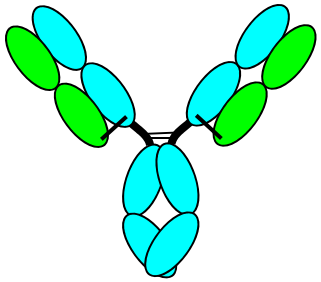
Enhanced Analytical Development for Complex Antibody Formats

Markus Wild, F. Hoffmann – La Roche Ltd., Switzerland

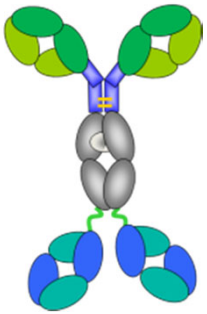
Outline

- Introduction
- Tools for Enhanced Method Development
- Case Study 1
- Case Study 2

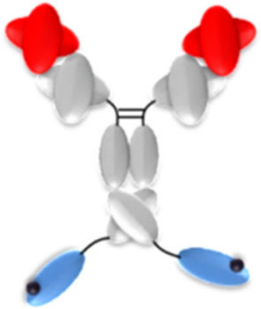
More Complex Antibody Formats

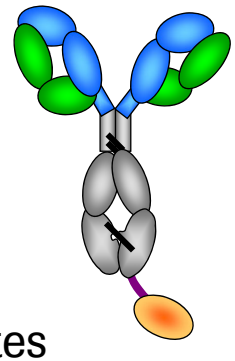


MAb

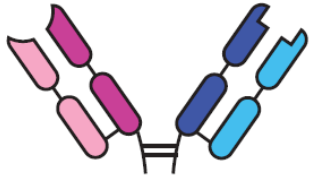


Appended BsAb

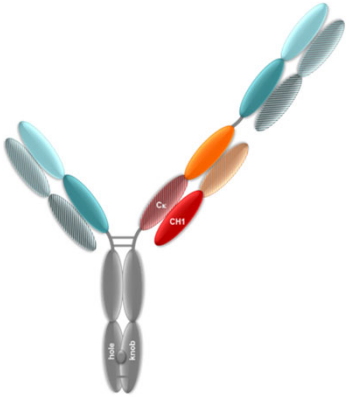


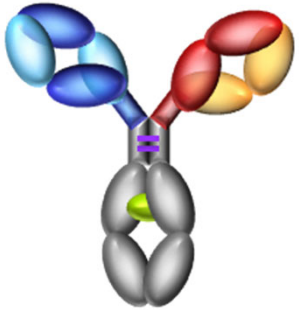


Conjugates

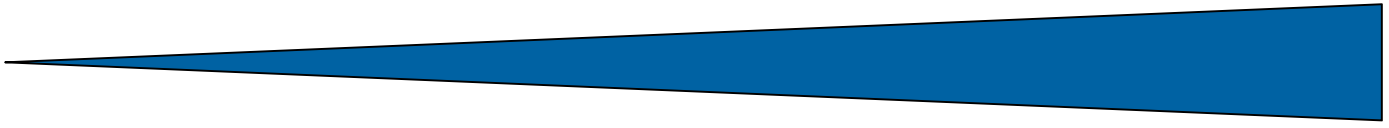


BsAb Fragments





Bispecific CrossMAb



Increase of complexity within the last 10 years

Complex Antibody Formats – Consequences

- New product related substances and impurities
- New critical quality attributes (CQAs)
- Less prior knowledge and more unknowns
- More analytical challenges have been observed with platform methods
  - Insufficient resolution
  - Carry over
  - Insufficient sample stability
  - Insufficient robustness
  - Method induced artifacts

-> Need to switch from platform methods to product specific methods

-> Increased investment in analytical development

-> Enhanced analytical development is needed in order to adress complexity and unknowns

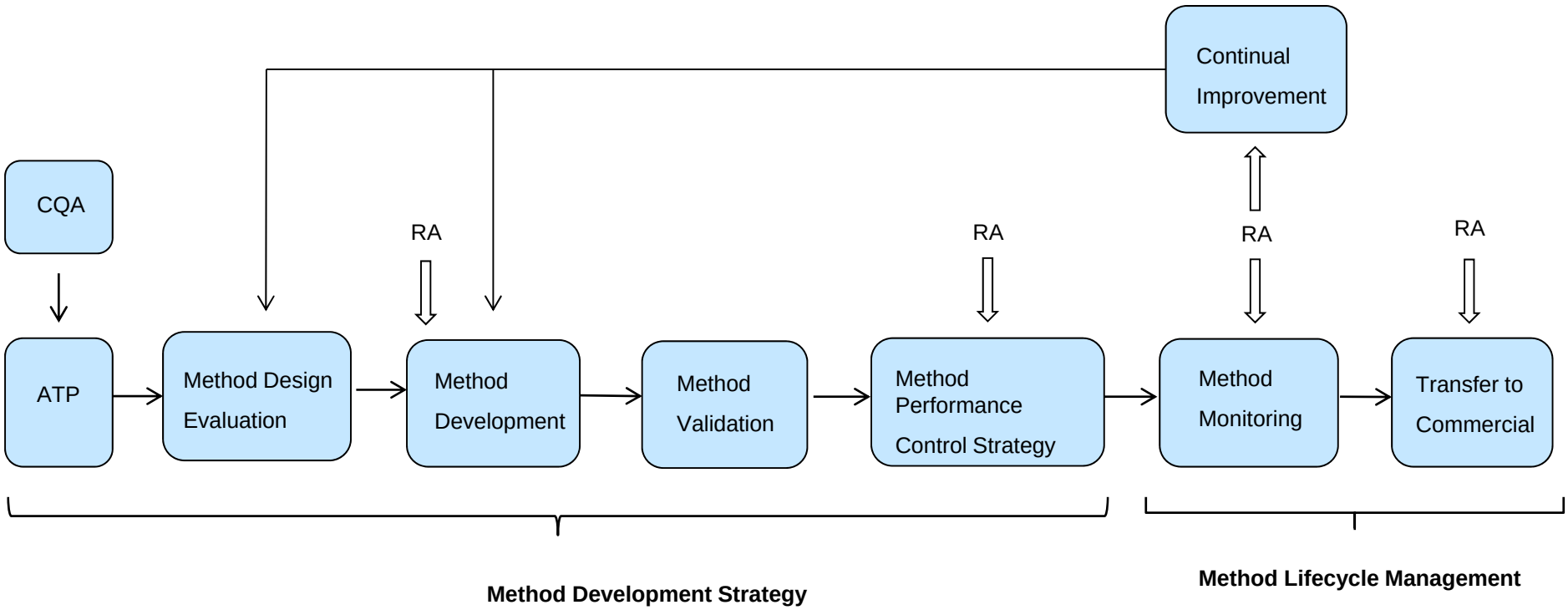
Analytical Quality by Design Principles (AQbD)

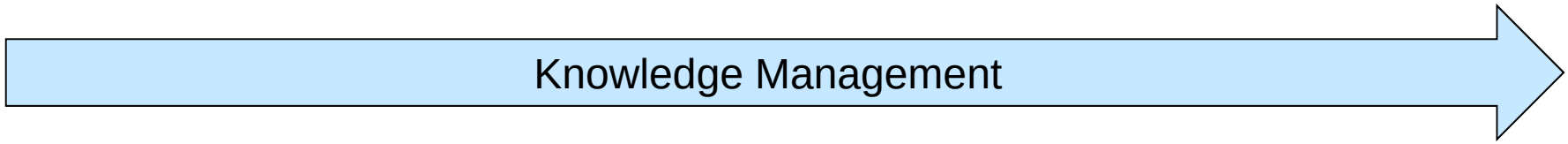
- Use of quality by design (QbD) principles according to the guidelines of international conference on harmonization ICH Q8-11
  - predefined objective
  - science and risk-based development
  - use multivariate DoE studies to define method operational limits
  - control strategy
  - lifecycle management
  - continual improvement

-> Analytical quality by design (AQbD) helps to develop robust methods which are applicable throughout the lifecycle of the method.

