Analytical lifecycle management within etherna

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With the establishment of Quality by Design in support of pharmaceutical development (ICH Q8 (R2)), a similar approach has been proposed over the recent years to support analytical procedure development (AQbD).

To this end, two new official guidelines have been issued: USP General Chapter <1220> Analytical Procedure Life Cycle (May 1st, 2022) and ICH Q14 Guideline on analytical procedure development (June 14th, 2024). This insight article provides an overview of how these QbD principles are implemented within etherna to support both process/product and analytical lifecycle activities, as each lifecycle is inherently connected to the other. This is illustrated in Figure 1.

Process/product lifecycle

The aim of pharmaceutical drug substance and drug product development (ICH Q11 and ICH Q8 (R2)) is to design a quality product and its manufacturing process to consistently deliver the intended performance of the product with respect to safety and efficacy. This goal can be achieved by choosing either a traditional, empirical approach or a more enhanced, systematic approach to product development, or a combination of both. The systematic approach implies applying QbD principles to ensure that product quality is built in by design throughout the lifecycle of the product. This is achieved by incorporating scientific knowledge, experimental design, and quality risk and knowledge management, using a Pharmaceutical Quality System.

Pharmaceutical development starts with defining the **Quality Target Product profile (QTPP)**. This is a prospective summary of the quality characteristics of a drug product that will ideally be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product. It forms the basis of design for the development of the product and can be updated throughout the product lifecycle.

Next, potential **Critical Quality Attributes (CQAs)** are identified based on their impact towards Safety, Quality, Identity, Purity and Potency (SQIPP) so that these product characteristics can be studied and controlled. Figures 2 and 3 show the potential CQAs for mRNA drug substance and drug product where the drug product is mRNA encapsulated in lipid nanoparticles.

CQAs are derived from the QTPP and/or are based on prior knowledge; the rationale for designating the product characteristic as a CQA should always be clearly explained. Identifying potential CQAs are at the basis of production process development, design, monitoring, control, and lifecycle management.[1] Early identification of potential CQAs reduces the risk of product development failures and ensures alignment of product testing and control with regulatory agency expectations. Potential CQAs may be adjusted based upon additional product and process knowledge or regulatory guidance during development.

Risk assessments can be performed to rank or prioritize quality attributes when identifying CQAs for complex products. Analytical procedures must be established for

