CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

216903Orig1s000

NON-CLINICAL REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	NDA 216903
Supporting document/s:	SDN 3, 4, 5, 10
Applicant's letter and CDER stamp date(s):	April 25, 2022; June 29, 2022; July 29, 2022; September 16, 2022
Product:	Neostigmine and Glycopyrrolate
Indication:	The reversal of the effects of non-depolarizing neuromuscular blocking agents (NMBAs) after surgery, (b) (4)
Applicant:	Slayback Pharma, LLC
Clinical Review Division:	Division of Anesthesiology, Addiction Medicine, and Pain Medicine (DAAP)
Pharm/Tox Division:	Division of Pharm/Tox for Neuroscience (DPT-N)
DPT-N Reviewer:	Alexander I. Son, PhD
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1 Executive Summary

1.1 Introduction

NDA 216903 submitted by Slayback Pharmaceuticals is for a fixed-dose combination product consisting of neostigmine methylsulfate (1.0 mg/mL) and glycopyrrolate (0.2 mg/mL) in a 3 mL prefilled syringe. The Applicant has submitted this NDA via the 505(b)(2) regulatory pathway and has cited NDA 204078 (BLOXIVERZ) and NDA 017558 (ROBINUL) as the Reference Drugs (RDs) for neostigmine methylsulfate and glycopyrrolate, respectively. The Maximum Daily Dose (MDD) for this product is 5 mg/day neostigmine, 1 mg/day glycopyrrolate.

1.2 Brief Discussion of Nonclinical Findings

In support of their drug product, the Applicant submitted a literature search from the time of approval of the RD to the date of submission regarding pharmacology, pharmacokinetic, ADME, toxicokinetic, and toxicology data for each API. There were no data from published studies that warrant changes to the proposed labeling. The Applicant also submitted a comparison of physiochemical properties between their proposed product, BLOXIVERZ (Neostigmine Methylsulfate), Glycopyrrolate Injection, USP (0.2 mg/mL), and a combined mixture between these products in a 1:1 ratio. The proposed drug product is isotonic and within the pH range of the respective RDs.

The Applicant's proposed specifications for drug substance and drug product impurities are within the levels outlined in ICH Q3A(R2) and Q3B(R2). Residual solvent specifications are within the levels as stated in ICH Q3C(R8). Elemental impurities are below the control threshold of 30% as per ICH Q3D. To support the safety of the container closure system, the Applicant provided extractables and leachables studies. One leachable above the SCT of (b) (4) were identified and was properly qualified, and therefore there are no concerns with the safety of the prefilled syringe container closure system.

To support the local safety of the combination product, the Applicant conducted an in vitro hemolysis study and a local tolerance (IV/PV) study. It is noted that while the hemolysis study was GLP, the local tolerance was non-GLP and utilized only male rabbits. In response to an information request (IR), the Applicant informed the Division that the local tolerance study was conducted in the spirit of GLP. The Applicant also provided literature indicating that sex-dependent differences regarding local toxicity from parenteral drugs are unlikely, which this Reviewer is in agreement. In discussions with the Clinical Review Team, it is noted that neostigmine methylsulfate and glycopyrrolate are used in combination. Taking into consideration the clinical experience with the individual APIs and with the combination, there are no outstanding safety concerns with the nonclinical data submitted in support of the fixed-dose prefilled syringe combination product.