-> The objective is to understand, reduce and control sources of variability.

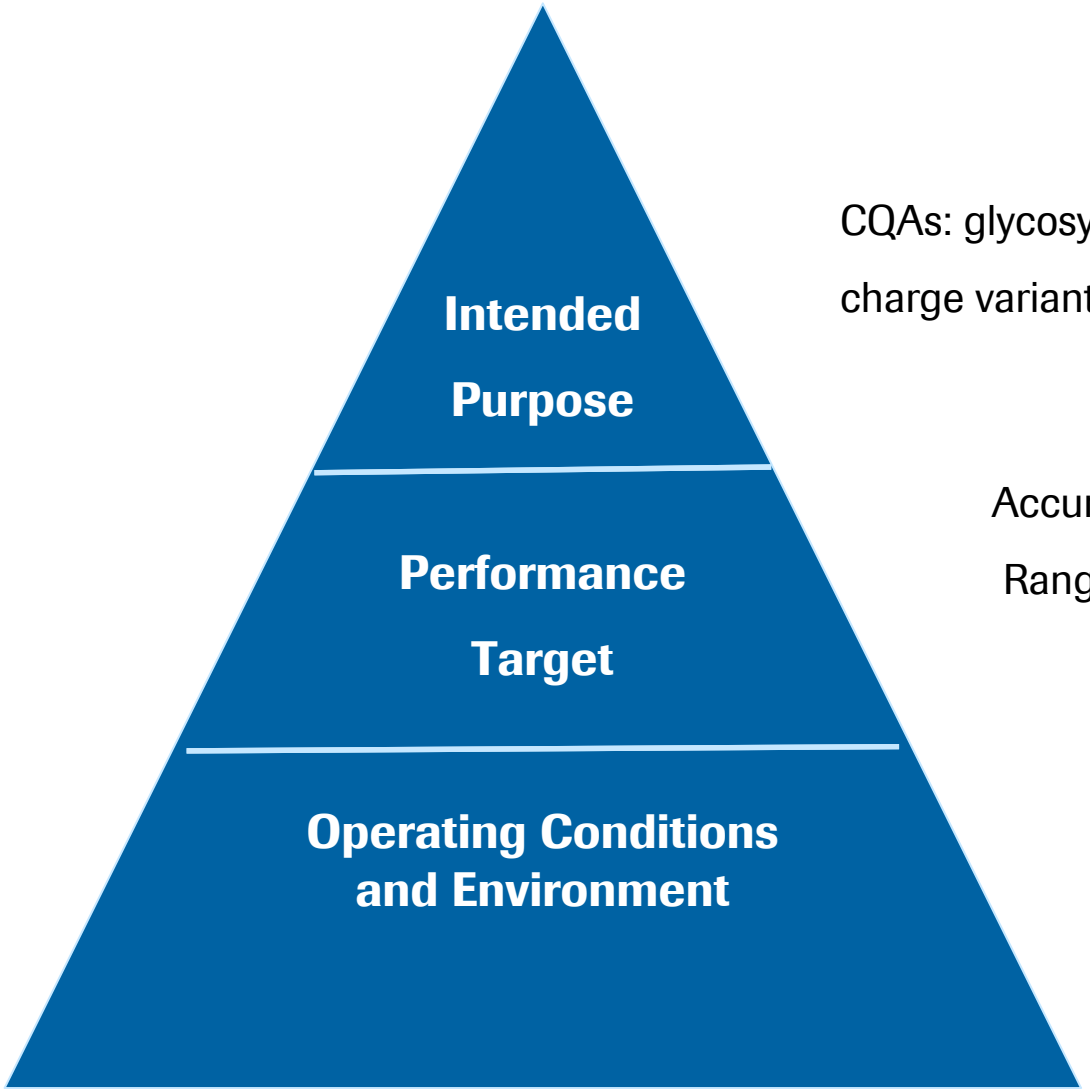
Method Lifecycle





CQA: critical quality attribute, ATP: Analytical Target Profile, RA: risk assessment

Analytical Target Profile (ATP)



CQAs: glycosylation, size variants

charge variants, oxidation, potency etc

Accuracy, Precision, Sensitivity,

Range etc / ICH Q2

Stable test procedure (≥48h)

No harmful ingredients

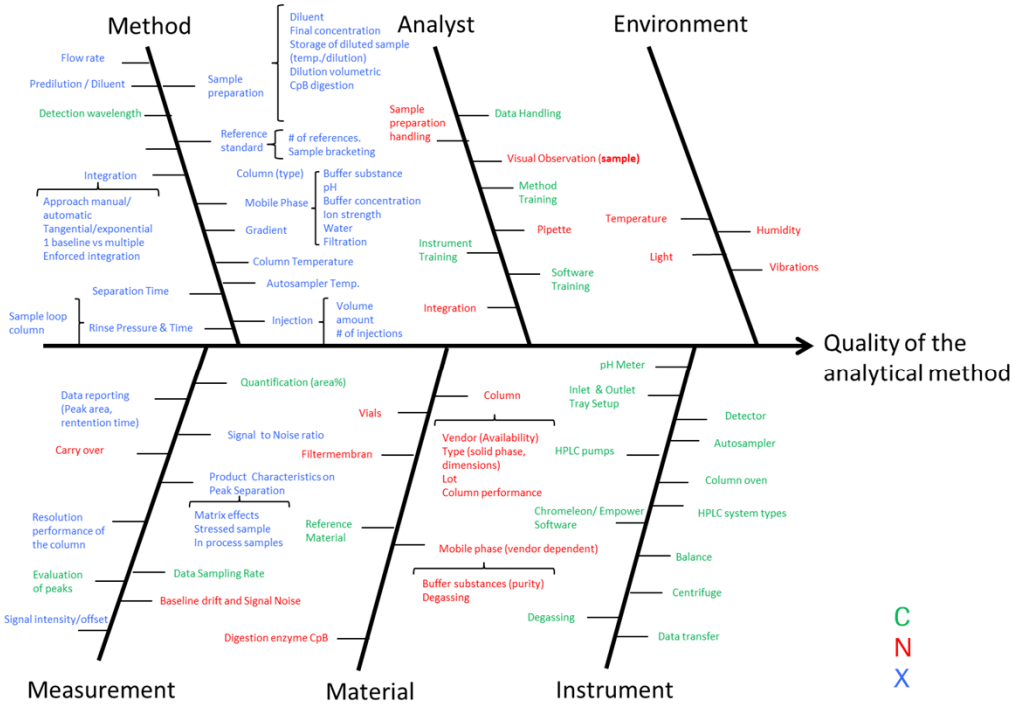
Operator safety

User requirements: low costs, speed etc

Risk Assessment

Risk assessments are performed in order to identify and assess critical method variables and parameters that can impact the ATP. These structured risk assessments are used to guide experimental plans.

