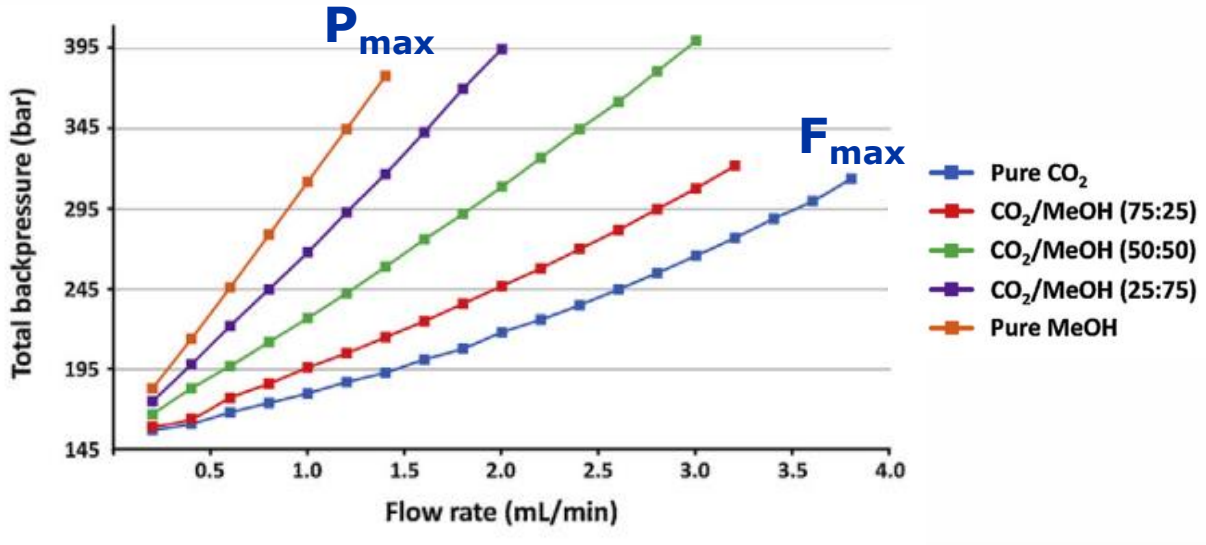
50cm, 2.1mm ID, 1.8µm at 1.3mL/min



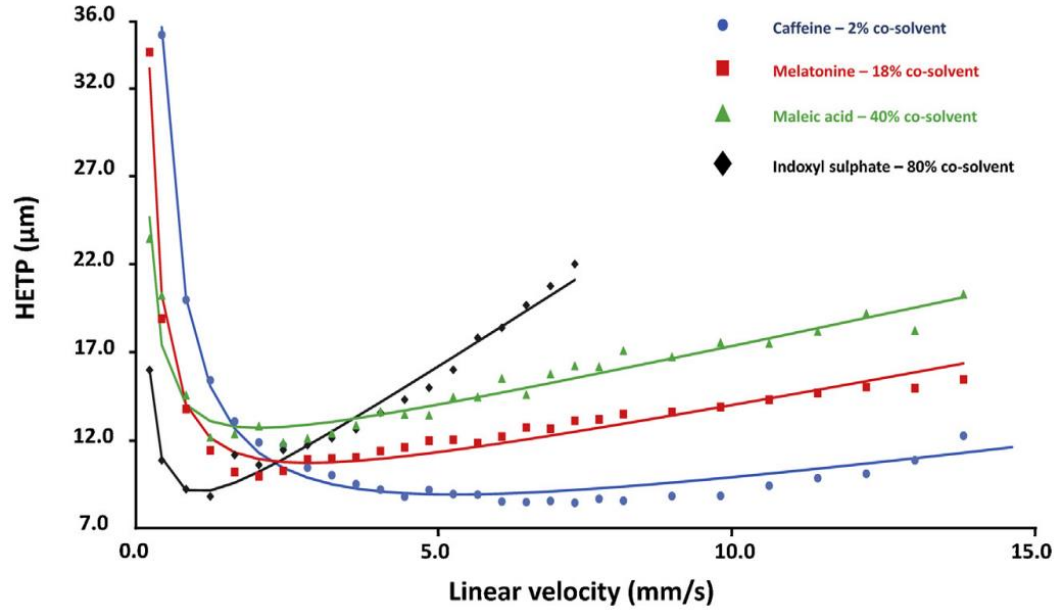
2.7µm poroshell, 3mm ID

LIMITS OF SEPARATION POWER FOR SFC

- Bamba and co-workers in 2014 proposed wide elution gradients: SFC => EFLC => HPLC, i.e. from CO_2 to MeOH
- Elution of a wide range of analytes: least to most polar
- Referred to as "unified chromatography" (UC)



Nucleoshell HILIC 2.7µm, 100 × 3.0 mm column



K. Taguchi et al., J. Chromatogr. A 1362 (2014) 270–277
V. Desfontaine et al., J. Chromatogr. A 1562 (2018) 96–107

LIMITS OF SEPARATION POWER FOR SFC

- West and co-workers applied combined flow rate and backpressure gradients:

