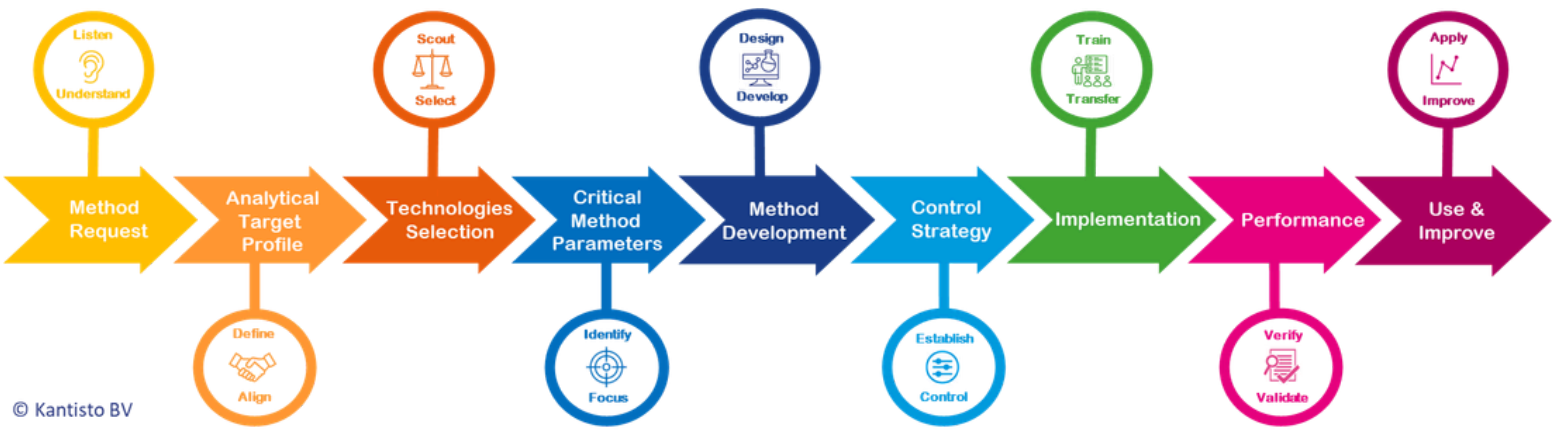
Analytical Quality by Design

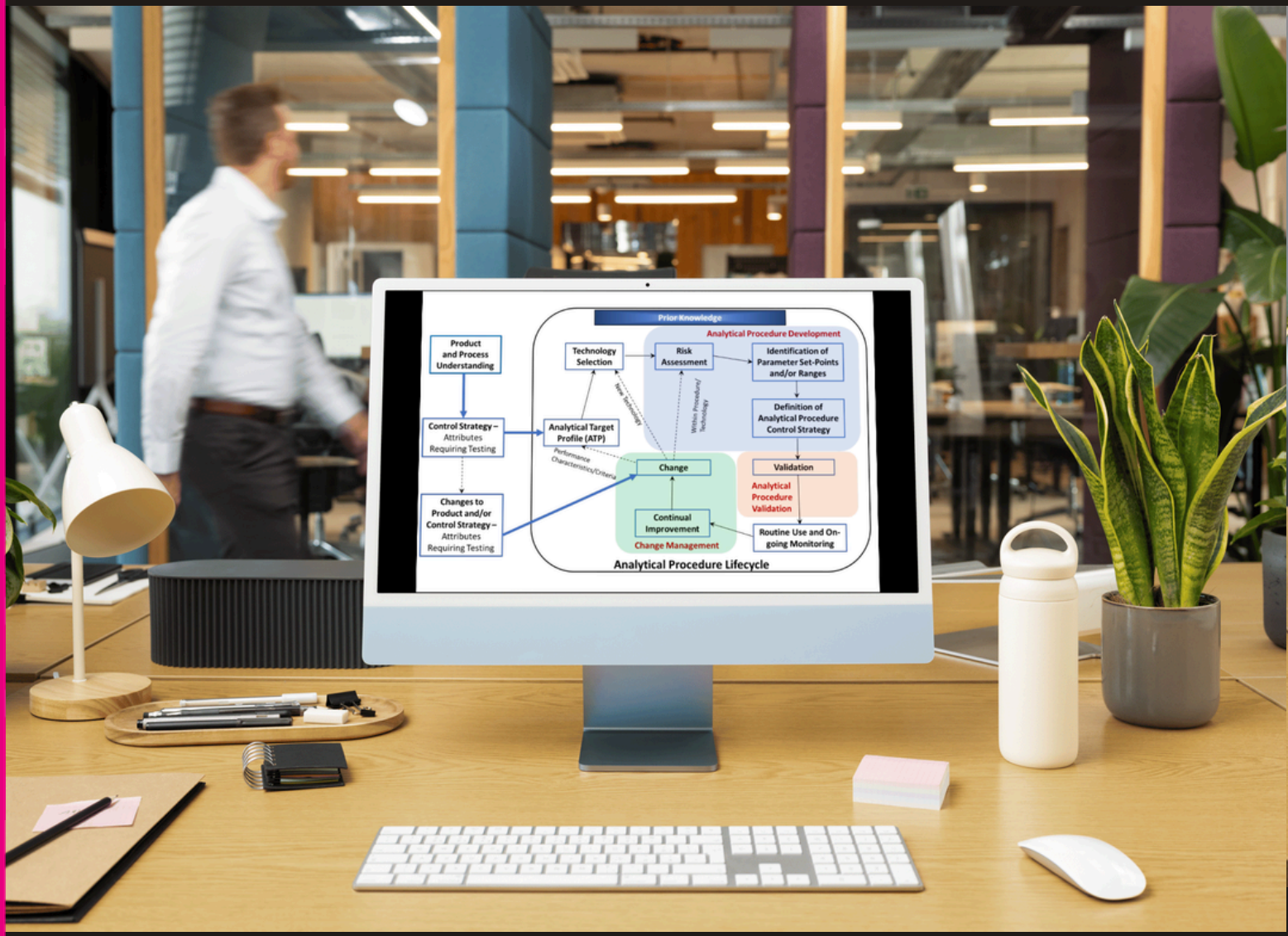
A Practical Guide to Implementing ICH Q14



