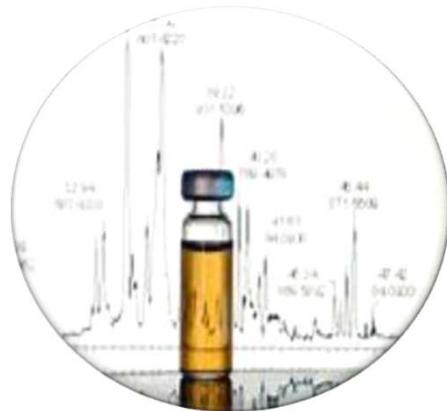


Quality by Design in der Pharmaanalytik:

Anwendung von QbD in der RP-HPLC-Methodenwicklung kleiner Moleküle



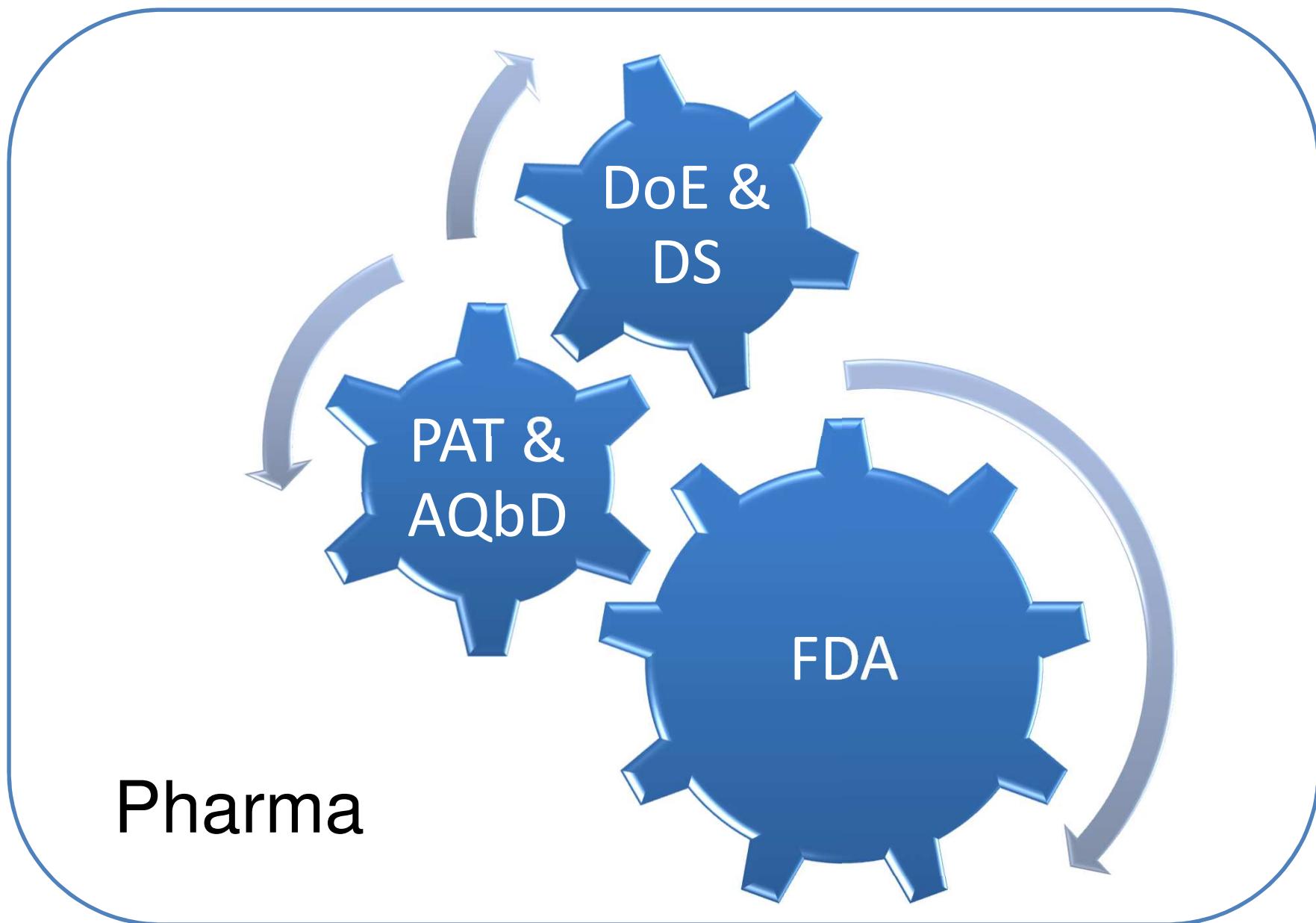
Dr. Hans-Werner Bilke

*LC-Pharm-RP-HPLC Expert Service
Brannenburg*

Dipl. Ing. (FH) Stefan Moser

Process Optimization Nußdorf am Inn

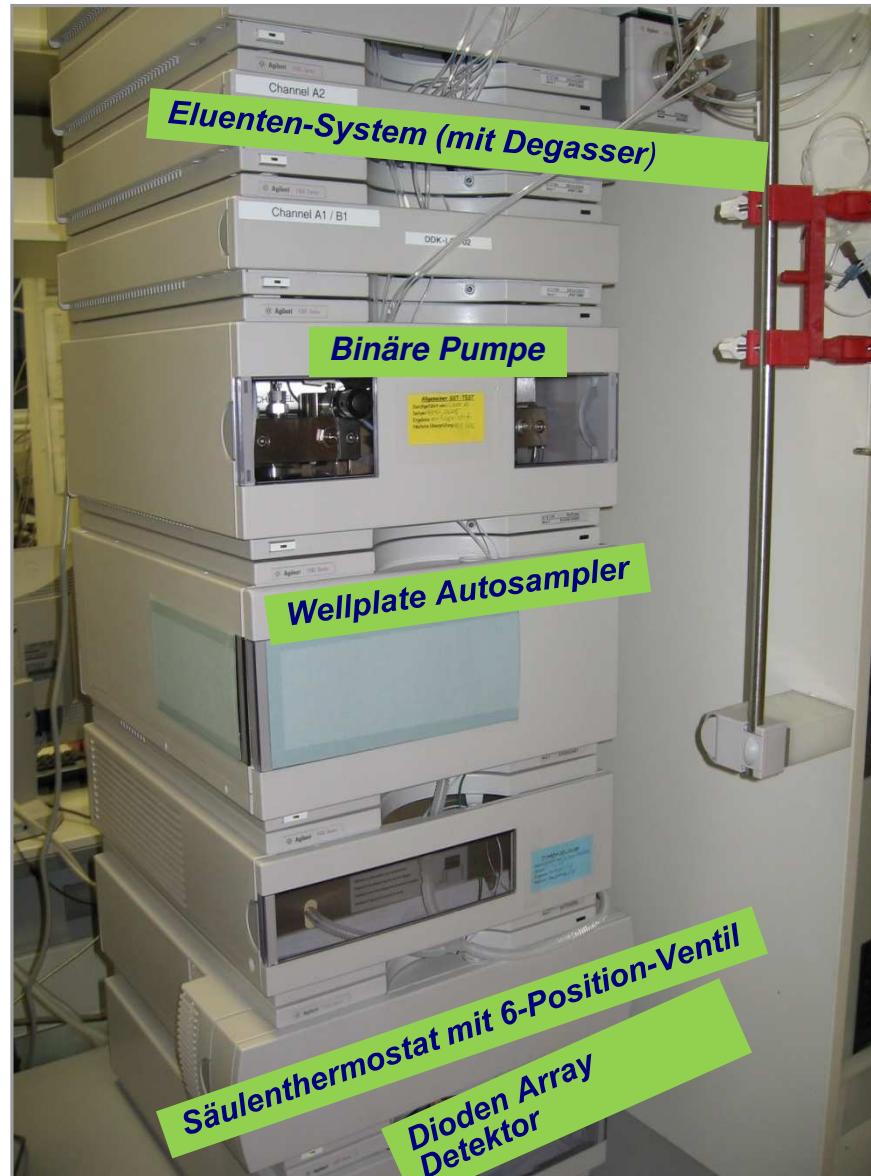
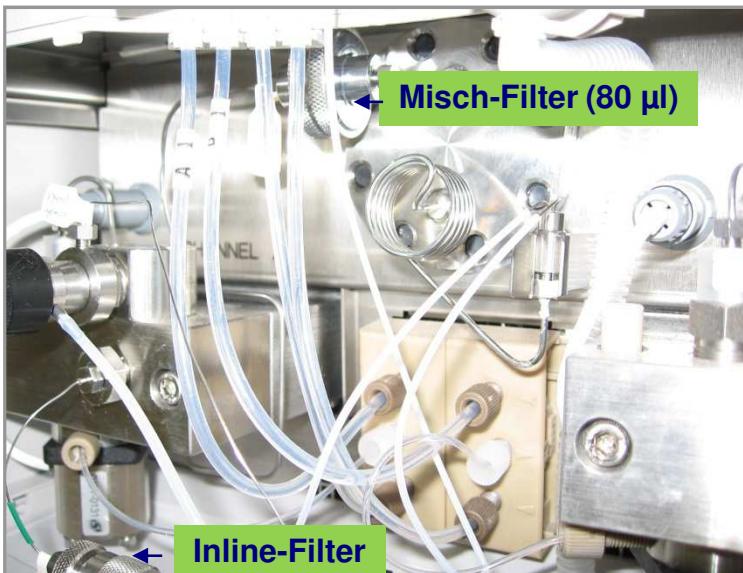
QbD, DoE und DS in der Pharmanalytik ?



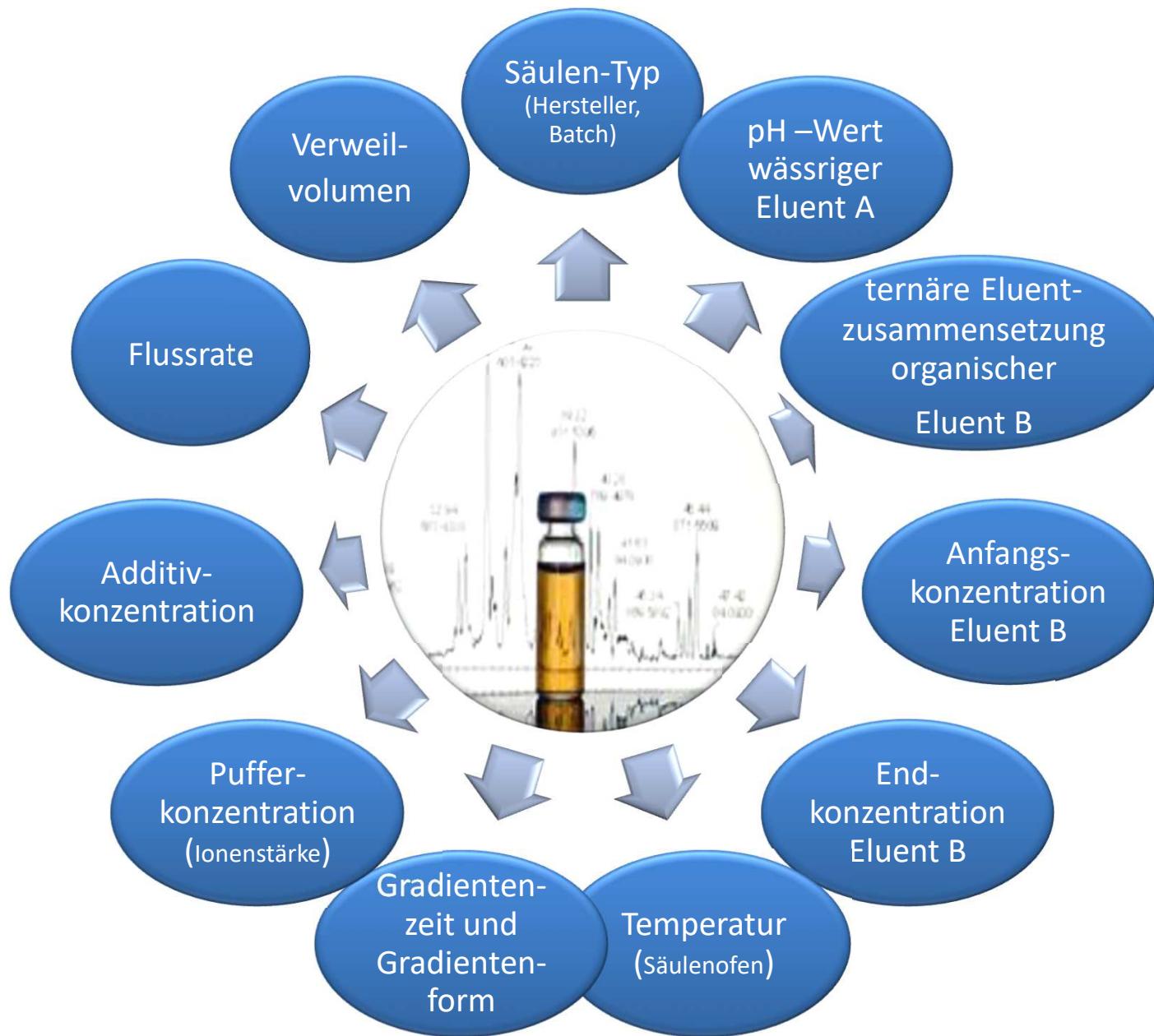
RP-HPLC – Analytik in der Arzneimittel Entwicklung?



