Computerized Design of Robust Separations in HPLC

Applica, Olten
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Introduction

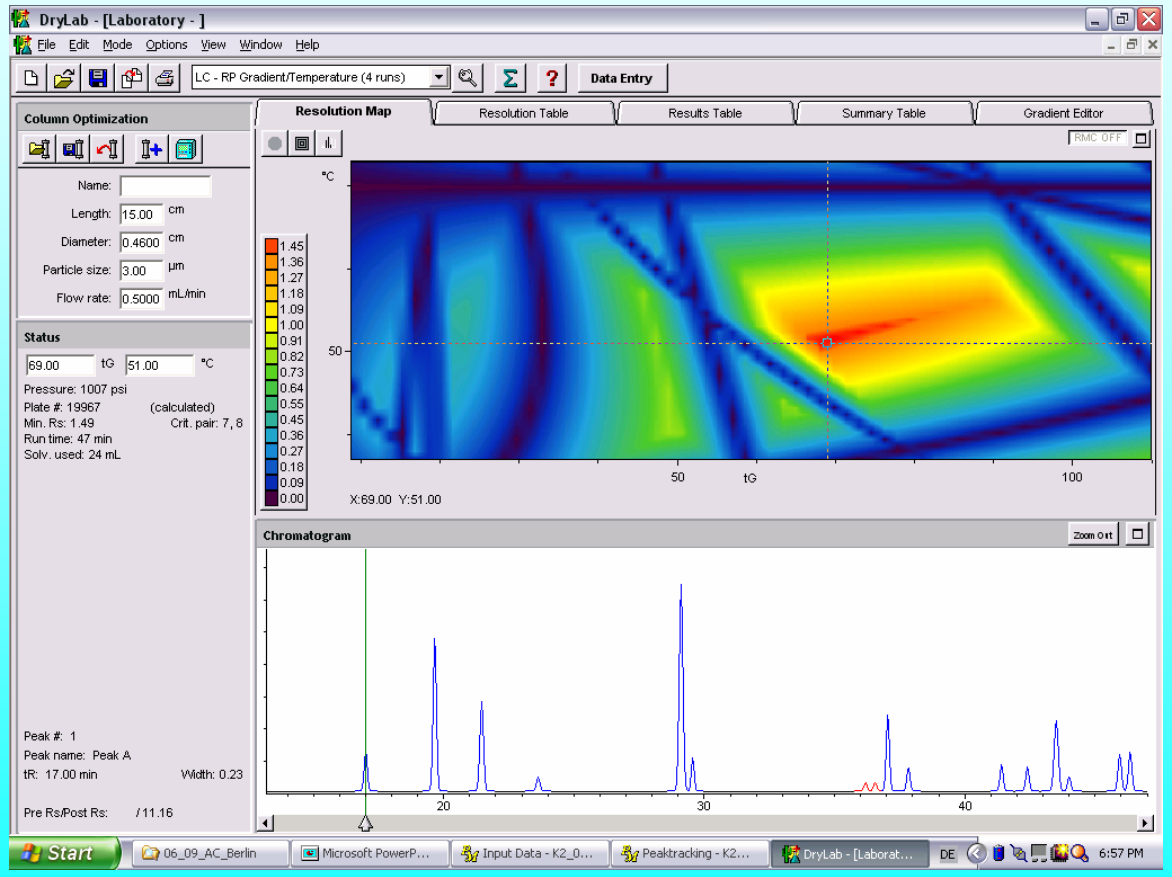
- Csaba Horváth, Yale, RP-HPLC, 1976 -> pH-retention-calculations - ***moving bands***
- Lloyd Snyder, John Dolan, 1986 - present -> Development of DryLab -> plattform of communication about HPLC-methods
- Resolution Maps = Method quality
- Method quality -> Product quality

Themes

- A. Success in HPLC
- B. Systematic RPC
- C. Moving Peaks
- D. Peak Match
- E. Precision
- F. Robustness
- G. Exploiting Column Selectivities

A. Success in HPLC

- Don‘t make lots of experiments ***slow*** but
- reduce the no.of bad experiments ***fast***



30x100 = 3000
bad experiments
can be substituted

better

by
5-10 promising
experiments

B. Systematic RPC

$$\ln k = A + B\mathcal{D} + C\Delta A +$$
$$+ D(K^e - 1)\, V^{2/3}\gamma + E +$$
$$+ \ln (RT/P_oV)$$

$$C = N\gamma /RT \qquad (\gamma \sim \%A = \%H_2O)$$

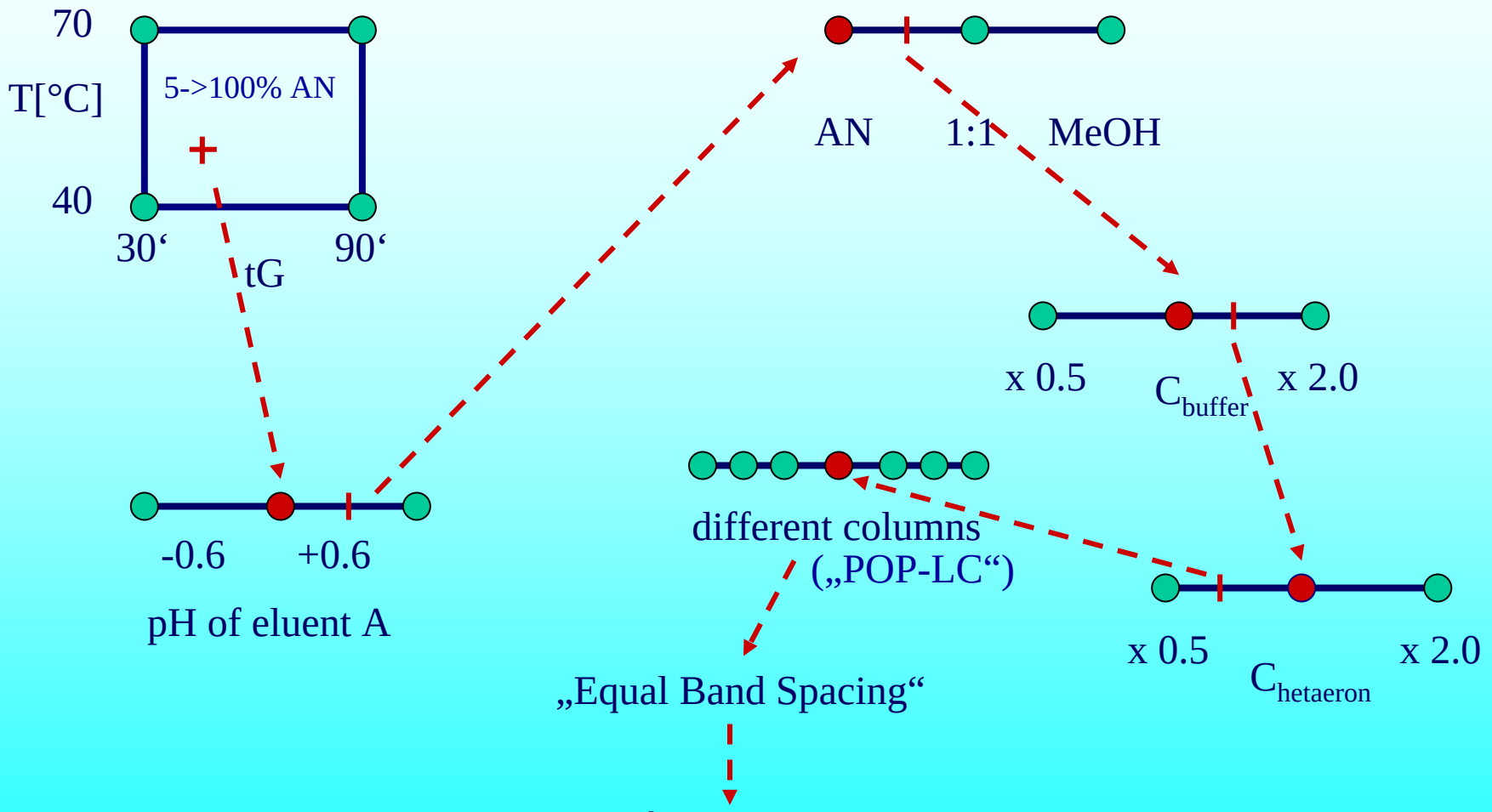
"Solvophobic Theory"

