BNT162b2 (PF-07302048) Comparability Report for Commercial Drug Substance Manufacturing Process Transfer to BioNTech Marburg

INX100451011

4.1(b) [redacted]	4.1(b) [redacted]

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INTRODUCTION

BioNTech and Pfizer have been granted a marketing authorization by EMA and Emergency Use Authorization (EUA) by FDA in December 2020 on a vaccine (Comirnaty) to prevent Coronavirus Disease 2019 (COVID-19) caused by the virus SARS-CoV-2 for adults older than 16 years. The vaccine is based on SARS-CoV-2 spike (S) glycoprotein antigen encoded in RNA and formulated in lipid nanoparticles (LNPs), referred to as COVID-19 mRNA Vaccine (BioNTech code number BNT162b2, Pfizer code PF-07302048).

The development of the commercial drug substance (DS) manufacturing process ("Process 2") includes linearized plasmid DNA as a template and uses tangential flow filtration (TFF) for RNA purification. This process was established in parallel at two sites i) BioNTech, Mainz, Germany (incl. Rentschler, Laupheim, Germany for RNA purification) and ii) Pfizer, Andover, USA (Andover Clinical Manufacturing Facility, ACMF), with the previous comparability assessments described in MAA Section 3.2.S.2.6 Development History and Comparability (INX100439833).

In order to increase production capacities in response to the demand in a global pandemic and to ensure business continuity, an additional DS supply site will be established. This process is executed by a tech transfer for BNT162b2 from the above-mentioned sites to BioNTech Manufacturing Marburg GmbH, Emil-von-Behring-Strasse 76, 35041 Marburg, Germany.

This report summarizes the heightened characterization testing for analytical comparability assessment of commercial BNT162b2 DS manufactured at BioNTech/Rentschler and at Marburg. The results demonstrate comparable DS quality for batches manufactured at Marburg and BioNTech Mainz/Rentschler facilities.

1. SCOPE AND APPROACH FOR COMPARABILITY

The scope of this report includes the heightened analytical characterization assessments of BNT162b2 DS batches for analytical comparability. Comparison of the DS manufacturing processes, process parameters and attributes, equipment, primary packaging materials, composition and DS specifications are described in Part A of the comparability protocol. Results from batch release testing are not in scope for this report.

1.1. Batch Selection

Comparability before and after process transfer is demonstrated through a comparison of the release test results for all available batches, as well as side-by-side testing and heightened characterization for a subset of representative DS batches relevant to process changes and process performance qualification (Table 1).

Table 1. Batches used for drug substance comparability

Manufacturing Site	Drug substance Batch(es)	Process	Batch Scale (L)	Date of Manufacture	Purpose of Material
Pfizer, Andover ACMF	20Y513C201	DS Process 2	4.2 1st ind.	13-AUG-2020	Emergency supply, Stability
BioNTech Manufacturing GmbH, Mainz / Rentschler Laupheim	20E162001 / 1071539			24-SEP-2020	PPQ 1, Stability
	20E162002 / 1071542			30-SEP-2020	PPQ 2, Stability
	20E162003 / 1071544			06-OCT-2020	PPQ 3, Stability
BioNTech Manufacturing Marburg GmbH, Marburg	MB0001			20-FEB-2021	PPQ 1, Stability
	MB0002			23-FEB-2021	PPQ 2, Stability
	MB0003			28-FEB-2021	PPQ 3, Stability

1.2. Analytical Comparability Testing Strategy

The analytical comparability assessments evaluated a combination of release and heightened characterization testing (Table 2). Additional heightened characterization using mass spectrometry and spectroscopic characterization methods was performed to compare drug substance primary and higher order structure elements.

Supportive profiles, data and detailed discussions of the heightened characterization analyses performed side-by-side are provided in the sections below. The data demonstrate that all the DS batches are comparable and no facility-specific trends were noted for any of the attributes tested.

Table 2. Summary of Analytical Comparability Assessment of BioNTech Mainz/Rentschler and Marburg Drug Substance PPQ Batches

Quality Attribute	Analytical Procedure	Release / Heightened Characterization
Appearance (Clarity)	Appearance (Visual)	Release
Appearance (Coloration)	Appearance (Visual)	Release
pH	Potentiometry	Release
Identity of encoded RNA sequence	RT-PCR	Release
Content (RNA concentration)	UV absorption spectrophotometry	Release
RNA Integrity	Capillary gel electrophoresis	Release
Residual DNA Template	qPCR	Release
Double stranded RNA (dsRNA)	Immunoblot	Release
5'-Cap	LC-UV	Release
Capped-Intact RNA	Capillary gel electrophoresis and RP-HPLC	Heightened Characterization
Poly(A) Tail	ddPCR	Release

Table 2. Summary of Analytical Comparability Assessment of BioNTech Mainz/Rentschler and Marburg Drug Substance PPQ Batches

Quality Attribute	Analytical Procedure	Release / Heightened Characterization
Poly(A) Tail: Length and Distribution[a]	RP-HPLC	Heightened Characterization
5'-Cap[a]	LC-UV/MS	Heightened Characterization
Oligonucleotide Mapping[a]	LC-MS/MS	Heightened Characterization
Higher order structure[a]	Circular dichroism	Heightened Characterization
Expressed Protein[a]	Western blot	Heightened Characterization

a. Tested side-by-side

2. COMPARABILITY RESULTS AND DISCUSSION

2.1. Side-by-Side Comparability Evaluation of Drug Substance Batches

In addition to batch release testing, side-by-side heightened characterization testing was performed to compare the Marburg PPQ batches to PPQ batches from BioNTech Mainz/Rentschler and to reference batch 20Y513C201. Additional characterization tests and associated comparability acceptance criteria are shown in Table 3. Batch 20Y513C201, manufactured at Pfizer ACMF, provides a bridge to previous comparability studies.