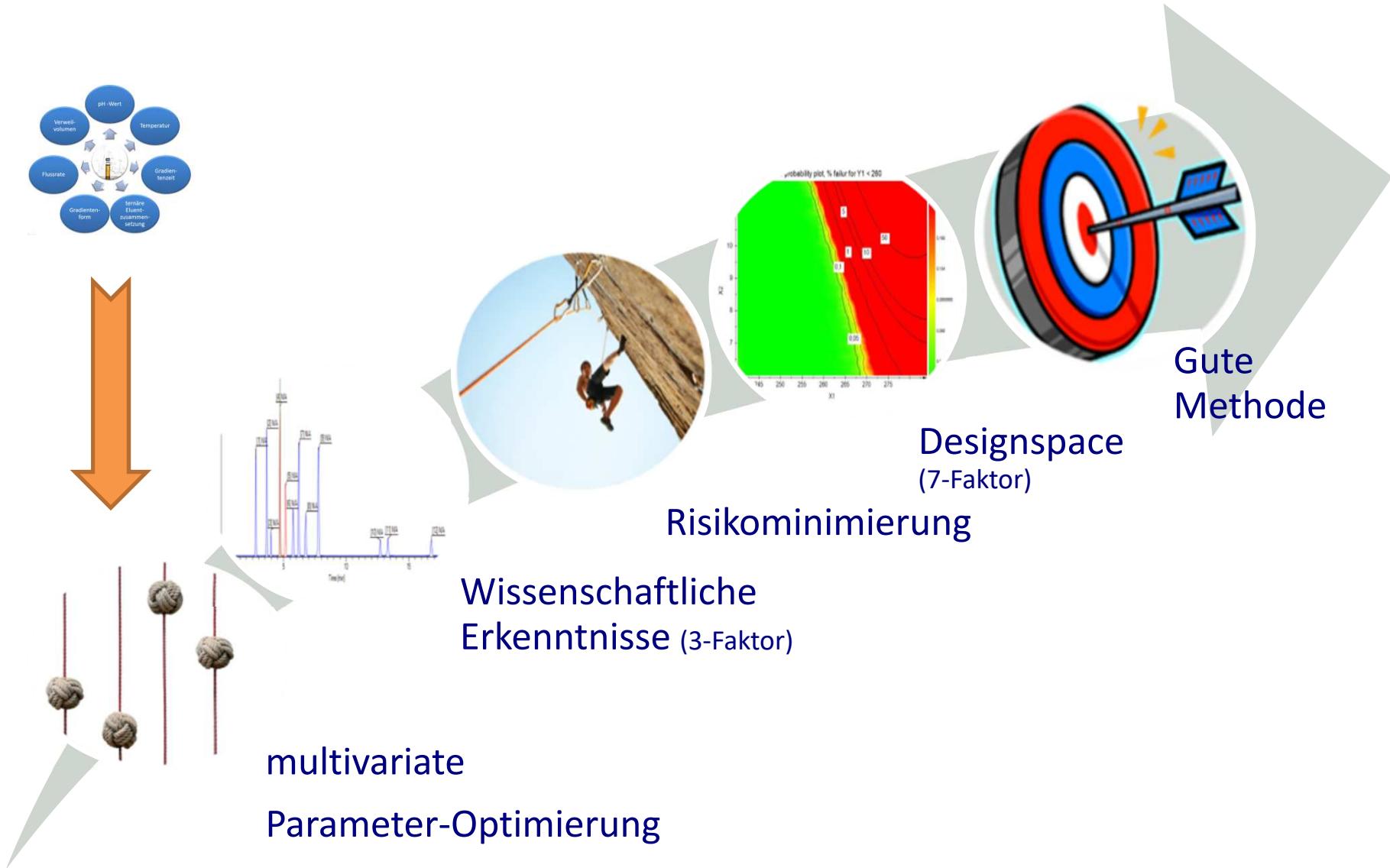
Schema HPLC Aufbau



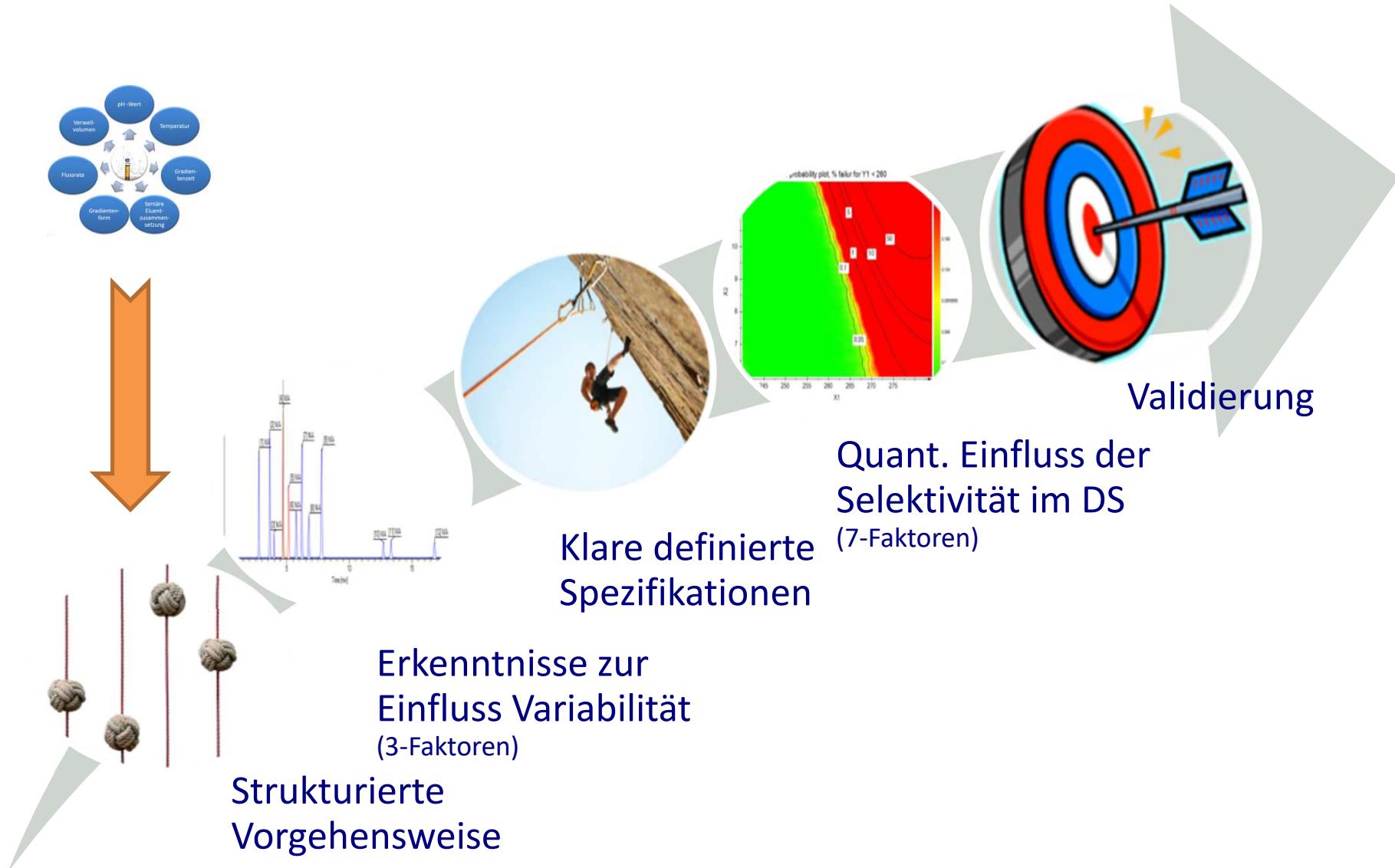
Multifaktorielle RP-HPLC-Trennungsoptimierung kleiner Moleküle



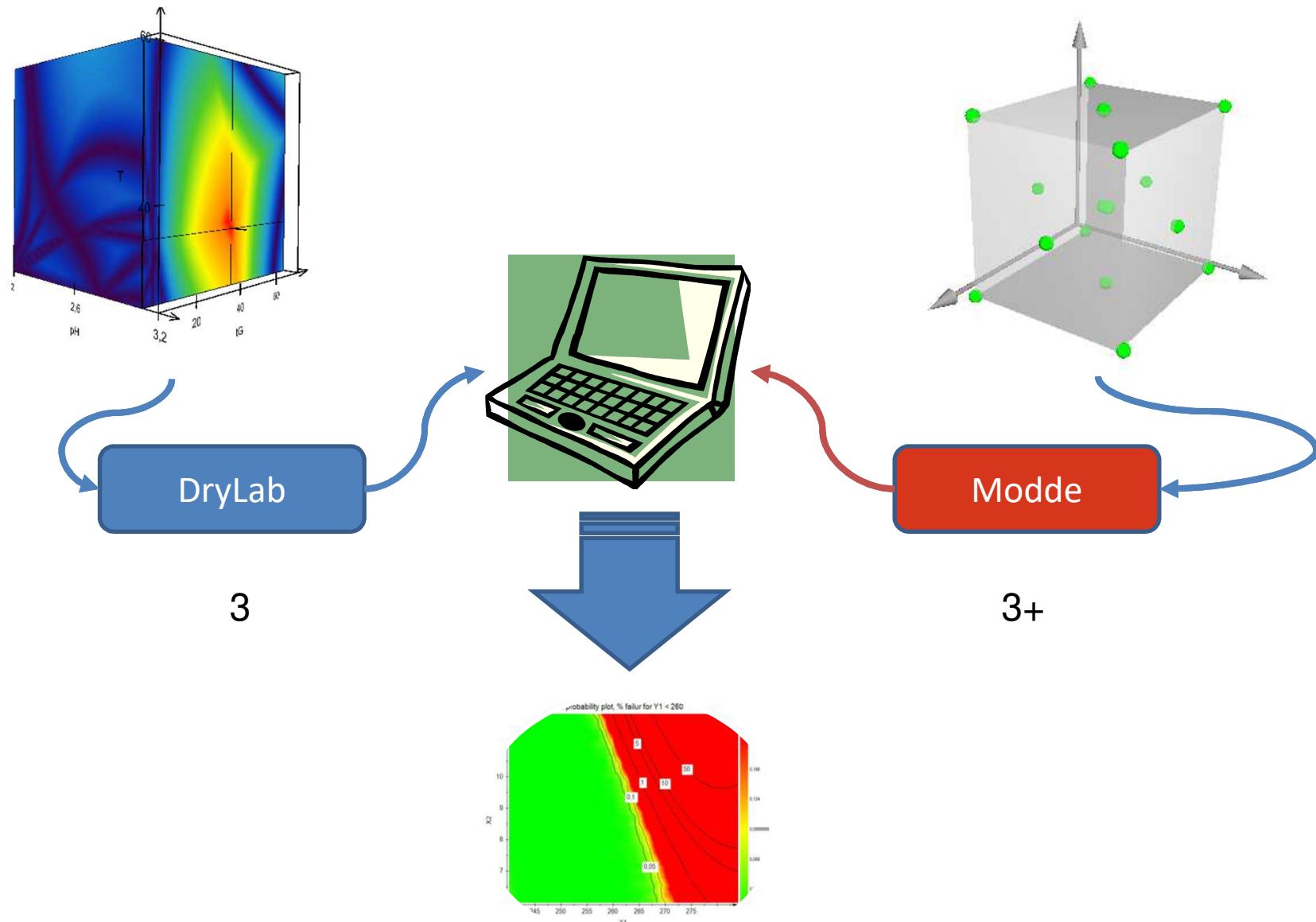
Quality by Design in der RP-HPLC



QbD, DoE und DS in der RP-HPLC ?



QbD-Konzept der RP-HPLC-Trennungsoptimierung

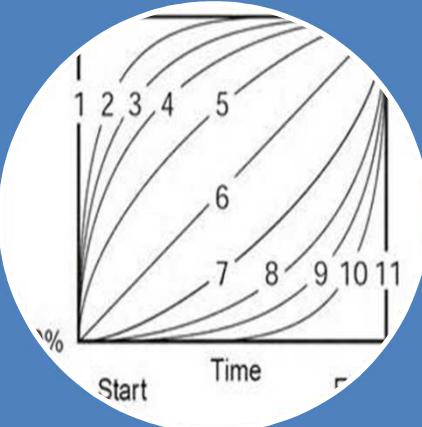


DryLab® - Modellierungsoptionen



Isokratische Arbeitweise

- Type Eluent %B
- Temperatur
- pH-Wert
- Eluent A
- Pufferkonzentration
- Additivkonzentration)
- ternären Eluent-zusammensetzung (B1:B2)



Gradientenarbeitsweise

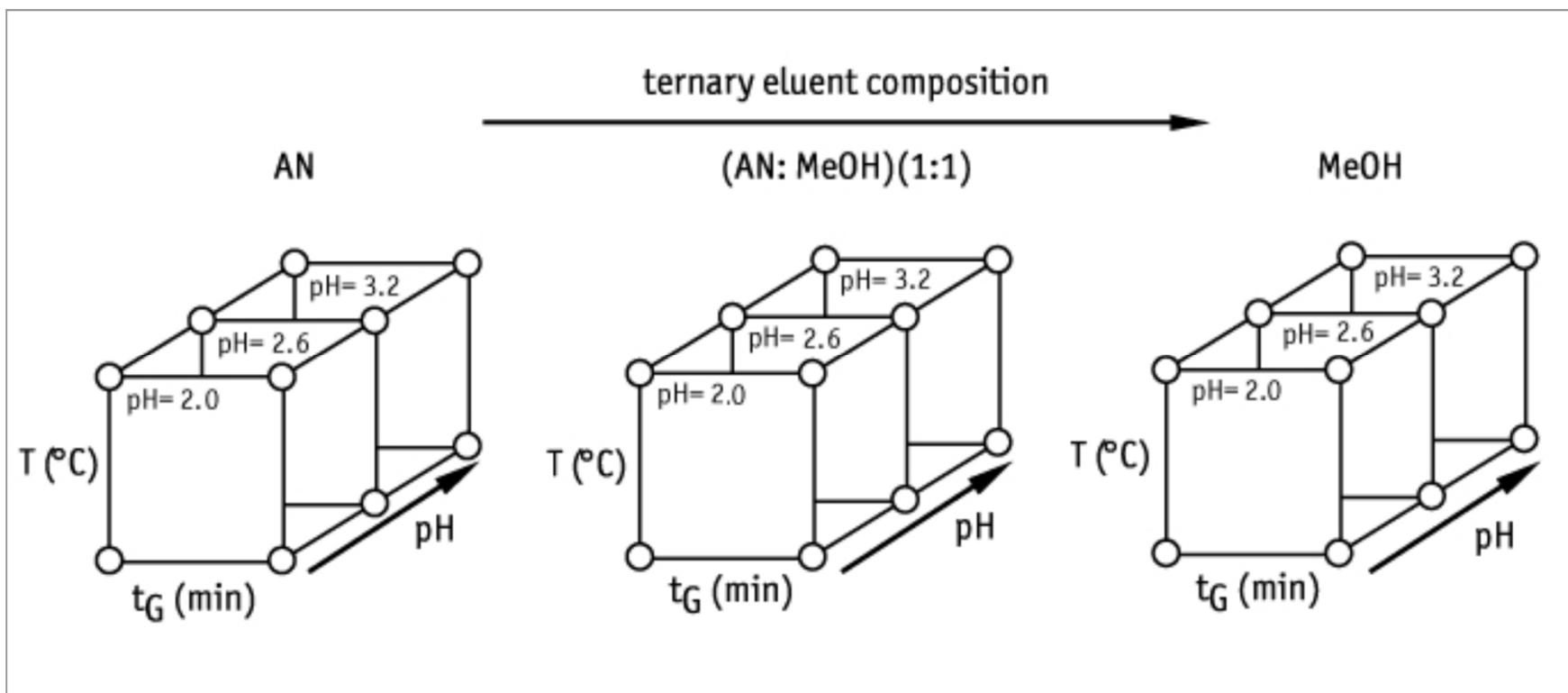
- alle Methodenparameter der isokratischen Arbeitweise
- Start und Ende %B
- Gradientenstufen
- Gradientenzeitz



Sonstiger Einfluss

- Säulendimensionen (Säulenlänge, Säuleninnendurchmesser)
- Partikelgröße
- Flussrate
- Verweilvolumens

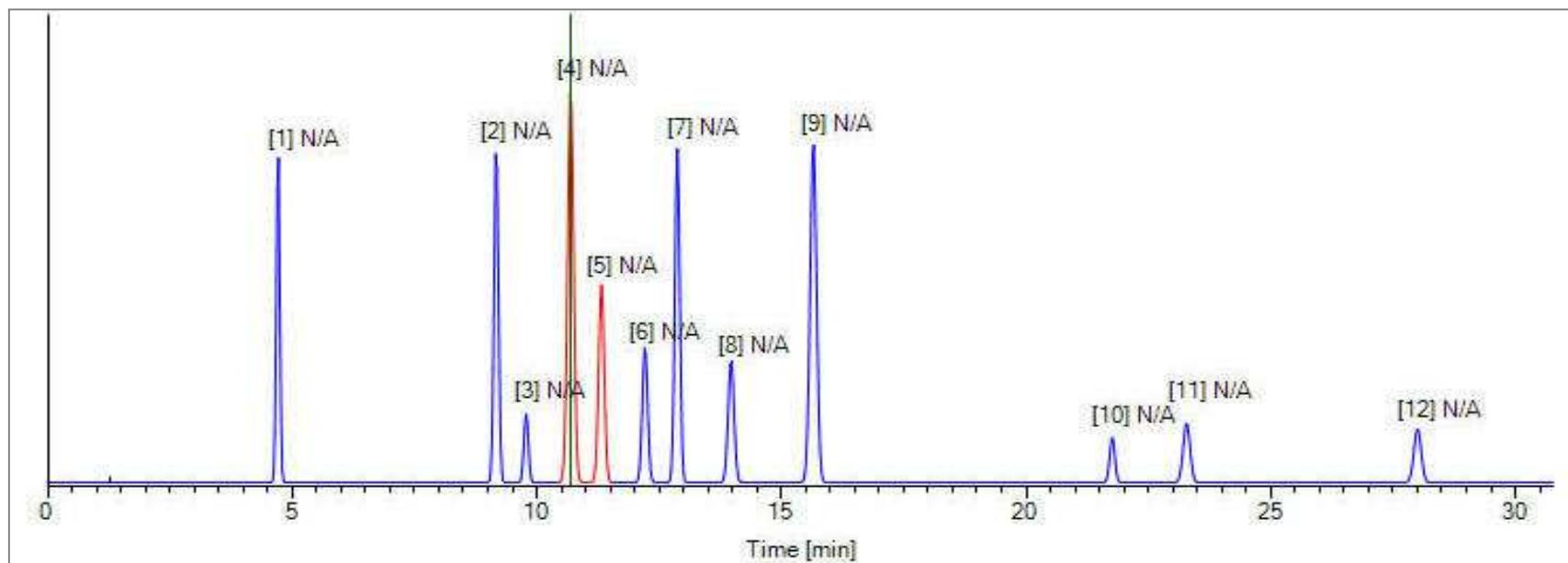
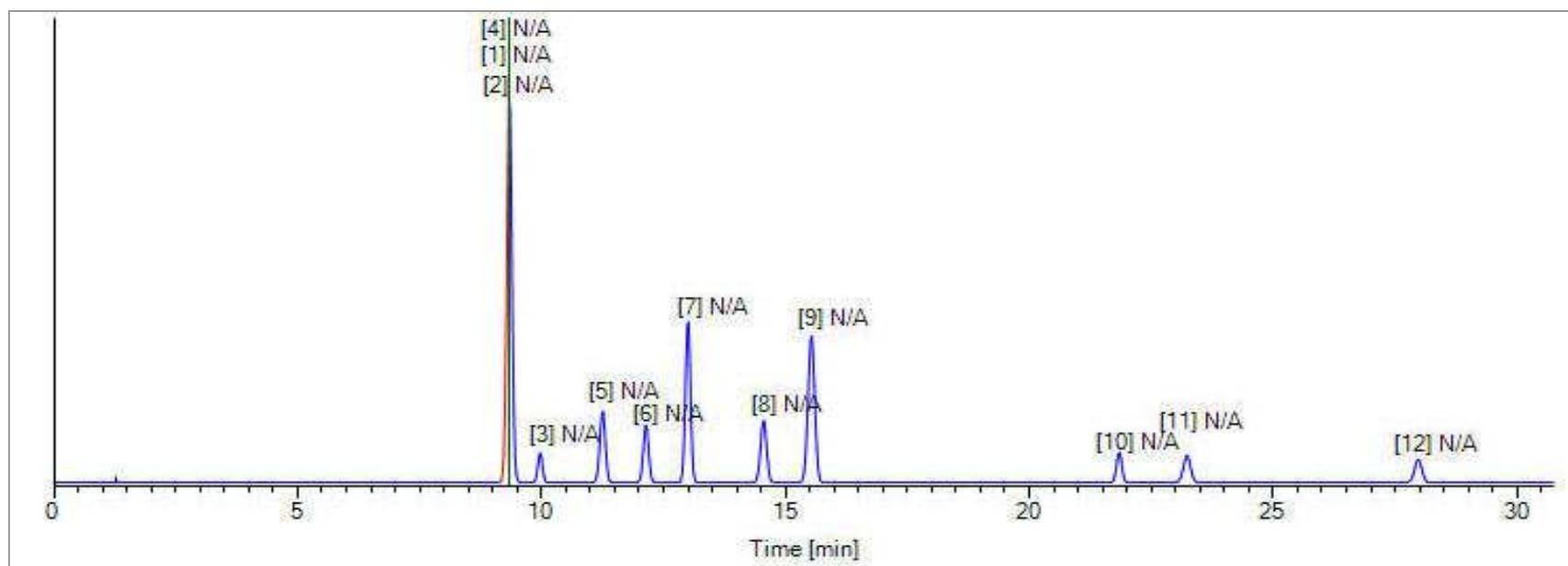
Daten-Basis: Drei-Faktor-Optimierungs - Strategie