Cs.Horváth, W.Melander, I.Molnar,
J.Chromatogr., **125** (1976) 129.

Systematic RPC

- Learn more with less experiments
- 4x (tG vs T) + 2x pH + 2x ternary composition + 6x diff.columns
- Automated generation of experiments
- Coordinate data in PeakMatch
- Transfer data to DryLab
- Evaluate DryLab-model

Systematic MD-Strategies require for reduced analysis time maximized critical resolution



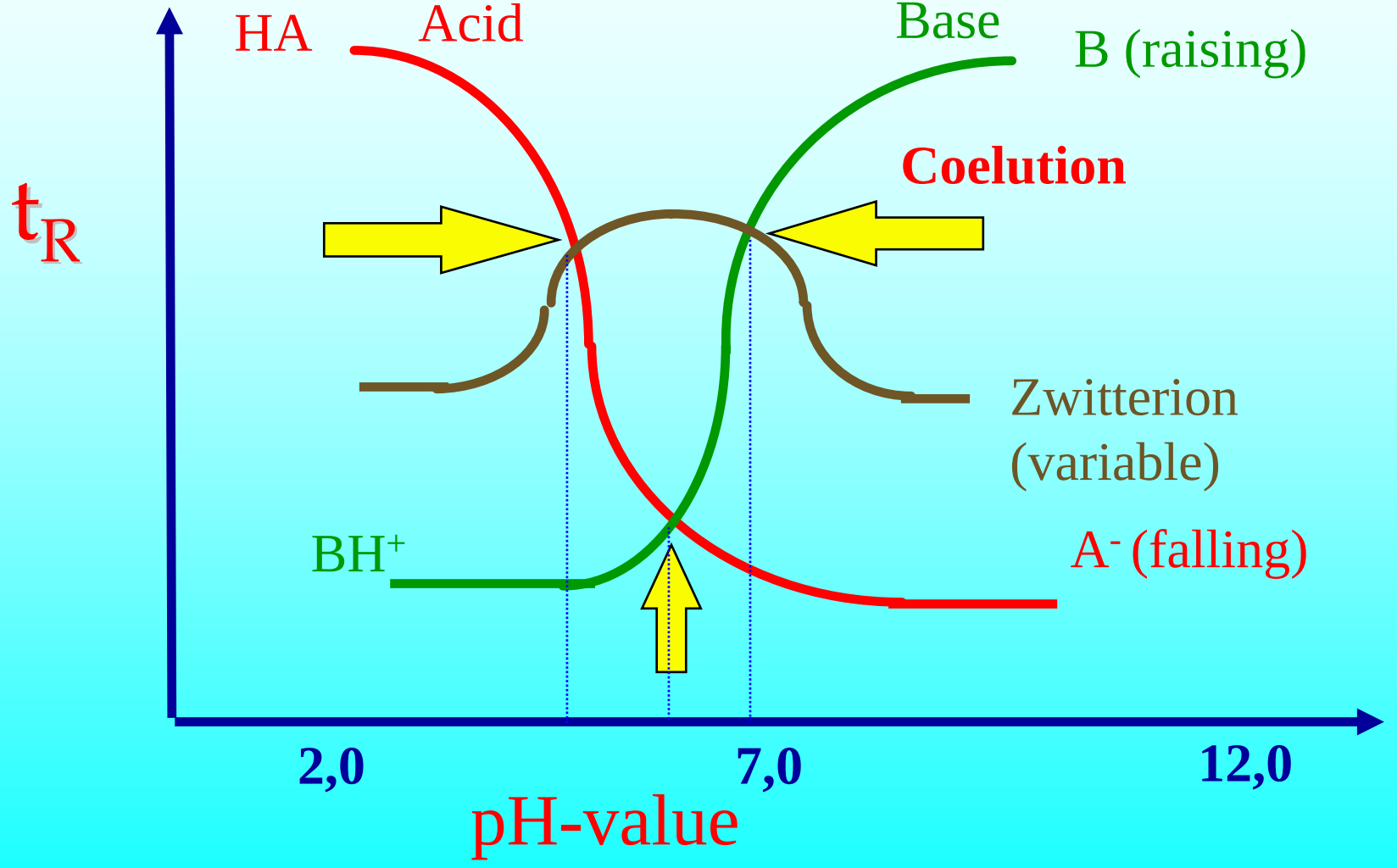
C. Moving peaks: Examples

- Moving peaks with pH - isocratic model
- Moving peaks with pH - gradient model

Controlling a method means to control peak movements

Download examples from homepage: www.molnar-institut.com

Parameter-changes cause ***continuous*** selectivity changes in RPC



Advantages of computerized models of separations

- Transparence of good and bad choices
- Saving the time for unnecessary runs
- Maximized resolution of separations
- Improved communication about working conditions and better development reports
- Method transfer greatly enhanced