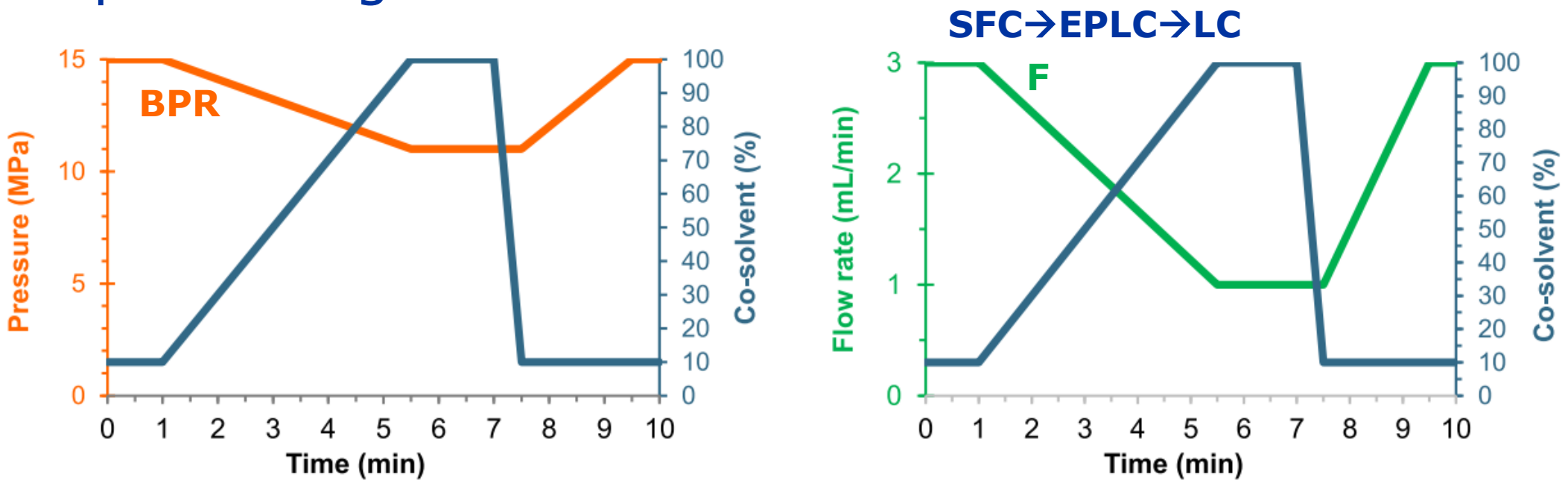


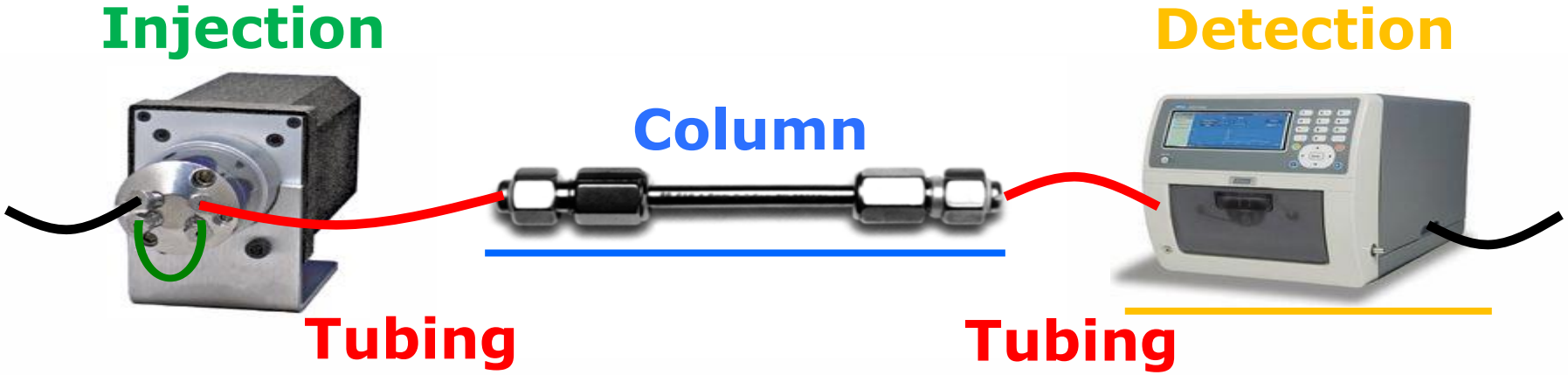
Fig. 1. Gradient profile in the optimized method showing the variation of co-solvent (blue lines), back-pressure (orange lines) and flow rate (green lines).

Chiralpak ZWIX(+), 150 × 3.0 mm, 3 µm

A. Raimbault et. al., J. Chromatogr. A 1616 (2020) 460772

EXTRA-COLUMN BAND BROADENING

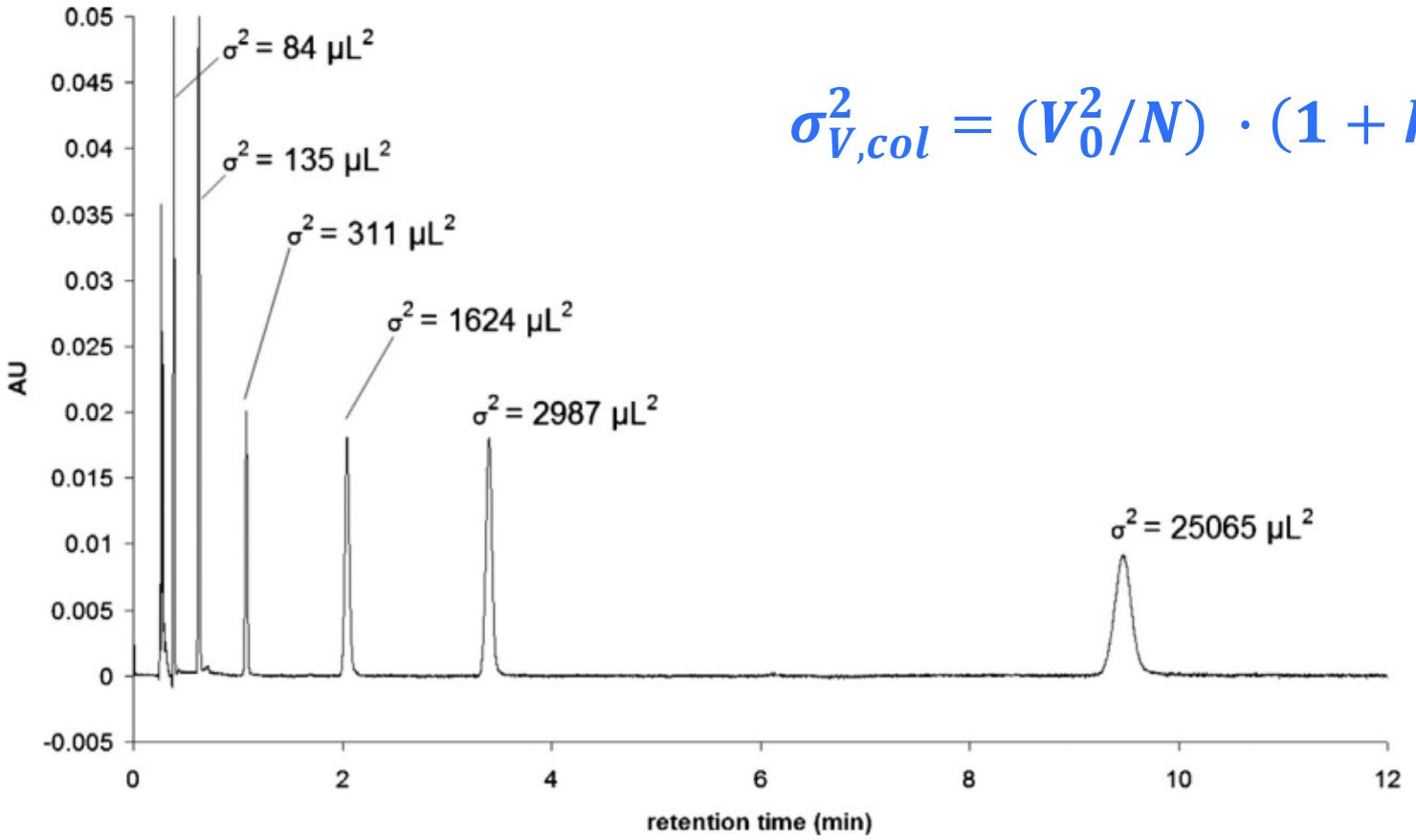
- Separation performance not only determined by mobile phase viscosity, columns and pump pressure
- Instrumental contributions to dispersion and pressure drop:



$$\sigma_{V,tot}{}^2 = \sigma_{V,inj}{}^2 + \sigma_{V,col}{}^2 + \sigma_{V,tub}{}^2 + \sigma_{V,det}{}^2$$

$$\sigma_{V,col}^2 = (V_0^2/N) \cdot (1 + k_e)^2$$

EXTRA-COLUMN BAND BROADENING



$$\sigma^2_{V,col} = (V_0^2/N) \cdot (1 + k_e)^2$$

Fig. 1. Representative chromatogram and peak variances for the estimation of system variance by using the "in presence of column" methodology. Instrument: Acquity UPC^2, column: Waters BEH 1.7 µm (100 mm × 3.0 mm) mobile phase: 10% MeOH in CO_2, temperature: 40 °C, flow rate: 2 mL/min. Test analytes: butylparaben, caffeine, theobromine, betamethazone, chlorthalidone and hydrochlorothiazide.