Table 3. Side-by-Side Heightened Characterization of BNT162b2 Drug Substance

Quality Attribute	Analytical Procedure	Acceptance Criteria	Comparability Assessment
5'- Cap (species ID)	RNase H digestion followed by LC-UV-MS	Pre- and post-change batches show visually comparable chromatograms. Observed masses are consistent with theoretical masses for 5'-Cap, 5'-ppp, 5'-pp, 5'-ppp minus A, and 5'-pp minus A species.	Criteria for comparability met. See Section 2.1.1
Poly(A) Tail: Length and Distribution	Enzyme digestion followed by RP-HPLC	Pre- and post-change batches show visually comparable chromatograms	Criteria for comparability met. See Section 2.1.2
Primary Structure	Oligonucleotide mapping by LC-MS/MS	Pre- and post-change batches show visually comparable chromatograms	Criteria for comparability met. See Section 2.1.3
Higher Order Structure	Circular Dichroism	Pre- and post-change batches show visually comparable spectra	Criteria for comparability met. See Section 2.1.4
Expressed Protein	Western blot	Pre- and post-change batches show visually comparable banding patterns	Criteria for comparability met. See Section 2.1.5

Supportive data profiles are provided below for side-by-side testing of poly(A) tail length and distribution and expressed protein size.

Additionally, a specifically selected series of state-of-the-art characterization analyses was performed to assess additional aspects and demonstrate comparability of the BNT162b2 DS structure. Side-by-side comparability studies were performed using mass spectrometry to characterize the 5'-cap and oligonucleotide mapping. Circular dichroism was performed to characterize higher order structure.

2.1.1. Comparative LC-UV/MS Analysis of the 5'-Cap in BNT162b2

[redacted]

4.2 1st ind.

[redacted]

[redacted] structures are the same across DS batches manufactured at Pfizer ACMF, BioNTech Mainz/Rentschler, and BioNTech Marburg.

