Improvement of Traditional HPLC Methods by Combining Temperature with a New Generation HPLC columns

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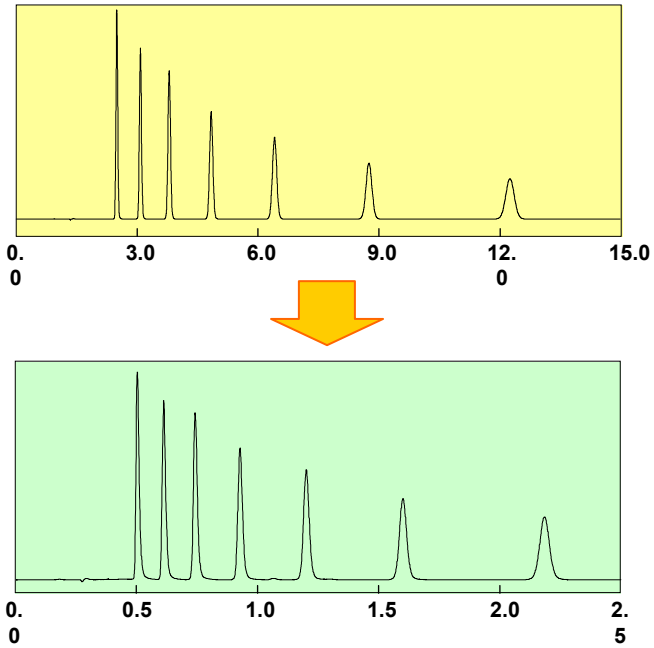
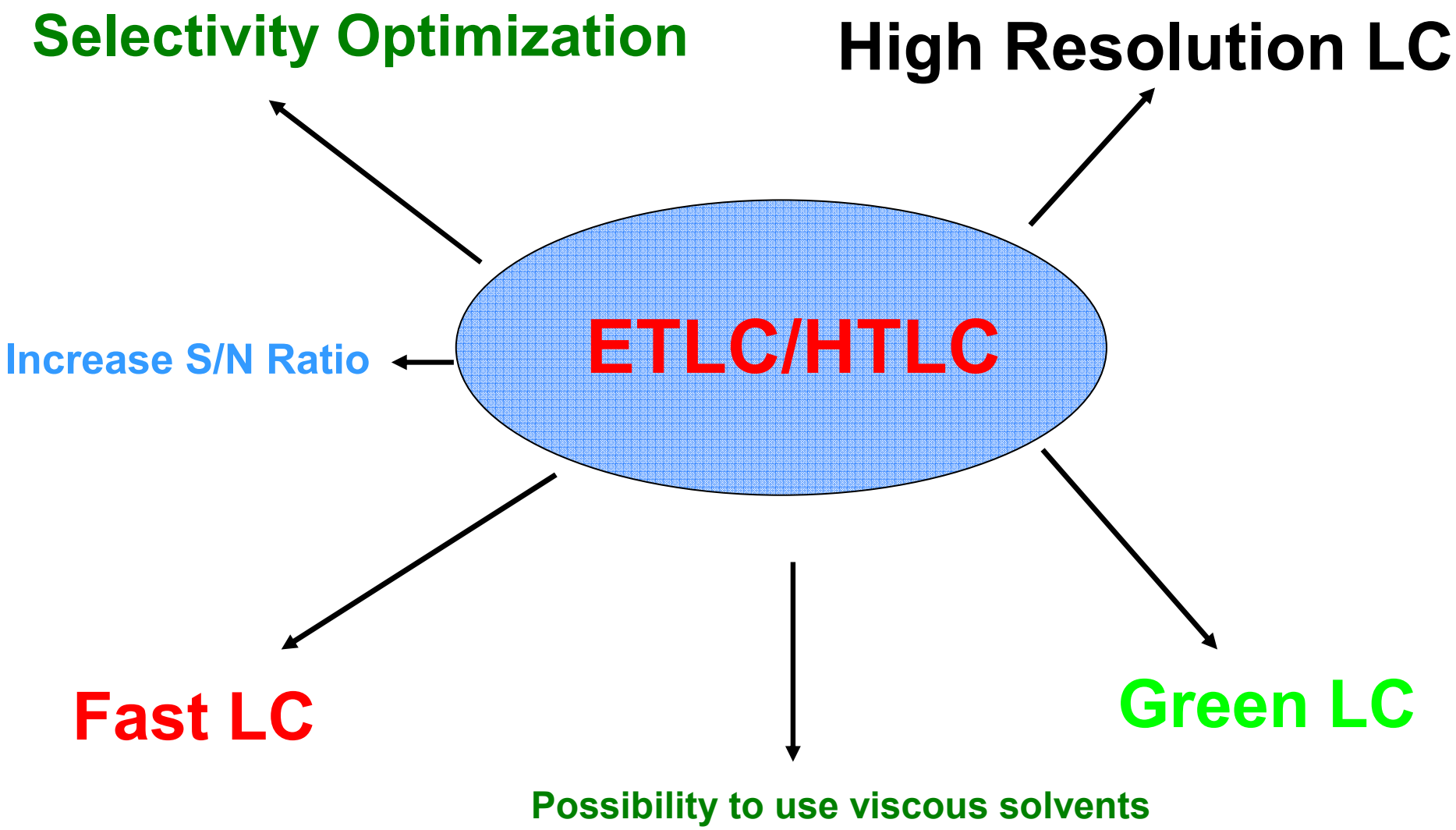
Goal: Enhancing Productivity

- **Faster - More speed**
  - → **Shorter run time while maintaining resolution**
    - **- Higher linear velocity**
    - **- Shorter column length**



- **Better - More resolution**
  - → **Better resolution while maintaining run time**
    - **- Higher theoretical plate numbers**
    - **- Different selectivity**

Temperature Driven LC



Why Fast LC?

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$$

$$\Delta G^\circ = -RT \ln\left(\frac{k}{\varphi}\right)$$

$$\mathrm{Ln}k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R}\mathrm{Ln}\varphi$$

K:	Retention Factor
ΔH°:	Enthalpy
ΔS°:	Entropy
R:	Gas Constant
T:	Temperature
Φ:	Phase Ratio

Why Fast LC?

Hydrophobic retention is in most cases exothermic, therefore both variables are negative

$$\mathrm{Ln}k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R}\mathrm{Ln}\varphi$$

