Chemometric optimization of LCxLC systems

when is two-dimensional chromatography profitable?

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"Resolving power is what it is all about in analytical separation science"[1]

[1]H. Poppe, J. Chromatogr. A 778 (1997) 3-21

Measuring resolving power

Introduction

One-dimensional chromatography

- Peak capacity in the hundreds

1 hr → 100 peaks

Two-dimensional chromatography

- Peak capacity in the thousands

1 hr → 1,000 peaks
1 day → 4,000 peaks
1 week → 8,000 peaks
1 month → 15,000 peaks
1 year → 20,000 peaks

(and patience)

"Resolving power is what it is all about in analytical separation science"

A trade-off between time and peak capacity

A Belgian dilemma…

Belgian chocolate or Belgian beer?

Introduction

Spend a maximum of 50€

Amount of Belgian beer (liters)

Pareto front

"Optimal" shop

Pareto-optimal experiments

Shop 2

Shop 1

Not an "optimal" shop

I could get more beer without getting less chocolate…

… or I could get more chocolate without getting less beer

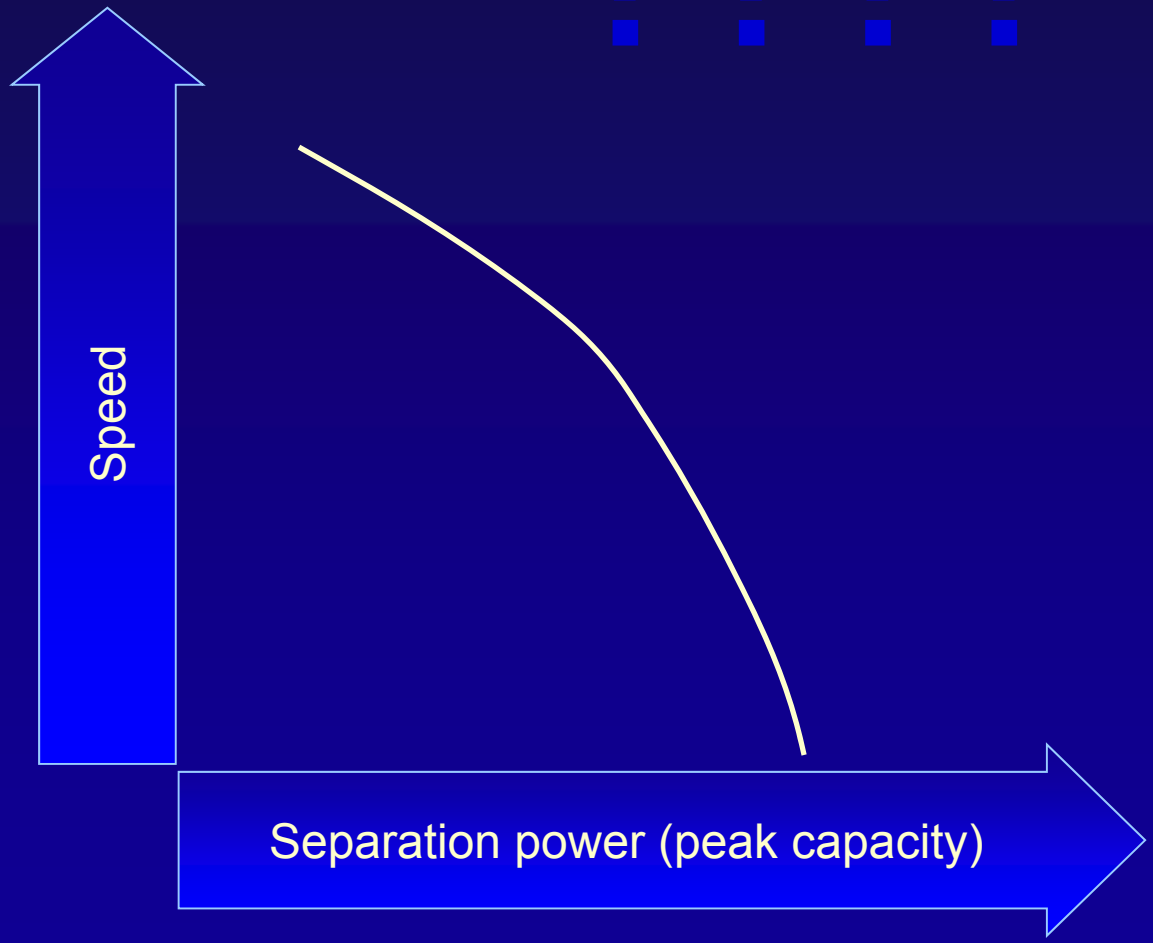
Amount of Belgian chocolate (Kg)

The chromatographer's dilemma…

Introduction

Time or peak capacity? ????

Spend 400 bar of pressure (in each chromatographic dimension)



The chromatographer's dilemma…

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Time or peak capacity? ????

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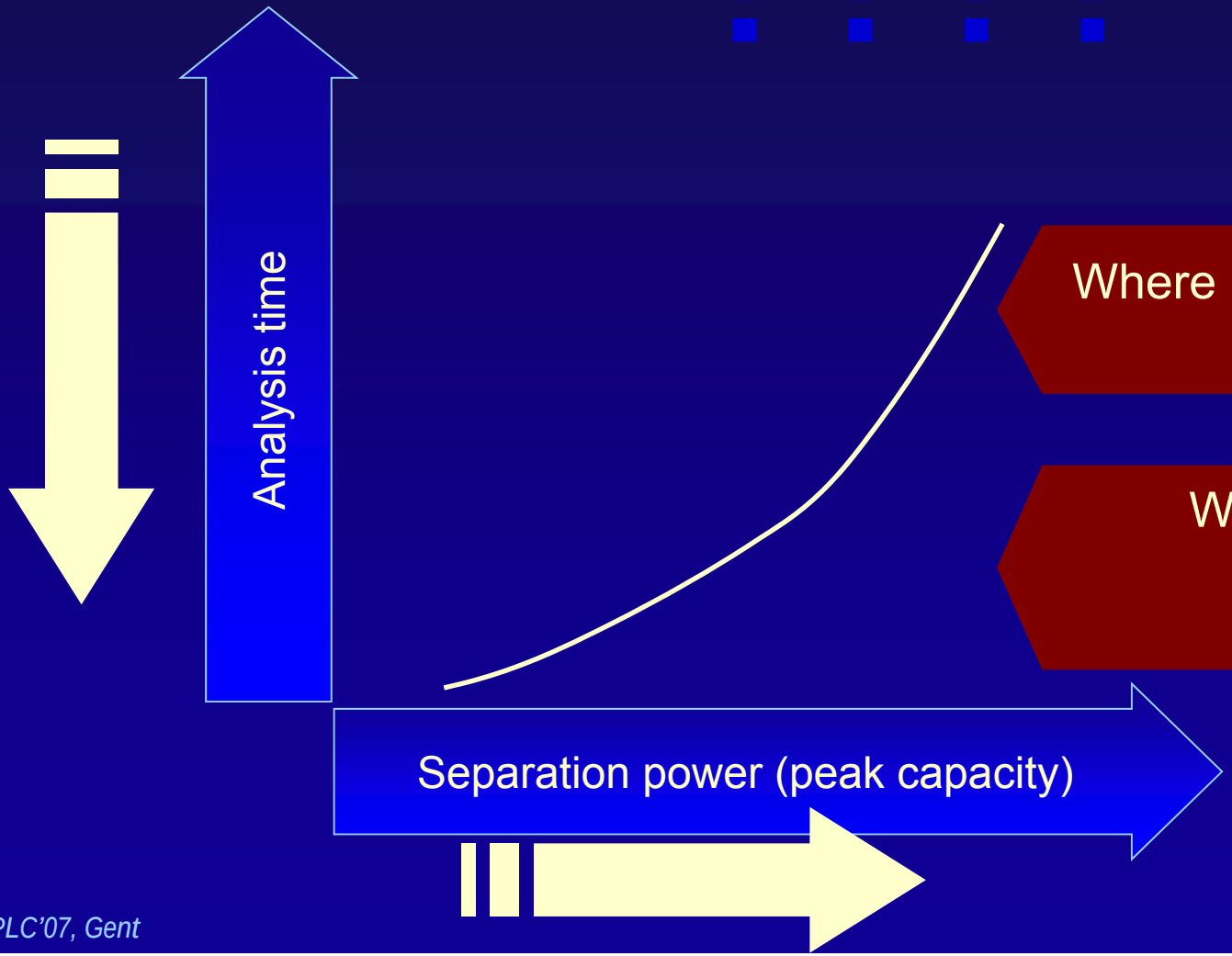