EXTRA-COLUMN BAND BROADENING

$$\sigma^2_{V,col} = (V_0^2/N) \cdot (1 + k_e)^2$$

- How large is column dispersion: (k_e = 2.16)
  - 4.6mm ID, 25cm, 5µm: 2500 µL²
  - 4.6mm ID, 15cm, 3.5µm: 1000 µL²
  - 2.1mm ID, 10cm, 2µm fully porous: 17 µL²
  - 2.1mm ID, 5cm, 1.5µm superficially porous particles: 6 µL²
- Evolution in extra-column dispersion in LC
  - HPLC systems: 20-100 µL²
  - UHPLC systems: < 10 µL², optimized systems < 2µL²
- Extra-column dispersion in SFC ?

EXTRA-COLUMN BAND BROADENING

$$\sigma^2_{V,col} = (V_0^2/N) \cdot (1 + k_e)^2$$

- Extra-column dispersion in SFC
  - Much less investigated than in LC
  - Estimated by Guillarme and co-workers (10% MeOH in CO_2)
    - 23 and 83 µL² for F = 1ml/min and 2ml/min on UPC²
    - 55 and 81 µL² for F = 1ml/min and 2ml/min on Agilent SFC

$$\sigma_{V,tot}{}^2 = \sigma_{V,ec}{}^2 + \sigma_{V,col}{}^2$$

$$N_{obs} = (V_0^2/\sigma_{V,tot}{}^2) \cdot (1 + k_e)^2$$

A. Grand-Guillaume Perrenoud et al.,
J. Chromatogr. A 1314 (2013) 288–297

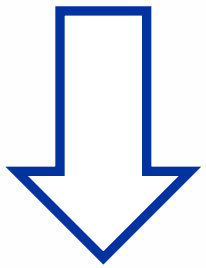
EXTRA-COLUMN BAND BROADENING

$$\sigma^2_{V,col} = (V_0^2/N) \cdot (1 + k_e)^2$$

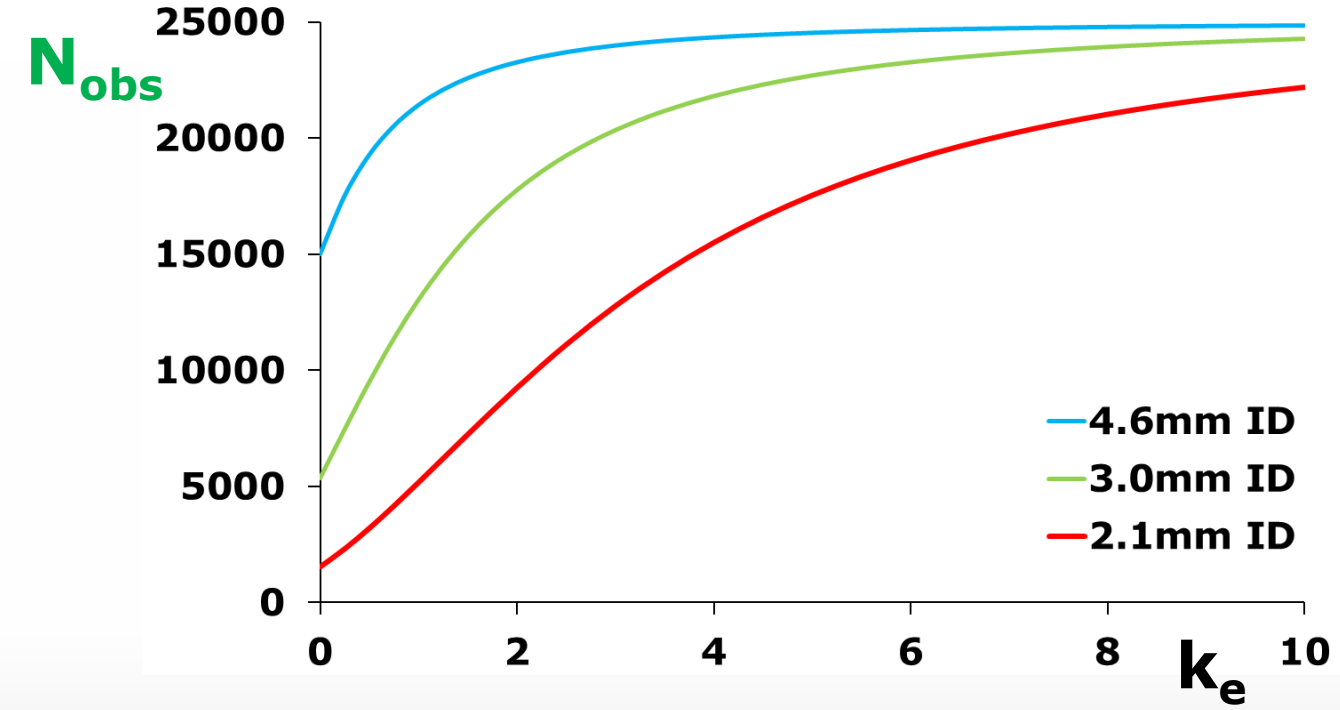
$$\sigma_{V,tot}{}^2 = \sigma_{V,ec}{}^2 + \sigma_{V,col}{}^2$$

$$N_{obs} = (V_0^2/\sigma_{V,tot}{}^2) \cdot (1 + k_e)^2$$

- Extra-column dispersion in SFC
  - 50 µL² extra-column dispersion for columns of 15cm long, with N = 25000, as a function of k_e for different column ID's
  - Observed efficiency



3.0 mm ID trade-off between ECBB and flow rate limitations



EXTRA-COLUMN BAND BROADENING

- Sources of extra-column dispersion in SFC
  - Analytes are dissolved in a strong(er) solvent (~mainly organic) than mobile phase (~ mainly CO_2)
  - Detector cells design and volumes not optimized as in UHPLC
  - Often larger ID tubing employed (170µm vs. 75-120µm in UHPLC)

