Regulations for Analytical development

USFDA/ICH/USP perspectives

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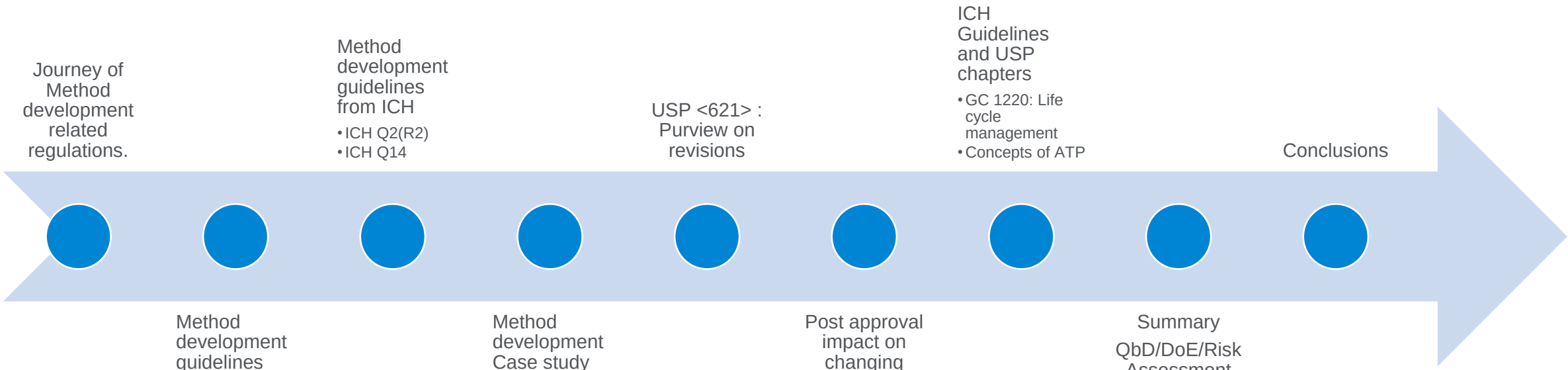
Agilent

Outline


Journey of Method development related regulations.
Method development guidelines from USFDA
• DoE based studies and Life cycle Management
• Handling Compendial methods
Method development guidelines from ICH
• ICH Q2(R2)
• ICH Q14
Method development Case study
• MODR and its various phases
USP <621> : Purview on revisions
Post approval impact on changing methods
ICH Guidelines and USP chapters
• GC 1220: Life cycle management
• Concepts of ATP
Summary
QbD/DoE/Risk Assessment
Conclusions

Regulations on Analytical Procedures by USFDA
An Overview

Analytical Procedures and Methods Validation for Drugs and Biologics

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

July 2015
Pharmaceutical Quality/CMC

III. ANALYTICAL METHODS DEVELOPMENT

An analytical procedure is developed to test a defined characteristic of the drug substance or drug product against established acceptance criteria for that characteristic. Early in the development of a new analytical procedure, the choice of analytical instrumentation and methodology should be selected based on the intended purpose and scope of the analytical method. Parameters that may be evaluated during method development are specificity, linearity, limits of detection (LOD) and limits of quantitation (LOQ), range, accuracy, and precision.

During early stages of method development, the robustness of methods should be evaluated because this characteristic can help you decide which method you will submit for approval. Analytical procedures in the early stages of development are initially developed based on a combination of mechanistic understanding of the basic methodology and prior experience. Experimental data from early procedures can be used to guide further development. You should submit development data within the method validation section if they support the validation of the method.

To fully understand the effect of changes in method parameters on an analytical procedure, you should adopt a systematic approach for a method robustness study (e.g., a design of experiments with method parameters). You should begin with an initial risk assessment and follow with multivariate experiments. Such approaches allow you to understand factorial parameter effects on method performance. Evaluation of a method's performance may include analyses of samples obtained from various stages of the manufacturing process from in-process to the finished product. Knowledge gained during these studies on the sources of method variation can help you assess the method performance.

VIII. LIFE CYCLE MANAGEMENT OF ANALYTICAL PROCEDURES

Once an analytical procedure (including compendial methods) is successfully validated (or verified) and implemented, the procedure should be followed during the life cycle of the product to continually assure that it remains fit for its intended purpose. Trend analysis on method performance should be performed at regular intervals to evaluate the need to optimize the analytical procedure or to revalidate all or a part of the analytical procedure. If an analytical procedure can only meet the established system suitability requirements with repeated adjustments to the operating conditions stated in the analytical procedure, the analytical procedure should be reevaluated, revalidated, or amended, as appropriate.

Over the life cycle of a product, new information and risk assessments (e.g., a better understanding of product CQAs or awareness of a new impurity) may warrant the development and validation of a new or alternative analytical method. New technologies may allow for greater understanding and/or confidence when ensuring product quality. Applicants should periodically evaluate the appropriateness of a product's analytical methods and consider new or alternative methods.

In anticipation of life cycle changes in analytics, an appropriate number of retention samples should be maintained to allow for comparative studies. The number should be based on scientific principles and an assessment of risk. For complex products that are sensitive to manufacturing changes, reserve samples can be an important tool to make these comparisons.

Regulations on Analytical Procedures USFDA
Robustness Studies and Life Cycle Management

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- To adopt a systematic approach to development its imperative to adopt a comprehensive risk assessment or screening protocol
- Move from Univariate to multivariate

- Understand factorial parameters
- Include samples from Various stages of manufacturing
- Record the sources of variations in the results

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- Constant adjustments to method to achieve system suitability calls for re-evaluation
- Periodic evaluation of trends and methods is imp.

- Life cycle management governs modernization
- Alternative methods can be developed and used

Regulations on Analytical Procedures USFDA
Managing Compendial Methods

C. Compendial Analytical Procedures

The suitability of an analytical procedure (e.g., USP/NF, the Official Methods of Analysis of AOAC International, or other recognized standard references) should be verified under actual conditions of use.[17] Information to demonstrate that USP/NF analytical procedures are suitable for the drug product or drug substance should be included in the submission and generated under a verification protocol.

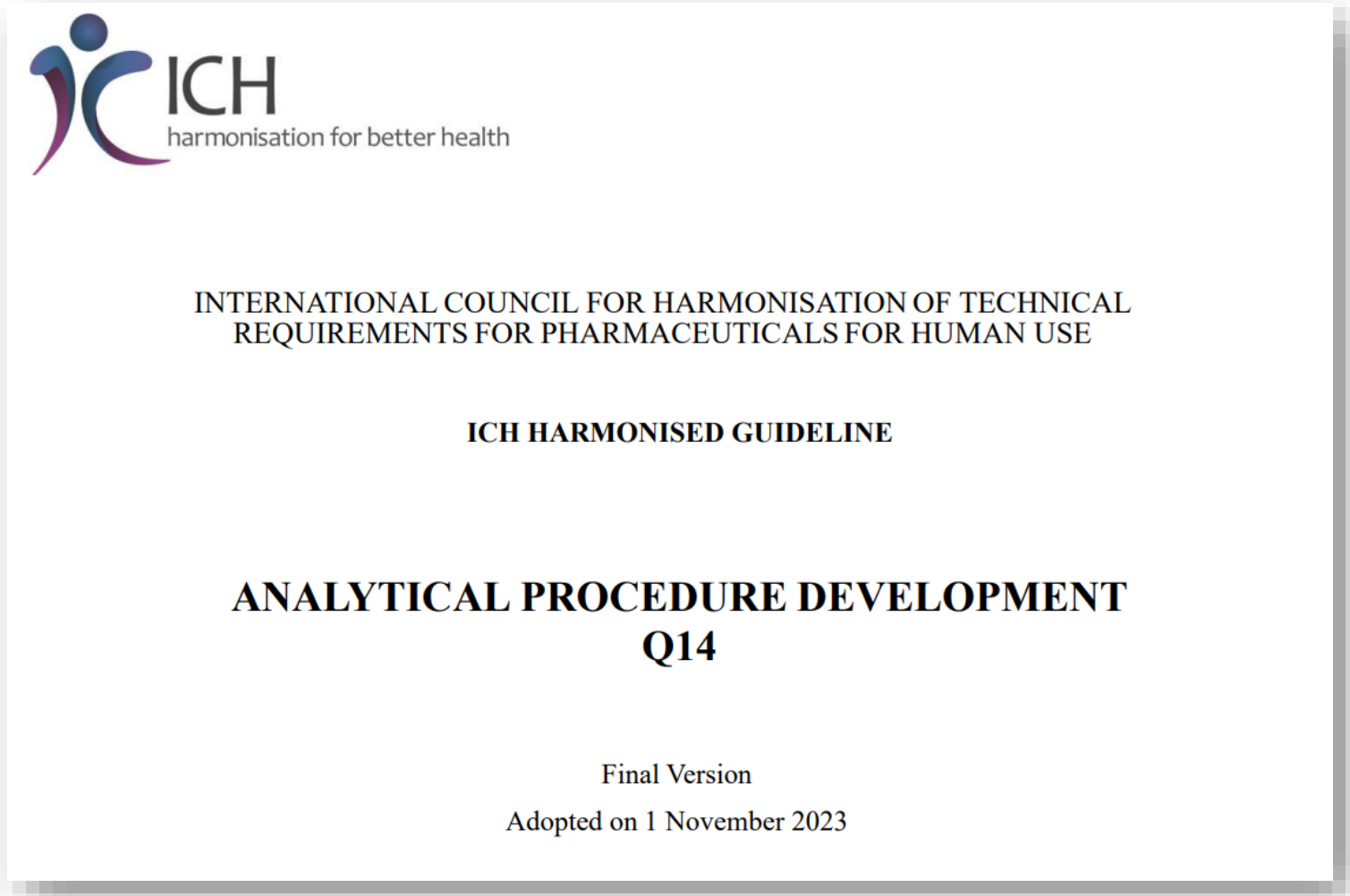
The verification protocol should include, but is not limited to: (1) compendial methodology to be verified with predetermined acceptance criteria, and (2) details of the methodology (e.g., suitability of reagent(s), equipment, component(s), chromatographic conditions, column, detector type(s), sensitivity of detector signal response, system suitability, sample preparation and stability). The procedure and extent of verification should dictate which validation characteristic tests should be included in the protocol (e.g., specificity, LOD, LOQ, precision, accuracy). Considerations that may influence what characteristic tests should be in the protocol may depend on situations such as whether specification limits are set tighter than compendial acceptance criteria, or RT or RRT profiles are changing in chromatographic methods because of the synthetic route of drug substance or differences in manufacturing process or matrix of drug product. Robustness studies of compendial assays do not need to be included, if methods are followed without deviations.

Require a verification protocol and extent of verification clarified

Verification under actual conditions of use.

No Robustness Studies required*

ICH Guideline Q14



ICH Q14 Guidelines

Guideline describes science and risk-based approaches for developing and maintaining *analytical procedures* suitable for the evaluation of the quality of drug substances and drug products.

When developing an analytical procedure, a minimal (also known as traditional) approach or elements of an enhanced approach can be applied.

Furthermore, the guideline describes additional considerations for the development of *multivariate analytical procedures* and for *real time release testing (RTRT)*. This guideline complements ICH Q2

The guideline also describes submission of analytical procedure development and related lifecycle information in the Common Technical Document (CTD) format (*ICH M4Q*). Information related to analytical procedure development and knowledge may be submitted to regulatory authorities to provide additional evidence that the analytical procedure is fit for the intended purpose.

Analytical Method Development in ICH Q14
Minimal Approach

Goal of Development: To obtain an analytical procedure fit for the intended purpose: to measure an attribute or attributes of the material with the needed *specificity/selectivity, accuracy, precision* over the *reportable range*.

Data gained during the development studies (*e.g.*, *robustness* data from a design of experiments (DoE) study) could be used as part of the validation data for the related analytical procedure performance characteristics and studies do not necessarily need to be repeated.

Minimal Approach:

Analytical procedure development should include the following elements as a minimum:

- Identifying the attributes of the product which need to be tested;
- Selecting an appropriate technology and related instruments or suitable apparatus;
- Conducting studies to evaluate analytical procedure performance characteristics such as specificity, accuracy and precision over the reportable range (including the *calibration model*, lower and/or higher range limits) and robustness;
- Documenting the analytical procedure including the analytical procedure control strategy.

Analytical Method Development in ICH Q14
Enhanced Approach

The approach offers a systematic way of developing and refining knowledge of an analytical procedure and demonstrating procedure understanding.

Product and process understanding informs the quality attributes to be tested. The anticipated *performance criteria* for relevant performance characteristics should be documented in an *analytical target profile* (ATP). In addition to the elements of the minimal approach, an enhanced approach may include the following elements as appropriate:

- Conducting risk assessment and evaluating prior knowledge to identify the *analytical procedure parameters* that can impact performance of the procedure;
- Conducting uni- or multi-variate experiments and/or modelling to explore ranges and interactions between identified analytical procedure parameters;
- Defining an analytical procedure control strategy including set-points and/or ranges for relevant analytical procedure parameters. These could include *proven acceptable ranges for analytical procedures* (PARs) and/or *method operable design regions* (MODRs).

