Reduce Time to market by Data Modeling in Analytics of Biopharmaceuticals

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Problems of Pharma Companies

- We have ca. 100,000 diseases, but only for 20,000 of them are drugs available
- Smaller patient populations, such as those with Citric Fibrosis (CF) are still suffering and need more help
- Vertex could develop 3 new drugs for CF in the last 3 years using modeling
- FDA supports Orphan Drug Development for Rare Diseases

Reduce Time in Analytics by Modeling

- HPLC separations take today btw. 10-30 min, Peptide Maps needs often > 60 min
- With modeling a run takes **one second only**
- The largest amount of time however is lost with Out of Specification (OoS) results, followed by „Change Management“, due to a **lack of understanding** the separation process

Biopharmaceuticals

- New Analytical Challenges in HPLC of Biopharmaceuticals
  - Higher complexity (mAb's, therapeutic Proteines),
  - HPLC of Antibody-Drug-Conjugates (ADC's)
- Quality by Design (QbD)
  - Visualizing Risk using Resolution Maps
  - Visualizing a Design Space by setting ATP
  - Calculate Robustness of methods
  - Predict OoS – Establish expectable Success Rate
- Knowledge Management Documentation for Data Integrity

Knowledge Management

Data Integrity in Analytics:

- Explain, why do you work at this set of conditions?
- How did you find this working point?
- Show your „solid Science“ with your experiments
- Create a model of your separation
- Collect everything in one signed document

Time to market

- The first company entering the market with a new drug receives 80% market share
- The second company receives ca. 10%, the 3rd 5%, etc.
- Therefore it is important to be the first one on the market – you have to speed up your work

Problems to get regulatory approval

- **Too many Analytical Method Variabilities**
- Insufficient Method Stability -> Results are too often **OoS**
  - due to **Variabilities of Parameters:** pH, temperature, gradient time, ternary composition, gradient shape, etc.,
  - and to **Variabilities of Instrument Factors:** Dwell Volume, Extra Column Volume, Flowrate, Pump Accuracy, Column Dimensions, etc.
- Post Approval Changes need better Understanding in Communication between Regulators and Applicants
- Modeling explains Characteristics of the **Design Space**, resulting in Robust Analytical Methods, incl. a Reporting Tool: the **Knowledge Management Document**

How can we speed up time to market?

- Eliminate Trial & Error by Modeling, especially in Analytics (HPLC)
- **Modeling** allows to make experiments in **seconds** instead of **days**
- Modeling is a bridge between applicant and regulatory professionals and allow faster process understanding and process approval
- **Easier dealing with Post Approval Changes**

Case Study 1: Method Optimization with DryLab® 4 Design Space

Ralf Hohl
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24.10.2016

SANOFI

DryLab®4-Chromatography Expert Team

General Principles of HPLC-Modeling

- **First Step:** Calibration - Modeling is based on initial experiments from at least two analytical runs
- **Second Step:** Peak Tracking , followed by Calculation of the Model - *In-silico* simulation to optimize method under different conditions
- **Third Step:** Verification of "Prediction" by performing final experiments

Optimization Parameters

- Slope of gradient, tG,
- Gradient steps
- Column temperature, T
- pH of mobile phase (for dissociated/charged molecules)
- Ternary Composition, tC, of mobile phase (for neutral molecules)(Ratio of AcN vs. MeOH in eluent B)
- Ionic strength of mobile phase

Initial Situation / Existing Method

- Purpose
  - Assay and purity testing of drug substance and drug product
    - Development project, small molecules
- Equipment
  - Conventional HPLC systems such as Agilent 1100 or Waters Alliance
    - System-(dwell)-volume ≥ 1.0 mL
- Goal
  - Optimization with DryLab®4 using a *"Gradient time, tG /Temperature, T/Ternary Eluent, tC" (tG-T-tC-Design)*

Initial Situation / Existing Method

- **HPLC instrument configuration**

Waters Alliance™	Separations Module 2695
	Dual Absorbance Detector 2487
	System volume = 1.0 mL

- **Procedure**

Flow rate:	1.0 mL/minute
Injection volume:	10 µL
Autosampler temperature:	8°C
Column temperature:	50°C
Detection wavelength(s):	256 nm
Typical total run time:	**42 minutes**

Initial Situation / Existing Method –

Analytical Conditions

- Column

Stationary phase:	Waters - XBridge Shield RP18, 3.5 µm
Column dimension:	4.6 mm x 150 mm

- Eluents

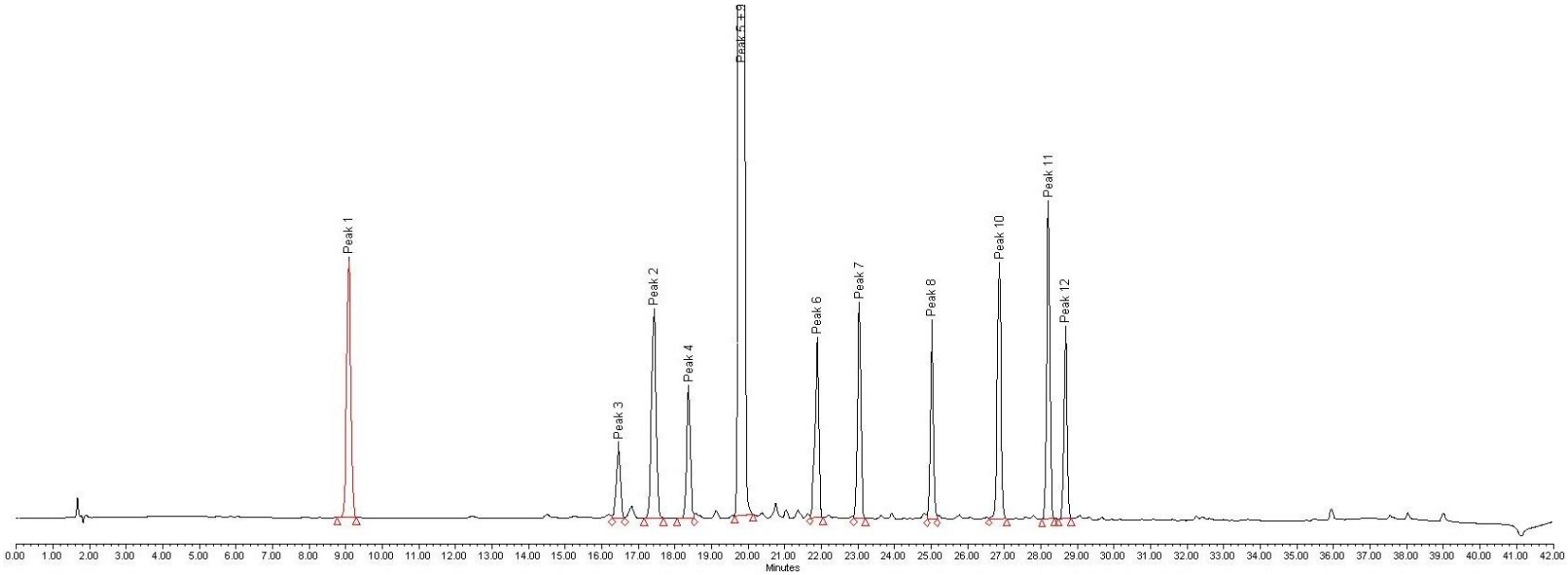
Mobile phase A		Mobile phase B	
Buffer pH 3.7 Acetonitril	800 mL 200 mL	Buffer pH 3.7 Acetonitril	100 mL 900 mL
Buffer: 2.21g ammonium formiate in 1000 mL water, pH adjustment with formic acid to pH 3.7			

- Gradient

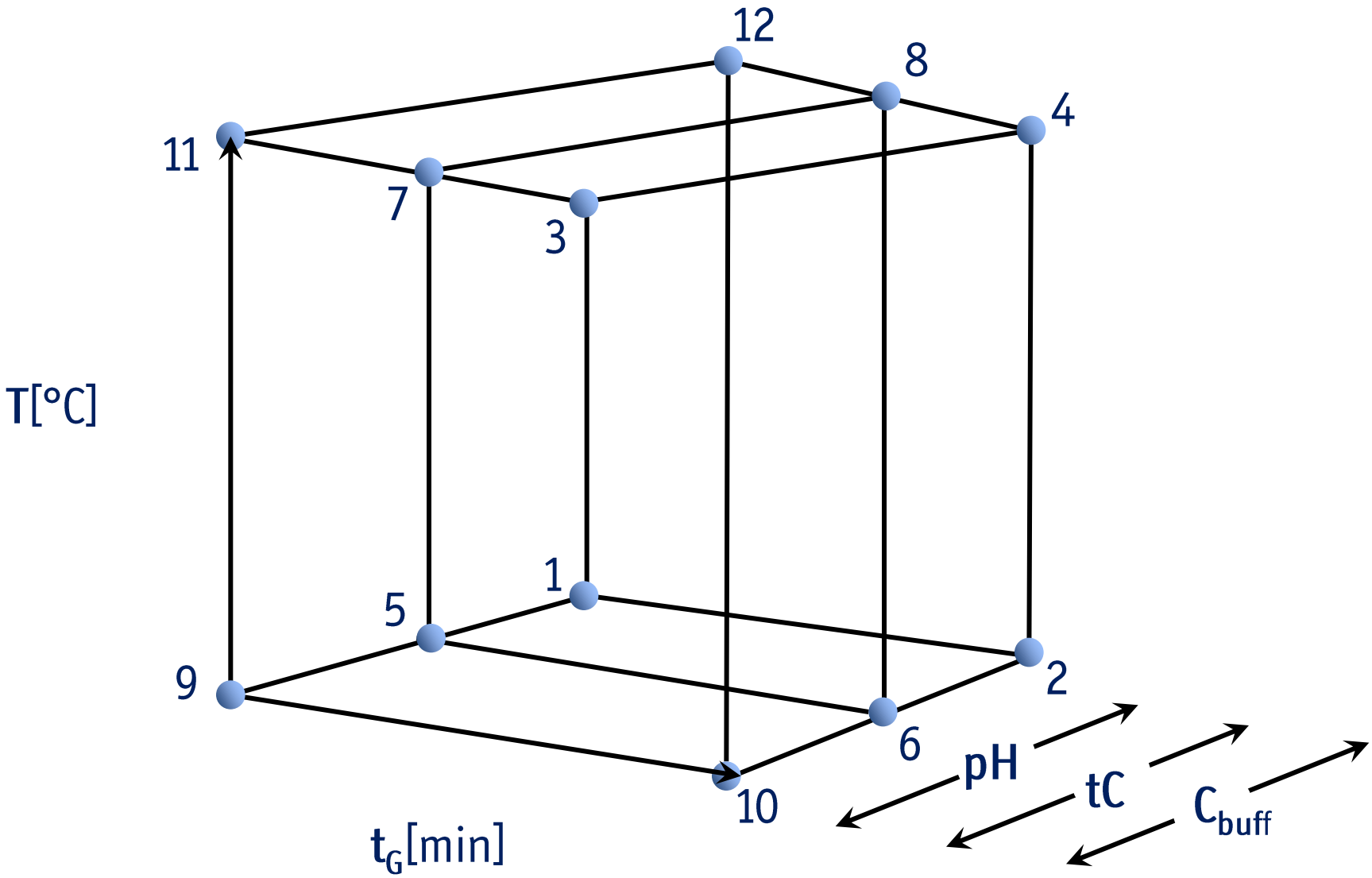
Time [minutes]	Mobile phase A [%]	Mobile phase B [%]
0	90	10
13	76	24
28	39	61
35	0	100
39	90	10
42	90	10

