



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

BioNTech COVID19 mRNA vaccine
(nucleoside modified)

EMA Quality Office CMC observations

BWP 24th November

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Quality Attribute	Analytical Procedure ^a	Acceptance Criteria
Composition and Strength		
Appearance	Appearance (Visual)	White to off-white suspension
Appearance (Visible Particulates)	Appearance (Particles) ^b	Essentially free from visible particulates
Subvisible Particles	Subvisible Particulate Matter ^{b, c}	Particles $\geq 10 \mu\text{m}$: ≤ 6000 per container ^{b, c} Particles $\geq 25 \mu\text{m}$: ≤ 600 per container ^{b, c}
pH	Potentiometry ^b	6.9 – 7.9
Osmolality	Osmometry ^{b, d, e}	425 - 625 mOsmol/kg
LNP Size	Dynamic Light Scattering (DLS)	40 to 180 nm
LNP Polydispersity	Dynamic Light Scattering (DLS)	≤ 0.3
RNA Encapsulation	Fluorescence assay	$\geq 80\%$
RNA content	Fluorescence assay	$0.50 \pm 0.13 \text{ mg/mL}$
ALC-0315 content	HPLC-CAD	4.50 to 9.25 mg/mL
ALC-0159 content	HPLC-CAD	0.55 to 1.20 mg/mL
DSPC content	HPLC-CAD	0.90 to 2.05 mg/mL
Cholesterol content	HPLC-CAD	1.80 to 3.90 mg/mL
Container content for injections	Volume of injections in containers ^{a, f}	Not less than the sum of the nominal volumes of 5 doses
Identity		
Lipid identities	HPLC-CAD ^e	Retention times consistent with references (ALC-0315, ALC-0159, Cholesterol, DSPC)
Identity of encoded RNA sequence	RT-PCR ^e	Identity confirmed
Potency		
In Vitro Expression	Cell-based flow cytometry	$> 30\%$ Cells Positive
Purity		
RNA Integrity	Capillary Gel Electrophoresis	$\geq 50\%$ intact RNA
Bacterial Endotoxin	Endotoxin (LAL) ^g	$\leq 12.5 \text{ EU/mL}$
Sterility	Sterility ^h	No Growth Detected
Container Closure Integrity	Dye incursion ^g	Pass

Company:
"The efficacy of the drug product is dependent on the expression of the delivered RNA, which requires a sufficiently intact RNA molecule."

DP site	Lot		RNA Integrity (%)	DS site
Polymun Scientific	COVVAC/270320	Tox, Stab	69	BioNTech, M
	BCV40420-A		75	BioNTech, IO Process <u>1</u>
	BCV40620-A		85	
	BCV40620-B		86	
	BCV40620-C		83	
	BCV40620-D	BNT162-02	77	
	BCV40620-E		85	
	BCV40720-A		71	
	BCV40720-B		72	
	BCV40720-C		69	
Puurs (S2S2)	ED3938		62	Pfizer, A Process <u>2</u>
	EE3813		63	
Puurs (WSL5)	EE8492 *		55	
	EE8493 *	BNT162-02, bridging	55	

*: Emergency Supply

ARE BATCHES
COMPARABLE ?

BioNTech, Mainz, DE

BioNTech, Idar Oberstein, DE

Pfizer, Andover, USA

Overall Batch Analyses %RNA integrity



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Company	Drug Substance batch number	%RNA integrity	Mean %RNA integrity	Drug Product batch number	%RNA integrity	Company	Mean %RNA integrity
	?			COVVAC/270320 69	69		
BNT	R427-P020.2-DS	77	81	BCV40420-A	75	Polymun	78
	R438-P020.2-DS	80		BCV40620-A	85		
	R438-P020.2-DS	80		BCV40620-B	86		
	R438-P020.2-DS	80		BCV40620-C	83		
	R438-P020.2-DS	80		BCV40620-D	77		
	R438-P020.2-DS	80		BCV40620-E	85		
	R443-P020.2-DS	81		BCV40720-A	71		
	R443-P020.2-DS	81		BCV40720-B	72		
	R443-P020.2-DS	81		BCV40720-C	69		
	R443-P020.2-DS	81		ED3938	62	Pfizer, Puurs (S2S2)	63
	R445-P020.2-DS	86		EE3813	63		
Pfizer, Andover	20Y513C101	62	62	EE8492	55	Pfizer, Puurs (WSL5)	55
	20Y513C101	62		EE8493	55		