Figure 1. 5'-Cap Assay UV Chromatograms of BNT162b2 DS

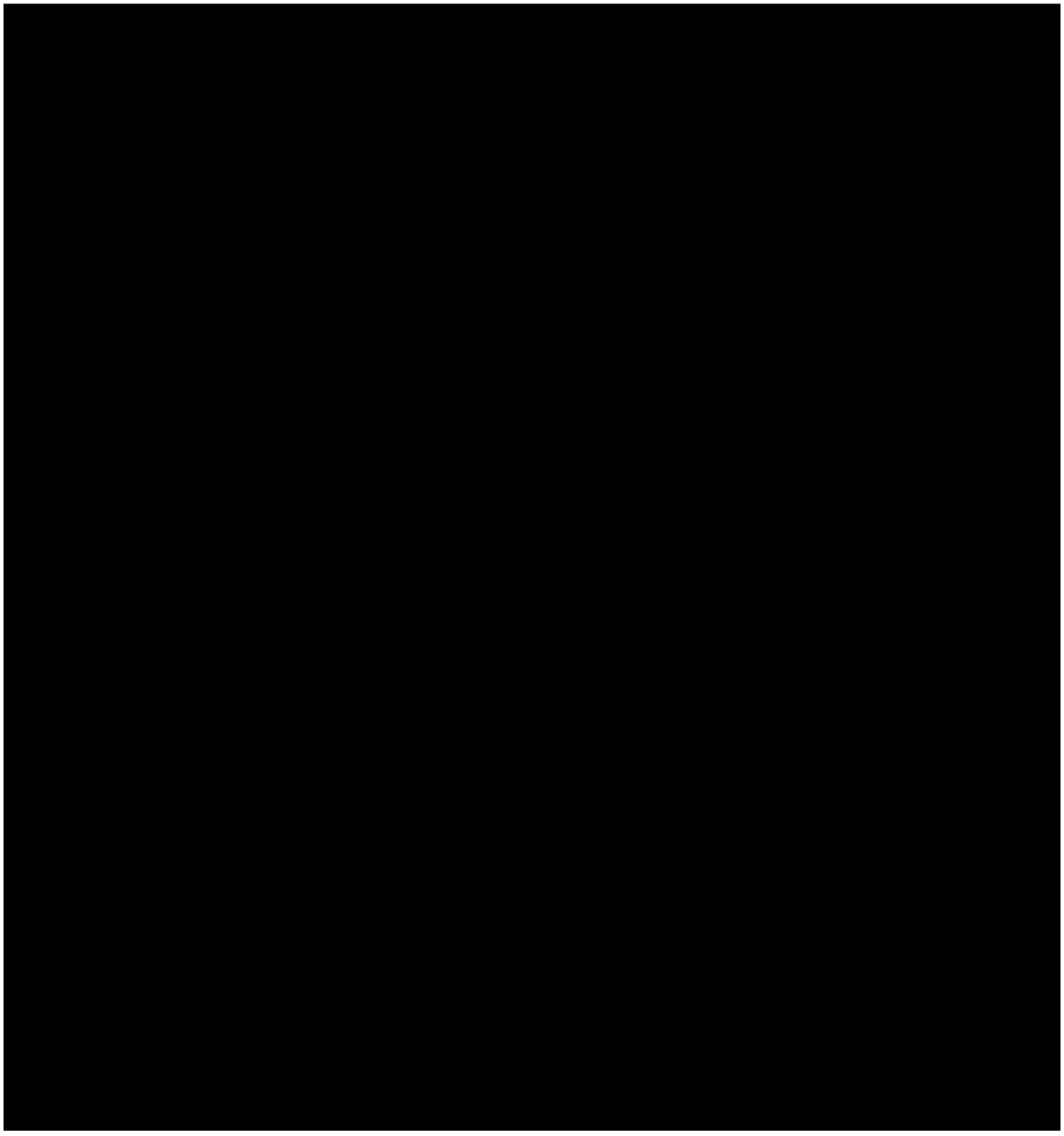


Table 4. 5'-Cap Percent Levels of BNT162b2 DS

Batch	5'-Cap %[a]
20Y513C201	4.2 1st ind.
20E162001	
20E162002	
20E162003	
MB0001	
MB0002	
MB0003	

4.2 1st ind.

Figure 2. Mass Spectra of 5'-Cap RNase Cleaved Fragments from BNT162b2 DS Batches

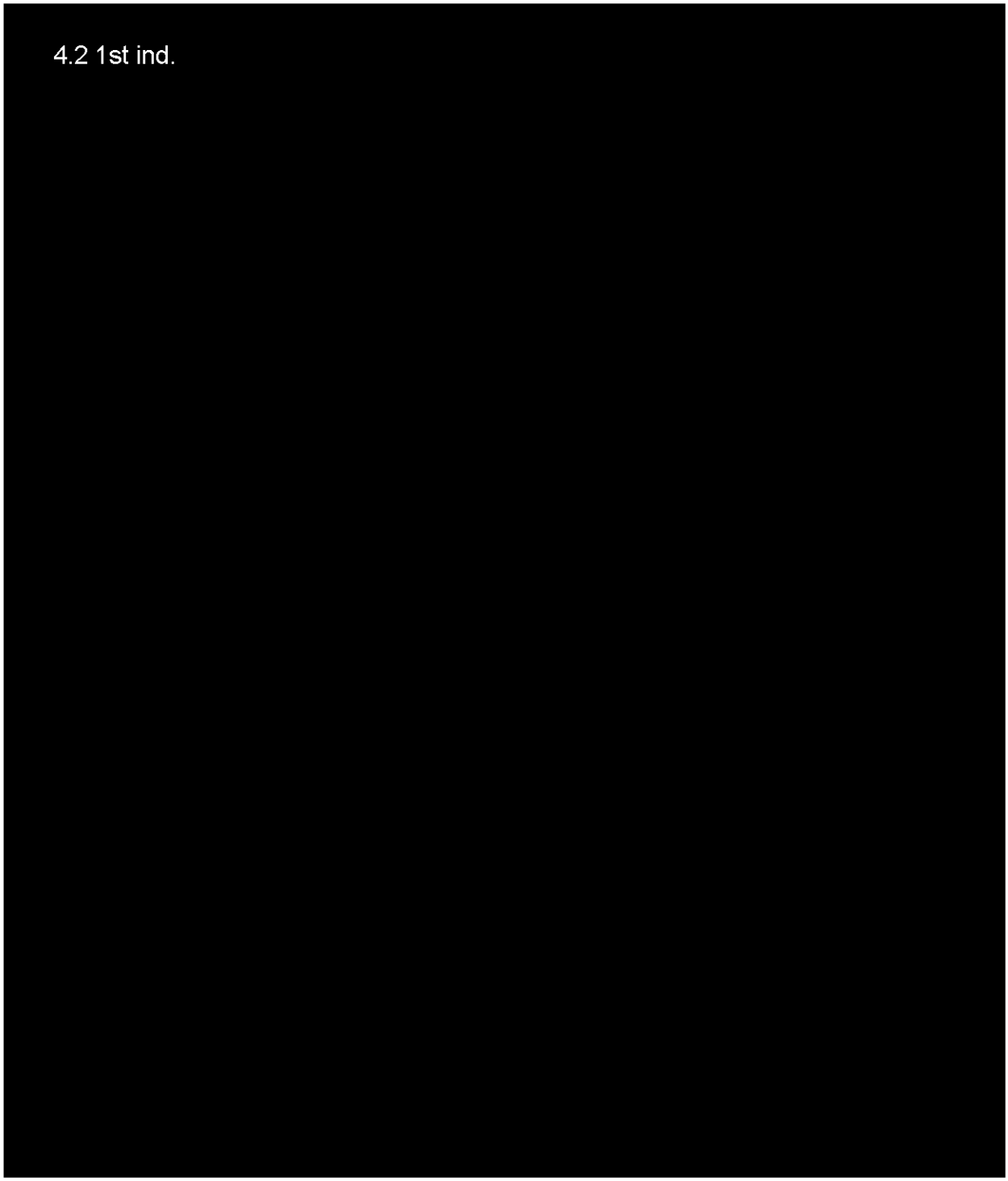


Figure 3. Mass Spectra of 5'-ppp and 5'-pp RNase Cleaved Fragments from BNT162b2 DS Batches

