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3.2.S.4.3 VALIDATION OF ANALYTICAL PROCEDURES

The analytical procedures used for testing CX-024414 have been validated (non-compendial test methods) by ModernaTX, Inc. and are summarized below.

The transfer, verification and validation of analytical methods to perform testing for CX-024414 at Lonza AG are currently on-going. Analytical Transfer Master Protocol, CHVI-361820, which describes the general process and activities to transfer test methods that are used to support testing at Lonza AG, has been provided. Lonza AG method transfer protocols and final report will be provided once available.

3.2.S.4.3.1 Verification of Compendial Microbiological Test Methods

3.2.S.4.3.1.1 Bioburden (Lonza AG)

The bioburden method verification is carried out according to current versions of the harmonized USP chapter <61>, EP chapter <2.6.12> and JP chapter <4.05> and is provided as CHVI-376286.

3.2.S.4.3.1.2 Bacterial Endotoxin (Lonza AG)

The method verification is carried out according to harmonized method in the current versions of USP chapter <85>, EP chapter <2.6.14>, and JP chapter <4.01> and is provided as CHVI-376210.

3.2.S.4.3.2 Validation of Non-Compendial Analytical Test Methods

The analytical procedures used for testing of CX-024414 were confirmed as suitable for their intended use through executed method validation experiments. The % purity method by RP-HPLC and the % polyA tailed RNA method by RP-HPLC were demonstrated to be stability indicating. The applicable validation parameters and method validation reports (provided as attachments) are provided in Table 1.

Table 1: Summary of Validation Parameters of the Analytical Methods for CX-024414

Attribute	Method	Method Parameter	Method Validation Report (Attached)
Identity (Table 2)	Reverse Transcription/ Sanger Sequencing	Specificity	QC-MVR-0015
Total RNA Content (Table 4)	UV	Specificity, linearity, accuracy, precision, intermediate precision, range, robustness	QC-MVR-0003
Purity (Table 5)	RP-HPLC	Specificity, linearity, precision, intermediate precision, accuracy, range, robustness, QL	QC-MVR-0005
% 5' Capped (Table 6)	RP-UPLC-UV	Specificity, accuracy, linearity, precision, intermediate precision, range, robustness, QL, sample stability	QC-MVR-0006
% PolyA Tailed RNA (Table 7)	RP-HPLC	Specificity, accuracy, linearity, precision, intermediate precision, range, robustness, QL, sample stability	QC-MVR-0007
Residual Plasmid Template (Table 8)	qPCR	Specificity, linearity, range, robustness, accuracy, precision, intermediate precision, DL, QL	QC-MVR-0016

QL = quantitation limit, DL = detection limit

3.2.S.4.3.2.1 Reverse Transcription/ Sanger Sequencing (Identity)

SOP-1019 and Microsynth: Confirmation of mRNA Sequence by RT-PCR and Sanger Sequencing has been validated and shown to be suitable for the purpose of determining the mRNA identity of CX-024414. The validation characteristic evaluated was specificity, as described in Table 2 and Table 3. Refer to QC-MVR-0015 and CHVI-383316 for details of the validation results.

Table 2: Overall Validation Summary for RT-PCR Sanger Sequencing (ModernaTX, Inc.)

Parameter	Acceptance Criteria	Pass/Fail
Specificity (QC-MVP-0015)	CCI [redacted]	Pass
Specificity (QC-MVP-0020)	CCI [redacted]	Pass

Table 3: Overall Validation Summary for RT-PCR Sanger Sequencing (Microsynth)

Parameter	Acceptance Criteria	Pass/Fail
Specificity	• Consensus sequence matches the reference sequence with 100% homology.	Pass

3.2.S.4.3.2.2 UV (Total RNA Content)

SOP-0995: mRNA Concentration by NaOH Digestion has been validated and shown to be suitable for the purpose of determining mRNA concentration of drug substance and in-process samples. The qualification characteristics evaluated were specificity, linearity, precision (repeatability and intermediate), accuracy, range, and robustness as described in Table 4. Refer to QC-MVR-0003 for details of the validation results.