Kantisto

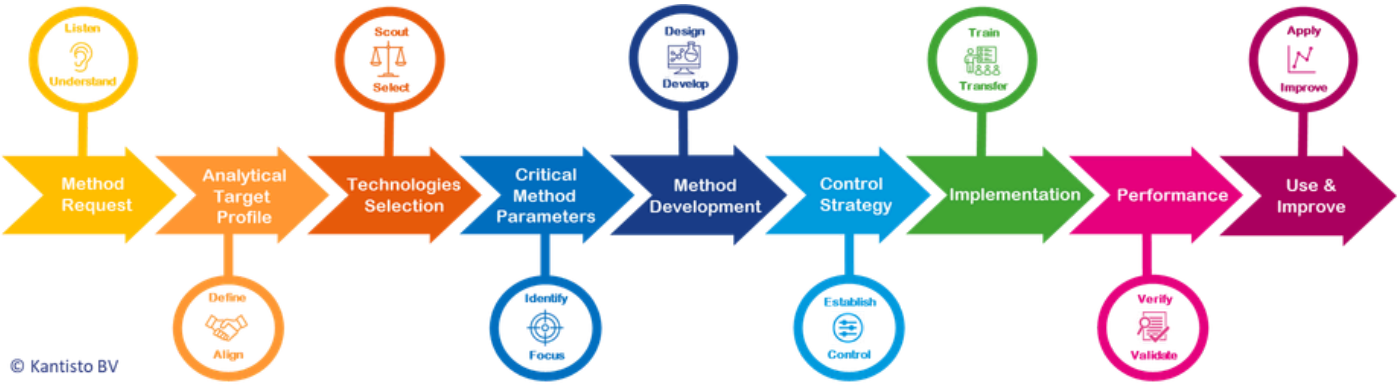
INNOVATING PHARMACEUTICAL ANALYSIS

Do you feel overwhelmed when reading **ICH Q14**?



