

		Category: Method Validation Report Title: Methodvalidierungsbericht Überprüfung der Integrität von 3' Poly(A) tail in BNT162b2 DS	
Version 01	State Effective	Effective Date 25-JUN-2021	Document ID 505545
Legacy Document ID N/A	Confidentiality Business Use Only		Owner Department QC

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Method Validation Report

Method Validation Report of the Verification of 3' Poly(A) tail integrity of BNT162b2 DS.

Quality Control Laboratory

4.2 1st ind

Document ID 505545

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EXECUTIVE SUMMARY

The purpose of this validation study in scope of CR-2021-047 was to validate the RP-HPLC method for verification of the 3' poly(A) tail integrity according to SOP-501868 (see validation plan 503423) for BNT162b2 DS.

For the verification of the 3' poly(A) tail integrity, a full validation according to SOP-8097462 was performed to show the suitability of the method. The method validation study was carried out with one batch to assess the suitability of this analytical method. All validation experiments have been performed using DS material (batch CZ13-P020.2-DS) provided by BioNTech SE Mainz under GMP conditions.

The validation took 9 days and defined test parameters were tested as per [Table 5](#).

The analytical method validation study showed that the analyses were executed consistently, correctly and reproducibly in the QC laboratory and the method is suitable to verify the 3' poly(A) tail integrity of BNT162b2 DS.

All final results fulfilled the predefined acceptance criteria and product specifications defined in the Validation Plan Version 1 (Doc ID 503423).

Based on these results, the method validation is evaluated as successful.

The validation parameters, requirements and results are summarized below (see [Table 6](#)).

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Revision History

Table 1: Revision History

Document No.	Approved	Change	Author
1.0	See approval date	first edition	4.1(b)

Superordinated Documents

Table 2: Superordinated Documents

Document ID	Title
SOP-8097462	Validation of Analytical Procedures
503423	Methodvalidierungsplan Überprüfung der Integrität von 3' Poly(A) tail in BNT162b2 DS
SOP-8093713	Bewertung, Bearbeitung und Genehmigung von Abweichungen oder OOX
SOP-8093567	Initiieren einer Abweichung oder OOX
SOP-7041507	Handhabung und Gebrauch von Referenzmaterialien
(SOP-)501868	Überprüfung der Integrität von 3' Poly(A) tail in BNT162b2 DS mittels RP-HPLC
(FRM-)501869	LTR Überprüfung der Integrität von 3' Poly(A) tail in BNT162b2 DS mittels RP-HPLC

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1 Scope and Objectives of the Validation Study

The scope and objectives of this validation study are summarized in Table 3: Scope and objectives of the validation study.

Table 3: Scope and objectives of the validation study

Topic	Description
Objective	The objective of this validation study was to provide documented evidence that the IP-RP-HPLC according to SOP-501868 to is suitable for the determination of the integrity of the 3' Poly(A) Tail of the BNT162b2 DS mRNA.
Execution based on	Validation protocol: 503423 „Methodvalidierungsplan Überprüfung der Integrität von 3' Poly(A) tail in BNT162b2 DS“
Supplementary studies	N/A
Interim report	N/A
Scope	Testing of 1 Batch BNT162b2 DS “CZ13-P020.2-DS”
Building	4.2 1st ind
CR-Reference	CR-2021-047

2 Description of method

BNT162 b2 contains a poly(A) tail at the 3' end consisting of approximately 4.2 1st ind adenosines with a small linker portion approximately 4.2 1st ind adenosines in. The method described in SOP-501868 “Überprüfung der Integrität von 3' Poly(A) tail in BNT162b2 DS mittels RP-HPLC” is used to confirm the length of poly(A) tail for samples compared to a reference material. The length of tail is determined by digestion of mRNA by the enzymes RNase T1 and RNase A followed by ion-pair-reversed phase high-performance chromatography separation (IP-RP-HPLC) and UV detection. 4.2 1st ind

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3 Execution of the Method Validation

The execution of the method validation is summarized in Table 4: Execution of the validation study.

Table 4: Execution of the validation study

Topic	Description
Validation study was executed according to	Validation protocol: 503423 „Methodenvalidierungsplan Überprüfung der Integrität von 3' Poly(A) tail in BNT162b2 DS“
No. of validation runs	1 DS Batch manufactured and provided by BioNTech SE Mainz
Validation Interim Report	N/A

4 Results

The validation study was performed between 07.06.2021 and 16.06.2021.

Table 5: Timetable of the validation study including reference to the respective documentation

Test Parameter	Date	LTR	Sequences
Specificity	07.06.2021 – 09.06.2021	004	BNT162B2-DS_501868V1_20210607 (invalid) ¹ BNT162b2-DS_501868v1_20210608
Robustness – 4.2 1st ind	11.06.2021 – 12.06.2021	007	BNT162b2-DS_501868v1_20210609 4.2 1st BNT162B2-DS_501868V1_20210611 4.2 1st
Robustness – 4.2 1st ind	09.06.2021 – 10.06.2021	006	BNT162b2-DS_501868v1_20210609
Robustness – 4.2 1st ind	09.06.2021 – 12.06.2021	006	BNT162b2-DS_501868v1_20210609
Robustness – 4.2 1st ind	14.06.2021 – 16.06.2021	008 009	BNT162B2-DS_501868V1_ID_20210614 (invalid) ¹ BNT162B2-DS_501868V1_ID_20210615
Robustness – 4.2 1st ind	14.06.2021 – 16.06.2021	008 009	BNT162B2-DS_501868V1_ID_20210614 (invalid) ¹ BNT162B2-DS_501868V1_ID_20210615

¹ Regarding invalid sequences see section 5.1.1

All listed test parameters were predefined and performed to show the suitability of the Poly(A) tail integrity method.

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The predefined acceptance criteria and the results of the validation tests according to the Validation Plan 503423 are shown in Table 6.

Table 6: Acceptance criteria for the Validation study

Validation attribute	Acceptance criterion	Pass/Fail	Chapter Reference
Specificity	The integrity of the poly(A) tail according to SOP-501868 of the BNT162b2 DS sample batch CZ13-P020.2-DS has to be evaluated as "pass".	pass	4.1
	The integrity of the poly(A) tail according to SOP-501868 of the challenge samples, the co-mix-samples and the DS-buffer sample has to be evaluated as "fail"	pass	
Robustness	4.2 1st ind		4.2.2
			4.2.1
			4.2.1
			4.2.3
			4.2.3

All results were obtained in the validation study executed according to SOP-501868 Version 1 and Validation Plan 503423 Version 1 and were documented in the LTRs and and Chromeleon Sequences as stated in Table 5.

