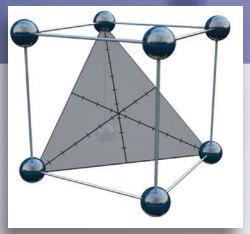


Fusion QbD®

Introduction

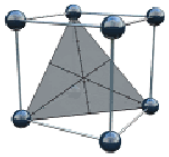
Application of QbD Principals
For the Efficient Development and Optimization of
Analytical Methods

cromingo e.K.
www.cromingo.com

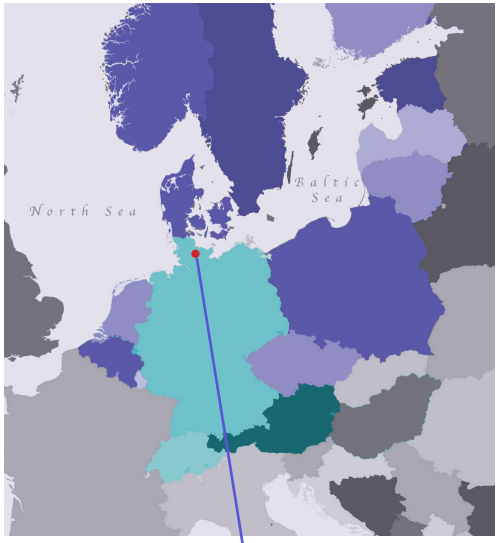
cromingo

Cromingo e.K:

- Represents the S-Matrix Corporation in the DACH Region and neighboring Countries
- Training, Support, Qualification, Consulting, Distribution for Fusion Pro and Fusion QbD (Remote & Onsite)
- Training, Consulting, Validation for Waters Empower CDS



Empower® 3



cromingo e.K.
Ingo Green, Owner and Managing Consultant
Altes Kreishaus, Klosterufer 2
24582 Bordesholm
www.cromingo.com
info@cromingo.com

Topics

1. Fusion QbD Software
2. Design of Experiments (DOE)
3. The QbD in Fusion
4. Practical Operation
5. Assessing Robustness
6. Special Features since Version 9.9.x

Fusion QbD® Software: DoE + Chromatographic Modelling

Difference to other DoE/Statistical Tools

- Option for fully automated design selection
- Option for fully automated data analysis
- Robustness simulation for the entire experimental region
- Interface to CDS systems for fully automated bidirectional data exchange
- Specific chromatography features

Difference to traditional Chromatographic Modelling

- Statistical equations and models based on the current experimental data set
- Characterization of interaction effects
- Unlimited selection of study factors and target responses
- Independancy on chemistry/separation technique of an analytical method
- Precise modelling of prediction chromatograms also considering peak shape

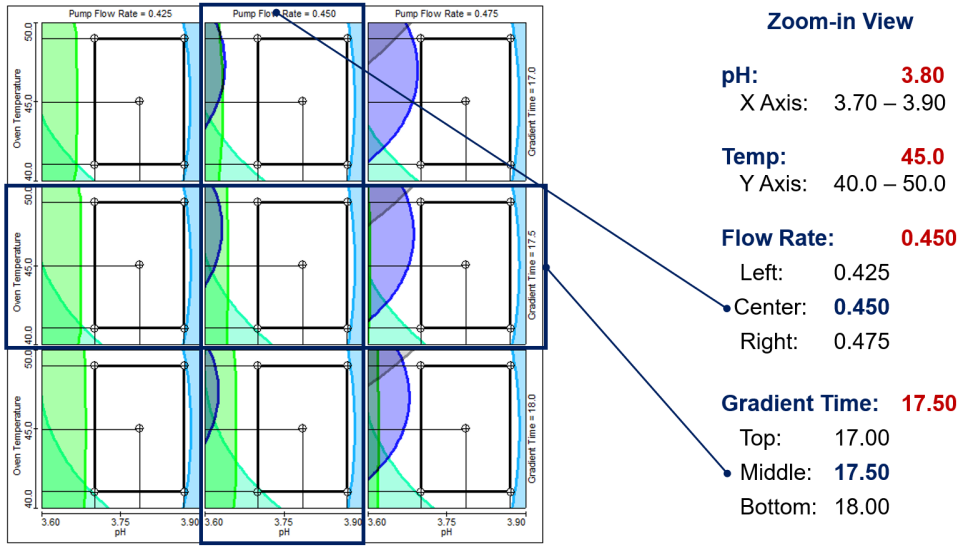
Software framework designed in allignment with regulatory requirements of customers in pharmaceutical industries:

user management, e-signature workflows, data security, audit trail, software qualification (IQ/OQ)

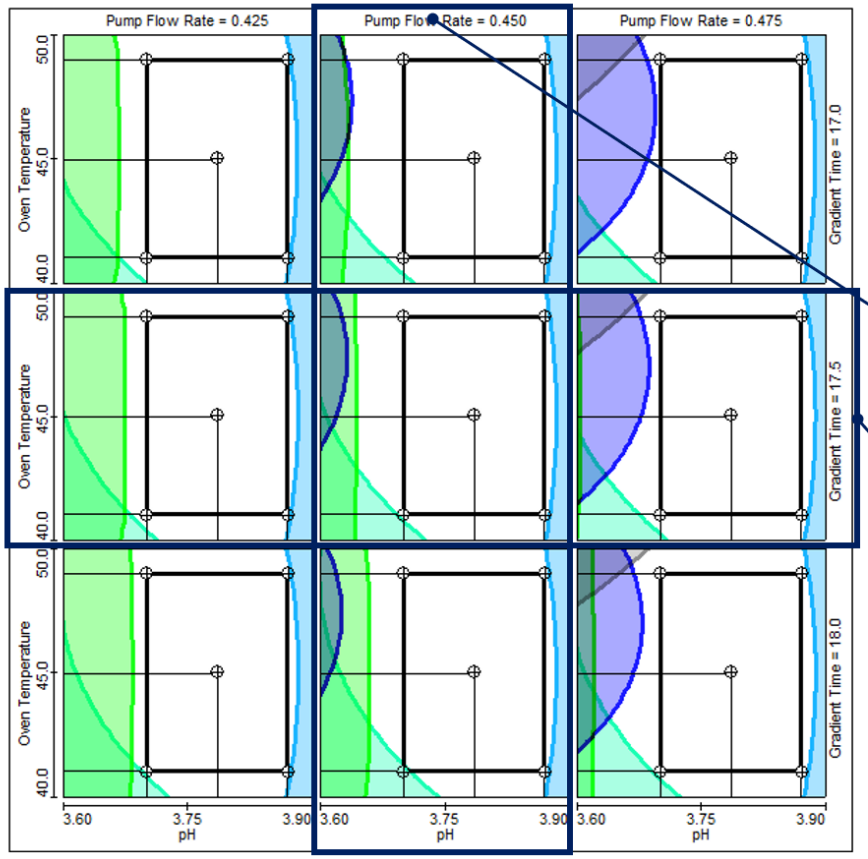
Expected in ICH Q14

An MODR consists of combined ranges for two or more variables within which the analytical procedure is shown to be fit for the intended use. In an enhanced approach, the ranges for the relevant parameters and their interactions can be investigated in multi-variate experiments (DoE).

Parameter ranges (e.g., PAR or MODR) can be proposed by the applicant based on development data and are subject to regulatory approval. Moving within an established parameter range does not require regulatory notification.



Expected in ICH Q14



Zoom-in View

pH: **3.80**
X Axis: 3.70 – 3.90

Temp: **45.0**
Y Axis: 40.0 – 50.0

Flow Rate: **0.450**
Left: 0.425
Center: **0.450**
Right: 0.475

Gradient Time: **17.50**
Top: 17.00
Middle: **17.50**
Bottom: 18.00

Within the MODR parameters can be modified **independently from each other**, without leaving the robust design region of the method.

Design of Experiments (DOE)

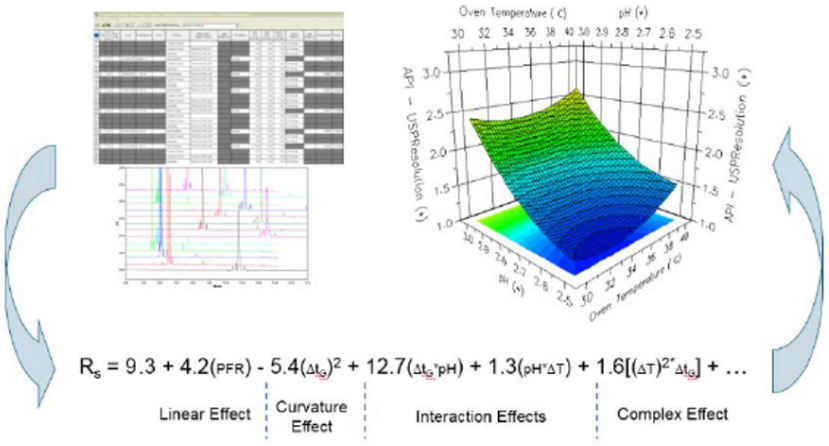
LC System as „Process in a Box“

Study Factors
(Critical Process Parameter, CPP)

Numerical
- Gradient Time
- Temperature
- pH
- $\%B_{start}$
- $\%B_{end}$
- $Konz_{Additive}$
- $Konz_{Buffer}$
- Flow
- Wavelength
- ...

Categorical
- mobile Phase
- stationary Phase
- Additive Type
- Buffer Type
- ...

- Independent from Method Chemistry
- Empirical Approach and therefore universally applicable



- hyper-accurate mathematic models, quantitativ characterization of all effects for a single CQA
- Prediction inside an outside the modelled region.
- The mathematical average of the Rs result corresponds to the expected average result at setpoint condition

Method Goals
(Critical Quality Attribute, CQA)

- Number of Peaks
- Number Peaks with Rs >/= 1.5
- Resolution
- Retention Time
- Tailing
- Area%
- Plate Count
- Robustness
- ....