Unsure where to begin?

We've successfully taught this simplified approach to **1000+** professionals:



Want to see how this simplified approach works?

Method Request

1

Listen to truly understand the analytical need

Translate it into a clear, actionable request

Make expectations explicit before you start

Name of Requester	Who is the contact person?
Department	Which department(s) needs the analytical method?
Project	For which project?
Purpose of study	What conclusions will be drawn based on the analytical result?
Clinical phase	Tox, pre-clinical, phase 1 – 2a, phase 2b – 3, commercial
Compound name	Which analyte needs to be analyzed?
Concentration	What is the concentration range of the analyte
Product description	What are the properties of the product?
Matrices	What is the composition of the matrix?
When needed?	Analytical method needed within 3 months

Analytical Target Profile (ATP)

Define all technical and business requirements
Align on measurable targets for method success

The **ATP** is a list of analytical and business requirements from the **Requester**, **method developer**, **end-user**, and the possible **future end-user**

The Analytical Target Profile (ATP)

	Summary of request		Priority	Targets and ranges	
REQUESTOR	Product quality attribute			Virus particle concentration determination (in VP/ml)	
	Clinical phase			Phase I – commercial	
	Aim of study/method			**1)** For adenovirus production process development and product characterization a precise, accurate, robust, degradation sensitive, and a high-throughput method is needed for the quantitation of adenovirus particles in all process intermediates, DS, and DP.	**2)** An accurate adenovirus amount should be loaded upon the AEX-filters, to avoid filter blockage, excessive viral dilution, and impurity break through and to avoid product degradation whilst testing, a precise, accurate, robust, and fast method for adenovirus quantitation is required for in-process control testing (IPC).
	Product description and/or process step		1	Crude harvest, Lysed harvest, Clarified harvest (IPC), Anion exchange product, Diafiltration product, Drug substance, Drug product	
	Analyte(s)		1	Adenovirus type 26 and 35	
	Matrices			The matrix could contain dell derbis, residual DNA, salt, protein and or formulation buffer components dependent on the process intermediate	
	Concentration range(s)		3	10^10 - 10^12 VP/ml	
	Requester requirements	Time-to-result	2	< 2 days for process development (1), < 4 h for IPC testing (2)	
METHOD DEVELOPER	Type of test			Content / potency	
	Required validation status			Qualified	
	ICH Q2-characteristics	Specificity		Detection of Ad26 and Ad35 without significant interference of sample matrix components	
		Range / linearity	3	$1x10^{10} – 1x10^{12}$ VP/ml	
		Accuracy	3	≤ 10% bias	
		Precision / Intermediate Precision	2	Intermediate precision ≤10% RSD	
END-USERS	End-user			Quality control	
	End-user requirements	Throughput	3	1 sample per run for IPC testing, > 7 samples per process development run. > 300 samples a month for process development.	

Technologies selection

Systematically evaluate suitable technologies

Select based on risk, performance, and timelines

STEP 1: Prioritize the ATP requirements

STEP 2: Map all possible technologies

STEP 3: List estimated figures of merit

STEP 4: Colour score technologies based on risk and feasibility (high, medium, low)