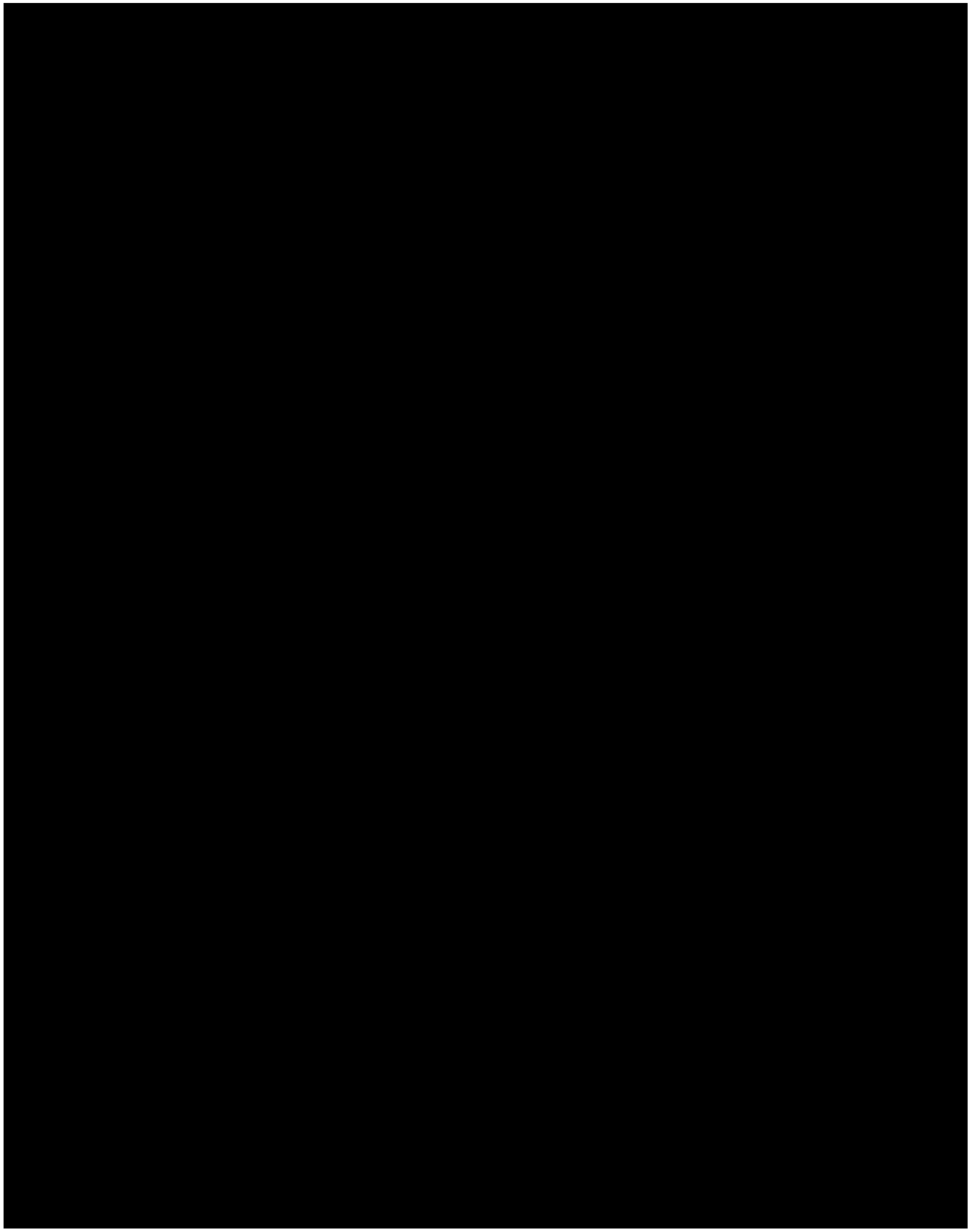


Table 5. Accurate Mass Assignments for BNT162b2 5'-Cap and non-Cap RNase Cleaved Fragments

[redacted]

2.1.2. Poly(A) Tail: Length and Distribution by RP-HPLC

To evaluate the distribution of the poly(A) tail segments of ~30 adenosine nucleotides (A30) and ~70 adenosine nucleotides (L70), BNT162b2 drug substance batches were digested with RNase T1 and RNase A, and subsequently analyzed by ion-pair reversed-phase HPLC (RP-HPLC). Figure 4 shows the chromatographic profiles of ACMF batch 20Y513C201 and the PPQ batches manufactured at BioNTech Mainz/Rentschler and Marburg facilities. Visual assessment of the chromatograms demonstrates comparable overall distributions of A30 and L70 species.