Initial Situation / Existing Method

Example of a Chromatogram / Spiked Sample



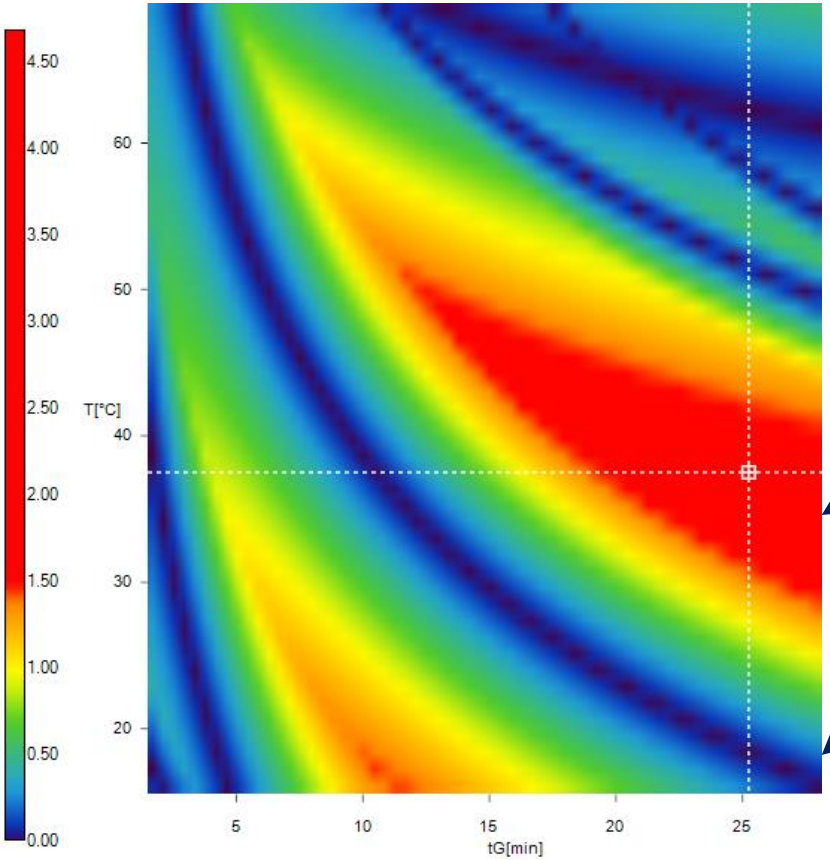
Design of Experiments (DoE)



Method Optimization
Initial Runs

	Gradient tG [min]	Temperatur T [°C]	Eluent %B
1	10 min	25°C	Methanol 100%
2	30 min	25°C	Methanol 100%
3	10 min	65°C	Methanol 100%
4	30 min	65°C	Methanol 100%
5	10 min	25°C	Methanol/Acetonitril 50%/50%
6	30 min	25°C	Methanol/Acetonitril 50%/50%
7	10 min	65°C	Methanol/Acetonitril 50%/50%
8	30 min	65°C	Methanol/Acetonitril 50%/50%
9	10 min	25°C	Acetonitril 100%
10	30 min	25°C	Acetonitril 100%
11	10 min	65°C	Acetonitril 100%
12	30 min	65°C	Acetonitril 100%

Method Optimization
DryLab® Resolution Map

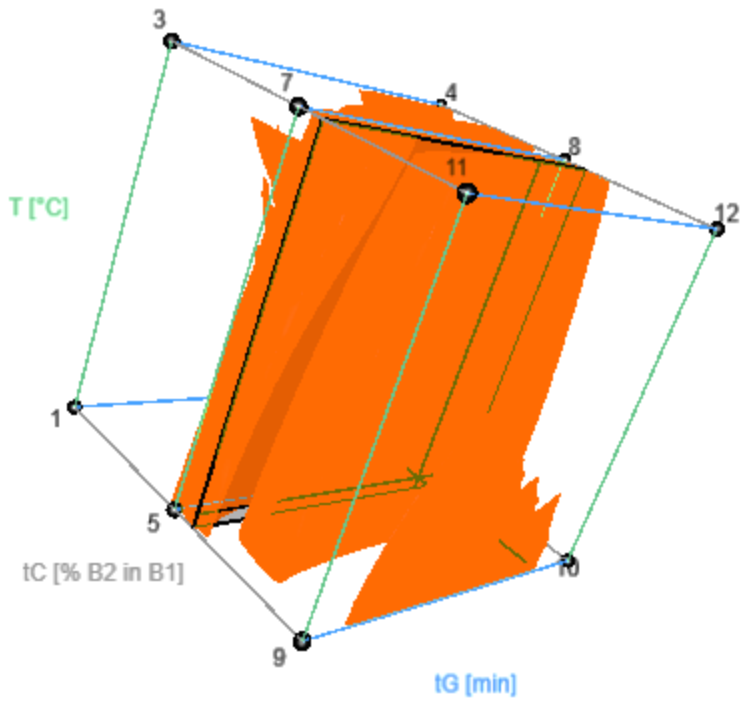


Method Operable Design Region

Coelution

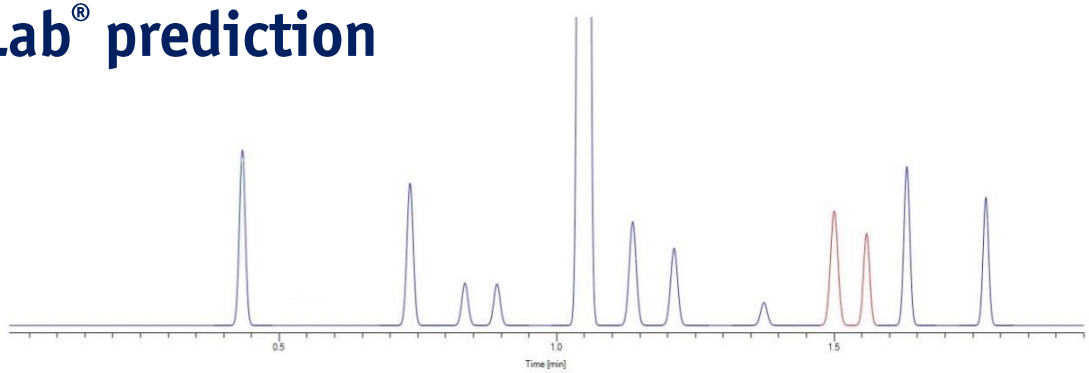
Method Optimization

- **Design Space (orange)** with critical resolution > 1.5 (= Baseline Resolution)

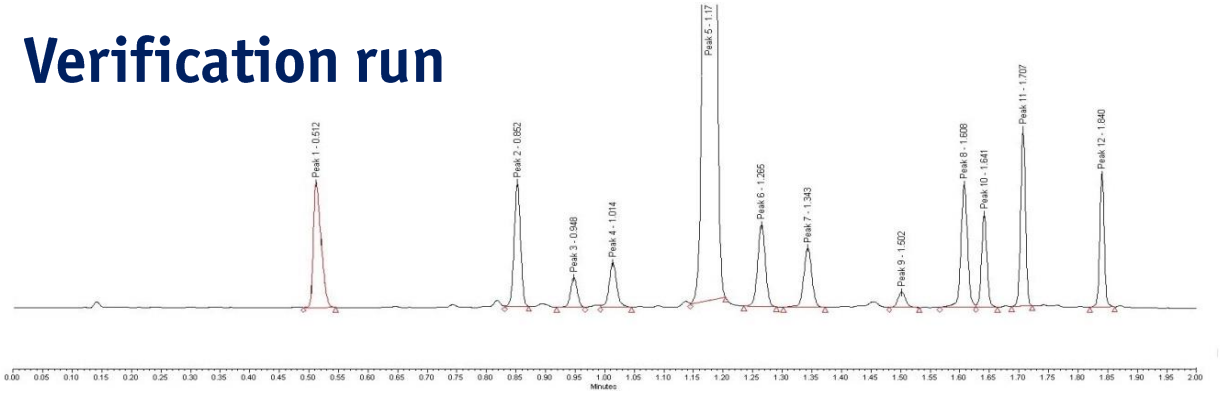


Final Method – Comparison and Verification

DryLab® prediction

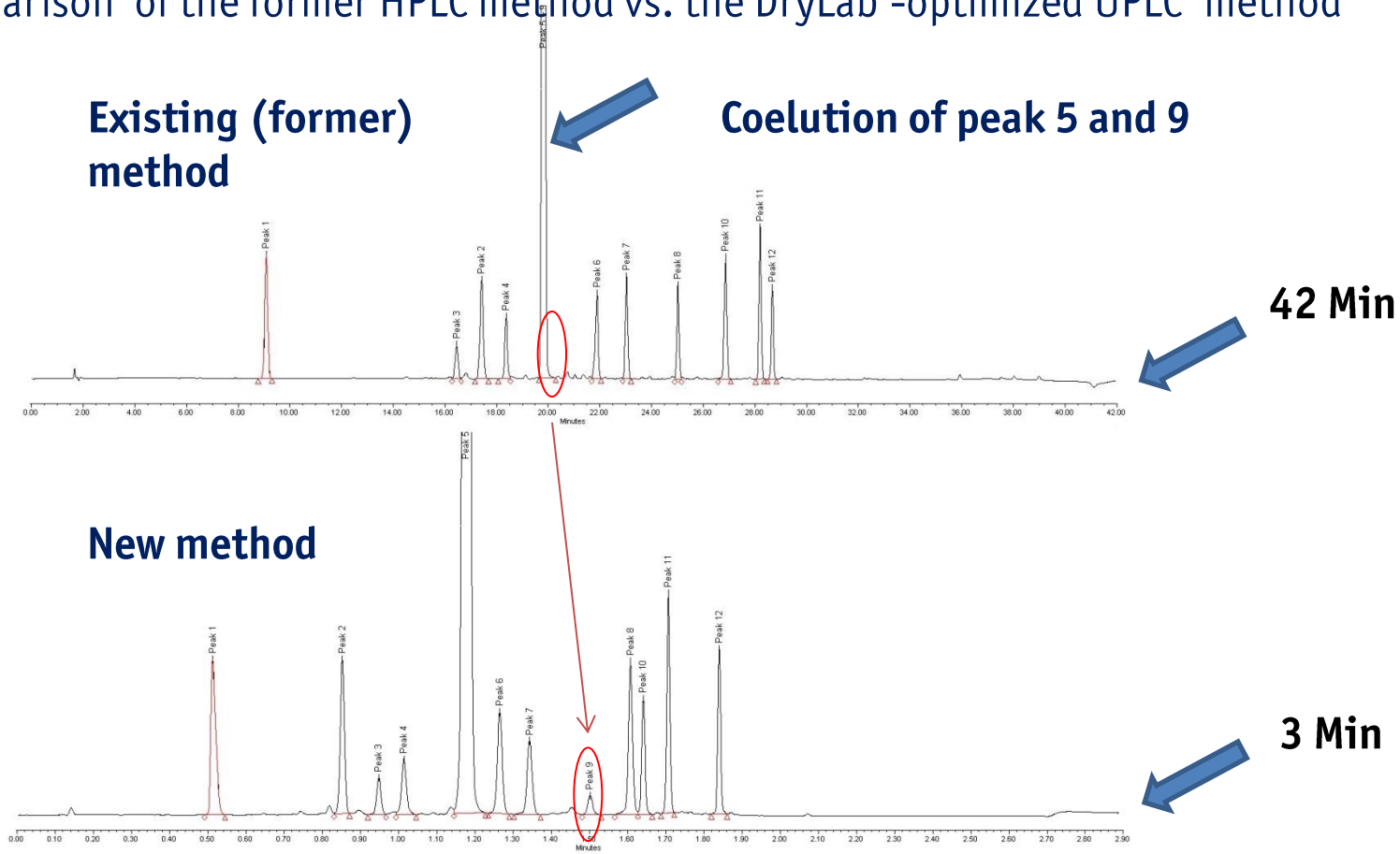


Verification run



Final Method

- Comparison of the former HPLC method vs. the DryLab®-optimized UPLC® method



Final Method

- Comparison of the existing HPLC method vs optimized DryLab® UPLC® method

	Former method	DryLab® method
System volume	1.0 mL	150 µL
Column dimension	4.6 mm x 150 mm, 3 um	2.1 mm x 50 mm, 1.7um
Total run time	42 min	3 min
Total number of peaks	11	12
Resolution critical peak pair	0	1.8

No separation of peak 5 (API) and peak 9 within the former method(!)
Improvement with the new and faster method.