Ishikawa Diagram



A tool known as CNX is used to classify the identified factors. It must be decided which factors can be controlled (C), which are potential noise factors (N) and which should be assessed experimentally (X) to determine acceptable conditions and ranges

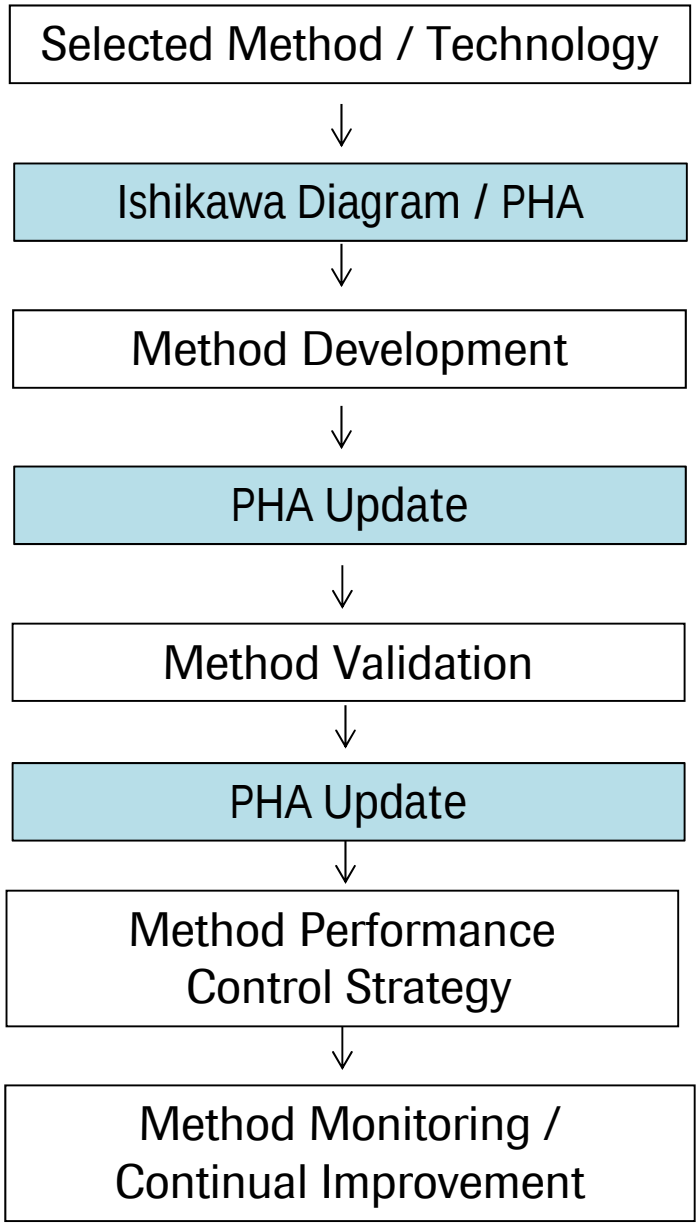
Preliminary Hazard Analysis (PHA)

			PHA1	PHA2	PHA1	PHA2
#	Category	Factor		Classif.	PRN	PRN
1	Method	flow rate	X	C	12	12
2	Method	predilution	X	X	12	12
3	Method	detection wavelength	C	C	12	12
4	Method	sample preparation: diluent	X	C	60	12
5	Method	sample preparation: final concentration	X	X	36	12
6	Method	sample preparation: storage of diluted sample - temperature	X	C	60	12
7	Method	sample preparation: storage of diluted sample - time	X	C	36	12
8	Method	sample preparation: volumentric dilution	X	X	12	12
9	Method	sample preparation: CpB digestion	X	X	12	12
10	Method	RS: # of references	X	X	12	12
11	Method	RS: sample bracketing	X	X	12	12
12	Method	integration: approach manual/ automatic	X	C	36	12
13	Method	integration: tangential/ exponential	X	C	12	12
14	Method	integration: one baseline vs. multiple enforced integration	X	C	60	36
15	Method	mobile phase: buffer substance	X	C	60	12
16	Method	mobile phase: pH	X	C	60	12
17	Method	mobile phase: buffer concentration	X	C	36	12
18	Method	mobile phase: ionic strength	X	C	60	12
19	Method	mobile phase: water	X	C	12	12
20	Method	mobile phase: filtration	X	C	36	36
21	Method	gradient	X	C	60	12
22	Method	column temperature	X	C	60	12
23	Method	autosampler temperature	X	C	12	12
24	Method	injection: volume	X	X	12	12
25	Method	injection: amount	X	C	36	36
26	Method	injection: No. of sample injections per sequence	X	X	12	12
27	Method	separation time	X	C	36	12
28	Method	column: rinse pressure & time	X	X	12	12
29	Method	sample loop: rinse pressure & time	X	X	12	12
30	Method	column (type)	X	C	60	12

A preliminary hazard analysis (PHA) is be used for the further risk assessment. Relevant factors like X-factors and critical N-factors which might have an influence on the method performance should be selected and assessed.

High risk factors should be evaluated during method development by using a Design of Experiments approach (DoE).

Risk Assessments



based on knowledge, experience and literature

Evaluation of high risk factors by uni-and multi-variate experiments (DoE)
Understand method performance and robustness

Test of high risk factors in the robustness study
Definition of acceptable range for those factors

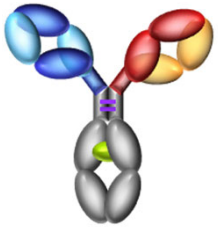
Establishment of control strategy for remaining risk factors (parameter set points and ranges, SST criteria and monitoring attributes)

Risk assessments as part of the evaluation of monitoring data

PHA: Preliminary Hazard Analysis

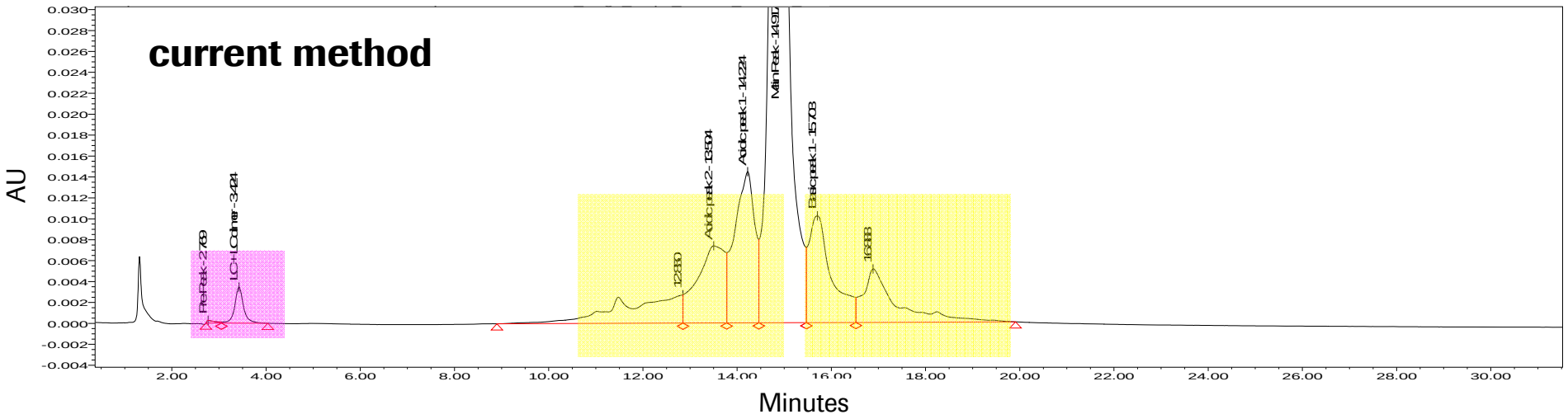
Case Study 1

- IEC method development for a BsAb



«Triggers» to develop a new IEC Method

- need for improved resolution of acidic and basic species
- need for increased method robustness regarding:
  - retention of LC/LC_2 signal
  - baseline / peak (group) delimiters
  - column lot-to-lot and system-to-system variability



Analytical Target Profile
Ion Exchange Chromatography

Topic	Examples
Method-operational Intent	Separation of Acidic Region (AR) and Basic Region (BR) from Main Peak (MP) Separation of impurity peaks among each other, detectable and quantifiable
Method Performance Criteria	• MP, AR, BR: ≤ 6.0 % RSD repeatability • MP, AR, BR: 94.0–106.0 % recovery
Range	• MP: ≥ 80%–120% of nominal protein concentration Other components: QL– 120% of upper spec limit