EXTRA-COLUMN BAND BROADENING

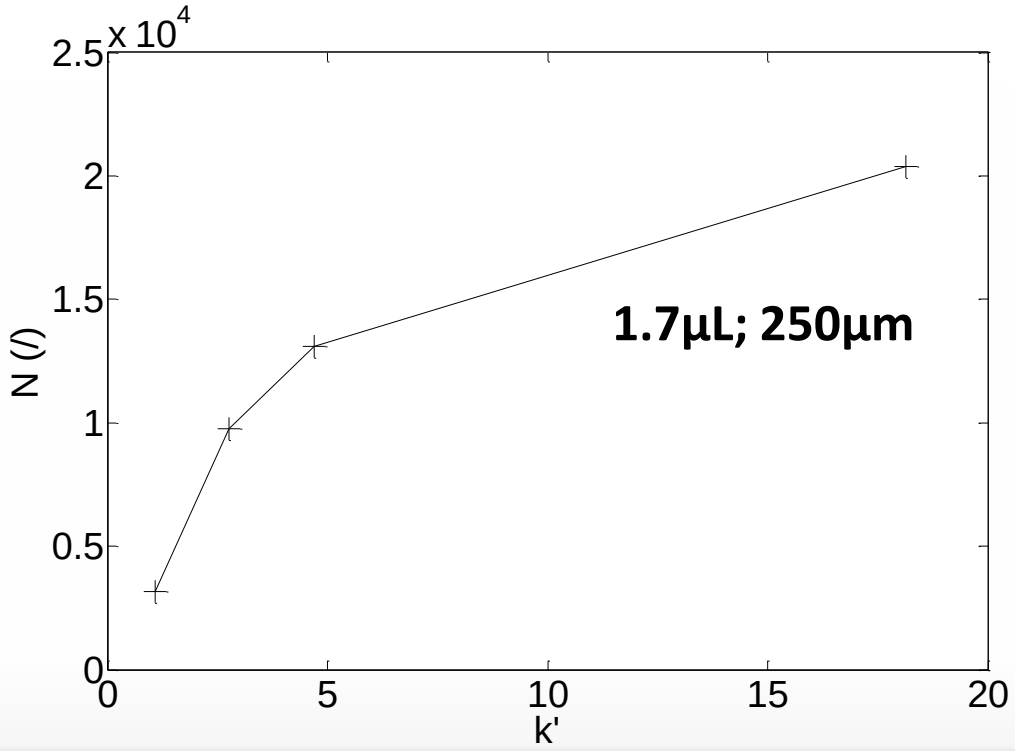
- Sources of extra-column dispersion in SFC: **detector**
  - Detector contribution: SFC 1.7µL flow cell vs. ultra-low dispersion max light cartridge

Agilent 1290 Ultra-Low Dispersion Max-Light Cartridge Flow Cell; V(σ)=0.6µL

Max. Press.: 6 MPa/60 bar/870 psi

EXTRA-COLUMN BAND BROADENING

- Sources of extra-column dispersion in SFC: **detector**
  - Sample solvent EtOH/IPA/hexane: 10/5/85, V_{inj} = 0.8µL
  - P_{back} = 130 bar, 2.1x100mm 1.8µm, F=1ml/min, 8 V% MeOH
  - Tubing
    - Length (50cm)
    - Inner diameter 250 µm ID



R. De Pauw et al., J. Chromatogr. A 1403 (2015) 132–137

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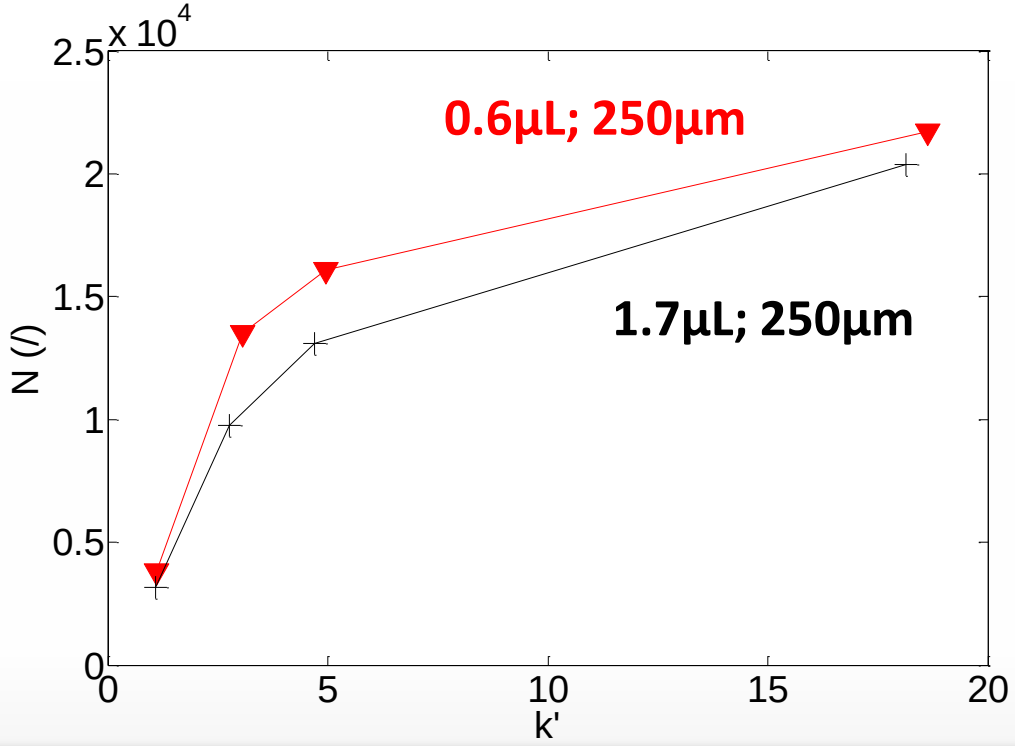

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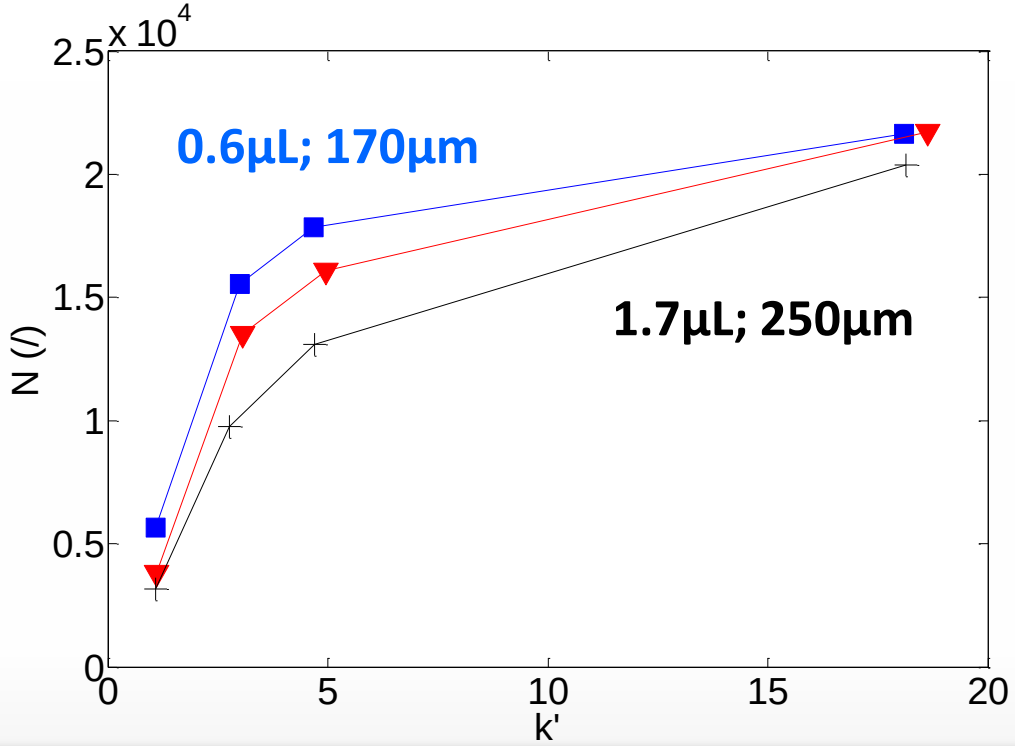
  - P_{back} = 130 bar, 2.1x100mm 1.8µm, F=1ml/min, 8 V% MeOH
  - Tubing
    - Length (50cm)
    - Inner diameter variable
      - **250µm**
      - **170µm**