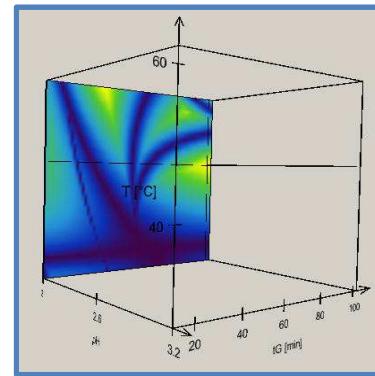
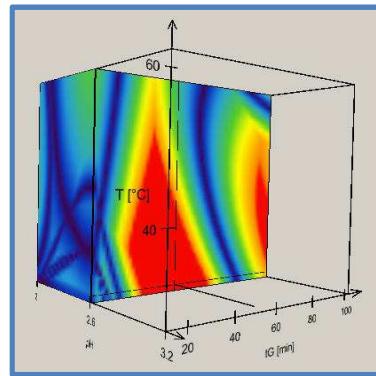
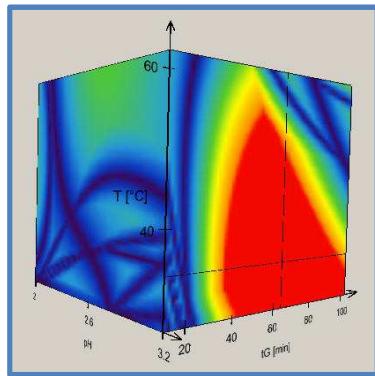
12 x 3 = 36

- Gradientenzeit (tG): 20 min und 60 min
- Temperatur: 30 °C und 60 °C
- Eluent A: 25 mM Phosphatpuffer pH-Werten 2.0, 2.6, 3.2
- Eluent B2 in B1: ACN:MeOH 100:0, 50:50, 0:100 V:V
- Säule: Länge 100, Innendurchmesser 4.6 mm
- Stationäre Phase: Halo C18, 2.7 µm
- Fluss: 0.8 ml/min

Repräsentative Vorläufe zur Generierung der Cubes



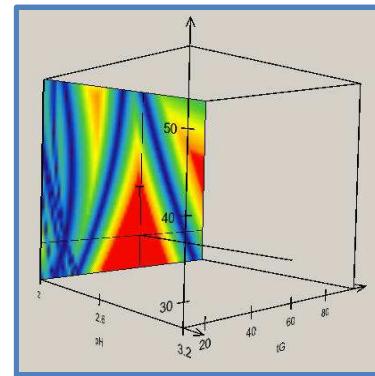
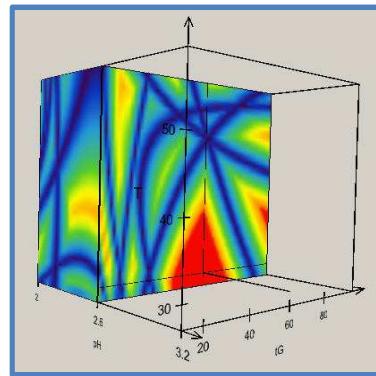
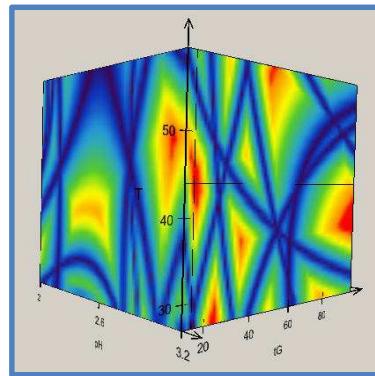
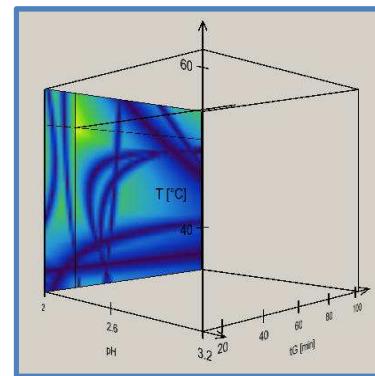
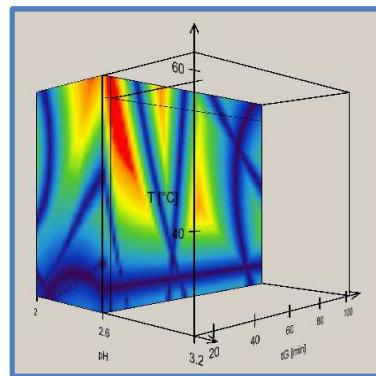
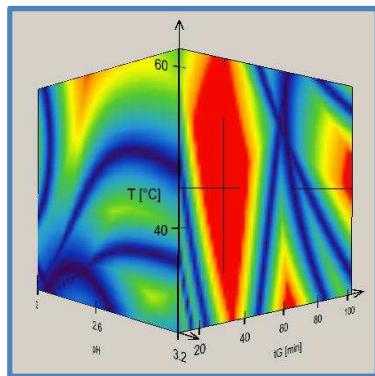
DryLab® - 3D-Resolution Maps / Cube - Modellierung t_G , T, pH, R_s



tC

ACN

ACN:MeOH(1:1)



pH

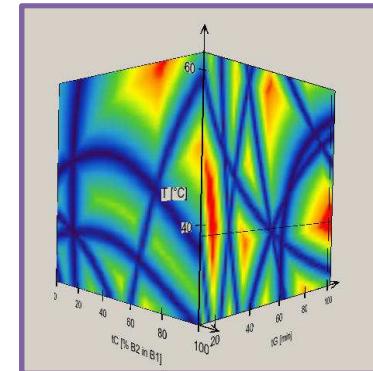
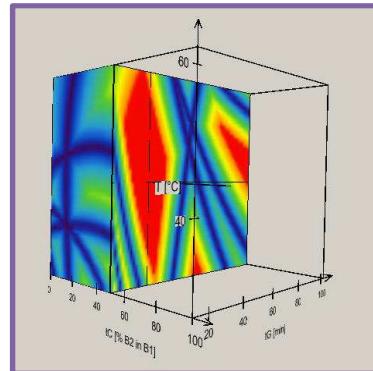
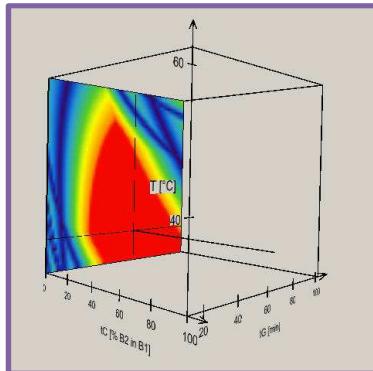
3.2

2.6

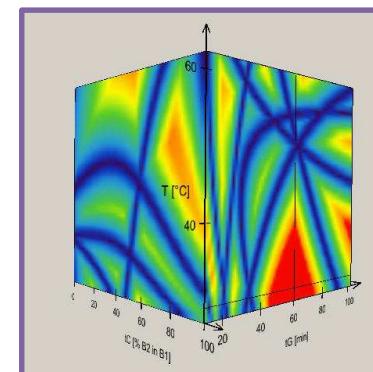
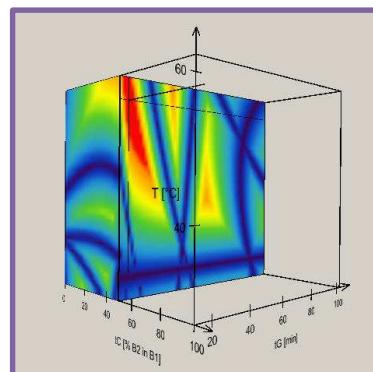
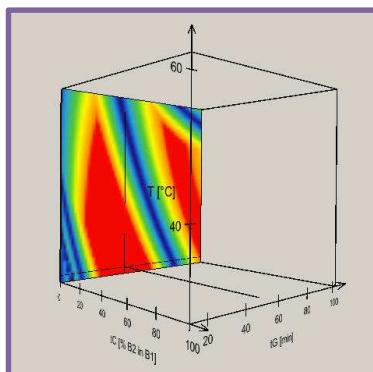
2.0

MeOH

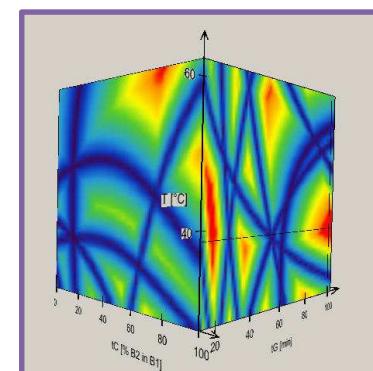
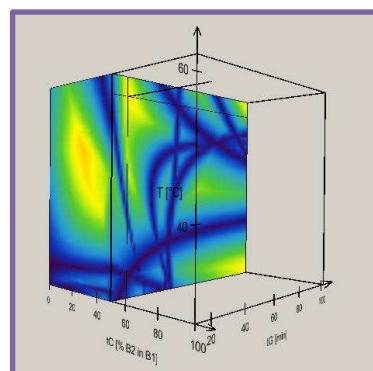
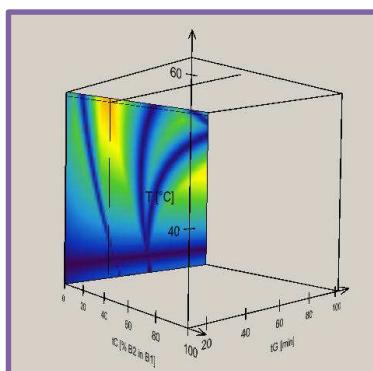
DryLab® - 3D-Resolution Maps / Cube - Modellierung t_G , T , t_C , R_s



pH 3.2



pH 2.6



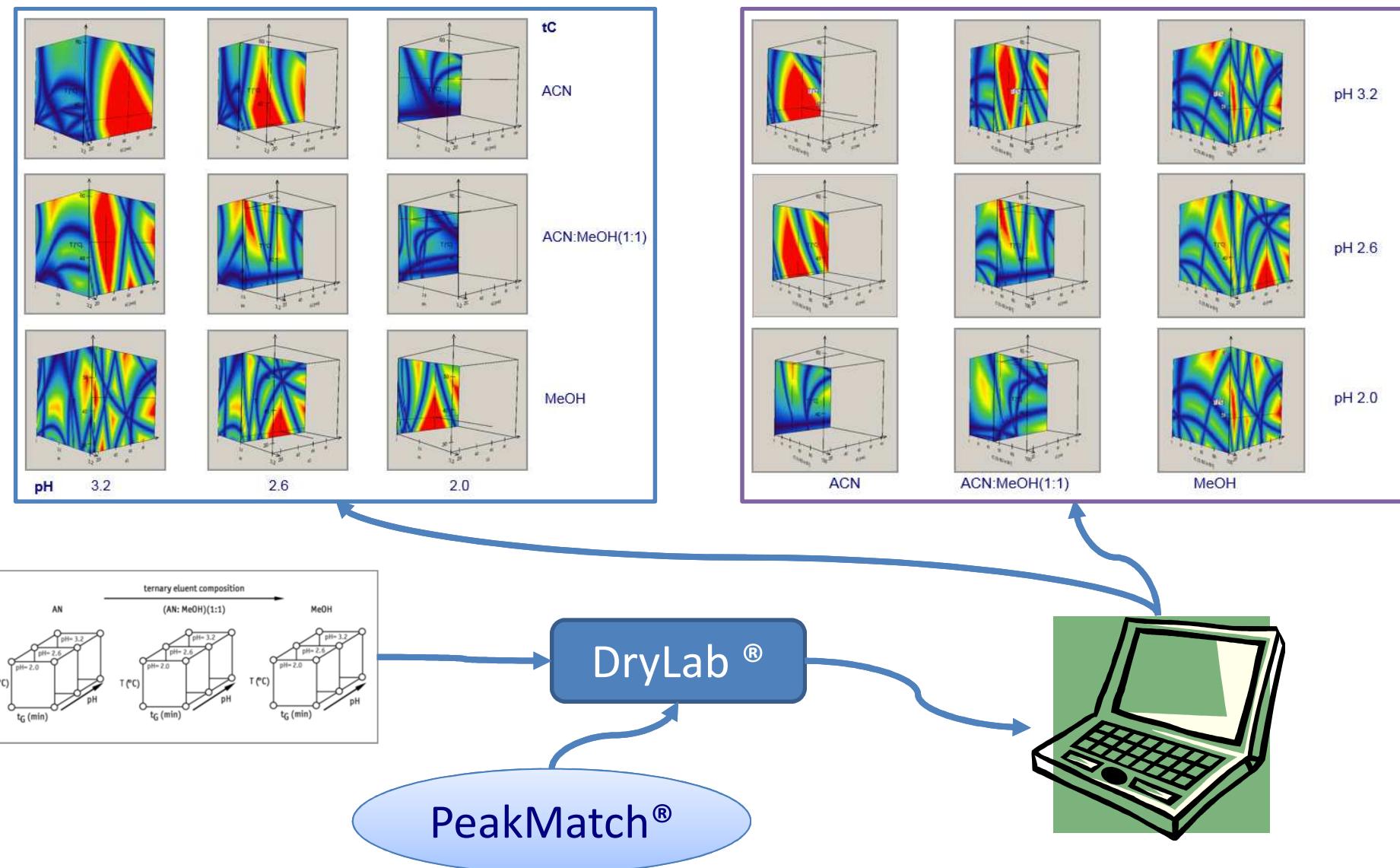
pH 2.0

ACN

ACN:MeOH(1:1)