STEP 5: Select 1 or more technologies based on risks and timelines

	Requirements		Virus particle concentration determination (in VP/ml)	Priority	Techniques							
					OD260	AEX-LC-UV	RP-UPLC-UV	SEC-LC	qPCR	CE-UV	Virocyt	AF4-UV
Requester	Process intermediate(s) and matrix		Crude harvest, Lysed harvest, Clarified harvest (IPC), Anion exchange product, Diafiltration product, Drug substance, Drug product. The matrix could contain dell derbis, residual DNA, salt, protein and or formulation buffer components dependent on the process intermediate	1	Only DS/DP	Only DS/DP	All	Only DS/DP	All	All	All	Only DS/DP
	Requester requirements	Time-to-result	< 4 h for IPC testing	2	< 4 h	4 h	4 h	4 h	4 h	1 h	1 h	4 h
Method developer		Specificity	Detection of Ad26 and Ad35 without significant interference of sample matrix components	1	Aromatic peptides and nucleotides	Adenovirus particles	Adenovirus protein	Adenovirus particles and protein aggregation	Adenovirus encapsulated DNA	Adenovirus particles	Adenovirus DNA and protein epitope	Adenovirus particles
		Accuracy	Accuracy (spiked recovery)	2	80 - 120%	80 - 120%	75 - 125%	80 - 120%	70 - 130%	90 -110%	Unknown	80 -120%
		Precision	Intermediate precison ≤10% RSD	2	20%	10 - 20%	10 - 20%	10 - 20%	10 - 35%	5%	9% (uncertain)	20%
		Concentration range	10^10 - 10^12 VP/ml	3	> 10^{10} VP/ml	> 10^{10} VP/ml	> 10^{10} VP/ml	> 10^{10} VP/ml	> 10^{9} VP/ml	> 10^{10} VP/ml	> 10^{7} VP/ml (uncertain)	> 10^{10} VP/ml
End-user	End-user		Quality control		Yes	Yes	Yes	Yes	Yes	Yes	Maybe	No
	End-user requirements	Throughput	1 sample per run for IPC testing, > 7 samples per process development run. > 300 samples a month for process development.	2	> 300	300	300	300	< 300	> 300	> 300	300
	Equipment availability		Yes		Available	Available	Available	Available	Available	Available	Not available	Not in QC

Technique of Choice

Critical method parameters (CMPs)

Identify parameters that impact ATP performance

Focus only on what truly drives method risk

Use a criticality assessment to turn 60+ parameters into 10–20 critical ones

1 Mapping parameters

2 Score effect and certainty

3 List of CMP, nCMP and pCMP

Method parameters	EFFECT (Low, Medium, High)	CERTAINTY (Low, Medium, High)	Proceed to risk assessment?	Score
Buffer pH	high	high	Yes	CMP
Buffer type	high	high	Yes	CMP
Capillary coating	high	high	Yes	CMP
Capillary conditioning	high	High	Yes	CMP
Sample type and matrix	high	high	Yes	CMP
Detection wavelength	high	high	Yes	CMP
Injection mode	high	high	Yes	CMP
Capillary storage	high	high	Yes	CMP
Cassette temperature	high	high	Yes	CMP
BGE degassing	high	medium	Yes	CMP
BGE filtration	high	medium	Yes	CMP
Injection volume	medium	high	Yes	CMP
Applied voltage	medium	high	Yes	CMP
Sample tray temperature	medium	high	Yes	CMP
Sample treatment (remove DNA)	High	low	Maybe	pCMP
Benzonase storage	High	low	Maybe	pCMP
CE vials	Low	high	No	nCMP
CE lamp equilibration time	Low	high	No	nCMP
Pipette step for sample transfer	Low	high	No	nCMP
Capillary length	Low	high	No	nCMP
Capillary diameter	Low	high	No	nCMP
Capillary alignment interface	Low	high	No	nCMP
... (50 additional rows)	...	...	...	...

Critical method parameters (CMPs)

Quantify risk for each parameter

Prioritize experiments where risk is highest

Use risk assessment to focus method development and experiments on the critical parameters that most impact your results.