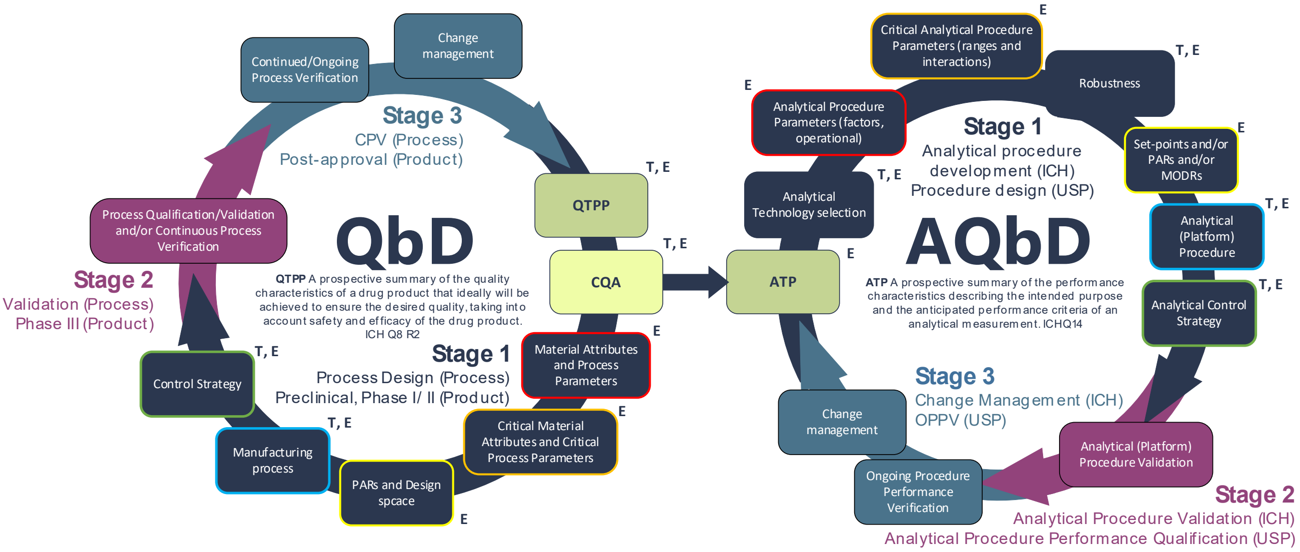


Figure 1: overview of the process/product lifecycle and analytical procedure lifecycle considering the traditional (T) (or minimal) and enhanced (E) development approach. In the enhanced approach Quality is built in by Design by applying QbD/AQbD principles (knowledge management, quality risk management (QRM) and performing design of experiments DoE as a statistical analysis tool.

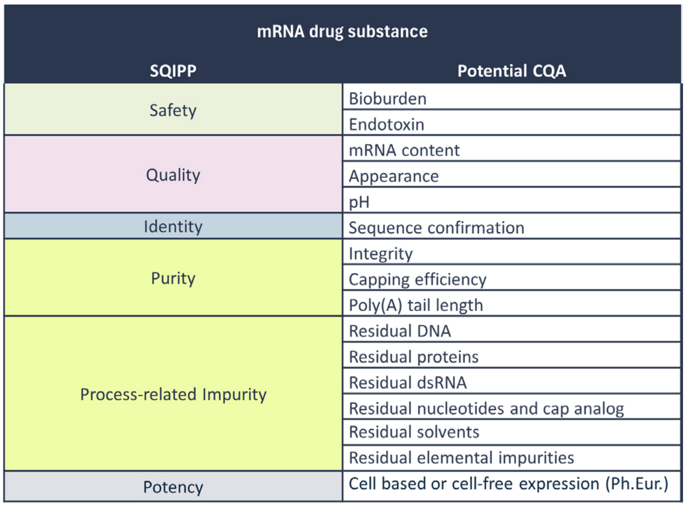
each CQA, and the analytical lifecycle activities should be managed within an **Analytical Lifecycle Program** to ensure control over the analytical procedure's suitability and compliance status throughout its use. For each CQA, product specific phase-appropriate acceptance criteria should be established based on available pre-clinical and clinical data. During a scientific advisory meeting both the sponsor and **etherna** as the reliable Manufacturing Services Provider can concurrently engage in scientific discourse with the required regulatory agency on the proposed strategy and plans during the development phase of new medicinal products.

In the **enhanced risk assessment** model, a risk-based approach is applied to determine/identify which material attributes and process parameters will potentially impact product CQAs and should therefore be considered as critical. Small-scale models can be developed and used to support process development studies. By performing **Design of Experiments** as a statistical tool, the relationship between process inputs (material attributes and process parameters) and the critical quality attributes can be determined and described in the **design space** when establishing the appropriate manufacturing process.

During this process, Proven Acceptable Ranges (PARs) can be determined for relevant process parameters. The design space might be determined per unit operation or as a combination of selected unit operations, and is subject to regulatory assessment and approval. Based on current product and process understanding, the control strategy is established to ensure that a product of required quality will be produced consistently. The control strategy should ensure that each CQA is within the appropriate range, limit or distribution to assure product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated methods and frequency of monitoring and control.

After the process design stage (Stage 1), **process qualification/validation** is performed (Stage 2) and documented before commercial distribution to provide evidence showing whether a process is capable of consistently delivering a quality product. As an alternative to the traditional process validation, continuous process verification can be utilized if an enhanced approach to development has been performed or where a substantial amount of product, process knowledge and understanding has been gained through historical data and manufacturing experience. A combination of traditional process validation and continuous process verification may be employed (hybrid approach).

Finally, in Stage 3, **product quality is monitored** during routine commercial production to ensure that a state of control is maintained throughout the product lifecycle with the relevant process trends statistically evaluated. Continual improvement is assured by applying product lifecycle management using a pharmaceutical quality system. Innovate approaches to improve product quality should be assessed towards their impact on product quality and might imply supportive comparability data and if applicable a verification of the design space should there be a change in the Normal Operating Range (NOR).



mRNA drug substance	
SQIPP	**Potential CQA**
Safety	Bioburden
	Endotoxin
Quality	mRNA content
	Appearance
	pH
Identity	Sequence confirmation
Purity	Integrity
	Capping efficiency
	Poly(A) tail length
Process-related Impurity	Residual DNA
	Residual proteins
	Residual dsRNA
	Residual nucleotides and cap analog
	Residual solvents
	Residual elemental impurities
Potency	Cell based or cell-free expression (Ph.Eur.)