K: Retention Factor

ΔH°: Enthalpy

ΔS°: Entropy

R: Gas Constant

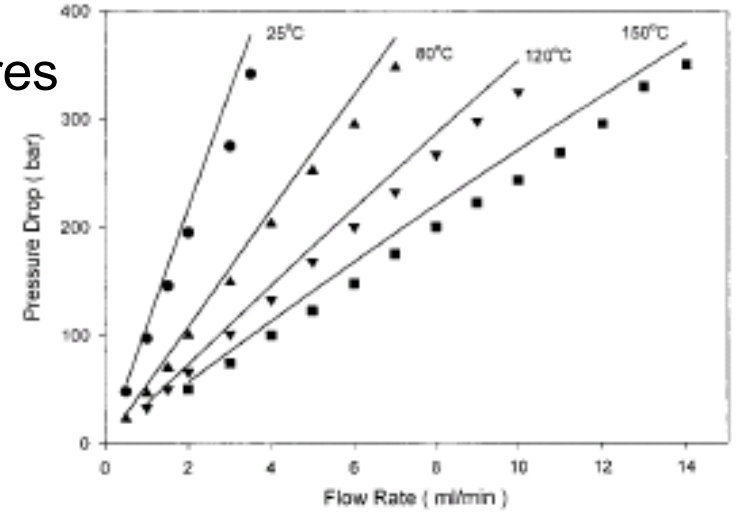
T: Temperature

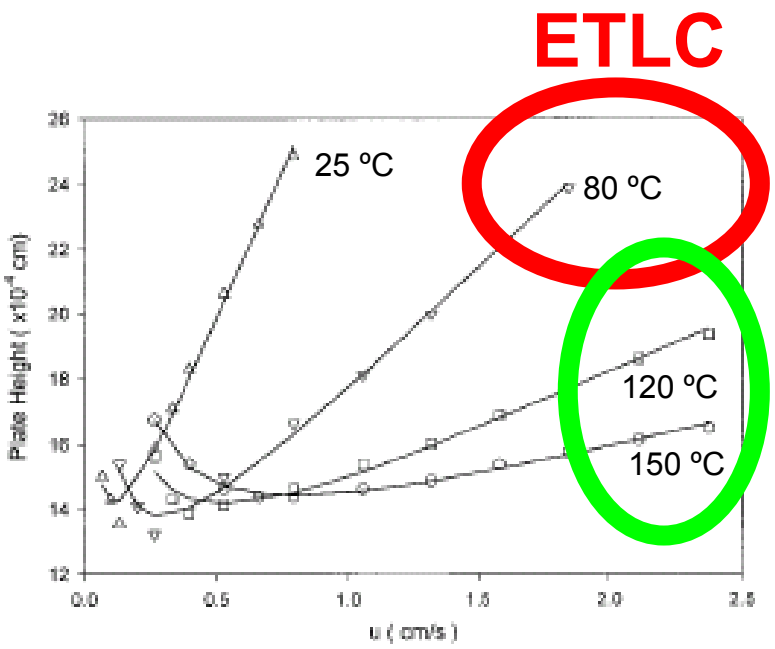
Φ: Phase Ratio

T ↑ = K ↓

Favorable Properties with Temperature Driven LC

Reduced backpressure at elevated Temperatures
(Increase your flow!!)



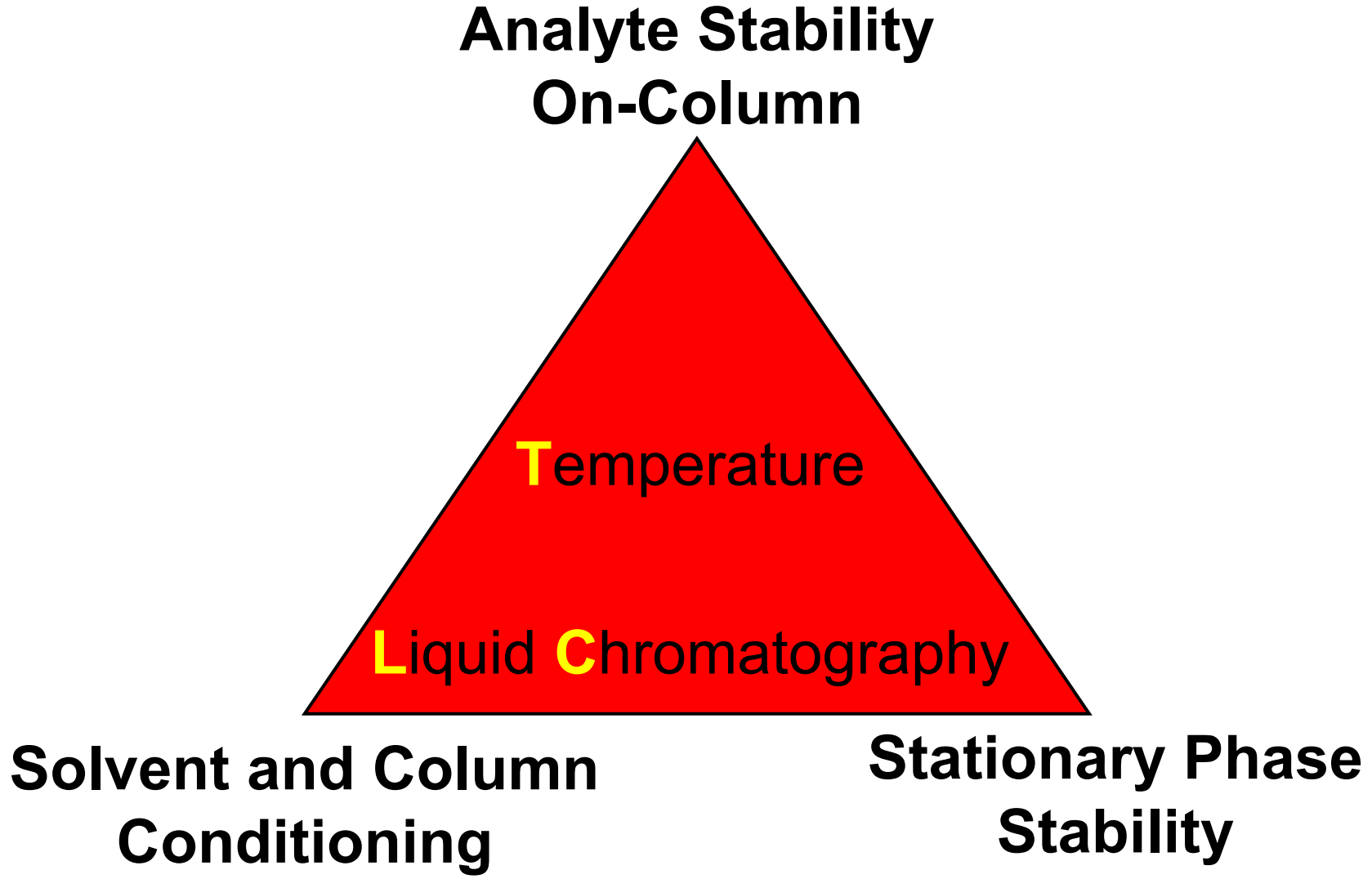


HTLC

Mass transfer increases as temperature increases (Increase your flow!!)

[P. W. Carr et al., Anal. Chem. 2000, 72, 1253-1262].

Requirements for Temperature Driven LC



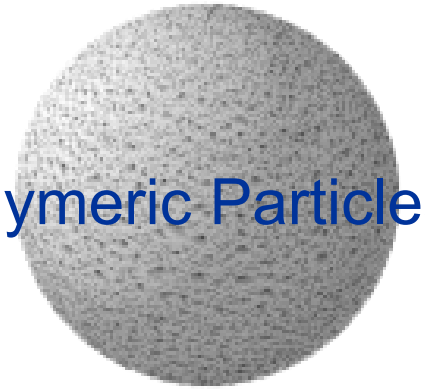
Column Technology
Is There A Solution?

A Wide Range Of Particle And Pore Sizes
High Chromatographic Efficiency
High Bed Stability

Silica Particles

Low Chemical Stability
Low Thermal Stability
Short Lifetimes

Flexibility In Chemical Design
High Chemical Stability
No Silanol Tailing



Polymeric Particles

Low Mechanical Stability
Low Mass Transfer Properties
Low Chromatographic Performance

Is There A Solution?

A Wide Range Of Particle And Pore Sizes
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Shant LABORATORIES

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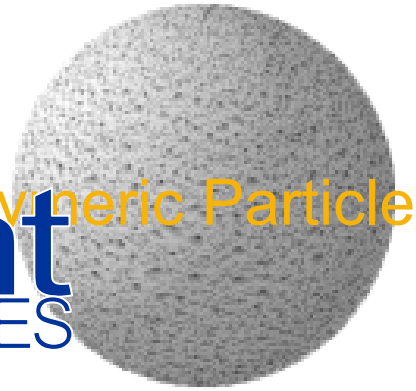
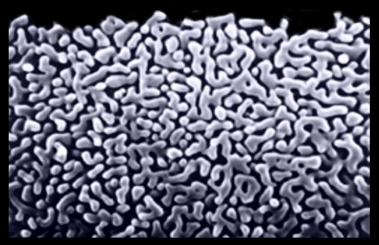


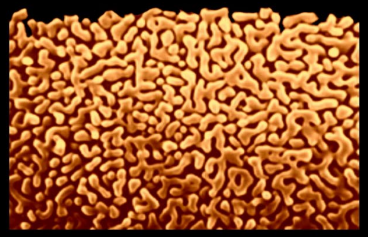
Polymeric Particles

Low Mechanical Stability
Low Mass Transfer Properties
Low Chromatographic Performance

