S-Matrix®

Fusion QbD

Powerful, Flexible, Automated Design of Experiments

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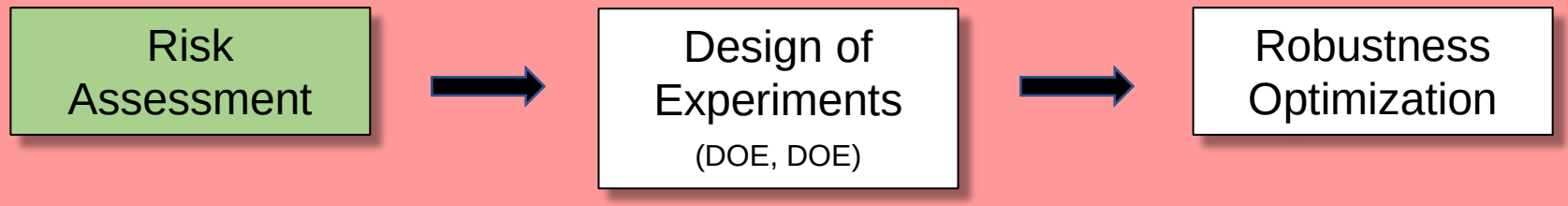
Risk Assessment



ICH Q14

Risk assessment and prior knowledge should be used to identify parameters, attributes and appropriate associated ranges to be investigated experimentally. (*Pg. 5*)

USP <1220>

For variables where there may be higher risk, one way to reduce risk is to gain additional knowledge about the influence of those parameters using modeling and/or experimentation. (*Pg. 8*)

Risk Assessment – Parameter Selection

Sources of Risk for Bias and/or Variation in Current Method

Element	Presumed CMPs*	CMAs						Category (C, N, X)
		Resolution USP	S/N	Tailing USP	Area % RSD - API	K-Prime - 1st Peak	K-Prime - Last Peak	
Chemistry	Column Type	5	1	1	3	5	5	X-S
	Strong Solvent	5	1	1	3	5	5	X-S
	Aqueous solvent	5	5	5	1	5	5	X-S
	pH	5	5	5	3	5	5	X-S-O
Process	Pump Flow Rate	3	1	5	3	5	5	X-O
	Injection Volume	3	5	3	5	1	1	C
	Oven Temperature	5	1	3	3	5	5	X-O
Gradient Program	Initial Hold Time	1	1	1	1	5	1	C or X-O
	Gradient Slope	5	1	5	3	5	5	X-S-O
Detection	Wavelength	5	5	1	5	1	1	C
	Sampling Rate	3	5	1	5	1	1	C
	Precision	1	3	1	3	1	1	C

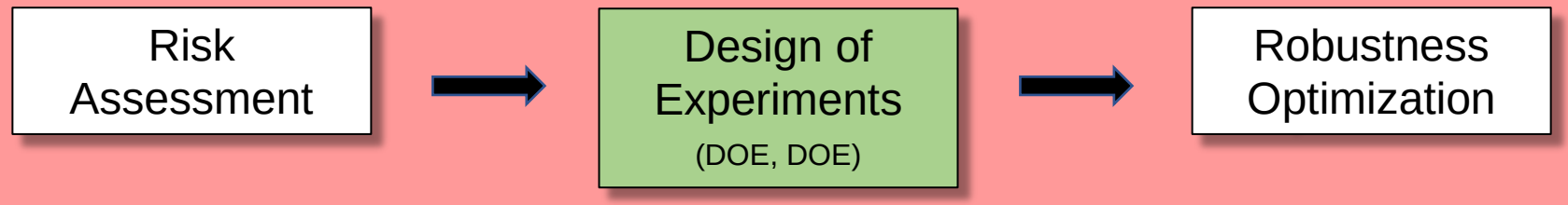
C = **C**ontrolled Factor, **X** = e**X**perimental Factor (**S** = Screening, **O** = Optimization)

Impact Severity

Low = 1
Medium = 3
High = 5

* – CMPs can change depending on the nature of sample compounds and the separation mode.

Design of Experiments (DOE, DoE)



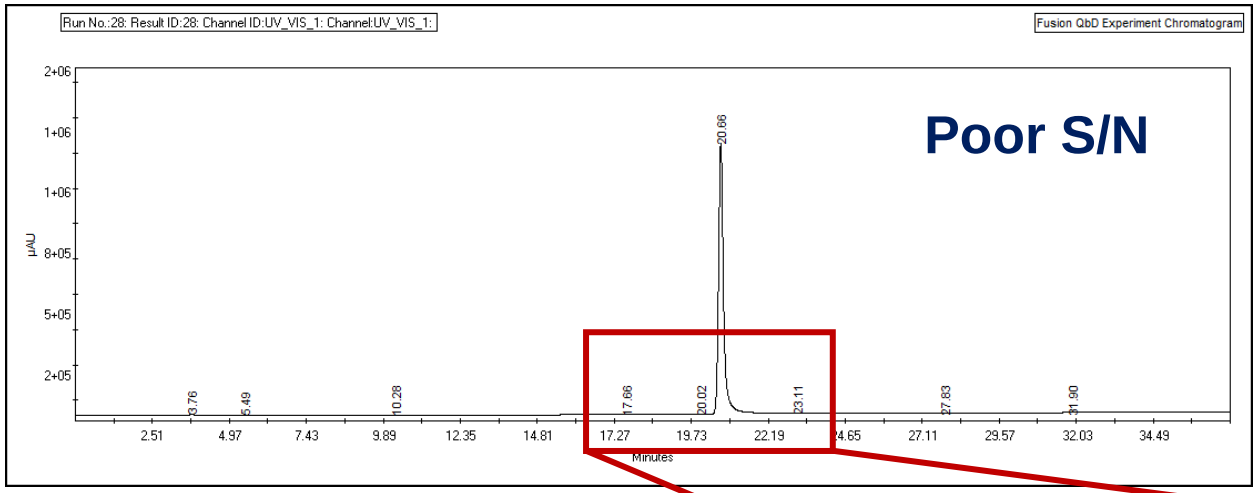
ICH Q14

In an enhanced approach, the ranges for the **relevant parameters and their interactions** can be investigated in multivariate experiments (DoE). (*Pg. 5*)

USP <1220>

Experimentation is a direct way of generating data that can be used to assess the impact of procedure parameters on performance, and **the use of statistical design of experiments (DOE) is an effective way to do this**. (*Pg. 8*)

DOE – The Critical First Step

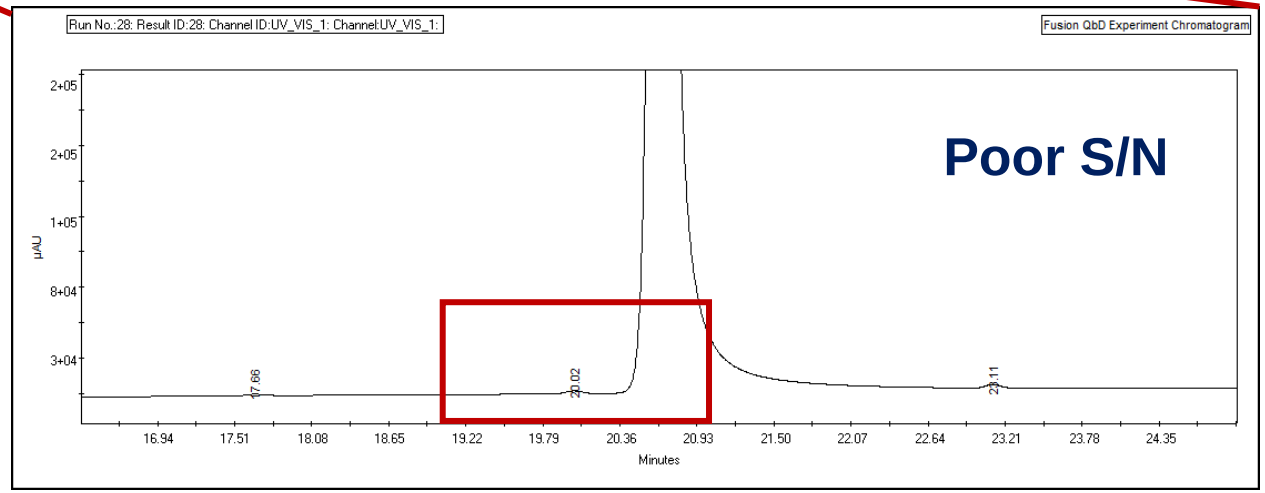


Problems with Determining the Cause of Peak Loss Corrupts the Data.

Co-elution or Just Poor Signal ?