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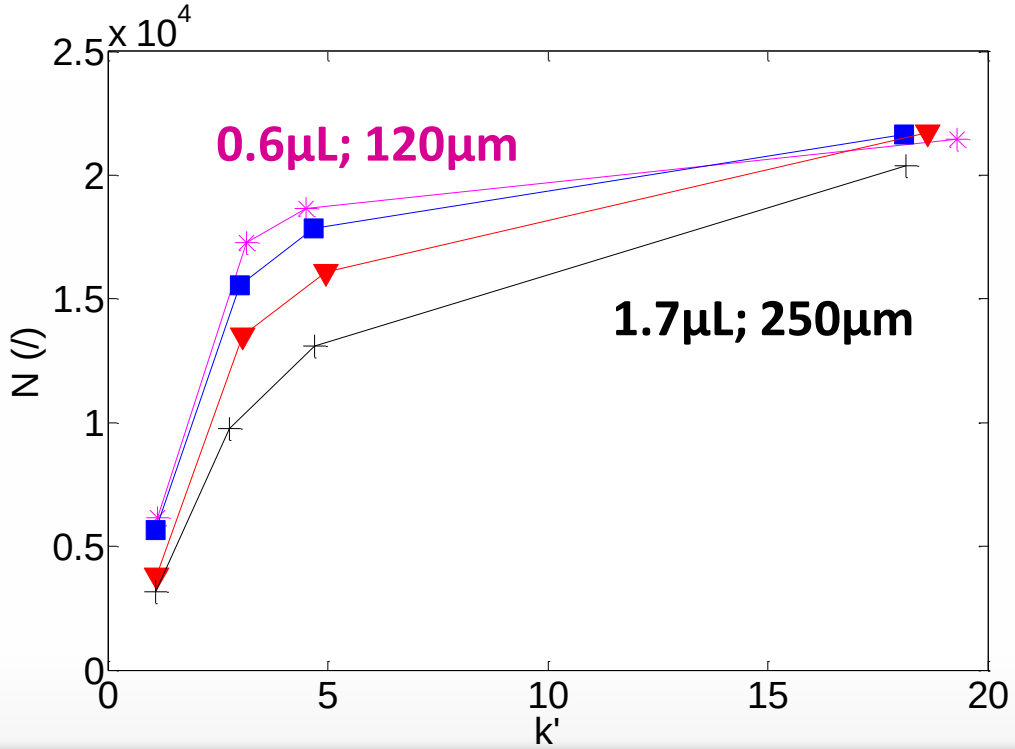
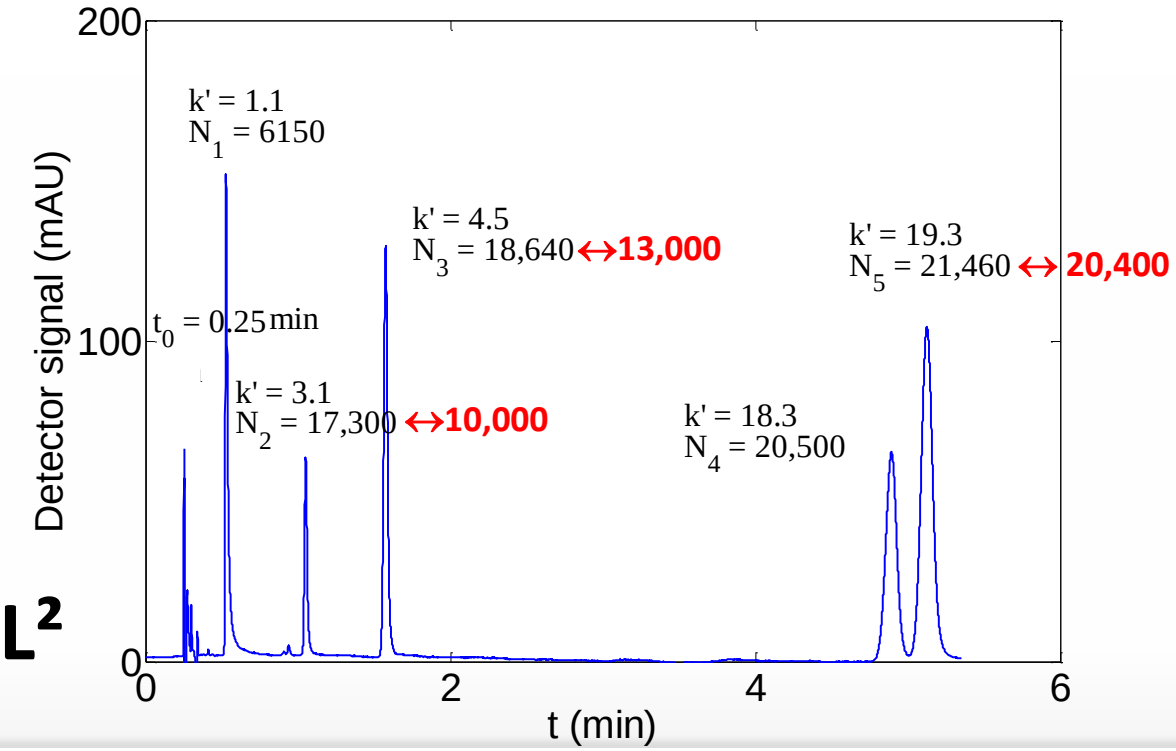
  - P_{back} = 130 bar, 2.1x100mm 1.8µm, F=1ml/min, 8 V% MeOH
  - Tubing
    - Length (50cm)
    - Inner diameter variable
      - **250µm**
      - **170µm**
      - **120µm**