Operating Conditions and Environment	• Suitable for HPLC platforms used in QC network
	• Column from established vendor and globally available
	• No carry over
	• Acceptable method performance for at least
	  • two column types
	  • 3 different resin batches
	• Short sample to sample run time, ≥ 48h of consecutive analyses
	• Preferably no harmful chemicals used

Selection of Parameters for Method Development derived from Risk Assessment (PHA)

				Potential Influence on Method Performance:																	
#	Category	Factor	Classification	accuracy	carry over	column performance	column robustness	method failure rate	method performance	method robustness	peak identification	peak resolution	peak signal	quantification (range)	reproducibility	sample integrity	sample stability	sensitivity	specificity		PRN
1	Method	flow rate	X			x	x					x									12
2	Method	predilution	X												x		x				12
4	Method	sample preparation: diluent	X	x								x			x		x		x		60
5	Method	sample preparation: final concentration	X		x							x	x	x				x			36
6	Method	sample preparation: storage of diluted sample - temperature	X	x							x			x			x				60
7	Method	sample preparation: storage of diluted sample - time	X	x										x			x				36
8	Method	sample preparation: volumentric dilution	X												x						12
9	Method	sample preparation: CpB digestion	X	x								x			x						12
10	Method	RS: # of references	X					x													12
11	Method	RS: sample bracketing	X																		12
12	Method	integration: approach manual/ automatic	X	x				x			x			x	x			x			36
13	Method	integration: tangential/ exponential	X												x			x			12
14	Method	integration: one baseline vs. multiple enforced integration	X	x											x			x			60
15	Method	mobile phase: buffer substance	X						x	x		x			x				x		60
16	Method	mobile phase: pH	X						x	x		x			x				x		60
17	Method	mobile phase: buffer concentration	X						x	x		x			x				x		36
18	Method	mobile phase: ionic strength	X						x	x		x			x				x		60
19	Method	mobile phase: water	X								x										12
20	Method	mobile phase: filtration	X			x					x										36
21	Method	gradient	X									x		x					x		60
22	Method	column temperature	X									x		x					x		60
23	Method	autosampler temperature	X	x							x				x		x				12
24	Method	injection: volume	X									x	x					x			12
25	Method	injection: amount	X									x	x					x			36
26	Method	injection: No. of sample injections per sequence	X	x	x	x															12
27	Method	separation time	X		x							x									36
28	Method	column: rinse pressure & time	X		x		x														12
29	Method	sample loop: rinse pressure & time	X		x																12
30	Method	column (type)	X					x	x												60

Selection of Parameters for Method Development derived from Risk Assessment (PHA)

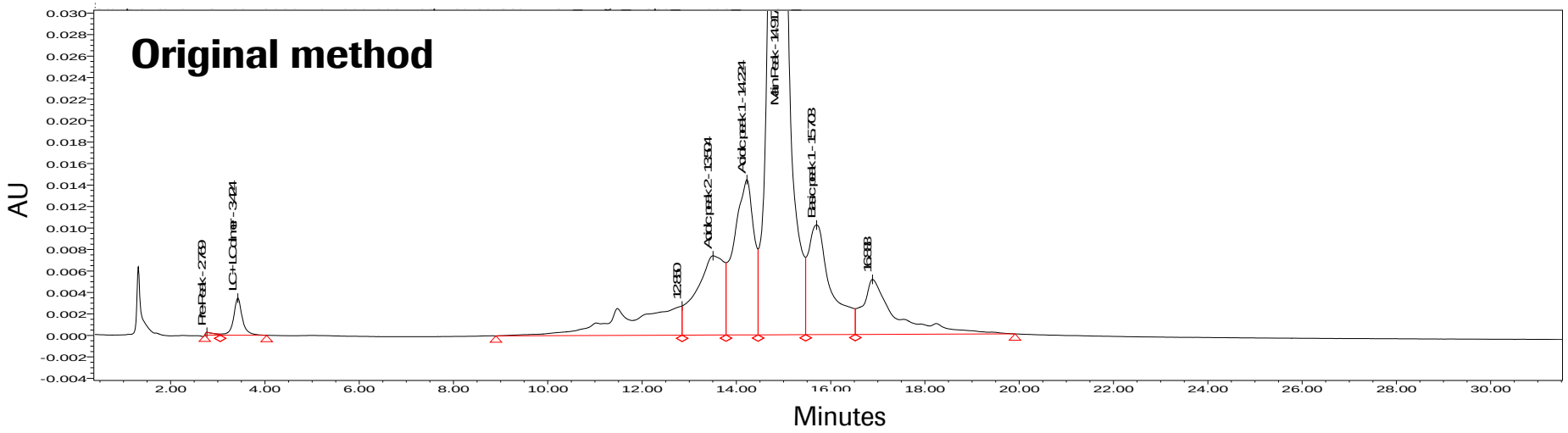
	Potential Influence on Method Performance:																
accuracy	accuracy	carry over	column performance	column robustness	method failure rate	method performance	method robustness	peak identification	peak resolution	peak signal	quantification (range)	reproducibility	sample integrity	sample stability	sensitivity	specificity	
Factor																	PRN
sample preparation: diluent	x								x			x		x		x	60
sample preparation: injection amount		x							x	x	x				x		60
sample preparation: storage of diluted sample - temperature	x							x			x			x			60
integration: one baseline vs. multiple enforced integration	x											x			x		60
mobile phase: buffer substance						x	x		x			x				x	60
mobile phase: pH						x	x		x			x				x	60
mobile phase: ionic strength						x	x		x			x				x	60
gradient									x		x					x	60
column temperature									x		x					x	60
column (type)					x	x											60

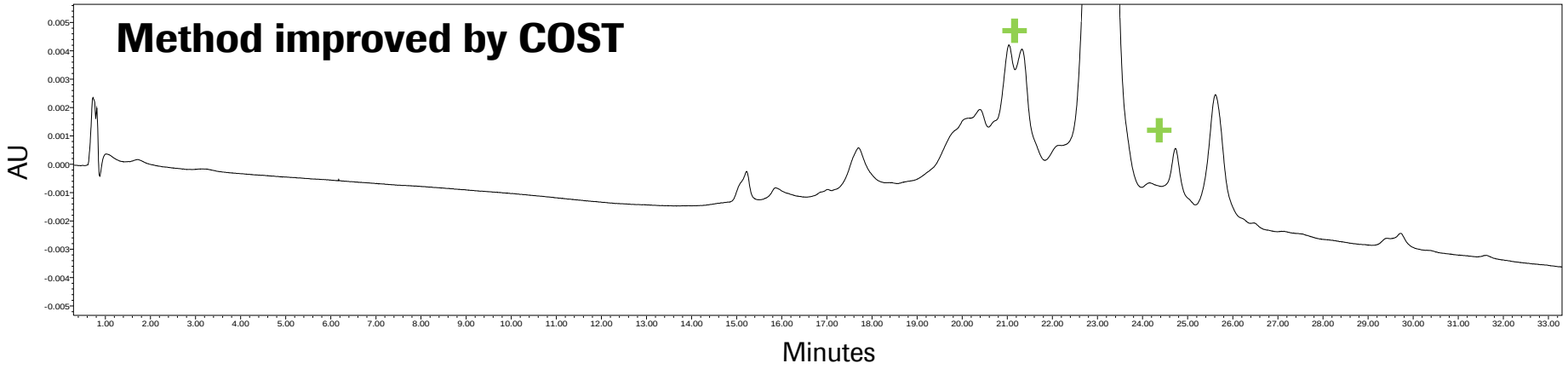
Method Development Approach

Factor	Screening with COST →	Optimization with DoE
stationary phase	• 10 different columns (weak/strong cation exchange, source, vendor, length, particle size etc.) • temperature	• Set to strong cation exchange (YMC, BioPro SP-F) • **temperature** range: 28 - 45°C
mobile phase	• buffer component (4 different buffer systems) • pH range • flow rate 0.7 - 1.1 • salt content (up to 750 mM)	• buffer component: BES • Buffer concentration: 15 – 25 mM • **pH** range: 6.6 – 7.2 • flow rate: 0.7 – 0.9 • salt content: 488 mM NaCl
gradient	• salt gradient • gradient slope	• **shape** • endpoint of slope 12 – 18%
injection		• **injection amount**: 20 – 210 µg