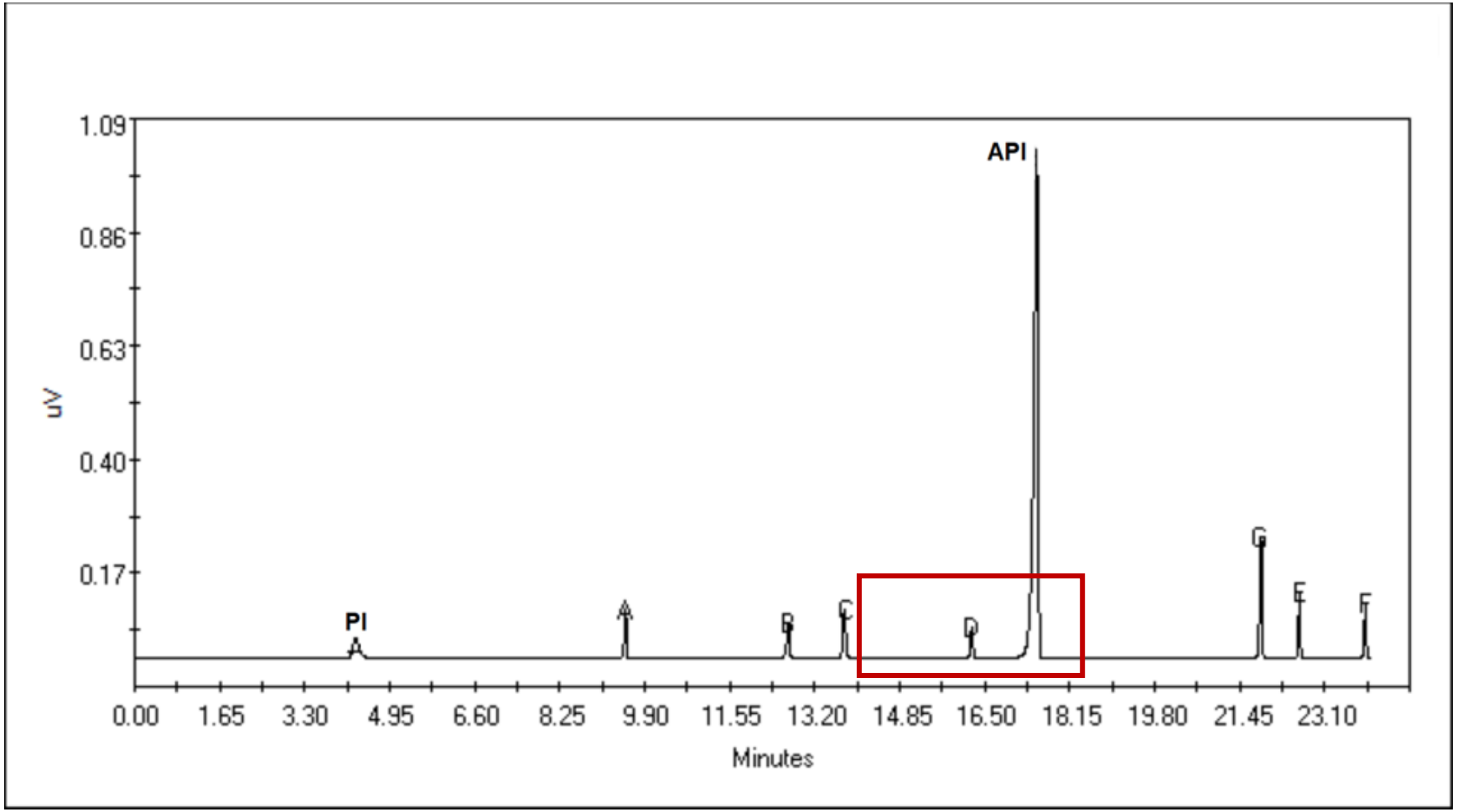
A Method Development Sample should not look like a final QC preparation – it should provide a very good S/N Ratio for all peaks of interest.

S/N Goal: Min = 20, Optimum ≥ 50



DOE – The Critical First Step

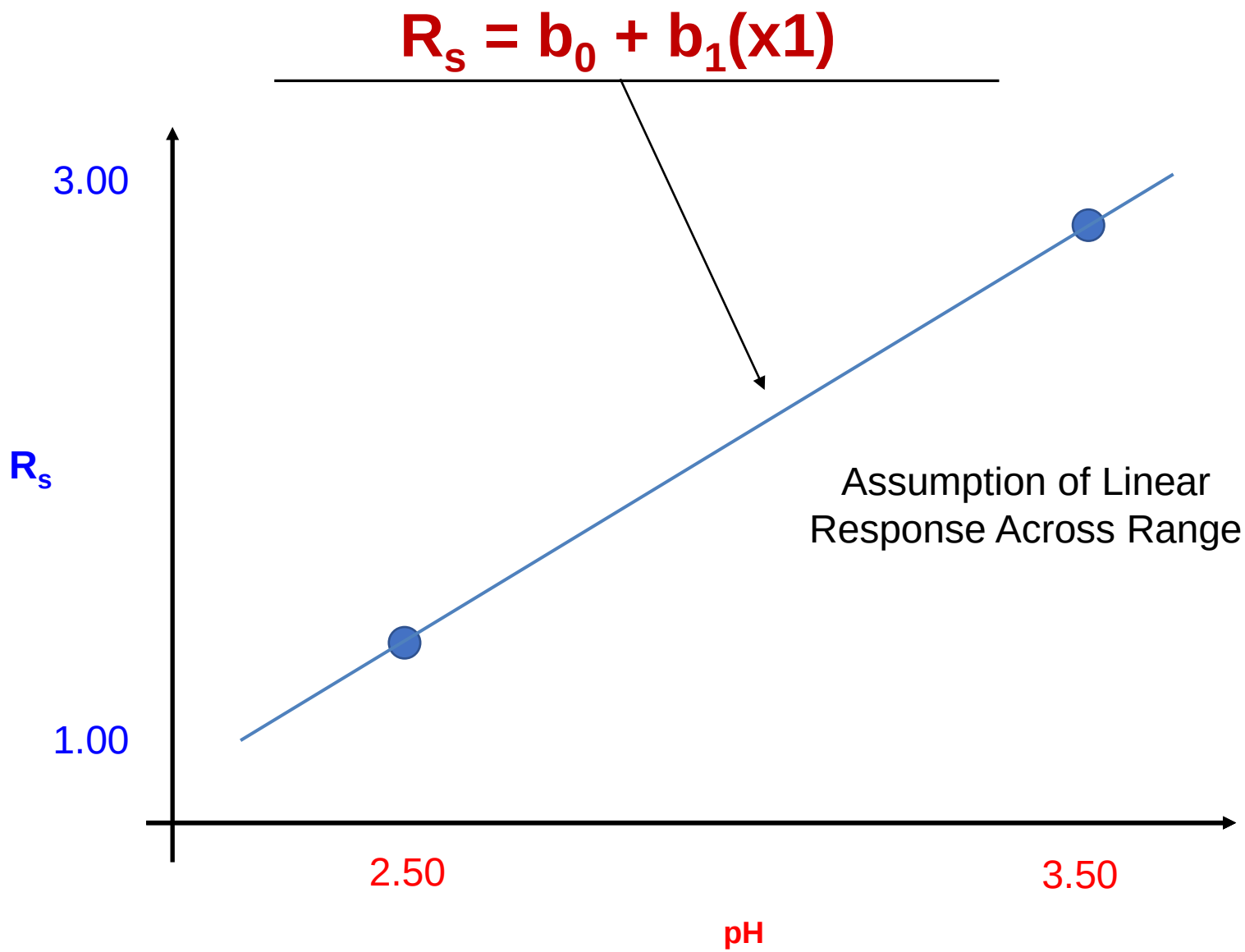
Final QC Preparation Should Provide a Very Good S/N Ratio for all Peaks of Interest