EXTRA-COLUMN BAND BROADENING

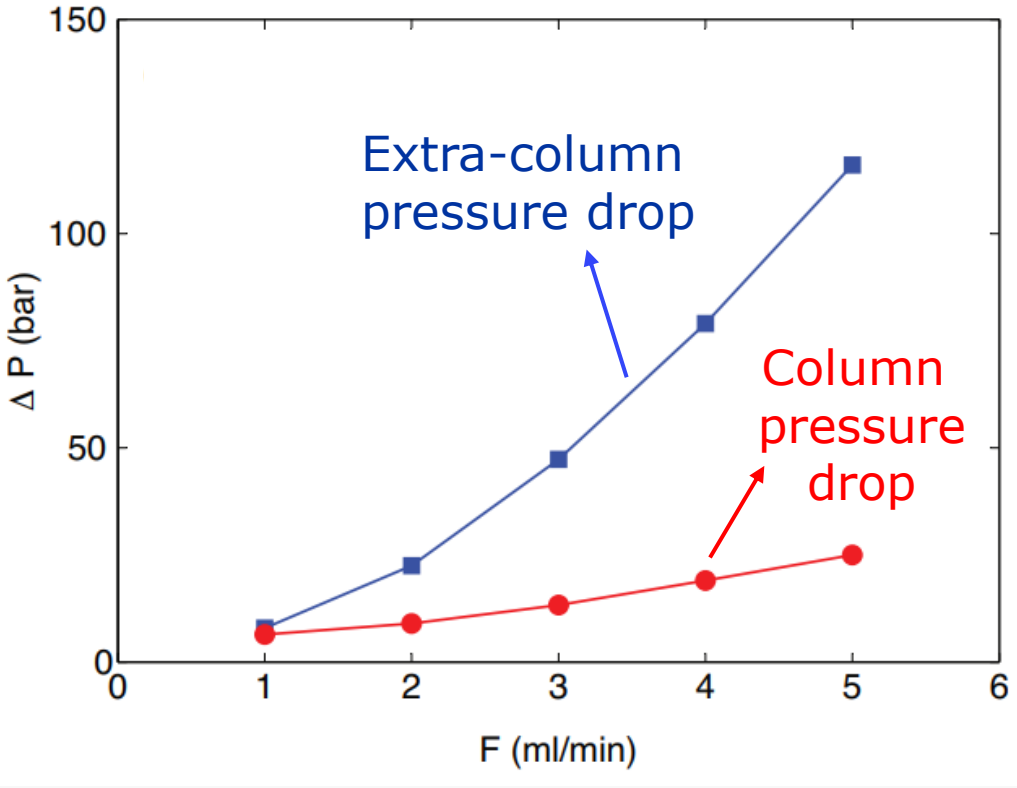
- Optimized system for minimal extra-column dispersion
  - Sample solvent EtOH/IPA/hexane: 10/5/85, V_{inj} = 0.8µL
  - P_{back} = 130 bar, 2.1x100mm 1.8µm, F=1ml/min, 8 V% MeOH
  - Tubing 50cm, 120µm

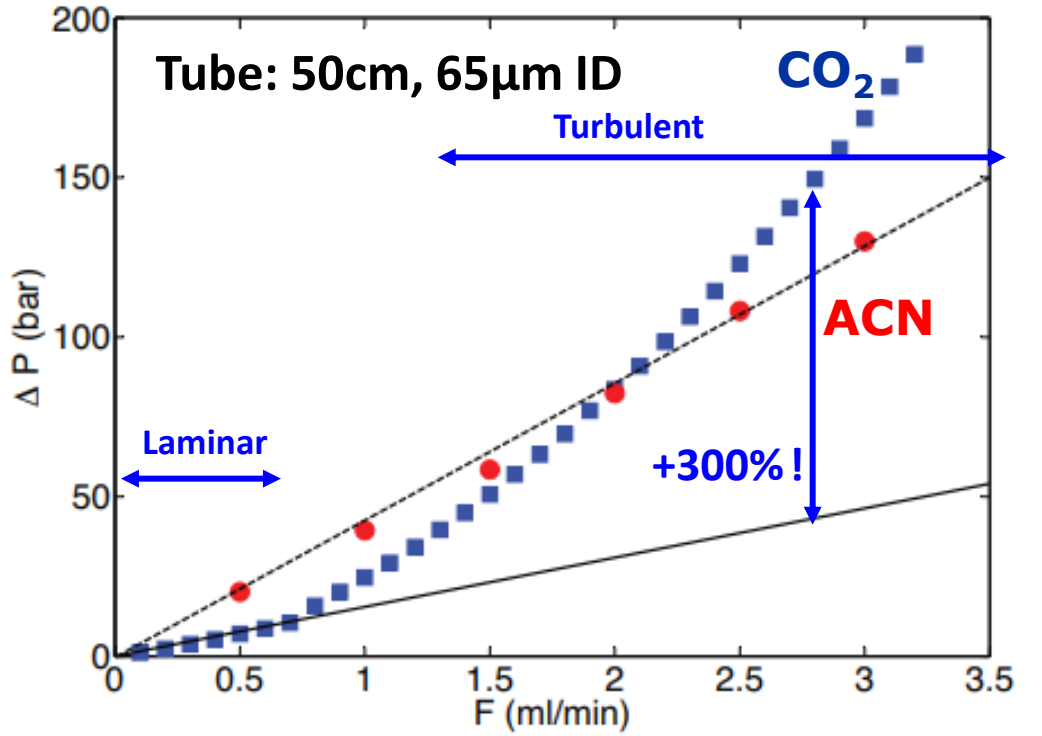


$\sigma_V^2 \approx$ 12-13 µL²

EXTRA-COLUMN PRESSURE

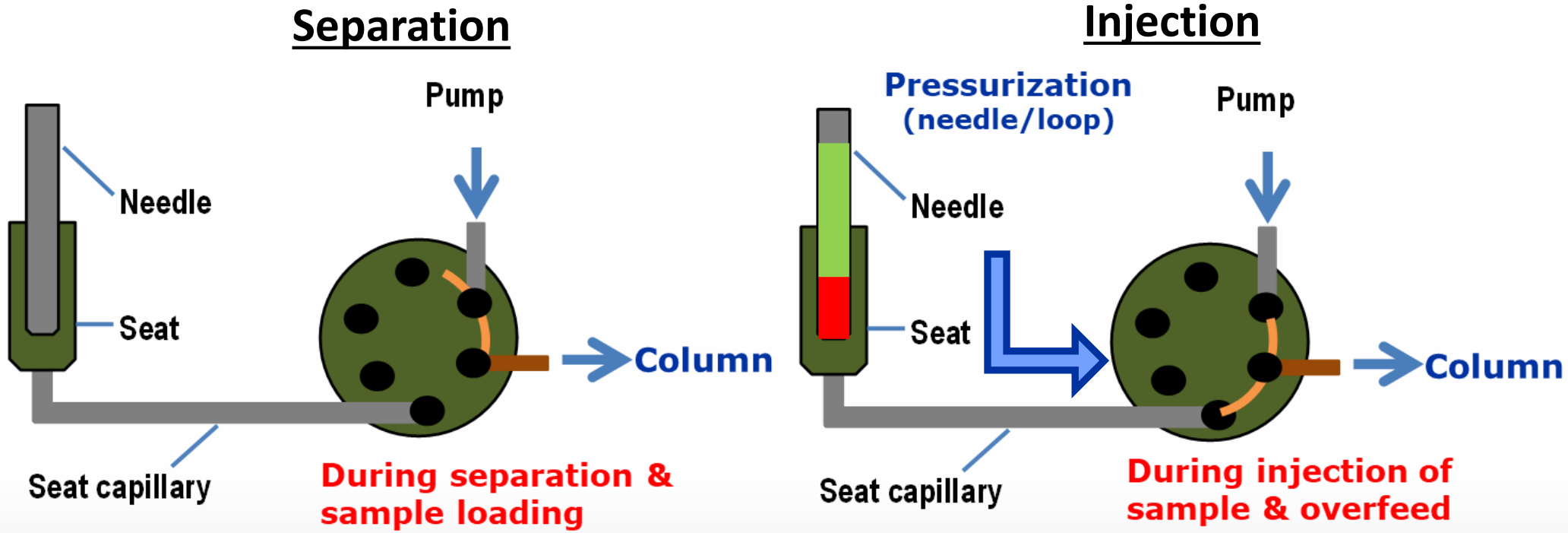
- Watch out for narrow ID tubing in SFC at high flow rate!
- 4.6 mm ID column (10% MeOH) and 120µm tubing in system