= Method Specifications

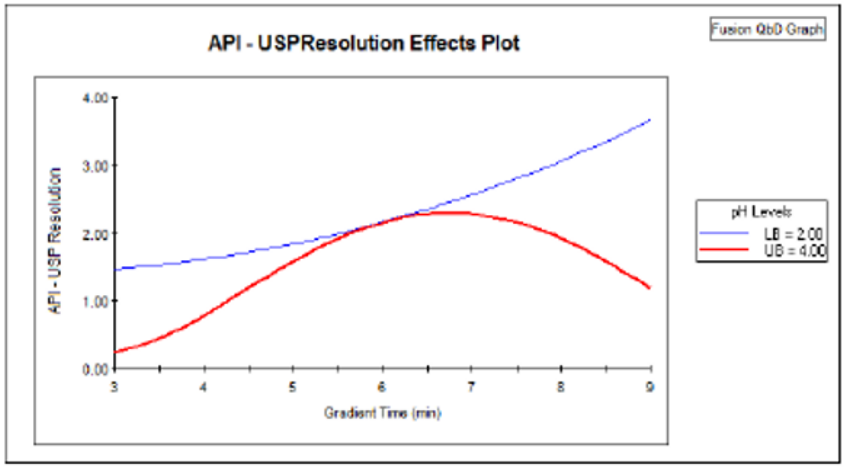
One Factor At a Time (OFAT)

OFAT (Trial and Error):

- Qualitative, visual inspection of experiment results , no understanding of overall methods effects

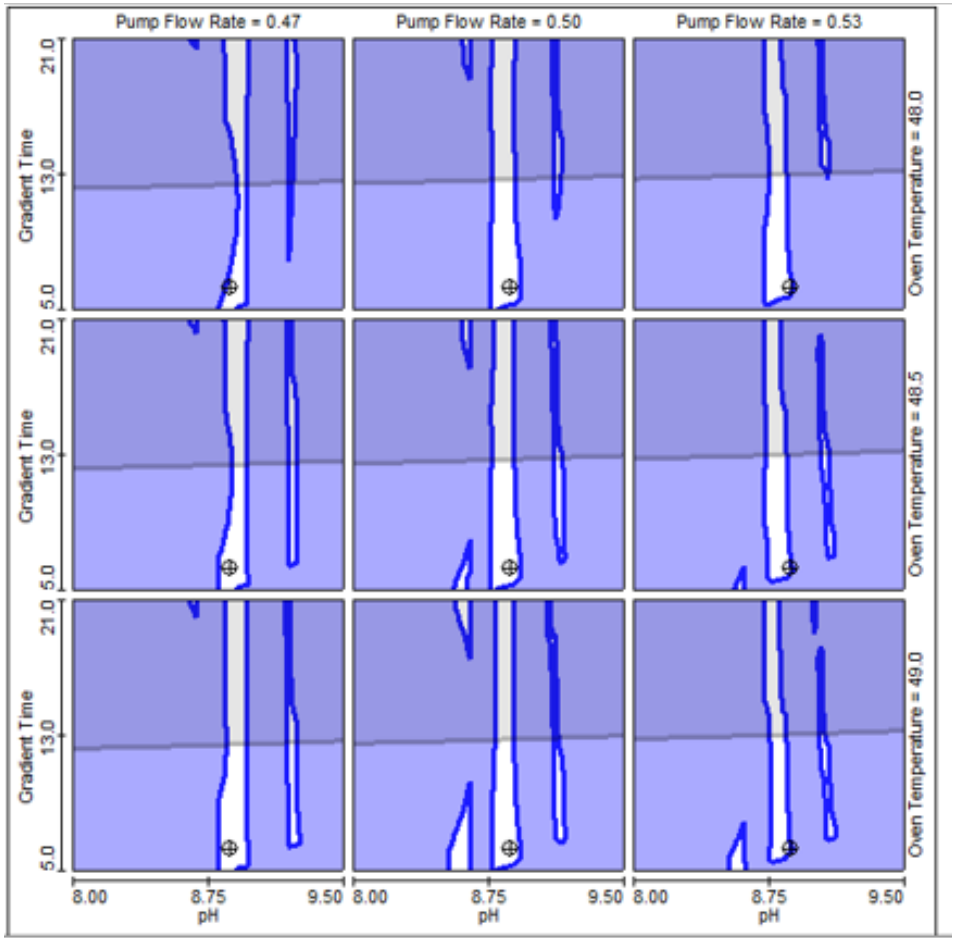
No investigation of interactive effects:

- E.g. combined effect of gradient time and pH value on the resolution of an API peak



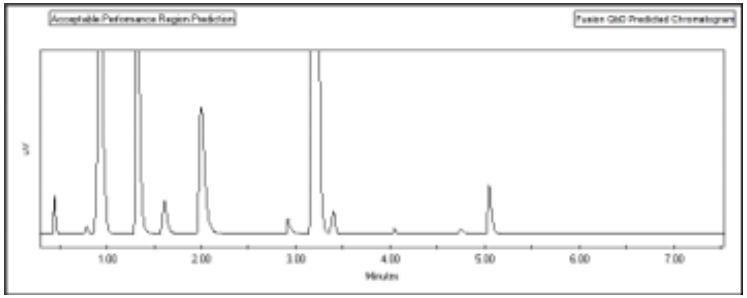
- **Interactive effect,** as the distance between the two curves changes and for pH 4 even decreases after 6.5 minutes.
- With an OFAT approach (change of the pH value at 6 minutes gradient time) the wrong conclusion could be drawn that the pH value has no impact.

Interaction Effects – Practical Significance



pH 8.8
RS Map Response >/= 1.8

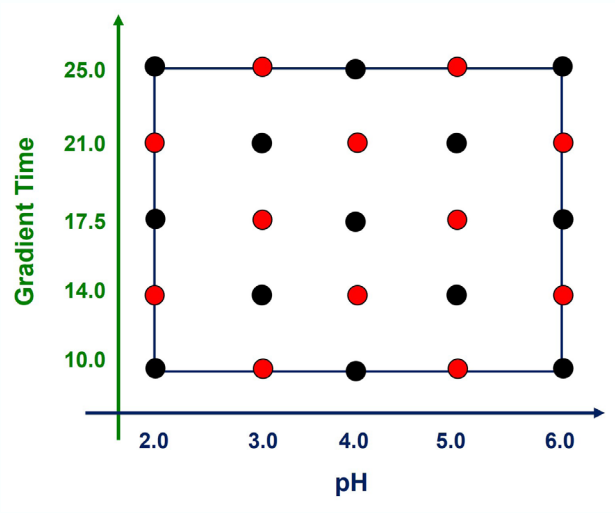
		Flow	Flow	Flow
GT	Temp	0.47	0.50	0.53
11	48.0	1.54	1.87	1.89
11	48.5	1.79	1.89	1.90
11	49.0	1.81	1.91	1.75
6	48.0	1.81	1.93	1.97
6	48.5	1.84	1.97	2.00
6	49.0	1.86	2.00	2.03



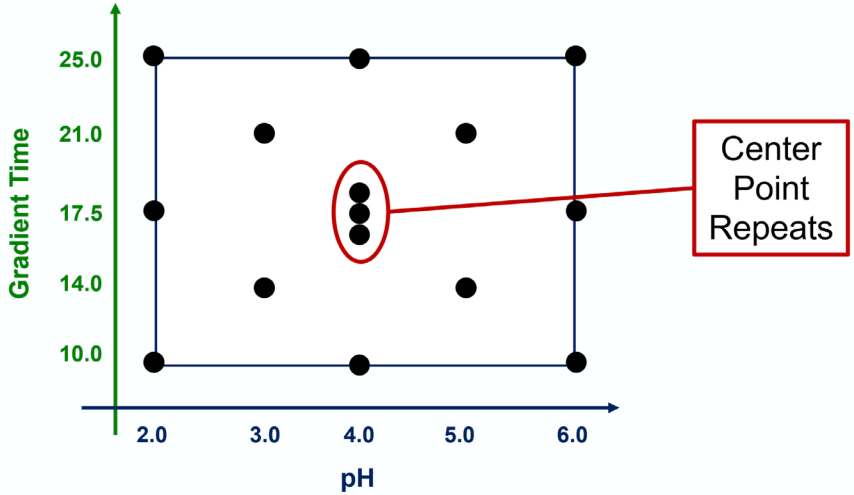
Study of the entire multivariate experimental Region

Consider two variables (pH and Gradient Time [min]) – five study levels each

Brute Force Approach
(All Possible Combinations)
5x5=25 Methods

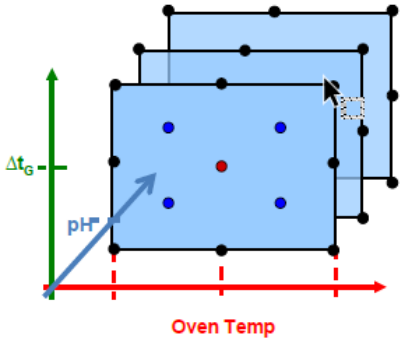


Design of Experiments
(„Statistical Sampling“)
15 Methods

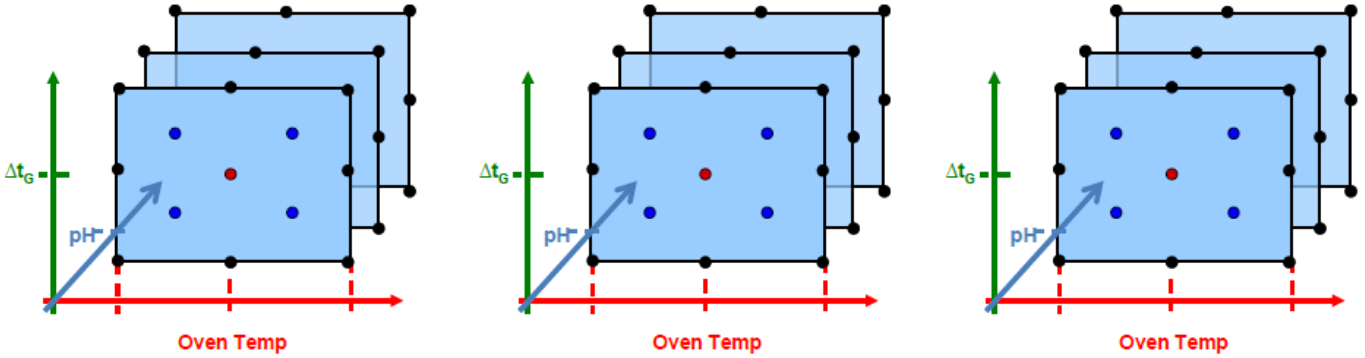


Study of the entire multivariate experimental Region

3rd Variable – 3 Levels, e.g. Temperature



4th Variable – 3 Levels, e.g. Column Type



Theoretically: 5x5x3x3 = 225 Methods

- With DoE: Reduction of study methods by a factor of 3 to 5

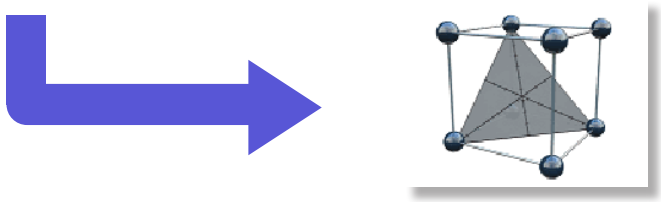
The QbD in Fusion

DOE as Part of Quality by Design (QbD)

Quality by Design:

- Model-based statistical design of experiments
- Multi-parameter study, simultaneous change of several influencing effects
- Quantitative analysis of both independant and interactive effects
- Calculation of statistical data models (mathematic equations), that describe the influence of the CCPs on the single CQAs in the experimental, multi-dimensional region
- **But:** Study includes multiple instrument parameters resulting in extensive designed experiments, numerical data analysis and modeling techniques

QbD no pragmatic approach for the analytical lab???