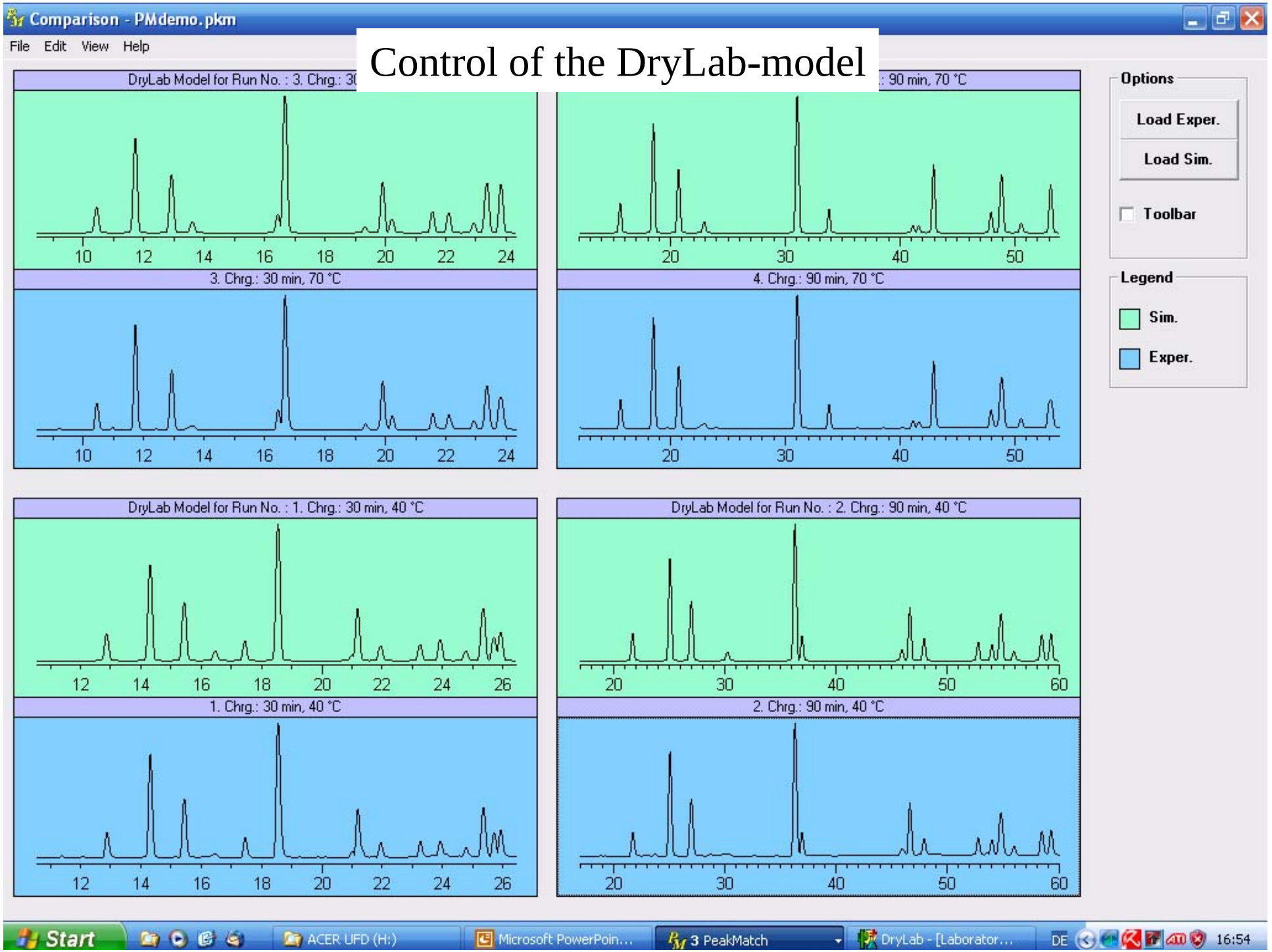
D. Peak Match

- Different runs give often confusing results - challange to go after
- Comparisons are complicated, if trial and error experiments were carried out - systematic work is more economic
- Semi- or full automation is needed to get the data organized and to simplify the process

		1. Chrg.: 30 min, 40 °C		2. Chrg.: 90 min, 40 °C		3. Chrg.: 30 min, 70 °C		4. Chrg.: 90 min, 70 °C		
	Peak Name	tR	Area	tR	Area	tR	Area	tR	Area	StdDev(%)
1	UNKNOWN2	12.83	156	21.63	139	10.40	152	15.50	142	4.7
2	Cpnd 1	14.27	581	24.99	566	11.68	572	18.38	572	0.9
3	Cpnd 2	15.40	371	26.90	366	12.88	370	20.59	368	0.5
4	Cpnd 3	16.43	59	30.14	51	13.57	60	22.81	60	6.6
5	Cpnd 4	17.41	110	36.84	113	16.40	105	33.71	109	2.6
6	Cpnd 5	18.51	873	36.20	857	16.64	898	30.94	874	1.7
7	Cpnd 6	20.96	31	45.80	28	19.30	34	41.04	35	8.6
8	Cpnd 6 Splitted	21.15	31	45.80	31	19.87	31	41.53	38	9.3
9	Cpnd 7	21.15	271	46.52	275	19.87	272	42.83	276	0.8
10	Cpnd 8	21.91	88	47.79	90	20.18	83	42.83	90	3.3
11	Cpnd 9	23.22	91	52.66	94	21.52	100	47.84	106	5.9
12	Cpnd 10	23.89	90	53.88	89	22.06	105	48.76	87	7.7
	Sum of peak areas		**3471**		**3390**		**3493**		**3426**	**1.2**

Peak Match (cont.)

- Chromatograms + Tables on the same page
- Tools and colours to reduce errors
- Automated data transfer to DryLab
- Comparison of original- w. model-chromatograms
- Automated location of optimum conditions
- Robustness calculations