All calculations for the evaluation of the documentation are documented in "Evaluationsheet of Poly(A) integrity validation (attachment to 505545)", stored in ZenQMS as a separate document-ID 512210.

All results are shown in chapter 4.

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4.1 Specificity

Table 7: Excerpt of Results of LTR-501869-004 and Sequence BNT162b2-DS_501868v1_20210608

	Ret. Time [min]	Ret. Time [min]	Resolution	Asymmetry				
Sample	A30_H1	L70	A30_H1 / A30_H2	L70	RRT (A30)	RRT (L70)	Poly(A)-Integrity acc. to SOP-501868	
4.2 1st ind	4.2 1st ind							n.a.
								n.a.
								n.a.
								pass
	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	fail	
	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	fail	
	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	fail	
	4.2 1st ind							fail
								fail
								n.a.
Average Retention Time A30 ("Kontrollen")	4.2 1st ind							
Average Retention Time L70 ("Kontrollen")								

For the assessment of specificity all tests have been performed according to method validation plan 503423 and SOP-501868 and have been documented in LTR-501869-004. According to method validation plan 503423 the BNT162b2 DS sample CZ13-P020.2-DS has to be evaluated as "pass" as per the criteria defined in SOP-501868 (i.e. the RRT of A30 and L70 has to be 4.2 1st ind). Furthermore, the 4.2 1st ind samples have to be evaluated as "fail" as per the criteria defined in SOP-501868 (i.e. 4.2 1st ind). The results are listed in Table 7. All SST-Criteria according to SOP-501868 have been fulfilled. All acceptance criteria according to 503423 have been fulfilled.

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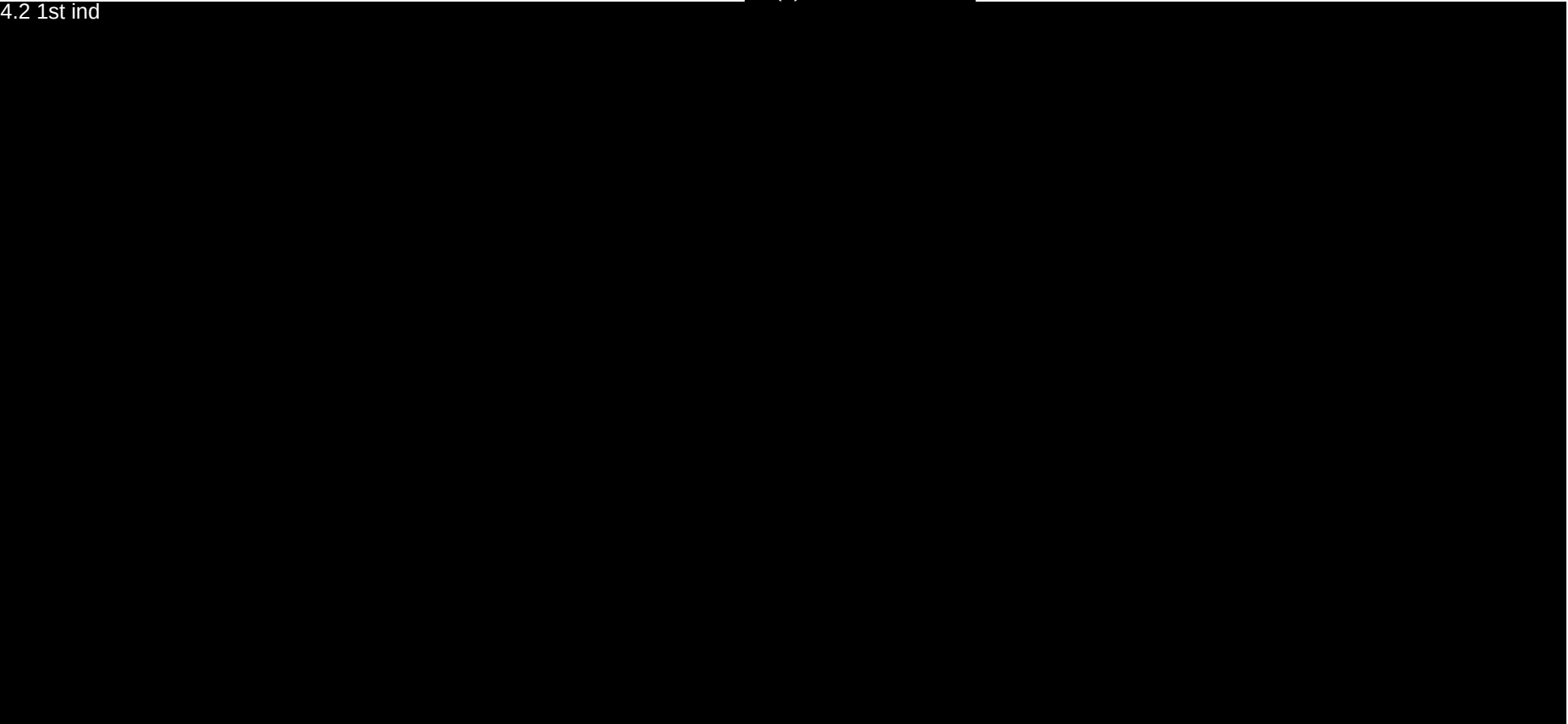


Figure 1: BNT162b2-DS_501868v1_20210608 (LTR 004) DS Reference sample (zoom)