OFAT (Univariate) Experiment

All Factors but One

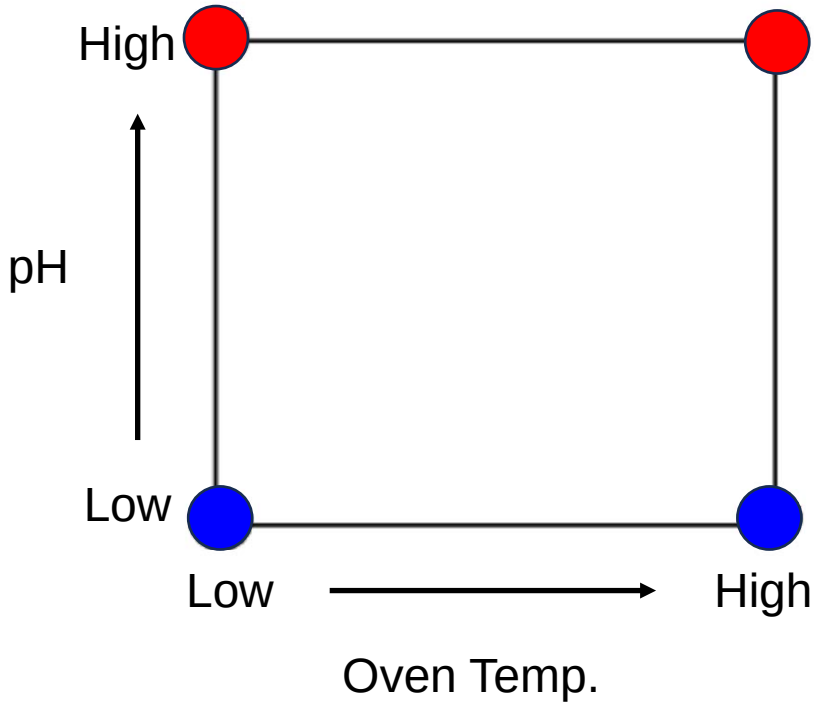
Held Constant



DoE – A Model Building Methodology

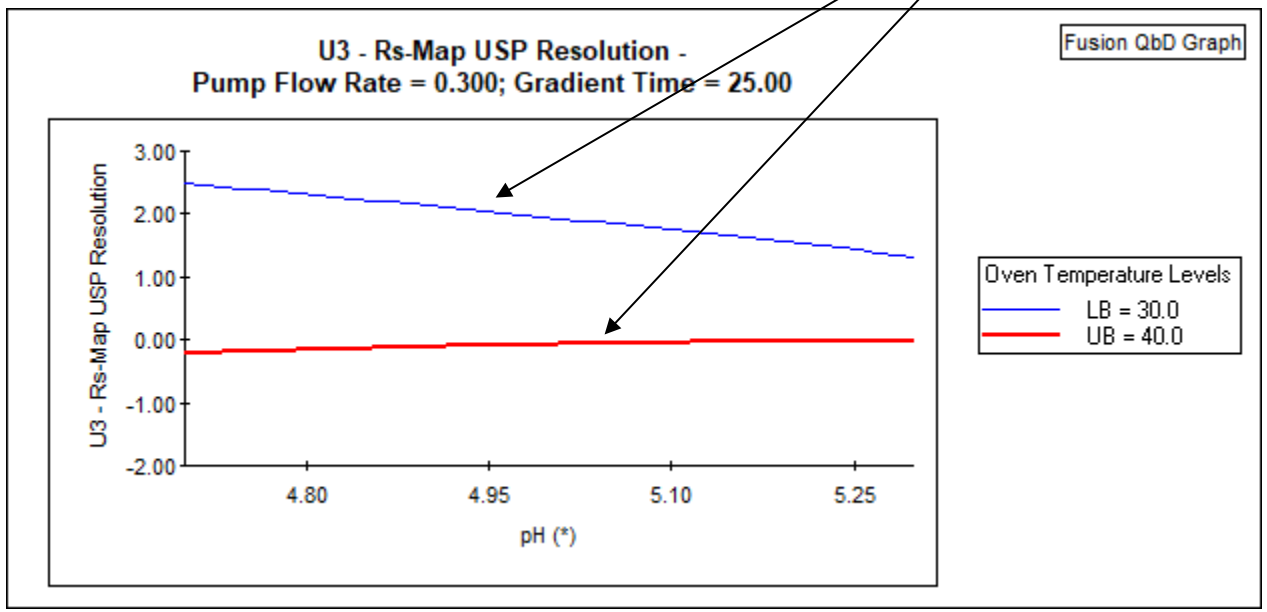
DoE for 2 Variables at 2 Levels –

Simplest Case



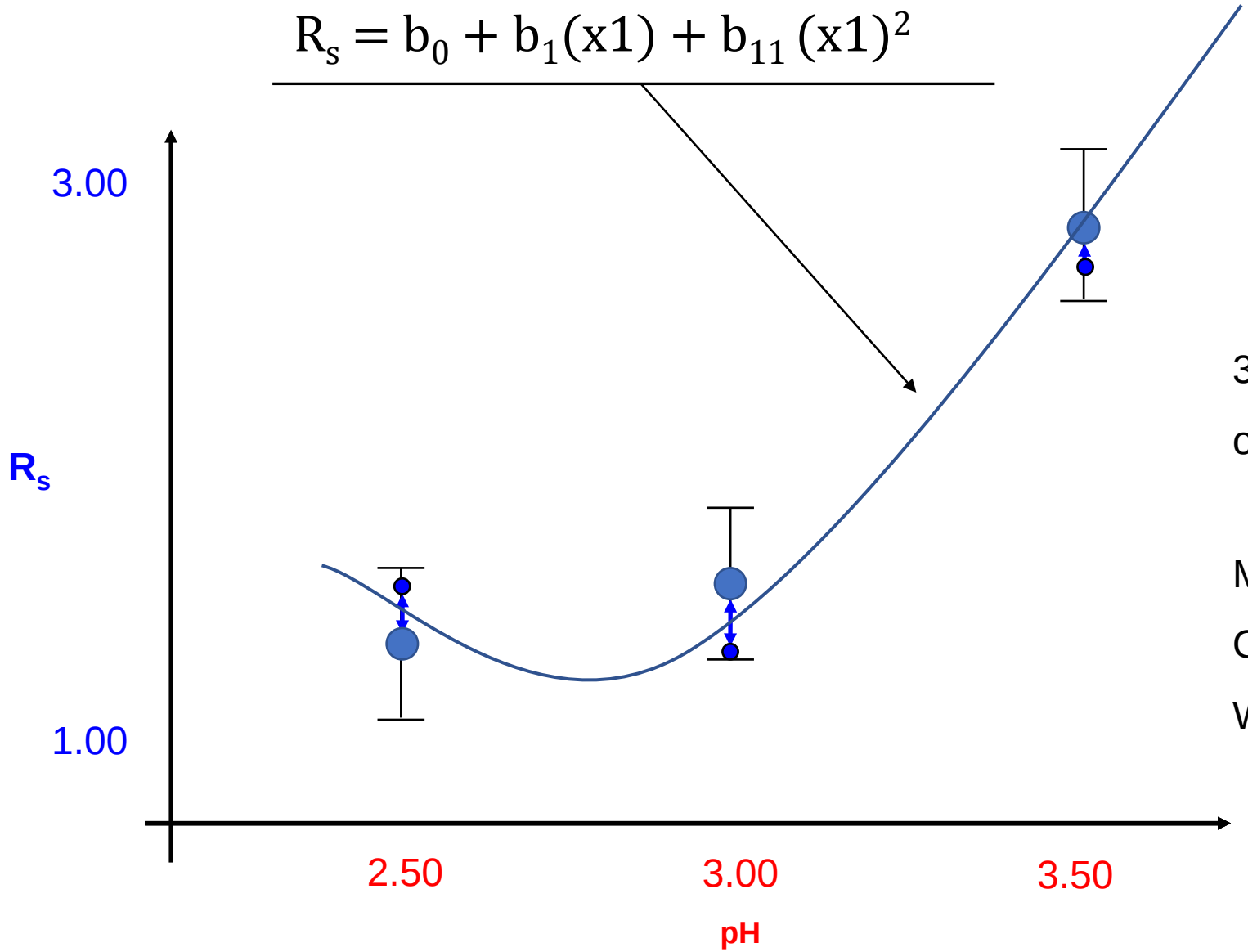
Interaction Effect: slope change

$$R_s = b_0 + b_1(x1) + b_2(x2) + b_{12}(x1 * x2)$$



DoE – A Model Building Methodology

$$R_s = b_0 + b_1(x1) + b_{11}(x1)^2$$



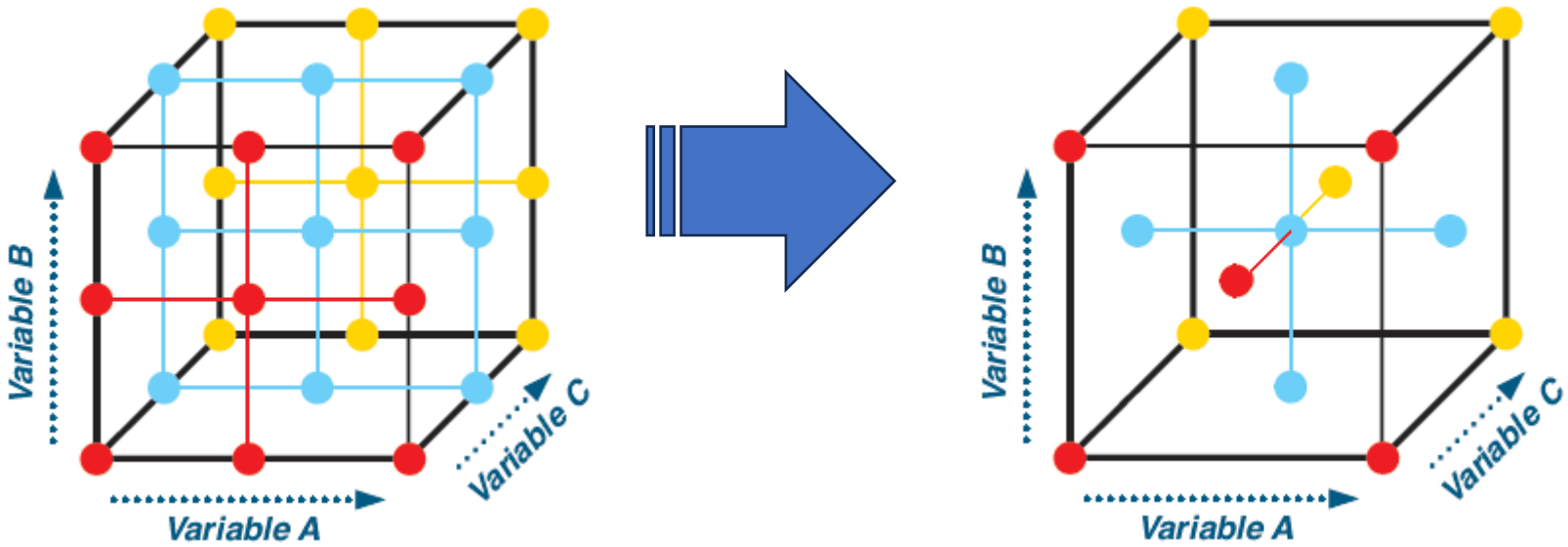
3rd Level Challenges Assumption of Linear Response Across Range