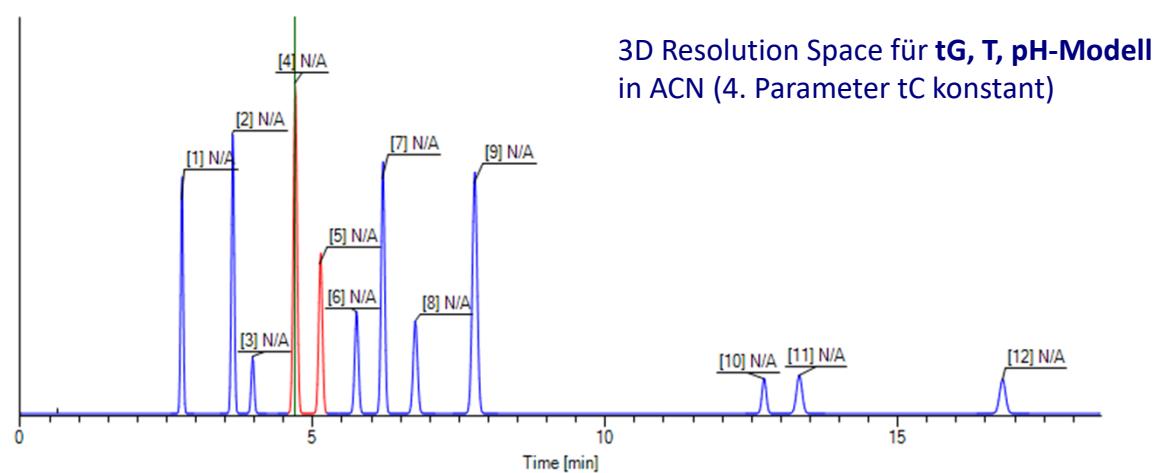
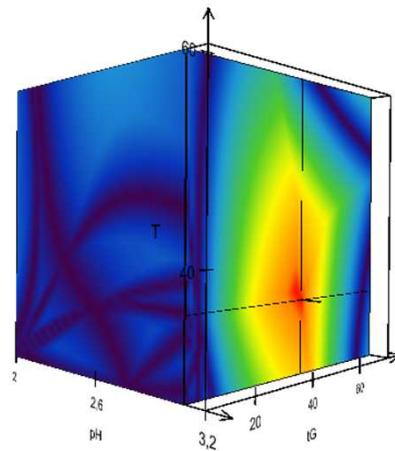
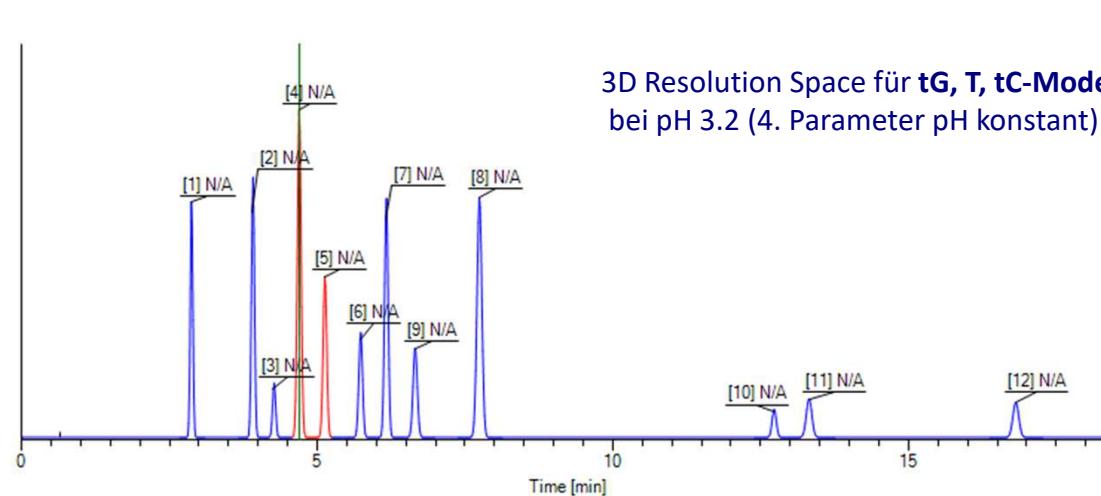
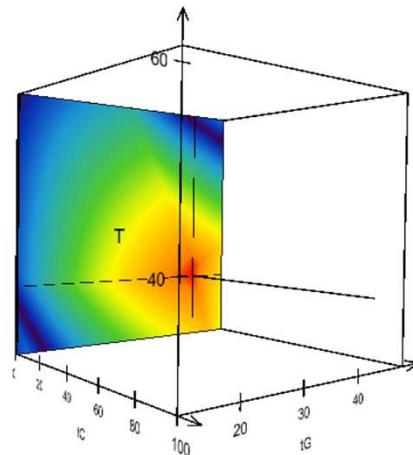
MeOH

DryLab® - 3-Faktor-Optimierung / Modelle tg,T,Ph und tg,T,tc



4 Peakzuordnung durch Molnar-Institut für angewandte Chromatographie, Berlin (2011)
 5 Molnar-Institut für angewandte Chromatographie, Berlin

DryLab® - 3-Faktor-Optimierung – Lokale Optima je nach 3F Model

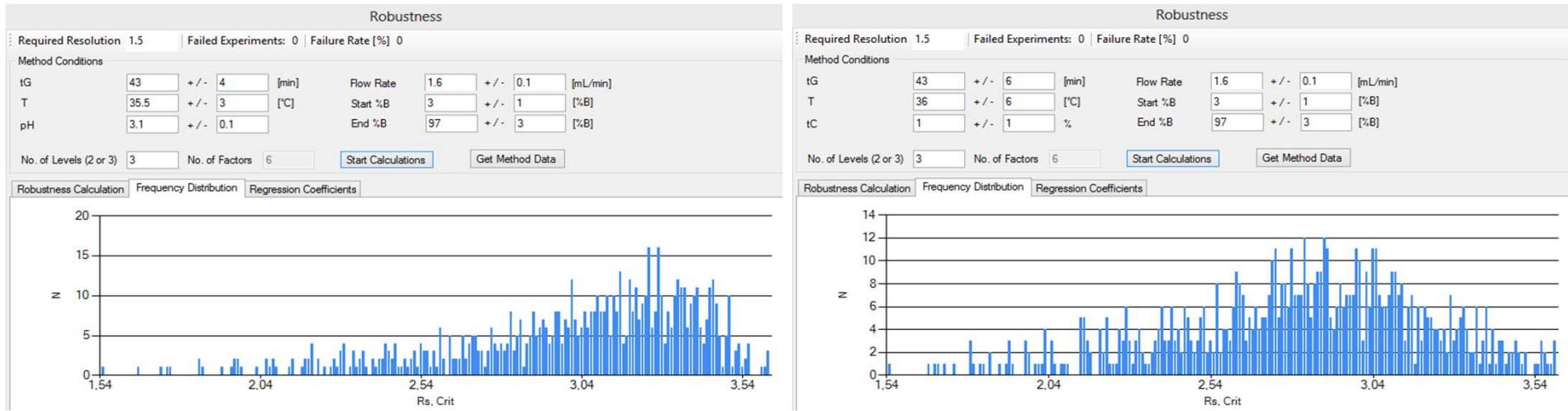


Optimierung Modelle	tG (min)	T (°C)	pH	tC (%B2:B1)		%Bs	%Be	Fluss (ml/min)	R _{s,krit}	tR _{max} (min)
				%ACN	%MeOH					
tG-T-pH	43	35.5	3.1	100	0	3	97	1.6	3.6	16.8
tG-T-tC	43	36	3.2	99	1	3	97	1.6	3.6	16.8

DryLab® - Robustheitsaussage opt. Trennung



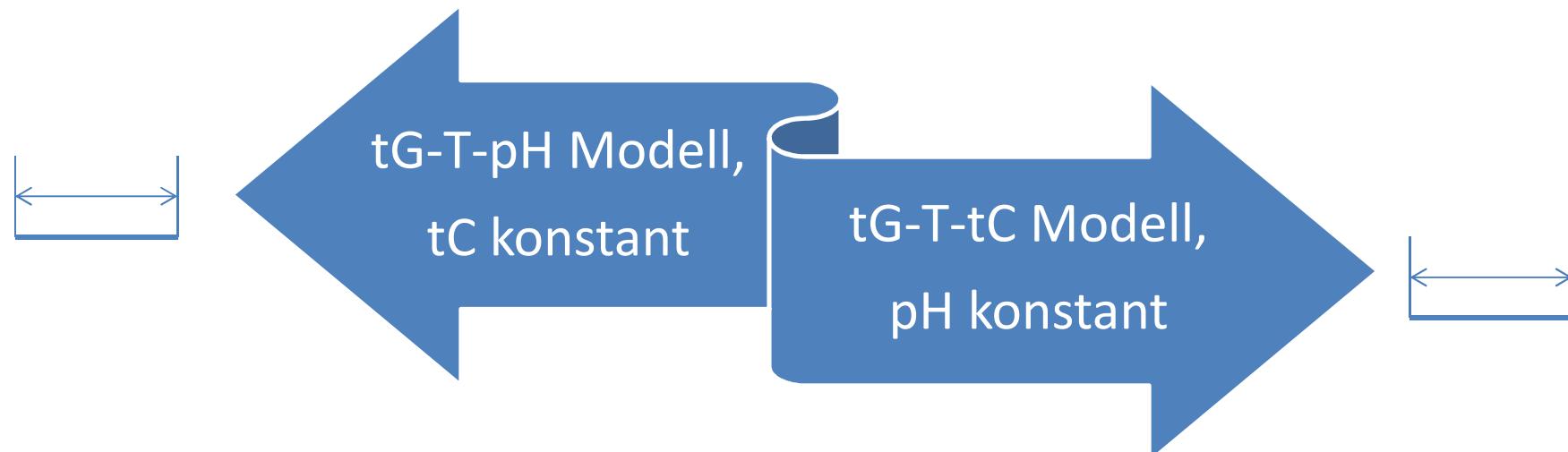
- „Robustness“-Tool⁶ von DryLab® auf Basis des 3-Faktor-Modelles



Faktoren-Einstellung für DryLab® tG-T-pH Modell und tG-T-tC Modell.

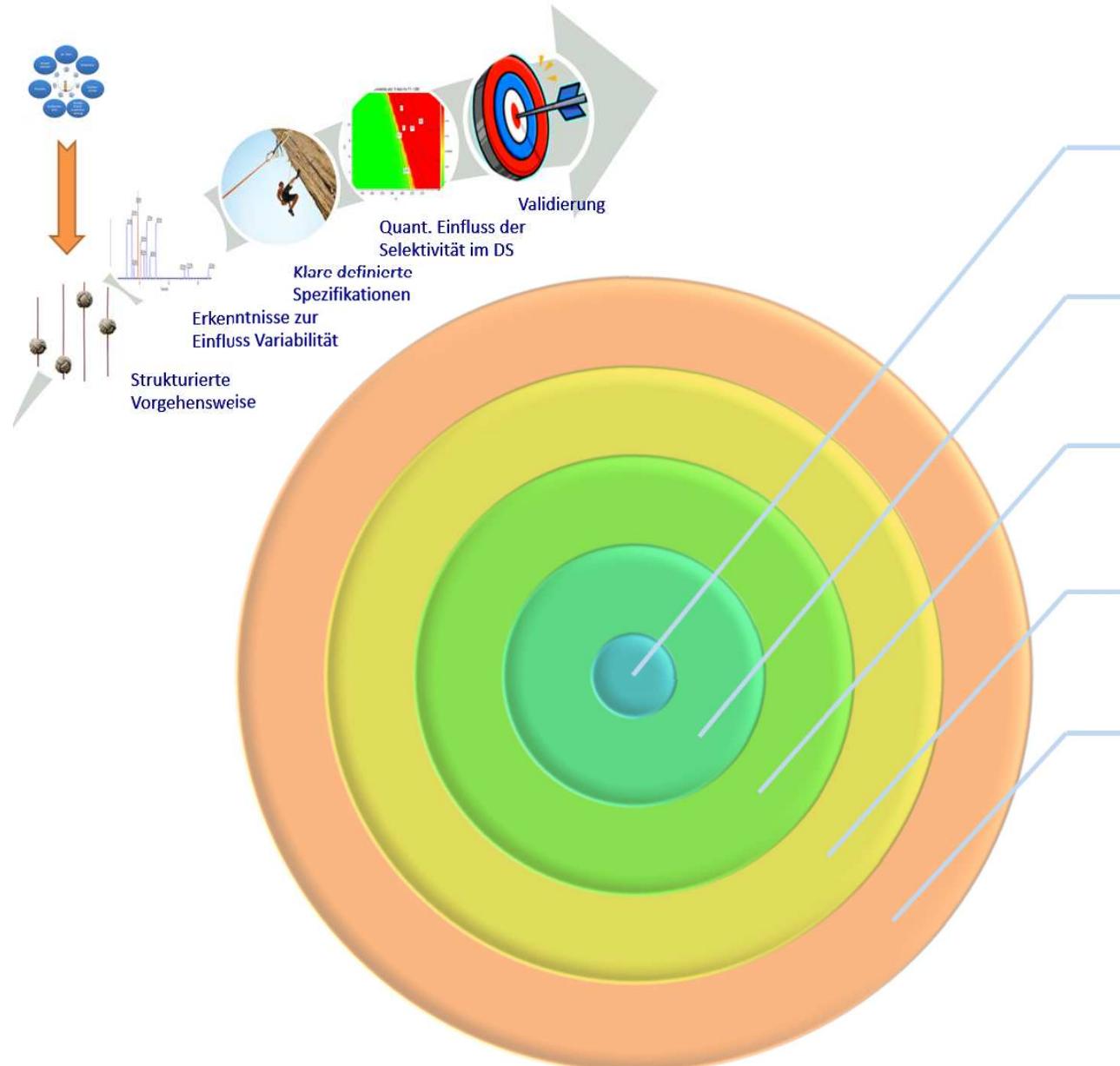
Faktoren	tG-T-pH Modell			tG-T-tC Modell		
	unten	optimal	oben	unten	optimal	oben
tG (min)	39	43	47	37	43	49
T (°C)	32.5	35.5	38.5	30	36	42
tC (Vol.% MeOH in ACN)	0	0	0	0	1	2
%Bstart (Vol.%B)	2	3	4	2	3	4
%Bend (Vol.%B)	94	97	100	94	97	100
pH	3.0	3.1	3.2	3.2	3.2	3.2
Fluss (ml/min)	1.5	1.6	1.7	1.5	1.6	1.7
Fehlerrate (%)	0			0		

DryLab® - Lokale Optima !



Faktoren	Lokale Optima der multifaktoriellen Optimierung einer RP-HPLC-Trennung								
	3 Faktor-Optimierung (tG, T, pH oder tC) Zielgrößen R_s^{16} Peakpaare								
	Faktoreneinstellung								
	tG-T-pH Modell, tC konstant				tG-T-tC Modell, pH konstant				
	MeOH:ACN 0:100 V/V	MeOH:ACN 50:50 V/V	MeOH:ACN 100:0 V/V	2.0	2.6	2.9	3.0	3.1	3.2
tG (min)	57	56	46	50	42	56	50	58	54
T (°C)	30	30	32	31	34	48	36	37	45
tC (Vol.% MeOH in ACN)	0	50	100	98	5	55	45	50	67
%Bstart (Vol.%B)	6	9.5	2	3.5	7	9.5	8.5	8	9.25
%Bend (Vol.%B)	95	100	97	96	95	96	96	94	94
pH	2.9	3.2	2.0	2.0	2.6	2.9	3.0	3.1	3.2
Fluss (ml/min)	1.2	1.5	1.5	1.5	1.1	1.4	1.4	1.3	1.2

Globales Optimum ?