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4.2 1st ind

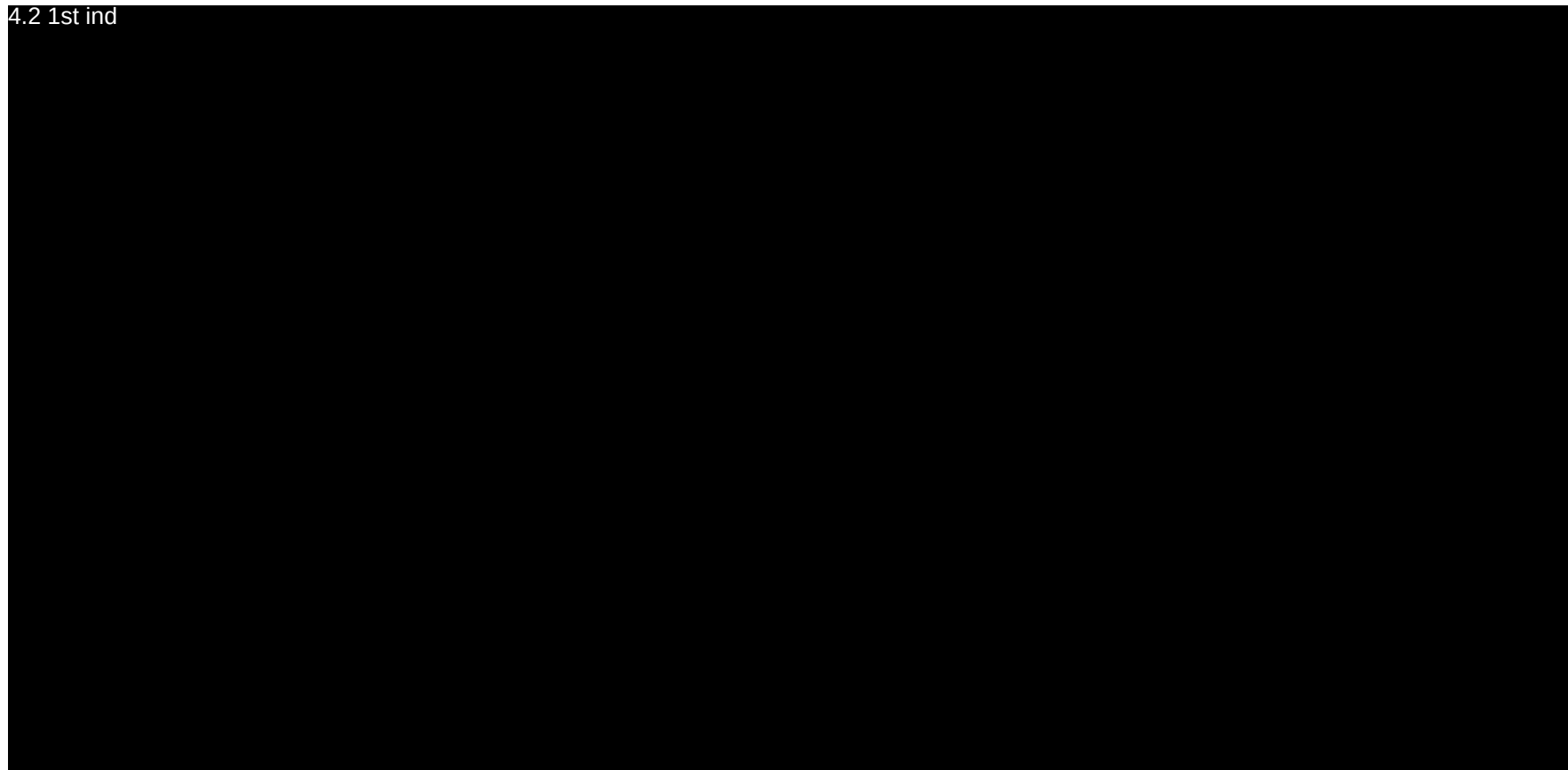


Figure 2: BNT162b2-DS_501868v1_20210608 (LTR 004); BNT162b2 DS sample CZ13-P020.2-DS (zoom)

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4.2 1st ind

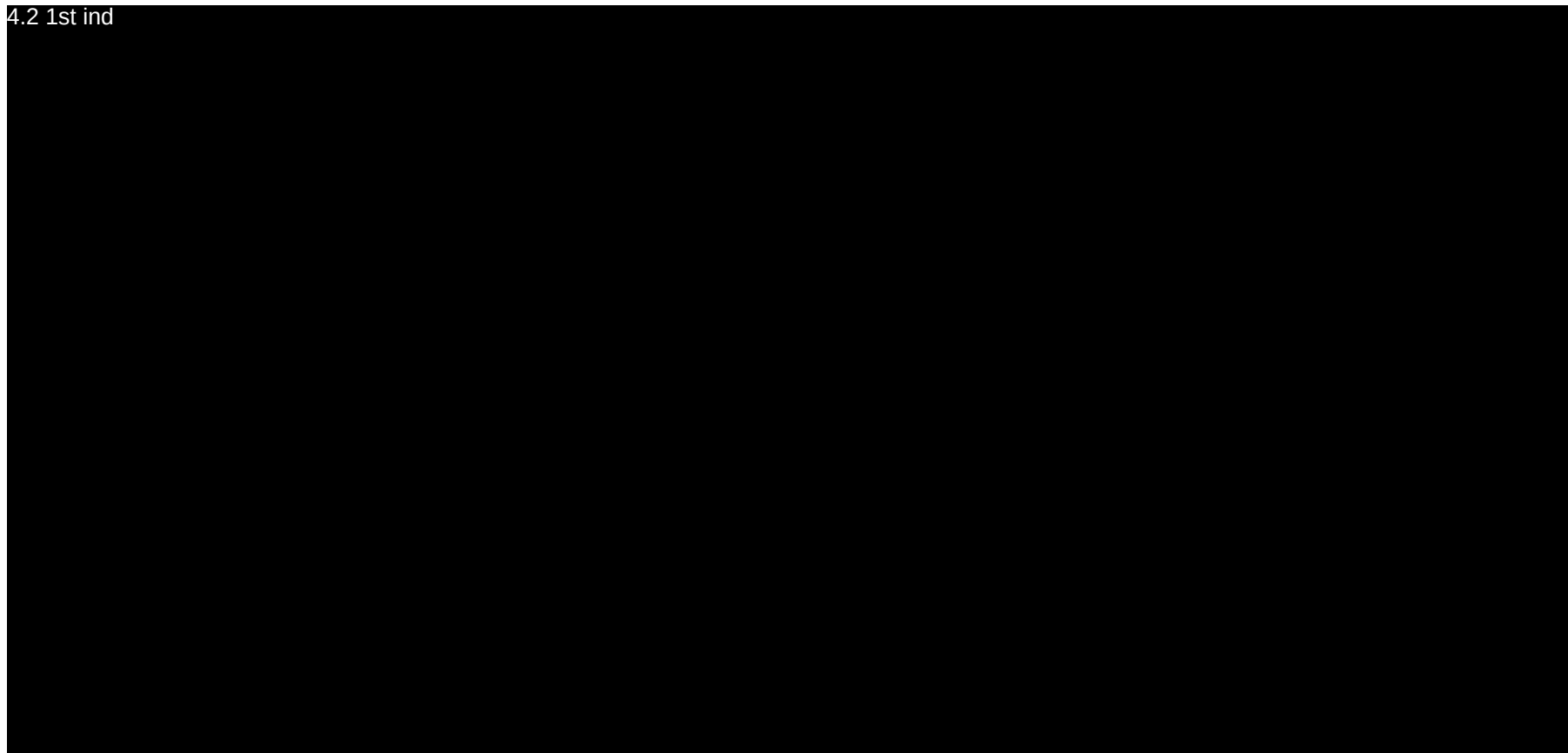


Figure 3: BNT162b2-DS_501868v1_20210608 (LTR 004); Overlay, DS Reference sample (blue) + DS-buffer (black) (zoom)