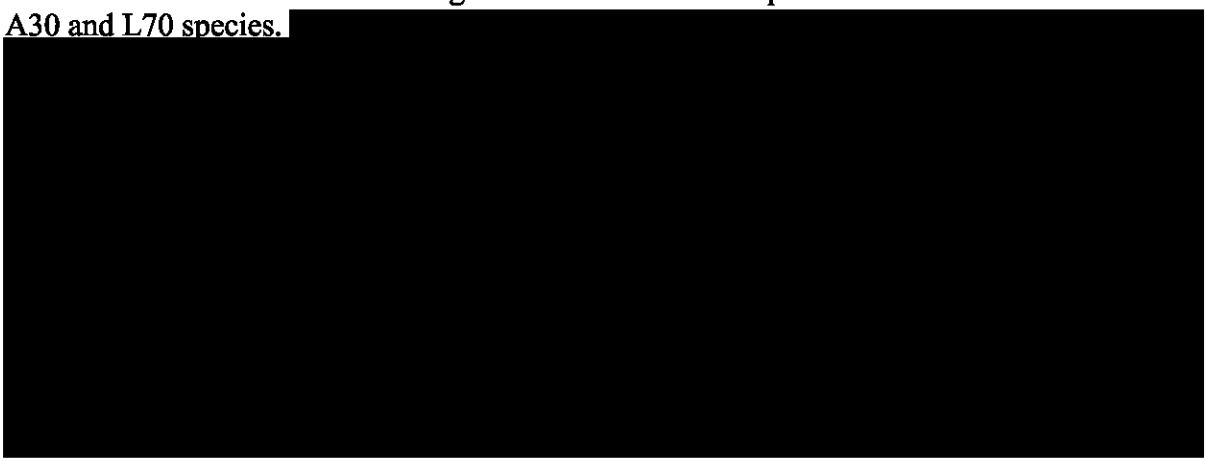
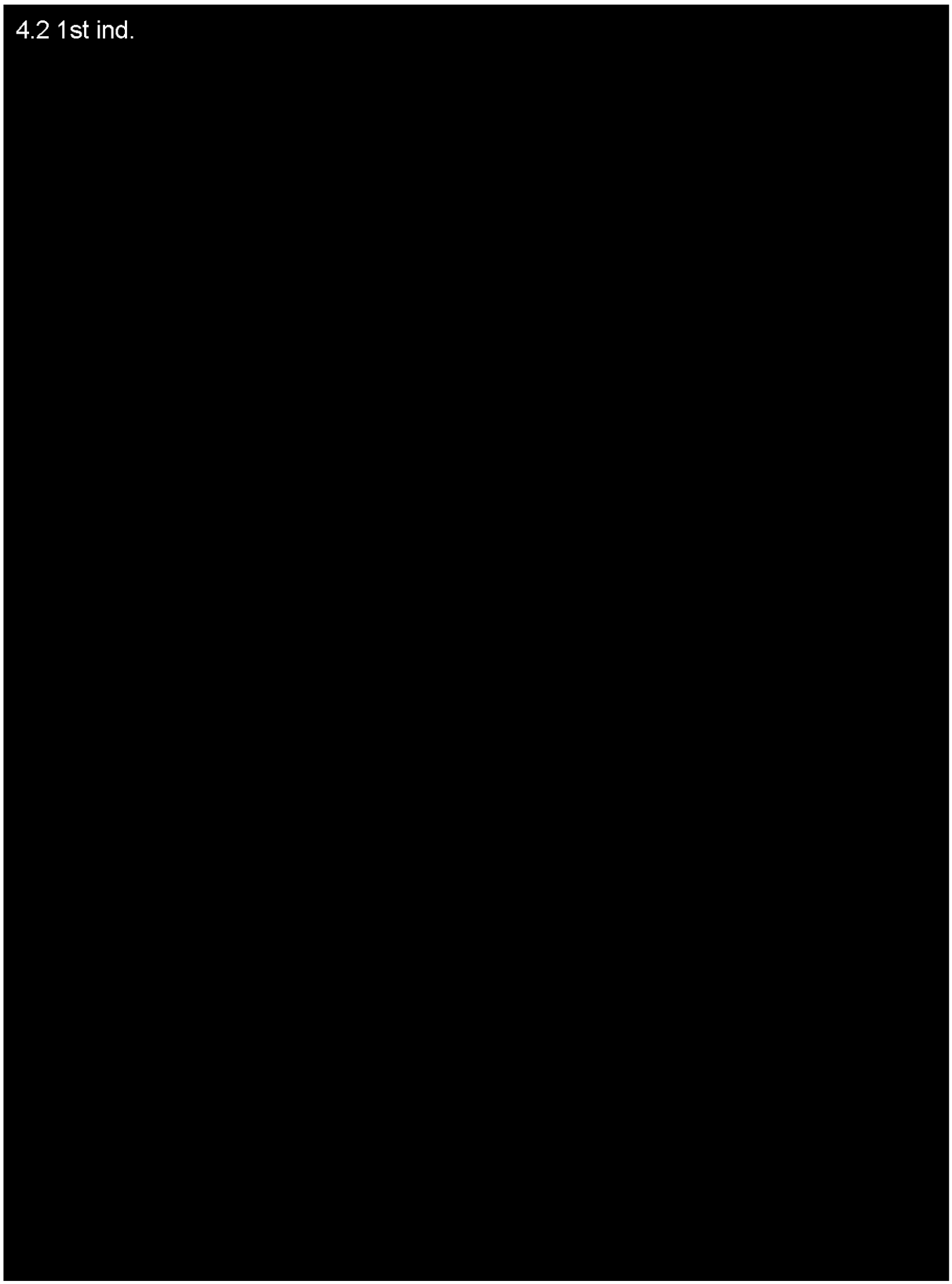


Table 6. BNT162b2 Poly(A) Tail: Length and Distribution

Batch	A30 Content (%)	L70 Content (%)	Other (%)
20Y513C201	4.2 1st ind.		
20E162001/ 1071539			
20E162002/ 1071542			
20E162003/ 1071544			
MB0001			
MB0002			
MB0003			

Figure 4. BNT162b2 Poly(A) Tail Analyzed by RP-HPLC



2.1.3. Comparative LC-MS/MS – Oligonucleotide Mapping of BNT162b2 [redacted]

[redacted]

4.2 1st ind.