Comparison - PMdemo.pkm
File Edit View Help
Control of the DryLab-model
DryLab Model for Run No. : 3. Chrg.: 3
: 90 min, 70 °C
3. Chrg.: 30 min, 70 °C
4. Chrg.: 90 min, 70 °C
DryLab Model for Run No. : 1. Chrg.: 30 min, 40 °C
DryLab Model for Run No. : 2. Chrg.: 90 min, 40 °C
1. Chrg.: 30 min, 40 °C
2. Chrg.: 90 min, 40 °C
Options
Load Exper.
Load Sim.
Toolbar
Legend
Sim.
Exper.
Start
ACER UFD (H:)
Microsoft PowerPoin...
3 PeakMatch
DryLab - [Laborator...
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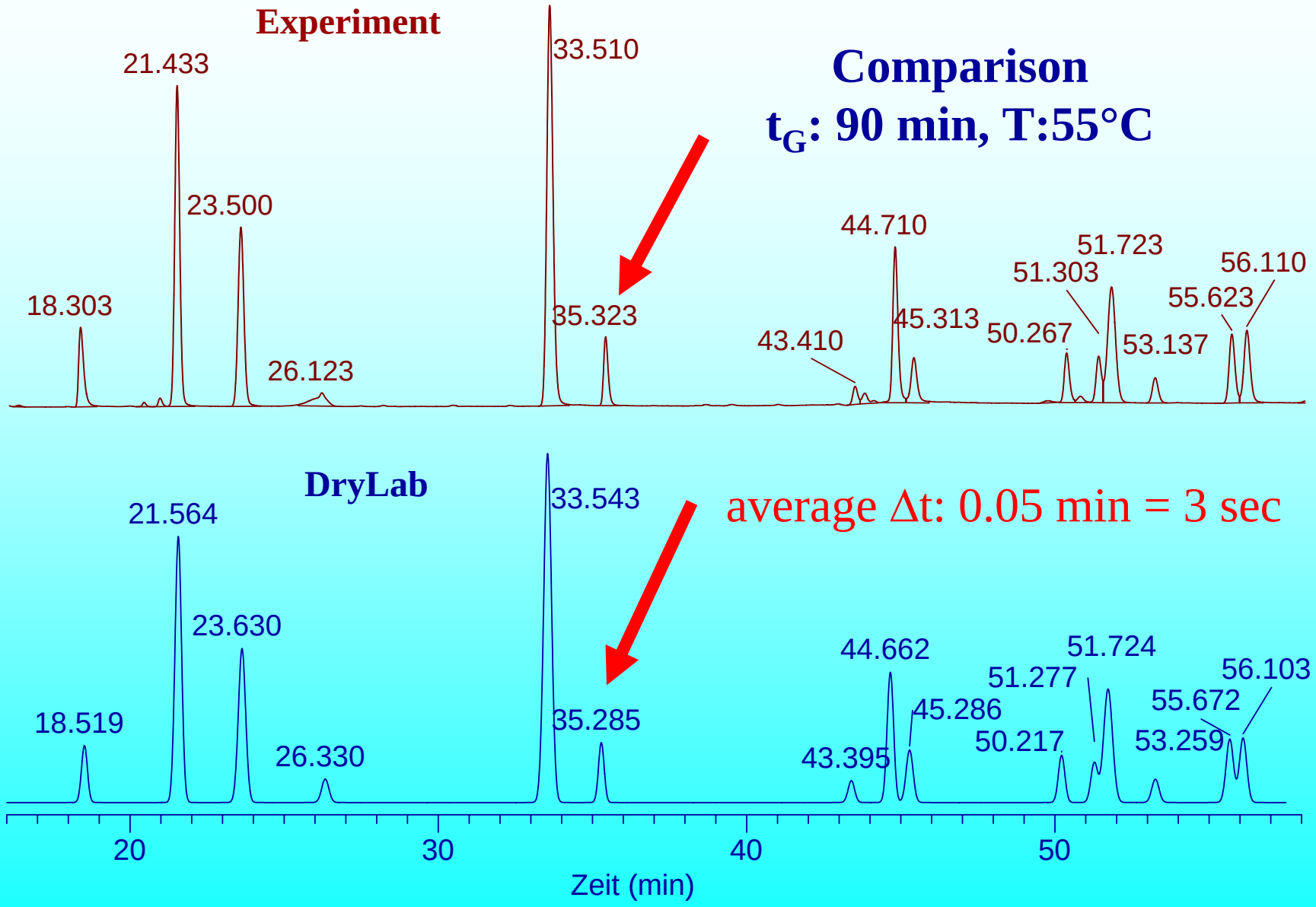
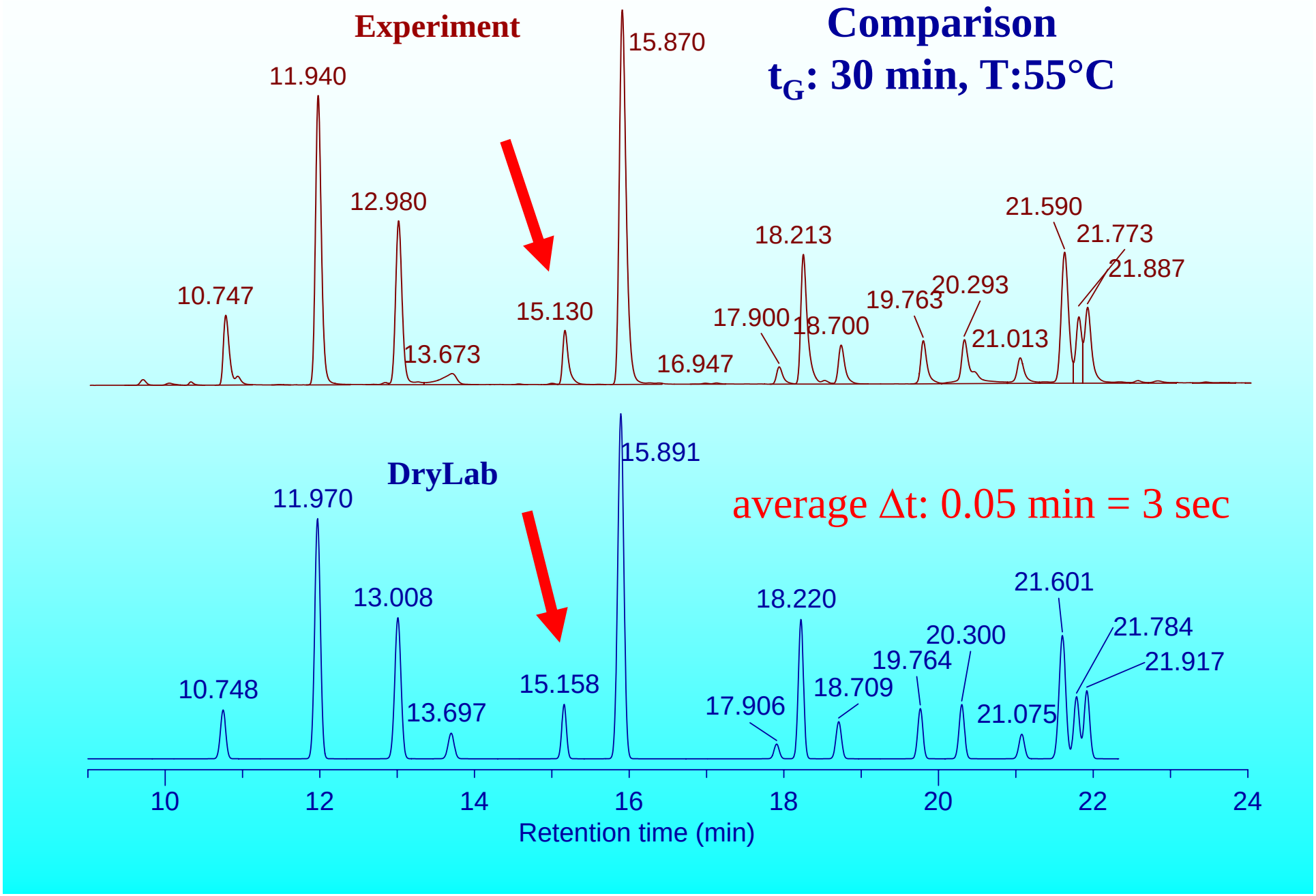
E. Precision of predicted data