Where is this pareto front located?

Which experimental conditions are pareto-optimal?

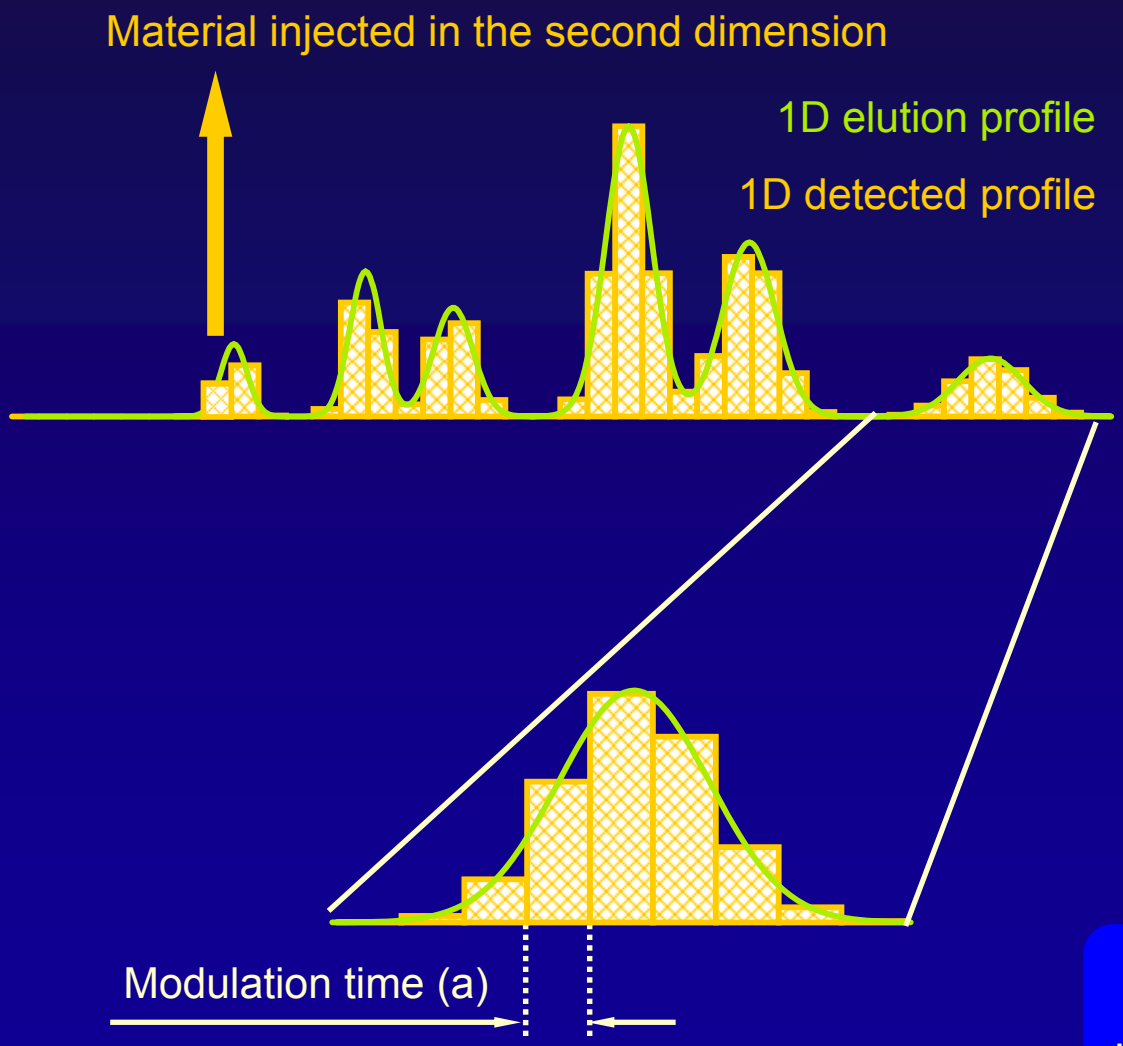
Factors affecting the total peak capacity, $n_c = {}^1n_c \times {}^2n_c$

Pareto optimization in 2D chromatography

First-dimension particle size (${}^1d_p$)	*Affects ${}^1n_c$ and thus n_c*
Second-dimension particle size (${}^2d_p$)	*Affects ${}^2n_c$ and thus n_c*
First-dimension column diameter (${}^1d_c$)	*Affects band broadening in the second dimension and thus n_c*
Second-dimension column diameter (${}^2d_c$)	*Affects band broadening in the second dimension and thus n_c*
Modulation time (a)	*Affects ${}^1n_c$ and ${}^2n_c$ and thus n_c*

Effect of modulation time (a)

Pareto optimization in 2D chromatography



Second-dimension separation operates as the detector for the first dimension

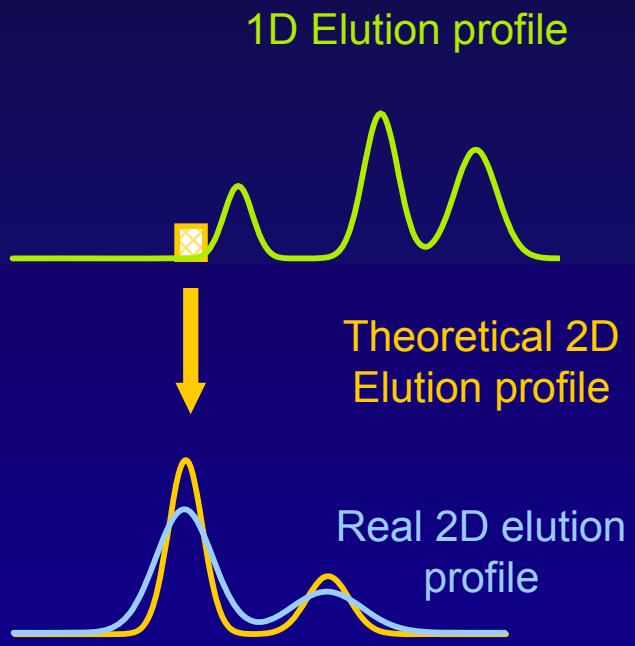
Total band broadening in the first dimension

$$ {}^1\sigma_{total} = \sqrt{\left({}^1\sigma_{peak}\right)^2 + \frac{a^2}{12}} $$