INJECTION/SAMPLE INTRODUCTION

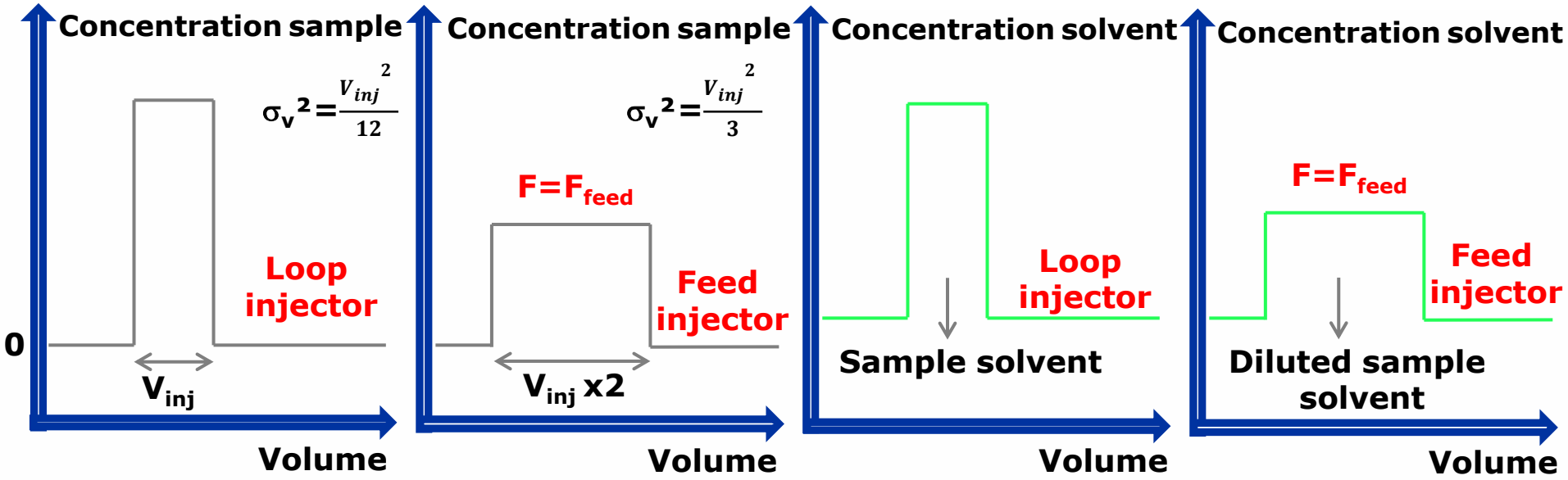
- Feed injector: dilute sample with mobile phase
- Injection flow rate can be varied (max. 1 ml/min)
- Overfeed volume needed to fully inject loaded sample volume