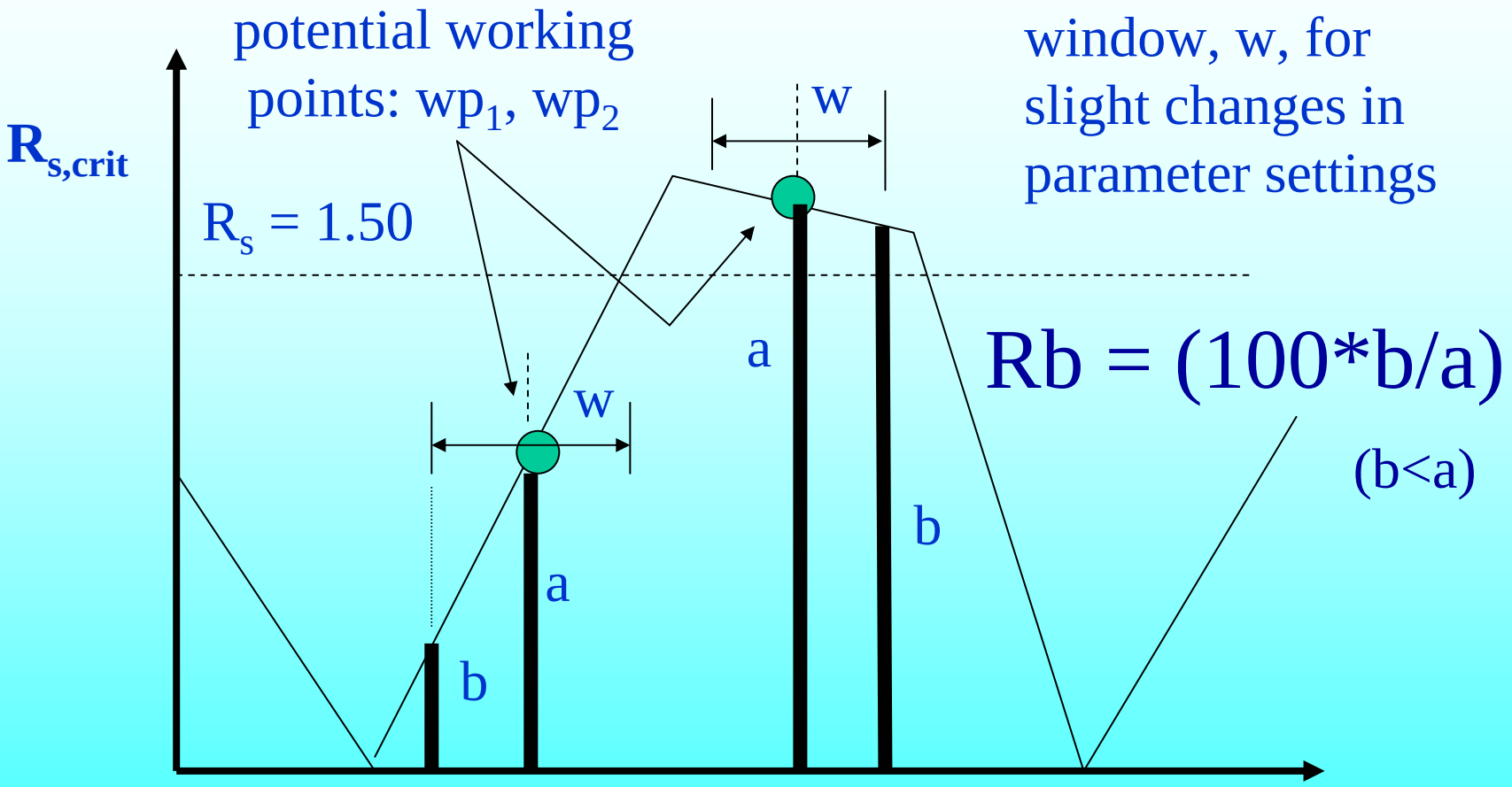



Experiment
Comparison
t_G: 30 min, T:55°C
10.747
11.940
12.980
13.673
15.130
15.870
16.947
17.900
18.213
18.700
19.763
20.293
21.013
21.590
21.773
21.887
DryLab
average Δt: 0.05 min = 3 sec
10.748
11.970
13.008
13.697
15.158
15.891
17.906
18.220
18.709
19.764
20.300
21.075
21.601
21.784
21.917
10
12
14
16
18
20
22
24
Retention time (min)

F. Robustness

- Capability of the method to remain stable at small variations of the experimental parameters
- A method is robust, if it is chromatographically safe, and gives us reliable results. ***Incorrect methods can not be robust***
- Robustness can be visualized by maps of the critical resolution in the vicinity of the working point

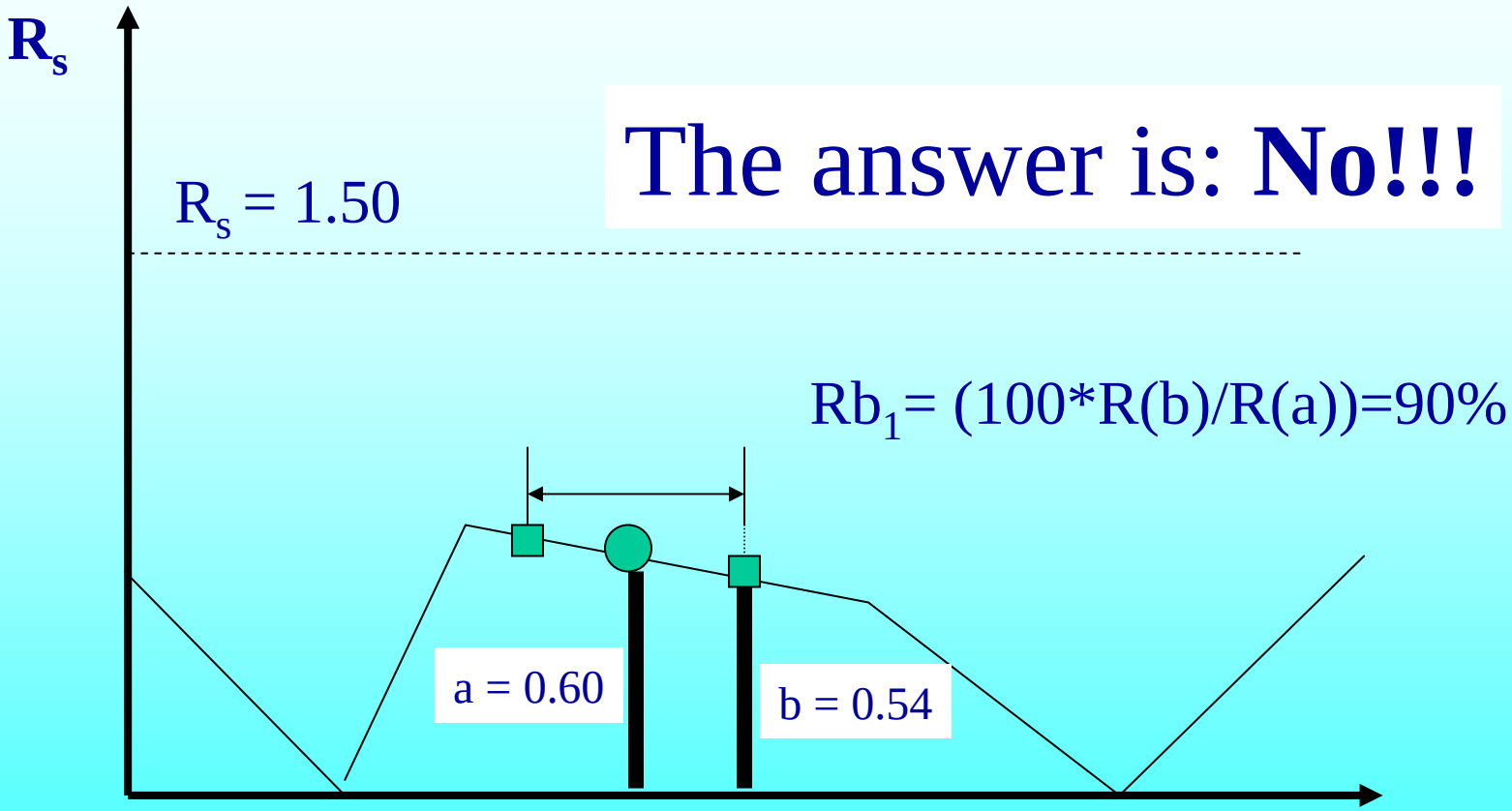
Estimation of Robustness



Chromatographic variables

(pH, t_G, %B, temp., buffer conc., etc.)