Benefits of Enhanced Approach

- Better understanding of the impact of analytical procedure parameters on the analytical procedure performance
- More flexibility for lifecycle management, such as wider operating ranges and a more appropriate set of *established conditions* (ECs) with associated reporting categories.

Risk Assessment as per ICH Q14

Relevance of the test

- Potential clinical impact of the measured attribute (efficacy, safety, pharmacokinetics and immunogenicity), e.g., controlling CQA vs. non CQA
- Extent of knowledge of the attribute
- Attribute ensured by other elements of the control strategy (testing or process control)

Complexity of the technology

- Platform technologies
- Novel vs. established technology (e.g., in pharmacopoeias)
- Several attributes reported as a sum (e.g., charge variants for large molecules)
- Biological assays, cell-based assays, immunochemical assays
- Multi-attribute analytical procedure
- Multivariate analytical procedure

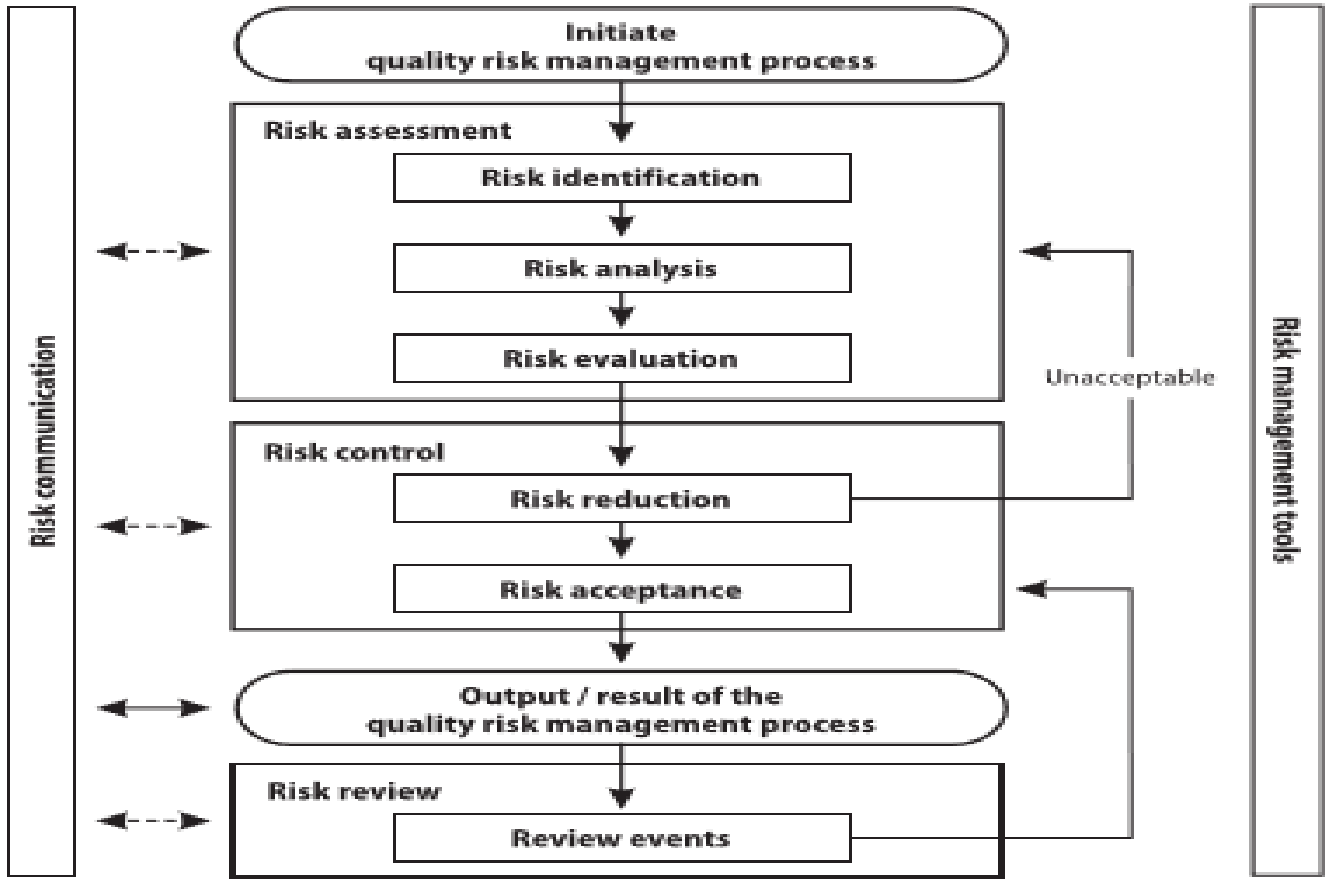
Extent of the change

- Change of one or several parameters outside the already proven acceptable ranges
- Change of the analytical procedure within existing analytical procedure performance characteristics and associated criteria
- Change to a new analytical procedure using a different technology
- Change to analytical procedure performance criteria (e.g., due to tightening a specification limit)

Guidelines on QRM: ICH Q9

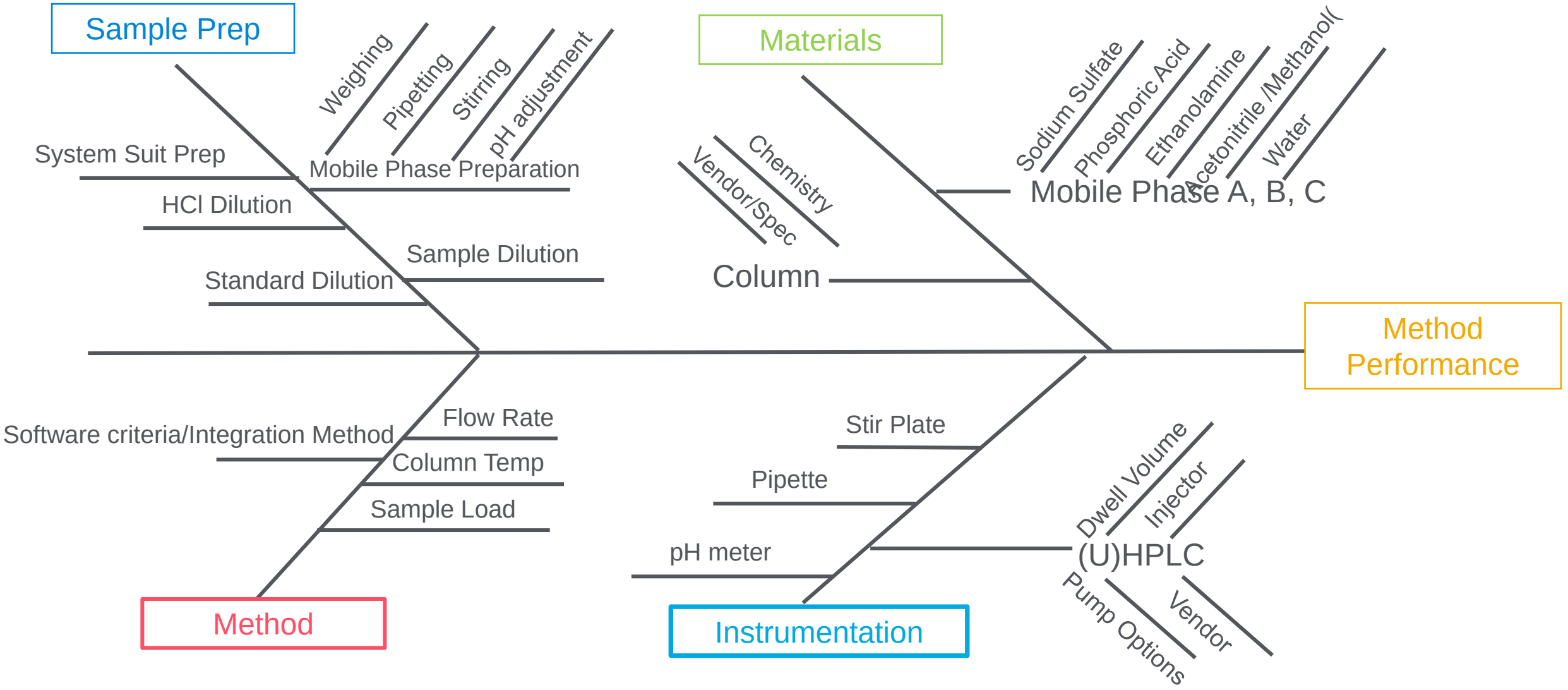
Quality Risk Management

Figure 1
Overview of a typical quality risk management process

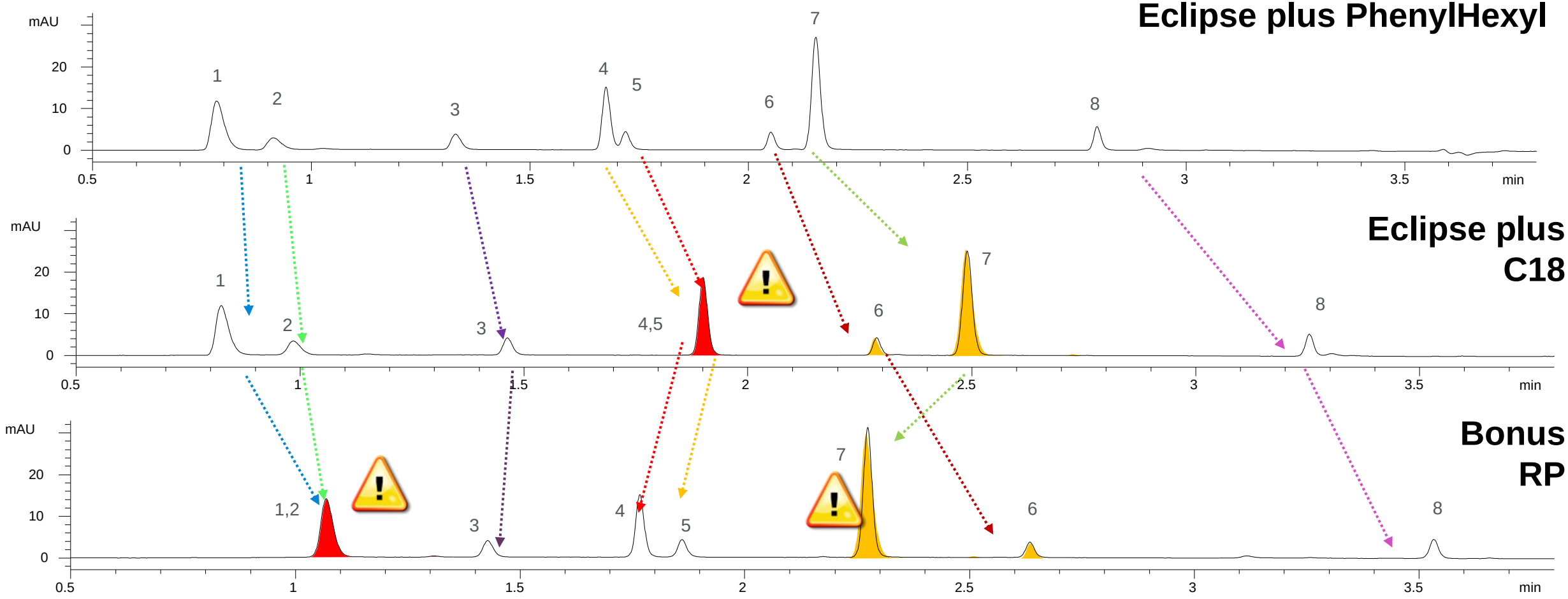


Reproduced from reference *5*: ICH Q9: Quality Risk Management.