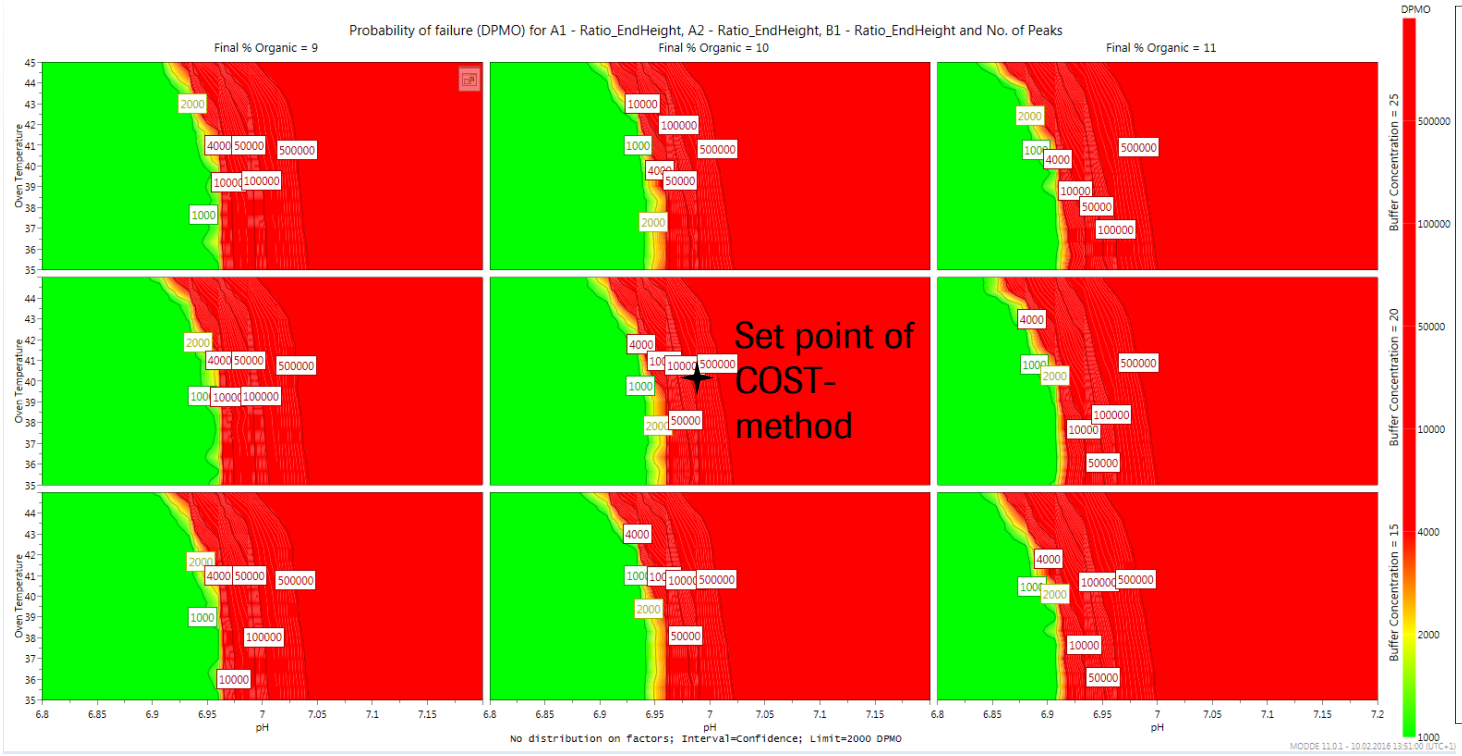
COST = **C**hange **O**ne **S**ingle Factor a **T**ime **high impact factors from PHA1**

Comparison Chromatograms: Original Method vs. Method improved by COST





Method optimization: COST or DoE-based?
Design Space Plot



DPMO plotted dependent on:
bottom: pH
left: temperature

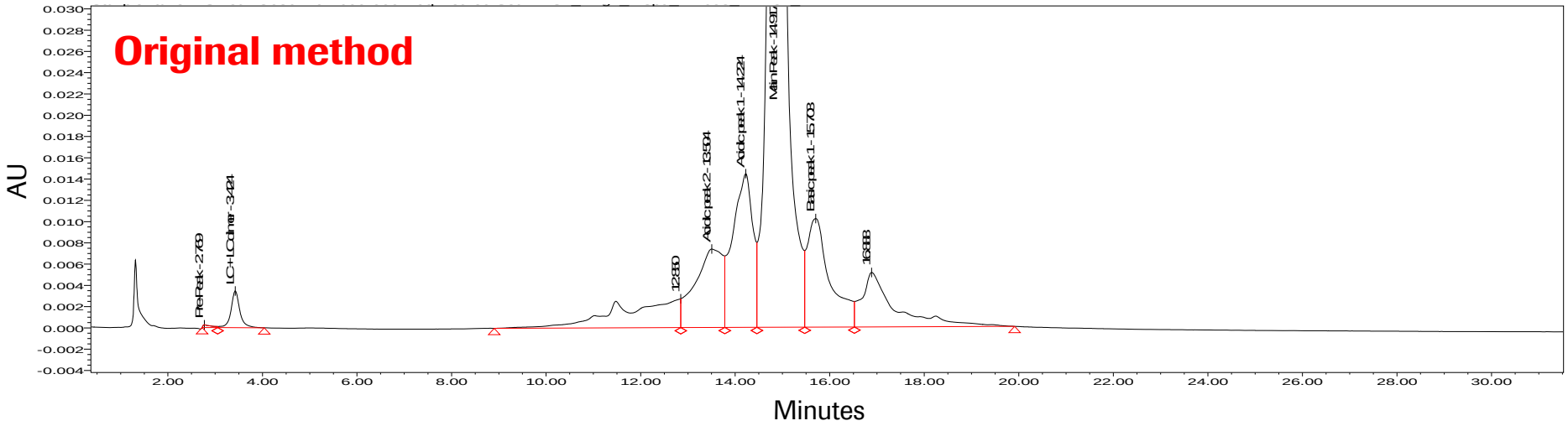
top: final %B
right: buffer conc.