[redacted] The observed chromatographic profiles for all drug substance batches are highly similar [redacted]

[redacted]

[redacted]

In conclusion, the highly comparable chromatographic profiles and consistent [redacted] between materials demonstrate that the BNT162b2 DS batches manufactured at BioNTech Marburg are comparable to those manufactured at previously established facilities and contain the correct, identical sequence as predicted from the linear DNA template.

Figure 5. LC-MS/MS – Oligonucleotide Mapping of BNT162b2 DS Batches ( )

Figure 6. LC-MS/MS – Oligonucleotide Mapping of BNT162b2 DS Batches ([redacted])

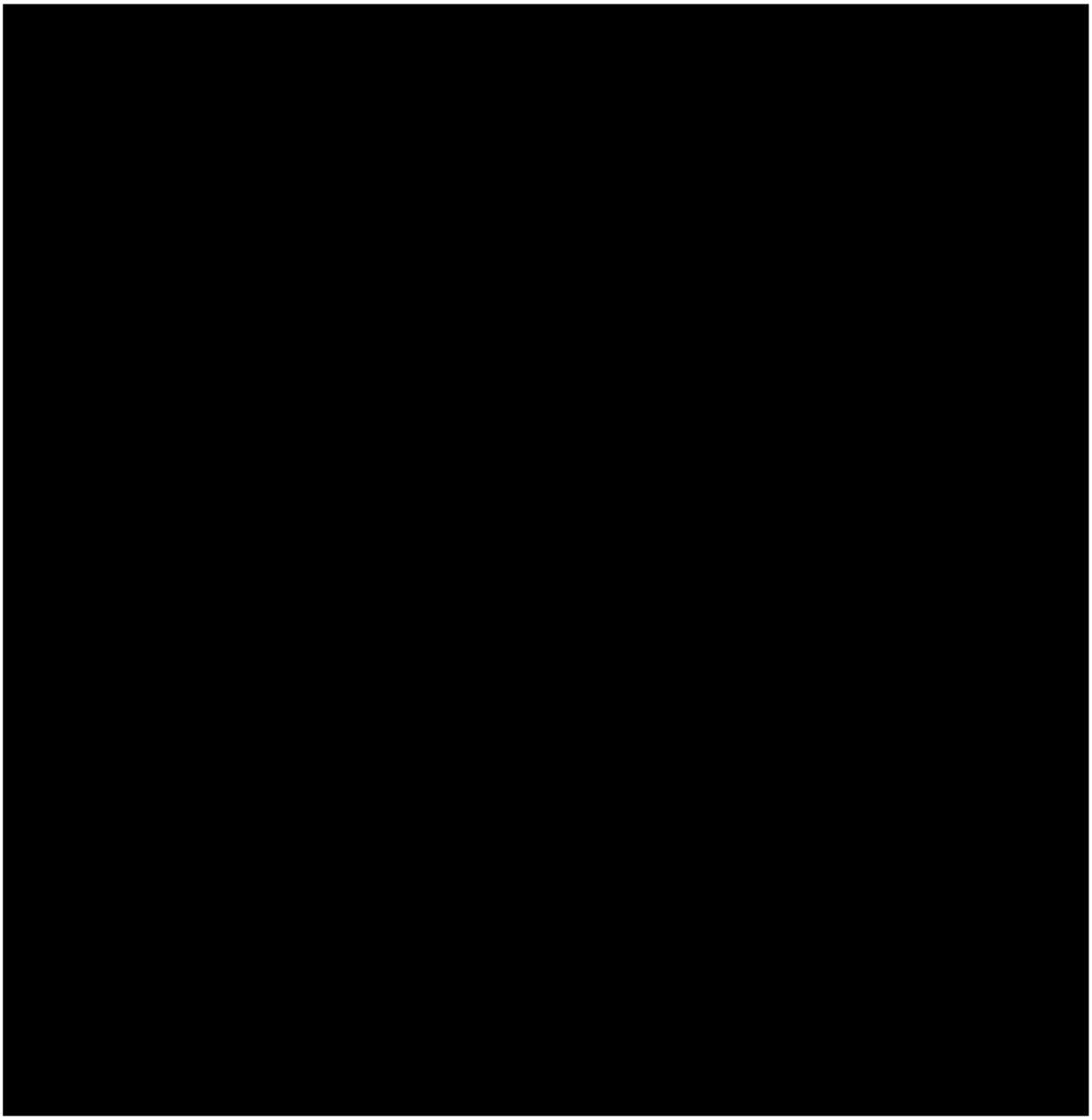


Figure 7. LC-MS/MS – Oligonucleotide Mapping of BNT162b2 DS Batches ([redacted])