Model Goal:

Observed Minus Predicted – Within Error at All Points

DoE Efficiency

DoE uses Statistical Sampling of All Possible Combinations to Support Accurate Estimation of Study Factor Effects



All Possible Combinations
= 27 Runs

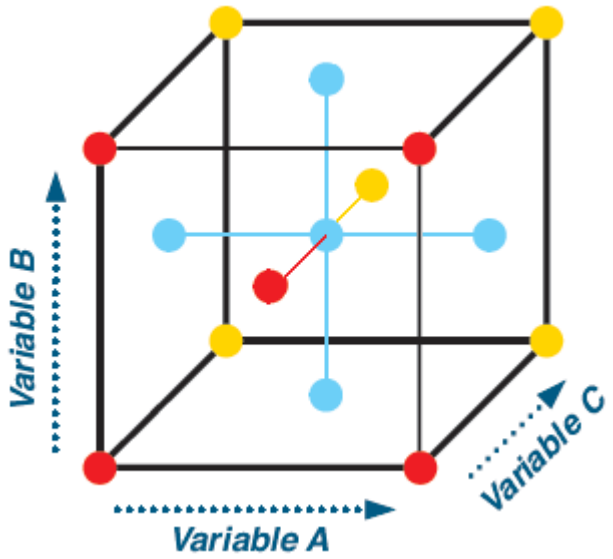
DoE: Central Composite Design
= 15 Runs

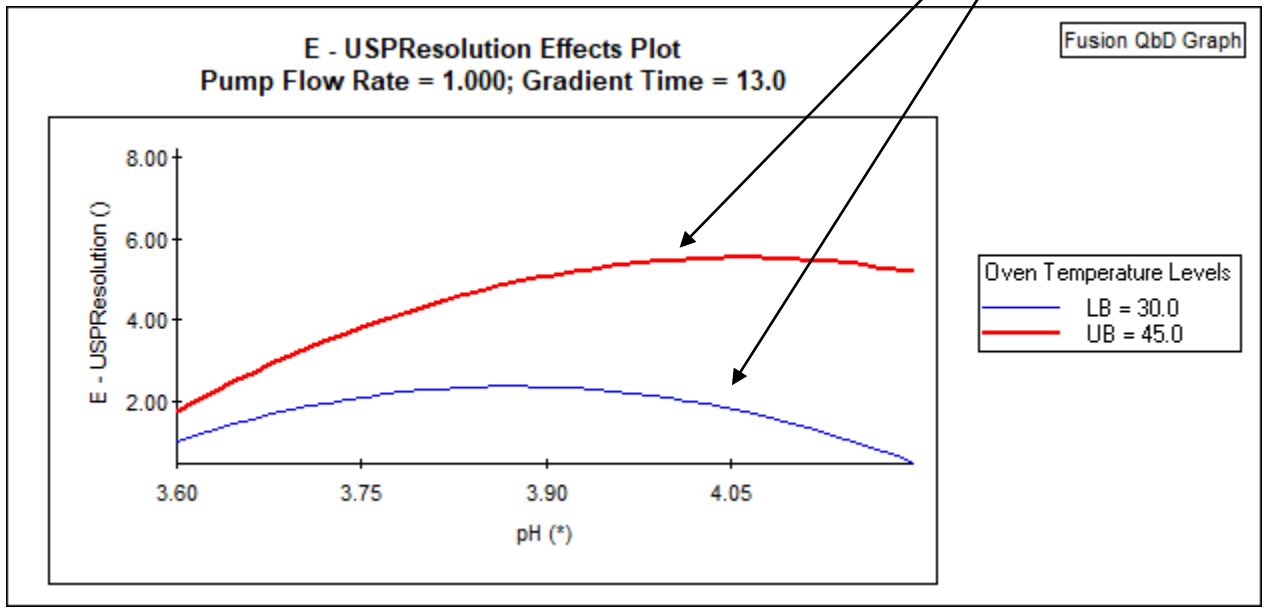
DoE – A Model Building Methodology

Squared Interaction Effect: Slope + Curvature Change

$$R_s = b_0 + b_1(x1) + b_2(x2) + b_3(x3) + b_{11}(x1)^2 + \cdots + b_{12}(x1 * x2) \ldots + b_{112}(x1)^2(x2) + \cdots$$