1 Cause and effect

2 Quantify the risk

3a Proposed experiments

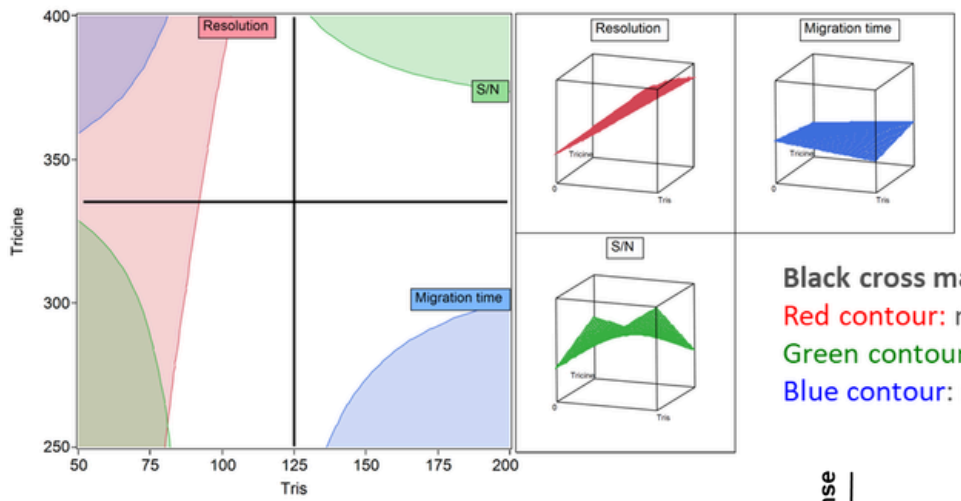
#	CMP	ATP requirement affected	Possible cause and effect	Risk score before mitigation: Effect (1-10)	Probability (1-10)	Score (before mitigation)	Proposed experiments
1	Capillary coating	Bias and precision	Virus adsorption to the capillary wall could impact bias and precision	10	8	80	Test multiple capillaries and coatings and select capillary + coating with good virus recovery
2	Separation Buffer pH	Bias	A pH that is too low or too high could cause virus degradation	10	8	80	Find the optimal pH range for adenovirus stability
4	BGE composition	Bias and precision	Inadequate BGE composition could cause the matrix components or adenovirus to adsorb, precipitate, or aggregate and cause an inaccurate and imprecise results	10	8	80	Screening of BGE and capillaries Screening to reduce adsorption Optimize conditions
5	Separation Buffer pH	Precision	Variations in the BGE pH might impact the migration time precision of the method	6	10	60	Prepare the BGE in such a way that the pH is reproducible
6	Sample type and matrix	Bias and precision	Sample matrix components like DNA, protein, or salts could impact the precision and bias	10	8	80	Evaluate whether a sample treatment is required for a set of representative samples from DSP and USP
7	Detection wavelength	LOD and LOQ	If the wrong detection wavelength is selected, this could result in low or no S/N values.	10	8	80	Evaluate and select a wavelength that allows for adenovirus detection and quantification
...	...	...	...	...	...	...	...

Method development

Understand parameter behaviour and interactions
Use DoE to define your design space

Design of Experiments (DoE) Multivariate approach

- Structured
- Systematic
- Planned
- Interaction of method parameters can be identified and quantified
- Identify true optimal settings and understand the design space.



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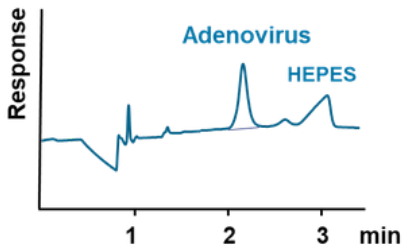
Red contour: resolution of 2.5
Green contour: Signal-to-noise of 150
Blue contour: run time of 6 min



Control strategy

Define, **describe**, and **control** your method parameters
Implement trending & monitoring, and system suitability controls

3 ways to mitigate and reduce the risk for your critical method parameters:

Define it	Describe it	Control it
• Buffer: 125 mM tris and 338 mM tricine • Detection at 214 nm	Mix the sample by pipetting up and down 5 times with a 100 µl pipette.	Adenovirus control sample at 1 x 10^{11} vp/ml Criterion: 0.85 – 1.15 x 10^{11} vp/ml

How?