Warning: the formula may be incorrect when $a >> \sigma$

Effect of modulation time (a)

Pareto optimization in 2D chromatography



Total band broadening in the second dimension

$$ {}^2\sigma_{total} = \sqrt{\left({}^2\sigma_{peak}\right)^2 + \left(\frac{{}^1F}{{}^2F}\right)^2 \frac{a^2}{\delta^2}} $$

^1F, ^2F, flow-rate in the first and second dimensions

δ^2, Injection parameter (normally = 4)

Injection band broadening

$$ {}^2\sigma_{total} = \sqrt{\left({}^2\sigma_{peak}\right)^2 + \frac{a'^2}{12}} \quad \Leftarrow \quad a' = \frac{{}^1F}{{}^2F}\frac{\sqrt{12}}{\delta}a $$

Effect of modulation time (a)

Pareto optimization in 2D chromatography

How is extra band broadening affecting gradient-elution peak capacity?

$$\sigma_{total} = \sqrt{(\sigma_{peak})^2 + \frac{a^2}{12}}$$

$$n_{c,gradient} = \frac{t_w}{R_s 4 \times \sqrt{(\sigma_{peak})^2 + \frac{a^2}{12}}}$$

Effect of modulation time (a)

Pareto optimization in 2D chromatography

How is extra band broadening affecting isocratic peak capacity?

After Taylor expansion (and rejecting higher-order terms)…

$$n_{c,isocratic} = 1 + \frac{\sqrt{N}}{4R_s}\frac{\sqrt{t^2 + \frac{1}{12}}}{t}\log\left(\frac{t_\omega + a\frac{\sqrt{N}}{12t}}{t_\alpha + a\frac{\sqrt{N}}{12t}}\right)$$

$$t = \frac{t_\omega + t_\alpha}{2a\sqrt{N}}$$

$$\lim_{a\to 0} n_{c,isocratic} = 1 + \frac{\sqrt{N}}{4R_s}\log\left(\frac{t_\omega}{t_\alpha}\right)$$

Effect of modulation time (a)

Pareto optimization in 2D chromatography

Increasing modulation time (a) affects…

- First-dimension peak capacity

$$ {}^1\sigma_{total} = \sqrt{\left({}^1\sigma_{peak}\right)^2 + \frac{a^2}{12}} $$

${}^1n_c$ *decreases*

- Second-dimension peak capacity

$$ {}^2\sigma_{total} = \sqrt{\left({}^2\sigma_{peak}\right)^2 + \frac{a'^2}{12}} $$