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4.2 1st ind

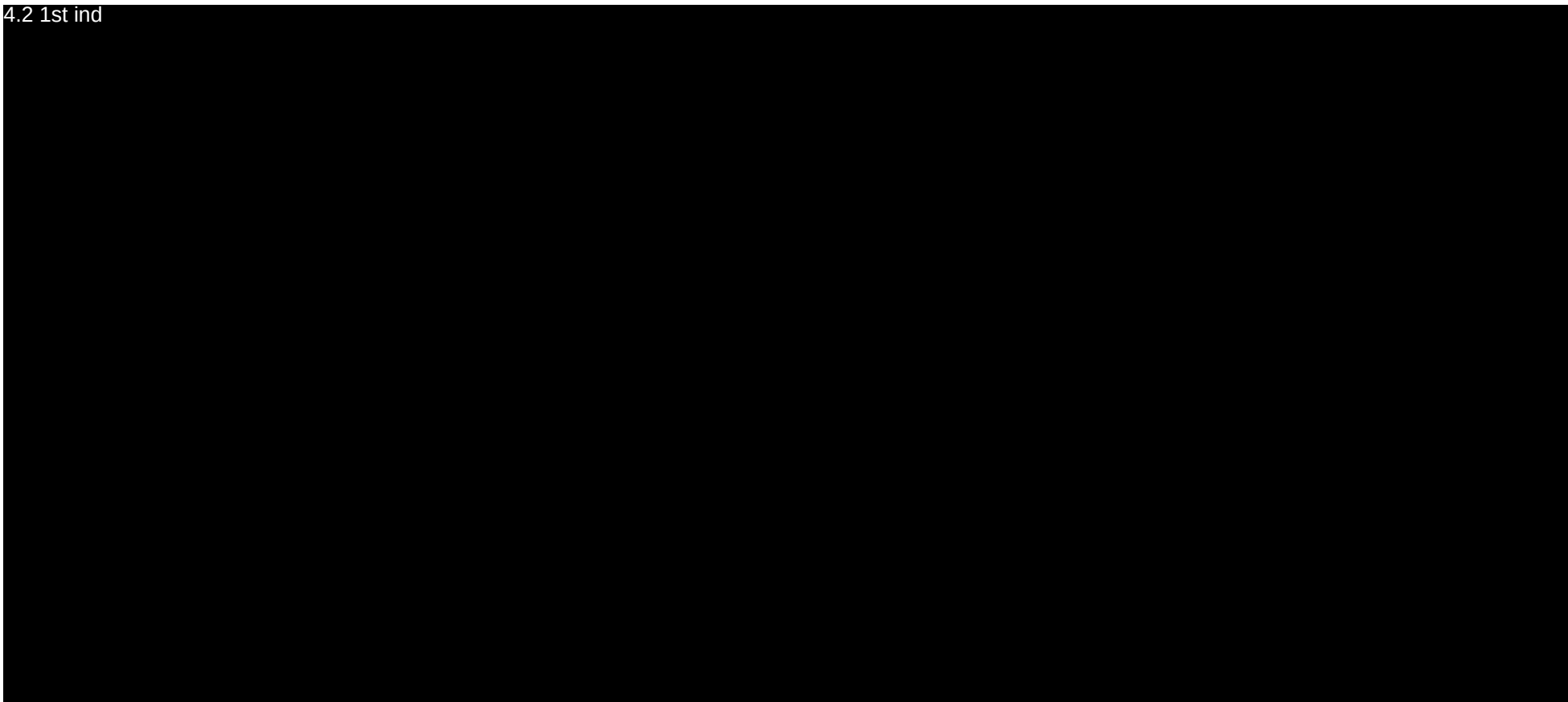


Figure 4: BNT162b2-DS_501868v1_20210608 (LTR 004); 25nt challenge sample (zoom)