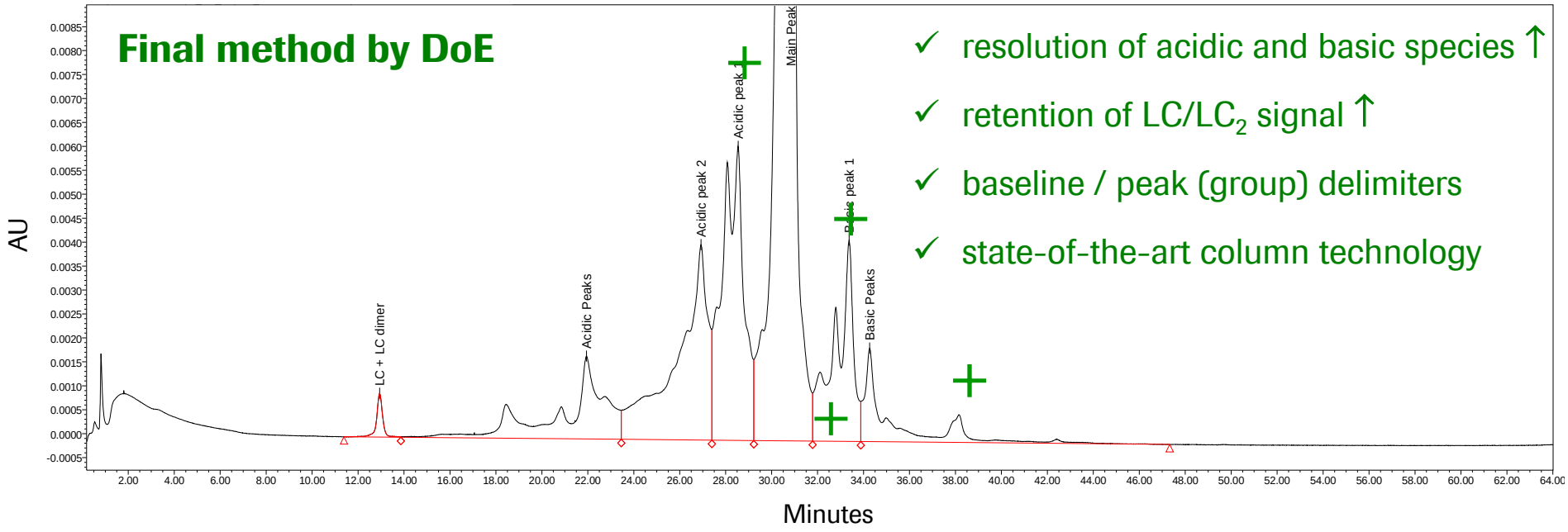
axis per box.

Flow rate, injection amount, column lot **fix at center point conditions.**

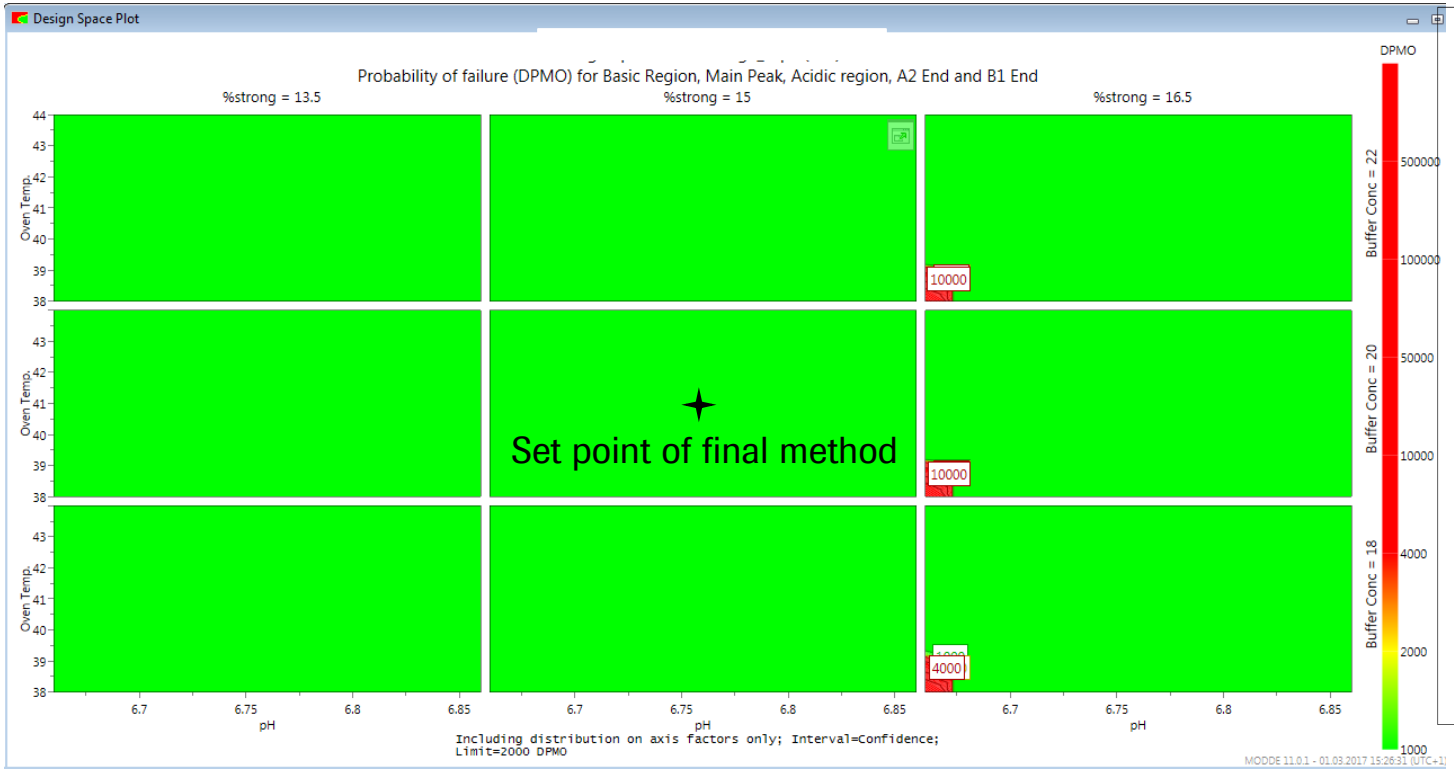
- Opportunity for further improvement elucidated by DoE modelling
- As result much better resolution could be obtained

Comparison Chromatograms: Original Method vs. Final Method improved by DOE





Final Method: *Design Space Plot*



DPMO plotted dependent on:
bottom: pH
left: temperature

top: final %B
right: buffer conc.

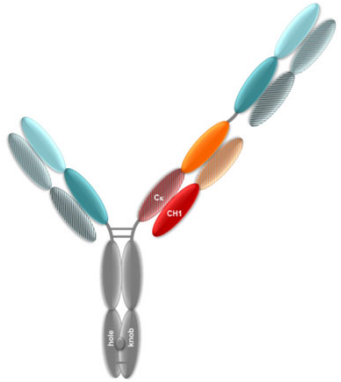
axis per box.