${}^2n_c$ *decreases*

- Second-dimension peak capacity

More "time" in the second dimension

${}^2n_c$ *increases*

- Second-dimension peak capacity

Peaks get broader

${}^2n_c$ *decreases*

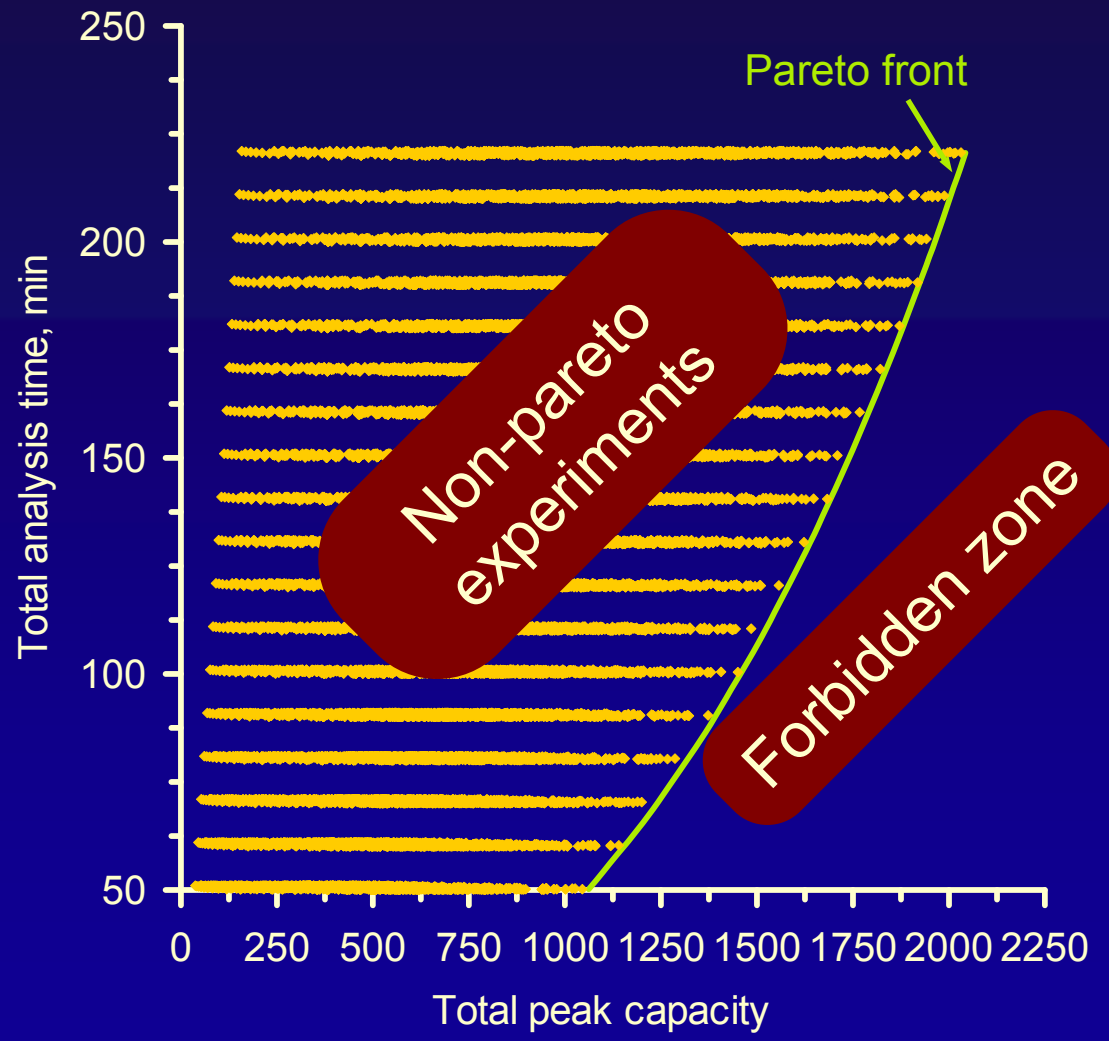
Pareto optimization, an example

Pareto optimization in 2D chromatography

1st dimension: gradient
2nd dimension: isocratic

Parameters

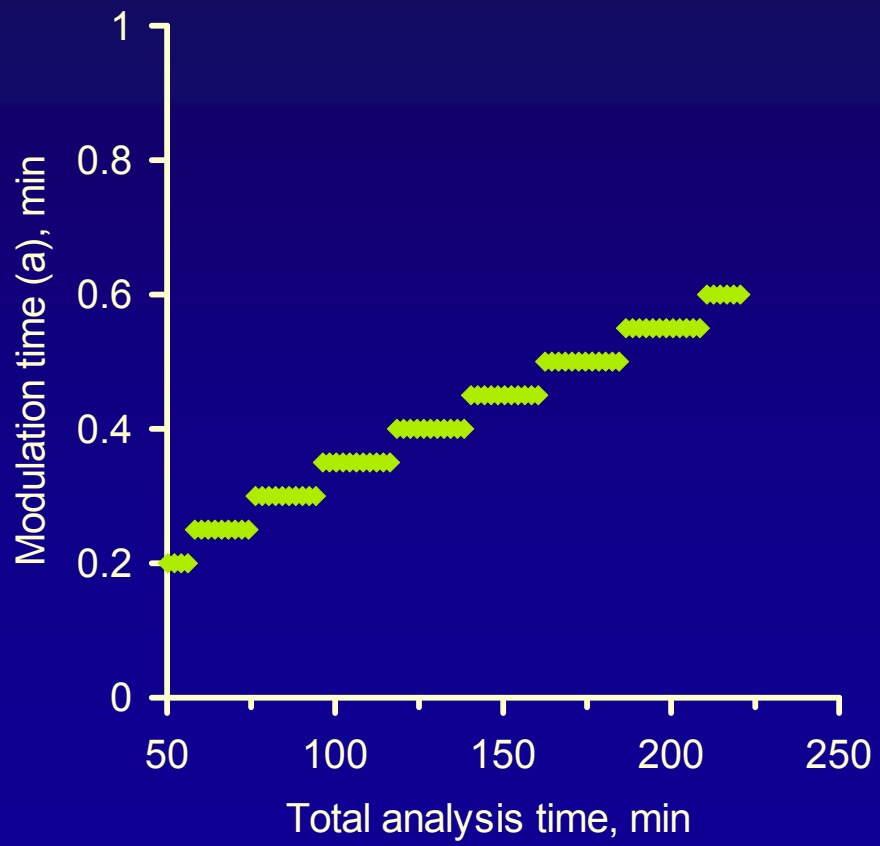
Parameter	Values
^1d_c, mm	[1,2,3]
^2d_c, mm	[5,10]
^1d_p, µm	[1,2,3,5,10]
^2d_p, µm	[1,2,3,5,10]
a, min	[0.1 – 1]



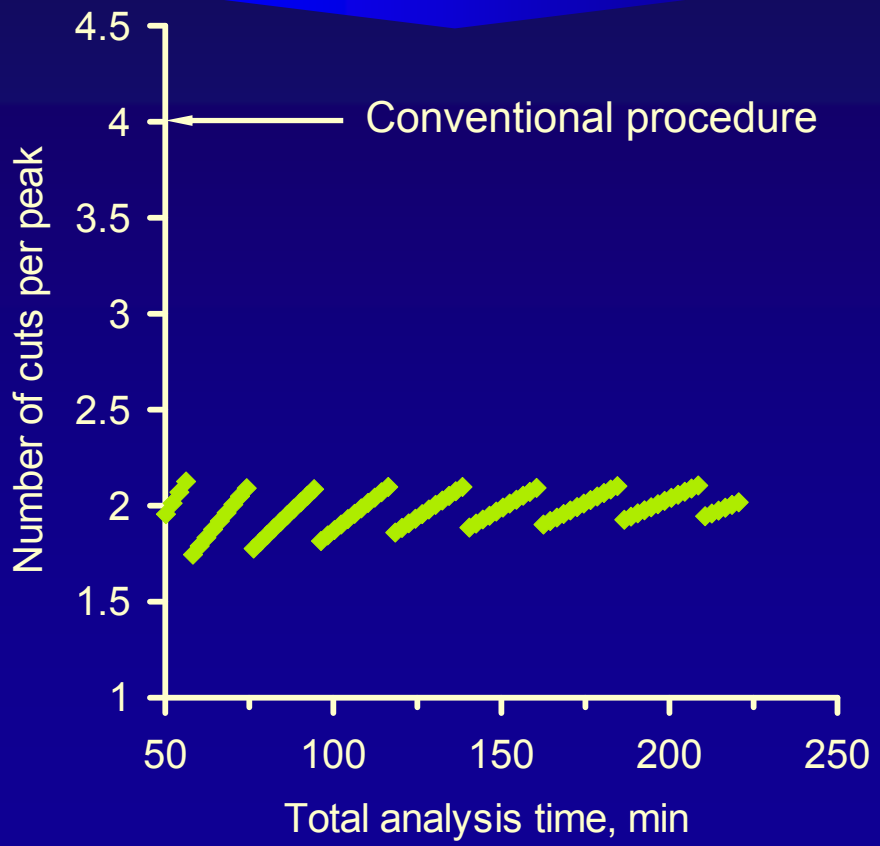
Pareto optimization, an example

Pareto optimization in 2D chromatography

(Pareto-optimal) modulation time



(Pareto-optimal) number of slices per peak



Pareto optimization, an example

Pareto optimization in 2D chromatography

Parameters

Parameter	Values
^1d_c, mm	[1,2,3]
^2d_c, mm	[5,10]
^1d_p, μm	[1,2,3,5,10]
^2d_p, μm	[1,2,3,5,10]
a, min	[0.1 – 1]

$$^2\sigma_{total} = \sqrt{\left(^2\sigma_{peak}\right)^2 + \left(\frac{^1F}{^2F}\right)^2 \frac{a^2}{\delta^2}}$$