Silica-Polymer Hybrid Technologies

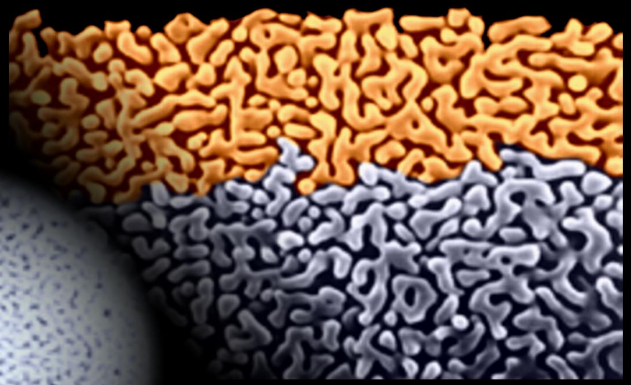


Silica Technology

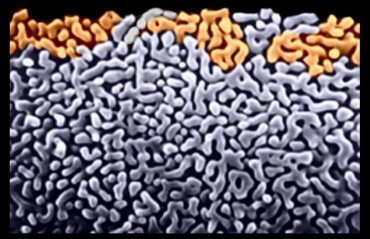


Polymer Technology

Hybrid Technology



Pathfinder Technology



Polymer
Grafted Technology

Polymer

Silica

Pathfinder Family

Pathfinder Standard®

Follow up the rules of traditional LC

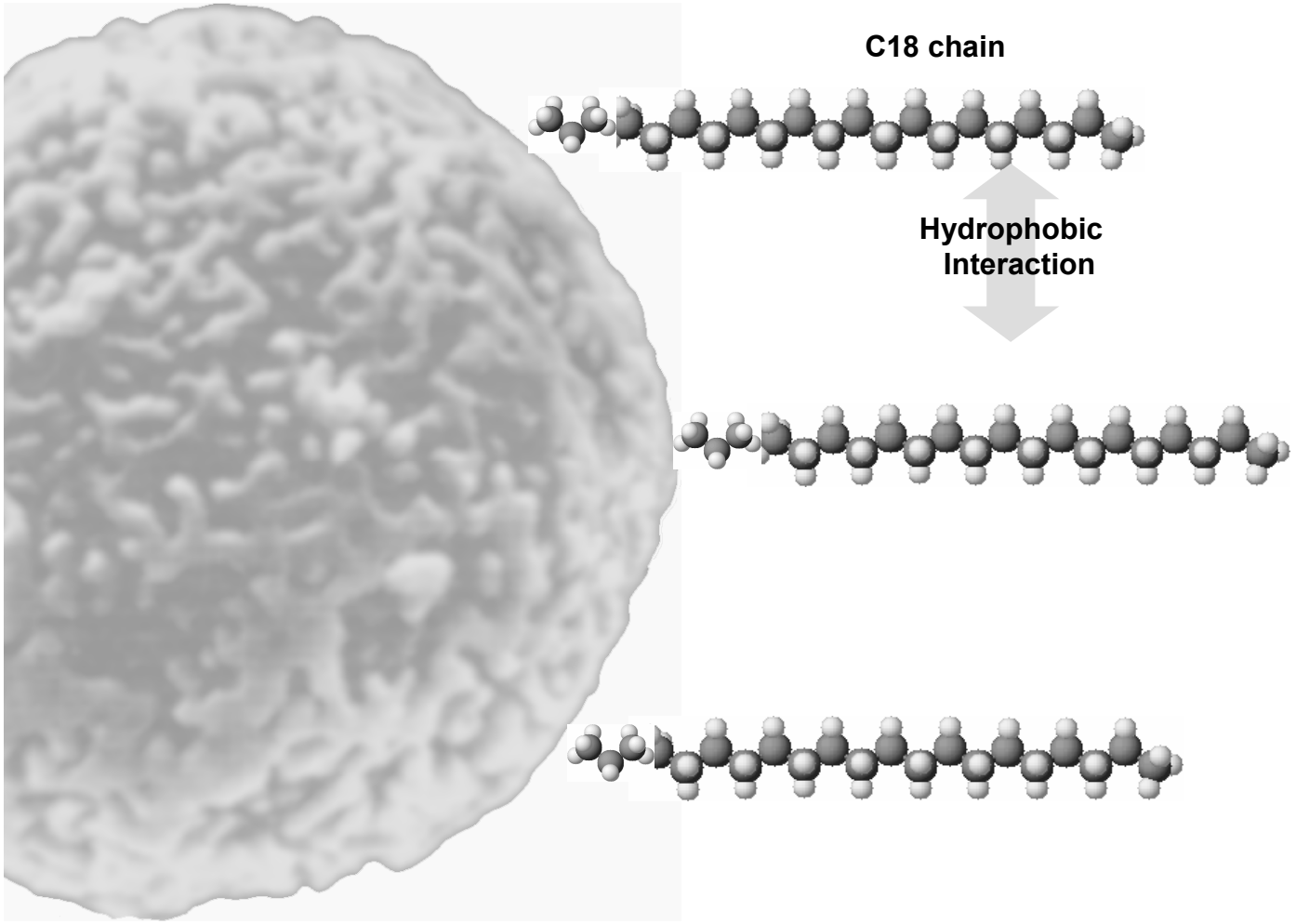
Mixed Mode Columns

MR EP PS AS AP

For difficult separations as alternative to traditional RP columns

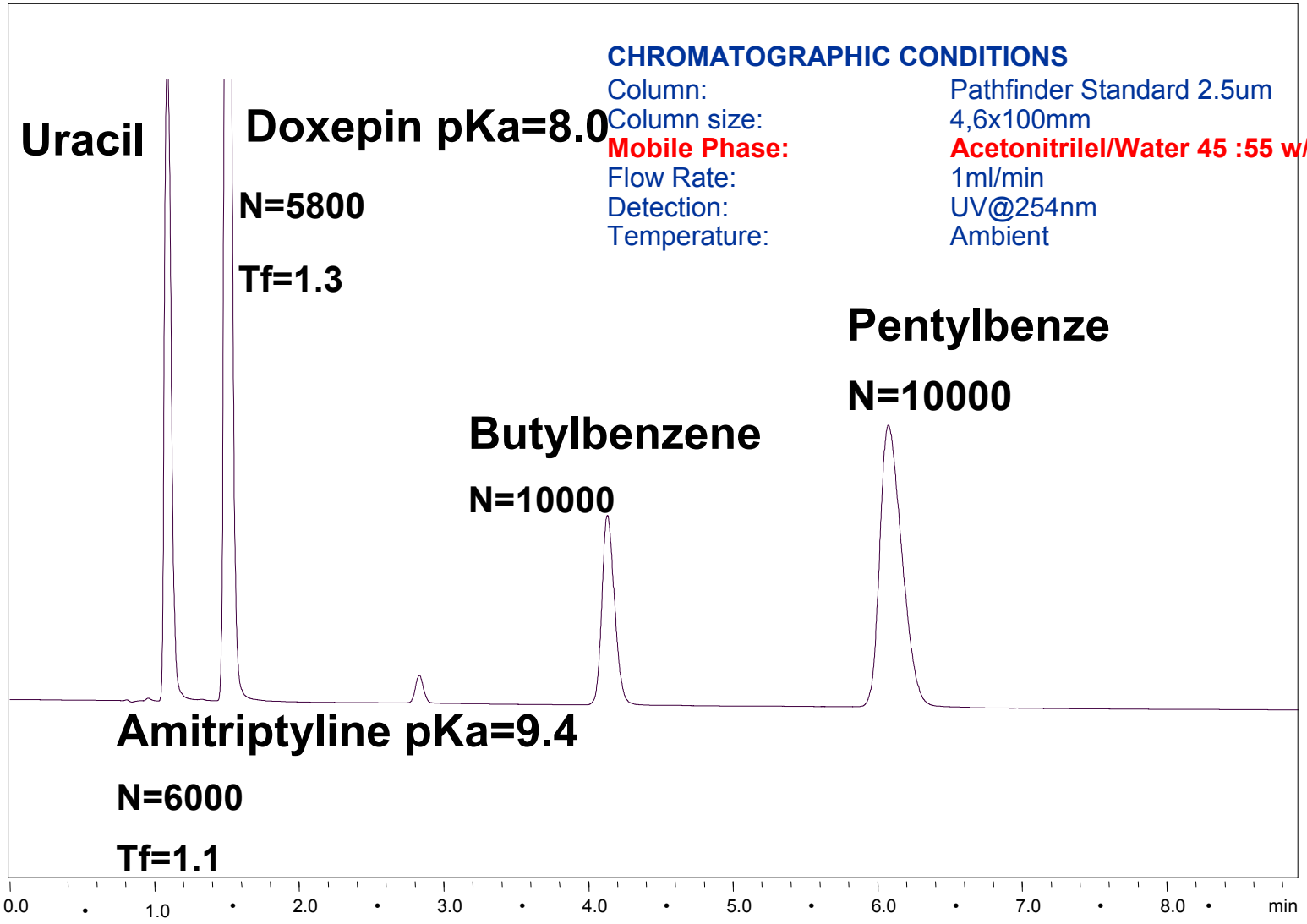