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4.2 1st ind

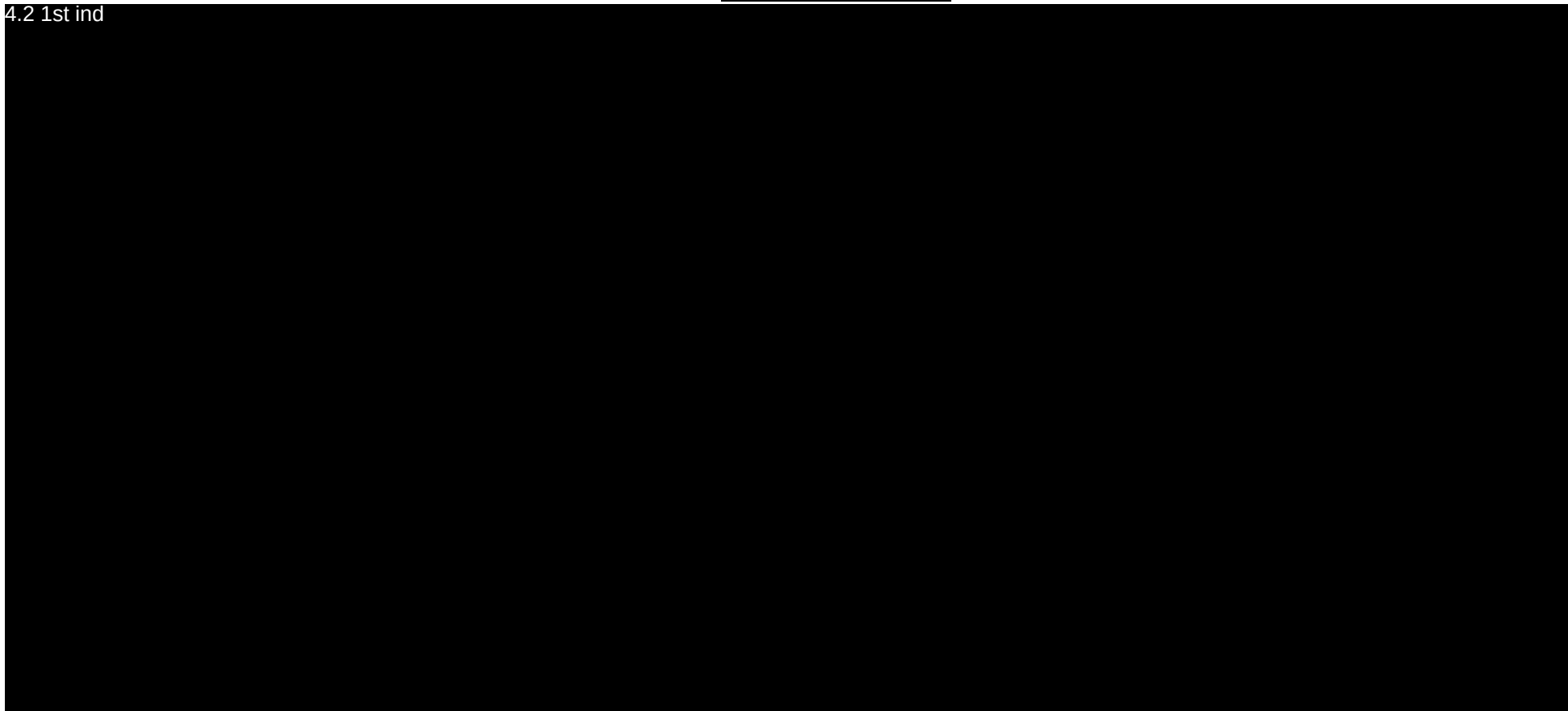


Figure 5: BNT162b2-DS_501868v1_20210608 (LTR 004); 40nt challenge sample (zoom)

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4.2 1st ind

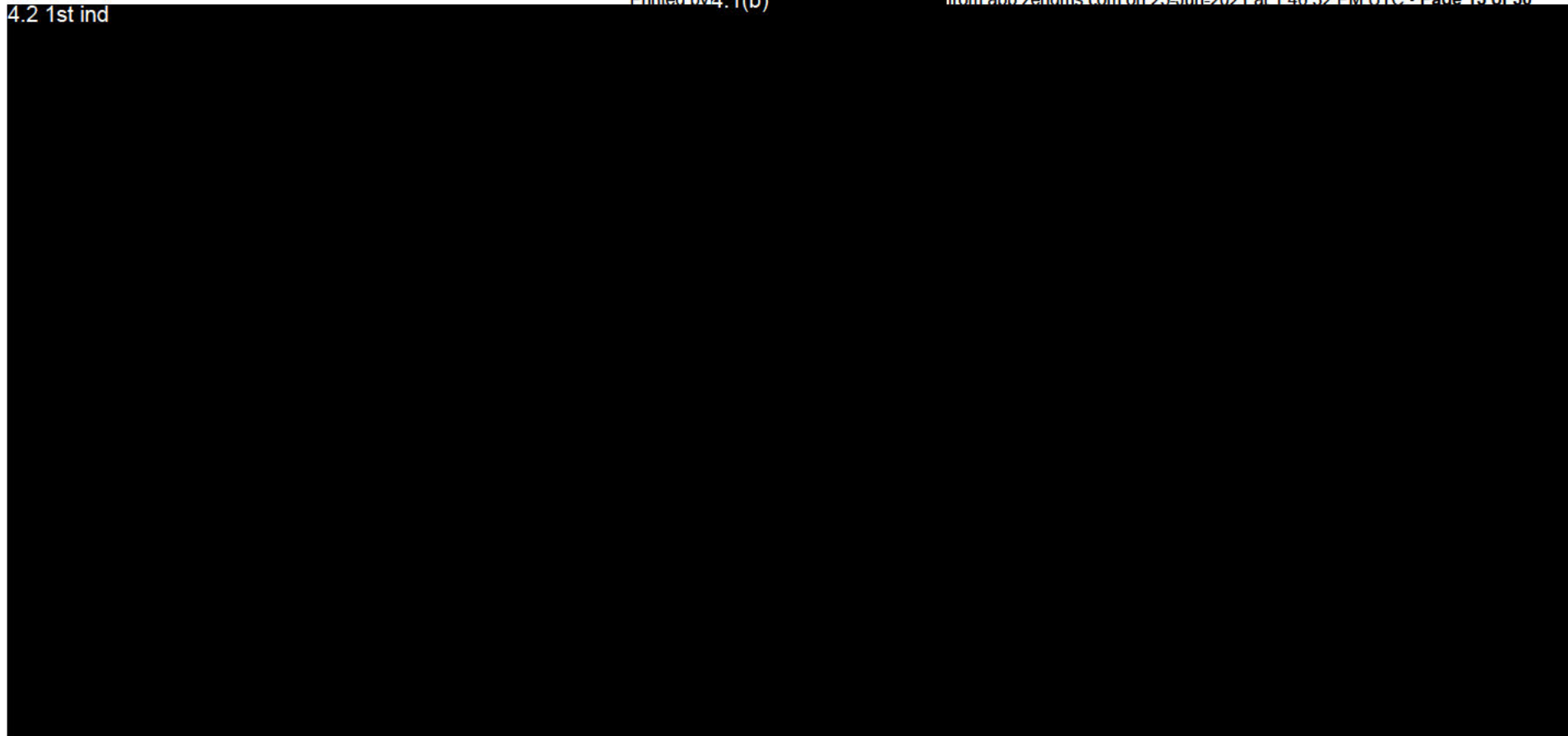


Figure 6: BNT162b2-DS_501868v1_20210608 (LTR 004); 25nt Co-Mix sample (zoom). Remark: the unknown peak derives from the 25nt challenge sample. It had to be integrated due to the run of the baseline.