Final Method

- **Comparison of the existing (original) HPLC method vs optimized DryLab® UPLC® method**

Peak	Retention time [min]		Symmetry		Resolution (USP)	
	Existing method	DryLab® method	Existing method	DryLab® method	Existing method	DryLab® method
Peak 1	9.09	0.51	0.98	1.60	-	-
Peak 2	17.43	0.85	0.95	0.99	34.46	14.96
Peak 3	16.46	0.95	0.92	0.98	4.34	4.61
Peak 4	18.37	1.01	1.03	0.99	4.47	2.98
Peak 5	19.82	1.18	0.94	1.09	8.20	6.78
Peak 6	21.89	1.27	0.81	1.02	11.69	3.60
Peak 7	23.04	1.34	0.96	1.03	6.32	3.12
Peak 8	25.02	1.61	1.00	1.03	12.12	4.82
Peak 9	19.82	1.50	n.a.	1.10	0 (co-elution)	6.51
Peak 10	26.86	1.64	0.94	1.03	10.84	1.84 (critical peak pair)
Peak 11	28.20	1.71	0.97	1.01	7.64	4.07
Peak 12	28.68	1.84	0.97	1.04	2.94	8.91

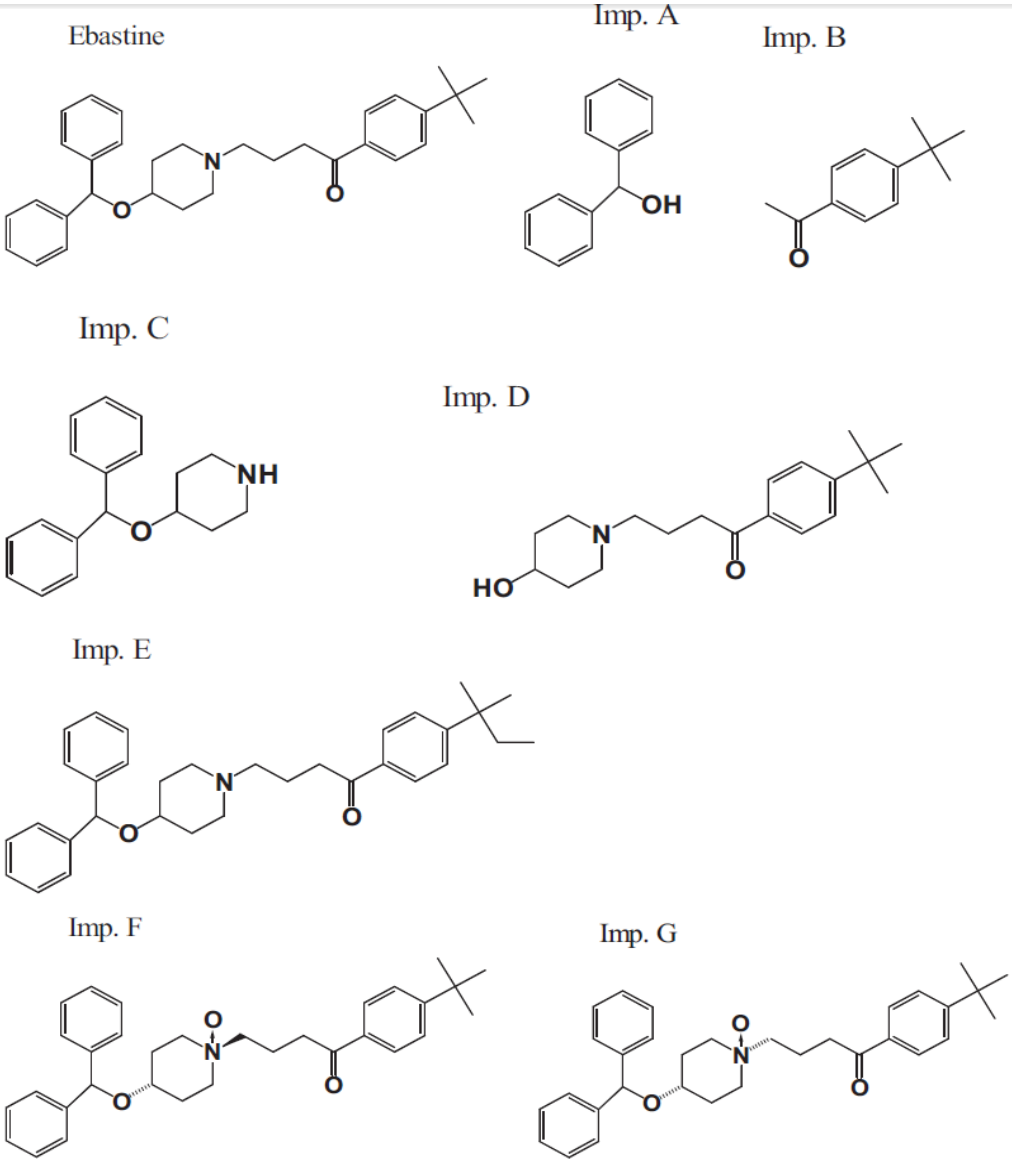
Fazit

Benefits of using Modeling Software

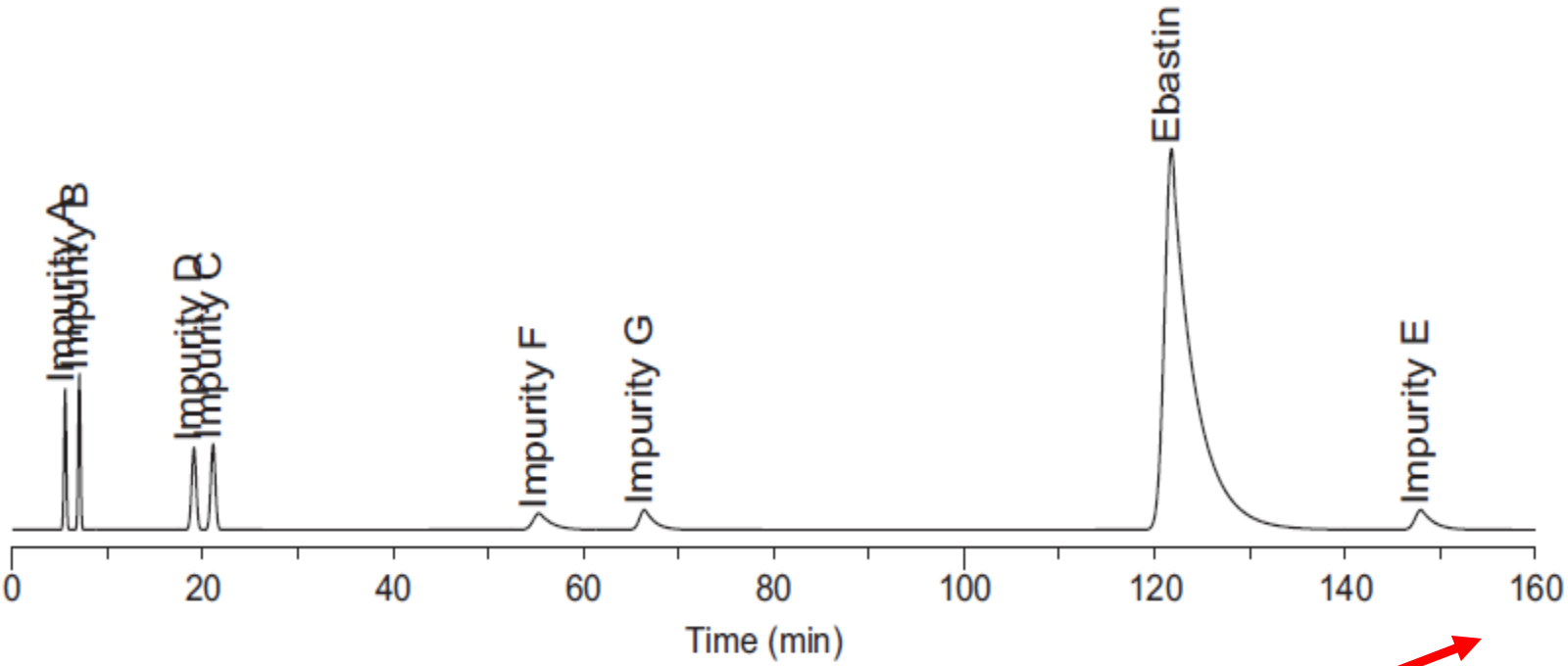
- Only a few HPLC runs were necessary to successfully predict a suitable and robust HPLC method
- Possibility of estimation of **Robustness** without additional runs
- **Method lifecycle management**
  - **Adjustments of HPLC method parameters** within the **Design Space are without further experiments possible**
- **Significant saving** of resources, time, equipment, and materials