Pathfinder Standard

Surface Chemistry



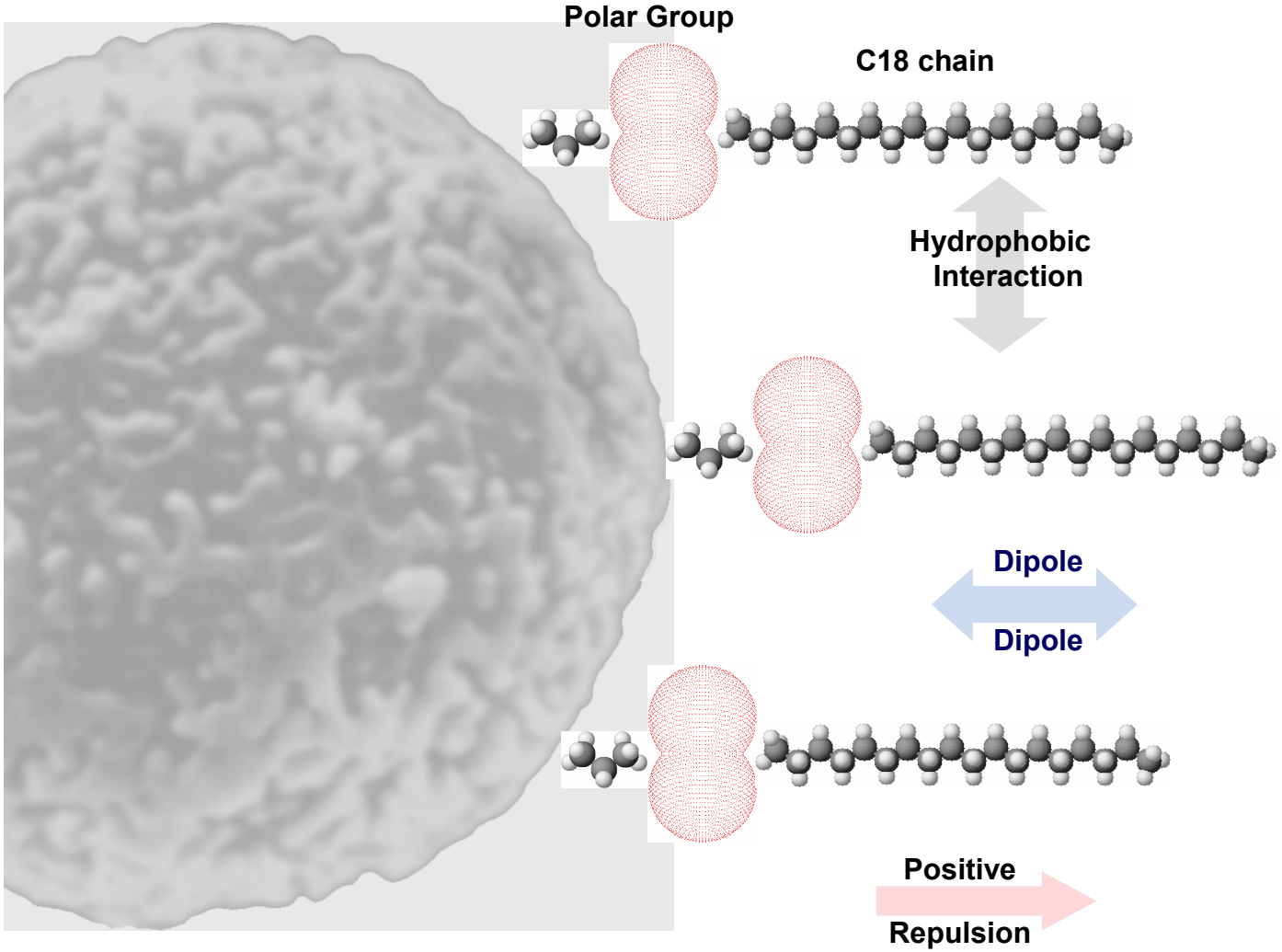
Compounds analysed largely by dispersive interaction of analyte and stationary phase

Pathfinder Standard
No Buffer Needed For Basic Molecules!!!



Pathfinder Mixed Mode

Surface Chemistry



Neutral compounds analysed largely by dispersive interaction of analyte and stationary phase

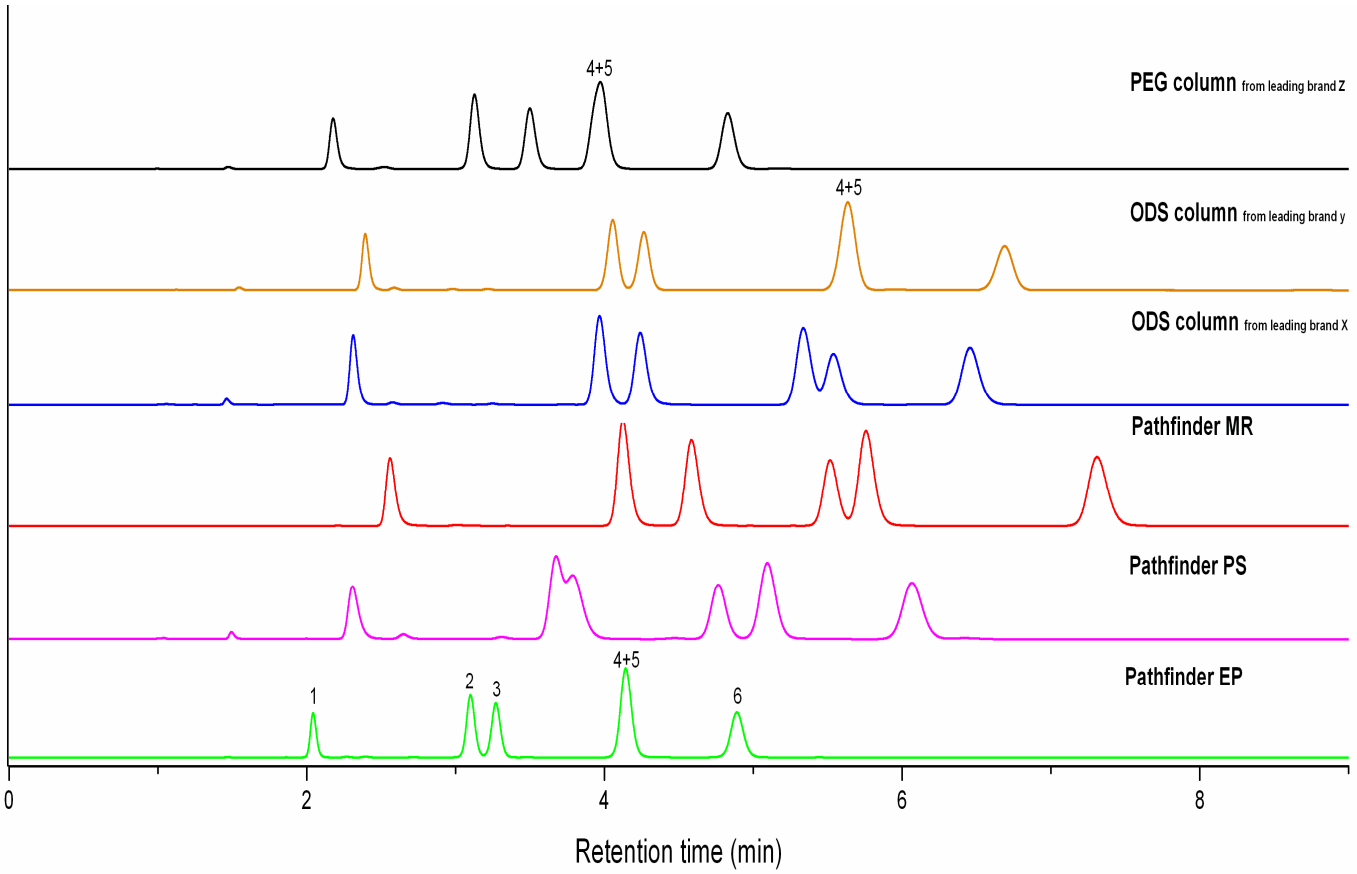
Polar interactions with analytes, these provide the greatest changes in selectivity.