Developing a DoE for Analytical Development



Risk Assessment
Evaluating Multiple Column Chemistries



Chemistries with Unique Selectivity: Zorbax Columns

Reliable totally porous columns for highest sample capacity and resistance to sample solvents

Best all around	Best for low pH mobile phases	Best for high pH mobile phases	Best for alternative selectivity	Best for more polar Analytes
Eclipse Plus C18 / C8 1.8 / 3.5 / 5 µm	**SB-C18** 1.8 / 3.5 / 5 / 7 µm	**Extend-C18** 1.8 / 3.5 / 5 µm	**Bonus-RP** 1.8 / 3.5 / 5 / 7 µm	**SB-Aq** 1.8 / 3.5 / 5 / 7 µm
Eclipse XDB C18 / C8 1.8 / 3.5 / 5 / 7 µm	**SB-C8** 1.8 / 3.5 / 5 / 7 µm		**SB-Phenyl** 1.8 / 3.5 / 5 / 7 µm	**SB-CN** 1.8 / 3.5 / 5 / 7 µm
Eclipse Plus Phenyl-Hexyl 1.8 / 3.5 / 5 µm	**SB-C3** 1.8 / 3.5 / 5 / 7 µm		**Eclipse PAH** 1.8 / 3.5 / 5 µm	**HILIC Plus** 1.8 / 3.5 µm
Eclipse XDB Phenyl 1.8 / 3.5 / 5 / 7 µm				**Eclipse XDB CN** 1.8 / 3.5 / 5 / 7 µm
Rx-C18 3.5 / 5 / 7 µm	**Rx-C8** 3.5 / 5 µm			**Rx-Sil/ NH2** 5 / 7 µm

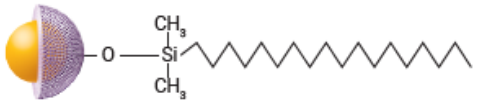
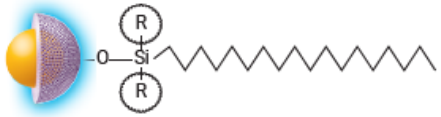
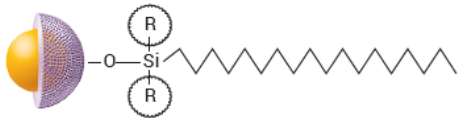
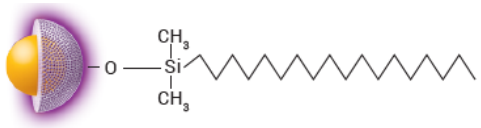
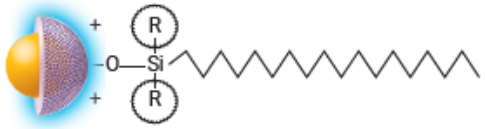
The InfinityLab Poroshell 120 Columns

Agilent Poroshell columns are designed for multiple separation modes

Best all around	Best for low pH mobile phases	Best for high pH mobile phases	Best for alternative selectivity	Best for more polar analytes	Chiral
EC-C18 [A] 1.9 µm, 2.7 µm, 4 µm	**SB-C18** [A] 1.9 µm, 2.7 µm, 4 µm	**HPH-C18** [A] 1.9 µm, 2.7 µm, 4 µm	**Aq-C18** [A,B] 2.7 µm	**SB-Aq** [A,B] 1.9 µm, 2.7 µm, 4 µm	**Chiral-V** [A,C,D] 2.7 µm
EC-C8 [A] 1.9 µm, 2.7 µm, 4 µm	**SB-C8** [A] 2.7 µm	**HPH-C8** [A] 2.7 µm, 4 µm	**Bonus-RP** [A,B] 2.7 µm	**EC-CN** [A,B,C,D] 2.7 µm	**Chiral-T** [A,C,D] 2.7 µm
Phenyl-Hexyl [A] 1.9 µm, 2.7 µm, 4 µm			**PFP** [A,B,D] 1.9 µm, 2.7 µm, 4 µm	**HILIC** [C,D,E] 1,9 µm, 2.7 µm, 4 µm	**Chiral- CD** [A,C,D] 2.7 µm
		←**CS-C18** [A] → 2.7 µm		**HILIC-Z** [C,D,E] 1.9 µm, 2.7 µm, 4 µm	**Chiral-CF** [A,C,D] 2.7 µm
				HILIC- OH5 [C,D,E] 2.7 µm	

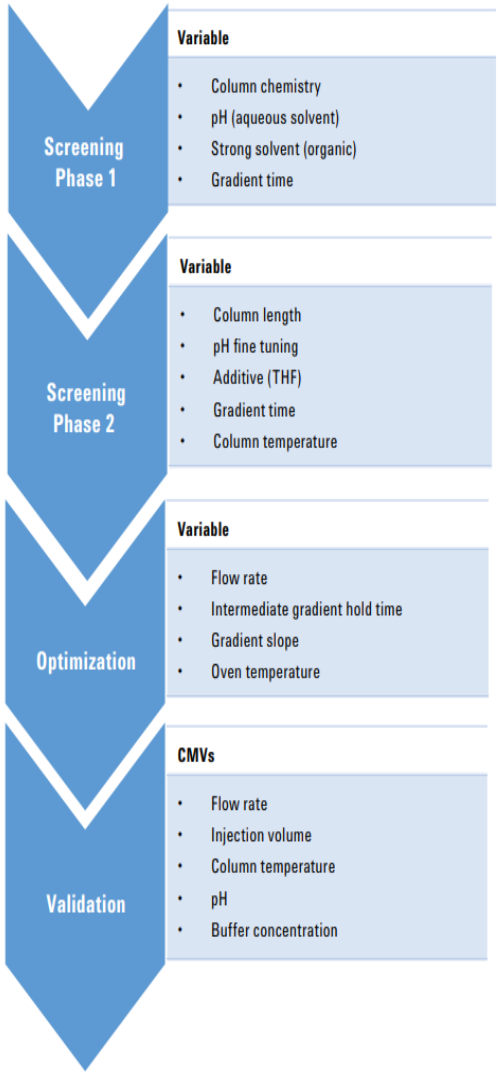
Legend
- [A] reversed phase
- [B] can be operated at 100% aqueous
- [C] Normal phase
- [D] SFC
- [E] HILIC

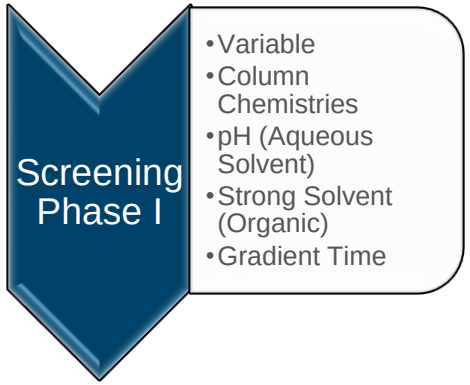
Choosing between C18s

InfinityLab Poroshell 120	Chemistry	Pore Size	Endcapped	Carbon Load	Surface Area	Best For
EC-C18 1.9 µm, 2.7 µm, 4 µm		120 Å	Yes	10%	130 m2/g	General Purpose Excellent peak shape and efficiency for acids, bases, neutrals
Aq-C18 * 2.7 µm		120 Å	Yes	Proprietary	130 m2/g	Enhanced retention for challenging polar compounds 100% aqueous mobile phase compatibility and low pH stability
SB-C18 1.9 µm, 2.7 µm, 4 µm		120 Å	No	9%	130 m2/g	Low pH Excellent stability and peak shape in highly acidic conditions
HPH-C18 1.9 µm, 2.7 µm, 4 µm		100 Å	Yes	Proprietary	95 m2/g	High pH capable Robust performance and long lifetimes
CS-C18 2.7 µm		100 Å	Yes	Proprietary	95 m2/g	Alternate selectivity Improved peak shape and sample capacity for basic compounds with low ionic strength mobile phases High pH capable

* - can be operated at 100% aqueous mobile phase conditions

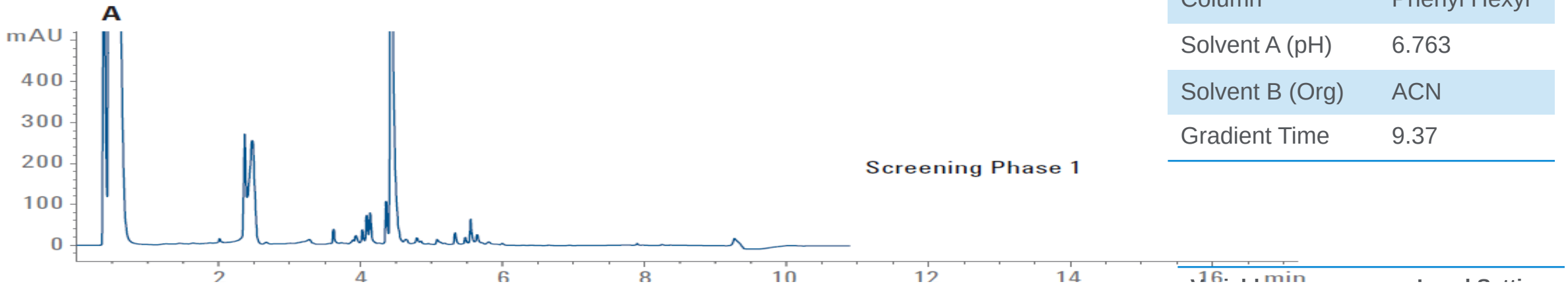
Case Study
Method Development and Validation of Oxidative Degraded Atorvastatin





Variable	
Columns	
Agilent ZORBAX RRHD SB Aq, 3.0 × 50 mm, 1.8 µm (p/n 857700-314)	
Agilent ZORBAX RRHD Bonus-RP 2.1 × 50 mm, 1.8 µm (p/n 857768-901)	
Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 × 50 mm, 1.8 µm (p/n 959757-306)	
Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl, 3.0 × 50 mm, 1.8 µm (p/n 959757-312)	
Agilent PLRP-S, 4.6 × 50 mm, 3.0 µm (p/n PL1512-1300)	
Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 × 50 mm, 1.8 µm (p/n 959757-306)	
Solvents	
A1	pH 3.0, 20 mM formic acid in water
A3	pH 4.0, 5 mM formic acid and 10 mM ammonium formate in water
A5	pH 5.0, 5 mM acetic acid and 10 mM ammonium accetate in water
A7	pH 7.0, 10 mM ammonium accetate in water
A9	pH 8.1, 10 mM ammonium hydrogencarbonate in water
B1	Acetonitrile
B2	Methanol
Gradient time	
3 to 10 minutes	
Constant	
Gradient	
Equilibration Initial hold Final hold Re-equilibration	3.0 minutes, at 5 % B 0.5 minutes, at 5 % B 0.5 minutes, at 95 % B 2 minutes, at 5 % B
Pump flow	0.6 mL/min
Injection volume	1 µL
Column temperature	40 °C
Wavelength	245 nm ± 4 nm (ref off)

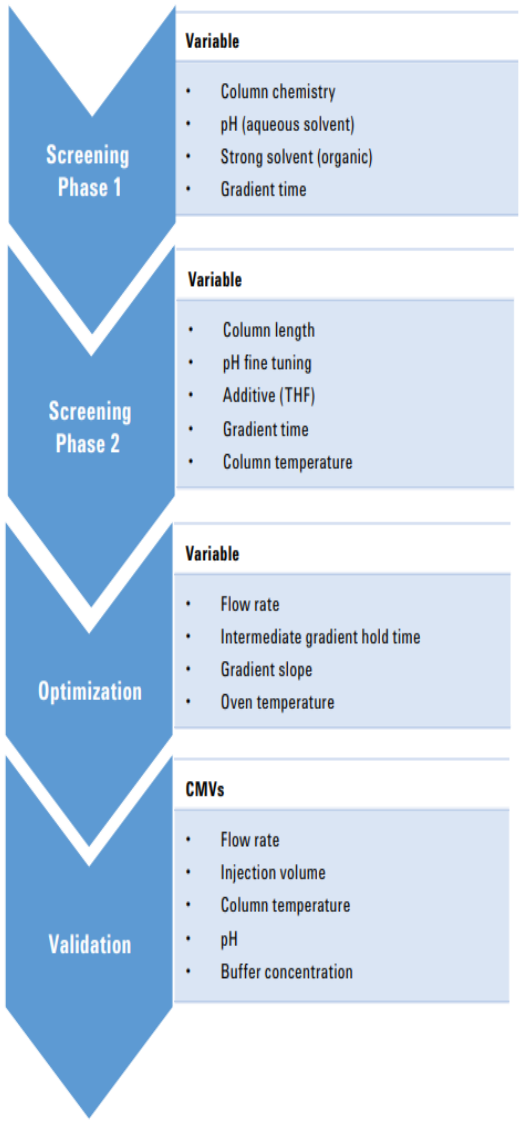
Chromatography Outcome
Screening Phase 1

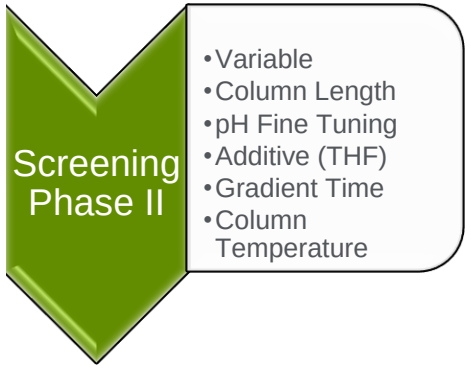


Variable	Level Setting
Column	Phenyl Hexyl
Solvent A (pH)	6.763
Solvent B (Org)	ACN
Gradient Time	9.37