Figure 8. LC-MS/MS – Oligonucleotide Mapping of BNT162b2 DS Batches ([redacted]

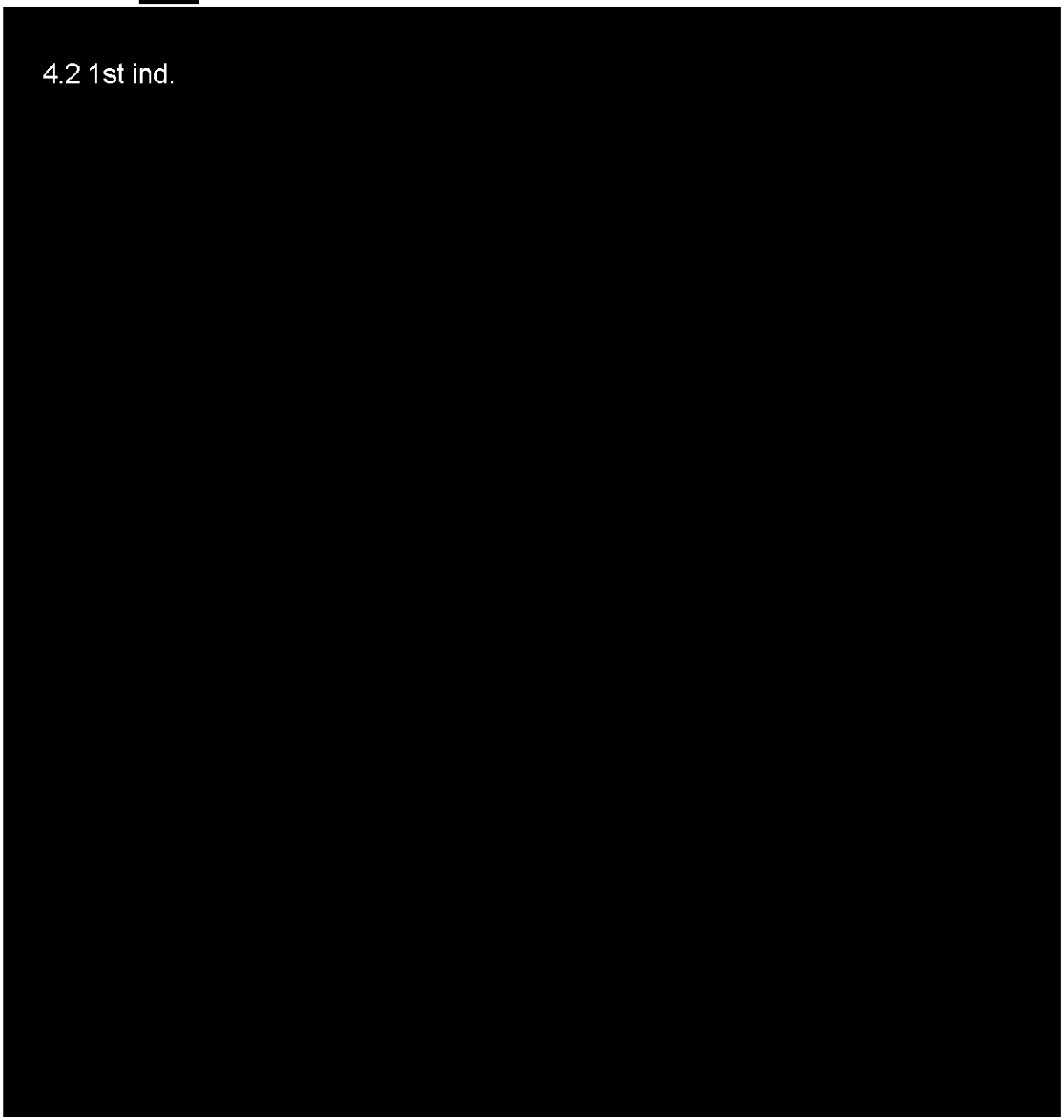


Figure 9. LC-MS/MS – Oligonucleotide Mapping of BNT162b2 DS Batches ([redacted]

Figure 10. LC-MS/MS – Oligonucleotide Mapping of BNT162b2 DS Batches ([redacted]

Table 7. LC-MS/MS – Oligonucleotide Mapping Summary of BNT162b2 DS Batches

Characteristics	20Y513C201	20E162001/ 1071539	20E162002/ 1071542	20E162003/ 1071544	MB0001	MB0002	MB0003
4.2 1st ind.							

2.1.4. Comparative Higher Order Structure Characterization of BNT162b2 DS Batches

Circular dichroism (CD) spectroscopy was used to assess the higher-order structure of BNT162b2 in solution for ACMF batch 20Y513C201 and PPQ batches from BioNTech Mainz/Rentschler and BioNTech Marburg.

4.2 1st ind.

Figure 11. CD Spectral Overlay BNT162b2 DS batches

4.2 1st ind.

Table 8. Spectral Similarity Scores between BNT162b2 Drug Substance Batches

	20Y513C201	20E162001	20E162002	20E162003	MB0001	MB0002	MB0003
20Y513C201[a]	4.2 1st ind.						