Is this method robust?



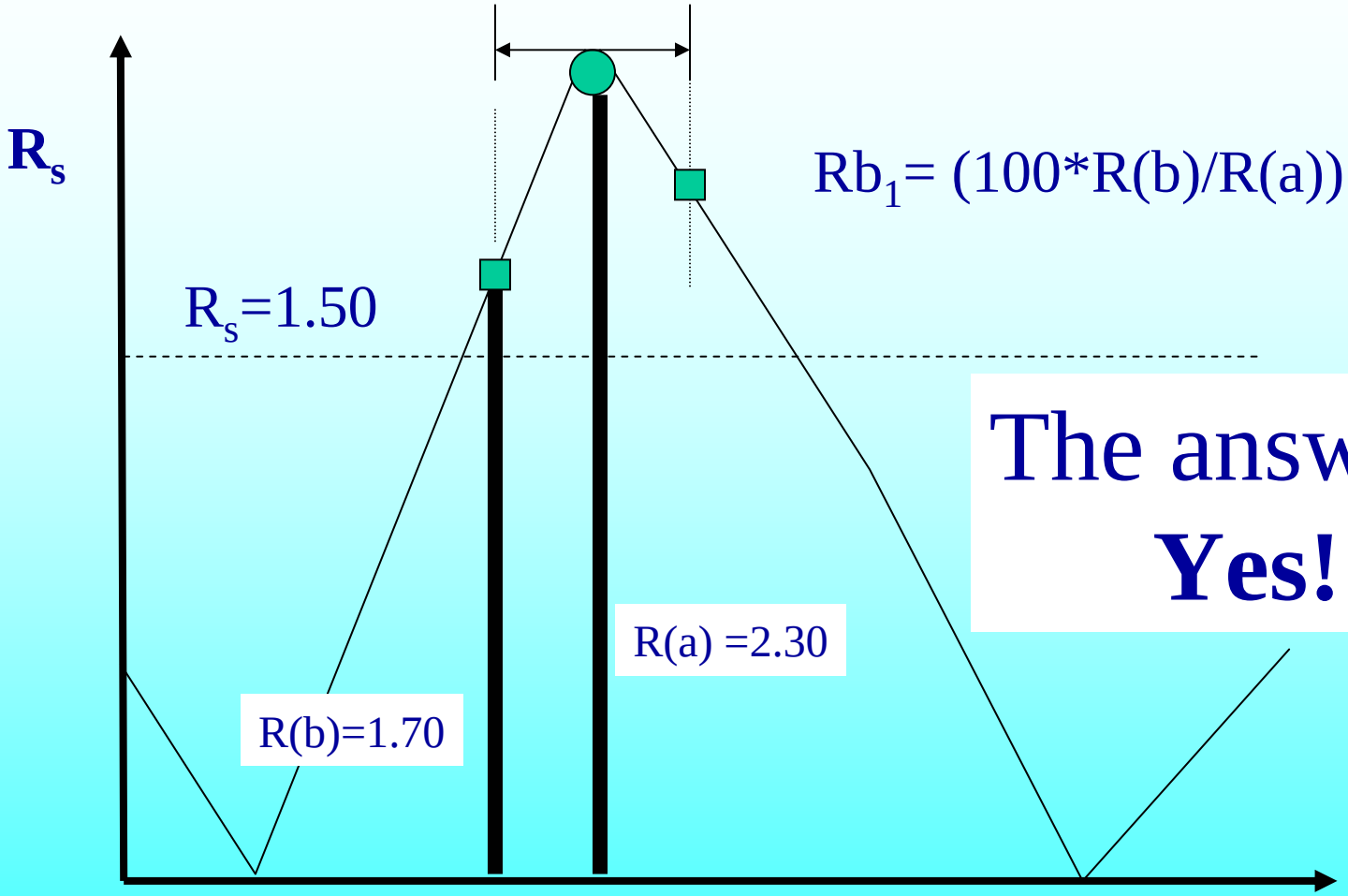
The answer is: **No!!!**

Rb_1= (100*R(b)/R(a))=90%

Chromatographic variables

(pH, tG, %B, temp., buffer conc., etc)

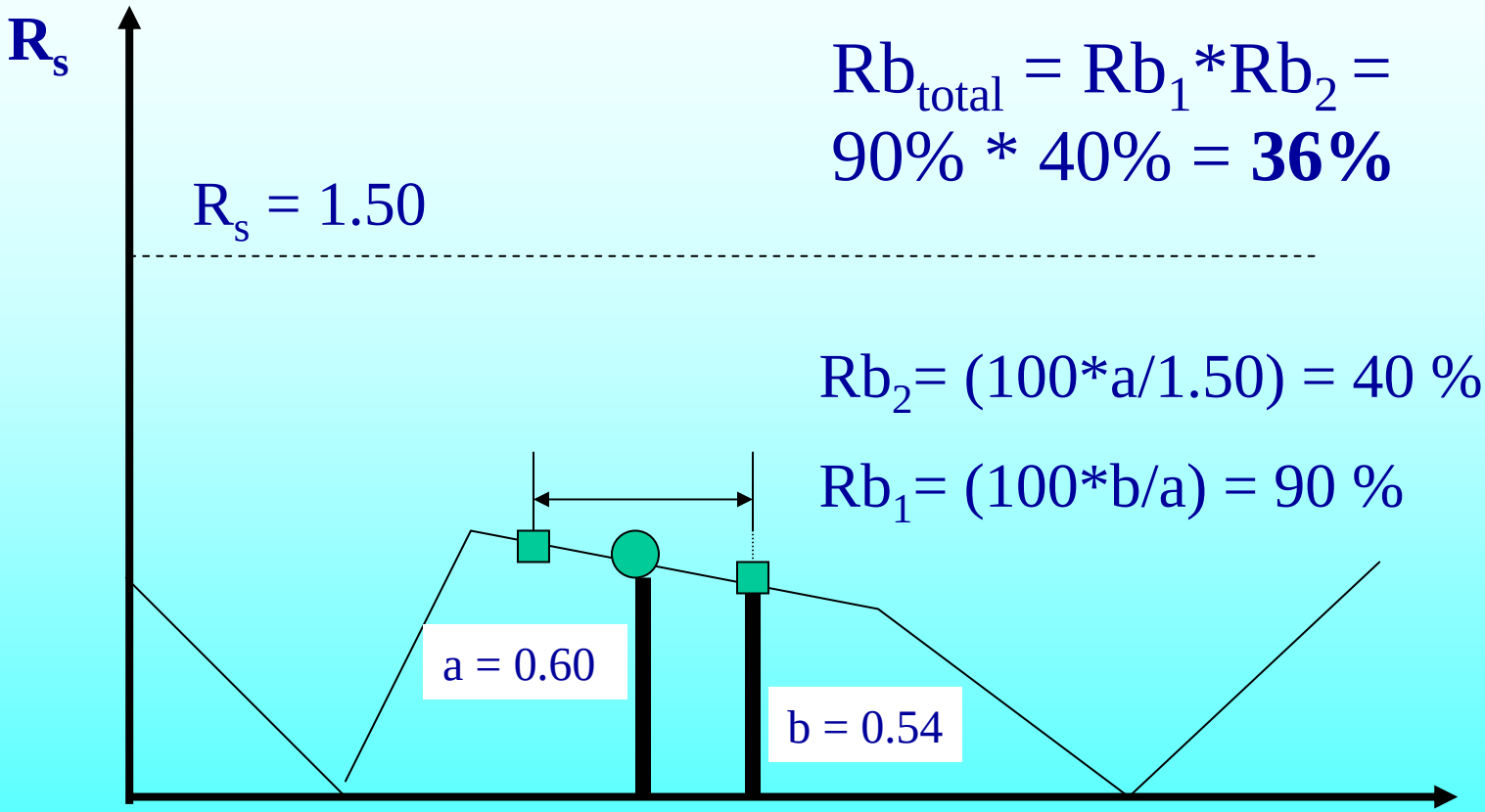
Is this method robust?