2. Case study: Ebastin

Ebastin
Impurities
In EUPharm



EuPharm Method for Ebastin



Analysis time: 160 min

Ebastin Design Space

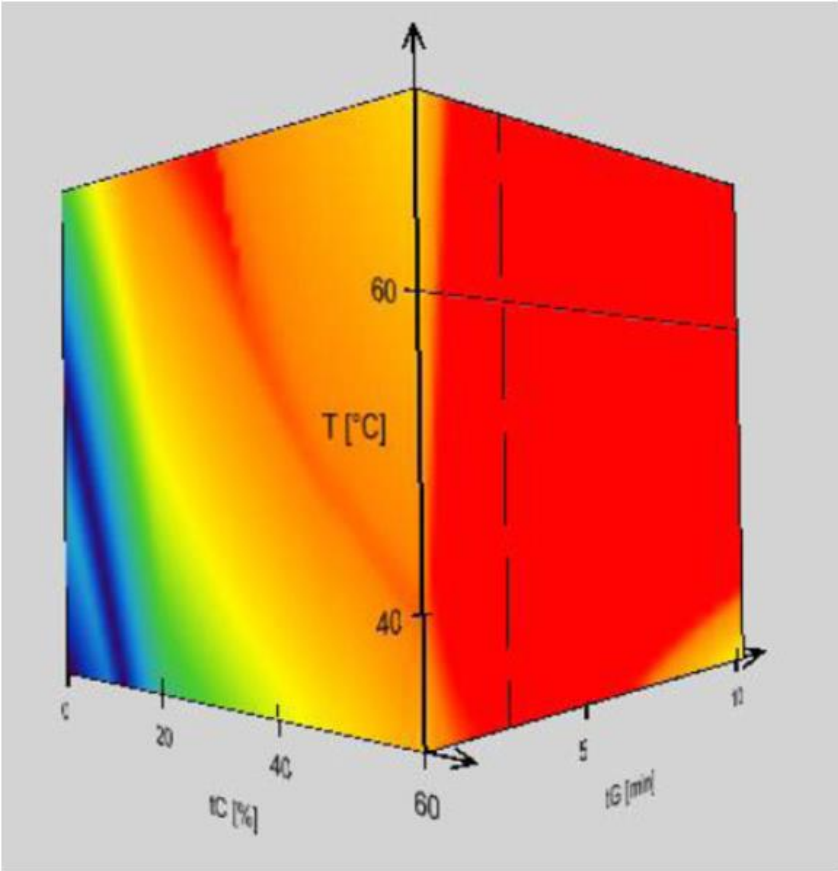
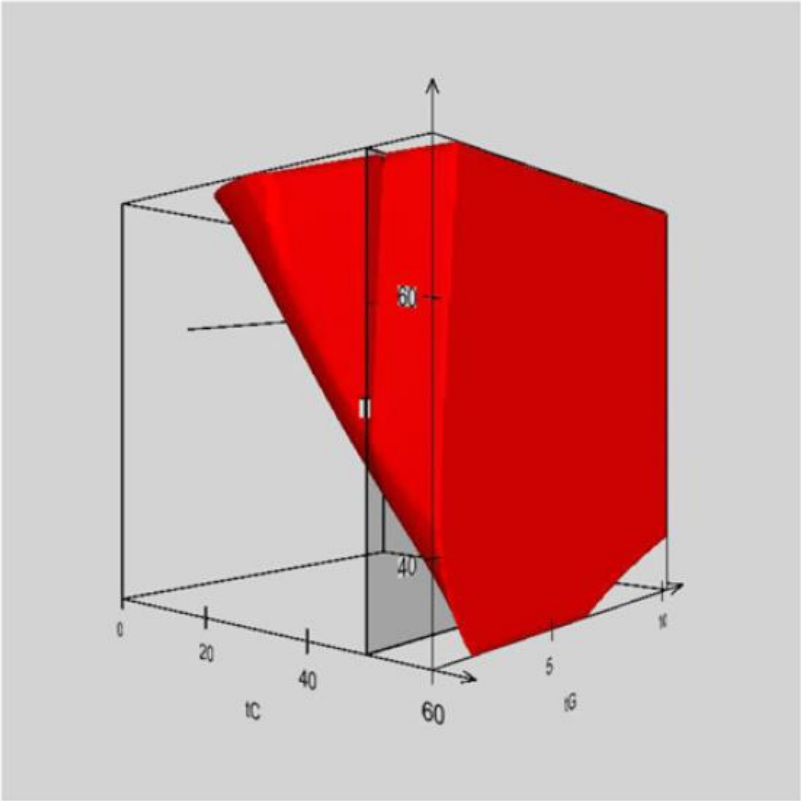
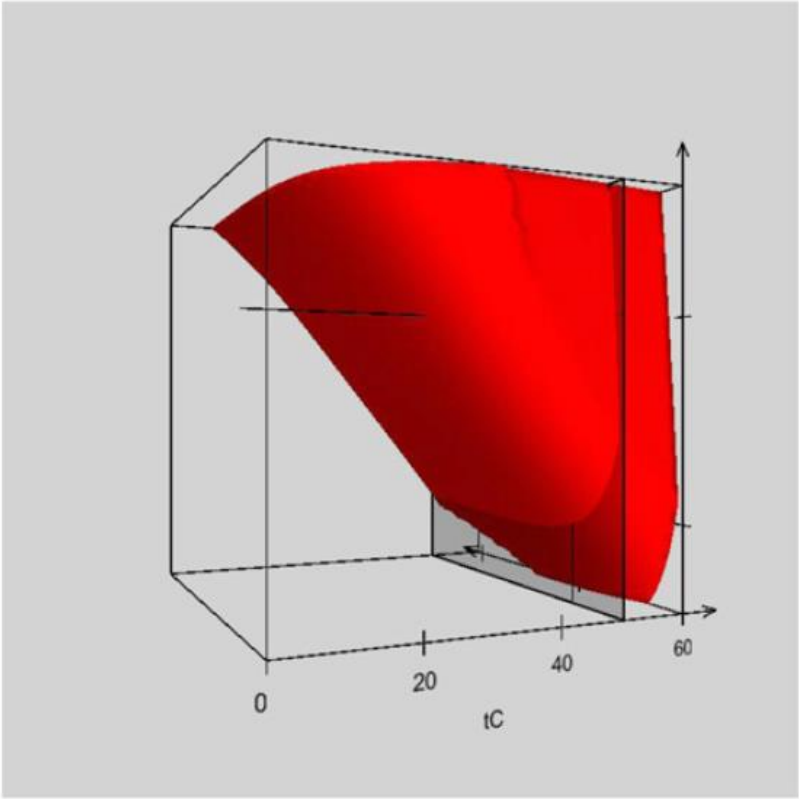


Fig. 5. The three 2-dimensional resolution maps were used to create a 3-D-resolution cube, in which the combined influences of the optimized parameters are visualized: Each point in these 3-D resolution cube stands for a highly accurate chromatogram. A comparison of predicted and experimental retention times for five validation points within the 3D resolution cube was found to be excellent.

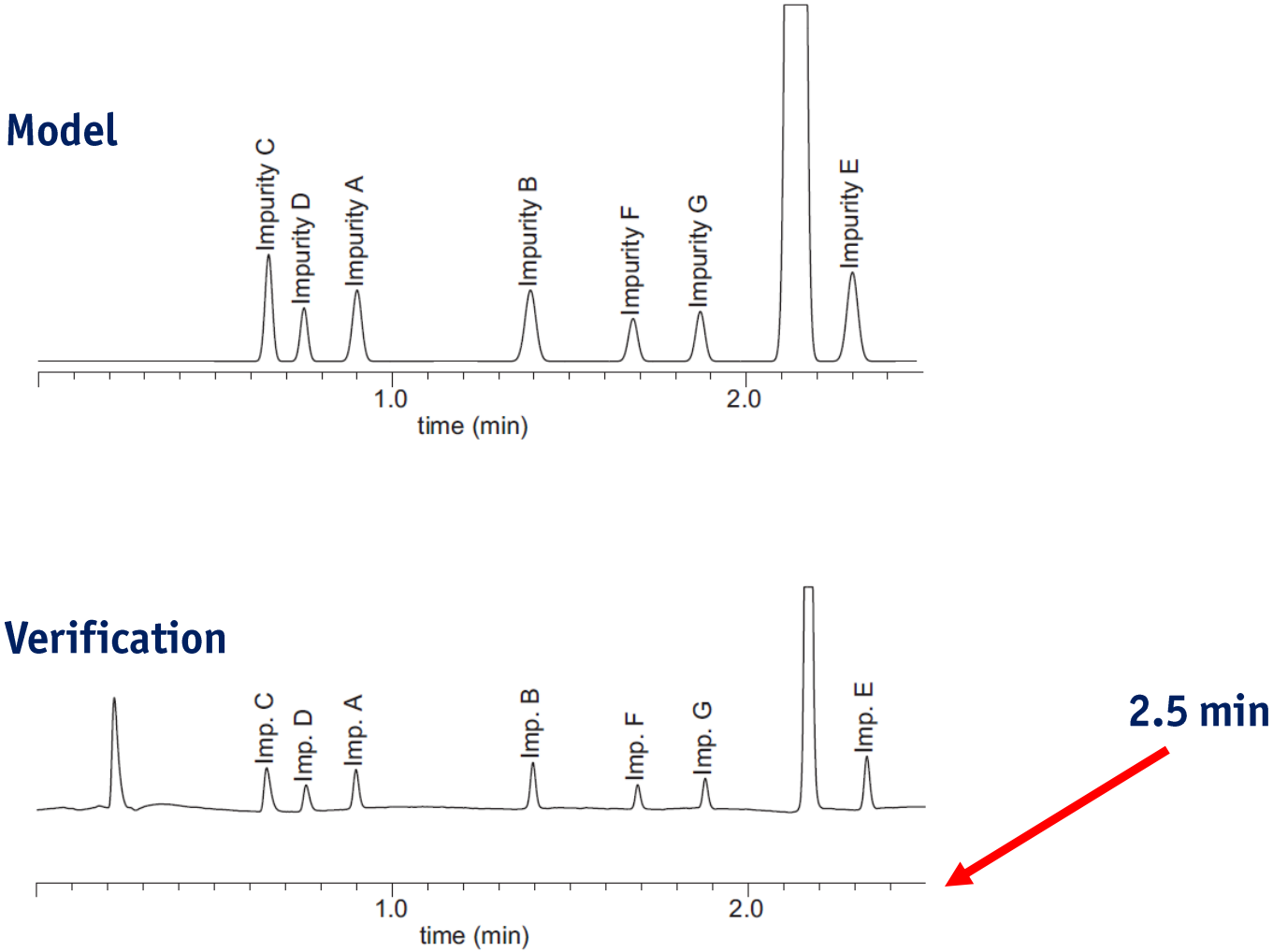
Ebastin Design Space





The red volume indicates baseline resolution regions (Rs,crit > 1.5) in a tG-T-tC-Cube
tC: ternary composition of AcN:i-PrOH in eluent B.