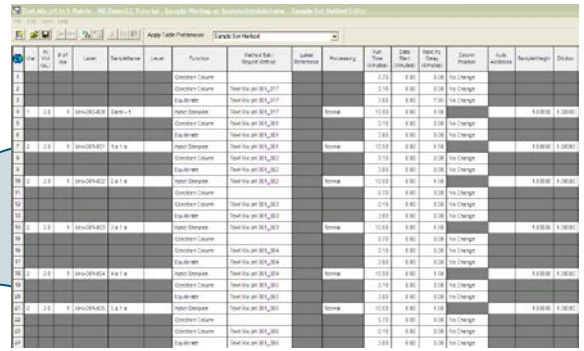
Central Composite Design

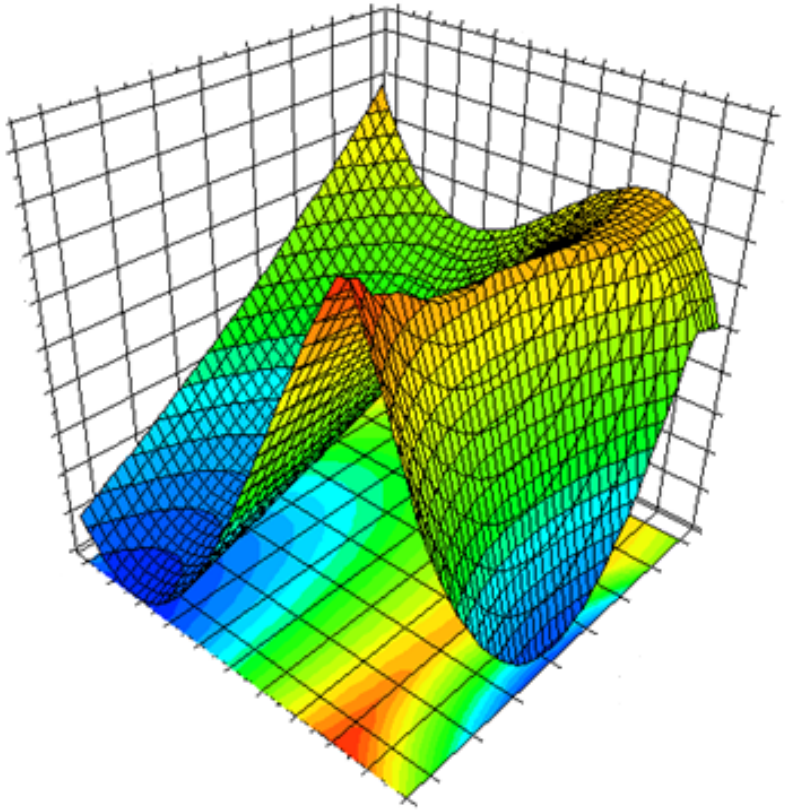




DoE (DOE) – A Model Building Methodology

Turning Chromatograms into Knowledge



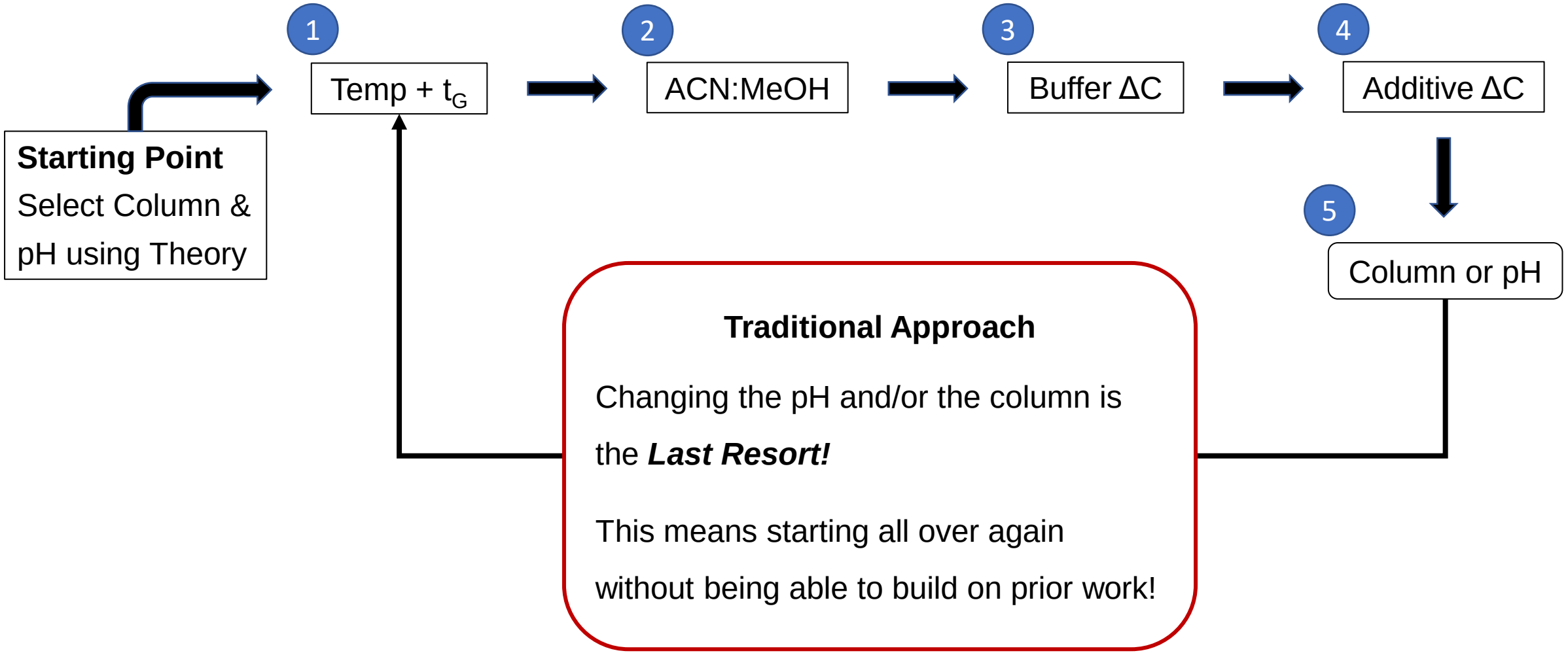


$$\#-R_s \geq 2.00 = 9.3 + 4.2(PFR) - 5.4(\Delta t_G)^2 + 12.7(\Delta t_G * pH) + 1.3(pH * \Delta T) + 1.6[(\Delta T)^{2*} \Delta t_G] + \ldots$$

Linear Effect | Curvature Effect | Interaction Effects | Complex Effect

Before Fusion QbD

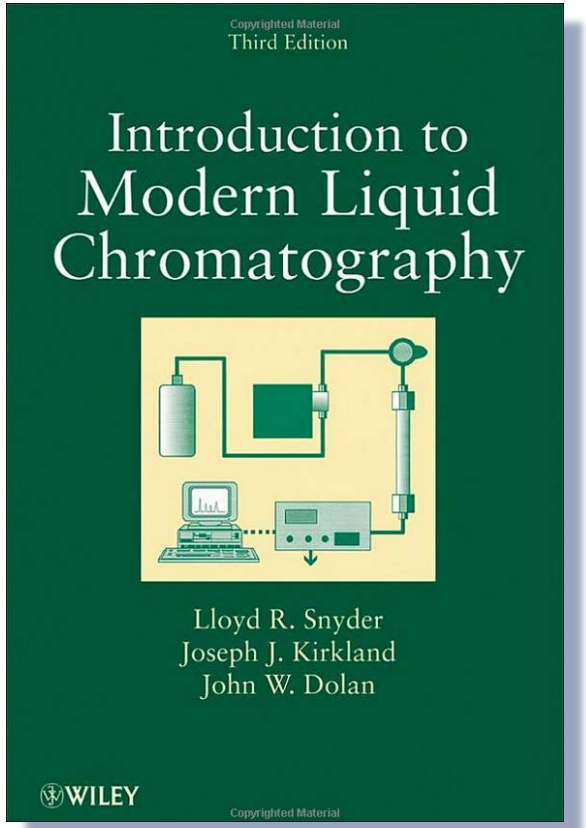
Before Fusion QbD: One-Factor-At-a-Time (OFAT) Approach



Current Thinking by Recognized Experts

"For methods involving a large number of samples, and where adequate resolution must be combined with run times that are as short as possible, **it can be profitable to spend more time initially on "scouting" experiments**.

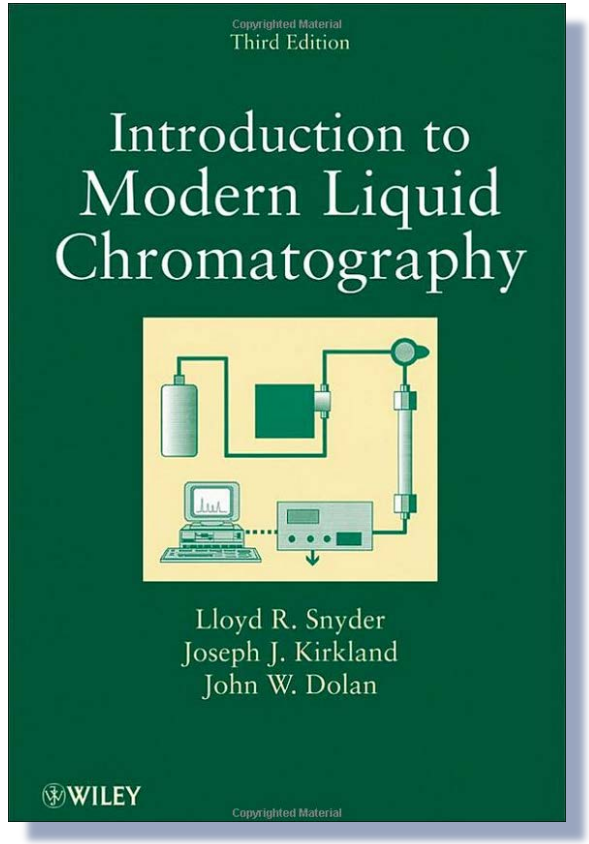
- Different **columns**
- Different **B-solvents**
- Variations in **pH** and **temperature**
- Use of **Gradient elution** during the experiments can help avoid the need to separately optimize values of %B for each variable studied."



Snyder, Kirkland, and Dolan. (2010). *Introduction to Modern Liquid Chromatography*, 3rd Edition; John Wiley & Sons, Inc., Hoboken, New Jersey (p. 67)

Current Thinking by Recognized Experts

"Still another approach is to **search the literature** for separation of the same or similar sample. **Trial-and-error** modifications of conditions are then followed until an acceptable separation is achieved. ***We do not recommend this approach**** because possible deficiencies in literature methods can delay subsequent attempts at achieving a final, acceptable separation."



***** – italics added by Snyder, Kirkland, and Dolan in book text to emphasize the point.

Snyder, Kirkland, and Dolan. (2010). *Introduction to Modern Liquid Chromatography*, 3rd Edition; John Wiley & Sons, Inc., Hoboken, New Jersey (p. 67)

DOE Supports Study Parameter Flexibility

Full utilization of Quaternary Pumps, Solvent Selection Valves, and Column Switching Valves.

Study **any combination** of LC parameters which can **interactively effect** method performance!

- Isocratic and Gradient Methods
- Strong Solvent Type
- Any pump program steps – e.g.
  - Equilibration Time & %
  - Isocratic Hold Time & %
  - Gradient Time / Slope
  - Initial / Final Hold Time & %
  - Re-equilibration Time & %
- Column Temperature
- Column Type
- Flow Rate
- Injection Volume
- pH
- Mobile Phase Blends
- Salt, Buffer, Additive – Type & ΔC
- Wavelength

Experiment Setup – Column Type

Column Settings

* - Sufficient Conditioning Time for 10 column volumes is recommended.