Figure 2: potential CQAs on mRNA drug substance

Analytical lifecycle

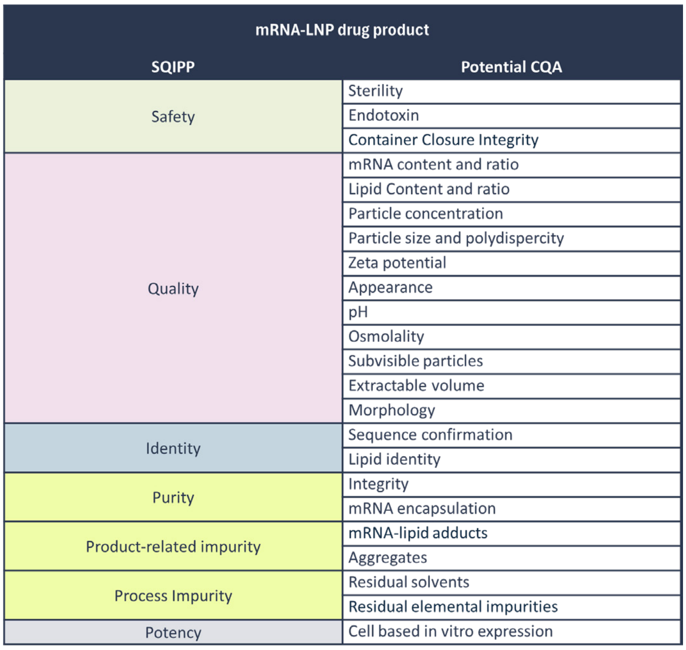
Both ICH Q14 and USP General Chapter <1220> provide a holistic framework that describes the concept of **Analytical Lifecyle Management (ALCM)** as a continuous process that is aligned with QbD concepts and manages the various stages of an analytical procedure to ensure it remains fit for its intended purpose and meets the regulatory expectations throughout its entire lifecycle. The principles of ALCM can be applied in a phase-appropriate manner to analytical procedures used during clinical development. In all cases, the analytical procedure requirements must meet the regulatory expectations at each stage of the product development lifecycle.

The Analytical Lifecycle includes three stages **(ICH/USP)**:

1. Analytical Procedure Development/Procedure Design
2. Analytical Procedure Validation/Analytical Procedure Performance Qualification
3. Change Management (incl. Routine Use and On-going Monitoring/Ongoing Procedure Performance Verification

Throughout the analytical procedure lifecycle, analytical methods transition from a development to qualification/validation status depending on their intended use and the product development phase. For characterization assays and phase I and phase II products, qualification may be the desired end point at that time. For release and stability assays in support of phase III testing, transition to validation is expected. The extent of effort associated with the analytical lifecycle activities should also be consistent with the complexity of the analytical procedure and the criticality of the quality attribute.

In the enhanced approach to analytical development, the lifecycle of an analytical procedure to assess the required CQA is initiated by establishing the **Analytical Target Profile (ATP)**. This is a summary of the performance characteristics describing the intended purpose and the anticipated performance criteria of an analytical measurement, and thus forms the basis for the development of the analytical procedure. Although the most benefit can be gained by establishing the ATP at the start of the product lifecycle, it may also be established retrospectively for established analytical procedures to facilitate ongoing and future lifecycle activities.[2]



mRNA-LNP drug product	
SQIPP	**Potential CQA**
Safety	Sterility
	Endotoxin
	Container Closure Integrity
Quality	mRNA content and ratio
	Lipid Content and ratio
	Particle concentration
	Particle size and polydispercity
	Zeta potential
	Appearance
	pH
	Osmolality
	Subvisible particles
	Extractable volume
	Morphology
Identity	Sequence confirmation
	Lipid identity
Purity	Integrity
	mRNA encapsulation
Product-related impurity	mRNA-lipid adducts
	Aggregates
Process Impurity	Residual solvents
	Residual elemental impurities
Potency	Cell based in vitro expression

Figure 3: potential CQAs on mRNA-LNP drug product

"The principles of ALCM can be applied in a phase-appropriate manner to analytical procedures used during clinical development. In all cases, the analytical procedure requirements must meet the regulator expectations at each stage of the product development lifecycle."