K. Vanderlinden et al., J. Chromatogr. A 1630 (2020) 461525

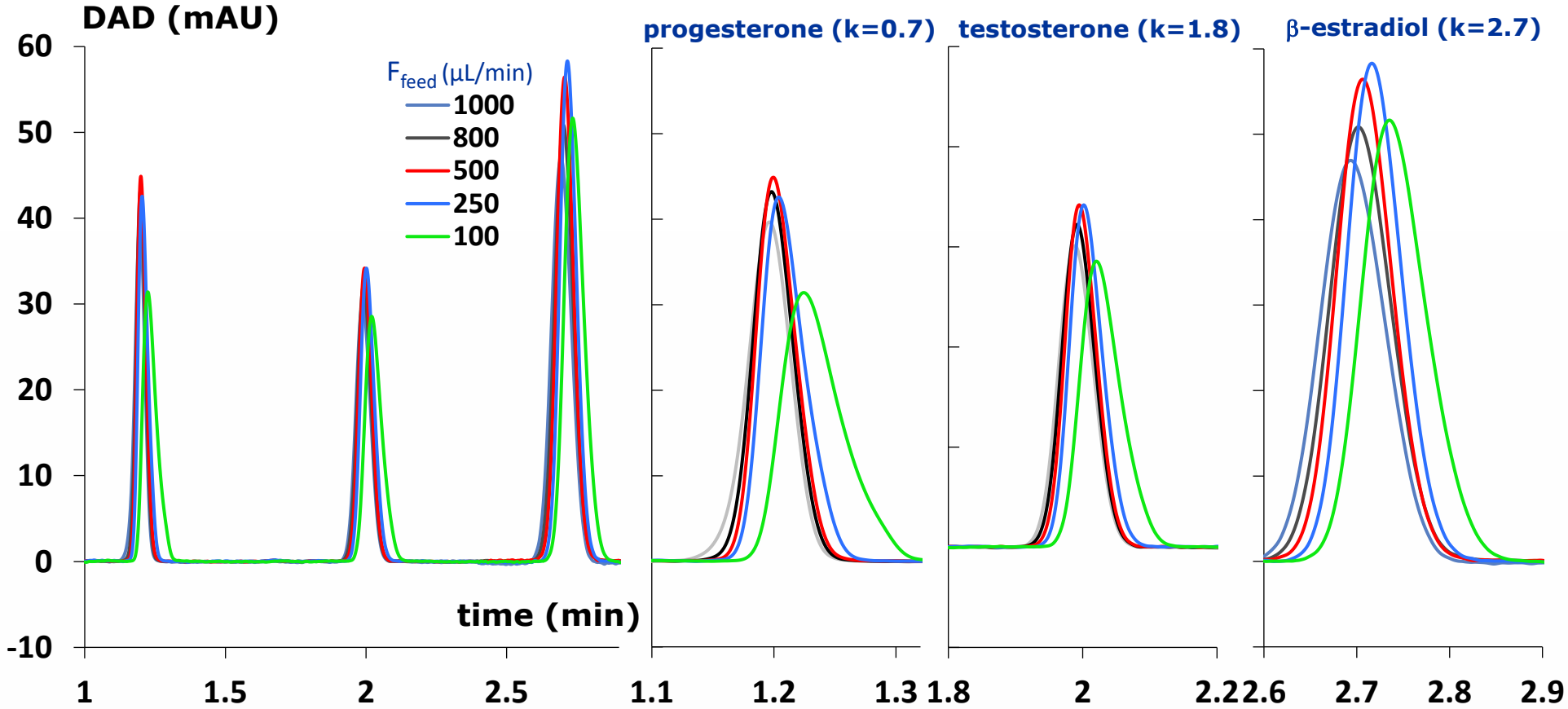
INJECTION/SAMPLE INTRODUCTION

- Feed injector: dilute sample with mobile phase
- Injected sample plug increases in volume ~ $1+F/F_{feed}$
- Sample solvent is diluted with mobile phase (~ mainly CO_2)



INJECTION/SAMPLE INTRODUCTION

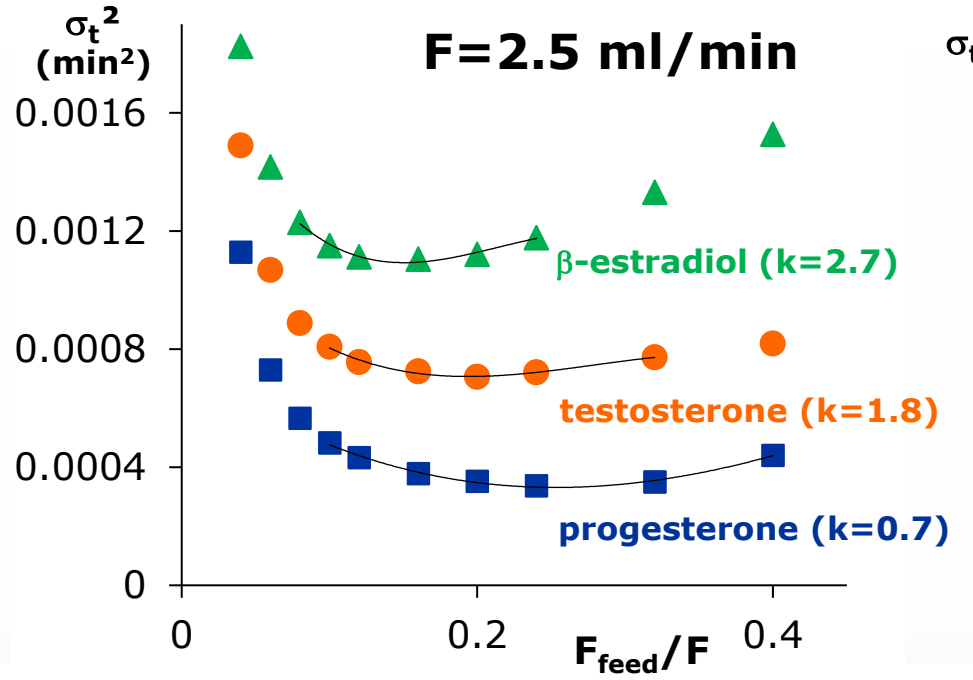
- Feed injector: effect of feed flow rate F_{feed} for F = 2.5 ml/min

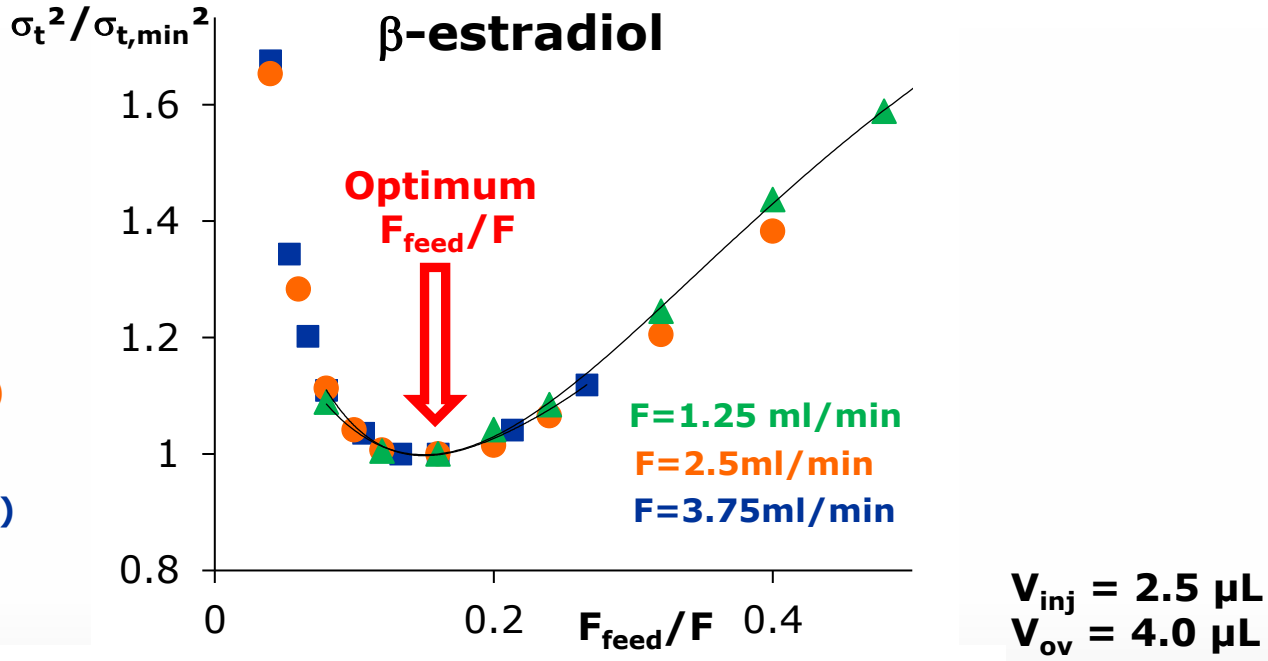


Agilent Zorbax RX-SIL, 4.6 x 150mm 5µm, 12% MeOH

INJECTION/SAMPLE INTRODUCTION

- Optimal feed flow rate F_{feed} found for strong dilution (3-6x)
- Optimal ratio F_{feed}/F independent on flow rate F
- Stronger dilution for solutes with larger dependency of k on φ





CONCLUSIONS

- CO_2-based mobile phases exhibit interesting properties but are often overstated for modern conditions and applications
- Kinetic separation performance of modern SFC instruments is similar to UHPLC
- Need for backpressure and lower maximum pump pressure decreases potential gains from low viscosity mobile phase and application of UC-gradients
- Extra-column band broadening can still be significantly improved for SFC to fully exploit advantages for small superficially porous particles and narrow ID columns

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