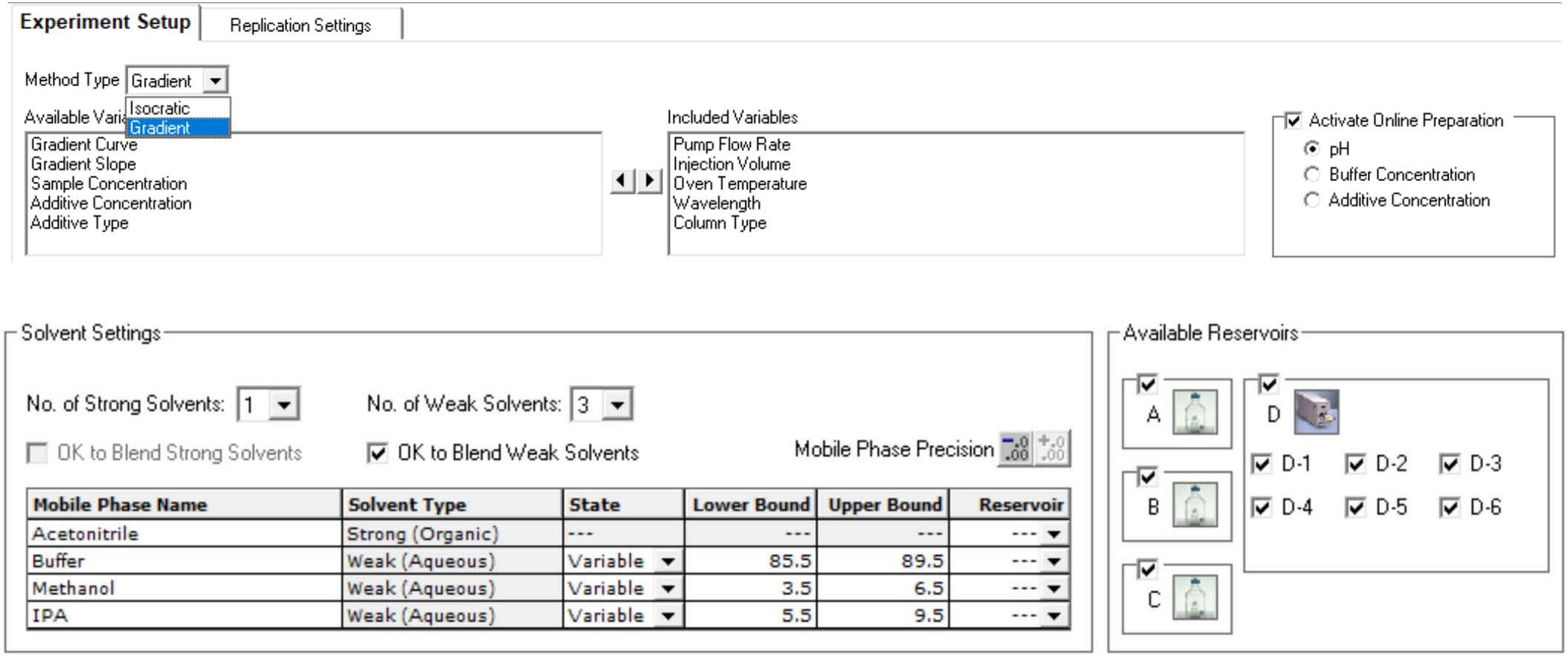
	Name	Valve Position	pH Upper Limit	Flow Rate	Diameter (mm)	Length (mm)	Time Required for One Column Volume (min)	Conditioning Time (min)*
1	HSS T3	Position 1	8.00	0.400	2.10	100.00	0.9	9.0
2	CSH Phenyl-Hexyl	Position 2	11.00	0.400	2.10	100.00	0.9	9.0
3	BEH C8	Position 3	12.00	0.400	2.10	100.00	0.9	9.0
4	BEH Shield RP18	Position 4	11.00	0.400	2.10	100.00	0.9	9.0

Chemistry Intelligence –

- Blocks design on Column Temp when it is a study factor
- Groups runs by MP Chemistry (e.g., pH, Strong Solvent)
- Incorporates column conditioning between MP Chemistry changes

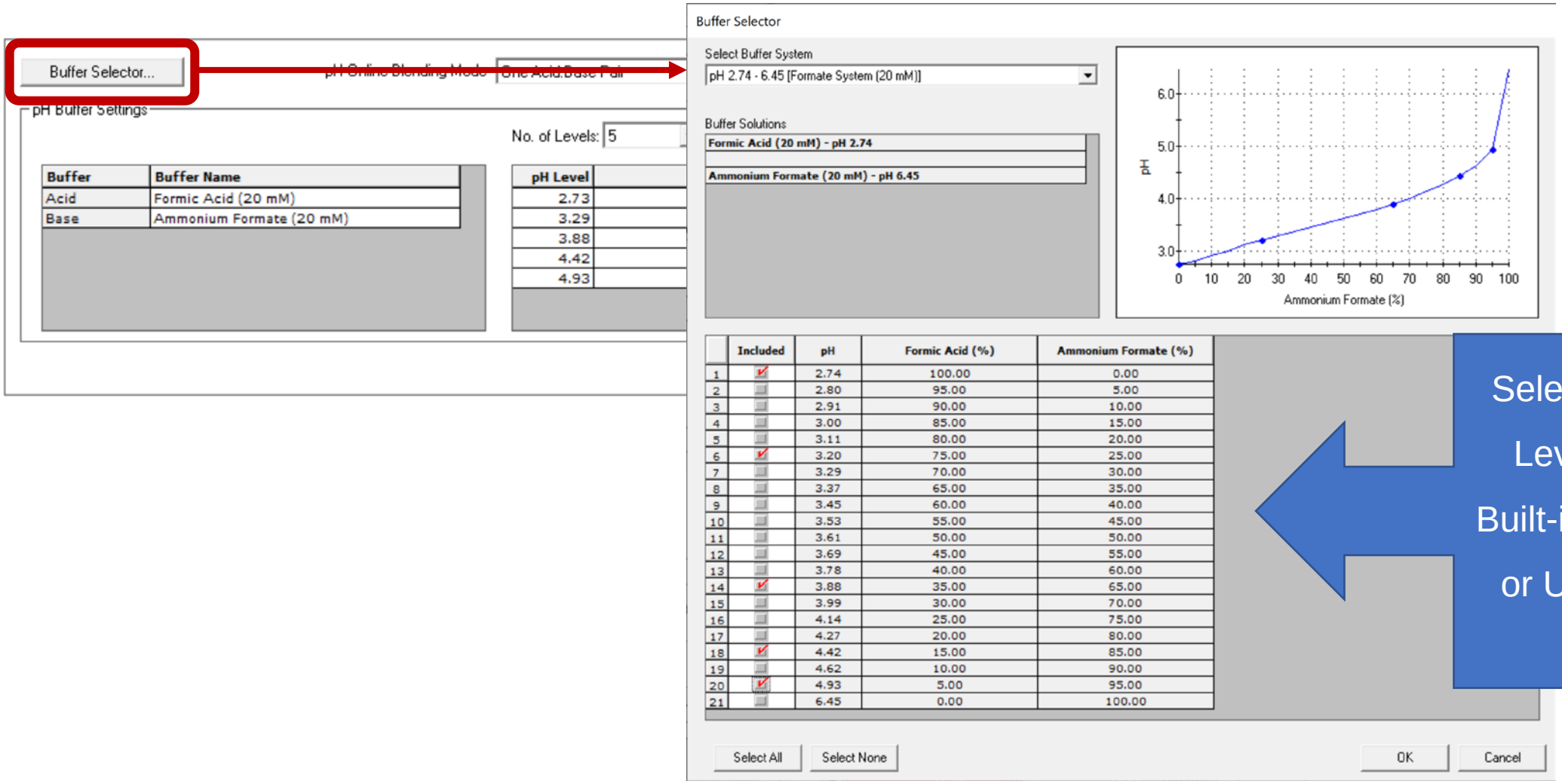
Valve Intelligence – Automatically generates multiple sequences as needed when # of columns in exceeds # of available valve positions.

Experiment Setup – Mobile Phase Blending



Experiment Setup – pH

Select One of the Built-in Buffer Systems or Enter Your Own



Select Desired pH Levels from the Built-in Buffer Curve or Use Your Own Curve

Experiment Setup – Gradient Time

Pump Program

No. of Gradient Steps: 1

Time Precision

	No.	Step Name	Time State	Time - Lower Bound	Time - Upper Bound	% Strong Solvent
☑	1	Equilibration	Constant	3.00	---	5.0
	2	Initial Hold	Constant	1.00	---	5.0
	3	Gradient	Variable	10.00	25.00	---
	4	Final Hold	Constant	1.00	---	95.0
	5	Ramp Up to Wash	Constant	0.50	---	---
☑	6	Column Wash	Constant	2.00	---	99.0
	7	Ramp Down from Wash	Constant	0.50	---	---
☑	8	Re-equilibration	Constant	2.00	---	5.0

Program duration: Min = 20.00 minutes, Max = 35.00 minutes

Gradient Study Factor: Gradient Time

Setting Mode
- (•) Time
- ( ) Slope

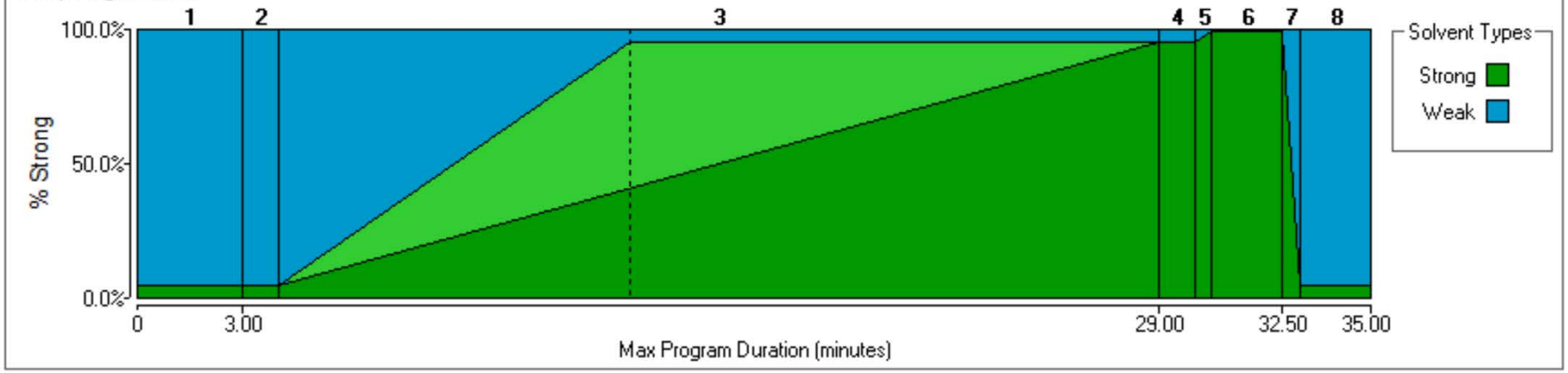
Update

Gradient

No. of Levels 3

Time (min)	Slope (%/min)
10.00	9.00
17.50	5.14
25.00	3.60

Pump Program Chart



Defining the Best Gradient Time Study Range

Whereas it is frequently recommended that the slopes of scanning gradients used to obtain retention data should vary by a factor of three or so, we do not see any evidence in our results that support this guideline. That is, similar retention prediction errors were obtained from models based on scanning gradients with slopes varying by a factor of three compared to models based on gradients with slopes varying by as little as 1.25. We also observe that the speed (i.e., absolute analysis or gradient time) does not have a strong impact on prediction error. On the other hand, the data show that the proximity of the slope of a gradient, for which retention will be predicted, to one of the scanning gradients, used to build the model, is far more determinant of retention prediction error. With decreasing proximity, it is more important that the slope of the target gradient lies between the slopes of the scanning gradients (i.e., interpolation is better than extrapolation, as one would expect).