Following the establishment of the ATP, the analytical lifecycle activities per analytical procedure to assess the required quality attribute are mapped out according **etherna**'s product specific Analytical Procedure Lifecyle Workflow (Figure 4). In alignment with the ATP, the most appropriate analytical technology is selected based on prior knowledge, best practices, state-of-the-art technologies, and regulatory expectations or by performing a feasibility study if required.

Any analytical technology that can meet the ATP criteria may be selected. In cases where an Analytical Platform Procedure is already available, this could accelerate or even reduce the required analytical procedure development activities. It is important to note that an Analytical Platform Procedure is an analytical procedure that is suitable to test quality attributes of different products without significant change to its operational conditions, system suitability and reporting structure. This type of analytical procedure may be used to analyze molecules that are sufficiently alike with respect to the attributes that the Analytical Platform Procedure is intended to measure.

Established Analytical Platform Procedures can be replaced by a new analytical technology if comparison as part of a feasibility study identifies that the use of the new analytical technology is preferred to meet the ATP criteria. These continuous analytical lifecycle improvements should be captured in a product specific Analytical Lifecycle Program. Feasibility studies performed to select the most appropriate analytical technology (Figure 4) to support mRNA drug substance (Figure 5) and mRNA-LNP drug product (Figure 6) testing illustrate how QTPP, SQIPP, CQA and ATP relate to each other when selecting the most appropriate analytical technology.

In cases where **Analytical Procedure Development** is required, a minimal (also known as traditional) approach or elements of an enhanced approach can be applied. The enhanced approach integrates the concept of applying Quality by Design in support of analytical procedure development (AQbD) which is quite similar to the QbD process flow as described in ICH Q8 (R2) and ICH Q11, and is therefore science-driven and risk-based to gain enhanced understanding of the analytical procedure throughout its lifecycle.

As part of the enhanced approach, the intention is to identify the analytical procedure parameters or factors (e.g., reagent quality) and operational steps (e.g., flow rate) and assess their potential impact on procedure performance and thus their impact toward meeting the ATP by applying Quality Risk Management tools. **Statistical Design of Experiments** can be further used to generate data with respect to assessing the impact of the analytical procedure parameters on procedure performance and to conduct analytical procedure robustness evaluation with the final intent of identifying set-points, proven acceptable ranges (PARs) and/or the **method operable design region (MODR)** to ensure the ATP is fulfilled and thus providing assurance of the quality of the measured value. Once all conditions and ranges are determined, the precision can be further improved by evaluating and selecting the most suitable sample and standard replication strategy.