7 Faktoren
Simultane Auswertung aller Faktoren Modde®

Simulierung der Ergebnisse
DryLab® und PeakMatch®

Erstellung eins Versuchsplan Modde®

Gutes Model
DryLab® und PeakMatch®



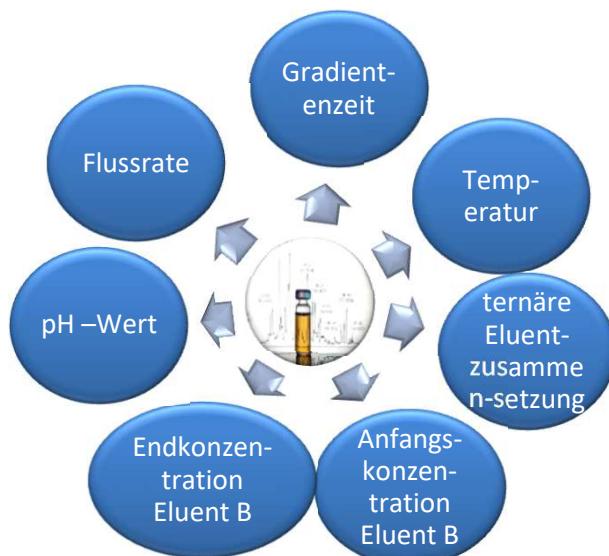
Gute Qualität der Basis Grundläufe



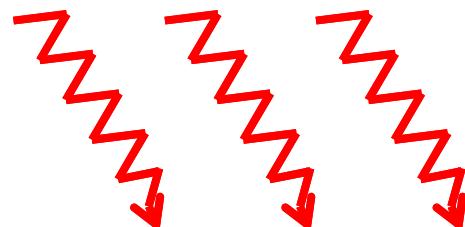
Je 3 Faktoren

Statistische Versuchsplanung (SVP) mit MODDE®

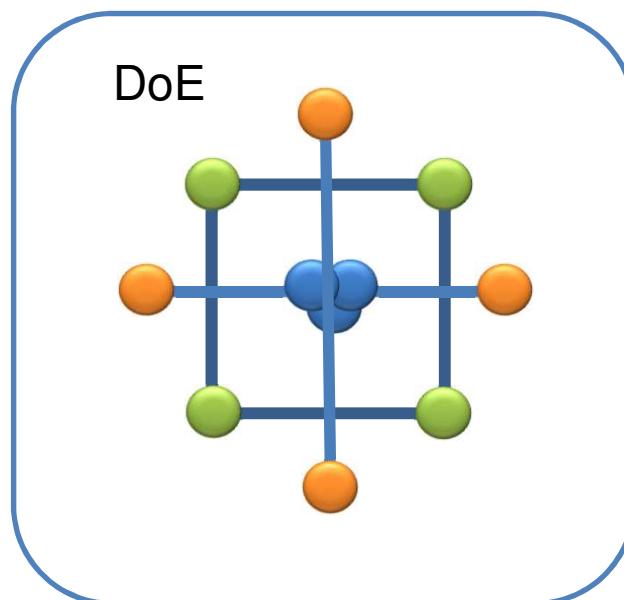
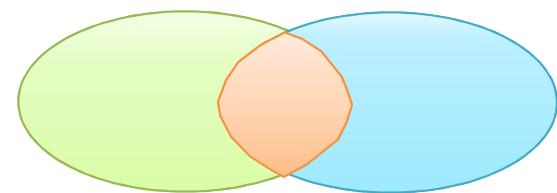
Signifikante Faktoren



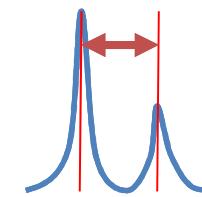
Störgrößen



Zielgrößen



Resolution

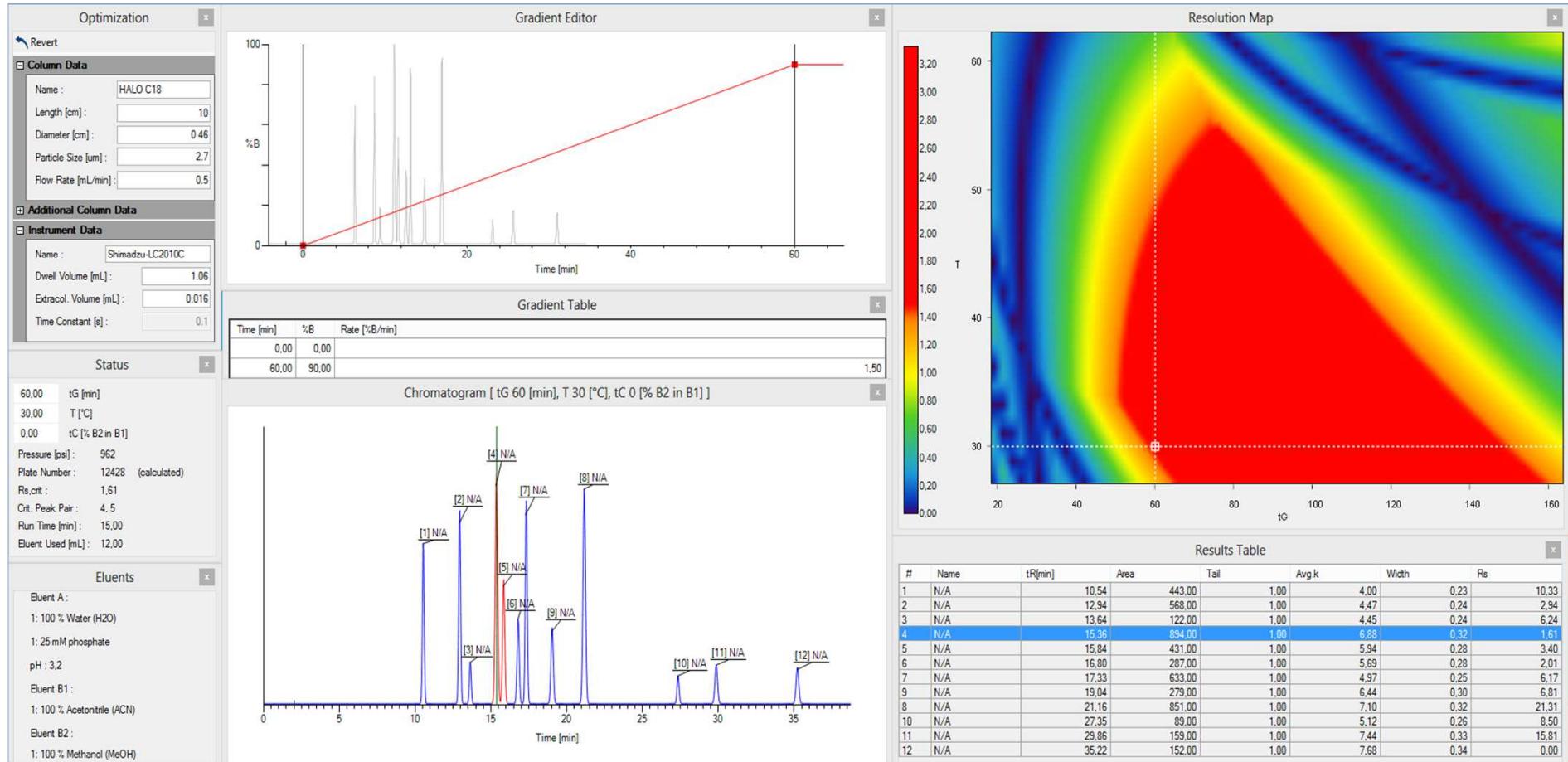


Minimale Analysenzeit



Transferfunktion $y_1, y_2 = f(x_1, x_2, \dots, x_n) + \varepsilon$

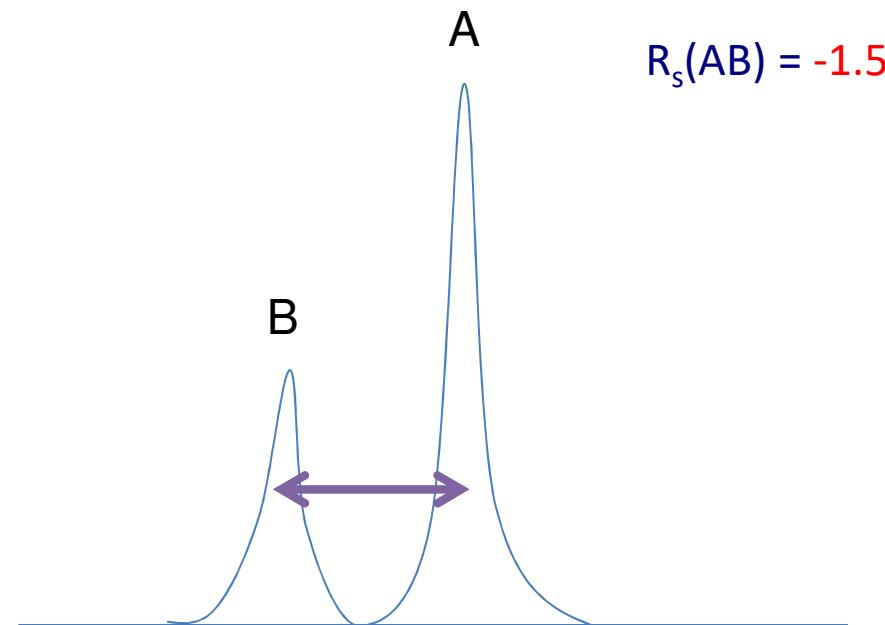
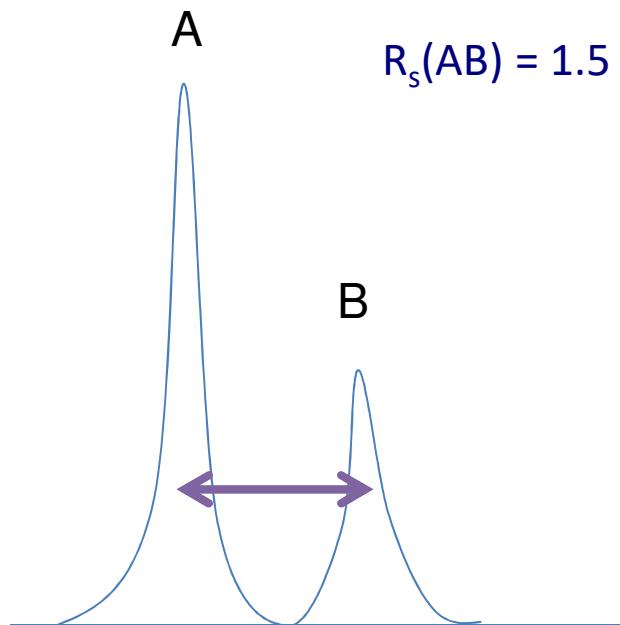
Versuchsplan Modde ® → Simulation der Experimente DryLab ®



Auflösungskarte für das SVP-Experiment 13:

- Abhängigkeit der kritischen Auflösung Gradientenzeit und Temperatur
- ternäre Eluentzusammensetzung tC = 100% ACN
- pH-Wert des wässrigen Eluenten = 3.2

Modellierung der Auflösung als Zielgröße I



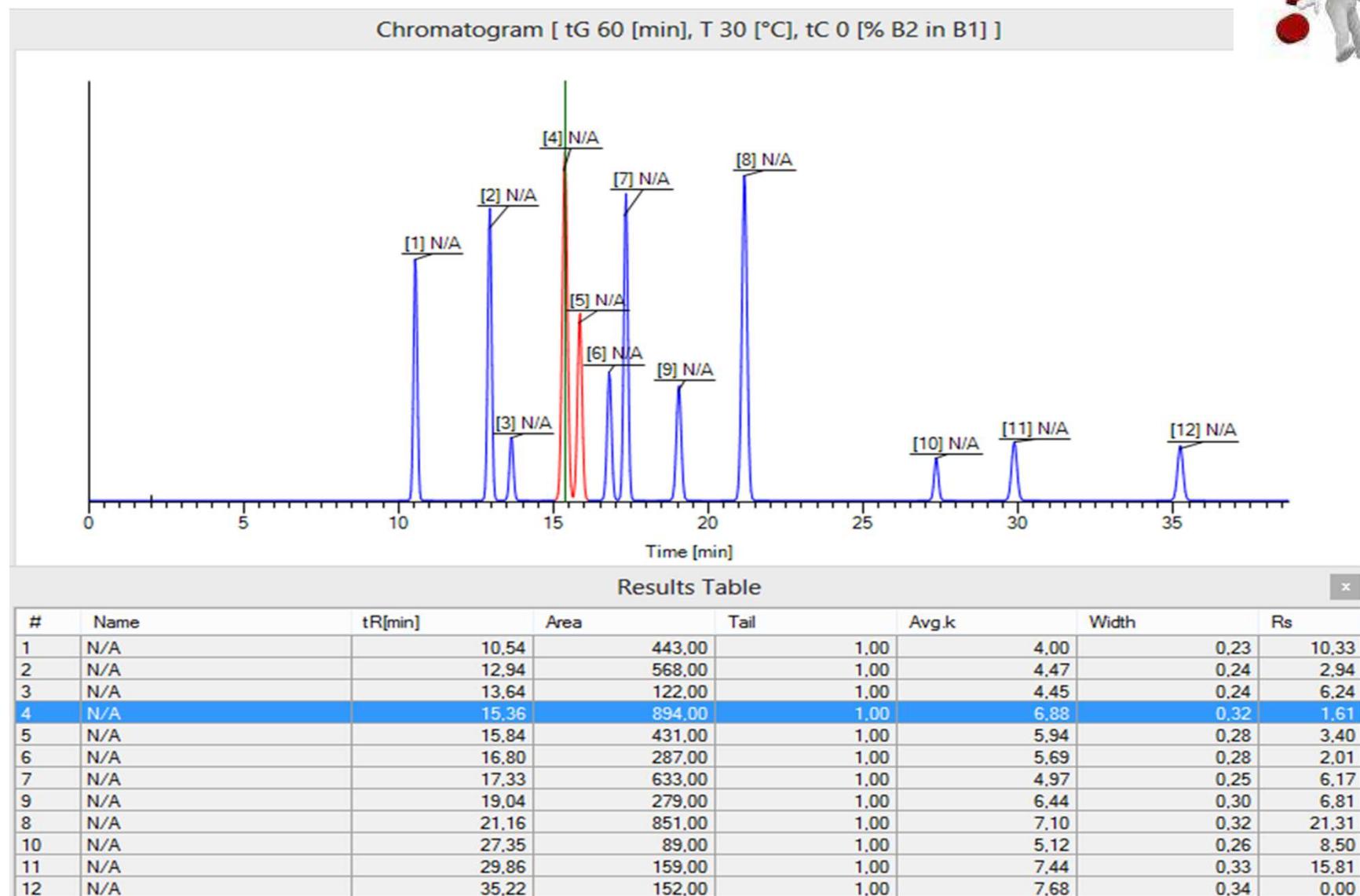
Peak-Umkehr

Einflussgrößen / Zielgrößen

Faktoren	Abkürzung	Einheit	Faktorstufen	Type
Gradientenzeit	tG	min	20 - 60	Quantitativ
Temperatur	T	° C	30 - 60	Quantitativ
Ternäre Eluentzusammensetzung	tC	(Vol.% MeOH in ACN)	0 - 100	Quantitativ
Startkonzentration Eluent B	%B _{start}	(Vol.%B)	0 - 10	Quantitativ
Endkonzentration Eluent B	%B _{end}	(Vol.%B)	90 - 100	Quantitativ
pH Eluent A	pH		2 - 3.2	Quantitativ
Flussrate	F	ml/min	0.5 - 1.5	Quantitativ