Table 4: Overall Validation Summary for UV

Parameter	Acceptance Criteria	Pass/Fail
Specificity	CCI [redacted]	Pass
Linearity		Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass
Accuracy		Pass
Range		Pass
Robustness		Pass

3.2.S.4.3.2.3 RP-HPLC (% Purity)

SOP-0996: Analysis of mRNA Purity by Size Based RPIP HPLC has been validated and shown to be suitable to assess mRNA purity of drug substance, mRNA containing bulk Lipid Nanoparticles (LNPs) and Drug Products. The validation characteristics evaluated were repeatability precision; intermediate precision; linearity; accuracy; specificity; determination of the quantitation limit; stability of standard and sample preparation solutions; range; and robustness as described in Table 5. Refer to QC-MVR-0005 for details of the validation results.

Table 5: Overall Validation Summary for RP-HPLC

Parameter	Acceptance Criteria	Pass/Fail
Specificity	CCI	Pass
Linearity		Pass
Accuracy (from Linearity)		Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass
Range		Pass
Robustness		Pass
Quantitation Limit (QL)		Pass
Prepared Solution Stability		Pass

Abbreviations: IG = impurity group

3.2.S.4.3.2.4 RP-UPLC-UV (%5' Capped)

SOP-0997: Determination of mRNA-1273 Determination of CAP species by Reverse Phase Ion Pari Ultra-High-Performance Liquid Chromatography, has been validated and shown to be suitable for the purpose of determining the mRNA % 5'CAP of drug substance samples. The validation characteristics evaluated were system suitability, specificity, linearity, accuracy, precision (repeatability and intermediate), sample stability, quantitation limit, range, and robustness as described in Table 6. Refer to QC-MVR-0006 for details of the validation results.

Table 6: Overall Summary for RP-HPLC-UV

Parameter	Acceptance Criteria	Pass/Fail
Specificity	CCI	Pass
Linearity		Pass
Accuracy		Pass
Precision (Repeatability		Pass
Precision (Intermediate)		Pass
Range		Pass
Quantitation Limit (QL)		Pass
Robustness		Pass
Sample stability		Pass

3.2.S.4.3.2.5 RP-HPLC (% PolyA Tailed RNA)

SOP-0994: Percent Poly-A Tailed and Tailed Variant mRNA by RP-HPLC, has been validated and shown to be suitable for the purpose of determining the percent tailed and tailed variant mRNA of drug substance samples. The qualification characteristics evaluated were system suitability, accuracy, precision, intermediate precision, sample and standard stability, specificity, quantitation limit, linearity, range, and robustness as described in Table 7. Refer to QC-MVR-0007 for details of the validation results.

Table 7: Overall Validation Summary for RP-HPLC

Parameter	Acceptance Criteria	Pass/Fail
Specificity	CCI [redacted]	Pass
Linearity		Pass
Accuracy (from Linearity)		Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass
Range		Pass
Robustness		Pass
Quantitation Limit (QL)		Pass
Sample and Standard Stability		Pass

3.2.S.4.3.2.6 qPCR (Residual Plasmid Template)

SOP-1020: Determination of Residual DNA by qPCR in mRNA Product Intermediate, has been validated and shown to be suitable for the purpose of determining quantity of residual plasmid in CX-024414 mRNA. The validation characteristic evaluated was accuracy, precision, intermediate precision as described in Table 8. Refer to QC-MVR-0016 for details of the validation results.

Table 8: Overall Validation Summary for qPCR

Parameter	Acceptance Criteria	Pass/Fail
Accuracy	CCI [redacted]	Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass
Linearity		Pass
Range		Pass
Detection Limit (DL)		Pass
Quantitation Limit (QL)		Pass
Sample Specificity		Pass
Robustness		Pass