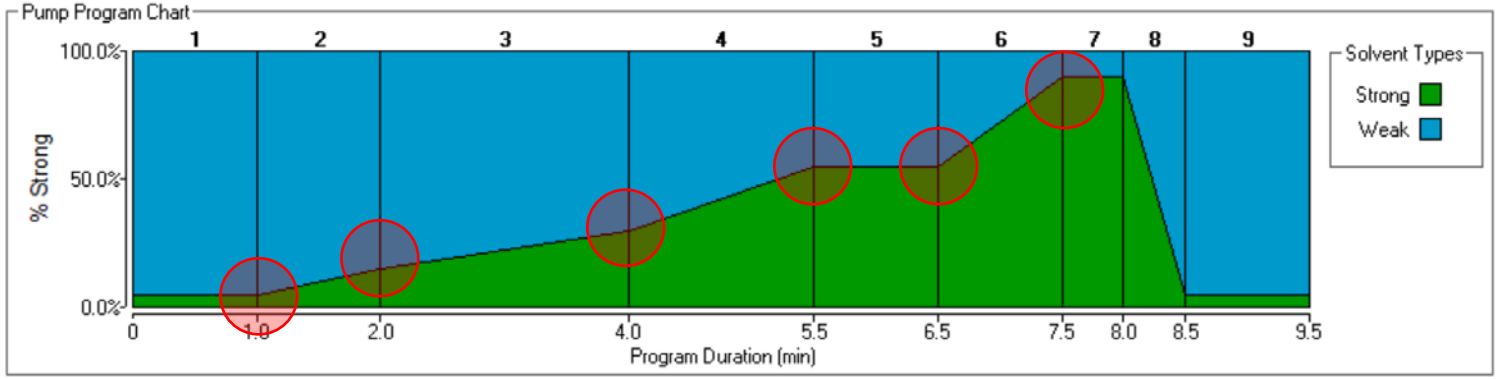
These findings have obvious implications for the design of experiments; using scanning gradients with a large variation in slopes is not required per se, but using a large range of slopes enables prediction of retention for a wider array of gradients without extrapolating.

J Chromatogr A. 2021 Jan 11:1636:461780.

What about Multi-segment Gradient Optimization

Most LC Method Development software relies primarily on localized gradient slope-based optimization. This drives the user to a multi-segment gradient method.

Multi-segment Gradients = Multiple Regions of POOR Robustness!



Localized Slope-Based Optimization is Now Recognized as High Risk.

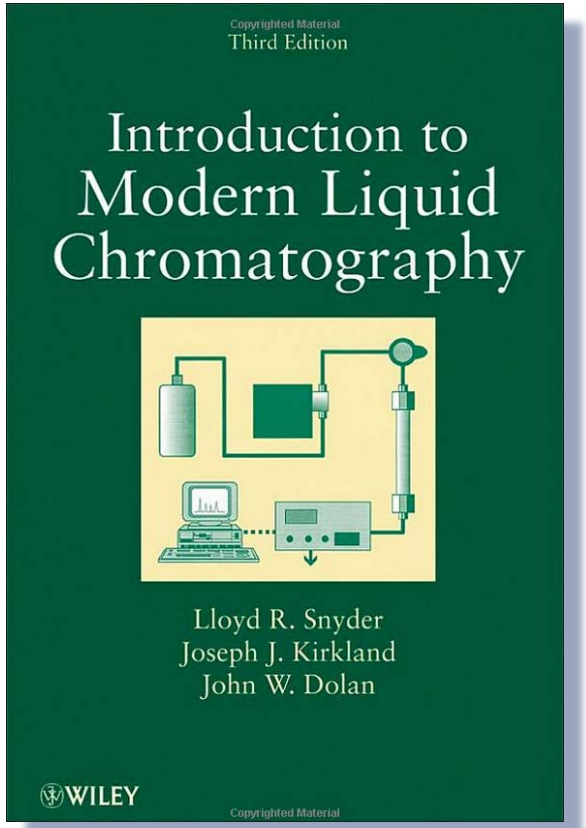
Fusion QbD Does Not Rely on This Approach!

(See the Next Slide →)

Current Thinking by Recognized Experts

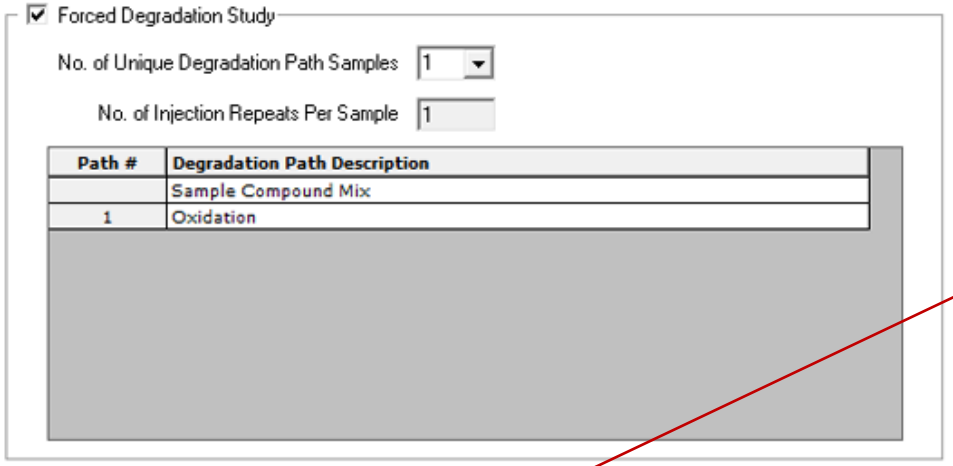
Issues with a Multi-step Gradient Approach to Method Optimization

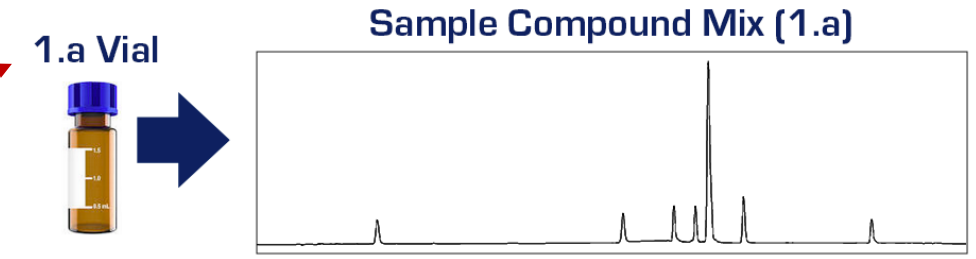
"Increasing resolution by adjusting selectivity for different parts of the chromatogram can sometimes be achieved with a segmented gradient; … **Segmented gradients are not often used for improving resolution … because their ability to enhance resolution without increasing run time is usually limited**… However, there are other – generally more useful – means of optimizing resolution by changing selectivity and relative retention. **Also, separations that use segmented gradients to improve resolution are likely to be less reproducible when transferred to another piece of equipment**."