DryLab Prediction vs. Experimental verification



A.Schmidt, I.Molnár, J. Pharm. Biomed. Anal., **78-79**, 65-74 (2013)

3. Case Study: QbD in Protein Research

J. Kochling et al. / Journal of Pharmaceutical and Biomedical Analysis 125 (2016) 130–139

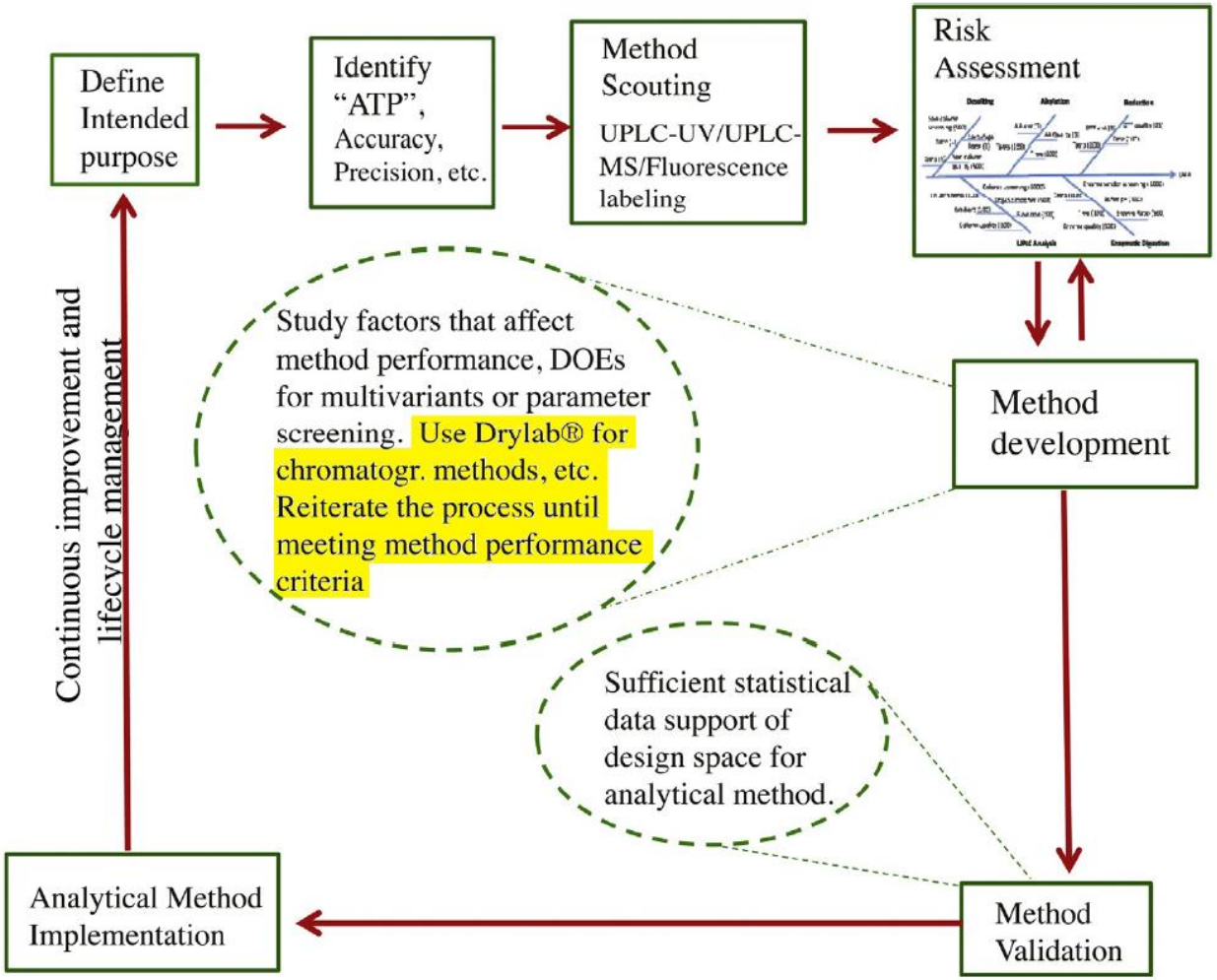


Fig. 1. A flow diagram illustrating the process of analytical quality by design (AQbD).

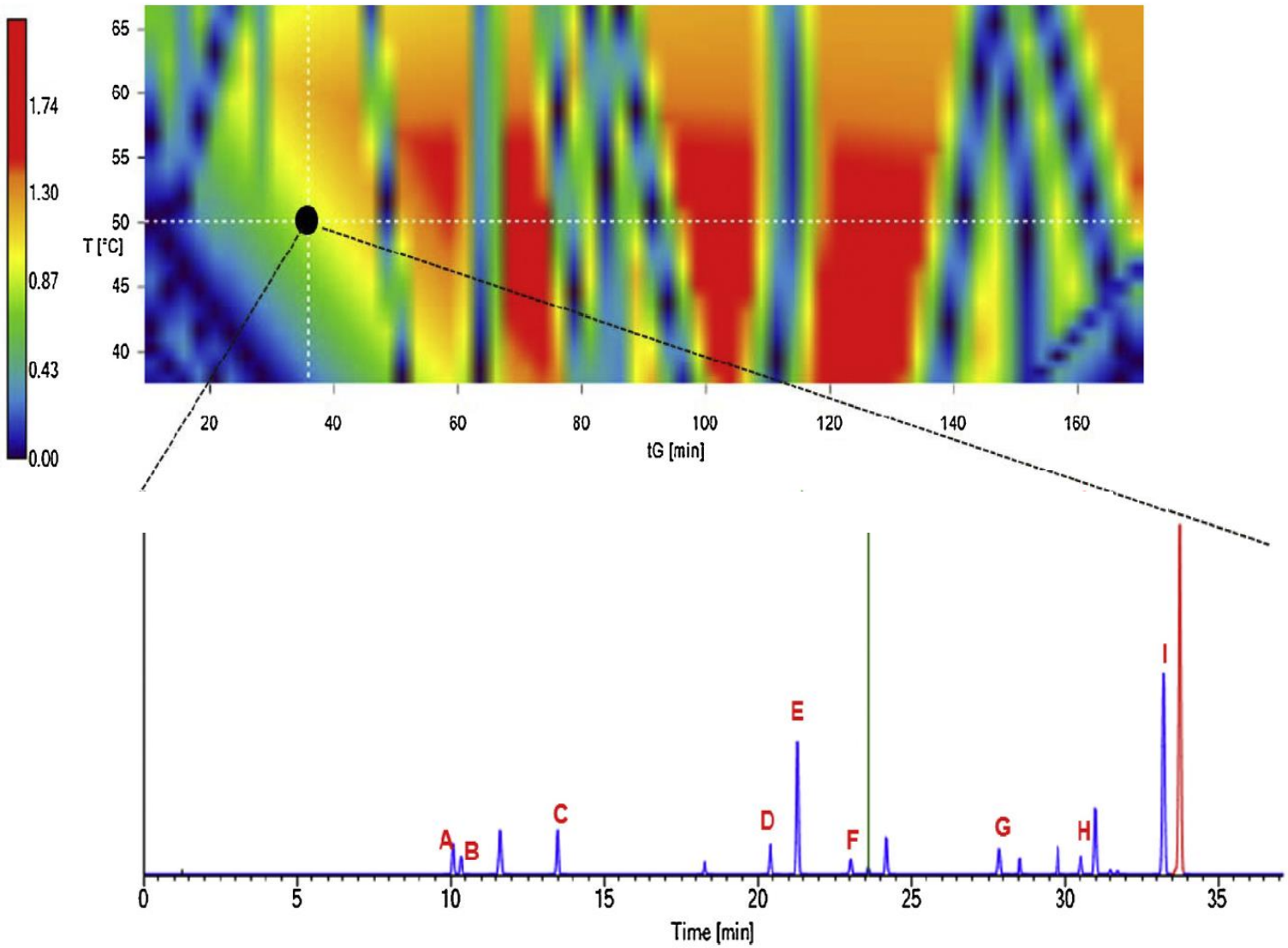


Fig. 7. A heat map generated with the Drylab® software which shows time vs resolution relationship. The bottom figure is a simulated chromatogram at a spot marked with a round dot on the top.

Statement of Dr. Jianmei Kochling, Sanofi-Genzyme

“Using DryLab the cost saving was tremendous. It has shortened the method development time from the typical **1–3 months to about 1 week. ...**The benefits are even more significant, since the **productivity of scientists can be increased with multiple factors.”**

„A platform analytical quality by design (AQbD) approach for multiple UHPLC-UV and UHPLC–MS methods development for protein analysis“

Jianmei Kochling, Wei Wu, Yimin Hua, Qian Guan, Juan Castaneda-Merced, J.Pharm.Biomed.Anal., **125**, 130-139 **(2016).**

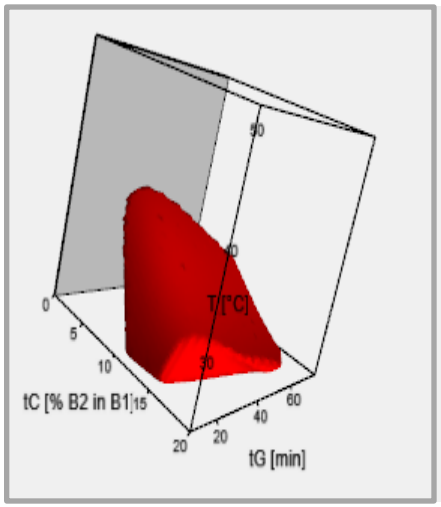
4. Case Study: searching for Equivalent Columns

Experimental details

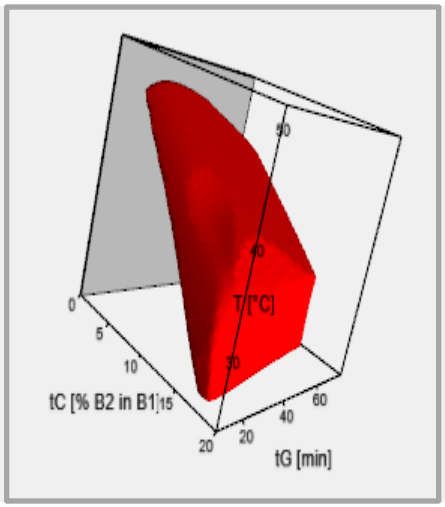
Colums	**Kinetex C18, 2,6 µm, 4,6 x150 mm** **Halo C18, 2,7 µm, 4,6 x150 mm** **Cortecs C18, 2,7 µm, 4,6 x150 mm**
Detection	220 nm + spektrum
A eluent	water
B eluents	B1: MeOH B2: ACN/MeOH=10/90 B3: ACN/MeOH=20/80
Flow rate	0.8 ml/min
Gradient	40-90%B
Gradient time	tG_1=15 perc tG_2=45 perc
Col.temperature	T_1=25°C T_2=50°C

Results

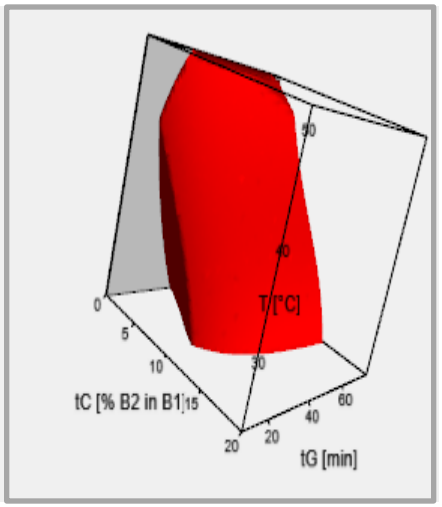
Robustness level of Critical Resolution: 1.5