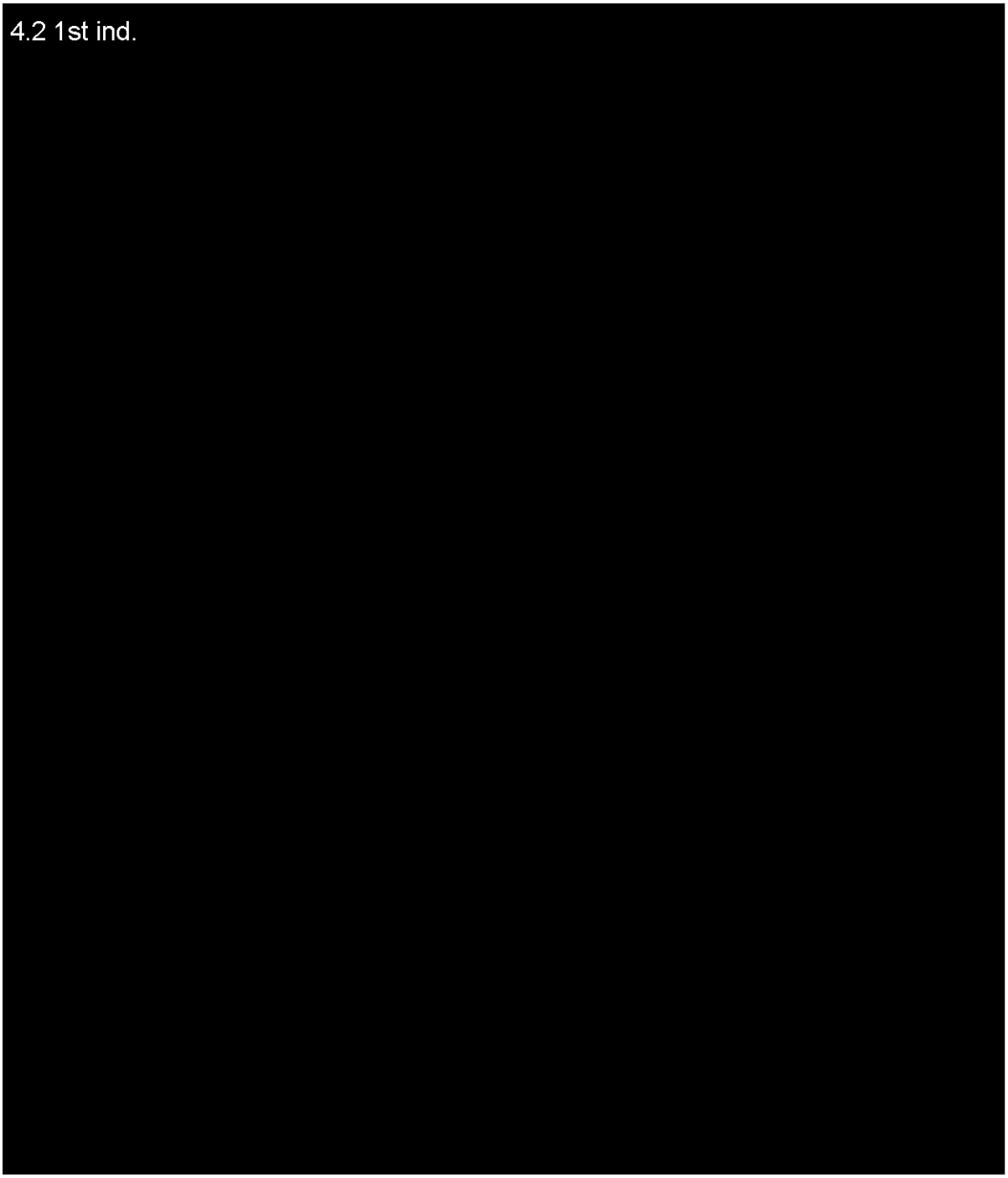
a. all lots are compared against batch 20Y513C201: 100% being identical

4.2 1st ind.
confirm that all BNT162b2 DS batches analyzed are comparable to each other with respect to higher order structure.

2.1.5. Expressed protein size by Western blot

The protein size after in-vitro expression of BNT162b2 drug substance was determined using Western blot. Expressed protein size was confirmed to be comparable for all analyzed DS batches. Figure 12 shows the expressed protein bands, 4.2 1st ind. The protein sizes observed are consistent with the expected sizes of the translated BNT162b2 drug substance and are comparable across all tested batches, with no additional bands observed for any samples.

Figure 12. BNT162b2 Expressed Protein Size by Western Blot



2.2. Overall Conclusions for Comparability

The comparability assessment presented here focused on an assessment of the BNT162b2 drug substance critical quality attributes for batches manufactured at BioNTech's

Mainz/Rentschler and Marburg facilities. Pfizer ACMF batch 20Y513C201 was included in side-by-side testing as a reference lot and as a bridge to prior comparability assessments that established Pfizer's DS manufacturing facilities. The heightened characterization and comparability study for the BNT162b2 drug substance employed several state-of-the-art techniques and methodologies to evaluate primary structure and higher order structure. The pre-determined acceptance criteria described in Table 3 were met for all side-by-side heightened characterization assessments. All results demonstrate that the drug substance batches manufactured at Pfizer ACMF, BioNTech Mainz/Rentschler, and BioNTech Marburg are comparable.

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