https://www.agilent.com/cs/library/applications/5991-4944EN.pdf

Screening Phase 2

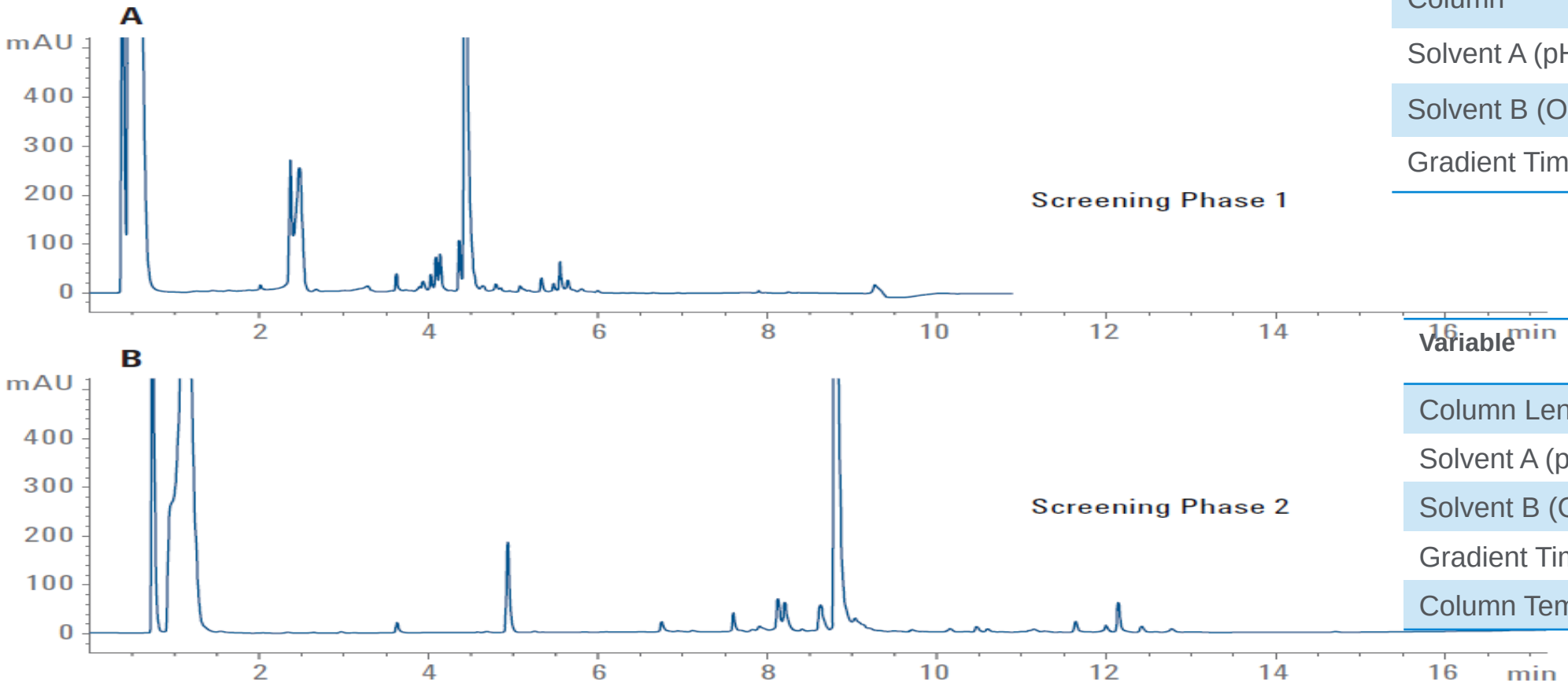




Variable	
Column Length (Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl)	
3.0 × 50 mm, 1.8 µm (p/n 959757-312)	
3.0 × 100 mm, 1.8 µm (p/n 959964-312)	
Solvents	
A1	pH 4.0, 5 mM formic acid and 10 mM ammonium formate in water
A2	pH 4.5, adjusted from pH 5 with acetic acid
A3	pH 5.0, 5 mM acetic acid and 10 mM ammonium acetate in water
A4	pH 5.5, adjusted from pH:7 with acetic acid
A5	pH 6.0, adjusted from pH:7 with acetic acid
A6	pH 6.5, adjusted from pH:7with acetic acid
A7	pH 7.0, 10 mM ammonium acetate in water
B1	Acetonitrile
B2	Acetonitrile: THF (88:12)
Gradient time	
9 minutes	
15 minutes	
Column temperature	
35 °C	
40 °C	
45 °C	
50 °C	
Constant	
Gradient	
Equilibration Initial hold Final hold Re-equilibration	1.0 minute, at 5 % B 1 minute, at 5 % B 2 minutes at 95 % B 4 minutes at 5 % B
Flow rate	0.6 mL/min
Injection volume	1 µL
Wavelength	245 nm ± 4 nm (ref off)
All other finalized parameters from Phase 1	

Chromatography Outcome
Screening Phase 1 & 2

Variable	Level Setting
Column	Phenyl Hexyl
Solvent A (pH)	6.763
Solvent B (Org)	ACN
Gradient Time	9.37

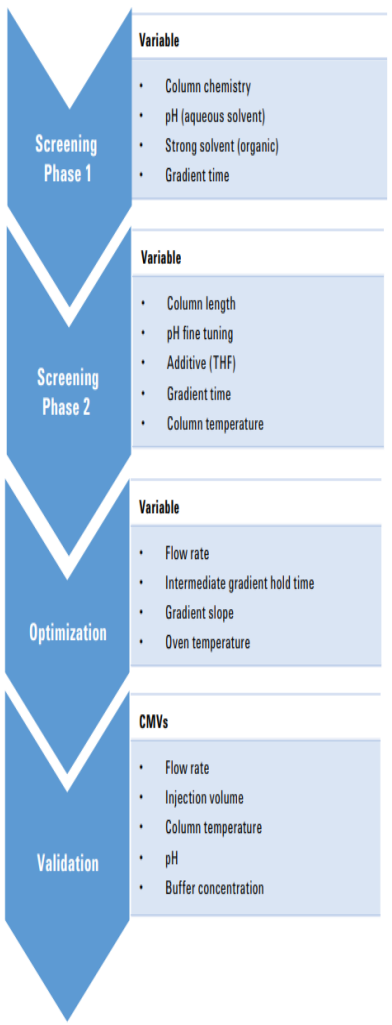


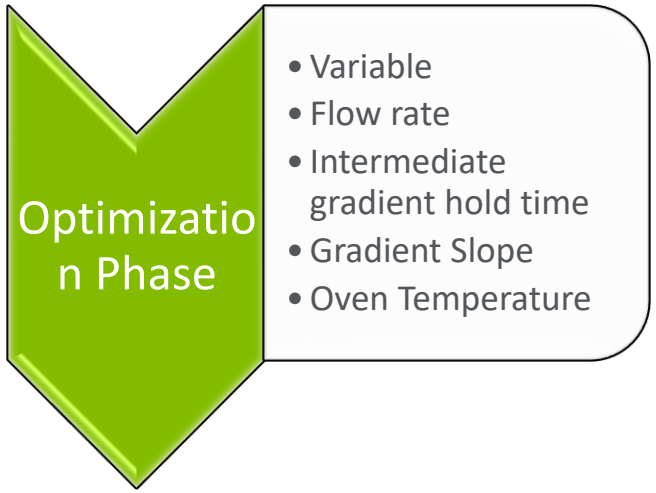
Variable	Level Setting
Column Length	100 mm
Solvent A (pH)	7.00
Solvent B (Org)	ACN
Gradient Time	15
Column Temp	35.1

Figure 3. Chromatograms showing the improvement in elution profile of degraded atorvastatin sample from screening Phase 1 (A) to Phase 2 (B).

https://www.agilent.com/cs/library/applications/5991-4944EN.pdf

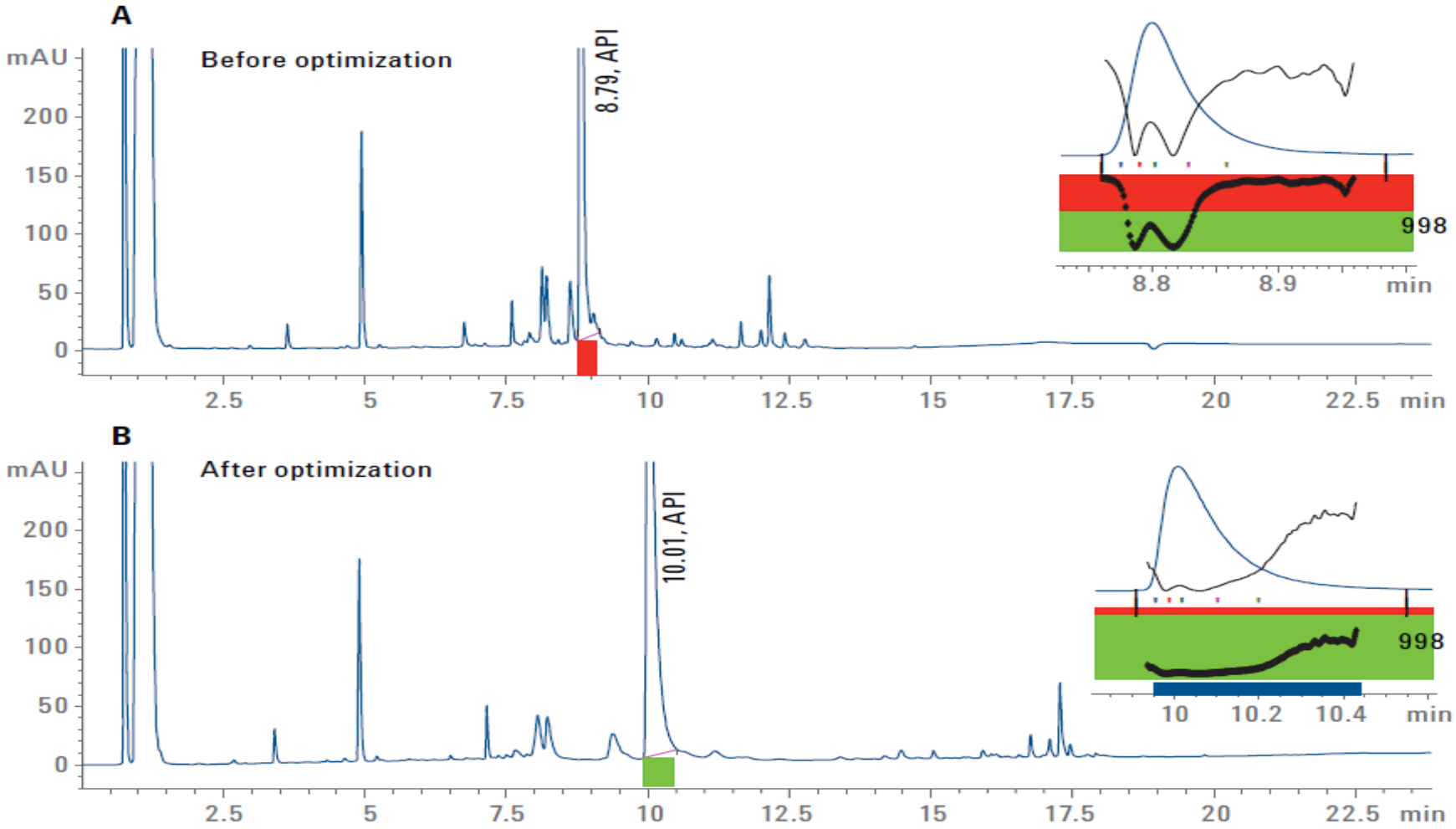
Optimization Phase





Variable Parameters	Study Range	Out Come
Pump Flow Rate (mL/min)	055	0.60 mL/min
	0.60	
	0.65	
Intermediate Hold Time (min)	3 to 7 min	5.52 min
Gradient Slope (Final % Gradient 1)	30 to 35%	35%
Oven Temperature	33, 36, 39 Deg. C.	33 Deg. C
Gradient 1	5% B to (30-35)% B	
Gradient 2	(30-35)% B to 90% B	

Chromatography Outcome –
Optimization Phase (Pre & Post)



Summary Post Optimization

- A total of 43 peaks were separated
- 35 of the 43 peaks achieving a tangent resolution ≥ 1.50.
- The number of peaks with narrow peak width and good peak tailing were 17 and 29 respectively.
- Fusion QbD software predicted response (CMA) values from the center point of PAR. The experimental results were compared with predicted values and found to be within Sigma confidence limit.