Fusion QbD – Integrated QbD Tools

Fully automated application of accepted QbD tools

Design of Experiment (DoE)

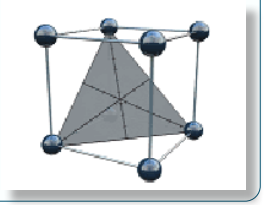
- **Statistical Design of Expermients**, to determine independent and interactive effects of instrument parameters affecting the performance of a process (method).
- Determination of the **Mean Performance** (average expected performance), but does not predict the variation of a particular characteristic that will be observed over multiple uses of the process.

Monte Carlo Simulation (Robustness)

- Well understood and accepted mathema-tical methodology for **predicting variation** in a CQA given joint variation in the CPPs expected during normal operation.

Process Capability (Cp, Cpk, Cpm, Cpkm)

- Determination of the multi-dimensional design space where **robustness** for a process (method) is reached.

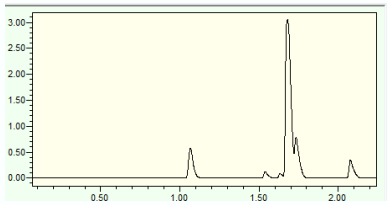


Fusion QbD
Practical operation

Fusion QbD - Method Development Phases

General Screening Design

- Starting point method (flow rate, concentration, wavelength, ...), it is solely important that all components clearly elute



Column/Solvent Screening

- Most important effectors (pH, gradient time, column, organic solvent, ...)
- Determination of a „region with stable chromatography“
- Defining materials for your methods, e.g. column type, strong solvent type

Method Optimization

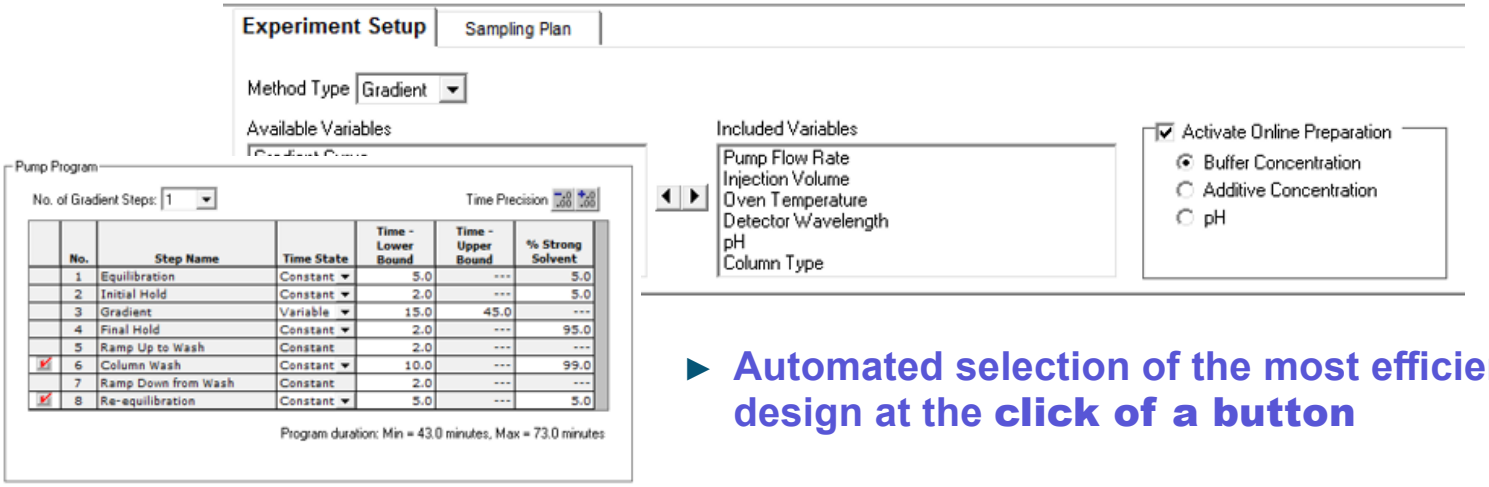
- Refinement of gradient time, pH value, column temperature in the determined experimental region, identification of a robust method
- 1. Best „Mean Performance“
- 2. Robustness

- Determination of the **MODR**

Fusion QbD - Create a Design

Flexible Experiment Setup

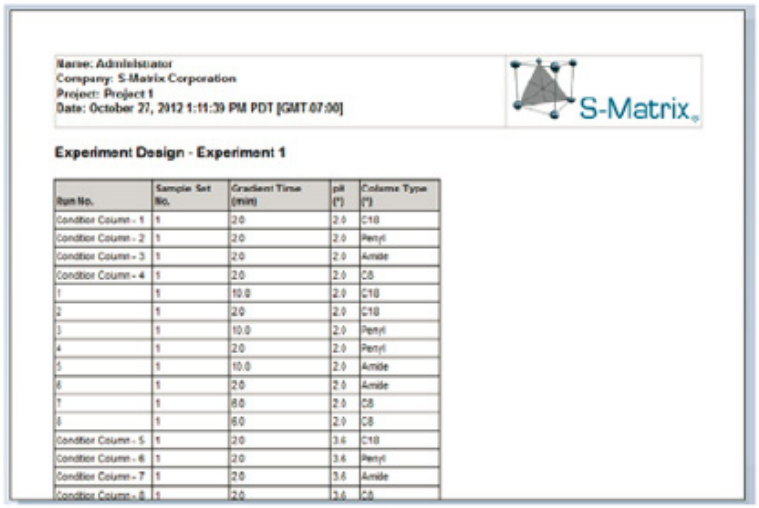
- Selection Gradient or Isocratic
- Selection of factors (**CPP's** – included variables) including ranges and levels for each instrument parameter
- Online Preparation mode for study factors such as pH or Buffer Concentration

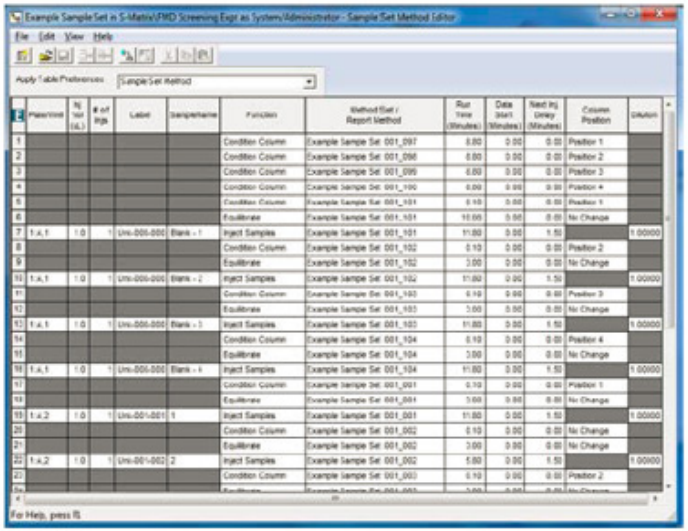


► **Automated selection of the most efficient experiment design at the click of a button**

Fusion QbD - Export to CDS

Automated reconstruction as ready-to-run methods and sequences within the CDS



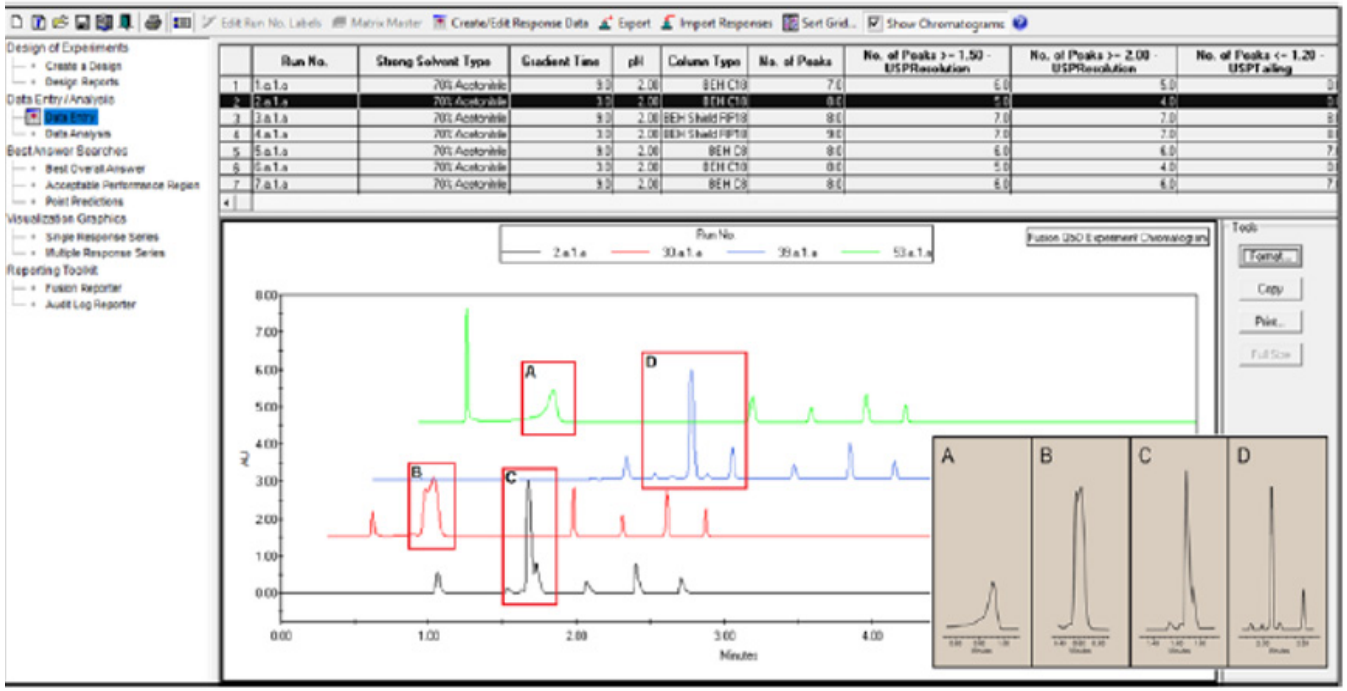


- Automatically builds sequence and ALL instrument methods, no further user interaction is required to start the analysis.
- Eliminate Transcription Errors & Maintain Data in Audited Environment.