$^2F >> ^1F$, 99% of the ^2n_c is maintained

With these conditions, however, the sample is diluted around 50 times

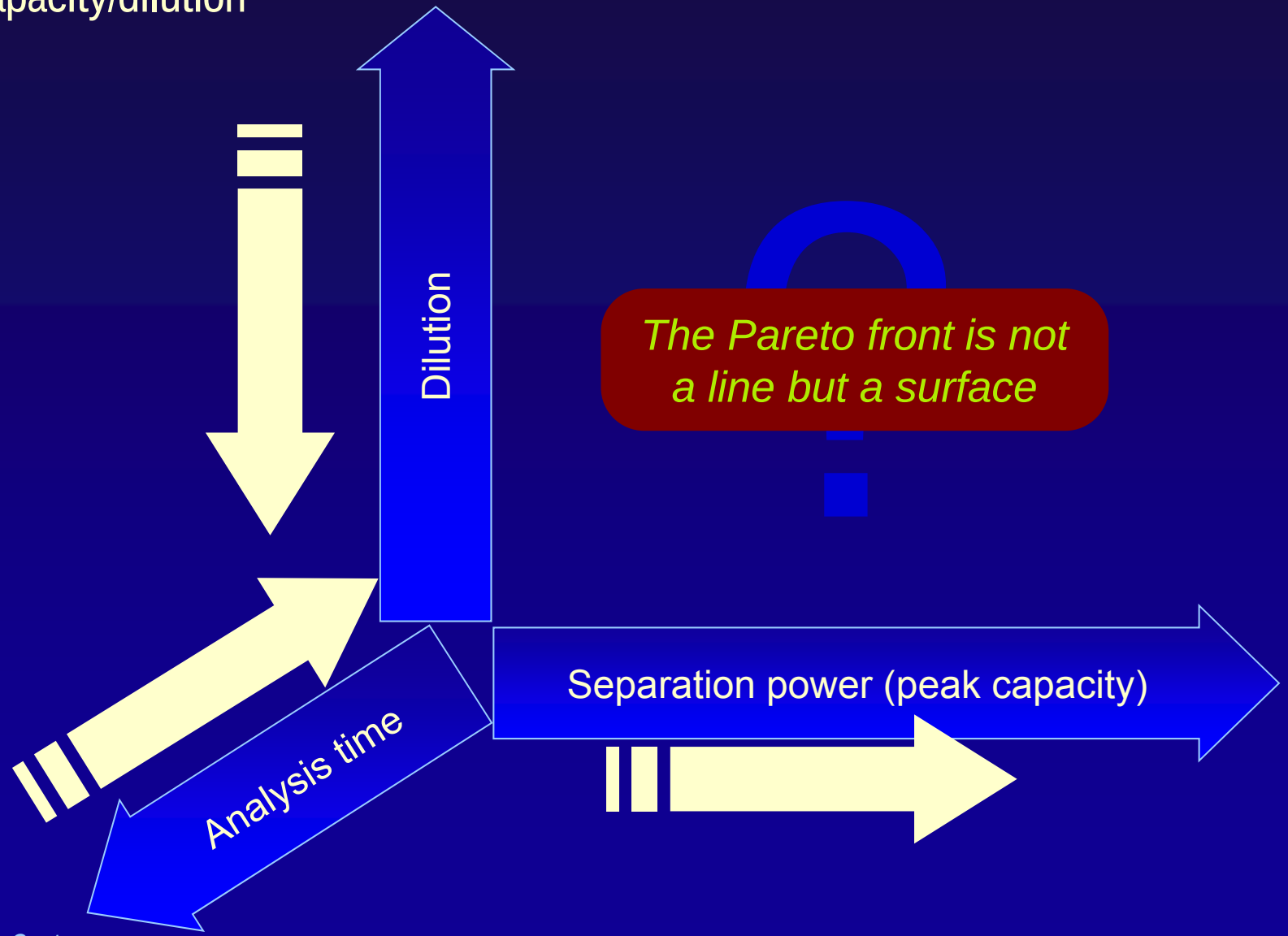
DF = 3.5 for the first dimension

DF = 15 for the second dimension

A trade-off between peak capacity, time and dilution

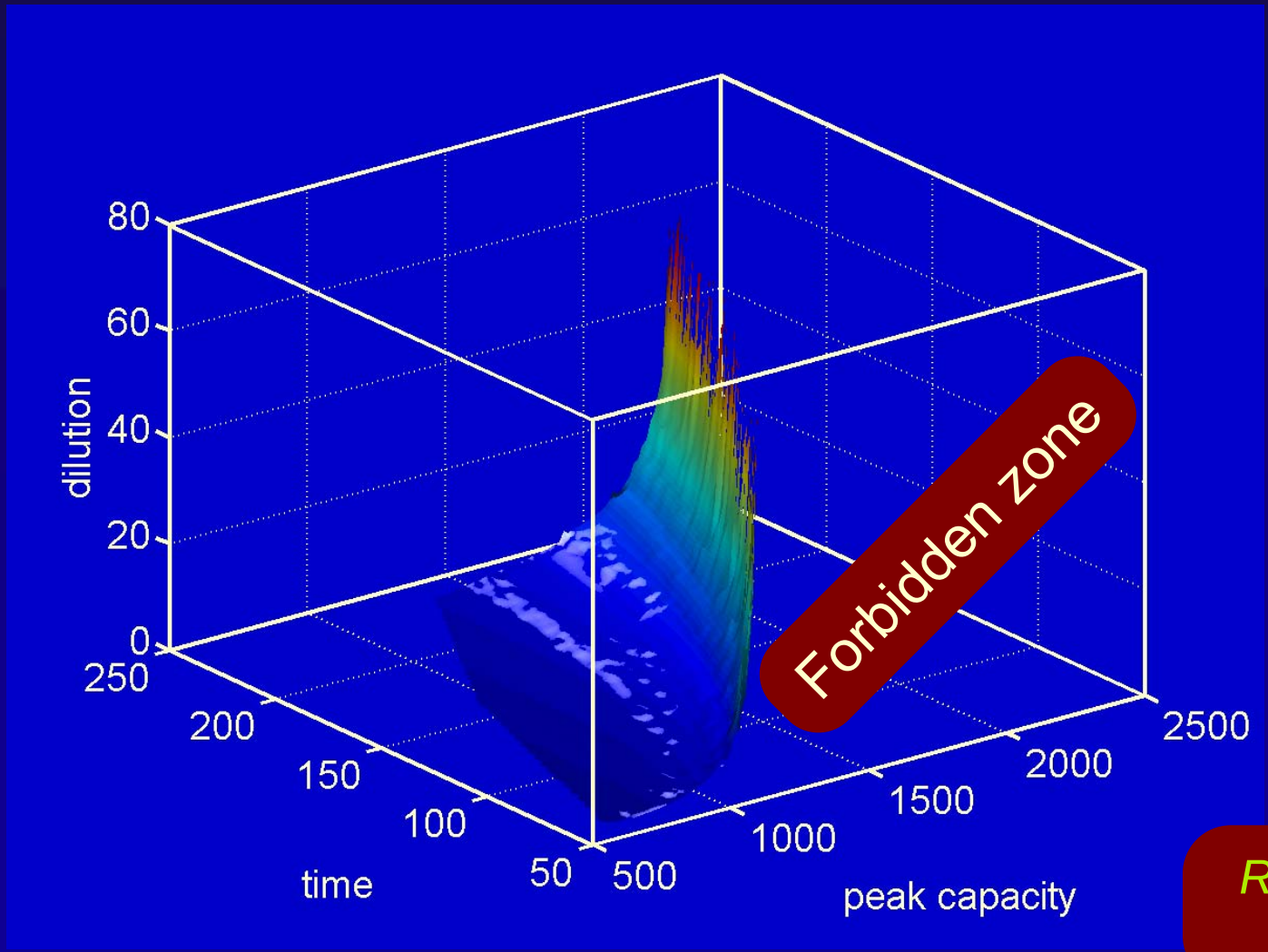
Optimization of time/peak capacity/dilution

Pareto optimization in 2D chromatography



Optimization of time/peak capacity/dilution

Pareto optimization in 2D chromatography



Running late?