Response Variable	Predicted Response Value	- 2 Sigma Confidence Limit	+ 2 Sigma Confidence Limit	Experimental
No Of Peaks	42.53	41.11	43.94	43
No. Of Peaks > 1.50 (Tangent Resolution)	35.3	33.87	36.73	35
No. Of Peaks < 0.05 (Width)	15.6	13.32	17.87	17
No. Of peaks < 1.6 (Tailing USP)	26.72	24.08	29.36	29

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Design Space Analysis

Screening Phase 1

Variable

- Column chemistry
- pH (aqueous solvent)
- Strong solvent (organic)
- Gradient time

Screening Phase 2

Variable

- Column length
- pH fine tuning
- Additive (THF)
- Gradient time
- Column temperature

Optimization

Variable

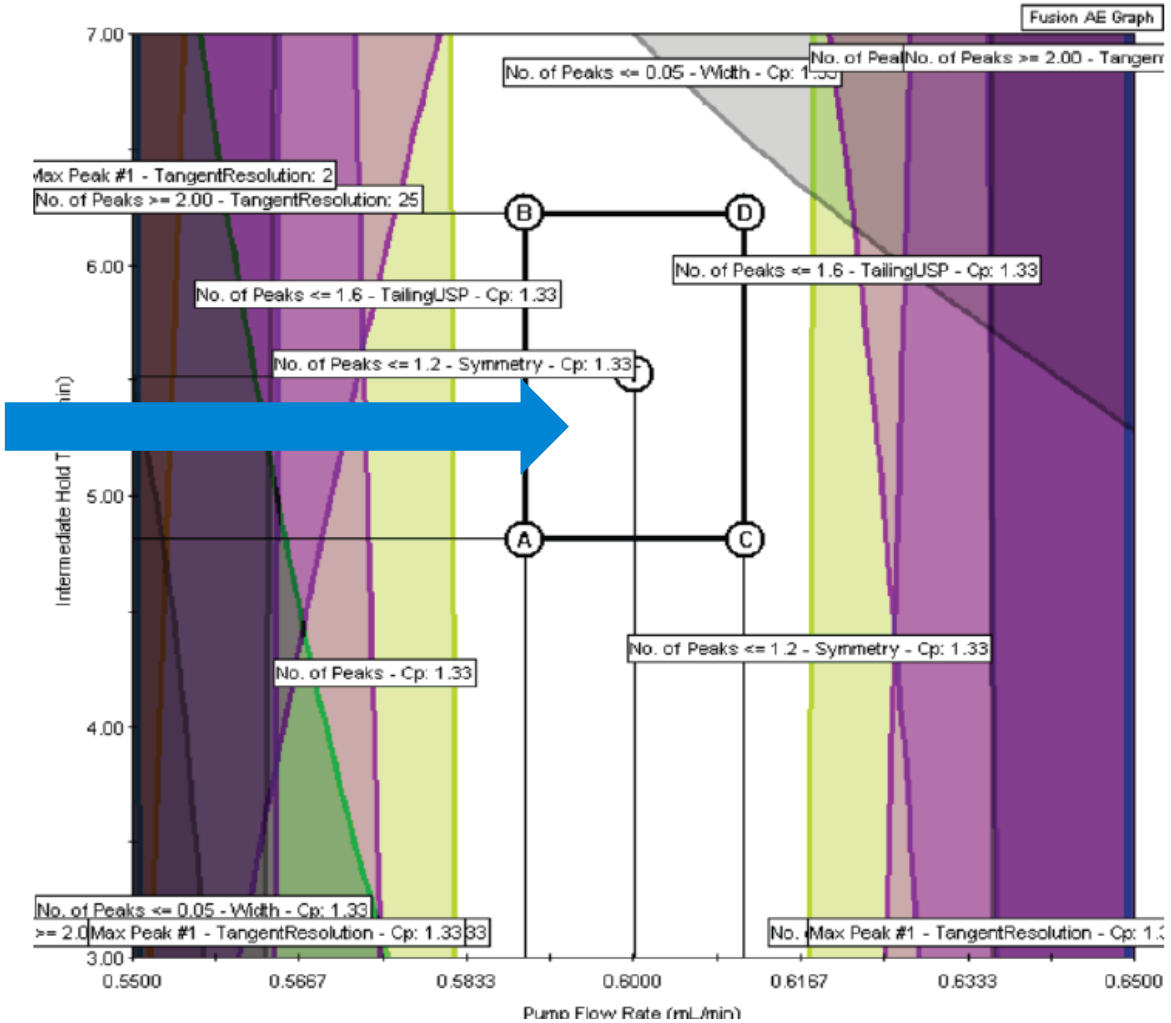
- Flow rate
- Intermediate gradient hold time
- Gradient slope
- Oven temperature

Validation

CMVs

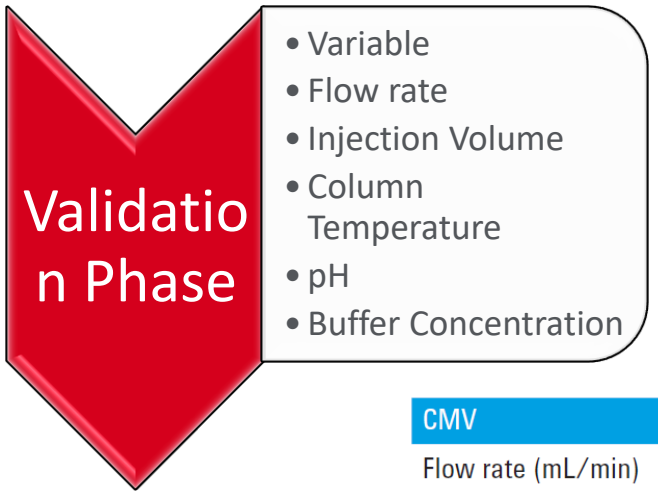
- Flow rate
- Injection volume
- Column temperature
- pH
- Buffer concentration

MODR



https://www.agilent.com/cs/library/applications/5991-4944EN.pdf

Validation Phase & Control Strategy



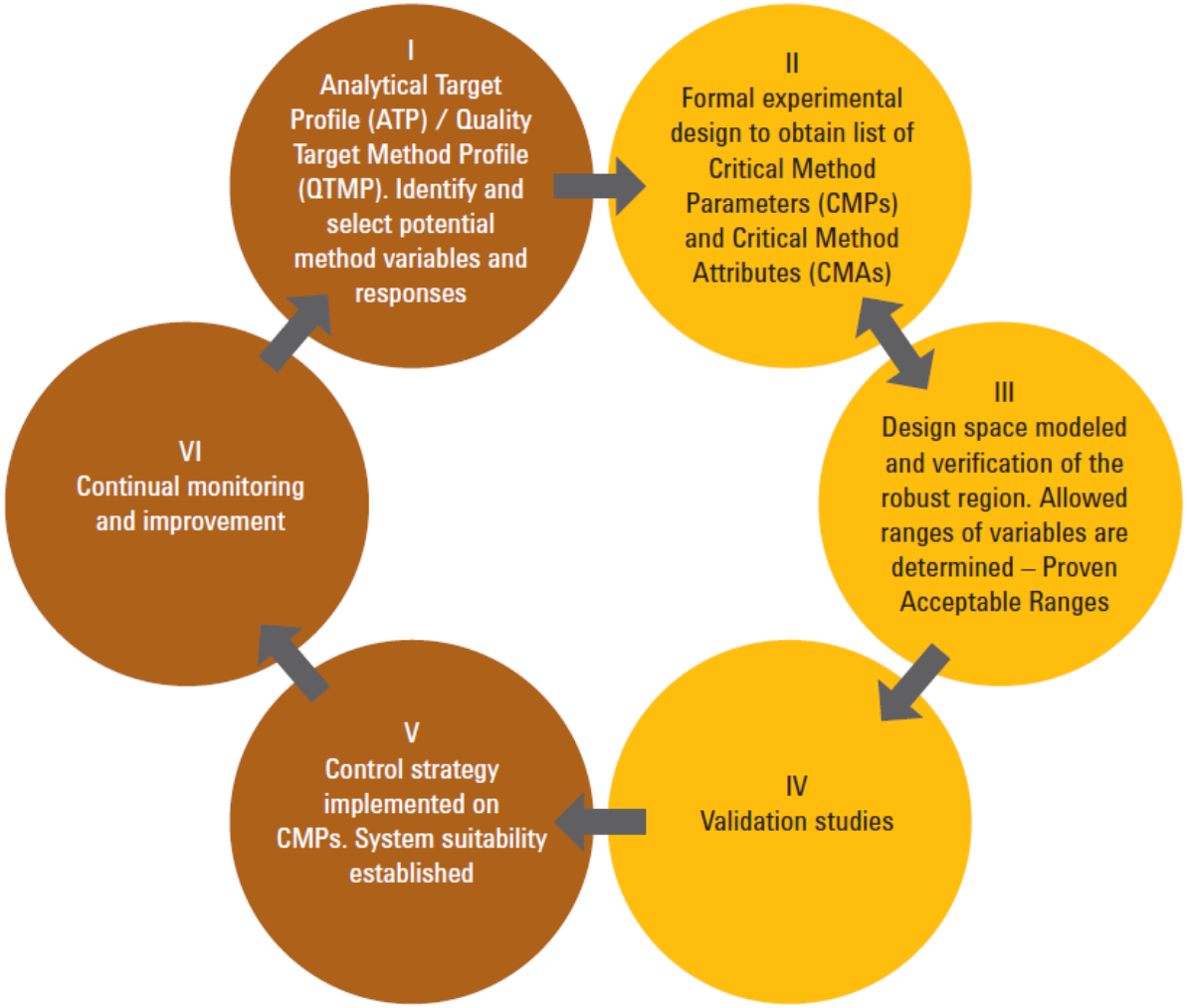
CMV	Coded name*	Range tested
Flow rate (mL/min)	A	0.59 0.60 0.61
Injection volume (µL)	B	0.9 1.0 1.1
Oven temperature (°C)	C	32 33 34
pH	D	6.66 6.76 6.86
Buffer concentration (mM)	E	9.5 10.0 10.5

*Coded names are used in robustness model displays.

CMV	Coded Name	Method Nominal	Robust Range
Flow Rate	A	0.6	± 0.01
Oven Temperature	B	33	± 1
pH	C	6.76	± 0.1
Buffer Concentration	D	10	± 0.5
Injection Volume	E	1	± 0.1

https://www.agilent.com/cs/library/applications/5991-4944EN.pdf

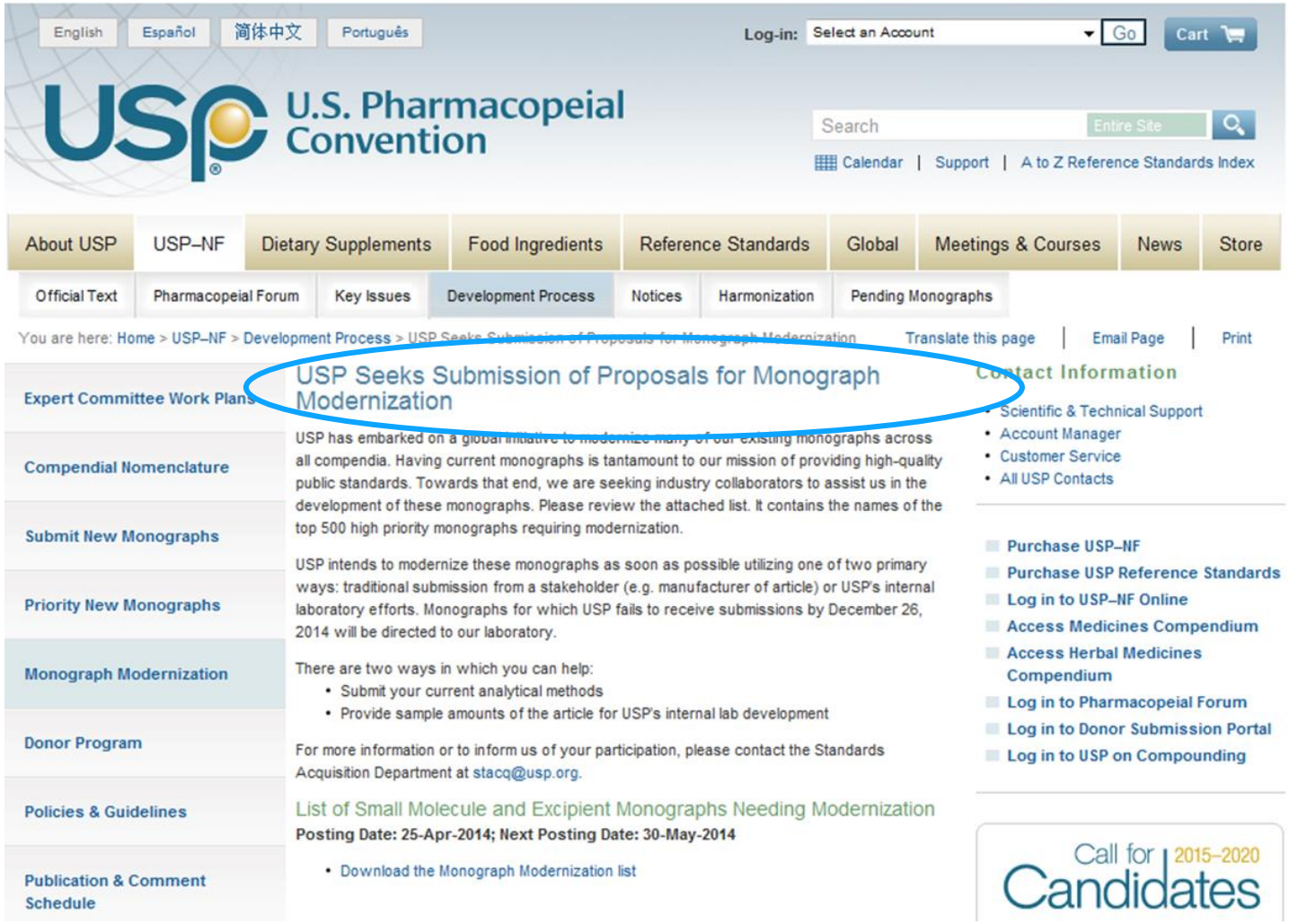
Workflow for Enhanced approach/QbD in Analytical Development



Typical workflow for QbD based analytical method development. Steps II, III, and IV represent software-assisted sections.