Fusion QbD - No Peak Tracking during Screening*

Selectivity in many runs often unfavorable:

Co-elution or Change of elution order



- Clear identification between experiment runs often difficult or impossible

**PeakTracking from Vers 9.9 with Waters PDA/QDa possible (MS/UV Spectra, in Chromeleon and ChemStation currently only traditional PeakTracking)*

- **Patented Trend Responses™ in Screening Experiments**

Fusion QbD - Trend Responses™

Import Wizard:

- Peak count based responses (e.g. Number of Peaks with an USP Resolution >/= 1.50)
- Peak property based responses (e.g. USP Resolution of the largest peak)

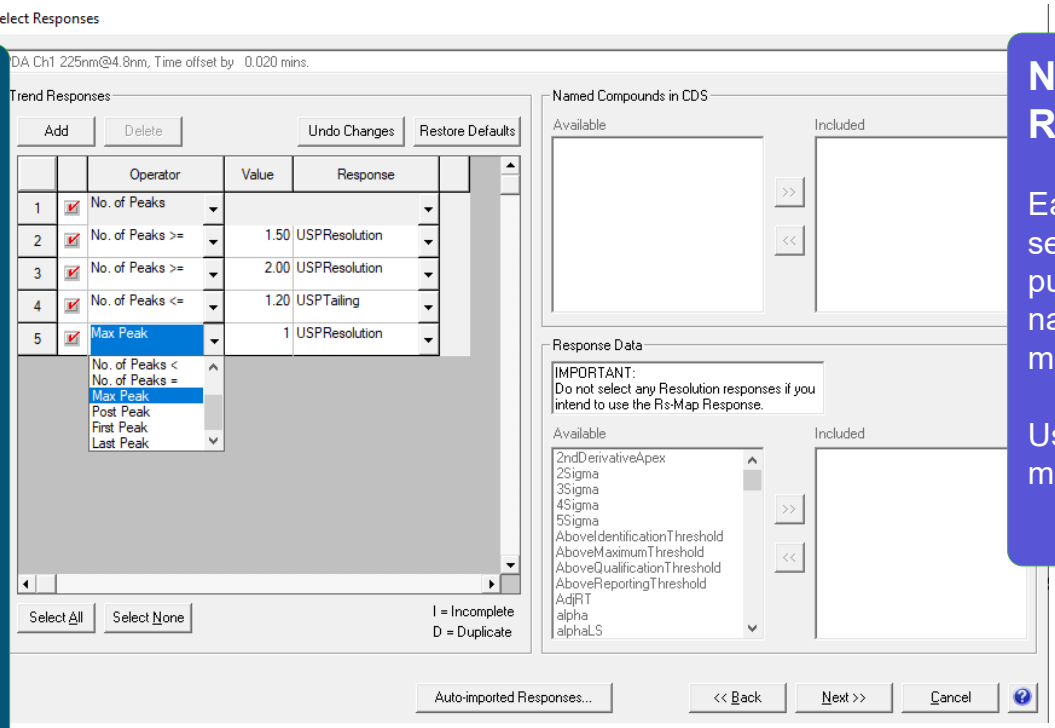
Trend Responses

Easily obtain critical separation metrics without the need for manual peak tracking.

Automated peak tracking gets all desired data for critical peaks:

- Max Peak #1
- Post Peak #1
- First Peak
- Last Peak

Use in screening experments

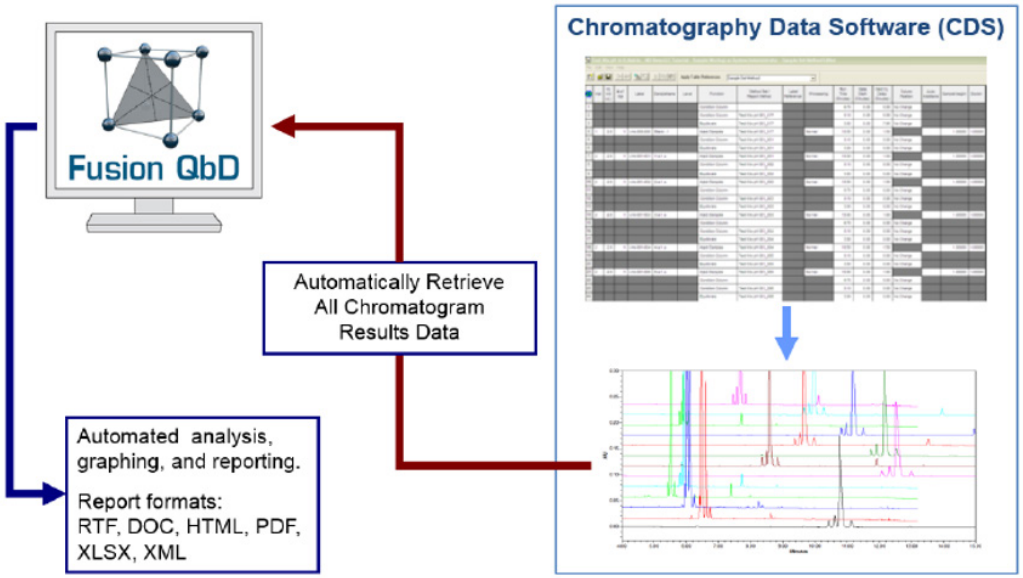


Named Compound Responses

Easily obtain any critical separation metrics computed by the CDS for any named peak in the experiment chromatogram

Use in optimzation experiments

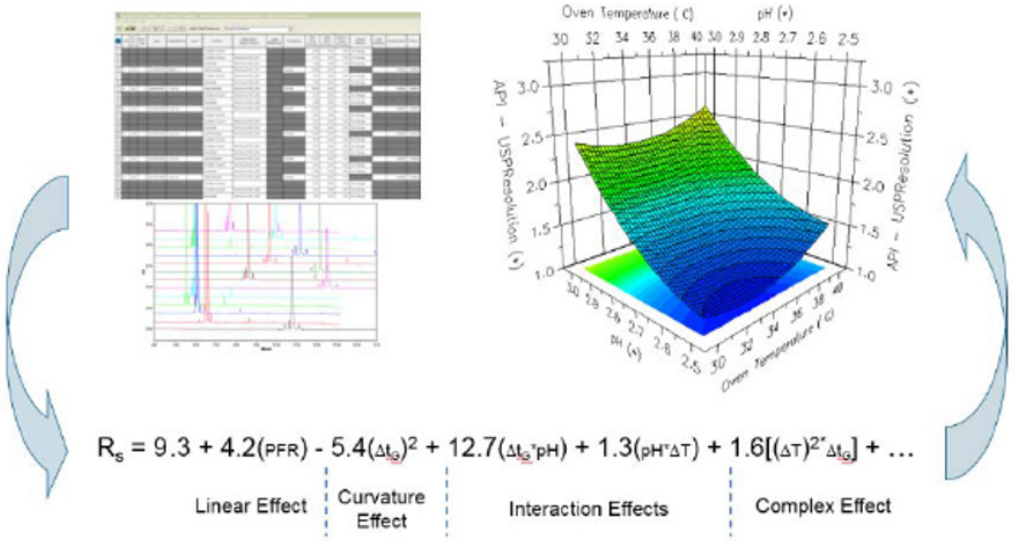
Fusion QbD - automated Import of defined Characteristics



Import of the desired experimental results for automated data modeling in Fusion QbD, no „pick-the-winner“ strategy.

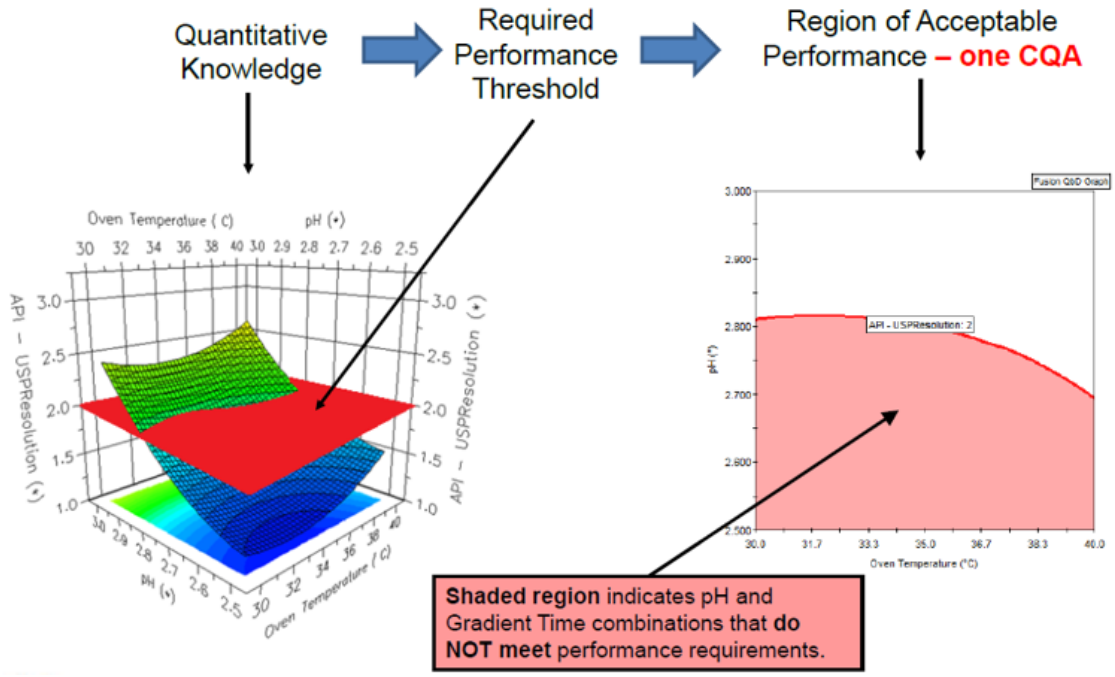
Fusion QbD - Automated Data Modeling

Turning Chromatograms into Knowledge



- Generation of models by a click on a button.