Drug Substance BNT162b2 Expressed Protein Size by Western Blot

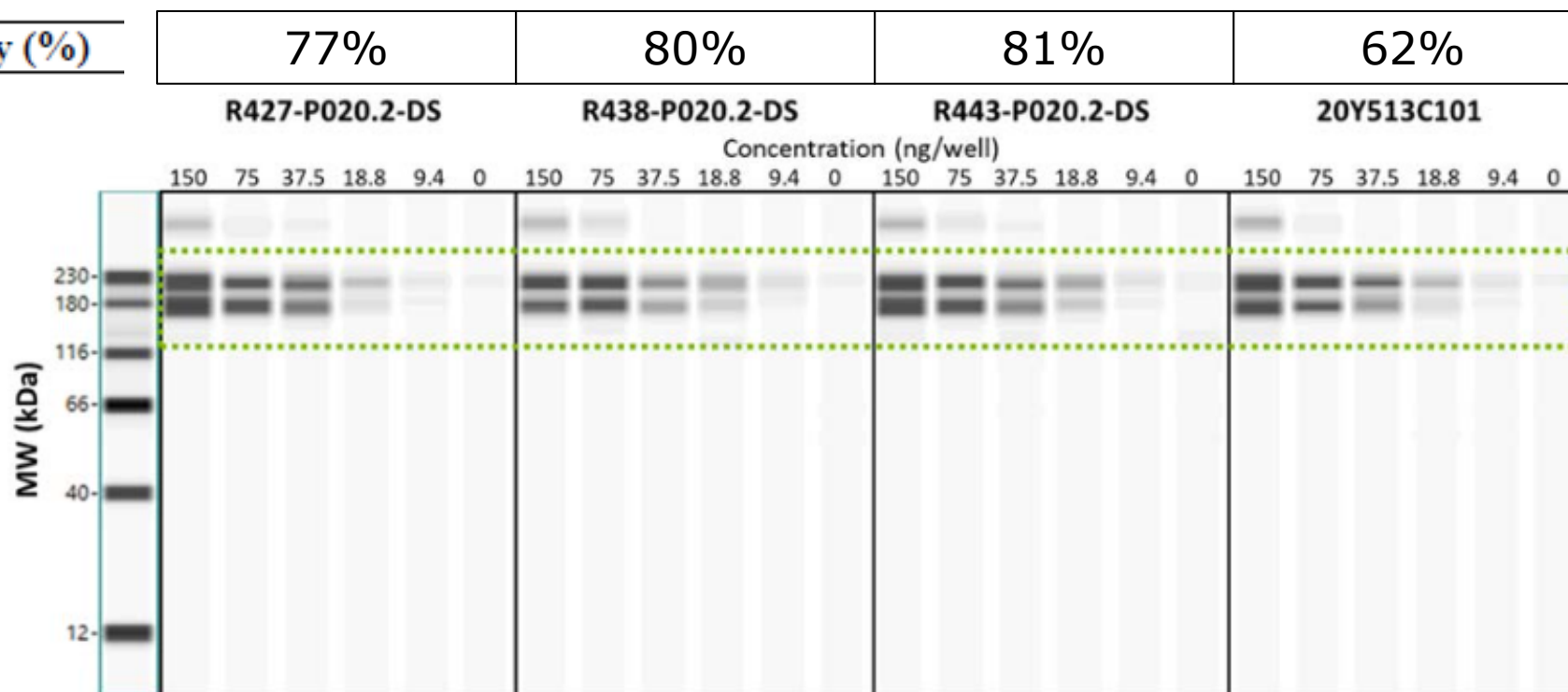


Figure 3.2.S.2.6-15. To evaluate expressed protein size, BNT162b2 DS was mixed with Lipofectamine and then transfected into HEK-293 cells. Following incubation, cell lysates were evaluated for the expressed protein antigen by Western blot using an antibody specific for the SARS-CoV-2 spike protein. The first lane shows a molecular weight (MW) marker. The concentrations shown for each DS batch correspond to the amounts of DS transfected per well of HEK-293 cells.

BNT162b2 drug product in vitro expression comparability and release data.

RNA Integrity (%)	Lot	(150ng) % Cells Positive	(100ng)
75	BCV40420-A	69 (comparability)	45
85	BCV40620-A	59 (comparability)	41
77	BCV40620-D	71 (comparability)	52
71	BCV40720-A	63 (comparability)	45
62	ED3938	50 (comparability)	?
63	EE3813	62 (comparability)	?
55	EE8492	63 (release)	?
55	EE8493	56; 65 (comparability; release)	21(!)



BACK-UP

Batch Analyses for Clinical COVID-19 Vaccine BNT162b2 Drug Substance Batches

Batch			R427-P020.2-DS	R438-P020.2-DS	R443-P020.2-DS	R445-P020.2-DS
Quality Attribute	Analytical Procedure	Acceptance Criteria ^a	Results			
RNA integrity	Capillary gel electrophoresis	≥ 85.0 % in the peak corresponding to intact RNA	100.0 (77) ^f	100.0 (80) ^f	99.4 (81) ^f	100.0 (86) ^f

Process **1**

Batch Analyses for Clinical, Emergency Supply and Process Performance Qualification COVID-19 Vaccine BNT162b2 Drug Substance Batches

Batch			20Y513C101	20Y513C201	20Y513C301	20Y513C401	20Y513C501
Quality Attribute	Analytical Procedure	Acceptance Criteria	Results				
RNA integrity	Capillary gel electrophoresis	≥ 50 % intact RNA	62	69	66	65	75

Process **2**

Batch Analyses for Nonclinical and Clinical BNT162b2 Drug Product Lots

Quality Attribute	Analytical Procedure	Acceptance Criteria ^a	Lot Number					
			BCV40420-A	BCV40620-A	BCV40620-B	BCV40620-C	BCV40620-D	BCV40620-E
			Results					
RNA integrity	Capillary gel electrophoresis	≥ 60% ^b	75	85	86	83	77	85

Batch Analyses for Clinical BNT162b2 Drug Product Lots

Quality Attribute	Analytical Procedure	Acceptance Criteria ^a	Lot Number				
			BCV40720-A	BCV40720-B	BCV40720-C	ED3938	EE3813
			Results				
RNA integrity	Capillary gel electrophoresis	≥ 60% ^b	71	72	69	62	63

Batch Analyses for Emergency Supply BNT162b2 Drug Product Lots

Quality Attribute	Analytical Procedure	Acceptance Criteria ^a	Lot Number	
			EE8492	EE8493
			Results	
RNA integrity	Capillary gel electrophoresis	≥ 50% in the peak corresponding to intact RNA	55	55