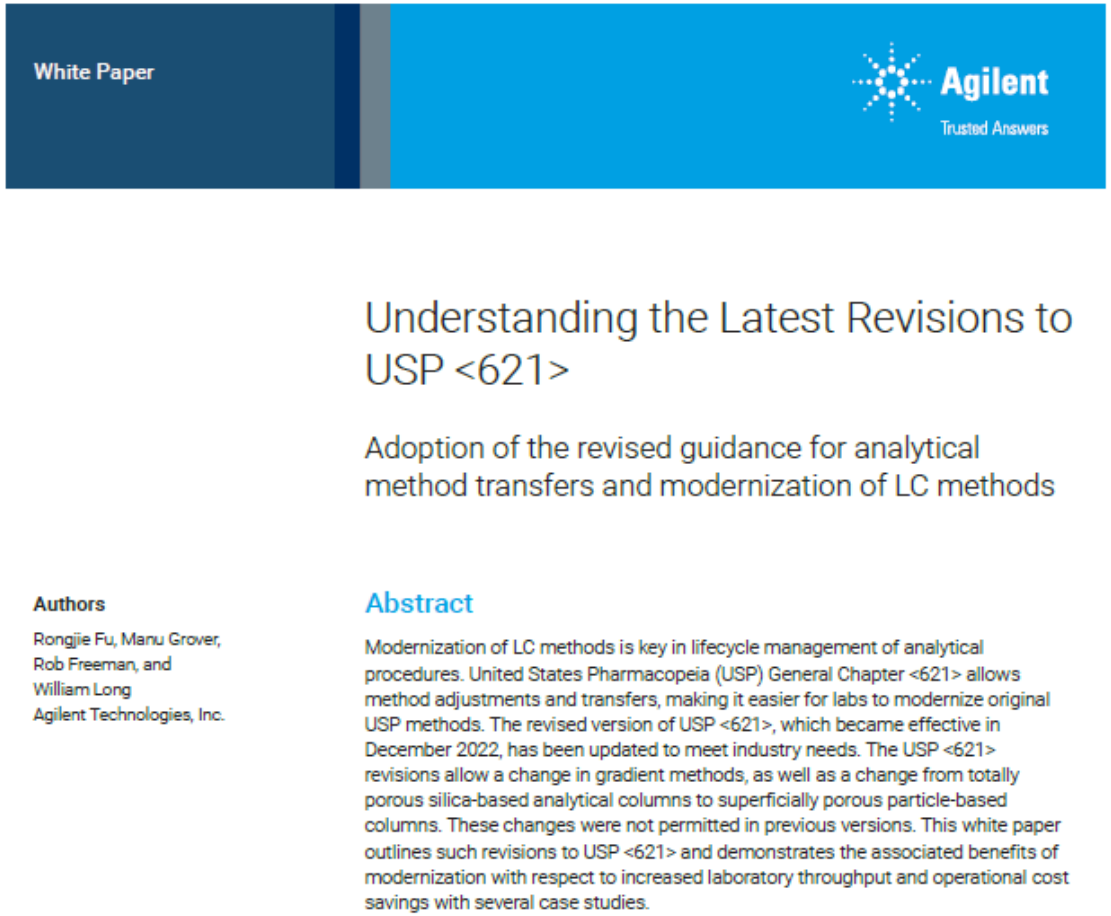
Analytical QbD terminology	Examples
Analytical Target Profile (ATP)	Accurate quantitation of API without interferences from degradants
Quality Target Method Profile (QTMP/CPQA)	pKa, Log P, solubility
Critical Method Parameters (CMP)	Flow rate, temperature, pH
Critical Method Attributes (CMA/CAPP)	Resolution, peak tailing, peak capacity
Control strategy	pH ± 0.1; Wavelength ± 2nm

General Chapter of Chromatography
USP <621> - Updates



Adjustments to the Chromatographic System

<621> provides allowable adjustments for HPLC to:


White Paper

Agilent
Trusted Answers

Understanding the Latest Revisions to USP <621>

Adoption of the revised guidance for analytical method transfers and modernization of LC methods

Authors

Rongjie Fu, Manu Grover, Rob Freeman, and William Long
Agilent Technologies, Inc.

Abstract

Modernization of LC methods is key in lifecycle management of analytical procedures. United States Pharmacopeia (USP) General Chapter <621> allows method adjustments and transfers, making it easier for labs to modernize original USP methods. The revised version of USP <621>, which became effective in December 2022, has been updated to meet industry needs. The USP <621> revisions allow a change in gradient methods, as well as a change from totally porous silica-based analytical columns to superficially porous particle-based columns. These changes were not permitted in previous versions. This white paper outlines such revisions to USP <621> and demonstrates the associated benefits of modernization with respect to increased laboratory throughput and operational cost savings with several case studies.

- Mobile phase composition
- Mobile phase pH
- Buffer concentration
- Column temperature
- Injection volume
- UV-VIS detector wavelength
- Particle size
- Column length
- Column inner diameter
- Flow rate

Chromatography: USP<621> NEW

Stage 4 Harmonization
Official: December 1, 2022

⟨621⟩CHROMATOGRAPHY

Change to read:

▲INTRODUCTION

Chromatographic separation techniques are multistage separation procedures in which the components of a sample are distributed between two phases, one of which is stationarywhile the other is mobile. The

- **Resolution** is based now on the peak width at half of the peak height.
- **Plate number** is based now on the peak width at half of the height.
- **Signal-to-noise** is now calculated using a range of noise 5 times the width at half-height of the peak.
- **Changes allowed to Gradient Chromatographic conditions.**

Allowed changes in Compendial methods as per USP<621>

Adjustment of chromatographic Conditions::

- The chromatographic conditions described have been validated during the elaboration of the monographs.
- The extent to which the various parameters of a chromatographic test may be adjusted without fundamentally modifying the pharmacopeial analytical procedures are listed below. Changes other than those indicated require revalidation of the procedure.

- Recommends **RISK ASSESMENT** to access cumulative effect of multiple adjustments.
- **SYSTEM SUITABILITY** is primary verification for compliance of satisfactory performance.
- **Dwell Volume:** Monographs preferably include an isocratic step before the start of the gradient program so that an adaptation can be made to the gradient time points to take account of differences in dwell volume between the system used for analytical procedure development and that actually used.

$$t_2 = t_1 - \{(D_2 - D_1)/F\}$$

1=monograph, 2=adjusted, t=gradient time, D=dwell volume & F=Flow

Link:https://www.usp.org/sites/default/files/usp/document/harmonization/gen-chapter/harmonization-november-2021-m99380.pdf

Parameter	ISOCRATIC Elution	GRADIENT Elution
Stationary Phase	NO Change in chemistry, Physiochemical allowed i.e. chromatographic support, surface modification and extent of chemical modification must be similar, a change from totally porous particle (TPP) columns to superficially porous particle (SPP) columns is allowed	
Column dimensions (dp, L)	Allowed wrt L/dp within −25% to +50%	
Adjustments from totally porous to superficially porous particles	Allowed wrt N (plate number) within −25% to +50%	
Internal diameter (iD)	Allowed wrt minimize extra-column band broadening $F2 = F2 \times [(dc2^2 \times dp1)/(dc1^2 \times dp2)]$ 1=Monograph 2=Adjusted F=Flow, dc=column internal diameter, dc=particle size factors instrument connections, detector cell volume and sampling rate and injection volume. (≥3-µm to <3-µm particles with performance drop within 20%; Flow ±50%) For GRADIENT; time must be adjusted to maintain constant ratio of volume using eq. $tG2 = tG1 \times (F1/F2)\,[(L2 \times dc2^2)/(L1 \times dc1^2)]$		
Column temperature	± 10°C	±5°C
MOBILE PHASE		
Composition	Allowed for minor component within ±30%. Also change in any component cannot exceed ±10% absolute.	principal peak(s) elute(s) within ±15% of the retention time(s).
pH	±0.2 pH units	
Buffer Concentration (Salt/s)	±10%	
Flow Rate	±50%	
DETECTOR		
Wavelength	NO Adjustments allowed	
Injection Volume	$Vinj2 = Vinj1\,(L2\,dc2)^2 / (L1\,dc1)^2$ 1=Monograph 2=Adjusted V=Volume, L=Length, dc=particle size NOTE: Equation may not be applicable to changes from TPP columns to SPP columns.	

Ratio of Column Length-to-Particle Size: L/dp Ratio
Maintaining Separation Power

Separation Index	Application Example	Efficiency (N) – *e.g.*	L/dp
Easy	Content Uniformity	5,000	15,000
Moderately Challenging	Related compound assay	12,000	30,000
Difficult	Impurity profiling	20,000	50,000
Extremely Difficult	Peptide Mapping/ Met IF	35,000	85,000

How to calculated L/dp ratio?

Typical HPLC Column - 4.6 x 150 mm, 5 µm

Column length (L) = 150 mm = 150,000 µm

d_p = 5 µm

$$\frac{L}{d_p} = \frac{150{,}000}{5} = \mathbf{30{,}000}$$

-25% → **22500 Count**

+50% → **45000 Count**

Permissible range for target column

How to modernize the chromatographic conditions as per USP <621>

Objectives:
Increase Productivity
Solvent Savings
Improving CQA's

Column Identification
Length
particle size
Zorbax /Pursuit
Poroshell etc.

Criteria for Column Selection
TPP to TPP (L/Dp)
TPP to SPP (N Or $\{tr/wh\}^2$

Method transfer steps
Step 1
Step 2
Step 3
Step 4

Step 1:

Selection of new column dimensions within allowed deviation: **L/dp: -25% to +50%**

(based on Liquid Chromatograph – Pressure specs

Step 2:

Adjust the flow rate according to new column dimensions

$F_2 = F_1 [(dc_2^2 \times dp_1)/(dc_1^2 \times dp_2)]$

Step 3:

Each segment of gradient timetable must be adjusted

$t_{G2} = t_{G1} \times (F_1/F_2) [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$

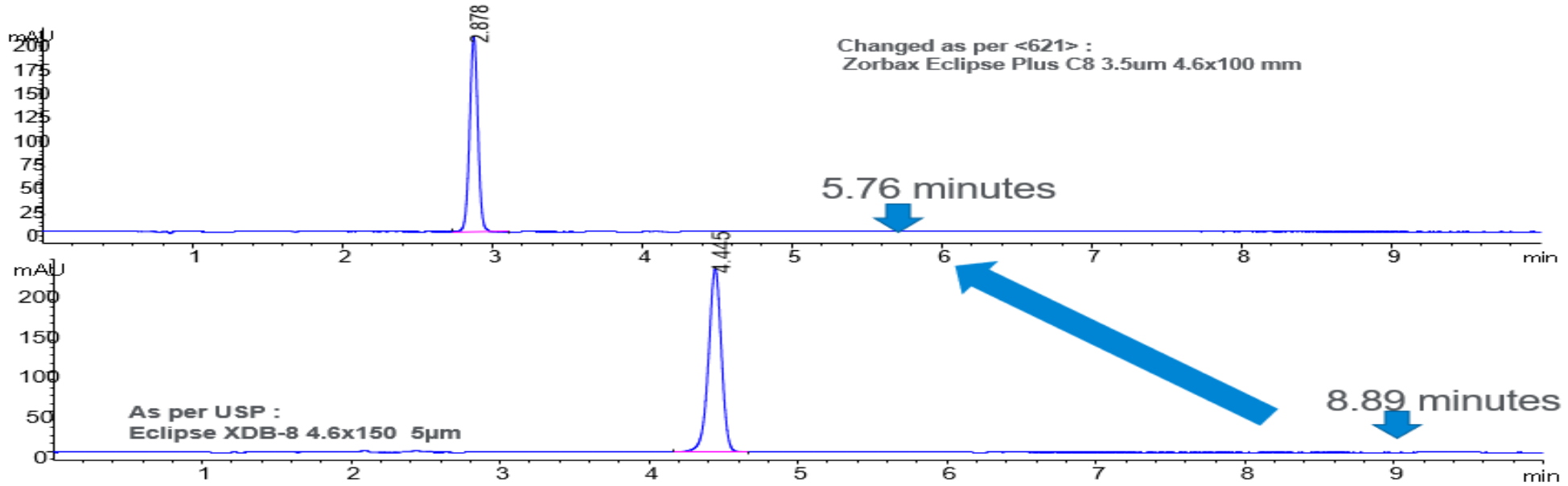
Not required for isocratic

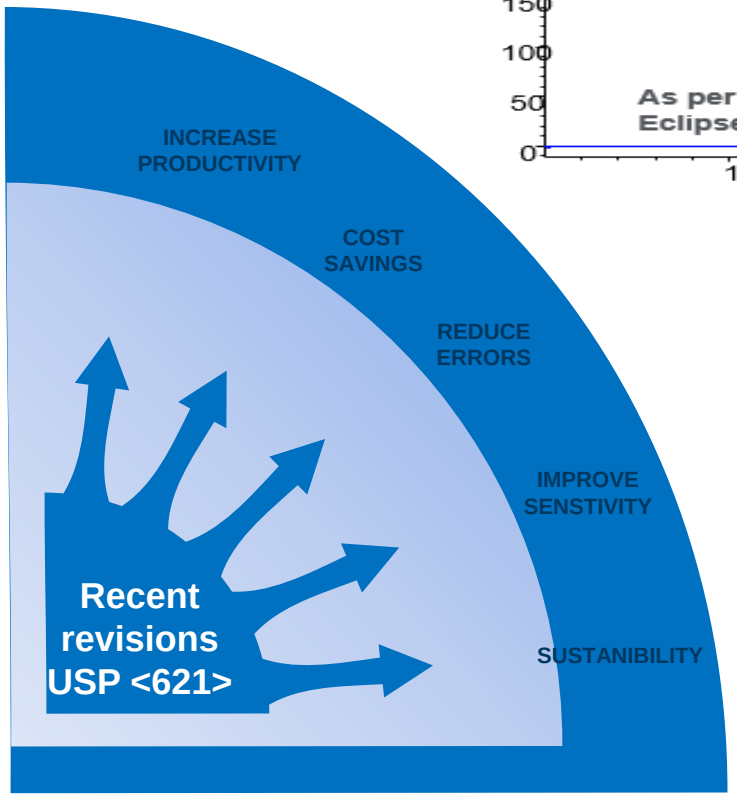
Step 4:

Injection volume adjustment

- $V_{inj2} = V_{inj1} \times [(L_2 \times dc_2^2)/(L_1 \times dc_1^2)]$

Impact of modernizations



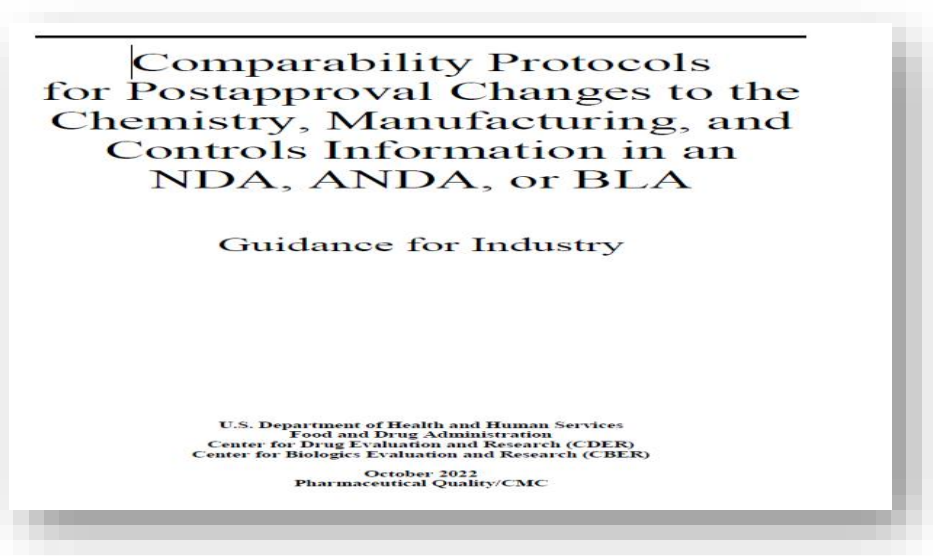


- Improve CQA's & Reduce errors
- No need for revalidation
- More Choice of chemistries

- Increase productivity
- Saves Up-to 80% time*
- Scope for upgrade to newer technologies and chemistries

- Adopt Lifecycle Management in analytical procedures
- Sustainability
- Product reach Faster to market

Post Approval impact on changing methods



Comparability Protocols for Postapproval Changes to the Chemistry, Manufacturing, and Controls Information in an NDA, ANDA, or BLA

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

October 2022
Pharmaceutical Quality/CMC

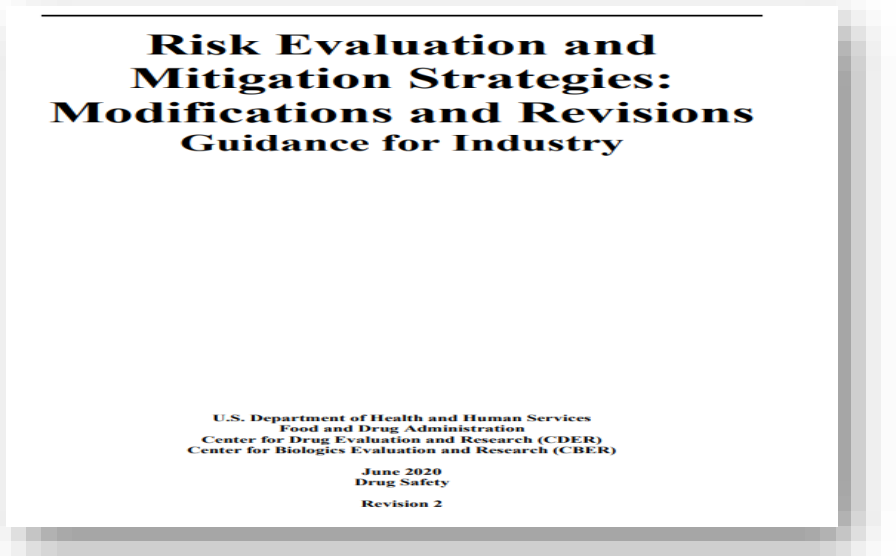
F. Specification, Including Analytical Procedure (Method) Changes

Does FDA have any recommendations or issues for industry to consider regarding specification changes in a CP?

Specifications are the quality standards (i.e., tests, analytical procedures, and acceptance criteria) provided in an approved application to confirm the quality of drug substances, products, intermediates, raw materials, reagents, components, in-process materials, container closure systems, and other materials used in the production of a drug substance or product.[78] Changes to the approved specification that provide the same or greater assurance of product quality can be included in a CP. A CP submission for a specification change should include a justification for the change.

For replacement or modification of an existing analytical procedure in an approved application, the new procedure must be scientifically sound[79] and should provide the same or greater assurance of product quality than the currently approved procedure.[80] The CP should include the specific plan, description of statistical method(s) to be used, and acceptance criteria to be achieved for evaluating the performance of the new procedure. Method validation data should be submitted with the notification of the change.

A CP can also be used for replacing a quality reference standard used in an analytical procedure(s).



Risk Evaluation and Mitigation Strategies: Modifications and Revisions

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

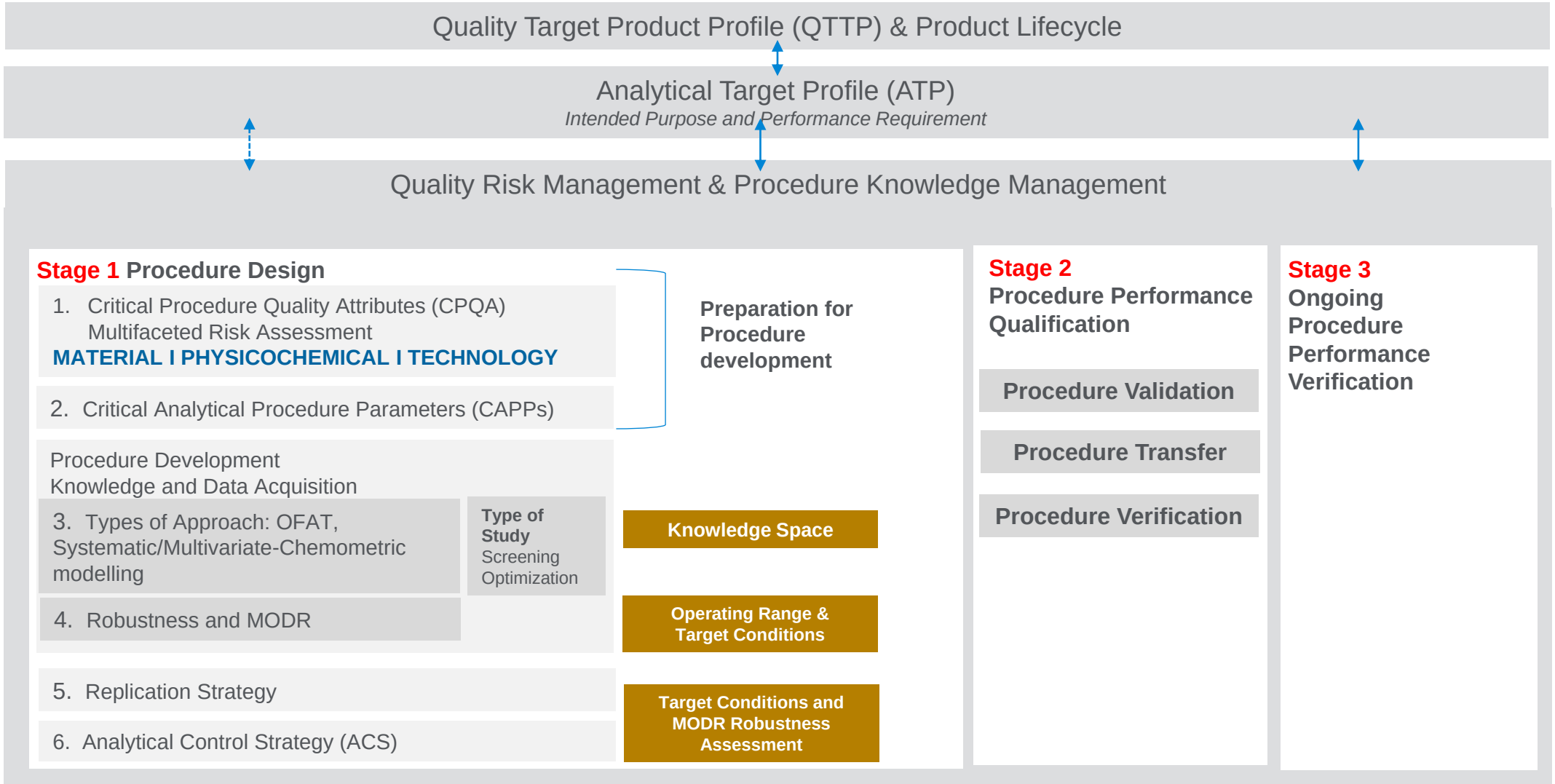
June 2020
Drug Safety

Revision 2

Existing FDA regulations describe how to make changes to approved applications and include a mechanism for rapid implementation of certain changes.[13] Some changes must be submitted as a prior approval supplement (PAS) and be approved before they are implemented. Changes-being-effected (CBE) supplements may be implemented at the time they are submitted or 30 days following submission.[14] If a supplement was inappropriately submitted as a CBE, the FDA will notify the application holder that the proposed change(s) require FDA approval before implementation. A description of how these existing submission requirements apply to proposed REMS changes is provided below in greater detail.

All proposed REMS modifications (minor or major) initiated by the application holder must include an *adequate rationale*.[25] The rationale may include, but is not limited to, the reason(s) why the proposed modification is necessary; the potential effect of the proposed modification on how the REMS addresses the serious risk(s) for which the REMS was required, on patient access to the drug, and/or on the burden on the health care delivery system; and other appropriate evidence or data to support the proposed change. If a REMS assessment was submitted in the previous 18 months and includes data to support the proposed modifications, then it can be referenced as the adequate rationale.

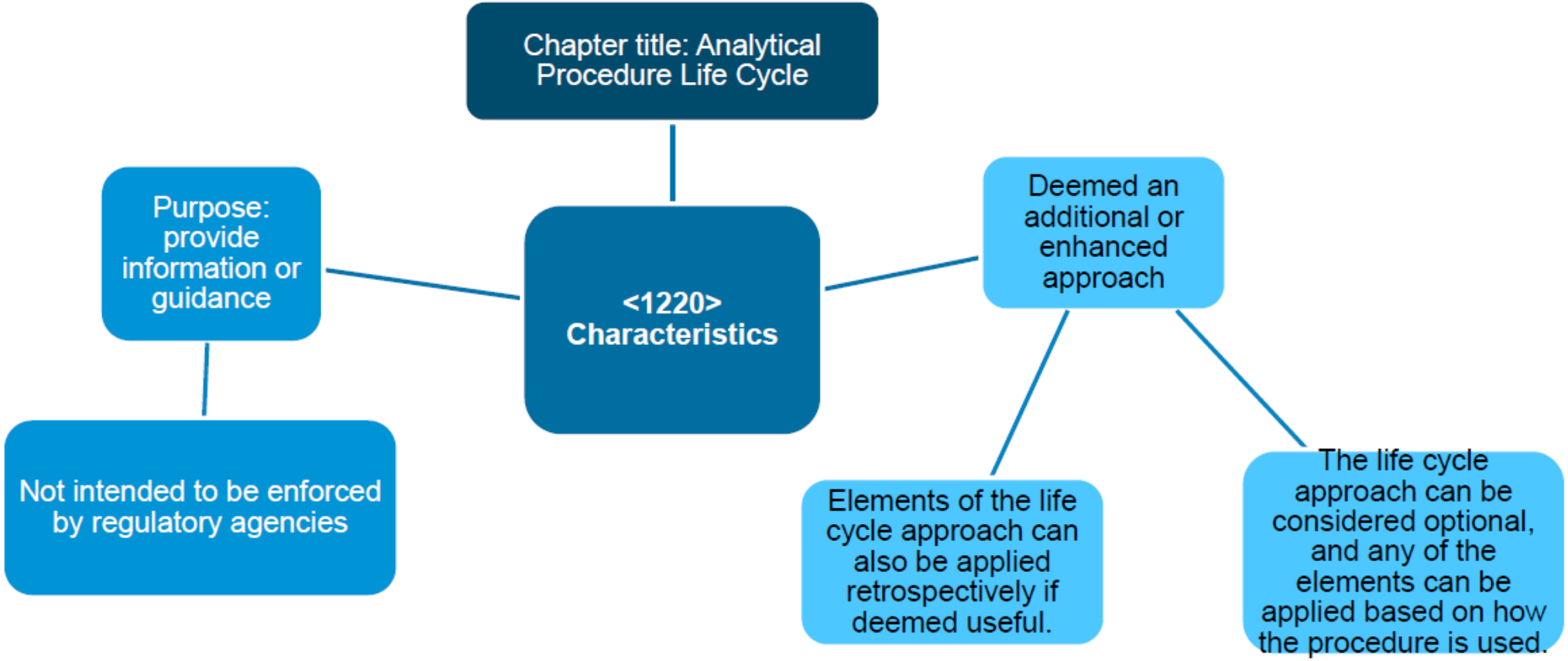
Analytical Procedure Life Cycle
USP <1220>