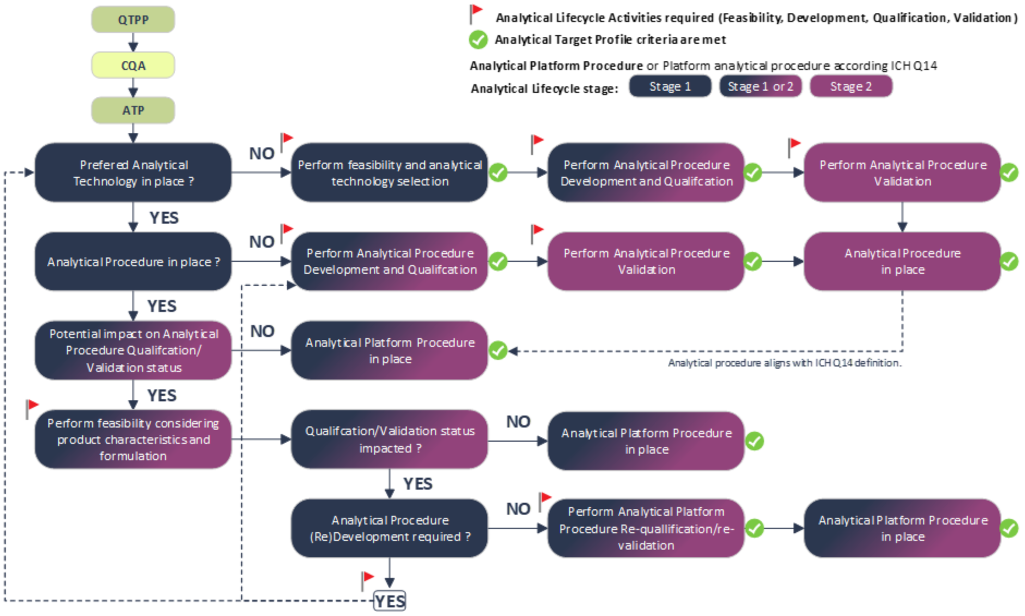
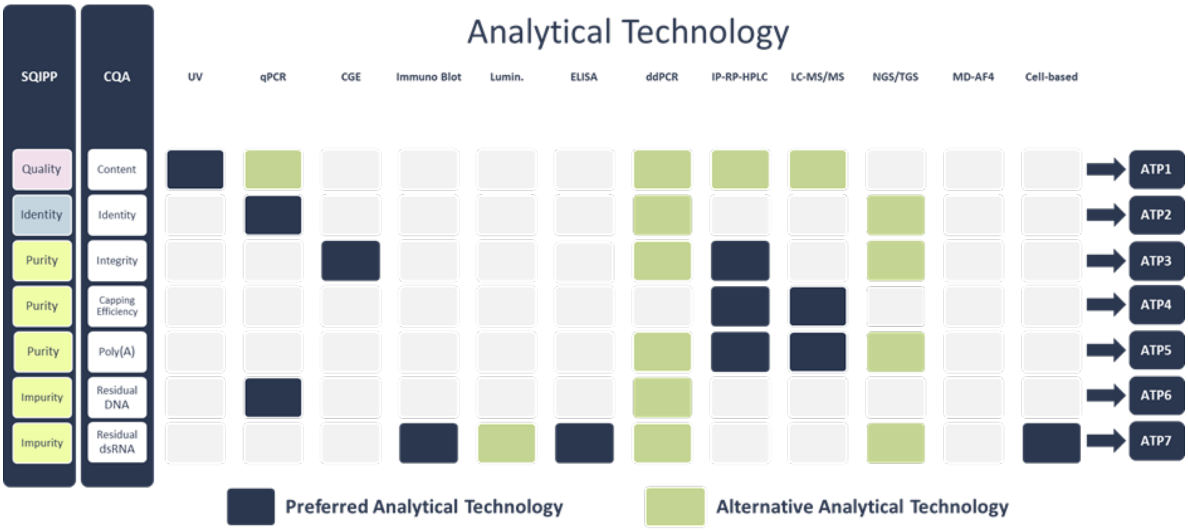


Figure 4: product specific Analytical Procedure Lifecycle Workflow



Analytical Technology

SQIPP	CQA	UV	qPCR	CGE	Immuno Blot	Lumin.	ELISA	ddPCR	IP-RP-HPLC	LC-MS/MS	NGS/TGS	MD-AF4	Cell-based	
Quality	Content	■	🟩					🟩	🟩	🟩				ATP1
Identity	Identity		■					🟩			🟩			ATP2
Purity	Integrity			■				🟩	■		🟩			ATP3
Purity	Capping Efficiency								■	■				ATP4
Purity	Poly(A)							🟩	■	■	🟩			ATP5
Impurity	Residual DNA		■					🟩						ATP6
Impurity	Residual dsRNA				■	🟩	■	🟩			🟩		■	ATP7