Flow rate, injection amount, column lot **fix at center point conditions.**

- Very low probability of failure
- Highly robust method developed by using DoE

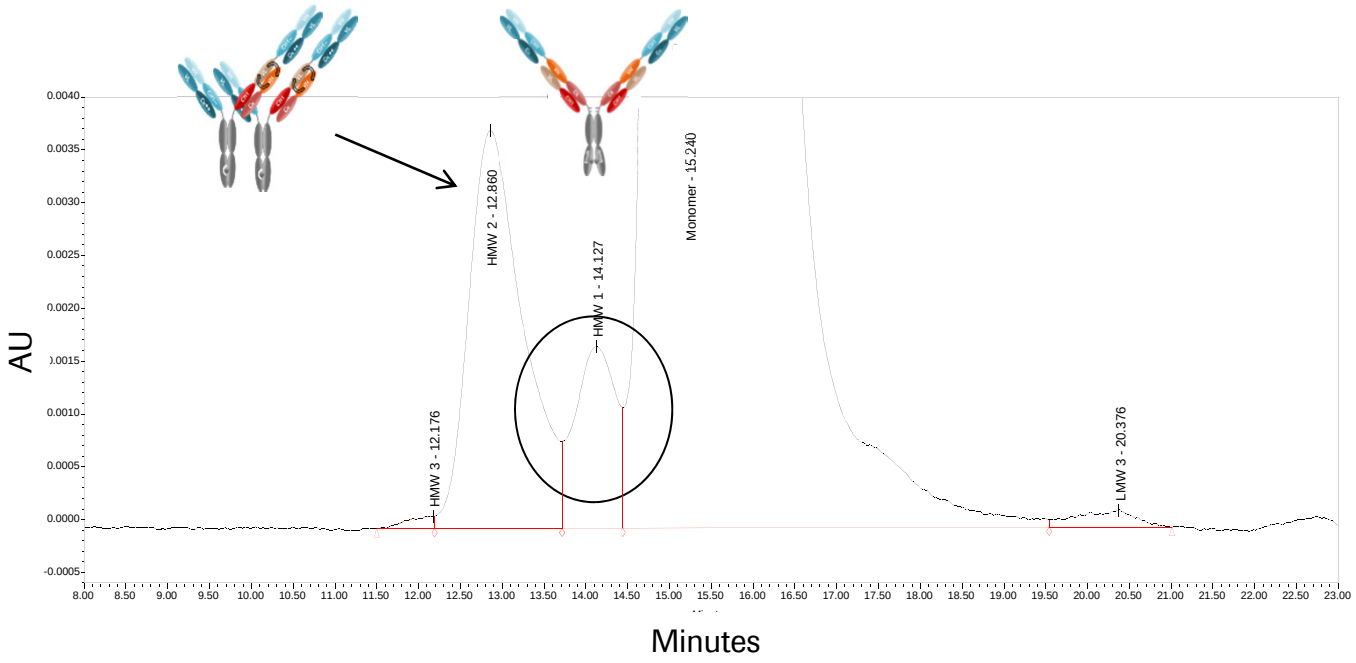
Case Study 2

- SEC Method development for a BsAb



«Triggers» to develop a new SEC Method

Starting point: platform SEC method



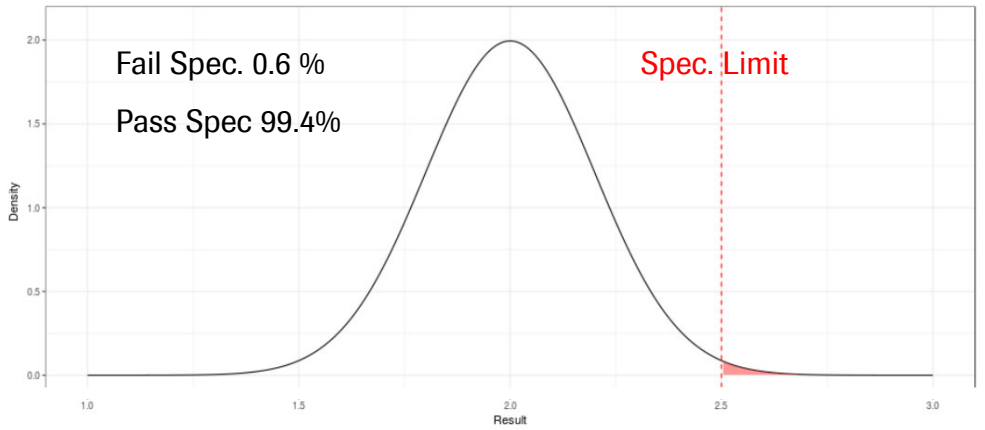
– need for improved resolution of potential critical species (HMW 1, HMW 2)

Analytical Target Profile
Size Exclusion Chromatography

Topic	Examples
Method-operational Intent	Separation of critical size variants i.e. High Molecular Weight species including HMW 1 from Main Peak detectable and quantifiable
Critical Quality Attribute (CQA)	• HMW species (e.g. HMW 1, HMW 2)
Method Performance Criteria	• Main Peak: ≤ 6.0 % RSD repeatability • **HMWs: ≤ 0.2 SD** repeatability • Main Peak: 94.0–106.0 % recovery • **HMWs: assumed true value ± 0.2 (area%)**
Range	• Main Peak: at least 80%–120% of nominal protein concentration HMWs: QL- 3.0 %

Limitations:

Target values and process variability usually unknown at time of method development!



Probability of iOOS for Normally Distributed Data

Selection of Parameters for Method Development derived from Risk Assessment (PHA)

				Potential Influence on Method Performance:																	
#	Category	Factor	Classification	accuracy	carry over	column performance	column robustness	method failure rate	method performance	method robustness	peak identification	peak resolution	peak signal	quantification (range)	reproducibility	sample integrity	sample stability	sensitivity	specificity		PRN
1	Method	flow rate	X			x	x					x									60
2	Method	predilution	X	x											x		x				60
4	Method	sample preparation: diluent	X	x								x			x		x		x		36
5	Method	sample preparation: final concentration	X		x							x	x	x				x			36
6	Method	sample preparation: storage of diluted sample - temperature	X	x							x			x			x				36
7	Method	sample preparation: storage of diluted sample - time	X	x										x			x				36
8	Method	sample preparation: volumentric dilution	X												x						12
9	Method	RS: # of references	X					x													12
10	Method	RS: sample bracketing	X						x												12
11	Method	integration: approach manual/ automatic	X	x				x			x			x	x			x			36
12	Method	integration: tangential/ exponential	X												x			x			12
13	Method	integration: one baseline vs. multiple enforced integration	X	x											x			x			12
14	Method	mobile phase: buffer substance	X						x	x		x			x				x		36
15	Method	mobile phase: pH	X						x	x		x			x				x		36
16	Method	mobile phase: buffer concentration	X						x	x		x			x				x		36
17	Method	mobile phase: ionic strength	X						x	x		x			x				x		60
18	Method	mobile phase: water	X								x										12
19	Method	mobile phase: filtration	X			x					x										36
20	Method	column temperature	X									x		x					x		60
21	Method	autosampler temperature	X	x							x				x		x				12
22	Method	injection: volume	X									x	x					x			36
23	Method	injection: amount	X									x	x					x			36
24	Method	injection: No. of sample injections per sequence	X	x	x	x															36
25	Method	separation time	X									x									36
26	Method	column: rinse pressure & time	X		x		x														12
27	Method	sample loop: rinse pressure & time	X		x																12
28	Method	column (type)	X					x	x												60

Selection of Parameters for Method Development derived from Risk Assessment (PHA)

	Potential Influence on Method Performance:																
	accuracy	carry over	column performance	column robustness	method failure rate	method performance	method robustness	peak identification	peak resolution	peak signal	quantification (range)	reproducibility	sample integrity	sample stability	sensitivity	specificity	
Factor																	**PRN**
flow rate			x	x					x								60
predilution	x											x		x			60
mobile phase: ionic strength						x	x		x			x				x	60
column temperature									x		x					x	60
column (type)					x	x											60

Method Development Approach

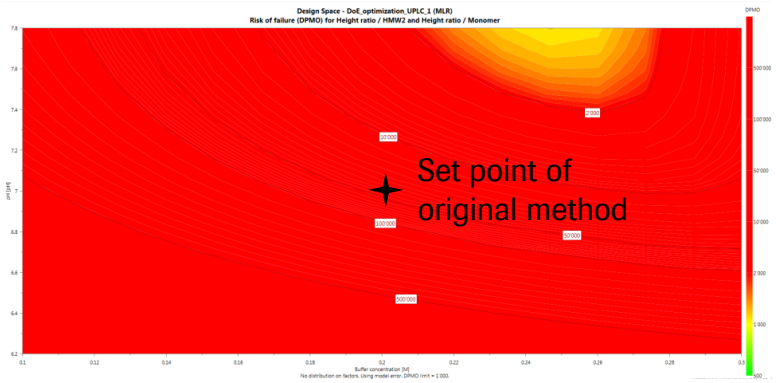
Factor	Screening with COST →	Optimization with DoE
Column	YMC Pack Diol 200 YMC Pack Diol 300 TSKgel Super SW mAb HR TSKgel Super SW mAb HTP TSKgel Ultra SW mAb Aggregate UPLC BEH200	UPLC BEH200
Column temperature		25°C – 45°C
Buffer composition	Sodium phosphate	
Buffer concentration	0.05 – 0.3 M	0.1 – 0.3 M
Mobile phase pH	pH range	6.2 – 7.8
Protein load	10 -150 µg	
Injection volume	2 – 30 µL	
Flow rate	0.1 – 0.5 mL/min	