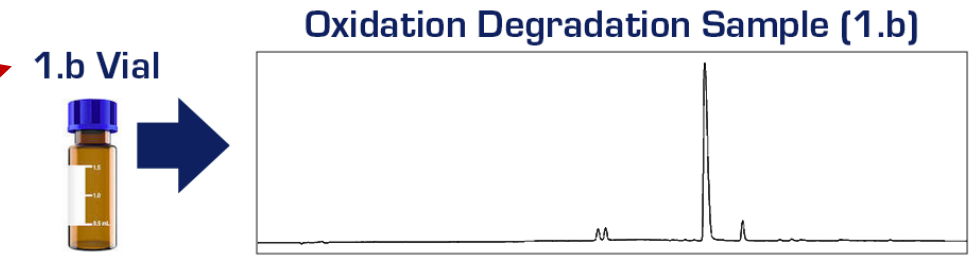


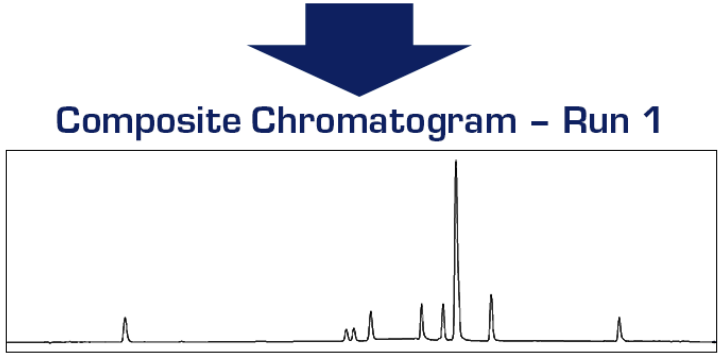
Snyder, Kirkland, and Dolan. (2010). *Introduction to Modern Liquid Chromatography*, 3rd Edition; John Wiley & Sons, Inc., Hoboken, New Jersey (p. 427-28)

Full Support for Forced Degradation Studies









Experiment Design Matrix

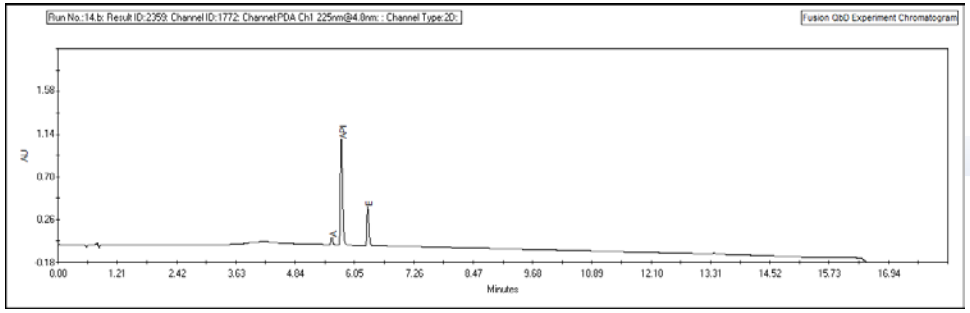
Run No.	Pump Flow Rate (mL/min)	Gradient Time (min)	Oven Temperature (°C)	pH
Conditioning_Run_1	0.400	2.0	30.0	4.60
1.a	0.300	8.0	30.0	4.60
1.b	0.300	8.0	30.0	4.60
2.a	0.500	8.0	30.0	4.60
2.b	0.500	8.0	30.0	4.60
3.a	0.300	16.0	30.0	4.60
3.b	0.300	16.0	30.0	4.60

Simple Setup integrates the replication scheme into the DoE Study, and automatically assigns a separate vial position to each replicate injection.

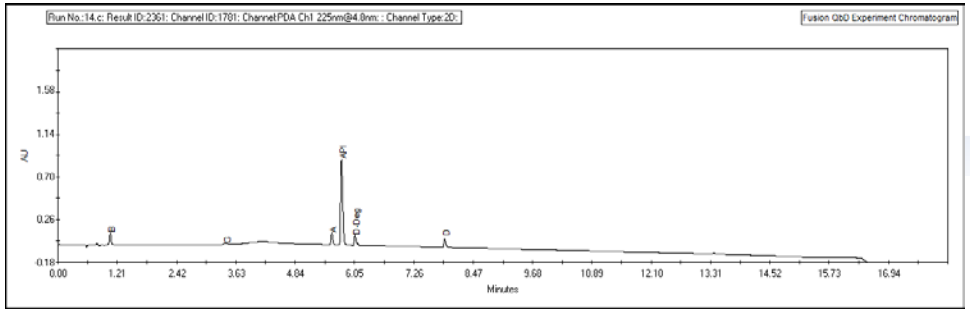
Fusion QbD tracks all peaks in all replicate chromatograms for each run and generates a *composite chromatogram* for each run containing all unique peaks from all replicate injections.

FDS Composite Chromatogram – 3 Degradation Path Example

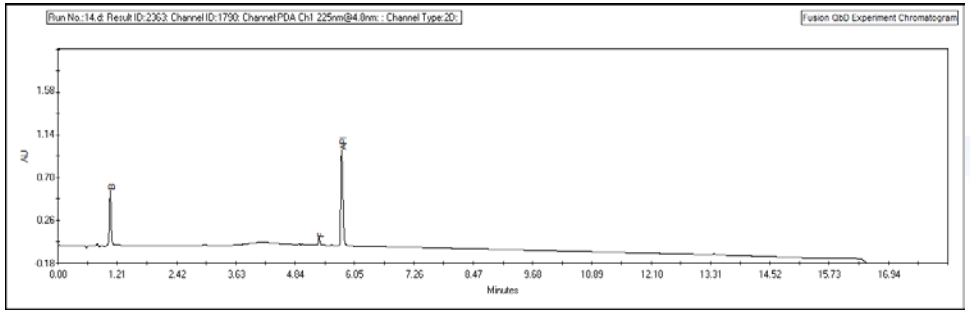
Acid Degradation Path

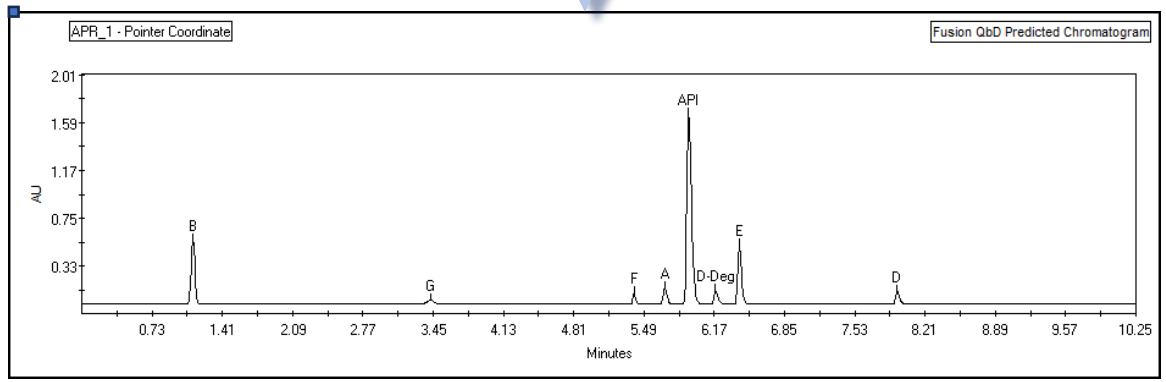


Base Degradation Path



Peroxide Degradation Path





Fusion QbD Composite Chromatogram

Fusion QbD – DoE Efficiency Example

5 levels of Gradient Time

5 levels of pH

4 levels of Column Type

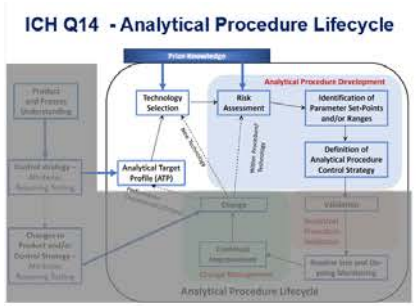
5 x 5 x 4 = 100 possible combinations

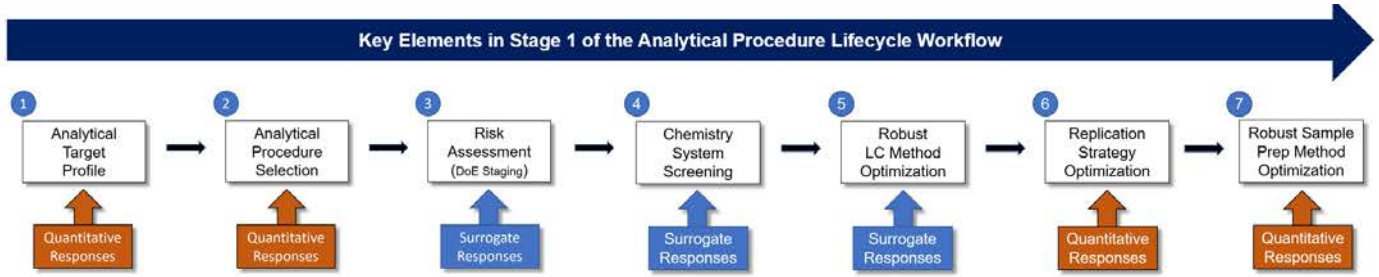
Fusion QbD design = 30 runs (plus 6 repeats)

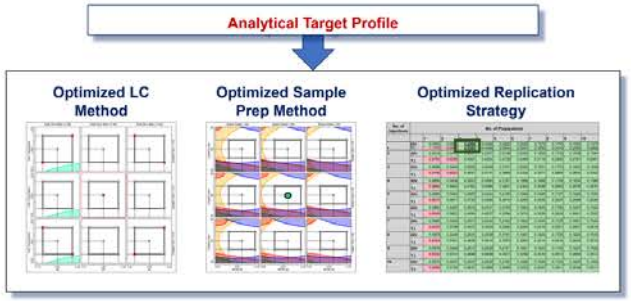
> 3x efficiency.

End of Presentation

Fusion QbD is the Only LC Method Development Software Which Completely Supports the AQbD / APLM Workflow in the Regulatory Guidances







ICH Q2(R2) / ICH Q14 / USP <1210> / USP <1220> / EP 11.60

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