Cortecs C18



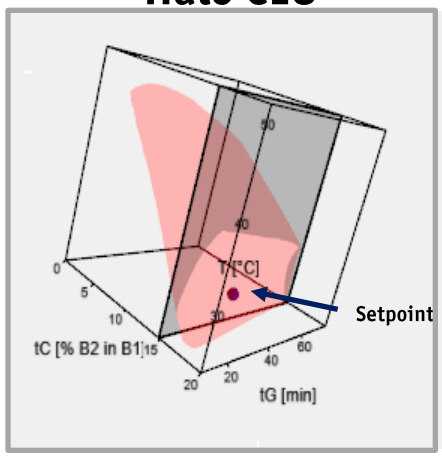
Halo C18



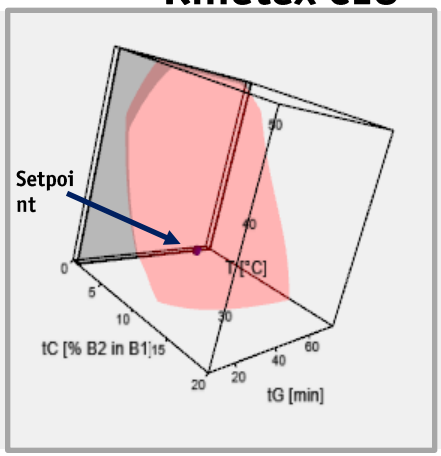
Kinetex C18

Intersection of robust spaces (blue) between Halo C18 and Kinetex C18

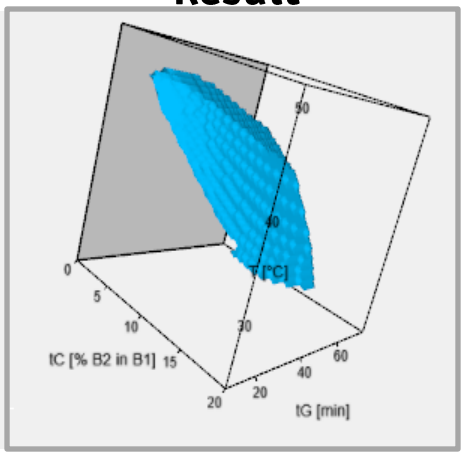
Halo C18



Kinetex C18



Result



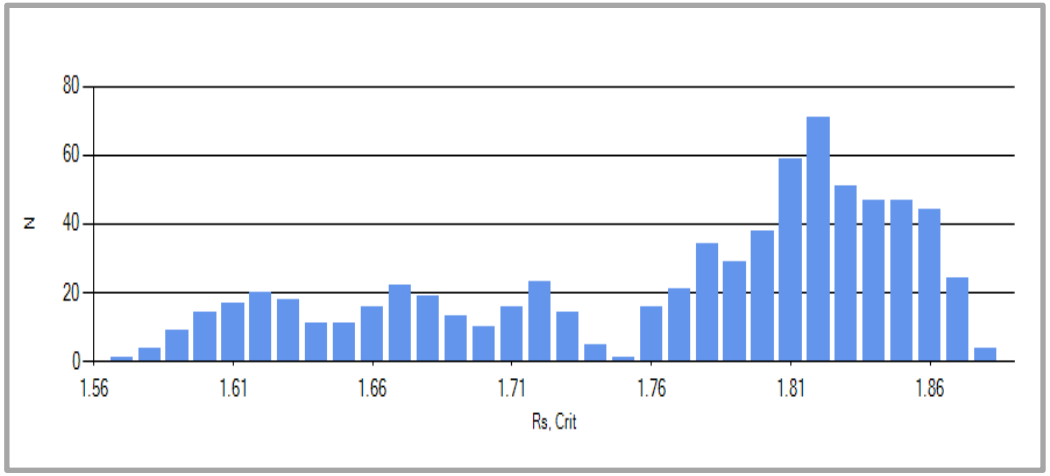
Robustness of Cortecs C18 at the point of maximum $R_{s,crit}$

Method Conditions

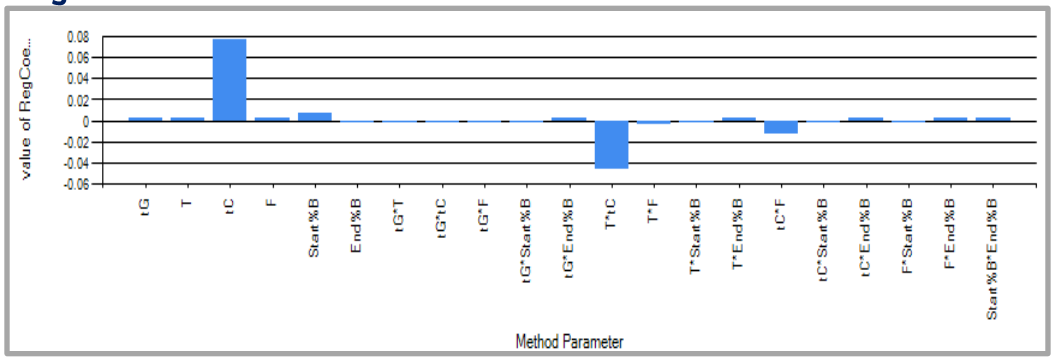
T[°C]:	22.5 ± 1
tC[%B2 in B1]:	9.6 ± 1
Flow Rate[mL/min]:	0.8 ± 0.1
Start[%B]:	40 ± 1
tG[min]:	36.8 ± 1
End[%B]:	90 ± 1
Dwell Volume[mL]:	1.06 ± 0.1

Required Resolution: 1.50
Number of Experiments: 729
Success rate: 100 %

Frequency Distribution

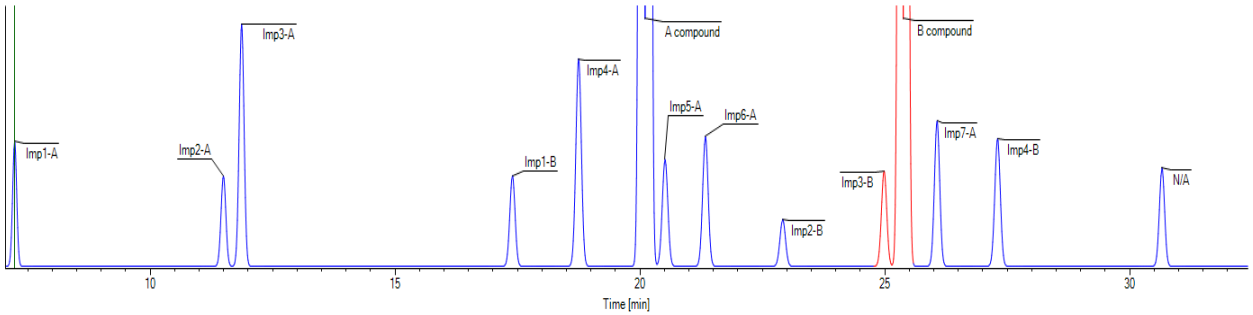


Regression Coefficients

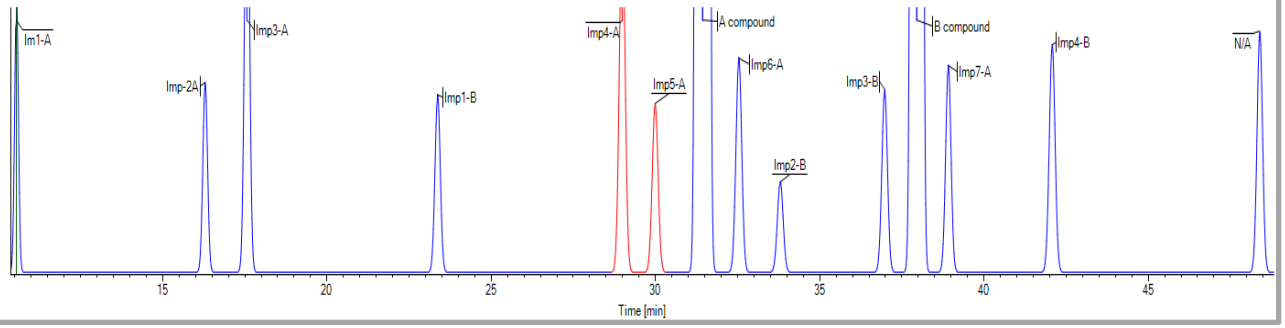


Chromatograms at the point of maximum $R_{s,crit}$

Cortecs C18
tG = 36.81 min
T = 22.5 °C
tC = 9.6 % B2 in B1
$R_{s,crit}$ = 1.85
Success rate: 100%



Kinetex C18
tG = 68.2 min
T = 22.5 °C
tC = 0.8 % B2 in B1
$R_{s,crit}$ = 2.93
Success rate: 100%