Positive repulsion of basic analytes provides protection of analytes from the surface silanols

Application:
Separation of Steroids with Pathfinder Mixed Mode

Compounds:

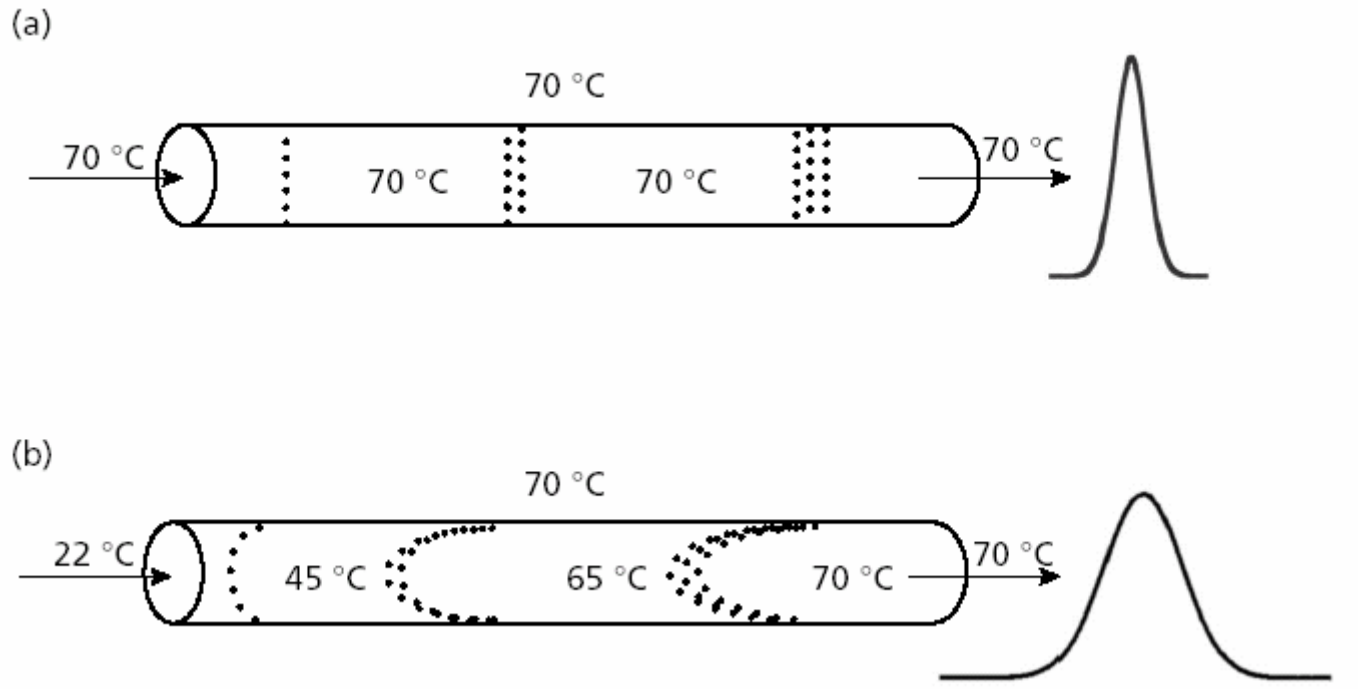
1. Hydrocortisone
2. 11-α-hydroxyprogestorone
3. Cortisone Acetate
4. Dioxycorticosterone
5. 11-ketoprogestorone
6. 17-α-hydroxyprogestorone



CHROMATOGRAPHIC CONDITIONS

Column size:	4,6x150mm 5µm
Mobile Phase:	Acetonitrilel/Water 50 :50 w/w
Flow Rate:	1ml/min
Detection:	UV@254nm
Temperature:	Ambient

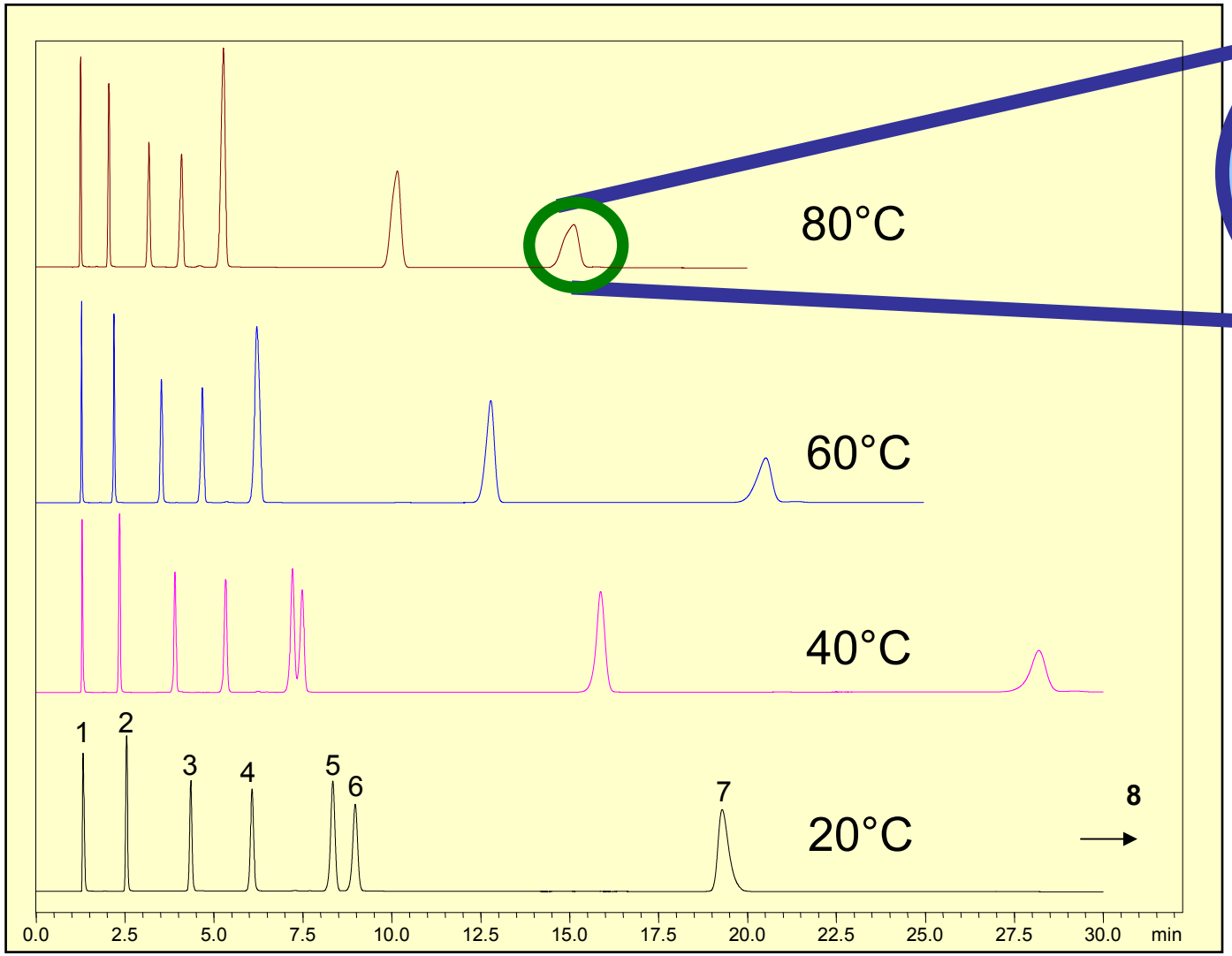
Mismatch due to Temperature Gradients

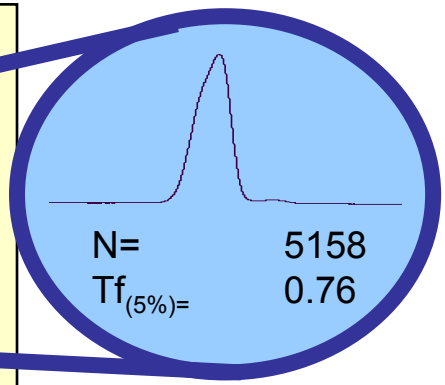


Peak shape decreases drastically when temperature gradients occurs inside the column.