■ Preferred Analytical Technology 🟩 Alternative Analytical Technology

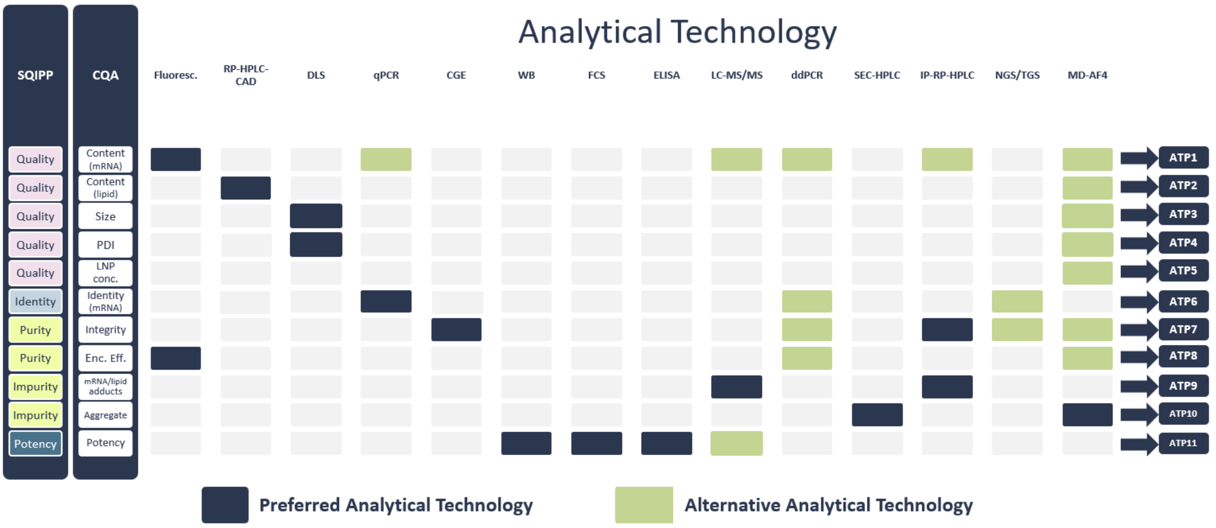
Figure 5: Analytical Platform Technology feasibility studies on mRNA drug substance

Finally, the analytical procedure control strategy is established to ensure that the analytical procedure is fit for the intended purpose during routine use throughout its lifecycle. It consists of a set of controls, derived from current understanding of the analytical procedure including development data, risk assessment, robustness and prior knowledge. The establishment of the analytical procedure control strategy is initiated during analytical procedure development and should be defined before validation (ICH Q2 (R2)) and confirmed once validation is finalized. It includes analytical procedure parameters which must be controlled and the **system suitability test (SST)** which are designed to verify selected analytical procedure attributes (e.g., peak symmetry factor). Additionally, it may also include a sample suitability assessment to ensure validity of the reported result.

The extent of Analytical Procedure Development and Validation activities required for an analytical procedure should be consistent with the product lifecycle stage and its intended use. The objective of validation of an analytical procedure is to demonstrate that the analytical procedure is fit for the intended purpose. For non-compendial analytical procedures, the extent of Analytical Procedure Validation may be based on the Analytical Procedure Performance Characteristics that should be demonstrated for the measured quality attribute using appropriate validation tests as described in ICH Q2 (R2). If an established Analytical Procedure is selected for the intended use, certain validation tests can be omitted based on a science- and risk-based justifications. Analytical procedure changes may be required during he lifecycle of a validated analytical procedure. In such cases, partial or full revalidation may be required. Transfer of a validated analytical procedure should be considered in the context of analytical lifecycle changes in line with ICH Q14. Compendial testing methods are considered validated and their suitability for testing should be verified under actual conditions of use.

The final **Ongoing Procedure Performance Verification (OPPV)** stage of the analytical procedure lifecycle implies the implementation of a continuous analytical procedure performance monitoring program to ensure analytical procedures remain fit for the intended purpose post validation. It provides the opportunity to use a more representative data set to evaluate analytical procedure performance as more analytical procedure in-use data is available over a longer period compared to the limited timeframe in which analytical procedure validation is performed. And although this stage in general is implemented once an analytical procedure is validated, there is added value of implementing Procedure Performance Verification principles in support of early phase analytical testing.

The monitoring program may include reporting and evaluating invalidated Out of Specifications with an analytical related root cause for lot release and long-term stability testing, laboratory quality events metrices, Analytical Procedure Attributes, Assay Control Samples by means of control charts for which the Assay Control Samples are chosen to be identical or representative of the sample to be analyzed. **Such reliable data provides valuable information** to ensure that the ATP is realized throughout the lifecycle and provides the opportunity to conduct investigations and take corrective and preventive actions if there is an indication the analytical procedure is not in control.