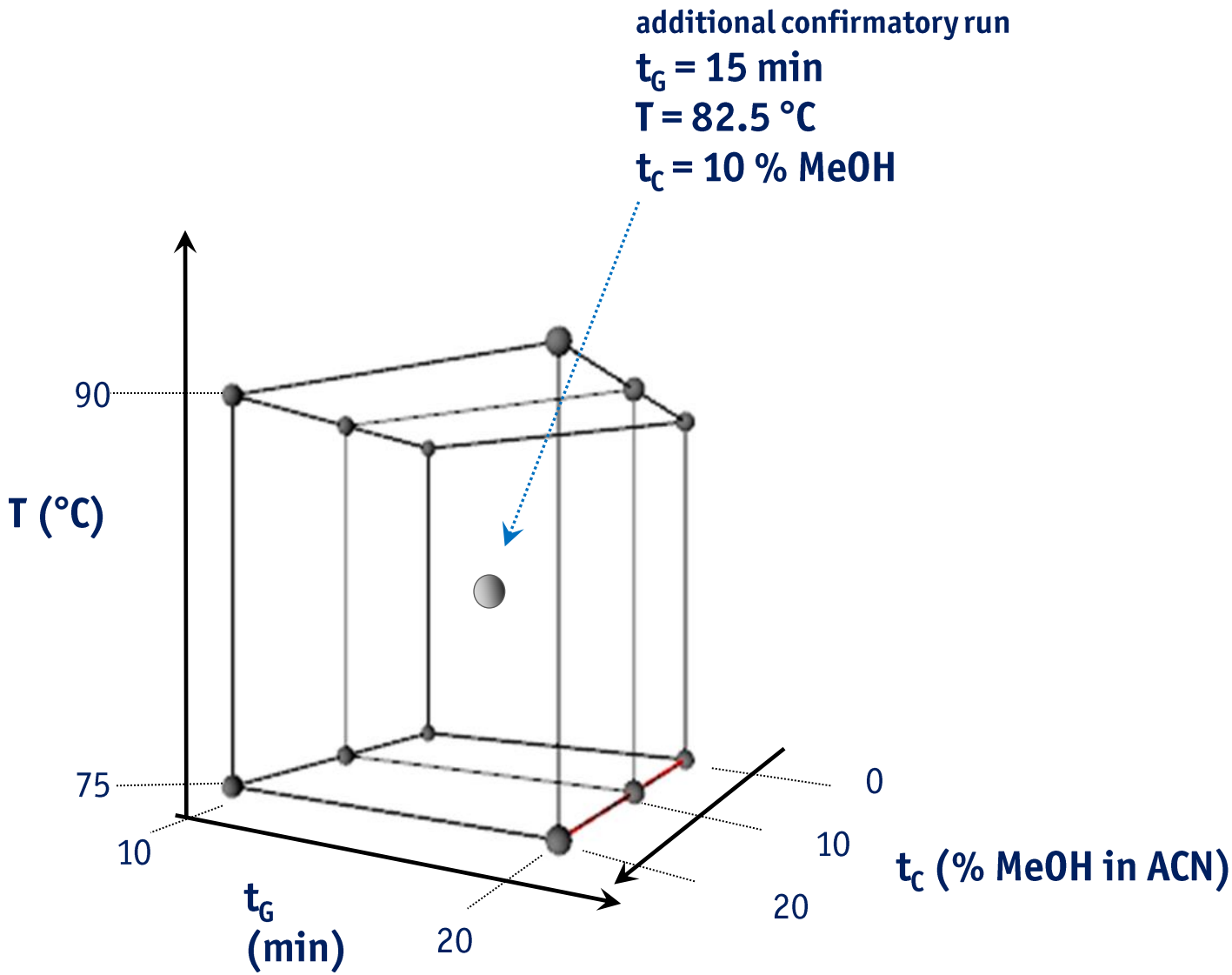


Column with the best robustness

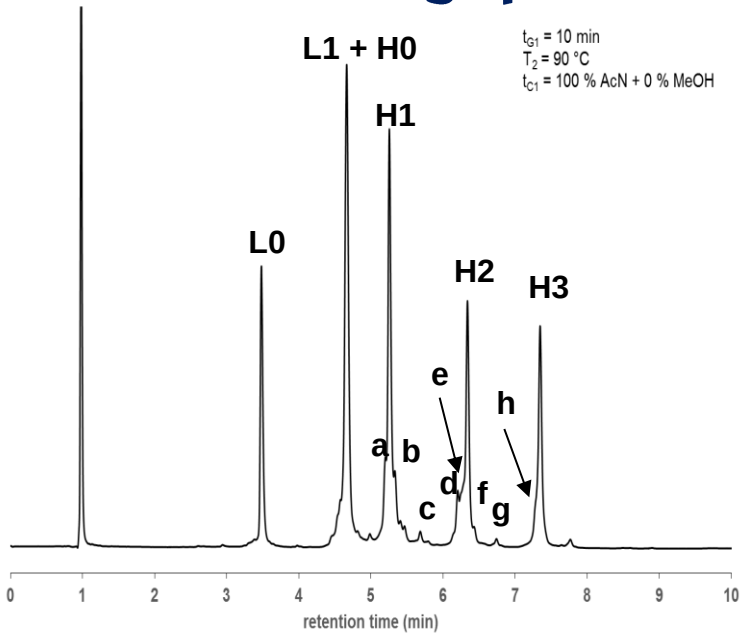
5. Case Study: Separation of Monoclonal Antibody subunits of reduced Brentuximab Vedotin (ADC)

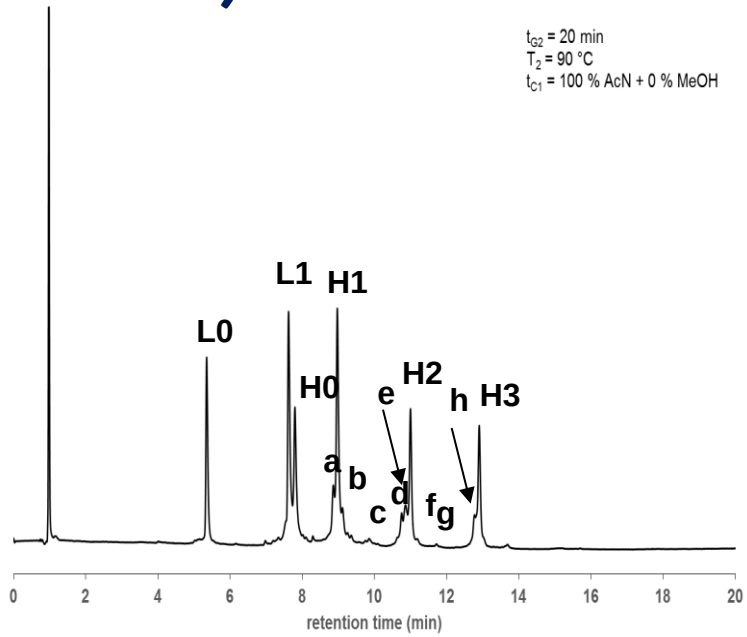
Column: Agilent AdvanceBioMab RP C4, 150 x 2.1 mm, 3.5 µm
A: 0.1 % TFA in water
B_1: 0.1 % TFA in 100% AcN + 0% MeOH
B_2: 0.1 % TFA in 90% AcN + 10% MeOH
B_3: 0.1 % TFA in 80% AcN + 20% MeOH
Flow: 0.3 mL/min, Gradient: 25 – 50 %B
tG_1 = 10 min, tG_2 = 20 min
T_1 = 75°C, T_2 = 90°C
Detection: 280 nm (UV)
Injected amount: 0.5 µL
System: Waters UPLC I Class

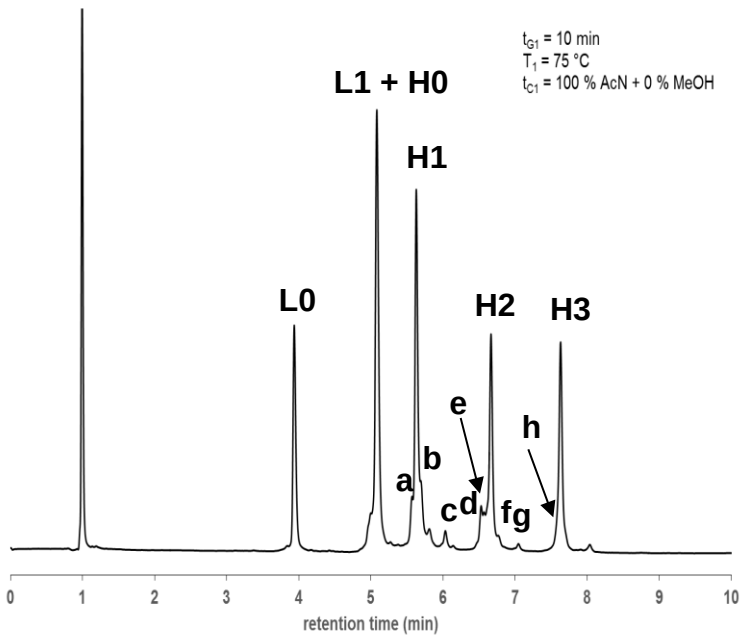
DoE for ADCs (mAbs) RP 3D retention model (12 + 1 runs)

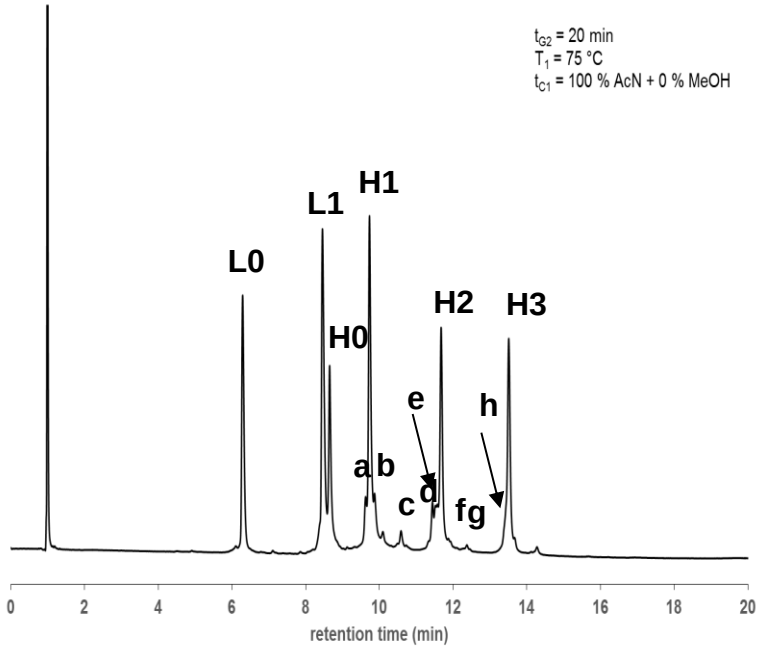


Peak tracking: plane 1 (100 % AcN)