The answer is:
Yes!!!

Chromatographic variables
(pH, tG, %B, temp., buffer conc., etc)

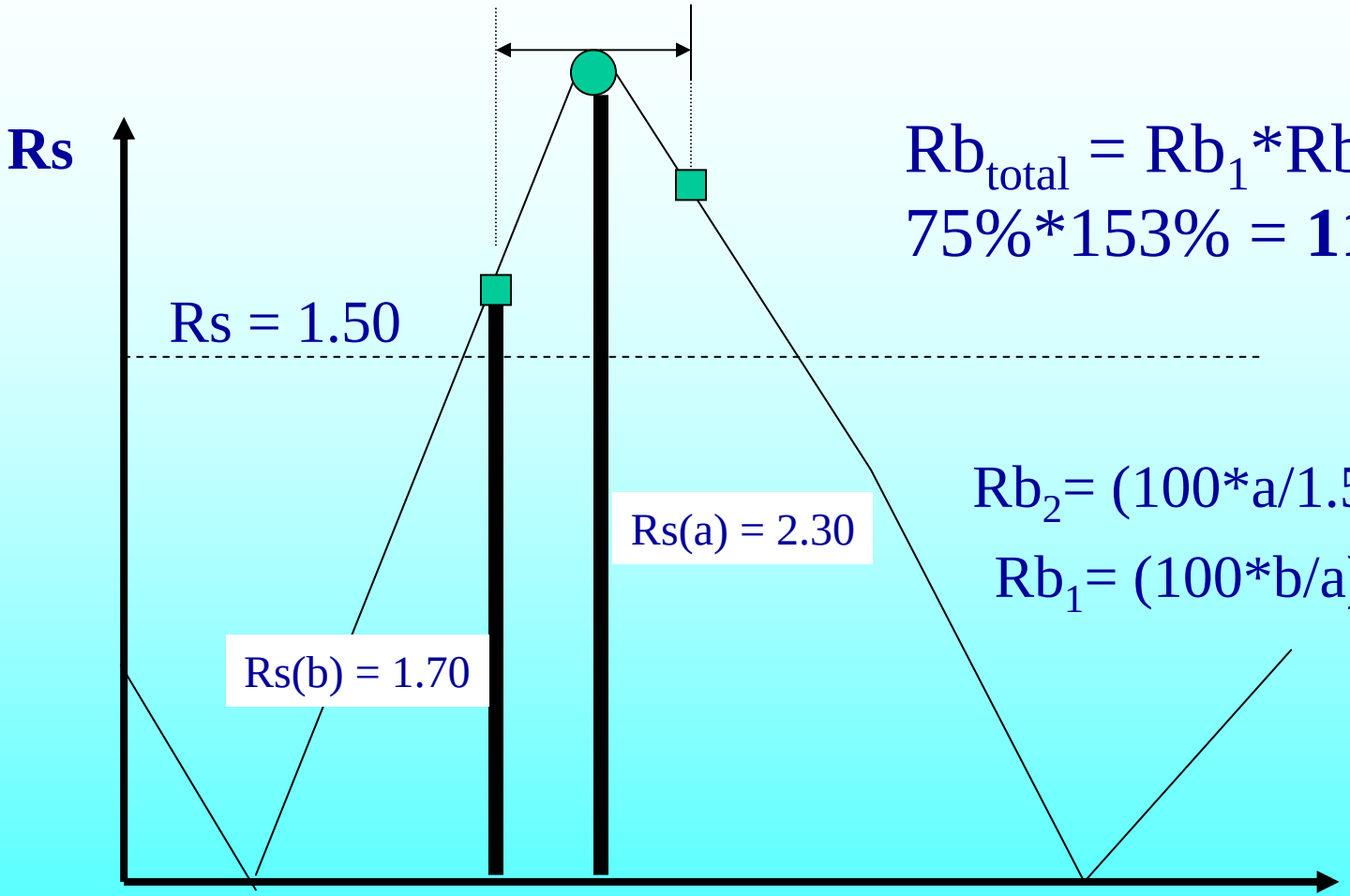
How robust is this method?



Chromatographic variables

(pH, tG, %B, temp., buffer conc., etc)

How robust is this method?



$Rb_{total} = Rb_1 * Rb_2 =$
$75\% * 153\% =$ **115%**

$Rb_2 = (100 * a/1.5) = 153\%$

$Rb_1 = (100 * b/a) = 75\%$

Chromatographic variables
(pH, tG, %B, temp., buffer conc., etc)