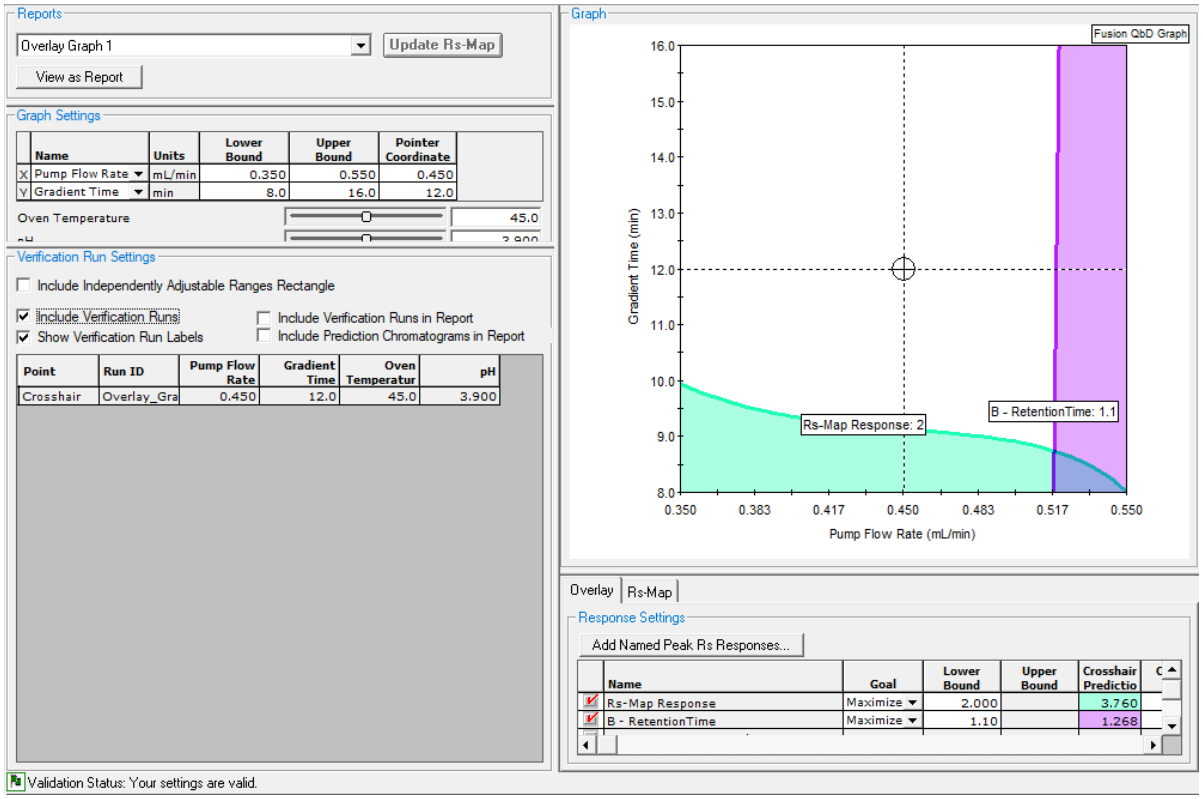
Fusion QbD - Visualizing Method Performance Characteristics



- Note: The threshold is considered as mean value (Mean Performance). Single values can be lower than 2.0.

- Illustrating regions of acceptable and not acceptable method performance (here for a single CQA)

Fusion QbD - Design Space – For all CQA‘s



- Plot of the Thresholds derived from the desirability function

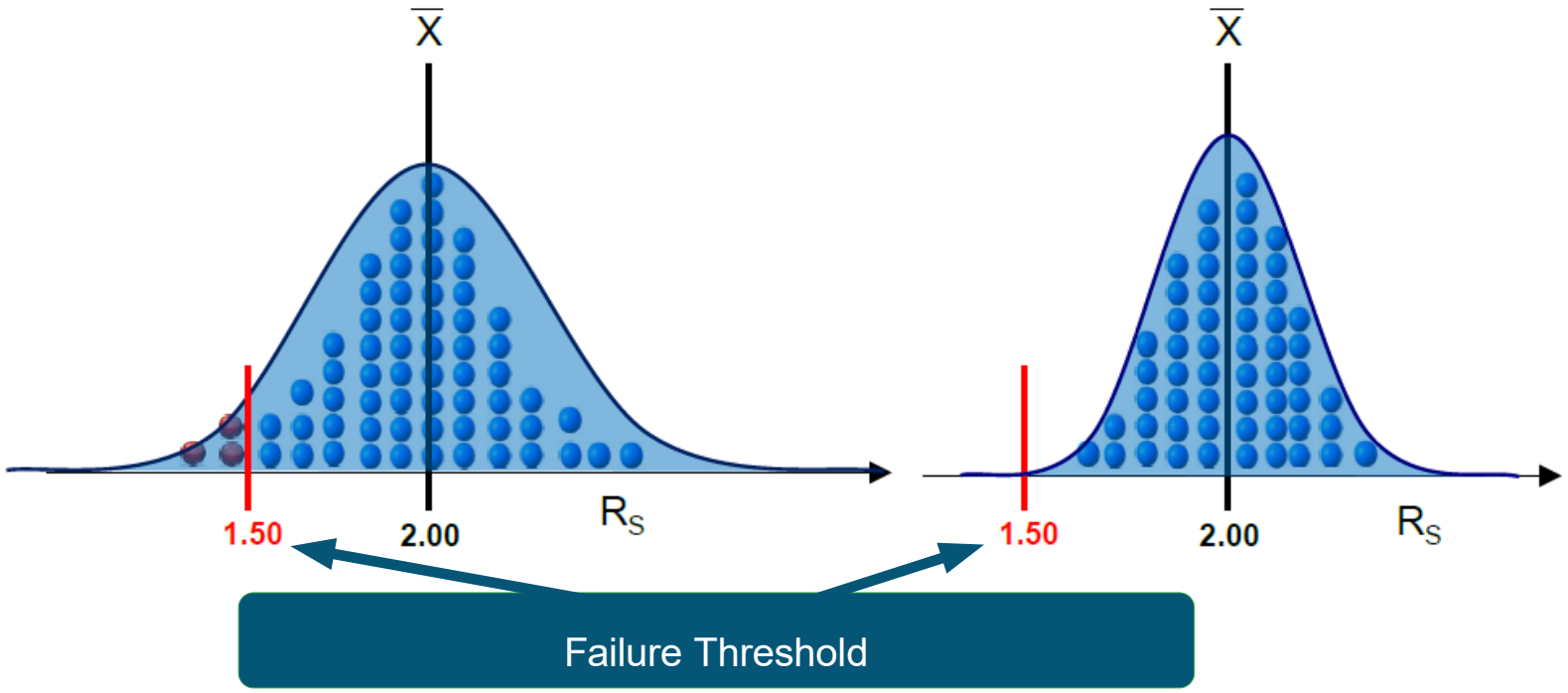
- Illustrating regions of acceptable and not acceptable method performance (here for all CQA‘s) -> Mean Performance
- Robustness ???

Assessing Robustness

Robustness

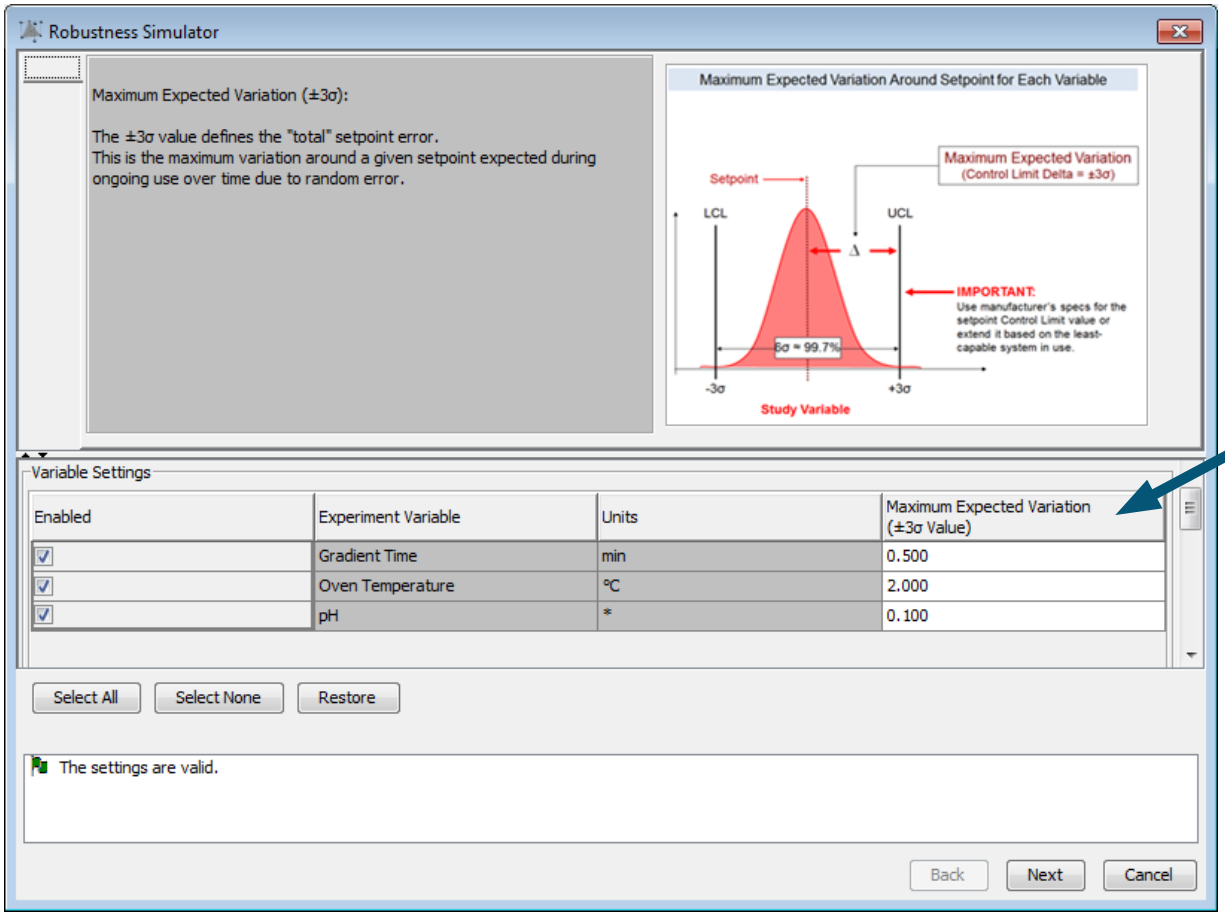
Goal for the average result: All single results ≥ 1.50