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4.2 1st ind

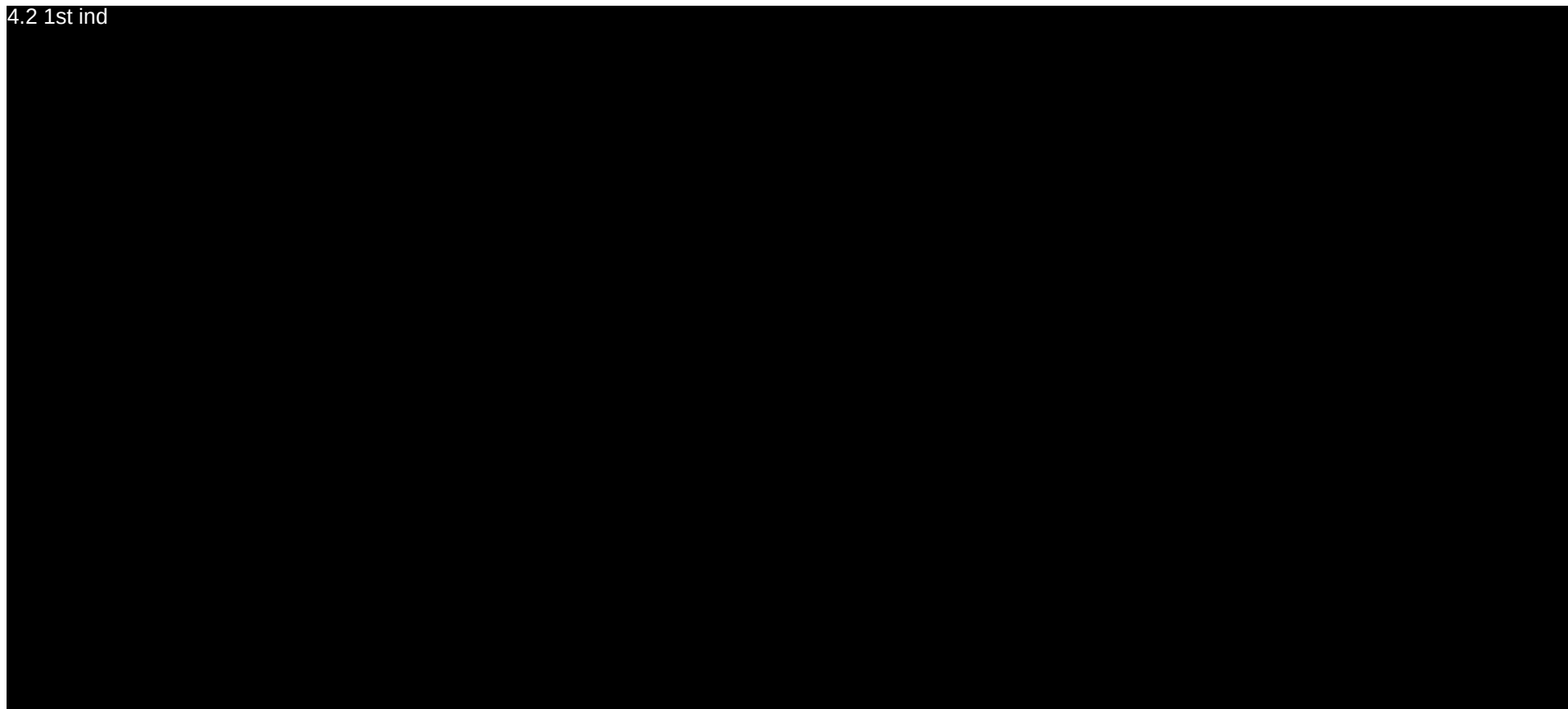


Figure 7: BNT162b2-DS_501868v1_20210608 (LTR 004); 40nt Co-Mix sample (zoom).

4.2 Robustness
4.2.1 In-vial sample stability & Incubation time robustness

Table 8: Excerpt of results from sequence BNT162b2-DS_501868v1_20210609 (LTR-501869-006). Not shown are blank injections and injections for column conditioning

Sample acc. to validation plan 503423	Purpose of injection	Ret. Time A30_H1	Re. Time L70	Resolution A30_H1 / A30_H2	Asymmetry L70	RRT (A30)	RRT (L70)	Injection Time
4.2 1st ind								09.06.2021 19:55
								09.06.2021 21:03
								09.06.2021 22:10
								09.06.2021 23:17
								10.06.2021 00:25
								10.06.2021 01:32
								10.06.2021 02:39
								10.06.2021 08:02
								10.06.2021 09:10
								10.06.2021 10:17
								10.06.2021 18:46
								10.06.2021 19:54
								10.06.2021 21:01
								11.06.2021 08:00
								11.06.2021 09:07
								11.06.2021 10:15
								11.06.2021 18:35
								11.06.2021 19:42
								11.06.2021 20:49
								11.06.2021 21:57

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Sample acc. to validation plan 503423	Purpose of injection	Ret. Time A30_H1	Re. Time L70	Resolution A30_H1 / A30_H2	Asymmetry L70	RRT (A30)	RRT (L70)	Injection Time
4.2 1st ind								12.06.2021 07:48
								12.06.2021 08:56
								12.06.2021 10:03

Table 9: Additional data from sequence BNT162b2-DS_501868v1_20210609 necessary for evaluation of the method validation acceptance criteria

Average Retention Time A30 (Kontrollen)	4.2 1st ind
Average Retention Time L70 (Kontrollen)	
Inj. Time Blank_PhaseA_1/1	
Timepoint of putting vials into Autosampler (LTR-501869-006)	09.06.2021 16:30

Table 10: Results of in-vial sample stability testing

	Sample	Injection Time	Time since start of Seq. [h]	RRT (A30)	RRT (L70)
4.2 1st ind		09.06.2021 23:17		4.2 1st ind	
		10.06.2021 09:10			
		10.06.2021 19:54			
		11.06.2021 09:07			
		11.06.2021 19:42			
		12.06.2021 08:56			
	Average	n/a	n/a		
	RSD [%]	n/a	n/a		

For the assessment of the in-vial stability the same sample preparation was injected 4.2 1st ind. According to the method validation plan 503423 the RSD of the relative retention times (RRT) of both peaks of interest (A30 and L70) had to be 4.2 1st This

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acceptance criterion has been fulfilled (see Table 10). All SST-Criteria according to SOP-501868 have been fulfilled. Therefore the prepared samples are stable 4.2 1st ind after the sample preparation if stored 4.2 1st ind

For the assessment of the incubation time robustness the same sample was prepared 4.2 1st. One aliquot was incubated for 4.2 1st ind one aliquot for 4.2 1st ind and the third aliquot was incubated for 4.2 1st. According to the method validation plan 503423 the RRT of both peaks of interest of the samples 4.2 1st ind were not allowed to differ more than 4.2 from the RRT of the respective peaks of the sample incubated for 4.2 1st ind. This acceptance criterion has been fulfilled (see Table 11).

Therefore the RNase incubation step during sample preparation can be performed between 4.2 1st ind However, it is recommended to limit the allowed incubation time to 4.2 1st ind to reduce potential inter-analysis variation.