1.3 Recommendations

1.3.1 Approvability

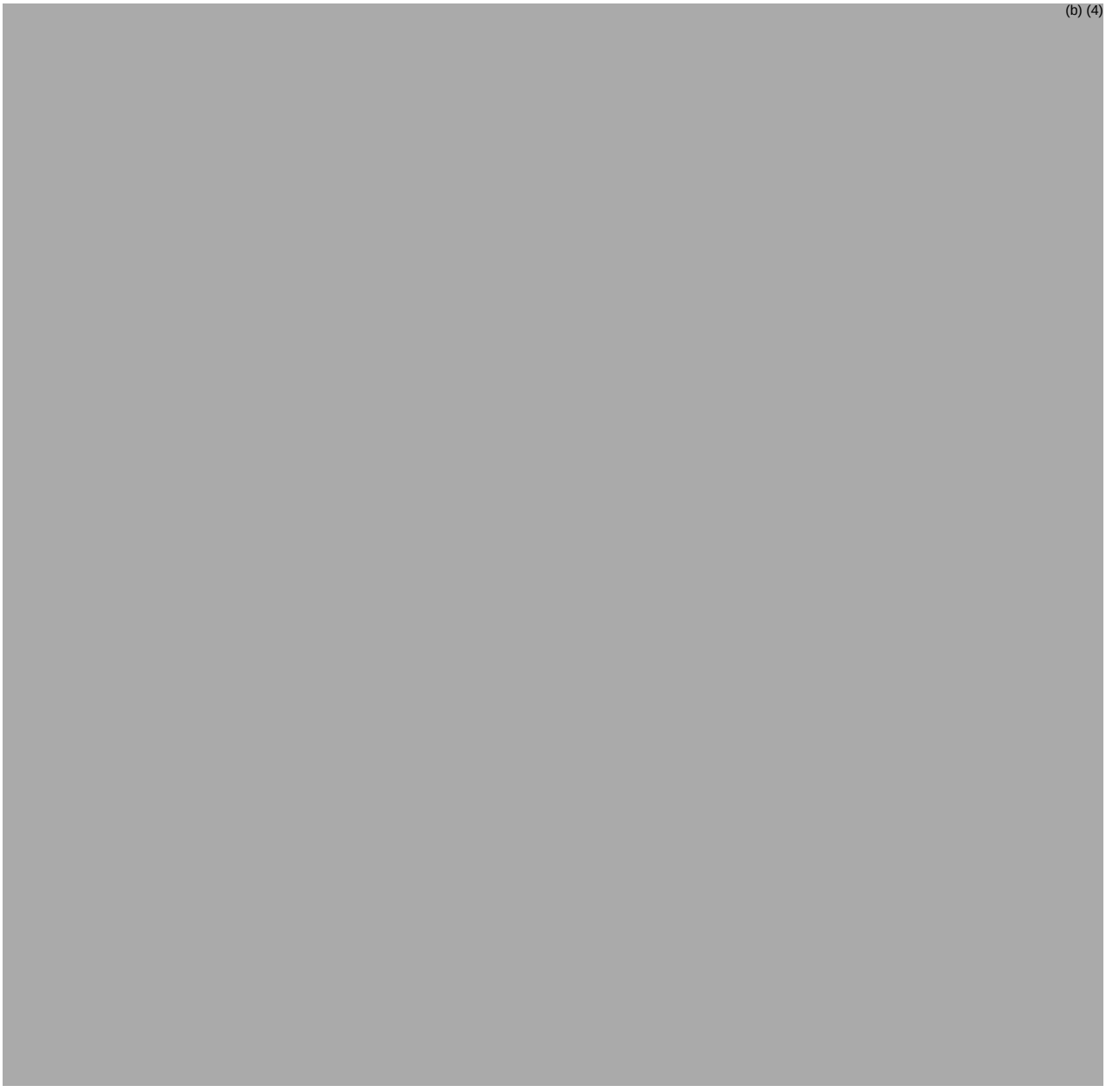
From a nonclinical pharmacology/toxicology perspective, NDA 216903 for the combination product housed in a prefilled syringe that consists of neostigmine methylsulfate and glycopyrrolate is recommended for approval.

1.3.2 Additional Nonclinical Recommendations

N/A

1.3.3 Labeling

(b) (4)



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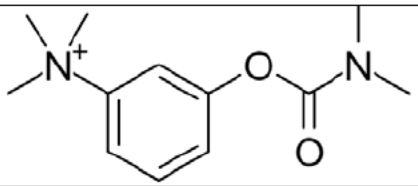
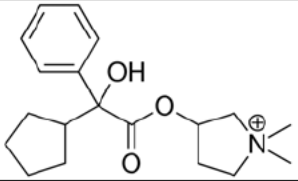
2 Drug Information

2.1 Drug

CAS Registry Number:	59-99-4	596-51-0
Generic Name:	Neostigmine Methylsulfate	Glycopyrrolate (b) (4)
Code Name:	Neostigmine	Glycopyrrolate
Chemical Name:	[3-(dimethylcarbamoyloxy)phenyl]-trimethylazanium	(1,1-dimethylpyrrolidin-1-ium-3-yl) 2-cyclopentyl-2-hydroxy-2-phenylacetate;bromide
Molecular Formula/Molecular Weight:	$C_{12}H_{19}N_2O_2$/223.3 g/mol	$C_{19}H_{28}BrNO_3$/398.3 g/mol
Structure or Biochemical Description:		
Established Pharmacologic Class (EPC):	Cholinesterase inhibitor	Anticholinergic