Analytical Technology

SQIPP	CQA	Fluoresc.	RP-HPLC-CAD	DLS	qPCR	CGE	WB	FCS	ELISA	LC-MS/MS	ddPCR	SEC-HPLC	IP-RP-HPLC	NGS/TGS	MD-AF4	
Quality	Content (mRNA)	■			🟩					🟩	🟩		🟩		🟩	→ ATP1
Quality	Content (lipid)		■												🟩	→ ATP2
Quality	Size			■											🟩	→ ATP3
Quality	PDI			■											🟩	→ ATP4
Quality	LNP conc.														🟩	→ ATP5
Identity	Identity (mRNA)				■						🟩			🟩		→ ATP6
Purity	Integrity					■					🟩		■	🟩	🟩	→ ATP7
Purity	Enc. Eff.	■									🟩				🟩	→ ATP8
Impurity	mRNA/lipid adducts									■			■			→ ATP9
Impurity	Aggregate											■			■	→ ATP10
Potency	Potency						■	■	■	🟩						→ ATP11

■ **Preferred Analytical Technology** 🟩 **Alternative Analytical Technology**

Figure 6: analytical platform technology feasibility studies on mRNA-LNP drug product

The need for ongoing periodic monitoring will depend on the number of analyses performed and should be risk-based taking the analytical procedure variability and complexity into account as well as its criticality towards the intended use.

References

ICH Q2(R2) Validation of Analytical Procedures.

ICH Q8 (R2) Pharmaceutical Development.

ICH Q9 Quality Risk Management.

ICH Q10 Pharmaceutical Quality System.

ICH Q11 Development and Manufacture of Drug Substances (chemical entities and biotechnological/biological entities).

ICH Q14 Analytical Procedure Development.

USP <1220> Analytical Procedure Life Cycle.

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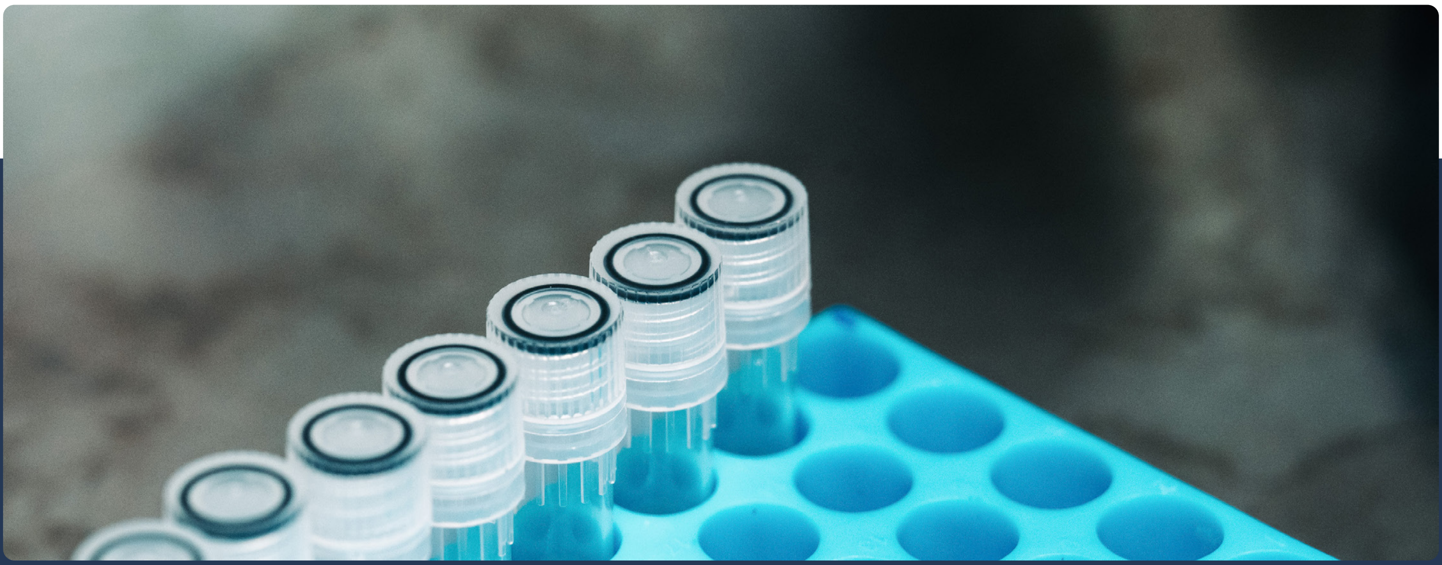

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