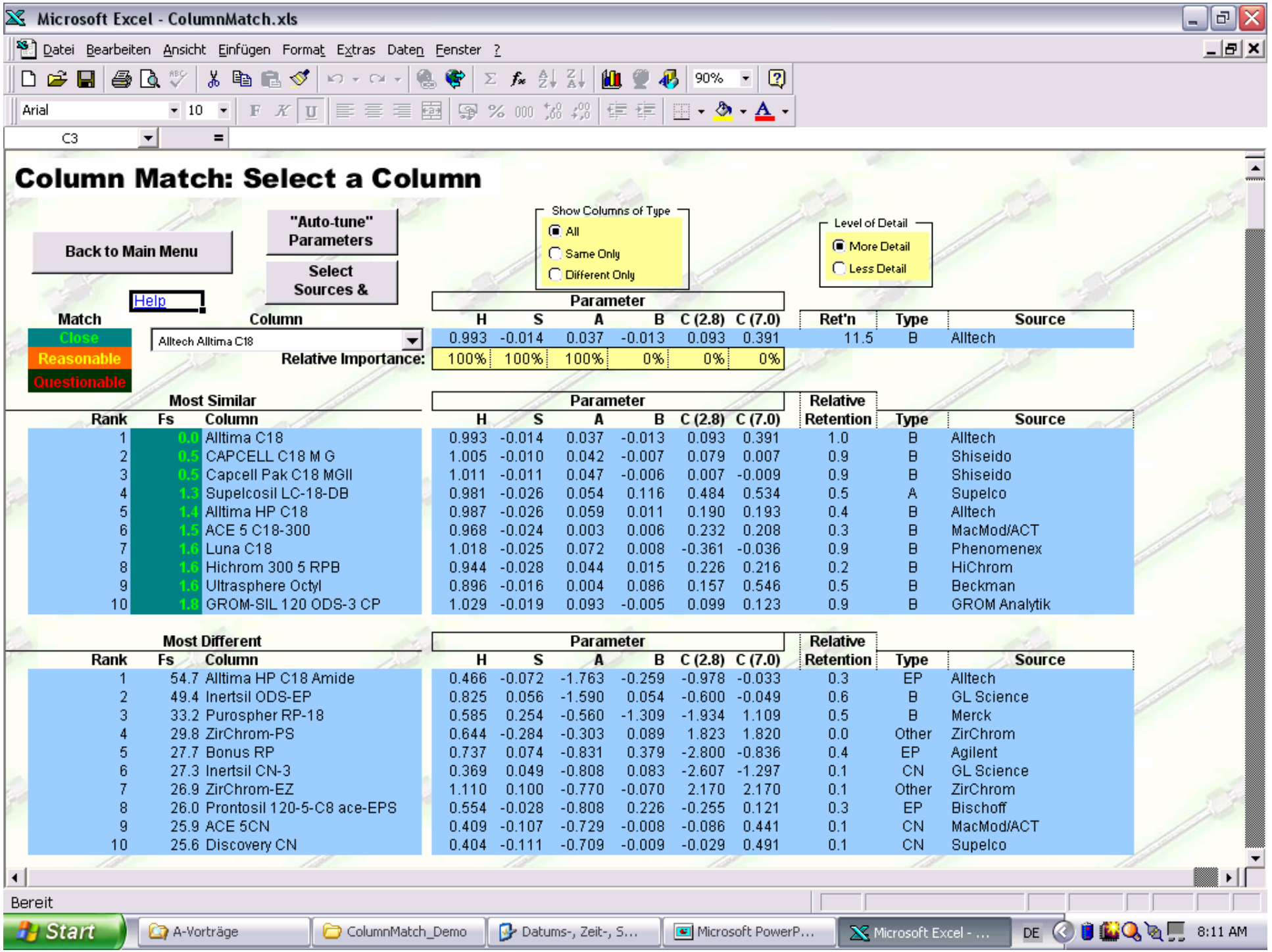
Contributions to robustness

- Contribution Rb_1 from the slope of $R_{s,crit}$ in the vicinity of the working point vs. the lower R_s-value in the working window
- Contribution Rb_2 from the absolute value of $R_{s,crit}$ at the working point. $R_{s,crit} = 1.50$ is equal to $Rb_2 = 100\%$

$$Rb_{total} = Rb_1 * Rb_2$$

G. Exploiting Column Selectivities
Column Match® Database

- Database with 300 columns using 6 Parameter
- Compares the 10 most similar and the 10 most different columns
- Selection of sample classes (acids, bases)


Microsoft Excel - ColumnMatch.xls
Datei Bearbeiten Ansicht Einfügen Format Extras Daten Fenster ?
Arial 10
C3 =
Column Match: Select a Column
Back to Main Menu
"Auto-tune" Parameters
Select Sources &
Help
Show Columns of Type
All
Same Only
Different Only
Level of Detail
More Detail
Less Detail
Match
Close
Reasonable
Questionable
Column
Alltech Alltima C18
Relative Importance:
Parameter
H S A B C (2.8) C (7.0)
Ret'n Type Source
0.993 -0.014 0.037 -0.013 0.093 0.391 11.5 B Alltech
100% 100% 100% 0% 0% 0%
Most Similar
Rank Fs Column
Parameter
H S A B C (2.8) C (7.0)
Relative Retention Type Source
1 0.0 Alltima C18 0.993 -0.014 0.037 -0.013 0.093 0.391 1.0 B Alltech
2 0.5 CAPCELL C18 M G 1.005 -0.010 0.042 -0.007 0.079 0.007 0.9 B Shiseido
3 0.5 Capcell Pak C18 MGII 1.011 -0.011 0.047 -0.006 0.007 -0.009 0.9 B Shiseido
4 1.3 Supelcosil LC-18-DB 0.981 -0.026 0.054 0.116 0.484 0.534 0.5 A Supelco
5 1.4 Alltima HP C18 0.987 -0.026 0.059 0.011 0.190 0.193 0.4 B Alltech
6 1.5 ACE 5 C18-300 0.968 -0.024 0.003 0.006 0.232 0.208 0.3 B MacMod/ACT
7 1.6 Luna C18 1.018 -0.025 0.072 0.008 -0.361 -0.036 0.9 B Phenomenex
8 1.6 Hichrom 300 5 RPB 0.944 -0.028 0.044 0.015 0.226 0.216 0.2 B HiChrom
9 1.6 Ultrasphere Octyl 0.896 -0.016 0.004 0.086 0.157 0.546 0.5 B Beckman
10 1.8 GROM-SIL 120 ODS-3 CP 1.029 -0.019 0.093 -0.005 0.099 0.123 0.9 B GROM Analytik
Most Different
Rank Fs Column
Parameter
H S A B C (2.8) C (7.0)
Relative Retention Type Source
1 54.7 Alltima HP C18 Amide 0.466 -0.072 -1.763 -0.259 -0.978 -0.033 0.3 EP Alltech
2 49.4 Inertsil ODS-EP 0.825 0.056 -1.590 0.054 -0.600 -0.049 0.6 B GL Science
3 33.2 Purospher RP-18 0.585 0.254 -0.560 -1.309 -1.934 1.109 0.5 B Merck
4 29.8 ZirChrom-PS 0.644 -0.284 -0.303 0.089 1.823 1.820 0.0 Other ZirChrom
5 27.7 Bonus RP 0.737 0.074 -0.831 0.379 -2.800 -0.836 0.4 EP Agilent
6 27.3 Inertsil CN-3 0.369 0.049 -0.808 0.083 -2.607 -1.297 0.1 CN GL Science
7 26.9 ZirChrom-EZ 1.110 0.100 -0.770 -0.070 2.170 2.170 0.1 Other ZirChrom
8 26.0 Prontosil 120-5-C8 ace-EPS 0.554 -0.028 -0.808 0.226 -0.255 0.121 0.3 EP Bischoff
9 25.9 ACE 5CN 0.409 -0.107 -0.729 -0.008 -0.086 0.441 0.1 CN MacMod/ACT
10 25.6 Discovery CN 0.404 -0.111 -0.709 -0.009 -0.029 0.491 0.1 CN Supelco
Bereit
Start
A-Vorträge
ColumnMatch_Demo
Datums-, Zeit-, S...
Microsoft PowerP...
Microsoft Excel - ...
DE
8:11 AM

Summary

- Systematic experiments are more economic than trial and error
- Semi-automation makes model setup easy
- Visible method quality in resolution maps
- Smooth method transfer
- Method robustness quantified
- Shorter analysis time
- More reliable product quality

Thanks to

Lloyd Snyder

John Dolan

Tom Jupille

Hans-Jürgen Rieger

More information:
www.molnar-institut.com