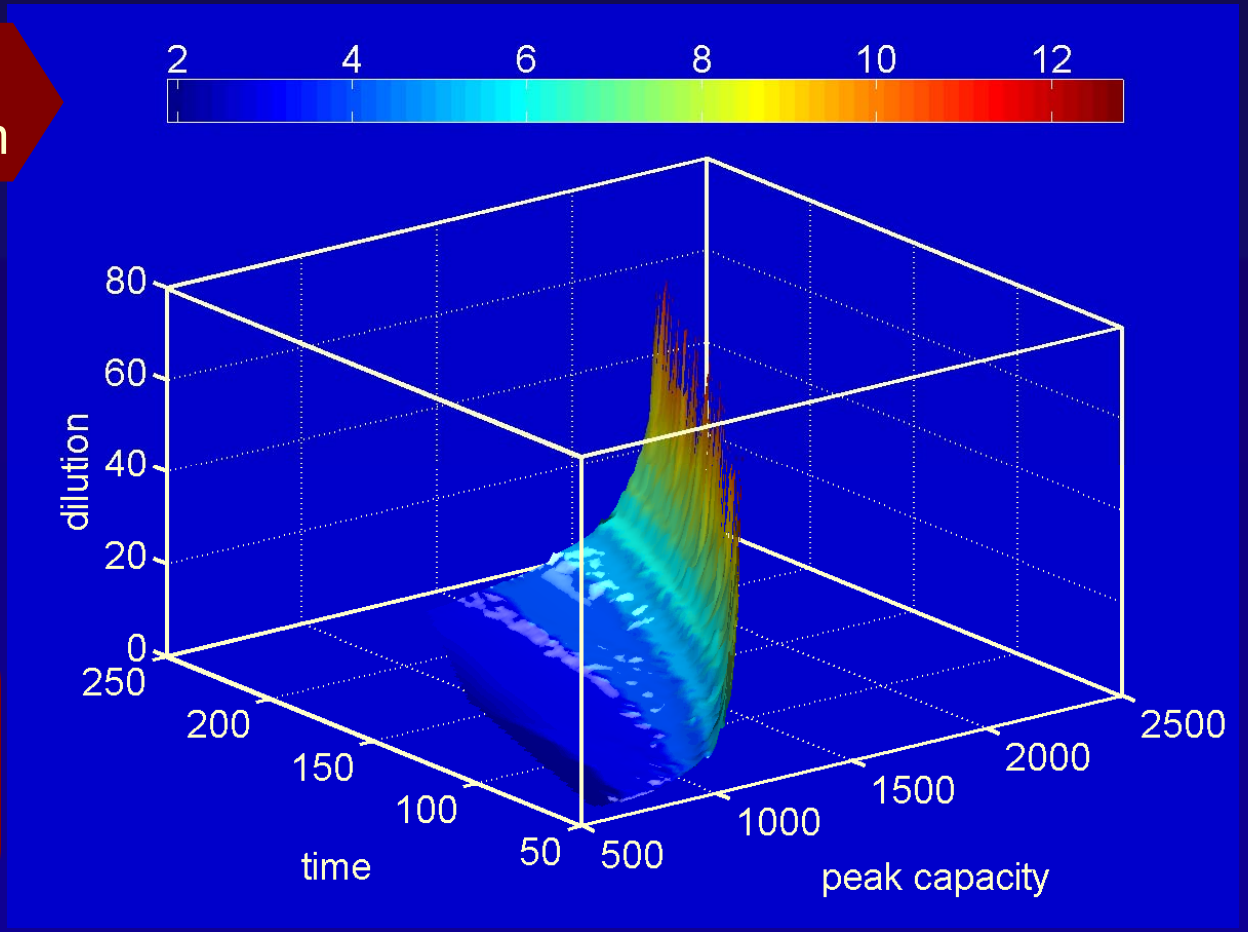
Optimization of time/peak capacity/dilution

Pareto optimization in 2D chromatography

Second-dimension column diameter, mm

Decreasing the column diameter from 10 to 6 mm

Dilution can be significantly reduced with little impact on peak capacity and time