- Experiments
- Theory/literature
- Common knowledge

Control strategy

Translate knowledge into an analytical control strategy

Define acceptance criteria and ongoing performance checks

Define acceptance criteria, SST, SSC, control samples, and reference standards

				Risk score before mitigation					Risk score after mitigation		
#	CMP	ATP requirement affected	Possible cause and effect	Effect (1-10)	Probability (1-10)	Score (before mitigation)	Proposed experiments	Mitigations	Effect (1-10)	Probability (1-10)	Score (after mitigation
1	Capillary coating	Bias and precision	Virus adsorption to the capillary wall could impact bias and precision	10	8	80	Test multiple capillaries and coatings and select capillary + coating with good virus recovery	A PVA coated capillary was selected to prevent adsorption of the adenovirus. Optimized capillary conditioning in between injections.	10	3	30
2	Separation Buffer pH	Bias	A pH that is too low or too high could cause virus degradation	10	8	80	Find the optimal pH range for adenovirus stability	The optimal pH range was determined as pH 6.0 – 8.5.	10	1	10
4	BGE composition	Bias and precision	Inadequate BGE composition could cause the matrix components or adenovirus to adsorb, precipitate, or aggregate and cause an inaccurate and imprecise results	10	8	80	Screening of BGE and capillaries Screening to reduce adsorption Optimize conditions	Use tris/tricine buffer. Add PS-20 to BGE. Set BGE to 125 mM tris and 338 mM tricine and 0.2% w/v PS-20. Introduce an adenovirus control sample to monitor accuracy and precision	10	1	10
5	Separation Buffer pH	Precision	Variations in the BGE pH might impact the migration time precision of the method	6	10	60	Prepare the BGE in such a way that the pH is reproducible	Tris and tricine at fixed concentrations (instead of titrated) to reduce the pH and ionic strength variation	6	1	6
6	Sample type and matrix	Bias and precision	Sample matrix components like DNA, protein, or salts could impact the precision and bias	10	8	80	Evaluate whether a sample treatment is required for a set of representative samples from DSP and USP	A sample treatment was implemented for USP samples to remove residual DNA. The precision and bias were determined and acceptable for three representative samples: CH, AEX, and DS	10	3	30
7	Detection wavelength	LOD and LOQ	If the wrong detection wavelength is selected, this could result in low or no S/N values.	10	8	80	Evaluate and select a wavelength that allows for adenovirus detection and quantification	A wavelength of 214 nm was selected to analyze adenovirus.	10	1	10
...	...	...	...	...	...	...	...	...	...	...	...

Implementation

Ensure robust method transfer

Train teams to execute consistently

What is often thought: ***We are done! Method is developed!***

However...

Implementation and lifecycle management typically take 30–40% of total development time.

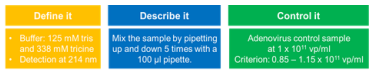
Implementation

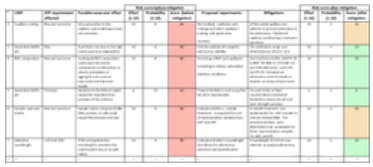
Ensure robust method transfer

Train teams to execute consistently

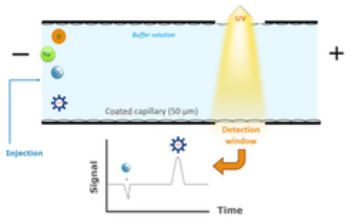
Key aspects of method implementation:

1. **The analytical procedure**
   a. Write the analytical procedure with maximum precision and clarity
   b. Focus on: Define, Describe and Control

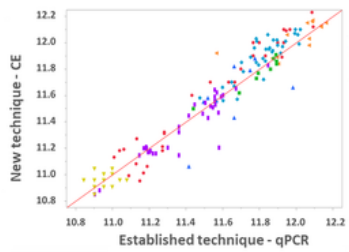




2. **Knowledge transfer and training**
   a. Risk assessment contains all critical knowledge
   b. Method specific training + theoretical and technology training



3. **Method comparison**
   a. Is the historical data still comparable?