- Average result (Mean Performance) OK in both cases, Robustness only in one case.
- CPP's needs to be set in a way that the outcome of the performance never falls below the failure threshold



Robustness

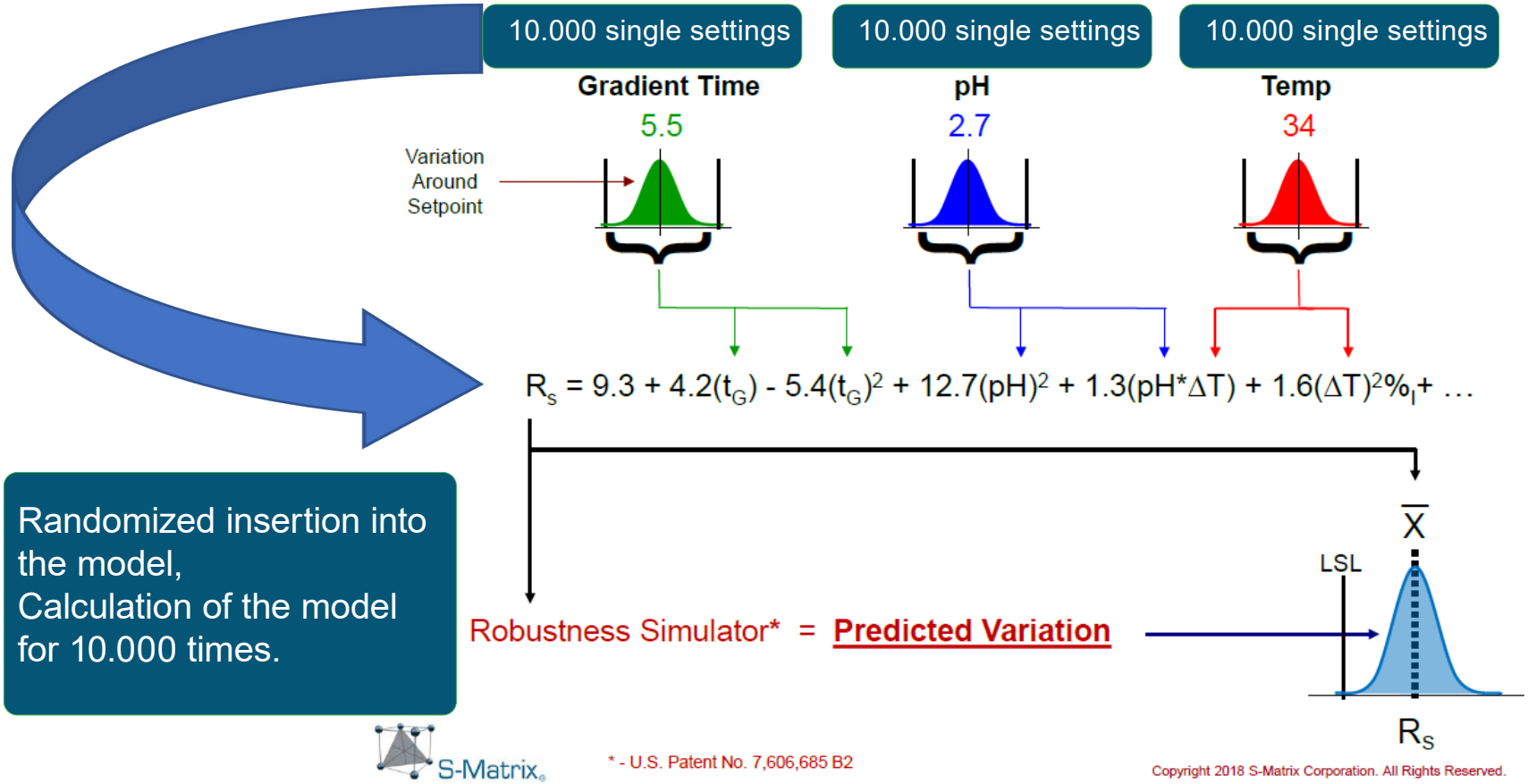
Monte Carlo Robustness Simulation - in silico assessment, no further measurements



Expected Variation Around Set-points for each CPP (e.g. manu-facturer's specs)