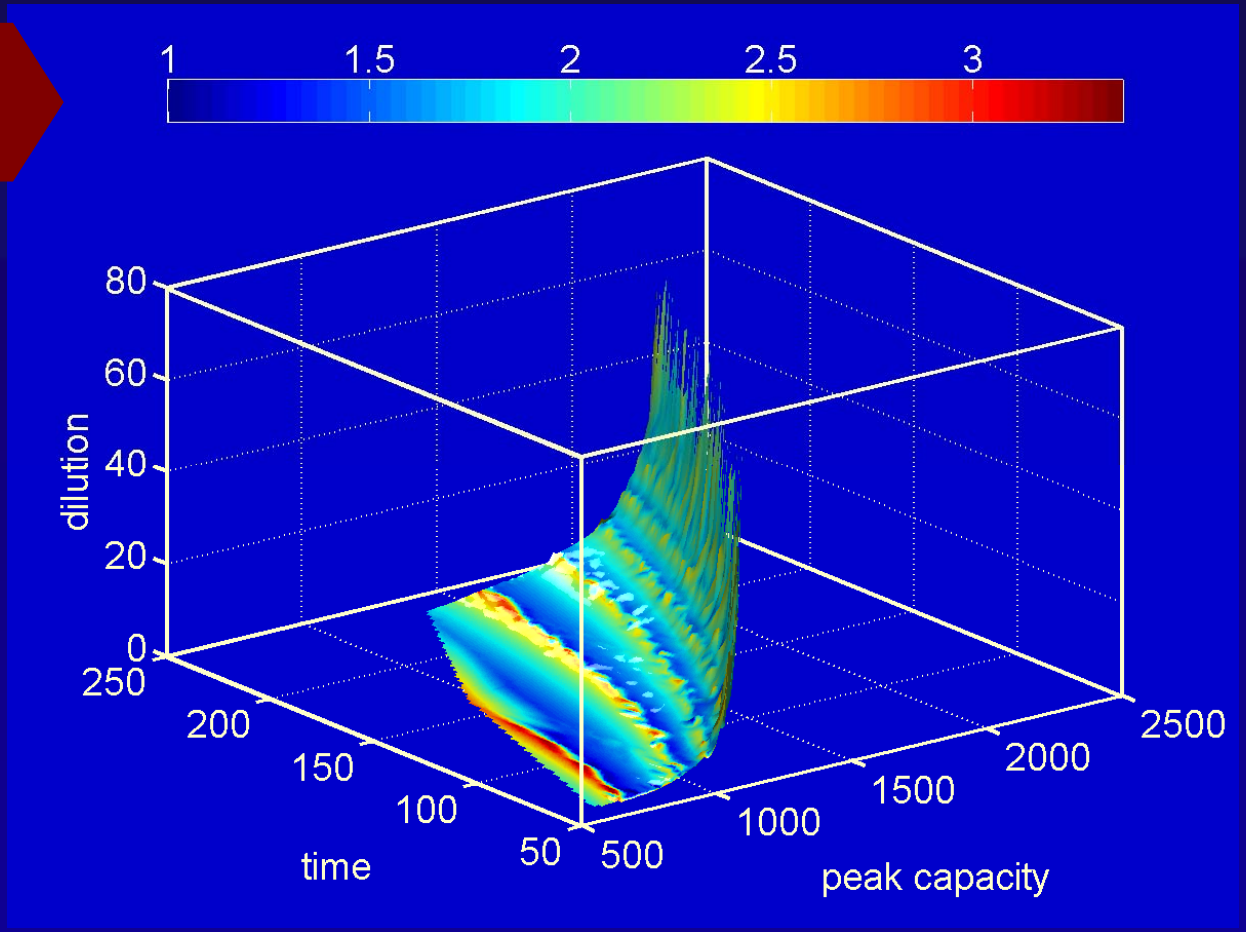


Optimization of time/peak capacity/dilution

Pareto optimization in 2D chromatography

Number of cuts per peak

Optimal number of cuts per peak between 3 and 1

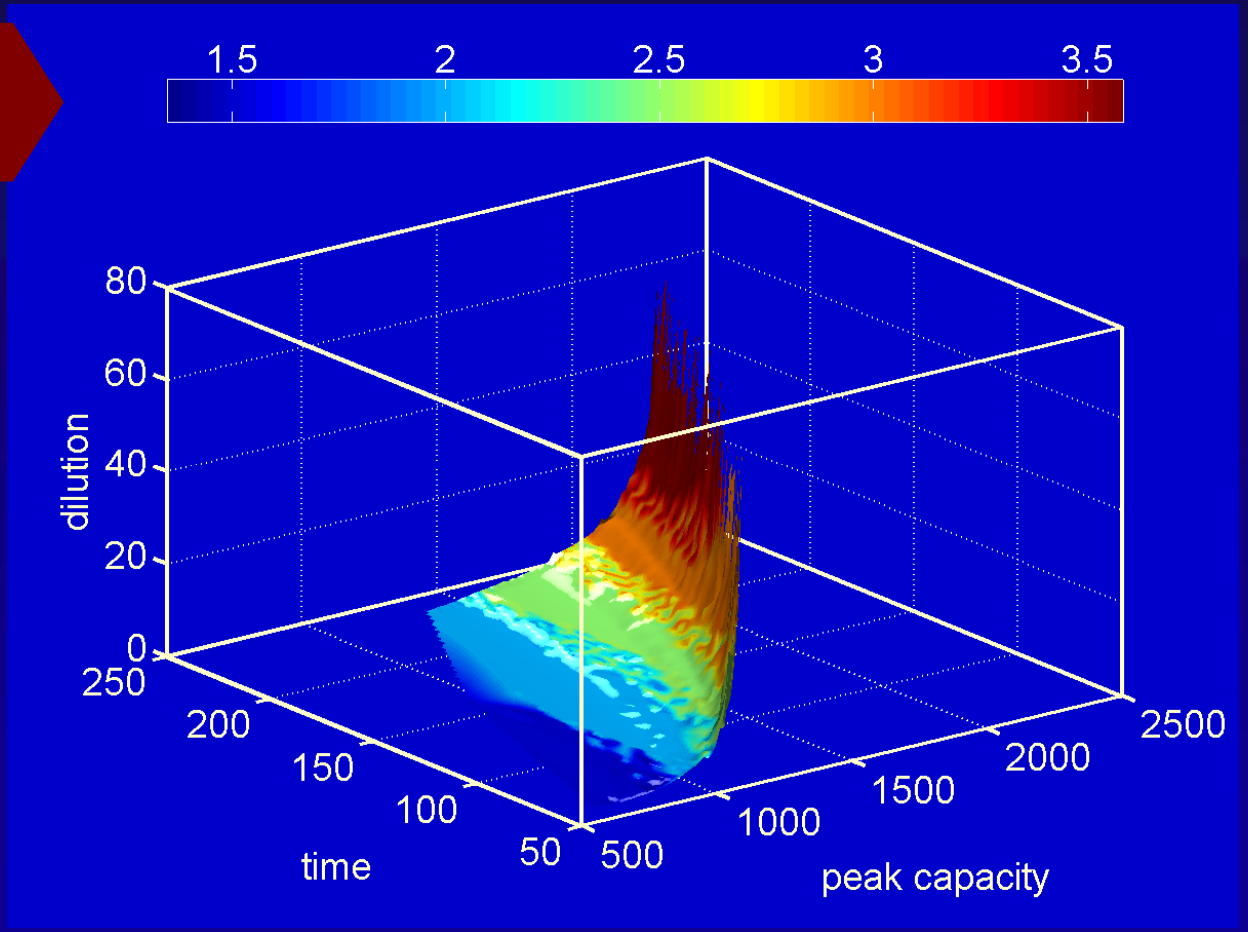


Optimization of time/peak capacity/dilution

Pareto optimization in 2D chromatography

Particle diameter in the first dimension

Dilution in the first dimension is also an issue



Focusing

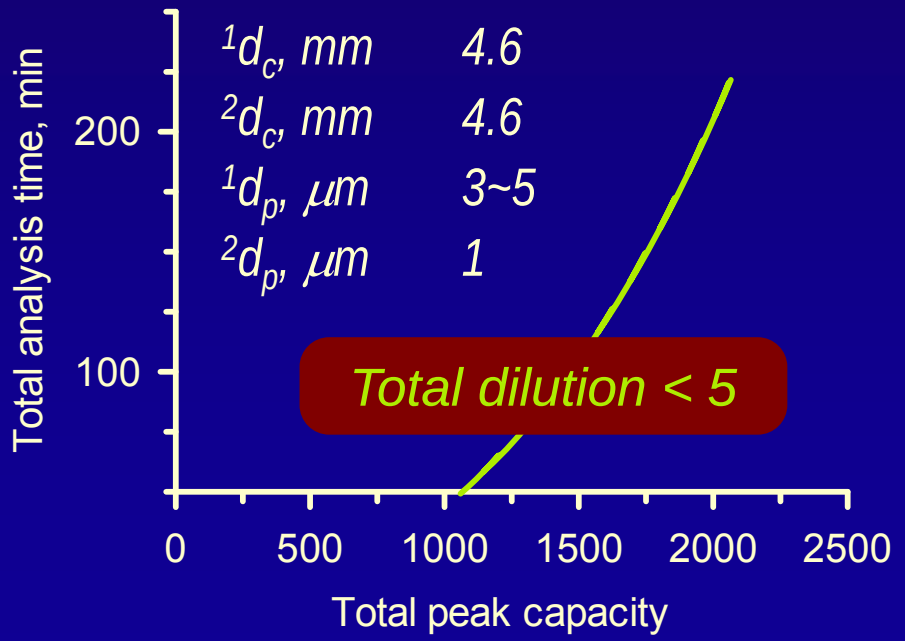
Pareto optimization in 2D chromatography

The benefits of focusing

- Dilution factor in the first dimension reduced
- Injection band broadening in the second dimension significantly reduced
- No need for $^2F >> {}^1F$ to maintain peak capacity in the second dimension → dilution factor in the second dimension reduced

$$ {}^2\sigma_{total} = \sqrt{ {}^2\sigma_{peak}{}^2 + \left(\frac{{}^1F}{{}^2F}\right)^2 \frac{a^2}{\delta^2}} $$