2.2 Relevant INDs, NDAs, BLAs and DMFs

Table 1: INDs and NDAs

Application ID	Drug	Route	Applicant	Status
PIND 139866	Neostigmine Methylsulfate and Glycopyrrolate (b) (4) Injection	Intravenous	Slayback Pharma LLC	PIND comments provided; no IND studies performed
NDA 204078 (RD)	BLOXIVERZ (Neostigmine Methylsulfate)	Intravenous	Exela Pharma Sciences	Approved
NDA 017558 (RD)	ROBINUL (Glycopyrrolate)	Intravenous, Intramuscular	Hikma Pharmaceuticals USA, Inc	Withdrawn, FR Effective
ANDA 09093 (RS)	Glycopyrrolate Injection	Intravenous, Intramuscular	Hikma Farmaceutica (Portugal) SA	Approved

RD = Reference Drug, RS = Reference Standard

Table 2: DMFs

Application ID	Product Name	Supplier/DMF Holder	Comment
DMF (b) (4) Type-II)	Neostigmine Methylsulfate USP	(b) (4)	N/A
DMF Type-II)	Glycopyrrolate USP		N/A

2.3 Drug Formulation

The Applicant provided a side-by-side comparison to the proposed drug product and reference drugs. It is noted that compared to the reference drugs BLOXIVERZ and ROBINUL, the proposed drug product does not contain phenol or benzyl alcohol but does include disodium edetate dihydrate, USP, which is a (b) (4)

Table 3: Drug Product Formulation and Comparison

Ingredient(s)	Quality Standard	Pharmaceutical Function	Quantity					
			Proposed Drug Product[7]		BLOXIVERZ[1]	ROBINUL[$,3]	GLYRX-PF[#,4]	Glycopyrrolate Injection[$,5]
			mg/mL	% w/v	mg/mL	mg/mL	mg/mL	mg/mL
Neostigmine Methylsulfate	USP	Active Pharmaceutical Ingredient	1.0 mg	(b) (4)	1.0 mg	--	--	--
Glycopyrrolate	USP	Active Pharmaceutical Ingredient	0.2 mg		--	0.2 mg	0.2 mg	0.2 mg
Disodium Edetate Dihydrate	USP	(b) (4)	0.5 mg		--	--	--	--
Sodium Chloride	USP		8 mg		--	--	(b) (4)	--
Sodium Acetate Trihydrate	USP		--		0.2 mg	--	--	--
Benzyl Alcohol	NF	Preservative	--		--	0.9%	--	0.9%
Phenol	USP	Preservative	--		4.5 mg	--	--	--
Acetic Acid	NF	pH Adjuster	--		q.s to adjust pH	--	--	--
Hydrochloric Acid	NF	pH Adjuster	q.s to adjust pH	--	--	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH
Sodium Hydroxide	NF	pH Adjuster	q.s to adjust pH	--	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH	q.s to adjust pH
Water for Injection	USP		(b) (4)					

Q.S = Quantity Sufficient; USP = United States Pharmacopeia; NF = National Formulary

#GLYRX-PF (Glycopyrrolate) Injection, 0.2 mg/mL, 0.4 mg/2 mL (b) (4) is a 505(b)(2) NDA # N210997 held by Exela Pharma Sciences LLC in solution dosage form for intramuscular & intravenous administration and it is approved against 505(b)(1) ROBINUL as RLD. GLYRX-PF differs from the Reference Listed Drug (ROBINUL) in osmolality, absence of benzyl alcohol and presence of sodium chloride.

$ROBINUL (NDA # N017558) is identified as RLD for Glycopyrrolate and has been discontinued. The Federal Register determination says that the product was not discontinued or withdrawn for safety or efficacy reasons. Subsequently, an approved Glycopyrrolate Injection, 0.2 mg/mL; ANDA # A090963 held by Hikma Farmaceutica (Portugal) SA has been designed as RS (Reference Standard) by the FDA.

From 2.3.P Drug Product QOS, pgs. 8-9/120.