Table 11: Assessment of the incubation time robustness

Sample	RRT (A30)	RRT (L70)	Relative difference [%]	
			A30	L70
4.2 1st ind				

The relative difference has been calculated as follows:

$$\text{Relative difference} = \frac{RRT_x}{RRT_y} * 100 - 100$$

with:

: 4.2 1st ind

4.2.2 Robustness: Alternative column

For the assessment of the alternative column 4.2 1st ind) on BNT162b2 DS sample (batch CZ13-P020.2-DS) was prepared. The sample preparation is documented in LTR-501869-007. This sample was injected once each in sequences BNT162b2-DS_501868v1_20210609 (LTR-501869-006) utilizing the regular column 4.2 1st ind) and BNT162B2-DS_501868V1_20210611 (LTR501869-007) utilizing the alternate column. 4.2 1st ind (see section 5.1.3 for

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details). According to the method validation plan 503423 the chromatograms of the 4.2 1st ind injections of the same sample on the different columns have to be visually comparable. The chromatograms are shown in [Figure 8](#). 4.2 1st ind

4.2 1st ind, i.e. the relative retention times (RRT) of both peaks of interest (A30 and L70) is 4.2 1st ind for both chromatograms in both sequences.

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4.2 1st ind

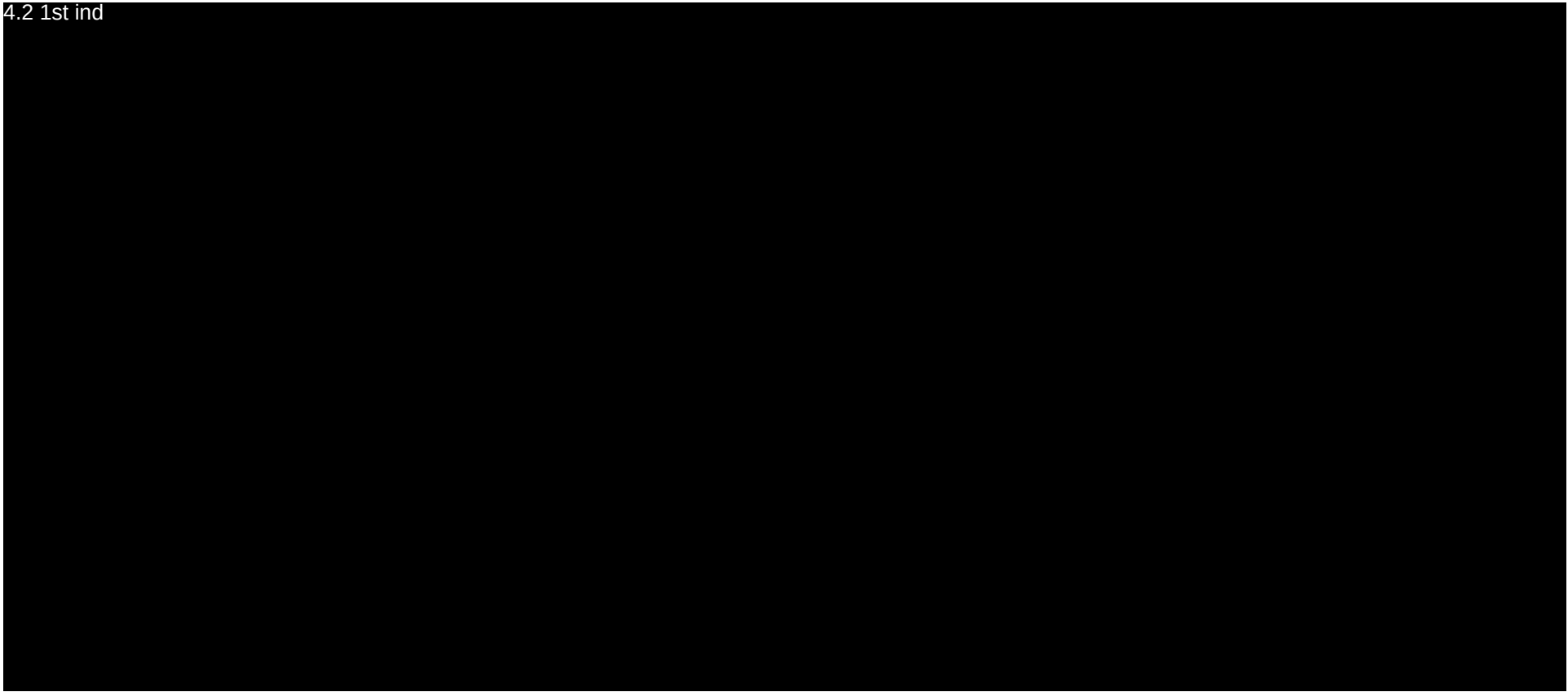


Figure 8: BNT162b2-DS (zoom, Overlay of injections of the same sample on different columns) from sequences BNT162b2-DS_501868v1_20210609 (LTR 006, black chromatogram, 4.2 1st ind) and BNT162B2-DS_501868V1_20210611 (LTR 007, blue chromatogram, 4.2 1st ind)

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4.2.3 Storage of sample intermediate and additional freeze/thaw cycle of DS reference standard

For the assessment of the stability of a sample preparation intermediate (of BNT162b2 sample CZ13-P020.2-DS) a prepared sample was stored at 4.2 1st ind. The sample was stored after the RNase incubation step before the addition of 4.2. The sample preparation was documented in LTR-501869-007 and a regular preparation (without the freeze, storage and thaw steps) of that sample was analyzed in sequence BNT162b2-DS_501868v1_20210609. After 4.2 the stored sample intermediate was thawed, 4.2 was added and it was analyzed in sequence BNT162B2-DS_501868V1_ID_20210615. According to the method validation plan 503423 the two resulting chromatograms have to be visually comparable. This acceptance criterion has been fulfilled (see [Figure 9](#)).

For the assessment of one additional freeze/thaw cycle (FT-cycle) of the DS reference standard PF-07305880, DS reference standard was thawed at room temperature on 07.06.2021 and subsequently stored at -20 °C. On 15.06.2021 the reference standard was thawed and prepared for analysis according to SOP-501868. The preparation was documented in LTR-501869-009. The reference standard was analyzed in Sequence BNT162B2-DS_501868V1_ID_20210615. 4.2 1st ind

This acceptance criterion has been fulfilled (see [Table 12](#)).