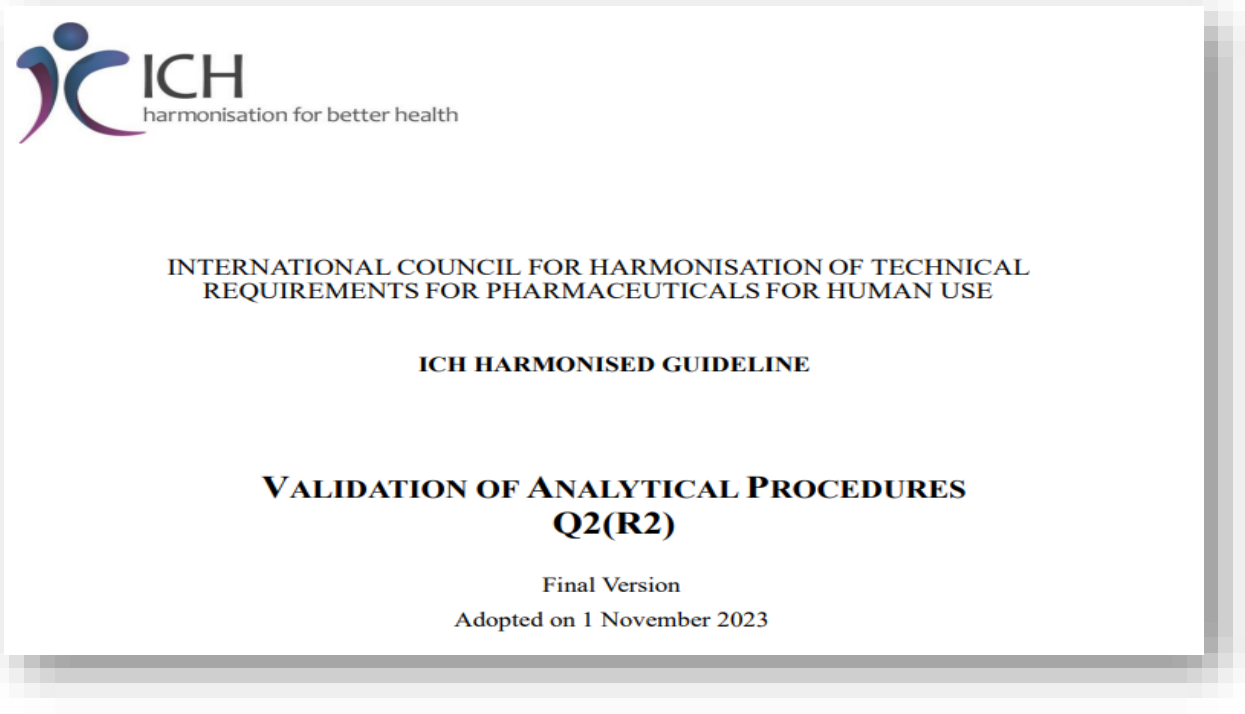
Analytical Quality by Design Fundamentals and Compendial and Regulatory Perspectives | SpringerLink

USP <1220>
Life Cycle Management

Characteristics



Regulations on Validation of Analytical Procedures by ICH


ICH
harmonisation for better health

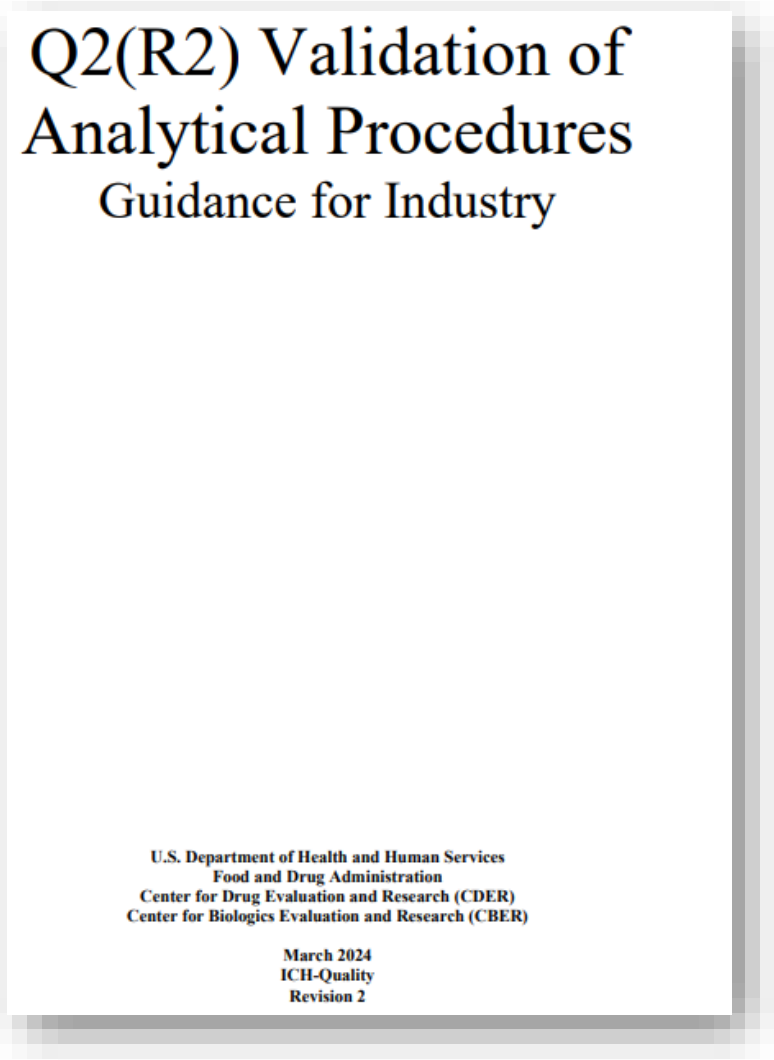
INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE

ICH HARMONISED GUIDELINE

VALIDATION OF ANALYTICAL PROCEDURES
Q2(R2)

Final Version

Adopted on 1 November 2023


Q2(R2) Validation of Analytical Procedures

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

March 2024
ICH-Quality
Revision 2

Introduction to

- Knowledge management,
- Multivariate approaches
- Lifecycle Management
- Non-linear responses

ICH Q2 (R2)

ICH Q2(R2) Guideline

Table 1: Typical performance characteristics and related validation tests for measured quality attributes

Measured Quality Attribute / Analytical Procedure Performance Characteristics to be Demonstrated (2)	IDENTITY	IMPURITY (PURITY) Other quantitative measurements (1)		ASSAY Content or potency / Other quantitative measurements (1)
		Quantitative Test	Limit Test	
Specificity (3) Specificity Test	+	+	+	+
Range Response (*Calibration Model*)	-	+	-	+
Lower Range Limit	-	QL†	DL	-
Accuracy (4) Accuracy Test	-	+	-	+
Precision (4) Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+ (5)	-	+ (5)

- signifies that this test is not normally conducted

+ signifies that this test is normally conducted

† in some complex cases DL may also be evaluated

QL, DL: quantitation limit, detection limit

(1) other quantitative measurements can follow the scheme for impurity, if the range limit is close to the DL/QL; other quantitative measurements can follow the scheme for assay (content or potency), if the range limit is not close to the DL/QL

(2) some performance characteristics can be substituted with technology-inherent justification in the case of certain analytical procedures for physicochemical properties

(3) lack of specificity of one analytical procedure should be compensated by one or more other supporting analytical procedures, unless appropriately justified

(4) alternatively, a combined approach can be used to evaluate accuracy and precision

(5) where reproducibility has been performed and intermediate precision can be derived from the reproducibility data set, an independent study for intermediate precision is not required

ICH Q2(R2) Guideline

Technique	**Separation techniques (*e.g.*, HPLC, GC, CE) for impurities or assay**	**Separation techniques with relative area quantitation (*e.g.*, product-related substances such as charge variants)**
Performance characteristic	**Validation study methodology**	
Reportable Range	Validation of calibration model across the range: Linearity: Dilution of the analytes of interest over the expected procedure range, at least 5 points Validation of lower range limits (for purity only): QL, DL through a selected methodology (*e.g.*, signal-to-noise determination)	Validation of calibration model across the range: Linearity: Between measured (observed) relative result *versus* theoretically expected relative result across specification range(s), *e.g.*, by spiking or degrading material Validation of lower range limits: QL (and DL) through a selected methodology (*e.g.*, signal-to-noise determination)
Robustness and other considerations (performed as part of analytical procedure development as per ICH Q14)	Deliberate variation of relevant parameters, *e.g.*, Sample preparation: extraction volume, extraction time, temperature, dilution Separation parameters: column/capillary lot, mobile phase/buffer composition and pH, column/capillary temperature, flow rate, detection wavelength Stability of sample and reference material preparations **Relative Response Factors** If the analyte has a different response from the reference material (*e.g.*, a different specific UV absorbance), relative response factors should be calculated using the appropriate ratio of responses. This evaluation may be performed during validation or development, and should use the finalised analytical procedure conditions and be appropriately documented If the relative response factor is outside the range 0.8-1.2, then a correction factor should be applied. If an impurity/degradation product is overestimated, it may be acceptable not to use a correction factor	

Table 3: Examples for quantitative separation techniques

Technique	**Separation techniques (*e.g.*, HPLC, GC, CE) for impurities or assay**	**Separation techniques with relative area quantitation (*e.g.*, product-related substances such as charge variants)**
Performance characteristic	**Validation study methodology**	
Specificity/ Selectivity	Absence of relevant interference: With product, buffer, or appropriate matrix, and between individual peaks of interest Spiking with known impurities/ excipients or By comparison of impurity profiles by an orthogonal analytical procedure Demonstration of stability-indicating properties through appropriate forced degradation samples, if necessary	Absence of relevant interference: With product, buffer, or appropriate matrix, and between individual peaks of interest Demonstration of stability-indicating properties through appropriate forced degradation samples if necessary
Precision	Repeatability: Replicate measurements with 3 times 3 levels across the reportable range or 6 times at 100% level, considering peak(s) of interest Intermediate precision: *e.g.*, different days, environmental conditions, analysts, equipment	
Accuracy	For Assay: Comparison with suitably characterised material (*e.g.*, reference material) or Comparison with an orthogonal procedure For impurities or related substances: Spiking studies with impurities or Comparison of impurity profiles with an orthogonal procedure	Comparison with an orthogonal procedure and/or suitably characterised material (*e.g.*, reference material) or Accuracy can be inferred once precision, linearity and specificity have been established or Spiking studies with forced degradation samples and/or suitably characterised material

Conclusions
Pharmaceutical Analysis is Changing..

	Traditional Approach	AQbD approach	Agilent Support in AQbD
Knowledge Space	Less Knowledge Not much scientific rationale	More Knowledge Strong scientific rationale	Field Expertise and Regulatory knowledge
Time consumed during Initial Screening and operations	Less Time consuming Easy operations	High Time consuming Need expertise	Poroshell Column Technology Method Development Systems Field Expertise
Evaluation of Risk Assessment, Method Robustness, &Reliability	High Time consuming Uncertain results	High Time consuming Certain results	Method Validation Kits Field Expertise HPLC Advisor app Method Development Systems
Analytical Method cycle *(Screening-optimization-Validation-Transfer-LCM)*	No Process	Process oriented, Defined ATP, CPQA etc.	Agilent Column Portfolio Method transfer tools : ISET Technology (LC, GC..) Portfolio Field Expertise
Failures at Q.C *(Tech Transfer/Routine)*	High	Low	Analytical Validation Kits Field Expertise

Link: https://www.hovione.com/knowledge-center/case-study/analytical-procedure-method-lifecycle-management-drives-method

Thank you

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Annex

Liquid Chromatography
Adjustments in Gradient elution

Variable	Original Conditions	Adjusted Conditions	Comment
Column length (L) in mm	150	100	user choice
Column diameter (dc) in mm	4.6	2.1	user choice
Particle size (dp) in µm	5	3	user choice
L/dp	30.0	33.3	(1)
Flow rate in mL/min	2.0	0.7	(2)
Gradient adjustment factor		0.4	(3)
Gradient conditions			
%B	Time (min)	Time (min)	
30	0	0	
30	3	(3x0.4)=1.2	
70	13	[1.2+(10x0.4)]=5.2	
30	16	[5.2+(3x0.4)]=6.4	

(1) 11% increase within allowed *L*/*dp* change of -25% to +50%
(2) calculated using $F_2 = F_1\ [(dc_2^2 \times dp_1) / (dc_1^2 \times dp_2)]$
(3) calculated using $t_{G2} = t_{G1} \times (F_1 / F_2)\ [(L_2 \times dc_2^2) / (L_1 \times dc_1^2)]$