The proposed drug product is a combination of neostigmine methylsulfate (1.0 mg/mL) and glycopyrrolate (0.2 mg/mL). The concentration of both APIs are identical to the respective RDs.

The Applicant has submitted a comparison of the physiochemical properties between their proposed product, BLOXIVERZ (Neostigmine Methylsulfate), Glycopyrrolate Injection, USP (0.2 mg/mL), and a combined mixture between these products in a 1:1 ratio (1.12.15 Pharma Equivalence Study Report). The pH of the proposed combination

drug product is between those of the reference drugs. It is noted that the osmolality of the drug product is above that of the RDs but is closer to the range of physiological blood than the RDs [1].

Table 4: Analytical results of Slayback's Neostigmine Methylsulfate and Glycopyrrolate Injection 1.0 mg and 0.2 mg per mL

Tests	Neostigmine Methylsulfate and Glycopyrrolate Injection 1.0 mg and 0.2 mg per mL		
	Batch No.: ATY101 Mfg. Date:03/2021	Batch No.: ATY102 Mfg. Date:03/2021	Batch No.: ATY103 Mfg. Date:03/2021
Description	Clear colorless solution, free of any visible particulate matter	Clear colorless solution, free of any visible particulate matter	Clear colorless solution, free of any visible particulate matter
pH	3.5	3.5	3.5
Osmolality mOsmol/Kg	267	267	267
Viscosity (cps)	1.32	1.39	1.38
Color of solution by UV-visible spectrophotometer (Abs)	0.001	0.000	0.000
Assay of Neostigmine Methylsulfate (by HPLC)	101.1 %	100.5 %	100.2 %
Assay of Glycopyrrolate (by HPLC)	99.9 %	99.5 %	100.0 %
Related Substances by HPLC			
			(b) (4)
Any unspecified degradation product	(b) (4) %	(b) (4) %	(b) (4) %
Total degradation products	%	%	%

From 1.12.15 Pharma Equivalence Study Report, pg. 10/13.

Table 5: Analytical results of BLOXIVERZ® (Neostigmine Methylsulfate) Injection, USP 1.0 mg per mL

Tests	BLOXIVERZ® (Neostigmine Methylsulfate Injection, USP) 1.0 mg per mL		
	Batch No.: AM3066D Exp. Date: 11/2022 Analysis Date: 08/21	Batch No.: AM6214B Exp. Date: 02/2023 Analysis Date: 08/21	Batch No.: AM8220B Exp. Date: 03/2023 Analysis Date: 08/21
Description	Clear colorless solution, free of any visible particulate matter	Clear colorless solution, free of any visible particulate matter	Clear colorless solution, free of any visible particulate matter
pH	5.50	5.57	5.56
Osmolality mOsmol/Kg	55	56	55
Viscosity (cps)	1.31	1.35	1.35
Color of solution by UV-visible spectrophotometer (Abs)	0.000	0.002	0.004
Assay of Neostigmine Methylsulfate (by HPLC)	100.0 %	99.9 %	100.3 %
Related Substances by HPLC			
			(b) (4)

From 1.12.15 Pharma Equivalence Study Report, pg. 7/13.

Table 6: Analytical results of Glycopyrrolate Injection, USP 0.2 mg per mL

Tests	Glycopyrrolate Injection, USP 0.2 mg per mL		
	Batch No.: 2005094.1 Exp. Date: 05/2022	Batch No.: 2005095.1 Exp. Date: 05/2022	Batch No.: 2105022.1 Exp. Date: 02/2023
	Analysis Date: 08/21	Analysis Date: 08/21	Analysis Date: 08/21
Description	Clear colorless solution, free of any visible particulate matter	Clear colorless solution, free of any visible particulate matter	Clear colorless solution, free of any visible particulate matter
pH	2.61	2.61	2.63
Osmolality mOsmol/Kg	86	86	87
Viscosity (cps)	1.34	1.33	1.31
Color of solution by UV-visible spectrophotometer (Abs)	0.000	0.002	0.000
Assay of Glycopyrrolate (by HPLC)	100.1 %	100.0 %	99.2 %
Related Substances by HPLC			
[redacted]			

(b) (4)

From 1.12.15 Pharma Equivalence Study Report, pg. 8/13.