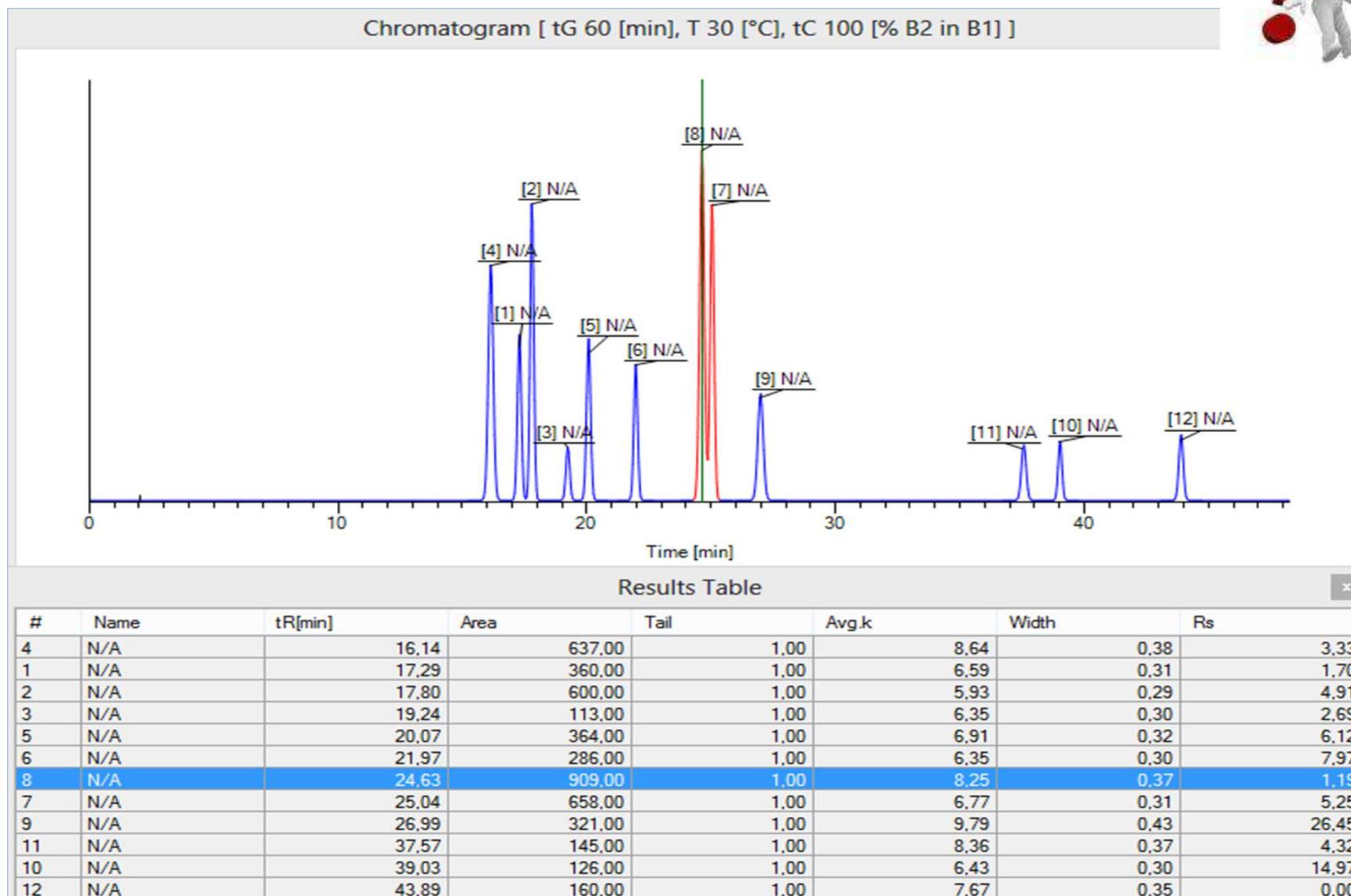
Zielgrößen	Abkürzung	Einheit	Min	Max	Type
Auflösung Peakpaar 1/2	R _s 1/2		1.5		Regulär
Auflösung Peakpaar 2/3	R _s 2/3		1.5		Regulär
Auflösung Peakpaar 2/4	R _s 2/4		1.5		Regulär
Auflösung Peakpaar 3/4	R _s 3/4		1.5		Regulär
Auflösung Peakpaar 3/5	R _s 3/5		1.5		Regulär
Auflösung Peakpaar 1/4	R _s 1/4		1.5		Regulär
Auflösung Peakpaare 4/5	R _s 4/5		1.5		Regulär
Auflösung Peakpaar 5/6	R _s 5/9		1.5		Regulär
Auflösung Peakpaar 6/7	R _s 6/7		1.5		Regulär
Auflösung Peakpaar 6/9	R _s 6/9		1.5		Regulär
Auflösung Peakpaar 7/8	R _s 7/8		1.5		Regulär
Auflösung Peakpaar 7/9	R _s 7/9		1.5		Regulär
Auflösung Peakpaar 9/8	R _s 9/8		1.5		Regulär
Auflösung Peakpaar 8/10	R _s 8/10		1.5		Regulär
Auflösung Peakpaar 10/11	R _s 10/11		1.5		Regulär
Auflösung Peakpaar 11/12	R _s 11/12		1.5		Regulär
Retentionszeit des letzten Peaks im Chromatogramm	tR _{max}	min	10	20	Regulär

Versuchsplan → Simulation der Experimente



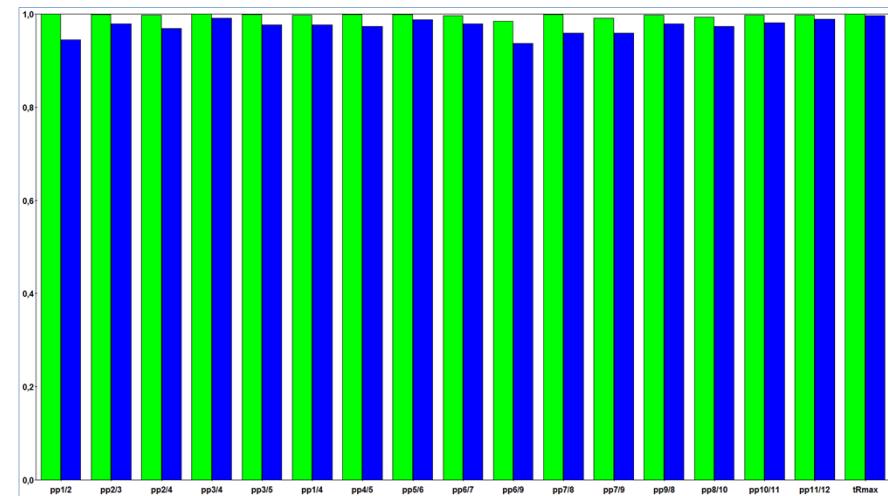
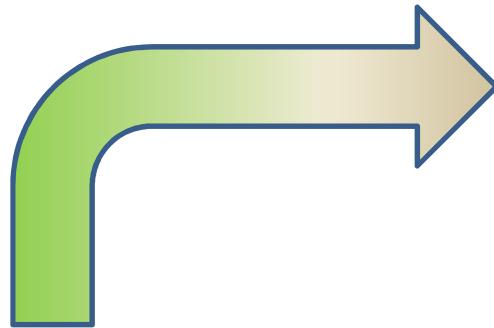
Wechsel bei krit. Peakpaar

Versuchsplan → Simulation der Experimente

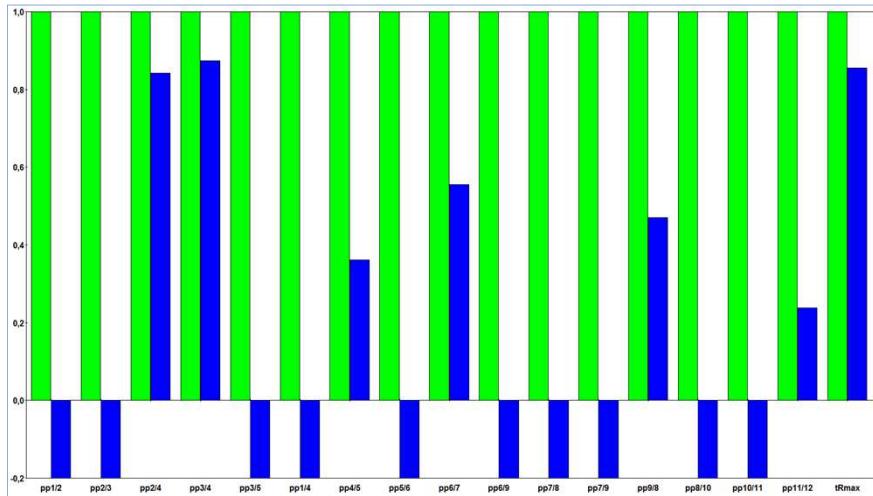


Wechsel bei krit. Peakpaar

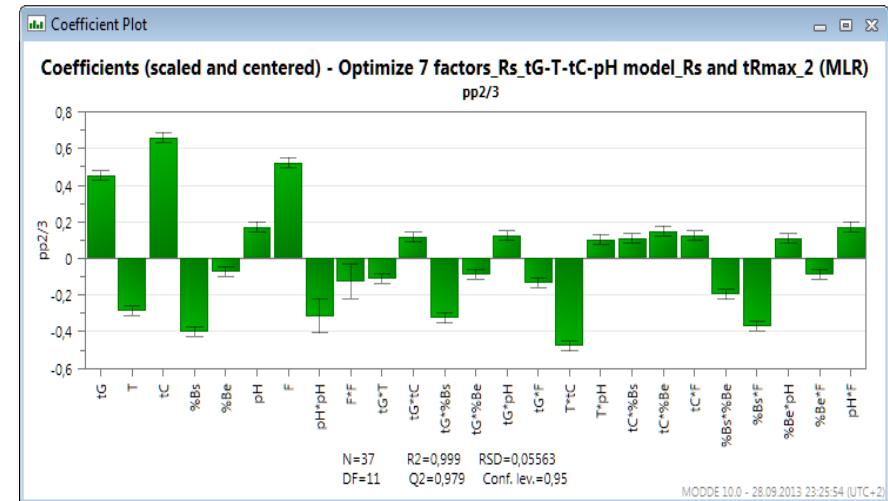
Versuchsplan → Modell und Modellanpassung



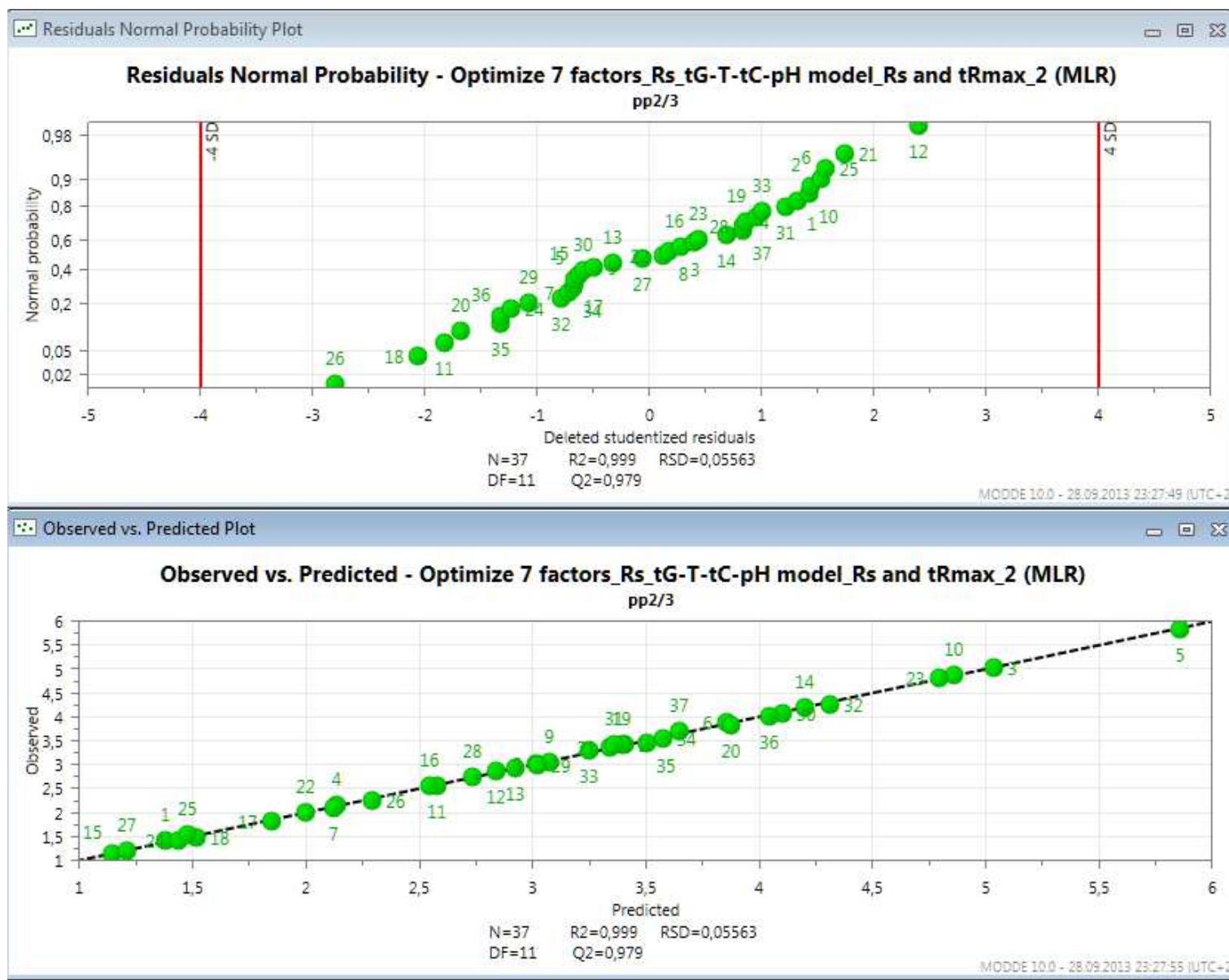
... → und nach Modellanpassung



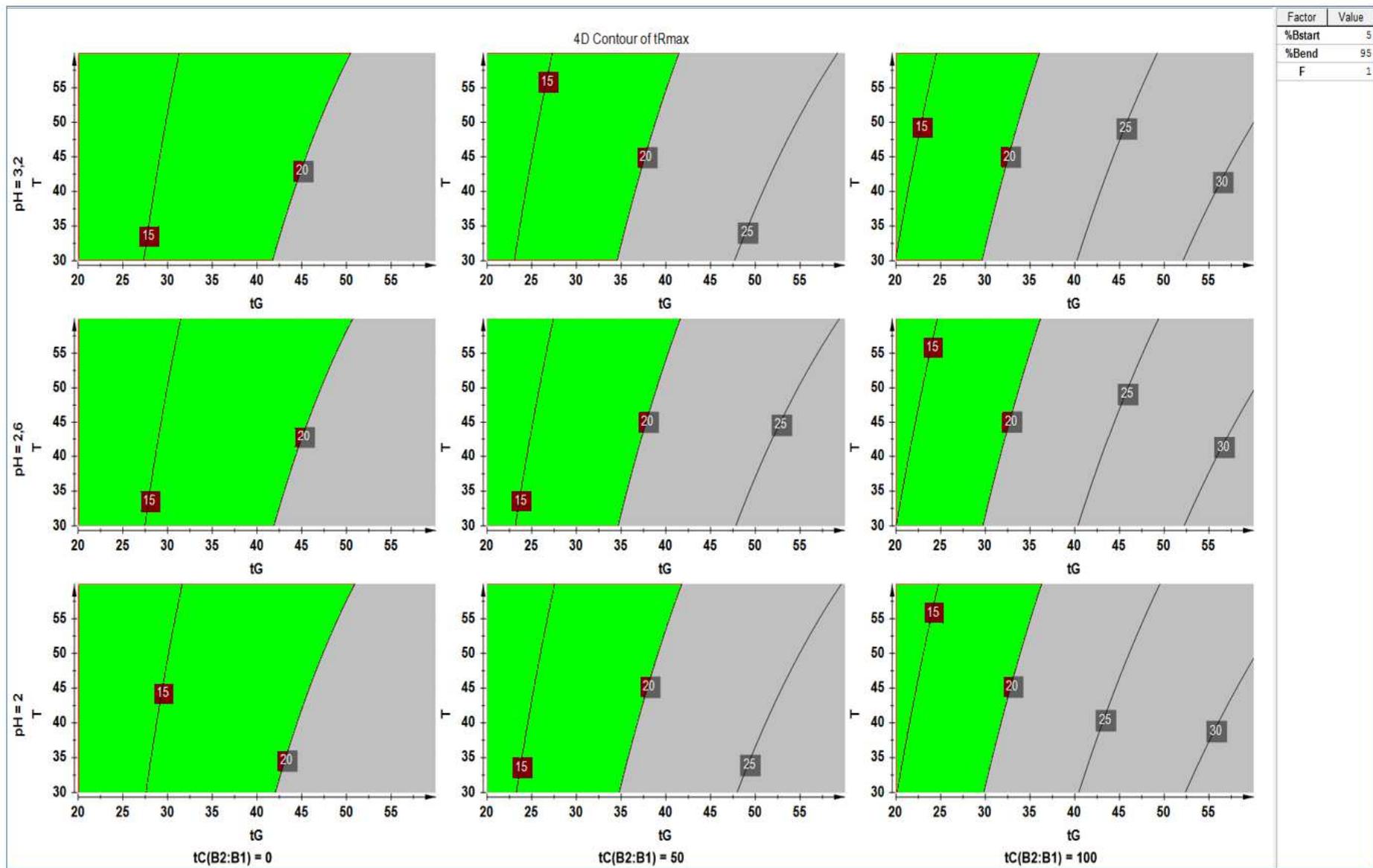
Vor Modellanpassung ...