Performance

Confirm performance against ATP targets
Validate according to **ICH Q2(R2)**

A validation experiment is a documented demonstration that an analytical method is suitable, reliable and reproducible for its intended purpose.

A successful validation, even with very tight results, does not guarantee future performance or demonstrate method robustness.

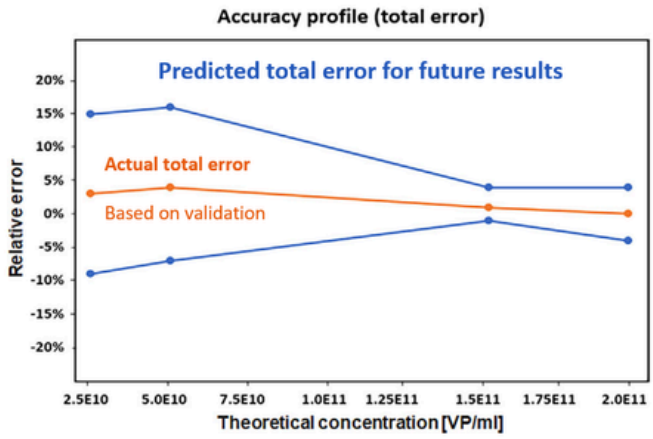
Traditional validation approach

Validation parameter	Acceptance criteria	Result
Method repeatability on concentration (vp/ml)	<10% CV	1.1 – 4.3% CV
Intermediate precision on concentration (vp/ml)	<10% CV	1.3 – 5.2% CV
Linearity	90% CI of slope between 0.9 and 1.1	0.93 – 0.99
Accuracy (spiked recovery)	90 - 110%	91 – 109%
Range	0.5 x10^{11} to 1.5 x10^{11} adenovirus particles per ml with sufficient accuracy and precision	Pass

How well was my method performing in that validation experiment

Total error approach

Total error = accuracy error + precision error



How well is method performing in the future

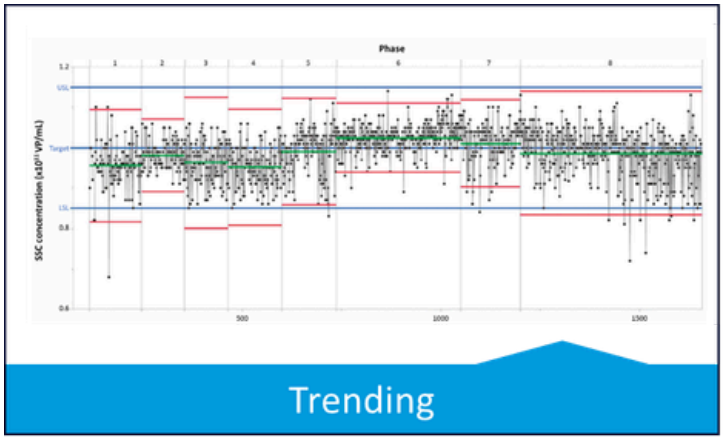
A maximum total error of 15%

Use and Improve

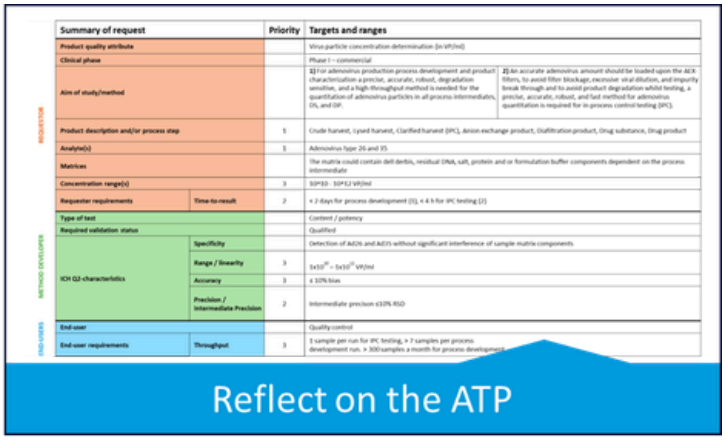
Apply the method in real use

Continuously monitor and improve performance

This ongoing use, trending, and review show whether the method is robust and performs as intended over time