[Reprinted from J.W. Dolan, LC•GC Eur., 15(4), 216–229(2002)]

Temperature Effects
No Solvent Pre Heating in ETLC



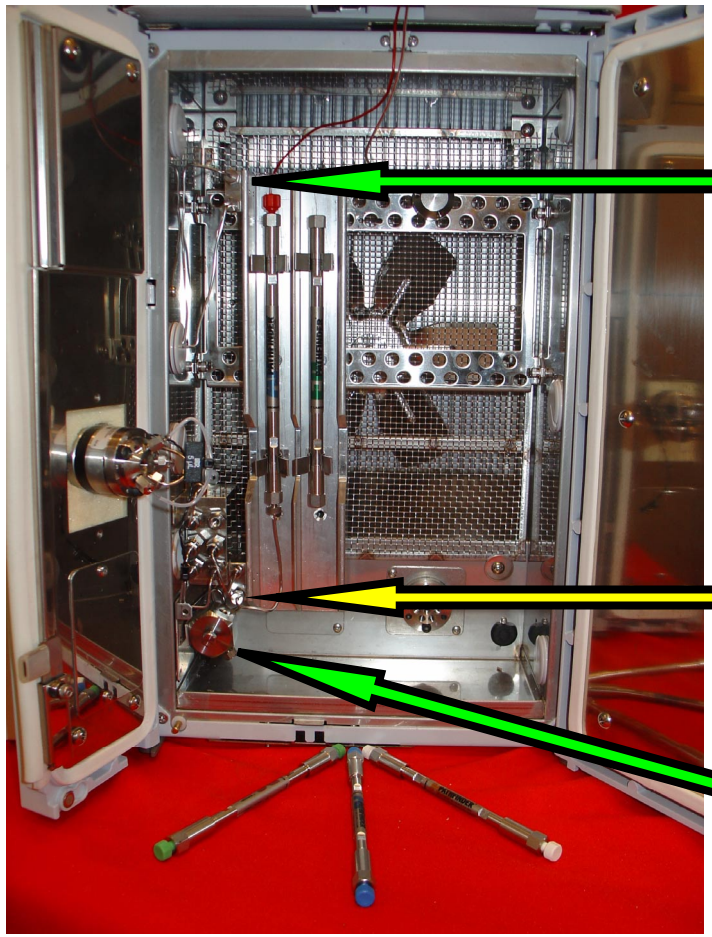


1. Uracil
2. Acetophenone
3. Benzene
4. Toluene
5. Ethylbenzene
6. Naphtalene
7. Butylbenzene
8. Pyrene

Heat Block Assembly CTO10ASvp Integrated in CTO20AC Column Oven

Points of attention:

- 0.13 mm i.d. SUS tubing
- Direct column connection with flow cell
- Ch.1/Ch.2 each 10µl d dead volume
- Assembly P/N 228-35158-91