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4.2 1st ind

Figure 9: BNT162b2-DS CZ13-P020.2-DS (zoom, 10% signal offset) from sequences BNT162b2-DS_501868v1_20210609 (black, regular sample preparation) and BNT162B2-DS_501868V1_ID_20210615 (blue, sample preparation with freeze, storage and thaw step of sample intermediate)

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Table 12: Excerpt of results from sequence BNT162B2-DS_501868V1_ID_20210615 (LTR-501869-009). Not shown are blank injections and injections for column conditioning

	Ret. Time	Ret. Time	Resolution	Asymmetry				
Sample acc. to validation plan 503423	A30_H1	L70	A30_H1 / A30_H2	L70	RRT (A30)	RRT (L70)	Rel. Diff. Retention Time A30	Rel. Diff. Retention Time L70
4.2 1st ind					n/a	n/a	n/a	n/a
					n/a	n/a	n/a	n/a
					n/a	n/a	n/a	n/a
					4.2 1st ind		n/a	n/a
					4.2 1st ind			
					n/a	n/a	n/a	n/a

Average Retention Time A30 (Kontrollen)

Average Retention Time L70 (Kontrollen)

4.2 1st ind

The relative difference has been calculated as follows:

$$\text{Relative difference} = \frac{\text{Retention time}_{\text{4.2 1st ind}}}{\text{Retention time}} * 100 - 100$$

with:

: 4.2 1st ind

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5 Deviations and conclusion

5.1 Deviations

This section documents all deviations to the validation plan. No deviations according to SOP-8093713 have been initiated in association with this analytical method validation.

4.2 1st ind

4.2 1st ind

4.2 1st ind

Chemical acc. to SOP-501868	Substitute
4.2 1st ind	

4.2 1st ind was used to substitute 4.2 1st ind of equivalent grade was used. All solutions (mobile Phase A, mobile Phase B and 4.2 1st ind) have been prepared with adjusted amounts of 4.2 1st ind added. The adjustments have been documented in the

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respective LTRs. 4.2 1st ind

4.2 1st ind was prepared in the lab according to SOP-7041028, dissolving 4.2 1st ind in 4.2 1st ind and adding 4.2 1st ind) to adjust the pH to 4.2 1st ind and afterwards adding 4.2 1st ind to reach the final volume. The in-house preparation for 4.2 1st ind is chemically identical to commercial available solutions. 4.2 1st ind

4.2 1st ind

4.2 1st ind

5.2 Conclusion

The analytical method validation study showed that the analyses were executed consistently and correctly in the QC laboratory and the method according to SOP-501868 is suitable to verify the 3' poly(A) tail integrity of BNT162b2 DS.

All final results fulfilled the predefined acceptance criteria and product specifications defined in the Validation Plan Version 1 (Doc ID 503423).

Based on these results, the method validation is evaluated as successful.

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6 General Information about the Validation Study

6.1 Method validation team

Name	Function	Role
4.1(b)		Author of method validation plan and report; Execution of validation experiments
		Execution of validation experiments
		Review & Approval of method validation plan and report
		Review & Approval of method validation plan and report

6.2 Review and Approval of this Method Validation Report

The Validation Plan 503423 and this Validation Report were reviewed and approved by the same validation team (i.e. representatives of the same functional units).

6.3 Training status of operators

All operators conducting validation experiments have been trained on the current version of SOP-501868 and the current version of the validation plan 503423 according to SOP-503717 or are regarded as trained due to writing, reviewing or approving the respective documents. All analysts involved in the execution of the method validation experiments are considered as trained on the method and may train new analysts according to SOP-7038718.

6.4 Relevant documents for Execution of Validation Study

Table 13 lists all documents relevant for the execution of the validation study, with indication of the version numbers valid at the time of execution:

Table 13: Relevant documents for Execution of the Validation Study

Document Number and Version	Document Type and Title
SOP-501868, V1	Überprüfung der Integrität von 3 Poly(A) tail in BNT162b2 DS mittels RP-HPLC
LTR-501869, V1	LTR Überprüfung der Integrität von 3 Poly(A) tail in BNT162b2 DS mittels RP-HPLC
503423, V1	Methodenvalidierungsplan Überprüfung der Integrität von 3 Poly(A) tail in BNT162b2 DS (v.01)
SOP-503717, V1	Personaltraining
SOP-7041028, V2.0	Handhabung von Materialien zu Testzwecken
SOP-7041507, V3.0	Handhabung und Gebrauch von Referenzmaterialien
SOP-8093567, V3.0	Initiierung einer Abweichung oder OOX

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Document Number and Version	Document Type and Title
SOP-8093713, V3.0	Bewertung, Bearbeitung und Genehmigung von Abweichungen und OOX
SOP-8097462, V1.0	Validation of Analytical Procedures

6.5 Raw Data of Validation

Raw data to be considered for this validation are summarized in Table 14: Raw data of Validation.

Table 14: Raw data of Validation

Documentation	Archive
Certificates of the materials	4.2 1st ind
Protocols of Execution	
Chromatography sequences	

6.6 Equipment and Material used during the Validation Study

6.6.1 Equipment used during the Validation Study

All equipments which were used during the validation are documented in the respective LTRs. 4.2 1st ind

6.6.2 Materials / Components / Starting Materials / Media used during the Validation Study

All materials, components, chemicals and references which were used during the validation are documented in the respective LTRs. 4.2 1st ind

7 Abbreviations / Definitions

7.1 Abbreviations

Table 15 Abbreviations

Abbreviation	Description
FT cycle	Freeze / Thaw cycle
IPC	In Process Control
mRNA	Messenger ribonucleic acid
N/A	Not applicable

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Abbreviation	Description
OOS	Out of Specification
OOX	Out-off-X (Summary of OOE=Out-of-Expectation Results, OOS= Out-of-Specification Results and OOT=Out of Trend Results)
QC	Quality control
RRT	Relative retention time
RSD	Relative Standard Deviation
SOP	Standard Operating Procedure
SST	System Suitability Test
WP	Working Procedure

7.2 Definitions

Table 16 Definitions

Term	Description
N/A	N/A

8 Attachments

- „Evaluationsheet of Poly(A) integrity validation (attachment to 505545)” (stored in ZenQMS as a separate document-ID 512210)

9 References

N/A



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REVISION HISTORY

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DOCUMENT ELECTRONIC SIGNATURES

DOCUMENT APPROVAL WORKFLOW
Author Approval

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Required Workflow Steps for this Category

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