Robustness

Variation of a Method– Simulation of multiple Injections



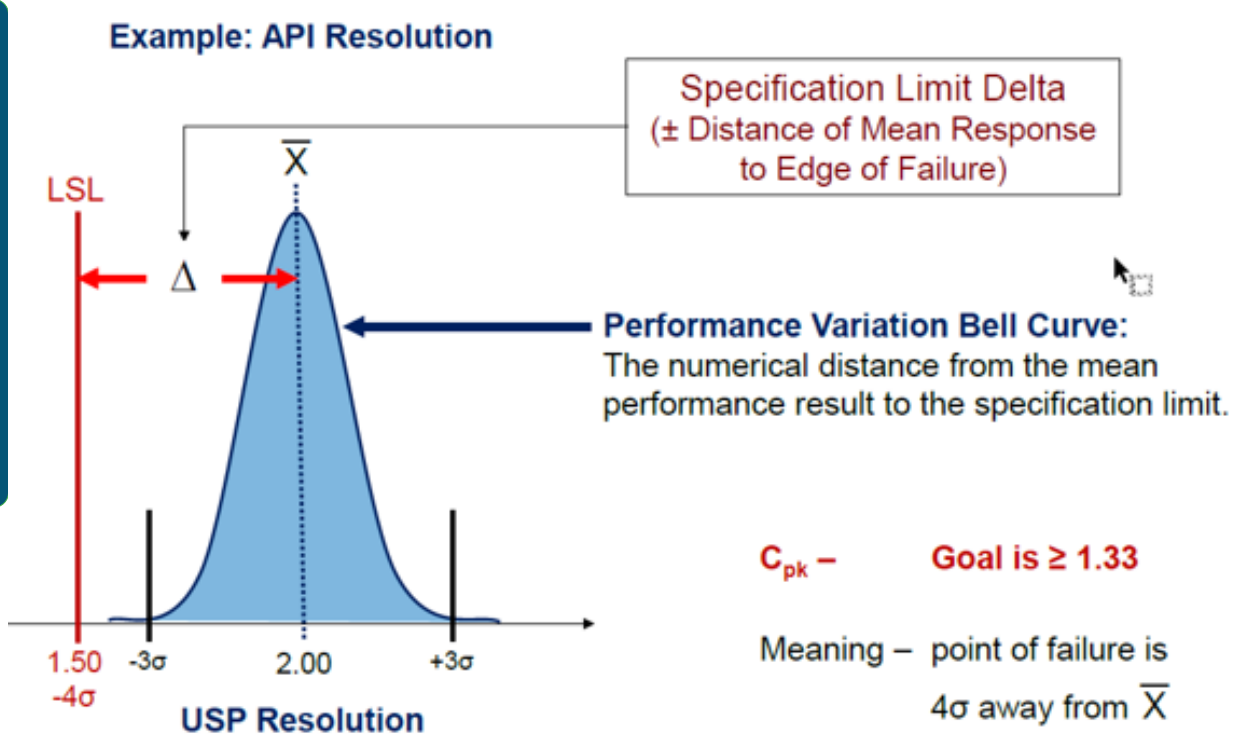
Robustness

Robustness Simulation – Statistical Robustness Metrics, Formulas

Cpk - one-sided specification limit

Calculation Cpk with LSL

$$\frac{\overline{X} - LSL}{3s}$$

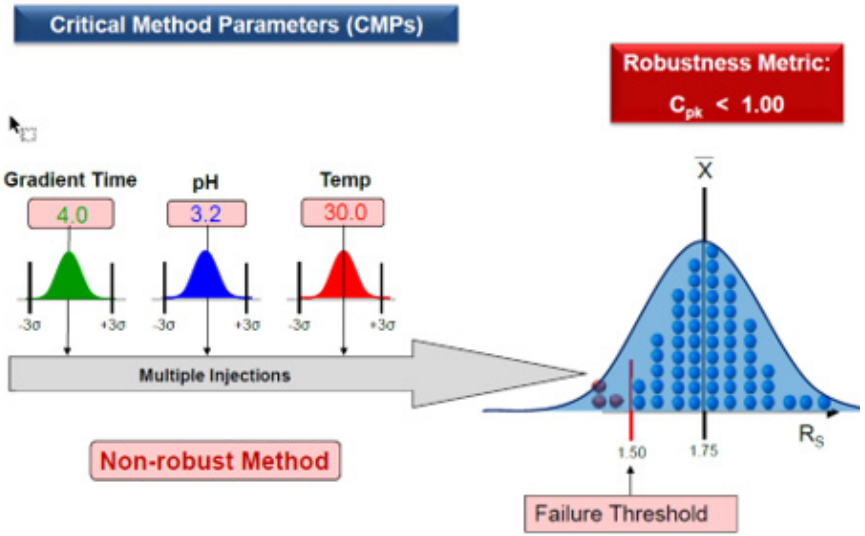


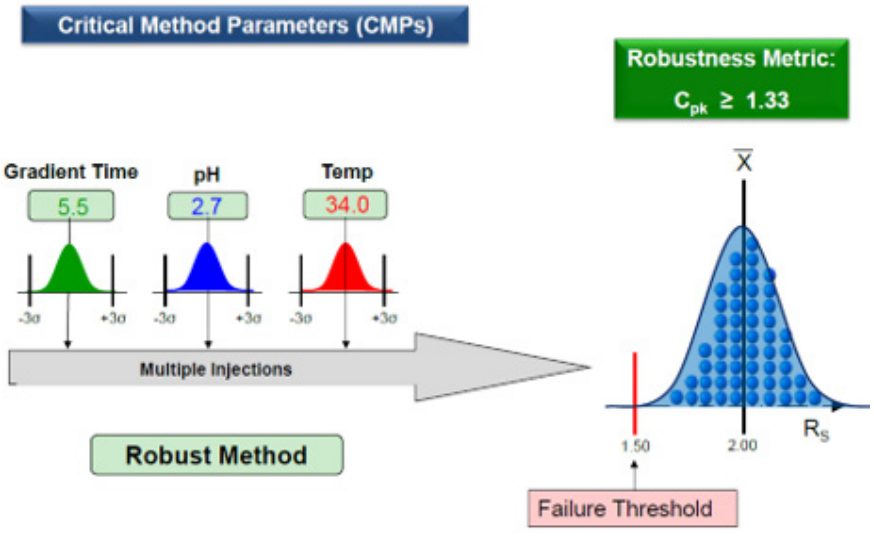
Robustness

Robustness Simulation – Statistical Robustness Metrics

Cpk below 1,00: Non-robust Method

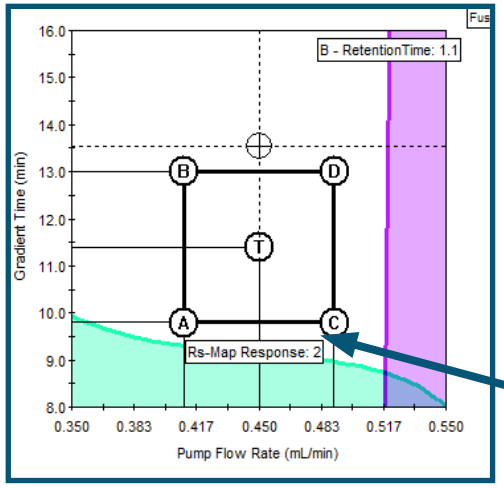
Cpk above 1,33: Robust Method

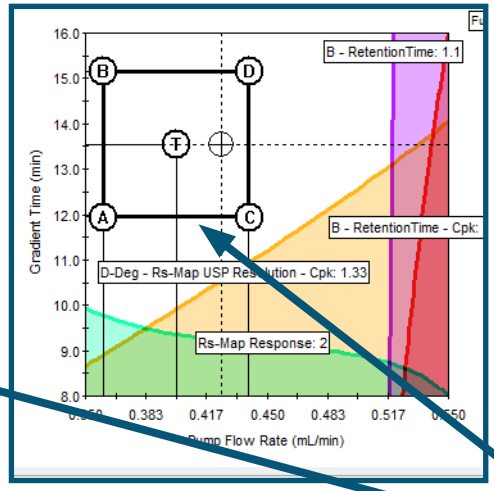




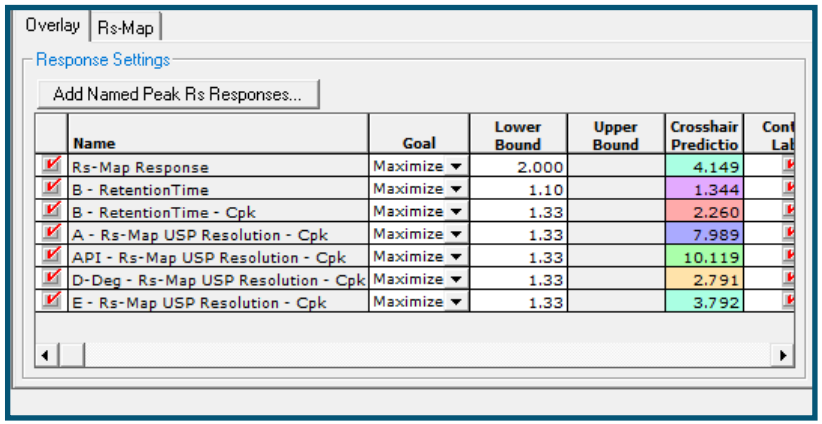
Robustness

Design Space (= UNshaded Region) and selection of the Final Robust **MODR** (Method Operable Design Region)





- Unshaded region indicates an space where target values for all CQA‘s are met.
- In the right diagramm robustness is included



Name	Goal	Lower Bound	Upper Bound	Crosshair Predictio	Cont Lal
Rs-Map Response	Maximize	2.000		4.149	
B - RetentionTime	Maximize	1.10		1.344	
B - RetentionTime - Cpk	Maximize	1.33		2.260	
A - Rs-Map USP Resolution - Cpk	Maximize	1.33		7.989	
API - Rs-Map USP Resolution - Cpk	Maximize	1.33		10.119	
D-Deg - Rs-Map USP Resolution - Cpk	Maximize	1.33		2.791	
E - Rs-Map USP Resolution - Cpk	Maximize	1.33		3.792	

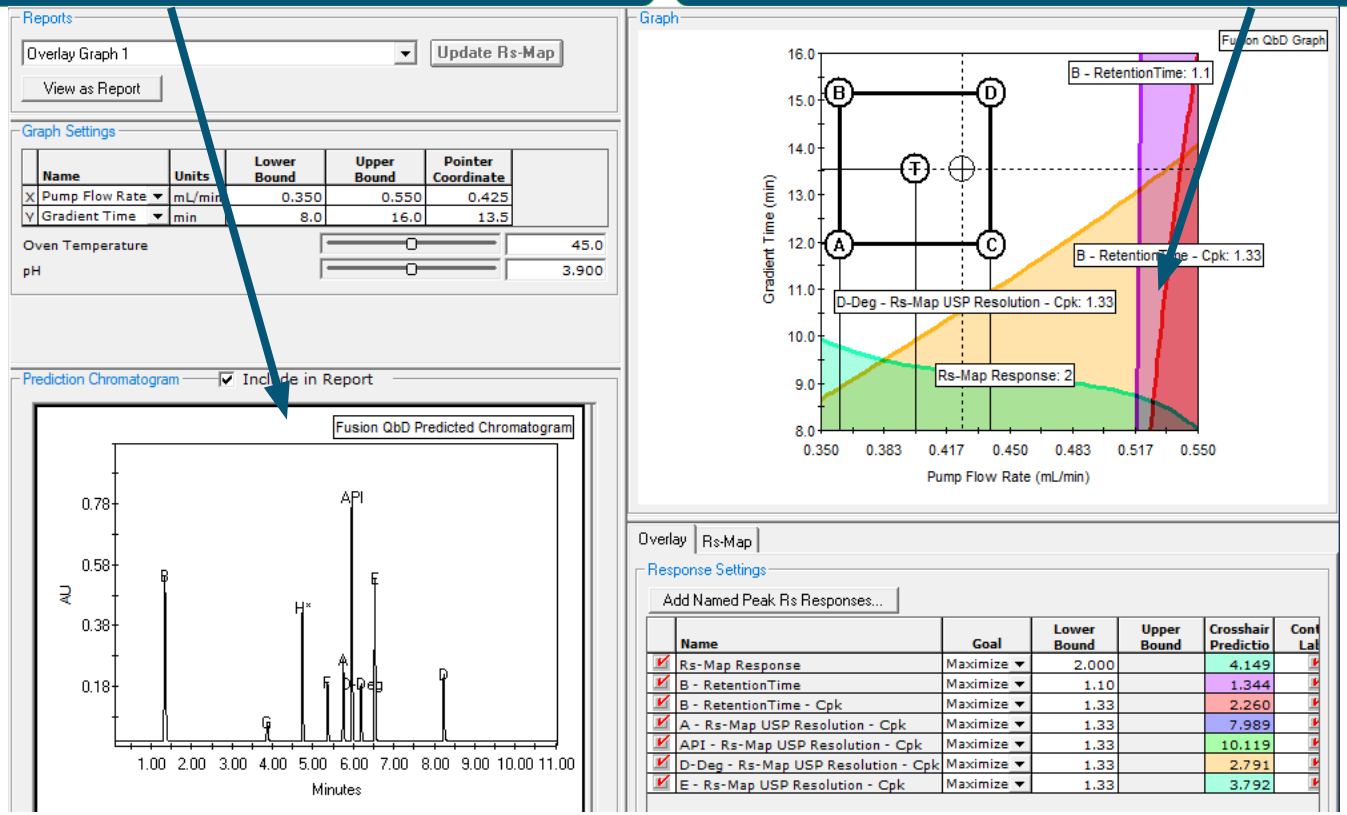
To ensure robustness the rectangle needs to be moved, else acceptance for D-Deg resolution will sometimes fail.