Modelldiagnose – Obs. Vers. Pred. & Diagnose der Residuen

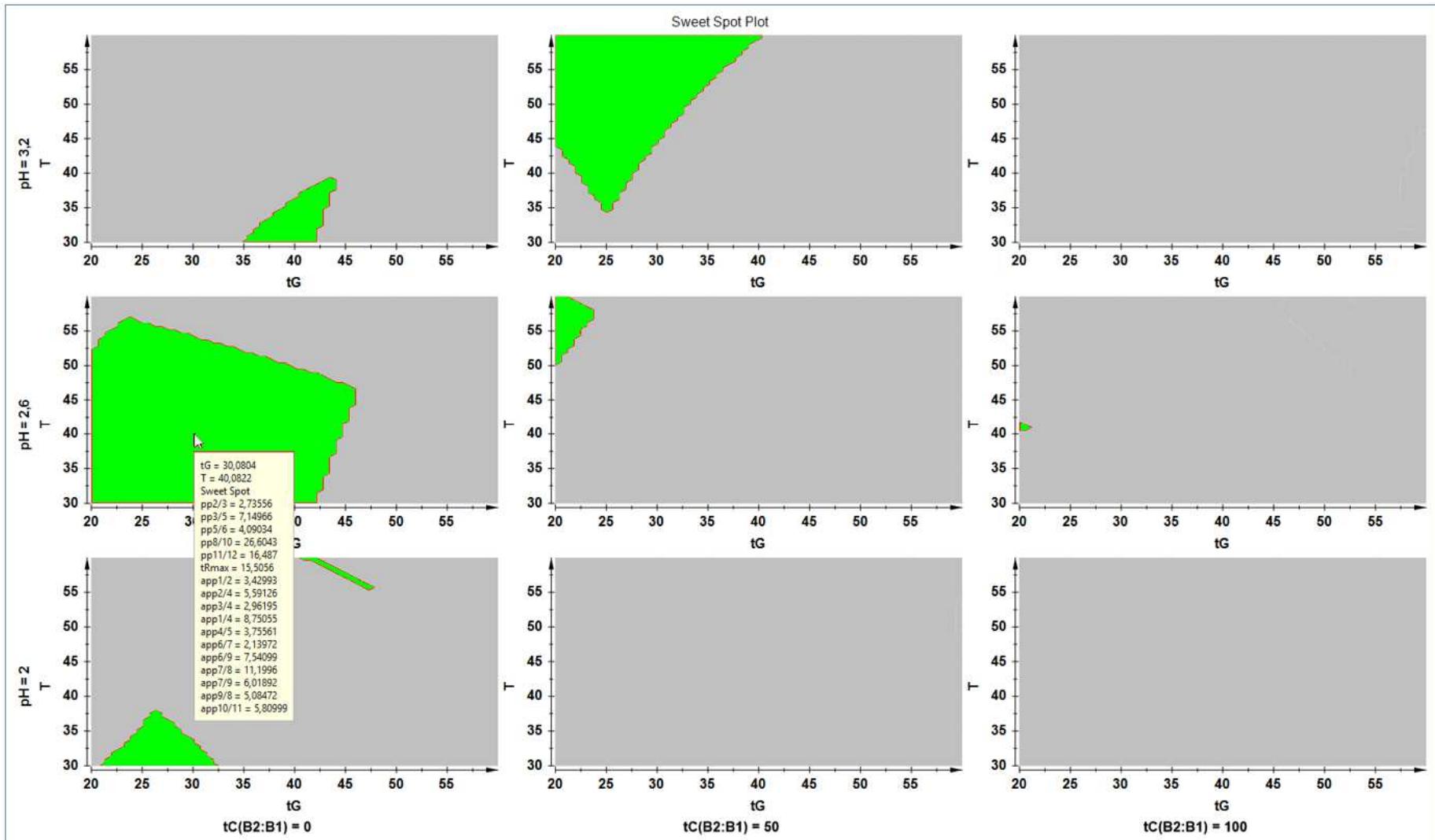


Modellinterpretation I – 4 Faktor-Kontourplot



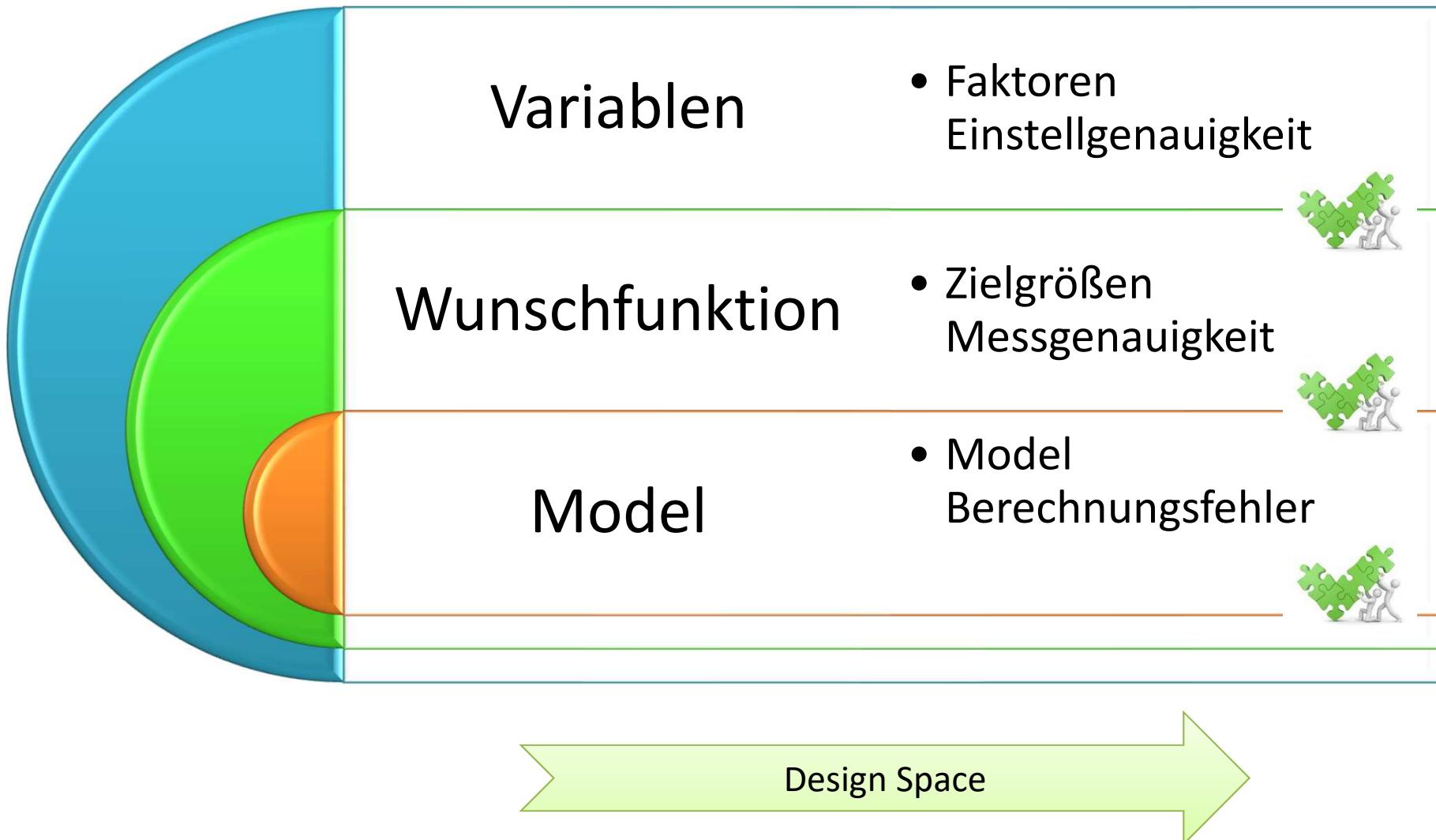
Retentionszeiten tR_{max} < 20 min

Modellinterpretation II – Sweet Spot Plot

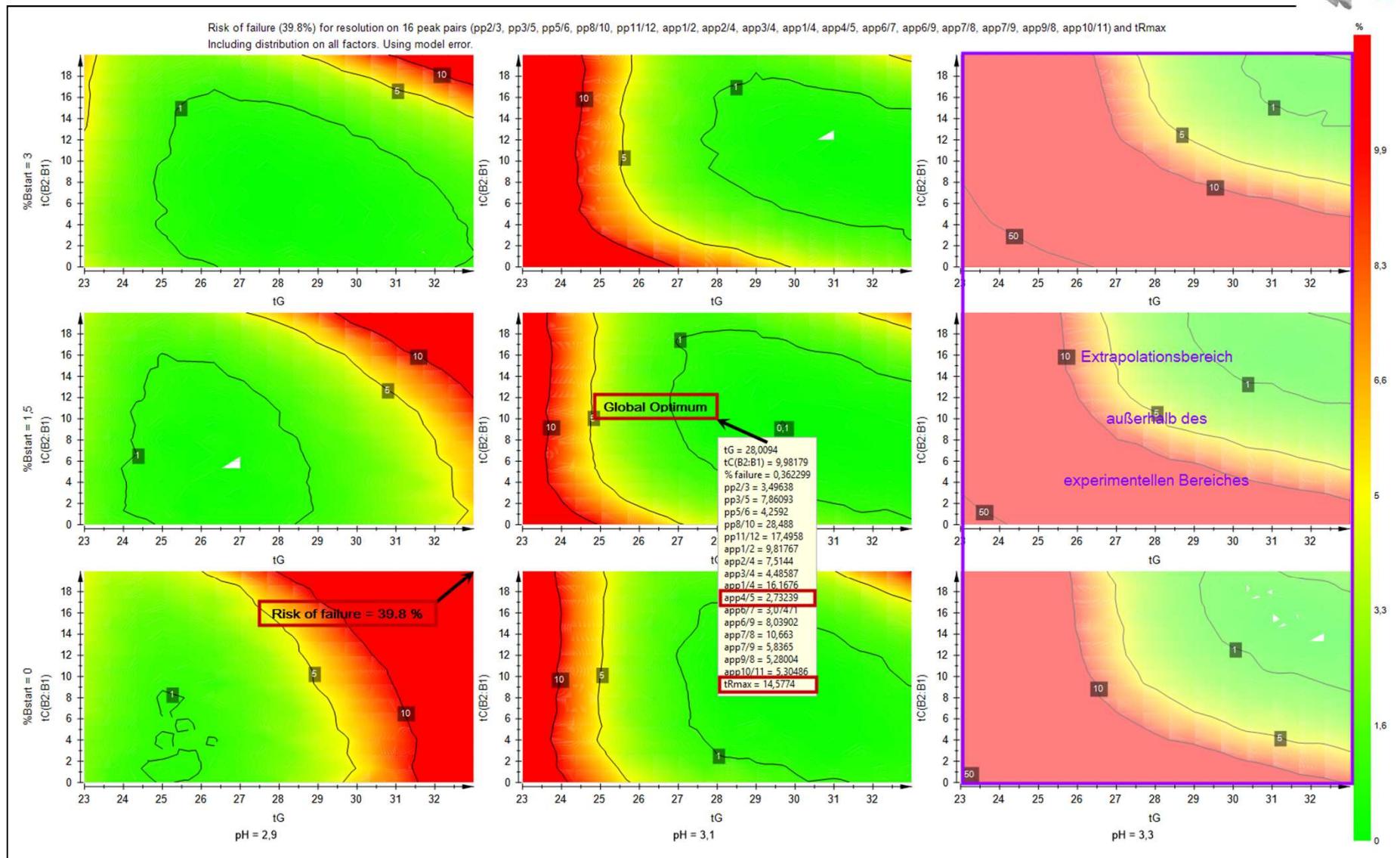


Zielgrößenspezifikationen ($R_{s,krit.} \geq 1.5$, $tR_{max} \leq 20$ min)

Modellinterpretation → Nächste Schritte



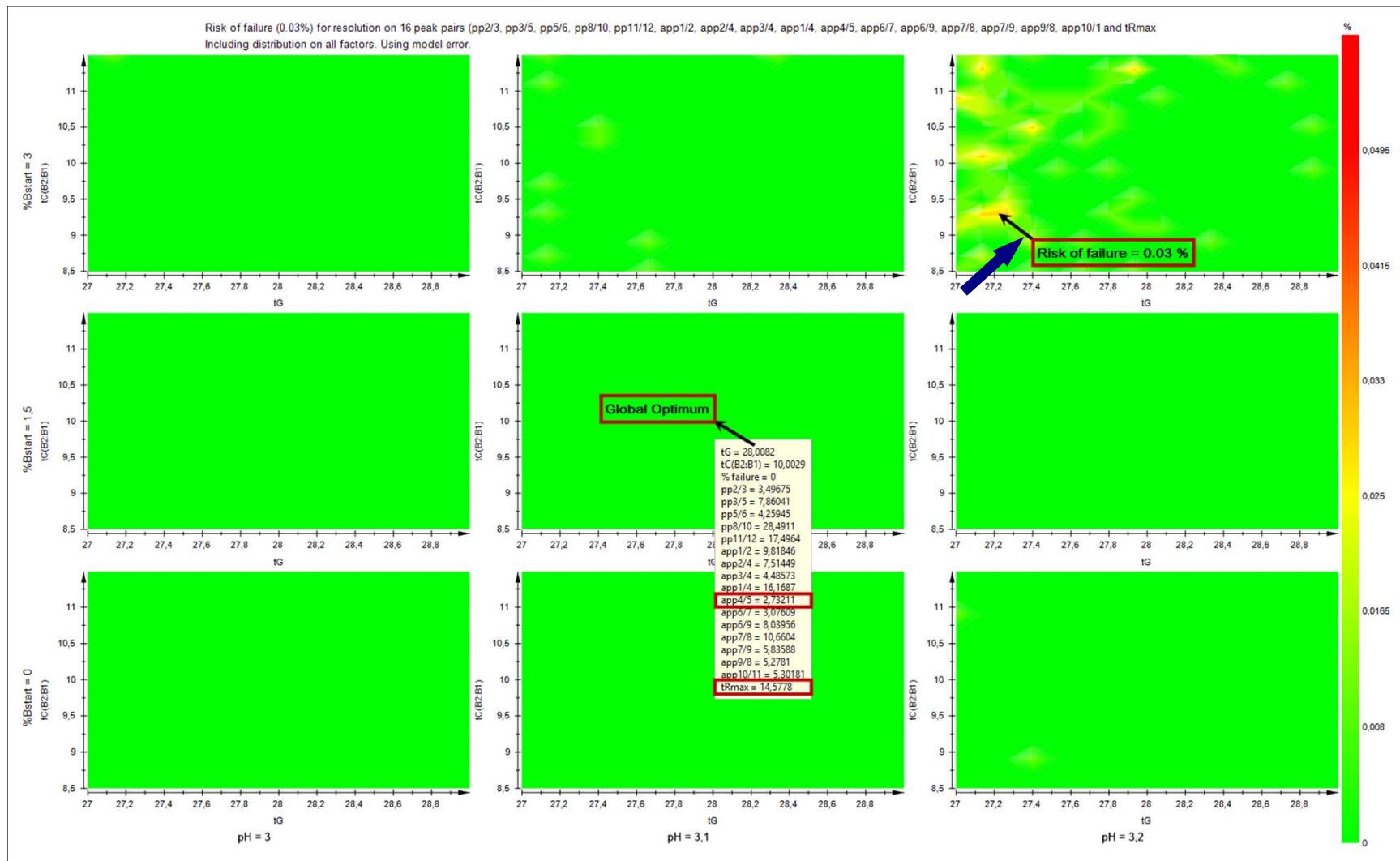
Modellinterpretation III Designs space Schätzung (DSE) - Probability Contour Plot



RP-HPLC-Designspace der 7-Faktor-Optimierung: Faktorenbereiche vor der Faktoranpassung

tG(28min±5min), T(30°C±5°C), tC(10%±10% MeOH in ACN, %B_s(1.5%±1.5%), %Be(90%±2%), pH(3.1±0.2Einheiten), F(1.5ml/min±0.1 ml/min), Verweilvolumen(1.06ml)

Modellinterpretation III Designspace Schätzung (DSE) - Probability Contour Plot



RP-HPLC-Designspace der 7-Faktor-Optimierung: Faktorenbereiche nach der Faktoranpassung
 tG($28\text{min} \pm 1\text{min}$), T($30^\circ\text{C} \pm 1^\circ\text{C}$), tC($10\% \pm 1.5\%$ MeOH in ACN, %B_s($1.5\% \pm 1.5\%$), %Be($90\% \pm 1\%$), pH(3.1 ± 0.1 Einheiten), F($1.5\text{ml}/\text{min} \pm 0.1 \text{ ml}/\text{min}$), Verweilvolumen(1.06ml)