Table 7: Analytical results of Mixture of BLOXIVERZ® (Neostigmine Methylsulfate) Injection, USP and Glycopyrrolate

Tests	BLOXIVERZ® (Neostigmine Methylsulfate Injection, USP) and Glycopyrrolate Injection, USP 1.0 mg per mL and 0.2 mg per mL (1:1 ratio mixture)		
	Batch No.: AM3066D and 2005094.1 Exp. Date: 11/2022 and 05/2022	Batch No.: AM6214B and 2005095.1 Exp. Date: 02/2023 and 05/2022	Batch No.: AM8220B and 2105022.1 Exp. Date: 03/2023 and 02/2023
	Analysis Date: 08/21	Analysis Date: 08/21	Analysis Date: 08/21
Description	Clear colorless solution, free of any visible particulate matter	Clear colorless solution, free of any visible particulate matter	Clear colorless solution, free of any visible particulate matter
pH	3.37	3.37	3.34
Osmolality mOsmol/Kg	71	70	69
Viscosity (cps)	1.36	1.34	1.37
Color of solution by UV-visible spectrophotometer (Abs)	0.004	0.004	0.001
Assay of Neostigmine Methylsulfate (by HPLC)	102.7 %	103.0 %	102.1 %
Assay of Glycopyrrolate (by HPLC)	100.7 %	100.9 %	99.2 %
Related Substances by HPLC			
[redacted]			

(b) (4)

From 1.12.15 Pharma Equivalence Study Report, pg. 9/13.

The maximum daily dose (MDD) of the proposed drug product is the same as the labeled use for the reference drug products. The labeling for the RD of glycopyrrolate, ROBINUL®, states the following:

The recommended dose of Glycopyrrolate Injection is 0.2 mg for each 1 mg of neostigmine or 5 mg of pyridostigmine.

The labeling for the RD of neostigmine methylsulfate, BLOXIVERZ®, states the following:

The recommended maximum total dose if 0.07 mg/kg or up to a total of 5 mg, whichever is less.

From the labeling, the MDD of the proposed product is 5 mg/day neostigmine, 1 mg/day glycopyrrolate.

2.4 Comments on Novel Excipients

The excipients for the proposed drug product are as follows:

Table 8: Quantitative Composition of Drug Product

Component	Quantity per Unit (3 mL Prefilled Syringe)	Maximum Daily Intake (MDI)	MDI in Inactive Ingredients Database (IID)	Comment
Disodium edetate dihydrate USP	0.5 mg	2.5 mg	19 mg	Within limits listed in IID.
Sodium Chloride	8 mg	0.8% w/v, 40 mg	1800 mg	Within limits listed in IID.
Hydrochloric Acid NF	q.s. to adjust pH 3.0-4.2	-	-	-
Sodium Hydroxide	q.s. to adjust pH 3.0-4.2	-	-	-
Water for Injection USP	q.s. to 1 mL	-	-	-

Data from 2.3.P Drug Product QOS, pgs. 2/120.

The excipients are within levels contained in approved drug products as listed in the FDA Inactive Ingredients Database (IID). Therefore, there are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

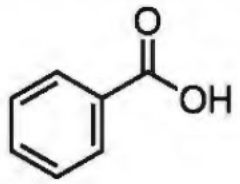
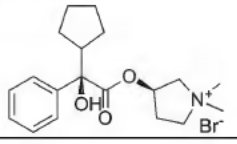
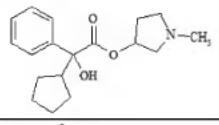
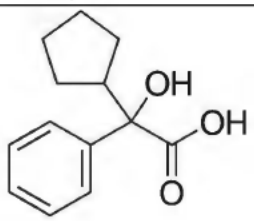
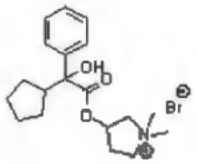
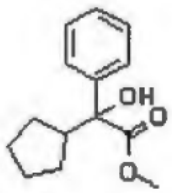
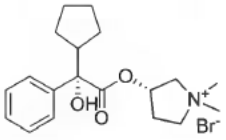
Based on the MDD of this product (5 mg neostigmine, 1 mg glycopyrrolate), the following are the relevant qualification thresholds:

- Drug Substance Impurities as per ICH Q3A(R2)
  = 0.15% or 1.0 mg/day, whichever is lower

- Drug Product Degradants as per ICH Q3B(R2)
  = 1.0% or 50 mcg TDI, whichever is lower