Method optimization – *Design Space Plot*

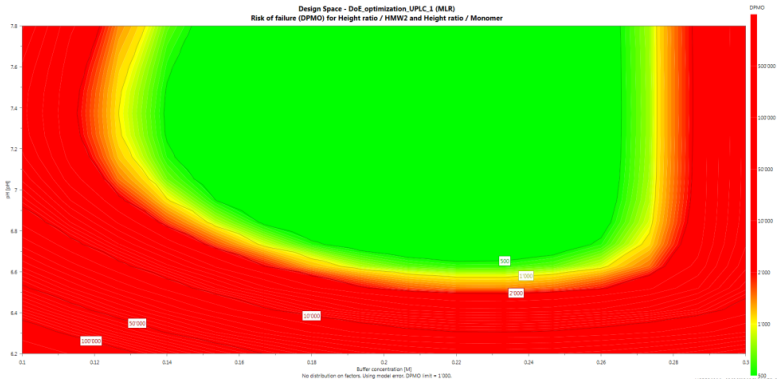
Response	Max
Height Ratio/ HMW2	0.4
Height Ratio/ Monomer	0.3

Factors at the plot axes:

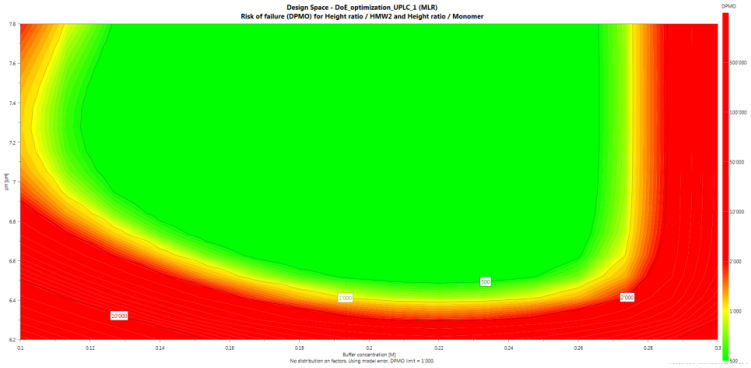
		Low:	High:
1st axis:	Buffer concentration	0.1	0.3
2nd axis:	pH	6.2	7.8



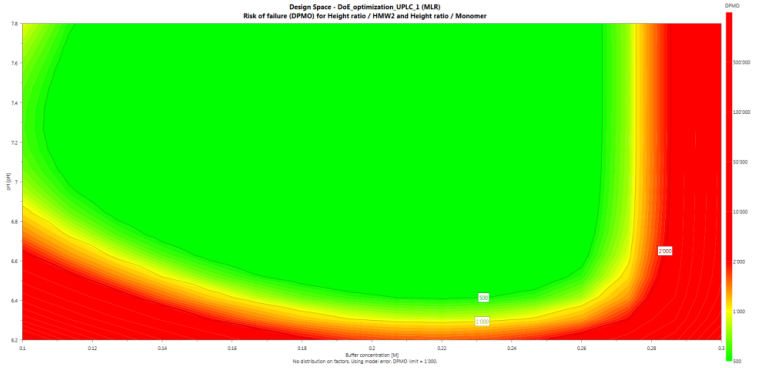
Column temperature = 25°C



Column temperature = 35°C



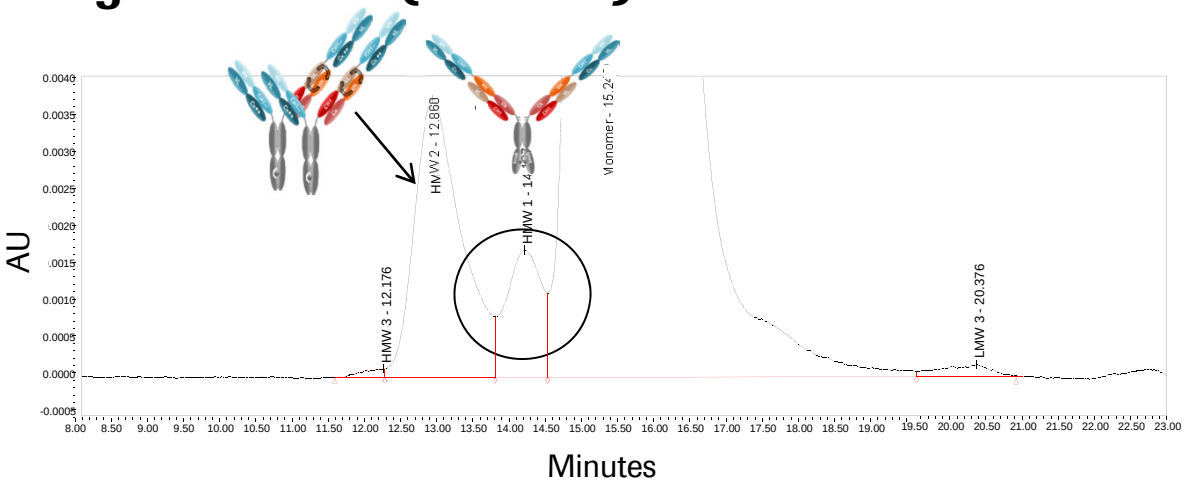
Column temperature = 40°C



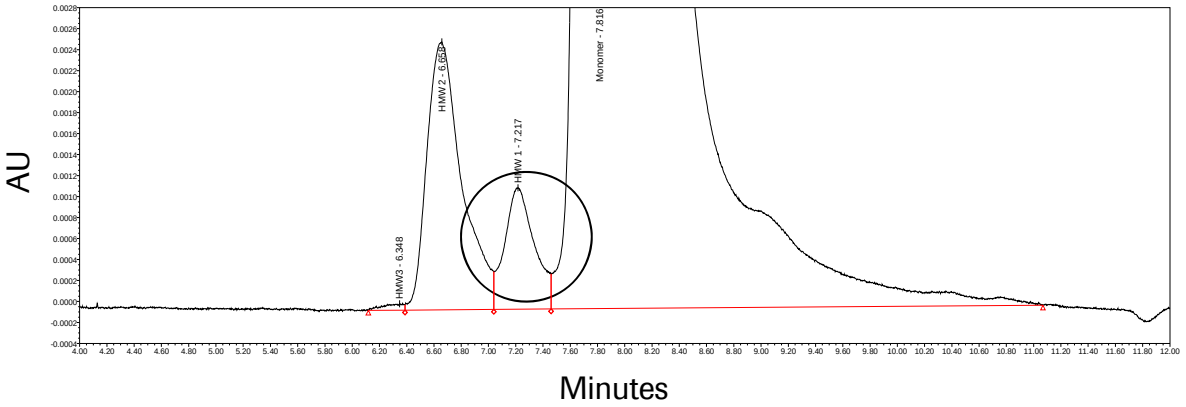
Column temperature = 45°C

Comparison Chromatograms: Original Method vs. Final Method improved by DoE

Original method (*SE-HPLC*)



Final method (*SE-UPLC*)



<u>Modified factors</u>

1. Column material & dimensions
2. Column temperature
3. Buffer concentration
4. Salt concentration
5. Flow rate
6. pH

Benefits of Enhanced Method Development

- AQbD tools provide a science- and risk-based framework for developing enhanced understanding of analytical methods
- Structured risk assessments are used to guide experimental plans
- Enhanced method robustness and ruggedness through the lifecycle
- Less analytical method related Out-of-Specification and failure investigations
- More robust method knowledge transfer due to enhanced analytical method understanding

Acknowledgements

- Alexandra Henriou
- Corinne Litzler
- Marion Krafft
- Steffen Kiessig
- Oxana Pester
- Katia Kolb
- Sébastien Violini
- Georg Hafenmair
- Volker Schaible
- Cecile Avenal
- Michael Grote
- Hans Beiderbeck
- Mahdi Adibzadeh

Doing now what patients need next