Zusammenfassung / Fazit

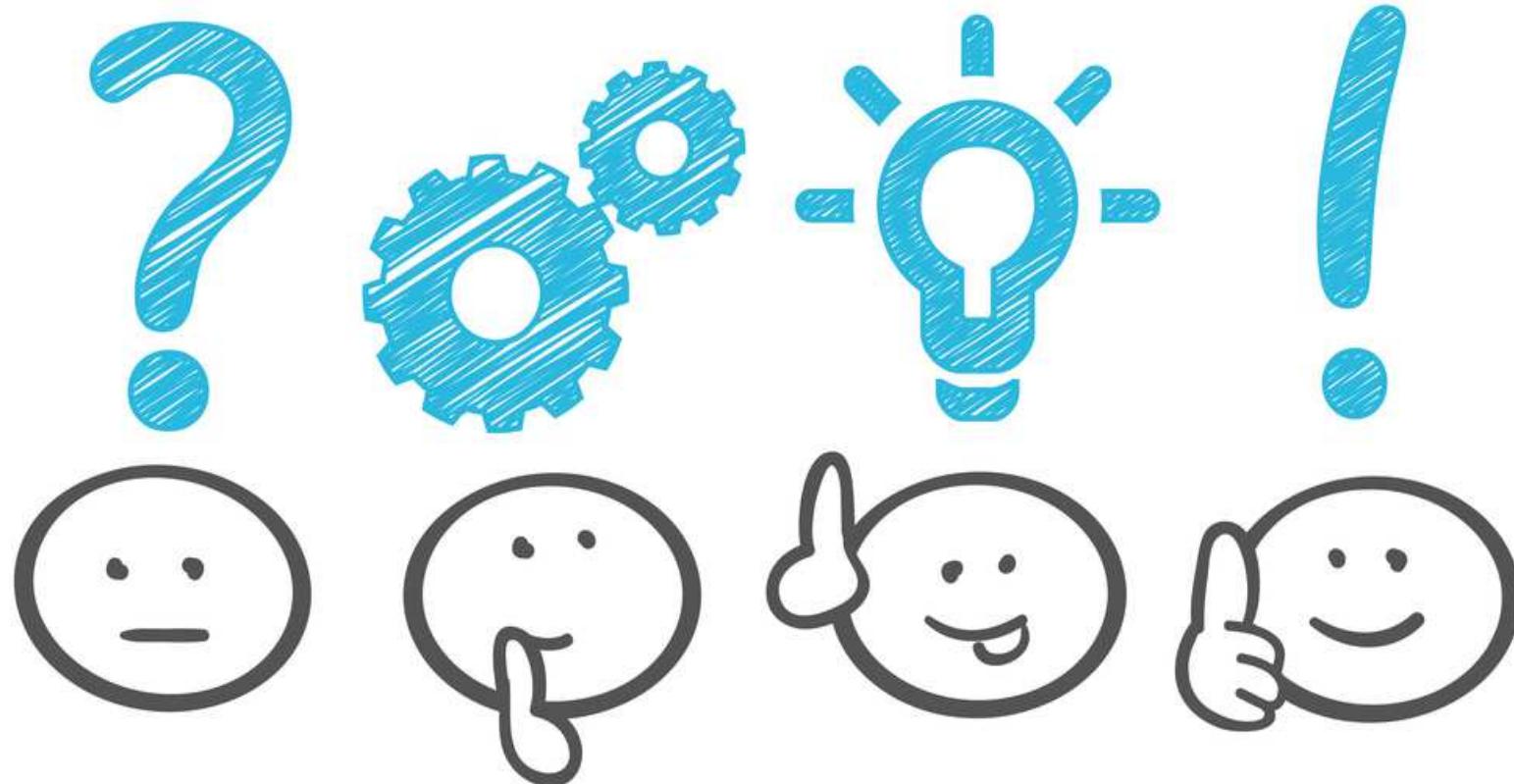


Danksagung

- dem Molnar Institut für angewandte Chromatographie für die Überlassung der Daten zur RP-HPLC-Trennungsoptimierung sowie für die Bereitstellung der
 - Chromatographie-Modellierungs-Software „DryLab® 4“ mit den Modulen „PeakMatch®“ und „3-D Cube®“.
 - bei Umetrics AB für die Überlassung der
 - DoE-Software „MODDE Vers. 9.1.1.0“.
 - Dr. Hans-Werner Bilke / LC-Pharm-HPLC Expert Service

A large, stylized word cloud centered around the words "Thank you". The word "Thank" is at the top in a dark green serif font, "you" is in the middle in a yellow serif font, and "Merci" is at the bottom right in a purple serif font. Surrounding these words are numerous other words in various colors (orange, red, purple, yellow) representing "Thank you" in different languages. Some of the visible translations include "raibh" (Irish), "Dziękuję" (Polish), "Obrigado" (Portuguese), "Teşekkür" (Turkish), "agat" (Malay), "ederim" (Korean), "Gracias" (Spanish), "Köszönöm" (Hungarian), "Gràcies" (Catalan), "Sipas" (Welsh), "Takk" (Norwegian), "fyri" (Icelandic), "baie" (French), "Dank" (German), "Mulțumesc" (Romanian), "Paldies" (Lithuanian), "Kiitos" (Finnish), "Tak" (Chinese), "Tack" (Swedish), "maith" (Irish), "Danke" (German), "Hvala" (Croatian), and "bér" (Hungarian). The background is white with a subtle grid pattern.

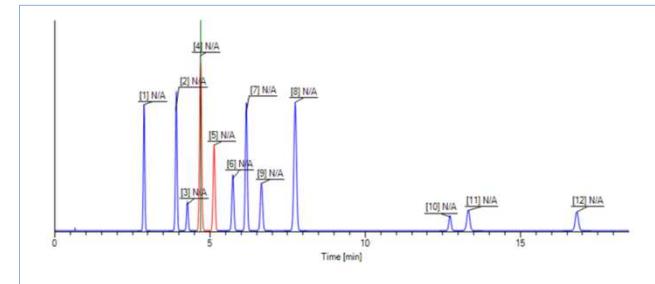
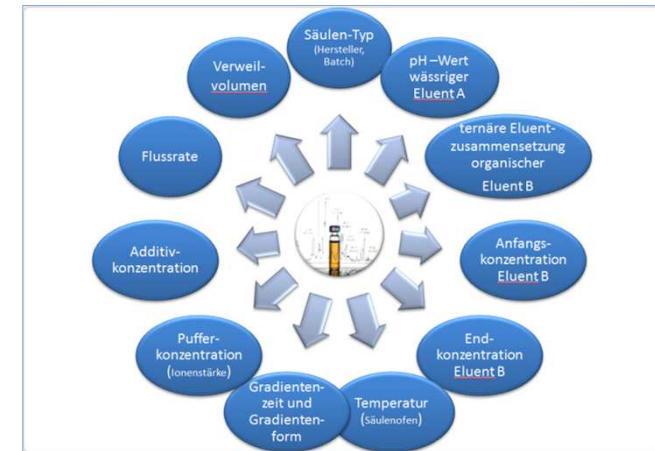
Danke fürs Zuhören !



Stefan Moser
Weberweg 3
D-83131 Nußdorf am Inn
Consultancy, Analysis & Training,
Email stefan_moser@web.de
Mobil: +49 (0) 170 811 240 3

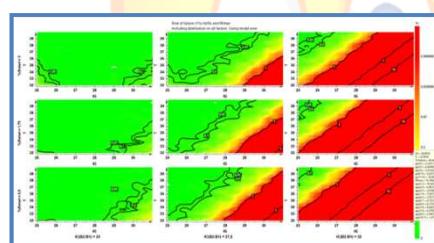
M O S E R S T E F A N
■ ■ ■ **PROCESS
OPTIMIZATION**

- DMAIC / DFSS Projects
- Design of Experiments
- Multivariate Data Analysis
- Product & Process development / optimization
- Rheology Training
- Validation



LC-Pharm-HPLC-Expert Service

Dr. Hans-Werner Bilke
Am Lutzfeld 9
D-83098 Brannenburg am Inn
Consultancy, Analysis & Training,
Email h.bilke@hplcanalytik.de
Mobil: +49 (0) 1525/3809608
Tel: +49 (0) 8034/6079305



- HPLC Method Development
- HPLC Method Optimization
- HPLC Robustness Test
- Quality by Design
- Design of Experiments
- Training and Consulting

Experiment-Nr.	tG	T	tC (B2:B1)	%B _{start}	%B _{end}	pH	F
1	20	30	0	0	90	2.0	0.5
2	20	60	100	10	100	3.2	1.5
3	60	30	100	10	100	3.2	1.5
4	60	60	0	10	100	3.2	1.5
5	60	60	100	0	100	3.2	1.5
6	60	60	100	10	90	3.2	1.5
7	60	60	100	10	100	2.0	1.5
8	60	60	100	10	100	3.2	0.5
9	60	60	0	0	90	2.0	0.5
10	60	30	100	0	90	2.0	0.5
11	60	30	0	10	90	2.0	0.5
12	60	30	0	0	100	2.0	0.5
13	60	30	0	0	90	3.2	0.5
14	60	30	0	0	90	2.0	1.5
15	20	60	100	0	90	2.0	0.5
16	20	60	0	10	90	2.0	0.5
17	20	60	0	0	100	2.0	0.5
18	20	60	0	0	90	3.2	0.5
19	20	60	0	0	90	2.0	1.5
20	20	30	100	10	90	2.0	0.5
21	20	30	100	0	100	2.0	0.5
22	20	30	100	0	90	3.2	0.5
23	20	30	100	0	90	2.0	1.5
24	20	30	0	10	100	2.0	0.5
25	20	30	0	10	90	3.2	0.5
26	20	30	0	10	90	2.0	1.5
27	20	30	0	0	100	3.2	0.5
28	20	30	0	0	100	2.0	1.5
29	20	30	0	0	90	3.2	1.5
30	60	45	50	5	95	2.6	1
31	40	60	50	5	95	2.6	1
32	40	45	100	5	95	2.6	1
33	40	45	50	10	95	2.6	1
34	40	45	50	5	100	2.6	1
35	40	45	50	5	95	3.2	1
36	40	45	50	5	95	2.6	1.5
37	40	45	50	5	95	2.6	1

Rechtschaffner-Versuchsplan - Ergebnisse

Exp.-Nr.	R _s 1/2	R _s 2/3	R _s 2/4	R _s 3/4	R _s 3/5	R _s 1/4	R _s 4/5	R _s 5/6	R _s 6/7	R _s 6/9	R _s 7/8	R _s 7/9	R _s 9/8	R _s 8/10	R _s 10/11	R _s 11/12	tR _{max}
1	0.0	1.4	6.7	5.4	7.0	6.7	1.2	0.6	0.6	7.4	11.2	7.1	4.2	7.5	9.2	9.9	17.4
2	8.8	3.4	4.8	1.5	5.7	13.1	4.0	6.3	4.0	6.1	5.5	2.6	2.5	39.4	0.3	19.6	10.9
3	16.7	5.0	2.5	-2.3	5.0	18.1	7.0	7.9	10.1	8.2	-2.4	-0.9	-1.3	60.6	-10.9	22.9	26.2
4	5.8	2.2	9.6	7.1	9.2	14.6	2.0	4.7	0.2	5.5	14.8	5.3	9.8	34.8	5.0	18.8	15.5
5	15.4	5.8	0.3	-5.3	6.2	15.1	11.3	11.3	4.0	-2.7	0.9	-6.3	6.9	41.0	-5.6	28.2	25.6
6	9.6	3.9	4.8	0.9	6.3	14.4	5.5	8.3	4.4	4.1	4.1	-0.2	4.1	40.3	-4.4	27.5	22.6
7	0.7	2.1	-3.2	-5.4	4.3	-2.6	9.7	8.2	6.4	9.0	2.4	2.6	-0.1	42.4	-5.0	26.9	21.0
8	8.0	3.0	4.3	1.4	5.2	12.0	3.7	5.8	3.6	5.6	4.6	2.3	2.2	33.2	0.2	16.2	32.7
9	1.3	3.1	0.9	-2.0	4.8	2.0	6.5	5.1	2.0	5.2	7.8	3.5	4.1	26.1	6.5	14.8	32.4
10	1.7	4.9	-5.0	-9.1	2.7	-3.3	11.2	6.1	10.1	13.8	-1.2	5.3	-5.9	43.0	-4.4	17.6	43.9
11	0.0	2.6	7.9	5.6	9.6	7.9	3.6	2.1	2.4	11.2	14.0	9.2	5.0	21.4	8.1	16.0	30.8
12	0.0	2.9	2.8	0.3	7.4	2.8	6.2	2.9	2.8	10.4	12.4	8.0	4.4	20.0	8.4	15.5	32.9
13	10.2	2.9	8.6	6.1	8.3	17.5	1.6	3.4	2.0	7.7	13.4	6.2	6.8	21.3	8.4	15.8	35.2
14	0.0	4.2	-3.9	-7.9	4.5	-3.9	11.4	5.9	6.2	10.8	8.1	5.1	3.0	37.0	0.2	18.3	23.9
15	0.4	1.1	-1.9	-3.3	2.7	-1.5	5.8	3.5	3.6	8.5	3.5	5.2	-1.7	25.9	2.8	8.0	18.9
16	0.8	2.6	4.8	2.3	4.9	5.5	2.5	2.7	0.5	7.1	12.7	6.6	6.1	11.4	8.3	10.2	15.1
17	0.7	1.8	2.7	1.0	3.8	3.4	2.7	2.4	0.4	6.0	11.0	5.6	5.4	9.4	7.9	8.8	15.5
18	5.4	1.5	5.2	3.8	5.3	10.2	1.4	2.5	-0.2	4.3	12.4	4.6	7.8	9.1	8.3	9.3	16.4
19	1.4	3.4	0.9	-2.3	5.2	2.3	7.0	5.6	2.1	5.9	8.5	3.9	4.8	29.6	7.3	17.1	10.8
20	-0.7	3.8	4.7	1.2	2.9	4.0	1.4	2.4	6.9	16.2	2.4	9.8	-7.1	23.1	1.0	10.5	19.5
21	-0.7	3.4	4.1	1.1	2.6	3.4	1.3	1.9	6.0	14.7	2.5	9.2	-6.7	19.9	1.6	8.8	18.9
22	9.7	2.0	5.6	3.7	3.8	14.5	-0.1	2.1	5.6	13.0	3.4	8.0	-4.5	20.6	1.9	9.6	20.3
23	1.7	4.8	-5.0	-9.4	2.7	-3.5	11.4	6.0	10.3	14.0	-1.3	5.4	-6.1	43.6	-4.5	18.4	14.6
24	0.0	1.4	8.1	6.8	7.5	8.1	0.3	0.3	0.5	7.6	11.6	7.4	4.3	7.3	9.3	9.9	15.2
25	6.9	1.5	11.5	10.1	8.5	17.9	-1.7	1.2	-0.7	6.3	13.0	7.1	5.6	8.3	10.3	10.7	16.2
26	0.0	2.3	7.5	5.4	9.1	7.5	3.4	2.0	2.3	10.8	13.3	8.6	4.9	20.4	7.9	16.3	10.3
27	5.6	1.2	9.6	8.7	7.4	15.1	-1.5	1.0	-0.7	5.5	11.3	6.2	4.9	6.2	9.3	9.4	16.3
28	0.0	2.8	2.8	0.3	7.3	2.8	6.2	2.8	2.7	9.9	11.9	7.4	4.3	19.7	7.8	14.7	11.0
29	10.0	3.0	8.5	6.0	8.1	17.0	1.5	3.2	1.9	7.5	12.7	6.0	6.7	20.7	8.3	16.3	11.7
30	4.7	4.1	3.5	-0.3	5.8	8.0	5.7	6.6	5.5	8.2	5.0	3.1	1.7	41.2	-2.6	20.7	26.9
31	4.5	3.4	3.8	0.5	5.6	8.3	4.9	6.4	3.5	6.2	6.3	3.0	3.0	34.6	0.2	20.4	19.5
32	6.1	4.3	-0.4	-4.5	3.7	5.5	8.0	7.6	7.8	9.7	0.4	2.7	-2.2	47.8	-4.5	20.3	23.3
33	3.9	3.3	7.0	3.9	6.6	10.5	2.4	5.1	4.7	10.8	8.0	6.5	1.5	37.5	-0.1	19.6	19.4
34	4.4	3.5	4.3	1.0	5.9	8.5	4.5	5.8	4.9	9.4	6.6	4.9	1.6	37.7	-0.1	19.3	20.2
35	11.1	3.5	6.4	3.2	7.2	17.2	3.7	5.6	3.9	6.5	7.5	3.0	4.2	36.6	0.2	19.4	20.9
36	4.8	4.0	3.4	-0.4	5.9	8.2	6.0	6.7	5.6	8.3	5.3	3.3	1.7	42.4	-2.6	21.4	18.0
37	4.6	3.7	4.3	0.9	5.7	8.7	4.5	5.7	4.9	9.2	6.4	4.7	1.6	37.5	-0.5	19.6	20.9