Drug Substance Impurities and Qualifications

Table 9: Specifications of Drug Substance Impurities

Drug Substance	Impurity	Structure	Proposed Specification	Acceptability
Glycopyrrolate USP (from 3.2.S.4.5 Justification of Specifications, pgs. 2-4/4)	Benzoic Acid		NMT (b) (4) %	Acceptable
	Didehydroglycopyrrolate			
	Glycopyrrolate Related Compound B			
	Glycopyrrolate Related Compound C			
	Glycopyrrolate Related Compound I			
	Glycopyrrolate Related Compound L			
	Erythro Isomer			
	(b) (4)			

	(b) (4)		NMT (b) (4) %	
			NMT (b) (4) % (b) (4) MDI = (b) (4)	
	Unspecified Impurity	N/A	NMT (b) (4) %	
Neostigmine Methylsulfate USP (from 3.2.S.4.5 Justification of Specifications, pgs. 2-3/4)	(b) (4)		NMT (b) (4) %	
			NMT (b) (4) %	

All specification limits for the drug substance impurities are within the qualification threshold outlined in ICH Q3A(R2).

Drug Product Degradants and Qualifications

Table 10: Specifications of Drug Product Degradants

Degradants	Structure	Proposed Specification	Acceptability
(b) (4)		NMT (b) (4) %	Acceptable
		NMT (b) (4) %	

The specification limits for the drug product degradants are set at (b) (4) %. All proposed levels are within the thresholds outlined in ICH Q3B(R2)

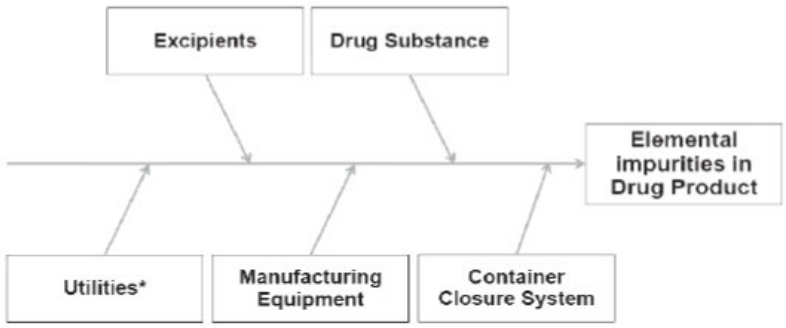
(b) (4)

Elemental Impurities

The Applicant provided an Elemental Impurities Risk Assessment study report (found in 3.2.P.2 Elemental Impurity Assessment Report).

The Applicant supplied an elemental impurities risk assessment with considerations of potential sources of elemental impurities in the drug substance, excipients, manufacturing equipment, utilities, and container closure system.

Figure 1: Elementals Risk Assessment



From 3.2.P.2 Elemental Impurity Assessment Report, pg. 8/304.

A risk assessment of the final drug product also took into considerations the leachables data. Three batches of the drug product (batch numbers ATY101, ATY 102, and ATY 103) were analyzed.

Table 12: Elementals detected in leachables assessment

(b) (4)

From 3.2.P.2 Elemental Impurity Assessment Report, pg. 14/304.

All elemental impurity levels were found to be below the control threshold of 30% PDE.

(b) (4) **Impurity Risk Assessment**

(b) (4)

2.6 Container Closure System

Neostigmine Methylsulfate and Glycopyrrolate [redacted] Injection is to be packaged in 1 mL luer lock syringes. A single package contains 5 syringes in a plastic tray.

Product Description	Container	Closure
3 mL Syringe with Luer Lock and Tipcap, 1-3 mL Plunger Stopper, Plunger Rod for 3 mL Syringe	[redacted] 3 mL Integrated Luer Lock with Tip Cap [redacted] Syringe	Plunger Stopper 1-3 mL, [redacted] Translucent [redacted] Plunger Rod

From 2.2.3.P Drug Product QOS, pg. 109/120.