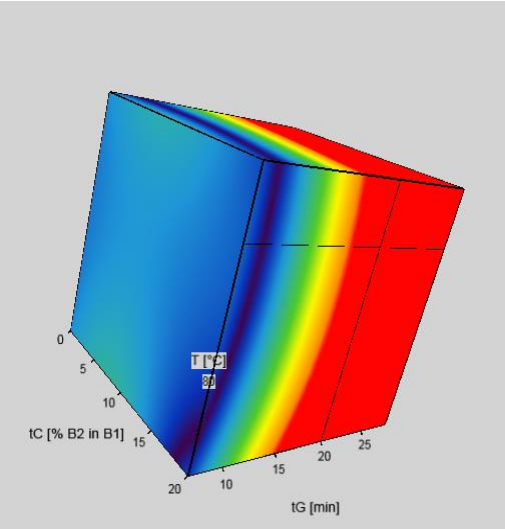


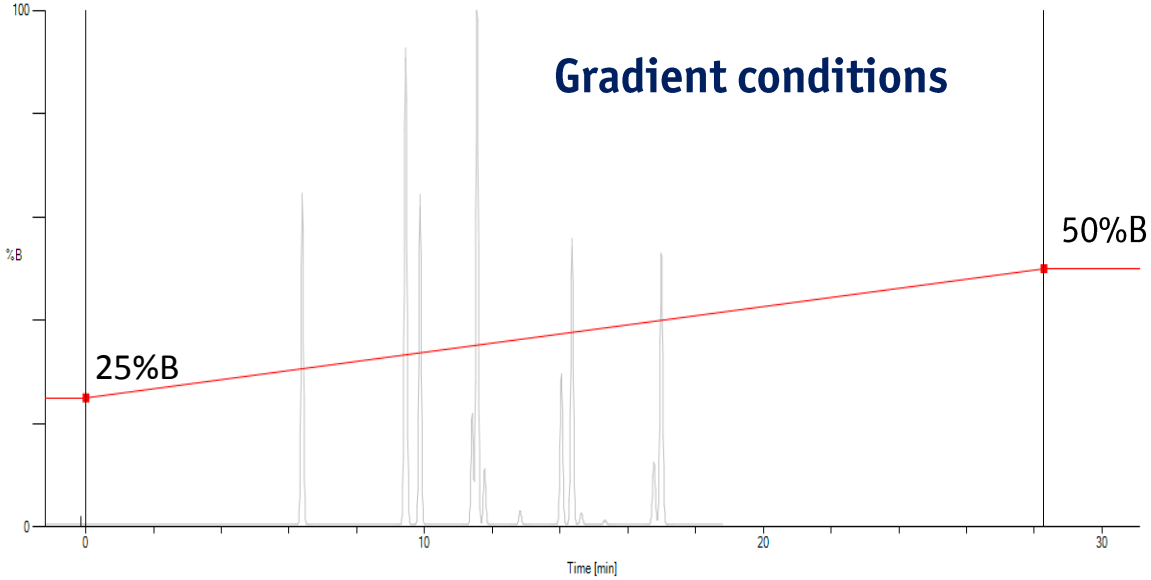


Peak tracking table input data

column: Advance Bio Mab RP C4																									
Flow: 0.3 mL/min																									
Grad: 25 - 50%B	tG	10 min		20 min		10 min		20 min		10 min		20 min		10 min		20 min		10 min		20 min		10 min		20 min	
A: 0.1 % TFA in water	T	75 C		75 C		90 C		90 C		75 C		75 C		90 C		90 C		75 C		75 C		90 C		90 C	
B1: 0.1 % TFA in ACN	tC	0% MeOH		0% MeOH		0% MeOH		0% MeOH		10% MeOH		10% MeOH		10% MeOH		10% MeOH		20% MeOH		20% MeOH		20% MeOH		10% MeOH	
B2: 0.1 % TFA in ACN+MeOH (90+10)		tr	area	tr	area	tr	area	tr	area	tr	area	tr	area	tr	area	tr	area	tr	area	tr	area	tr	area	tr	area
B3: 0.1 % TFA in ACN+MeOH (80+20)	L0	3.94	66734.5	6.29	58735	3.48	70947.6	5.35	61235.2	4.72	60823	7.79	57113	4.16	56403	6.71	54648	5.56	60398	9.44	58872	4.89	52132.5	8.15	53062.4
det: 280 nm	L1	5.08	90000	8.46	85496	4.67	90000	7.62	93939.2	5.9	90000	10	93987	5.39	90000	8.96	86501	6.73	90161	11.67	80298	6.11	83870	10.47	72556.8
inj: 0.5 uL	H0	5.08	50000	8.65	42060	4.67	50000	7.8	56483.2	5.9	50000	10.27	42960	5.39	50000	9.2	55579	6.79	46515	12	36589	6.14	53395	10.76	52000
sample: BV reduced	a	5.57	9227.6	9.63	9557	5.2	16220.4	8.86	18332.8	6.43	7620	11.28	9346	5.94	14727	10.28	19316	7.29	7466	13	8003	6.7	11637.5	11.83	18793.6
	H1	5.63	108762.4	9.73	82324	5.26	118696.8	8.97	94054.4	6.49	83967	11.39	81348	6	96598	10.39	84908	7.36	76178	13.12	69692	6.76	89997.5	11.96	85926.4
	b	5.69	12854.7	9.87	11456	5.33	12573.6	9.12	9628.8	6.55	11383	11.53	11566	6.08	10747	10.55	9626	7.41	13480	13.26	11334	6.83	8495	12.1	10294.4
	c	6.03	3877.8	10.59	2894	5.69	2376	9.86	1436.8	6.9	6377	12.26	4446	6.45	4740	11.3	2801	7.75	4496	13.96	3433	7.16	1515	12.83	2182.4
	d	6.53	9627.8	11.44	11220	6.21	10838.4	10.76	11113.6	7.4	10661	13.12	8935	6.96	10303	12.2	8589	8.25	8386	14.83	7363	7.7	8007.5	13.73	7996.8
	e	6.57	7042.6	11.58	8603	6.3	20565.6	10.86	21033.6	7.44	6827	13.2	14307	7.05	16617	12.29	17925	8.31	13167	14.91	13597	7.75	17000	13.81	19084.8
	H2	6.67	77581.3	11.68	58836	6.34	72984	11	57052.8	7.53	69190	13.35	57610	7.1	62865	12.44	50442	8.38	59330	15.05	53218	7.83	55255	13.96	49670.4
	f	6.77	3098.1	11.87	1362	6.43	2956.8	11.18	2156.8	7.61	3624	13.56	1295	7.2	3006	12.65	1966	8.47	3238	15.22	1359	7.91	2495	14.14	793.6
	g	7.05	1432.9	12.38	624	6.74	1323.6	11.72	758.4	7.92	1554	14.06	760	7.5	1878	13.17	732	8.78	1203	15.78	997	8.22	1545	14.71	400
	h	7.6	9241.4	13.45	10478	7.31	13180.8	12.78	12252.8	8.47	9352	15.13	8150	8.05	10599	14.22	12847	9.31	8914	16.79	6719	8.77	11782.5	15.7	15622.4
	H3	7.63	68096.1	13.51	57254	7.35	71445.6	12.91	58393.6	8.51	62931	15.19	55081	8.11	68880	14.35	49235	9.35	57581	16.88	54255	8.81	47397.5	15.84	46534.4

3D map (all peak of interest)

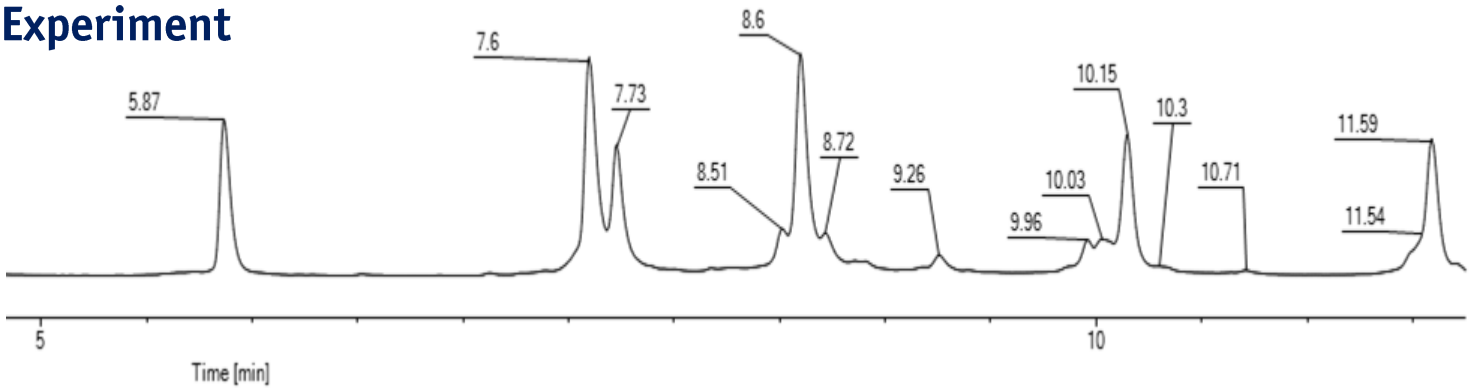




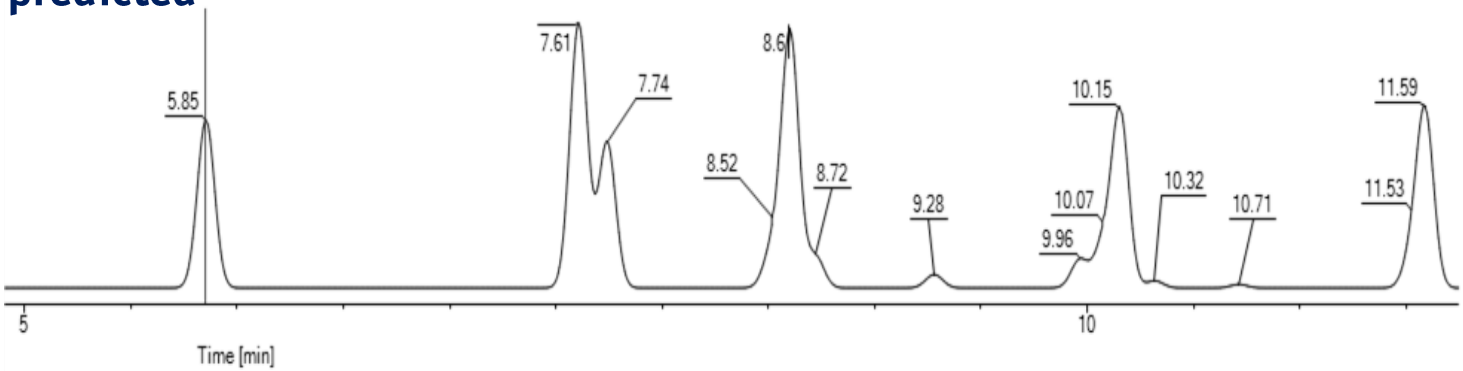
3D model: tG x T x tC center point verification

Comparison of experimental and model chromatograms at the center point (tG = 15 min, T = 82.5 C, tC = 10 % MeOH

Experiment

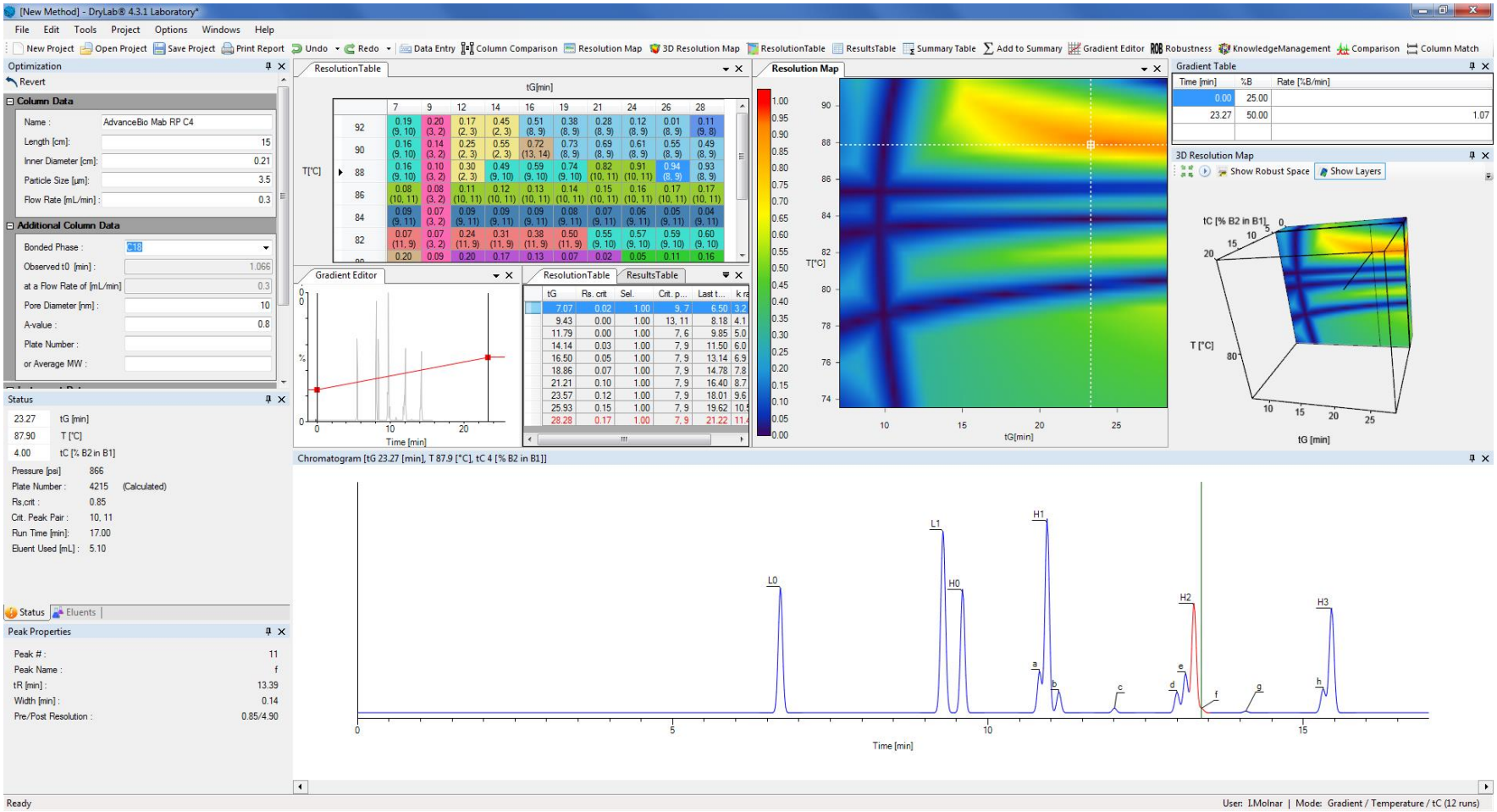


predicted



Very good agreement!
(peak widths can also be applied to make the model more accurate)

Data Integrity: Windows Management in DryLab®4



Summary

- Modeling helps to find optimum conditions for robust operation faster
- Cooperation with regulatory agencies is based more on solid science
- Knowledge Management Documents for better Data Integrity are helping to receive faster market authorization

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Thank you.

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