Trending



Reflect on the ATP

Issue logging

Method improvement and retraining

References

Electrophoresis

WILEY-VCH

ELECTROPHORESIS

RESEARCH ARTICLE OPEN ACCESS

A Practical Approach to Implementing ICH Q14: Tools for Analytical Quality by Design in Capillary Electrophoresis Method Development

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ABSTRACT

The ICH Q14 guideline introduces a structured framework for analytical method development based on Analytical Quality by Design (AQbD) principles, aiming to ensure robust, reliable, and fit-for-purpose methods throughout the product lifecycle. However, implementing ICH Q14 remains challenging due to the lack of complete examples and training resources, making it difficult for organizations to translate theory into practice. Although previous studies have applied AQbD to capillary electrophoresis method development, many have focused only on specific aspects such as the design of experiments (DoEs) or analytical target profile (ATP), leaving a gap in providing comprehensive, practical tools for the entire analytical lifecycle. This manuscript presents a novel, user-friendly approach to implementing ICH Q14 and AQbD, offering ready-to-implement tools and methodologies that simplify the process of method design, optimization, validation, and implementation. Through a stepwise process, the approach provides practical solutions for integrating AQbD principles into everyday workflows, bridging the gap between theoretical concepts and real-world applications. The approach has been thoroughly tested in diverse industrial settings, demonstrating its reliability and effectiveness. This work aims to facilitate the adoption of AQbD in analytical method development by providing structured tools, lessons learned, and best practices that align with ICH Q14 guidelines.

Abbreviations: Ad, adenovirus; Ad26, adenovirus serotype 26; Ad35, adenovirus serotype 35; AEX, anion exchange; AEX-HPLC, anion exchange HPLC; AF4, asymmetric flow field-flow fractionation; APCS, analytical procedure control strategy; AQbD, analytical quality by design; ATP, analytical target profile; BGE, background electrolyte; CCD, central composite design; CH, clarified harvest; CMP, critical method parameter; CQA, critical quality attribute; CR, crude harvest; CRS, chemical reference substances; DF, diafiltration/ultrafiltration product; DM-β-CD, dimethyl-β-cyclodextrin; DNA, deoxyribonucleic acid; DoE, design of experiments; DS, drug substance; DSP, downstream processing; FCCD, face centered central composite design; FDA, Food and Drug Administration; FMEA, failure-mode effect analysis; HA, hemagglutinin; HDMS-β-CD, hexadecyl-modified β-cyclodextrin; ICH, The International Council for Harmonization; IPC, in-process control; LH, lysed harvest; LSL, lower specification limit; MODR, method operable design region; nCMP, non-critical method parameter; OD260, absorbance spectrophotometry at 260 nm; OFAT, one factor at a time; PAR, proven acceptable ranges; pCMP, potential critical method parameter; QC, quality control; qPCR, quantitative polymerase chain reaction; QTPP, quality target product profile; rCE-SDS, capillary gel electrophoresis with sodium dodecyl sulfate under reducing conditions; RP-LC, reversed phase liquid chromatography; RSM, response surface model; SDS–PAGE, SDS–polyacrylamide gel electrophoresis; SRID, single radial immunodiffusion; SSC, system suitability control; SST, system suitability test; S-γ-CD, sulfated γ-cyclodextrin; TETA, triethylenetetramine; USL, upper specification limit; USP, upstream processing; UV, ultraviolet.

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