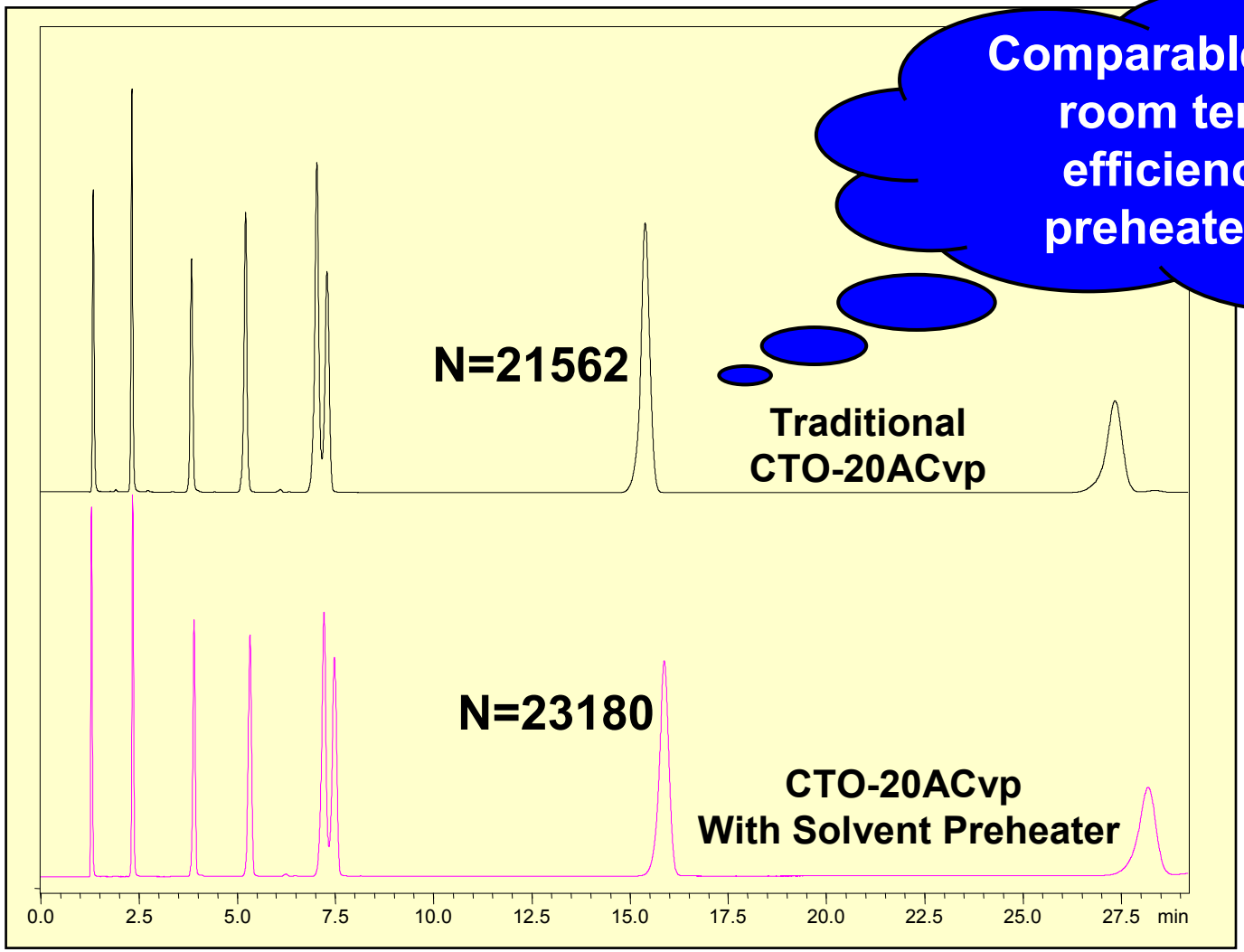


Ch 1. Solvent preheated prior to injection

Ch 2. Solvent/sample preheating before entering the column

Solvent preheating inside mixing chamber

Performance Comparison
CTO-20ACvp with and without Solvent Pre Heater



Experimental conditions:
Pathfinder AP
Temperature 40°C
Flow @ 1.0 ml/min

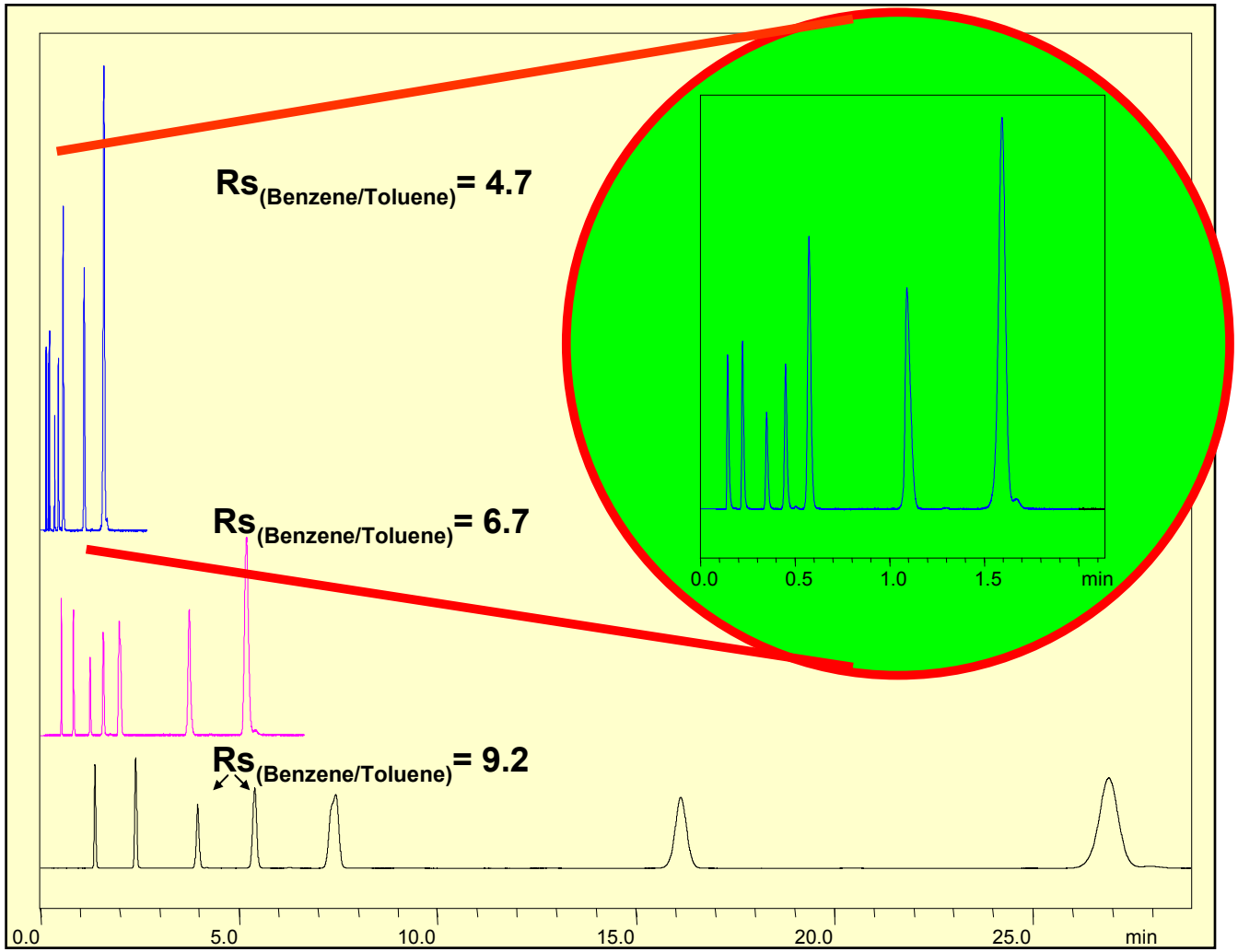
Pre Heater Capacity @ 85°C
~Plate number vs flow and temperature~

Name	40°C 1.0 ml/min	85°C 1.0 ml/min	85°C 1.5 ml/min	85°C 2.0 ml/min
Uracil	4672	7292	5972	5370
Acetophenone	12785	13465	12367	11299
Benzene	19417	17893	18479	17873
Toluene	21581	20183	20622	19636
Naphthalene	NA	NA	NA	NA
Ethylbenzene	NA	NA	NA	NA
Butylbenzene	23180	21796	23912	25246
Pyrene	23363	24187	25621	25909

No loss in efficiency over the entire flow and temperature range!!

Speed up your Analysis

18 Times Faster!!



Pathfinder AS
2.5 µm x 100Å
4.6 mm ID x 50 mm
Flow @ 3.0 ml/min
Temperature 85°C
Pressure 170 bar

Pathfinder AS
3,5 µm x 100Å
4.6 mm ID x 150 mm
Flow @ 2.5ml/min
Temperature 85°C
Pressure 120 bar

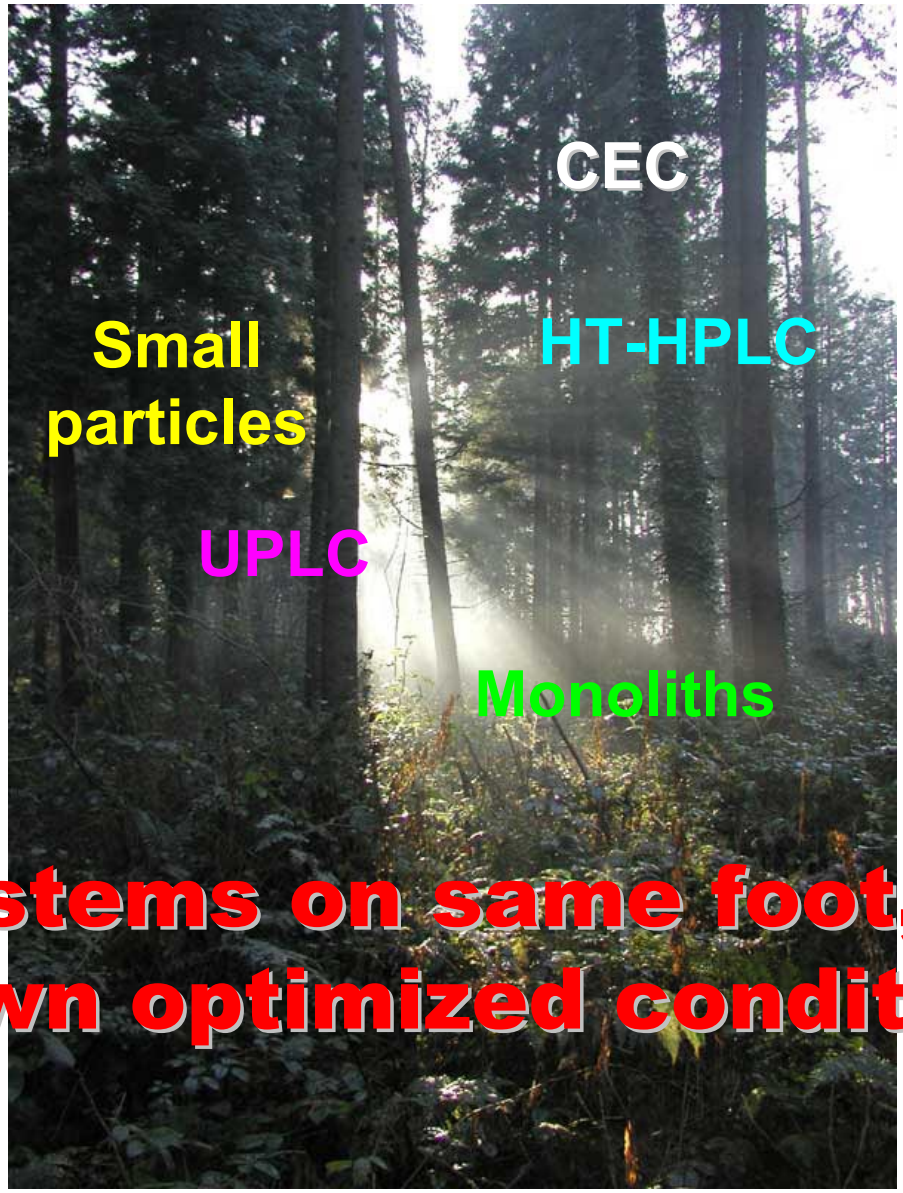
Pathfinder AS
5.0 µm x 100Å
4.6 mm ID x 150 mm
Flow @ 1.0 ml/min
Temperature 40°C
Pressure 80 bar

What About:

High Temperature

Liquid Chromatography

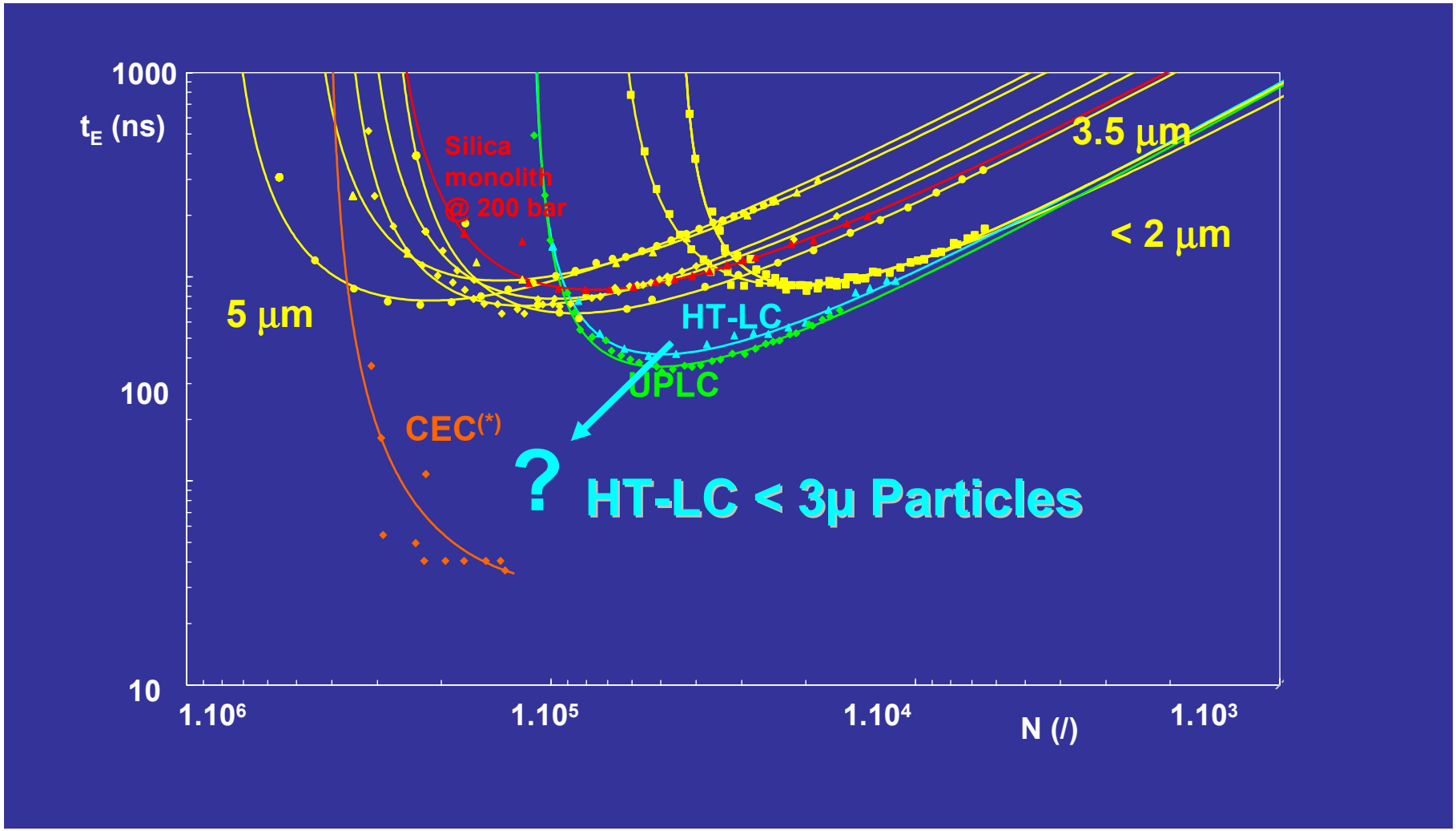
How keeping the overview ?



Compare systems on same foot, each for their own optimized conditions

Monoliths ↔ UPLC ↔ HT-HPLC ↔ CEC

Plots Provided by Prof. Desmet (VUB)

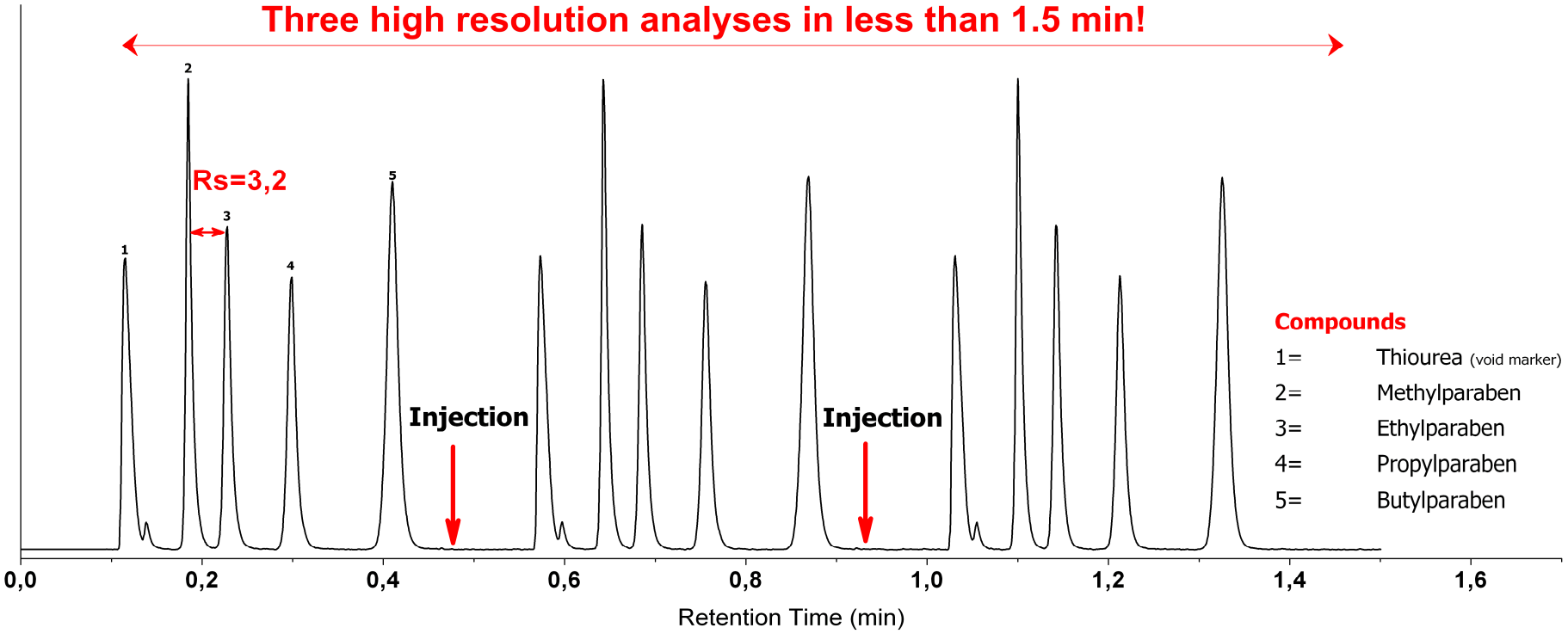


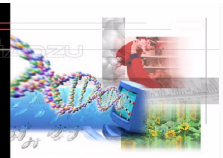
(*)R. Stol, W. Th. Kok and H. Poppe, J. Chrom A, 853 (1999) 45-54
Fluorene on 7 µm wide porous RP-HPLC beads (400 nm)

Application HTLC: Preservatives

CHROMATOGRAPHIC CONDITIONS

Column:	Pathfinder MR 2.5um
Column size:	4,6x50mm
Mobile Phase:	Acetonitrilel/Water 45 :55 w/w
Flow Rate:	5ml/min
Detection:	UV@254nm
Temperature:	**110 degrees**





Shimadzu's Temperature Approach

PATHFINDER®

ETLC with **Shimadzu Prominence**

- **Fast LC analysis**
- **Fast LC gradients**
- **No loss in efficiency below 85°C using** 4.6 mm ID **columns**
- **Appropriate heating capacity up to 3.0 ml/min**
- **Temperature & pH range compatible with Pathfinder column technology**
- **No need for special equipment, cost saving!**

Near future:

High Temperature Separations

SHIMADZU
Solutions for Science
since 1875

Acknowledgements

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Shimadzu Europe

Dr. Björn Erxleben

Robbert

For information and applications

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