Robustness

Graphical and Numeric Predictions in the Robust Design Space

Visualization of prediction chromatograms for each method in the design space

Visualization of the final method and robust operable region for all CQA's

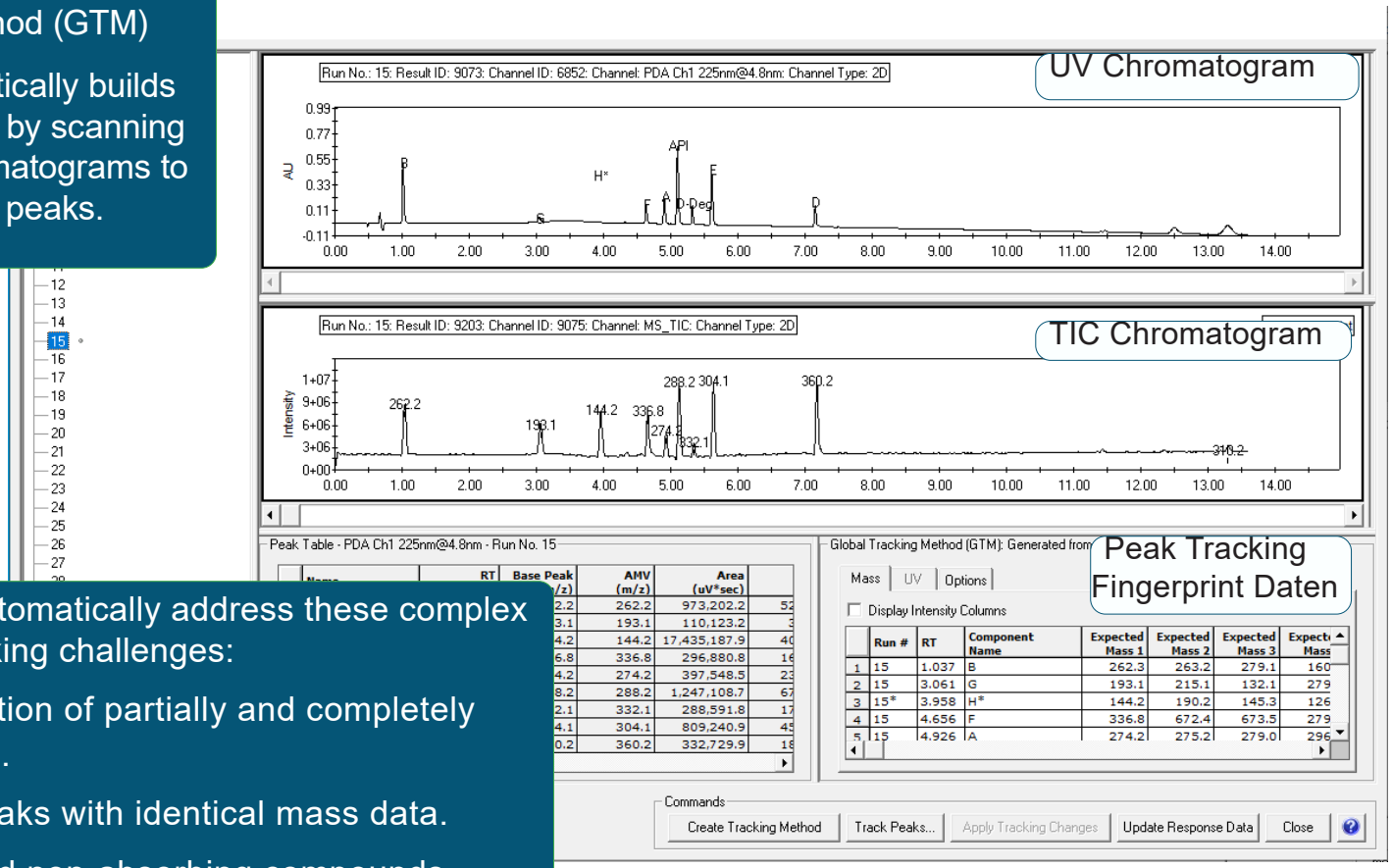


New Features since Fusion 9.9

PeakTracker™

Global Tracking Method (GTM)

PeakTracker automatically builds a customizable GTM by scanning all UV and TIC chromatograms to identify all integrated peaks.



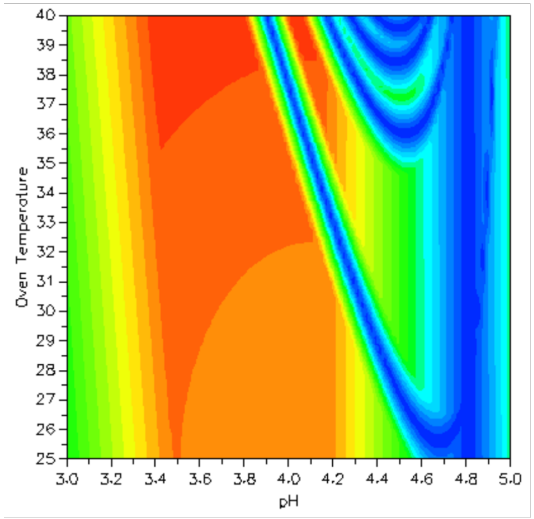
PeakTracker can automatically address these complex separation and tracking challenges:

- Auto-deconvolution of partially and completely co-eluted peaks.
- Two or more peaks with identical mass data.
- Non-ionizing and non-absorbing compounds.

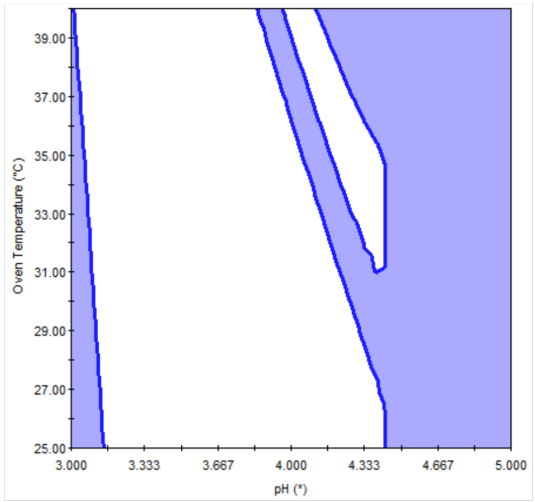
RS-Map Response

Fusion QbD now uses its hyper-accurate modeling technologies to predict USP or EP Resolutions for all peaks for any method conditions using standard Resolution equations. Graphical and numerical displays update in real time as you change method conditions.

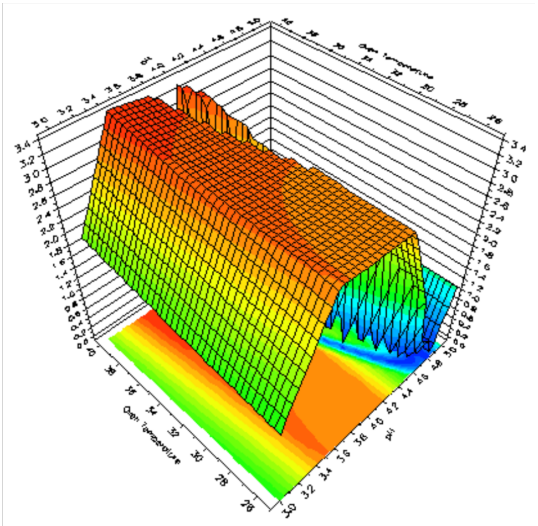
Contour Graph



Overlay Graph



Response Surface Graph



Forced Degradation Studies

Forced Degradation Studies – Full Automation Support

Forced Degradation Study

No. of Unique Degradation Path Samples: 3

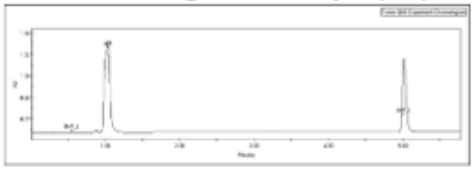
No. of Injection Repeats Per Sample: 1

Path #	Degradation Path Description
	Sample Compound Mix
1	Photo Degradation
2	Acid Degradation
3	Peroxide Degradation

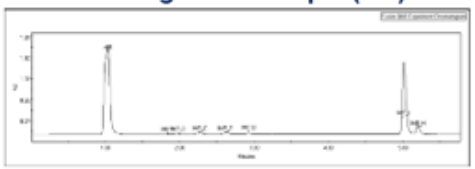
Experiment Design Matrix

Run No.	Pump Flow Rate (mL/min)	Gradient Time (min)	Oven Temperature (°C)	pH
Condition Column - 1	0.400	2.0	35.0	3.20
1.a	0.300	5.0	35.0	3.20
1.b	0.300	5.0	35.0	3.20
1.c	0.300	5.0	35.0	3.20
2.a	0.500	5.0	35.0	3.20
2.b	0.500	5.0	35.0	3.20
2.c	0.500	5.0	35.0	3.20
3.a	0.300	15.0	35.0	3.20
3.b	0.300	15.0	35.0	3.20
3.c	0.300	15.0	35.0	3.20

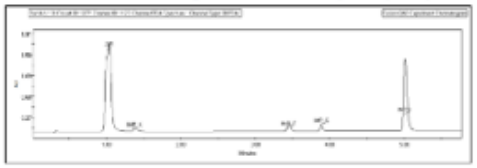
1.a

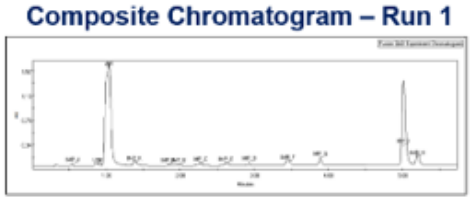


1.b

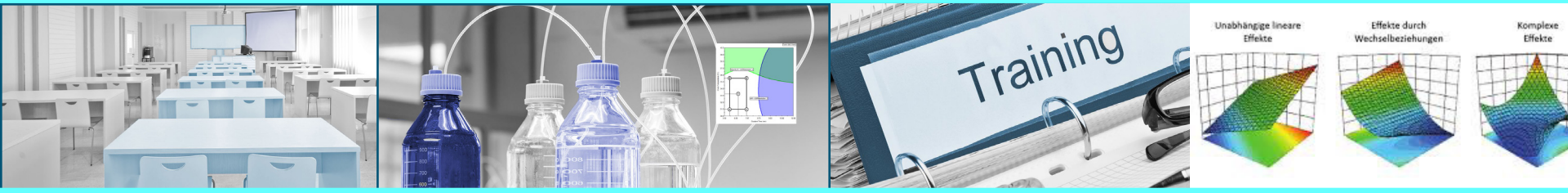


1.c





- Each experiment run is replicated for each degradation path sample
- Each peak is tracked in each degradation path sample chromatogram
- All peaks from all degradation path sample chromatograms are aggregated into a single composite chromatogram for the run



Fusion QbD - Introduction

Application of QbD Principals
For the Efficient Development and Optimization of
Analytical Methods

cromingo e.K.
www.cromingo.com
info@cromingo.com

cromingo