Again, around two cuts per peak are optimal

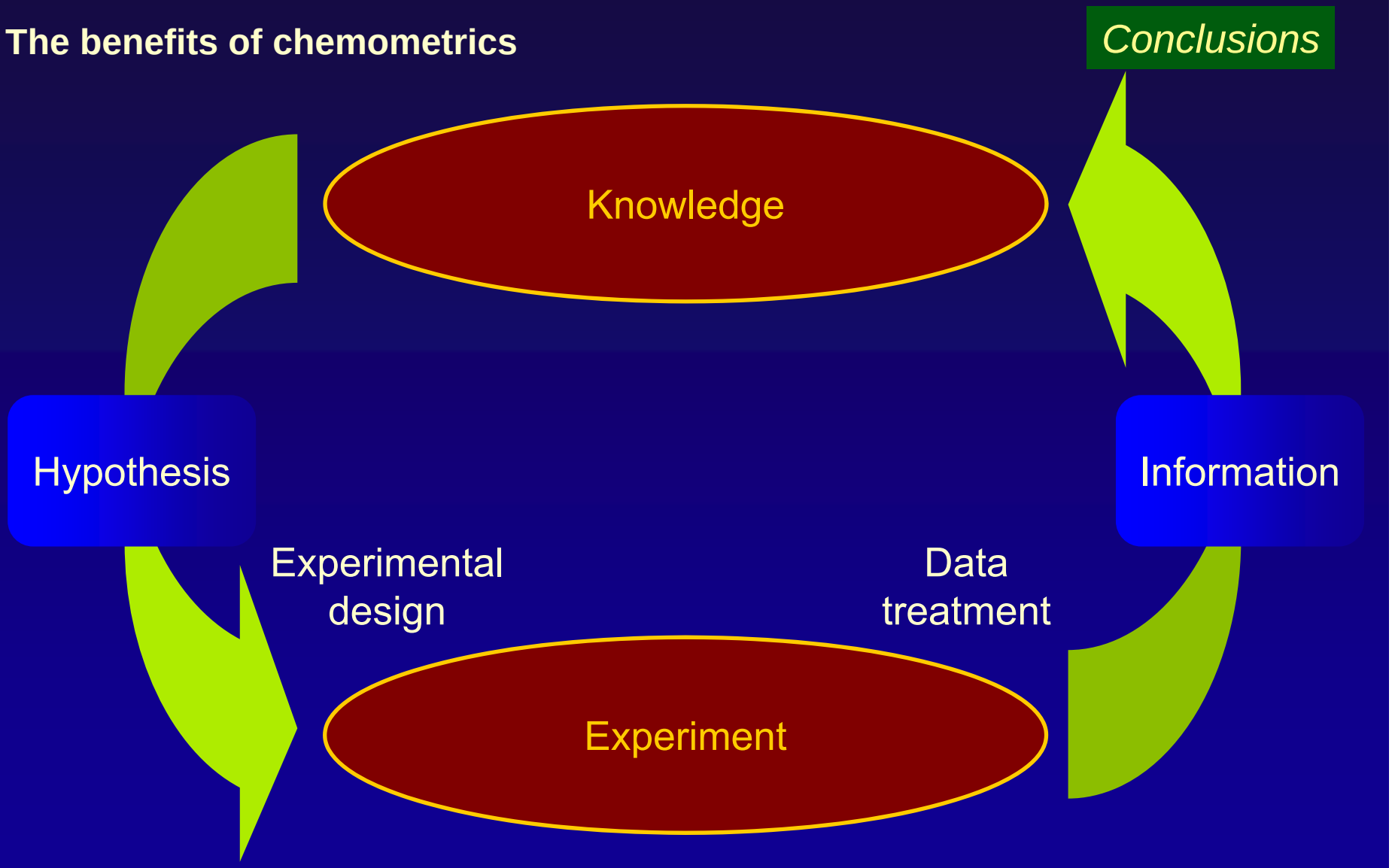


Conclusions

- Pareto optimization ensures that we get the best results from our two-dimensional separations.
- Modulation time has an impact on (first- and second-dimension) peak capacity.
- The optimal number of modulations per peak is around 2 (below the currently accepted values)[1]
- Sensitivity is one of the drawbacks of two-dimensional chromatography (→ work at sub-optimal conditions).
- Focusing should be applied to avoid a trade-off between peak capacity, time, and dilution.

K. Horie *et al*. Anal. Chem. 79 (2007) 3764-3770

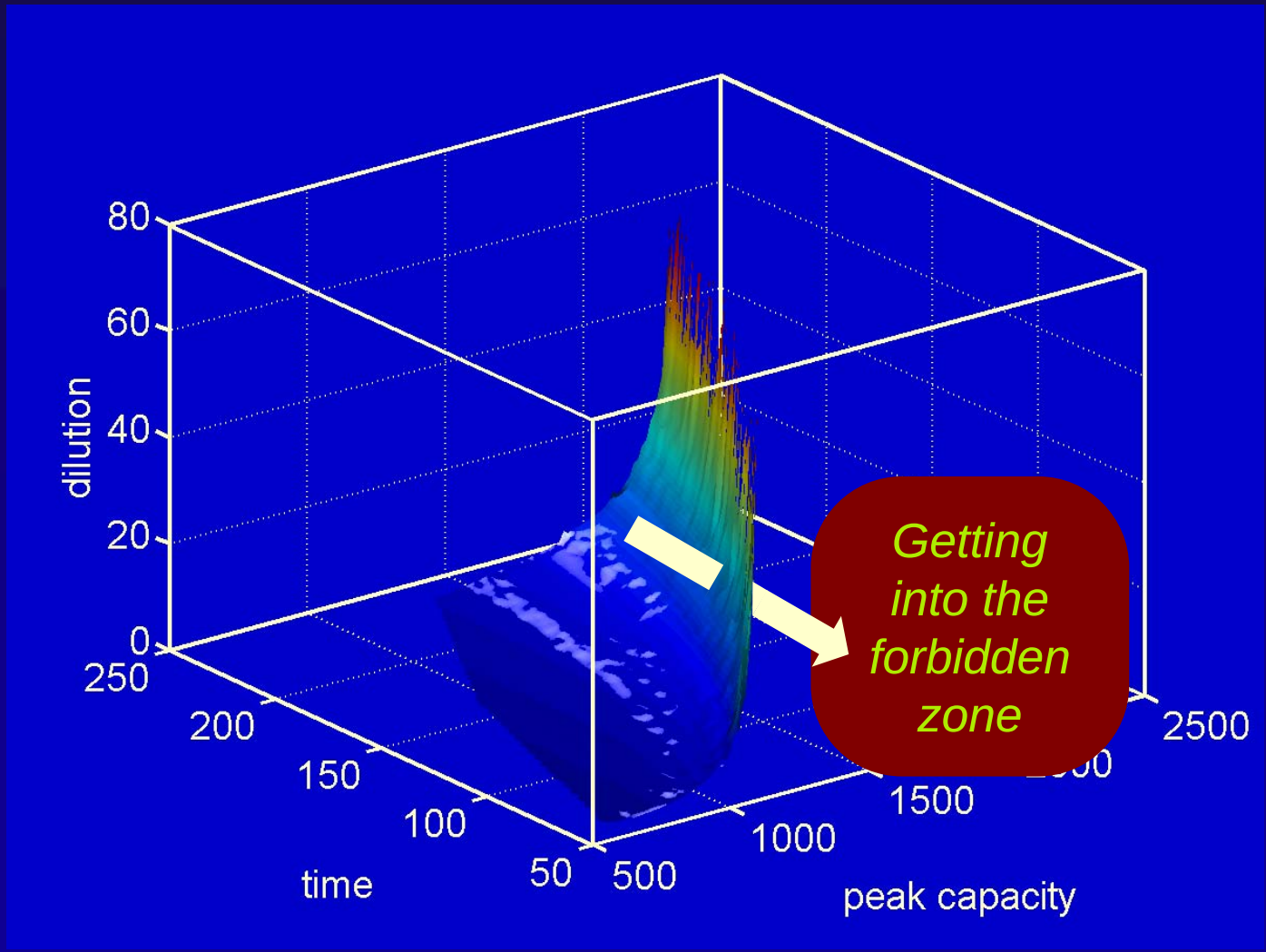
The benefits of chemometrics



In D.L. Massart *et al.*, "Handbook of Chemometrics and Qualimetrics, Part A", Elsevier, Amsterdam, 1997

Conclusions

Decreasing the need for high resolution between peaks: use data treatment (deconvolution) methods!



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