The Applicant submitted extractable and leachable information under section 3.2.P.2 Pharmaceutical Development:

Report Type	Report Title	Report #	Location
Extractables	Extractable Study Report for the Printed Label and Gum Adhesive	EXTR-21-034-00	EDR
	Extractable Study Report for the [redacted] Plunger Rubber Stopper	EXTR-21-030-00	EDR
	Extractable Study Report for the 3 ML Plastic [redacted] Syringe Barrel [redacted]	EXTR-21-029-00	EDR
	Extractable Study for [redacted]	[redacted]	EDR
	Extractable Study for [redacted]	[redacted]	EDR
Leachables	Report for the Leachable Study [redacted]	LCHR-22-008-00	EDR
	Report for the Leachable Study [redacted]	LCHR-22-025-00	EDR

Extraction Study of Container Closure System

The Applicant conducted extraction studies on the primary container-closure and manufacturing components. These include the [redacted] 3 mL Plastic [redacted] syringe Barrel, [redacted] plunger stoppers, and printed label and gum adhesive.

Table 13: Extraction Test Sample Preparation Summary

EXTRACTABLE TEST SAMPLE PREPARATION	
Extraction Method / Parameters	[redacted], 3 mL Plastic [redacted] syringe Barrel, [redacted] plunger stoppers, printed label and gum adhesive
	[redacted] extraction, 0 and/or 24 hours
Extraction Solvent	[redacted]

Component	Extraction Solvents	LOD and LOQ
(b) (4) plunger stoppers	(b) (4)	
Plastic (b) (4) Syringe Barrel		
(b) (4)		
(b) (4)		
Printed Label and Gum Adhesive		

(b) (4)

Reviewer Comment:
The analytical methods to detect volatile, semi-volatile, and non-volatile organic and inorganic compounds appear appropriate. The SCT utilized for these studies are appropriate. LOQ for these studies have the appropriate sensitivity to detect compounds at the SCT.

Reviewer's calculation of the AET:

Safety Concern Threshold (SCT) = (b) (4)

Maximum daily dose (MDD) of Neostigmine = 5 mg
MDD of Glycopyrrolate = 1 mg

Concentration of drug product = 1 mg/mL Neostigmine Methylsulfate, 0.2 mg/mL Glycopyrrolate

(b) (4)

Specification	Reviewer's AET	Applicant's AET	Adequacy
Safety Concern Threshold (SCT)	(b) (4)	(b) (4)	Adequate
MDD	5 mg Neostigmine/1 mg Glycopyrrolate	5 mg Neostigmine/1 mg Glycopyrrolate	
Extraction Conditions	(b) (4)		
AET			

The methods of the extraction studies appear adequate from a pharmacology/toxicology perspective; however, refer to the CMC review for the final determination on the adequacy of the extraction methods.

Extraction Study Results

The following are the reported extractables from 3 mL Plastic (b) (4) syringe Barrel, (b) (4) plunger stoppers, (b) (4), and printed label and gum adhesive. See extractables reports listed above for results.

The Applicant reports that all detected compounds which exceeded the AET of at least (b) (4) ppm were evaluated in the leachables assessment. AETs were calculated based on the (b) (4)

Reviewer Comment: *The following compounds within the extraction study exceeded the Reviewer's AET and should be targeted in the leachable studies.*

Compound	Max. extracted conc. (mcg/day)	Max. Source of Extractable
(b) (4)		(b) (4) plunger rubber stopper

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(b) (4)	
	Printed Label and Gum Adhesive

The Reader is referred to the CMC review to determine if the leachable studies adequately evaluate the compounds listed in the table above.

Leachables Study of Container Closure System

The Applicant provided two leachables reports (LCHR-22-008-00 and LCHR-22-025-00), which included evaluations at initial release (0 months), 6 months, and 12 month time points under long-term normal conditions (25°C±2°C /40%±5%RH).

	LEACHABLE TEST METHOD
	(b) (4)

(b) (4)

The Applicant states (b) (4) was used for the qualification threshold for all compounds that have been detected from the extractables studies.

Reviewer Comment: *Based on the information provided, the following calculation is made regarding the AET for the leachables study:*

(b) (4)

The limit of quantitation (LOQ) for organic and inorganic compounds appears adequate when compared to the AET calculated by this reviewer.

LEACHABLE STUDY
(b) (4)

Results of Leachable Study

The leachable study analyzed three batches of the drug product at release, 6 months, and 12 months. The targeted compounds in these leachable studies were based on all compounds from the extractables study above the AET of (b) (4) ppm based on the SCT of (b) (4)

Only one compound was identified to be above the AET, while two additional compounds were identified at 12 months but below the reviewer AET.

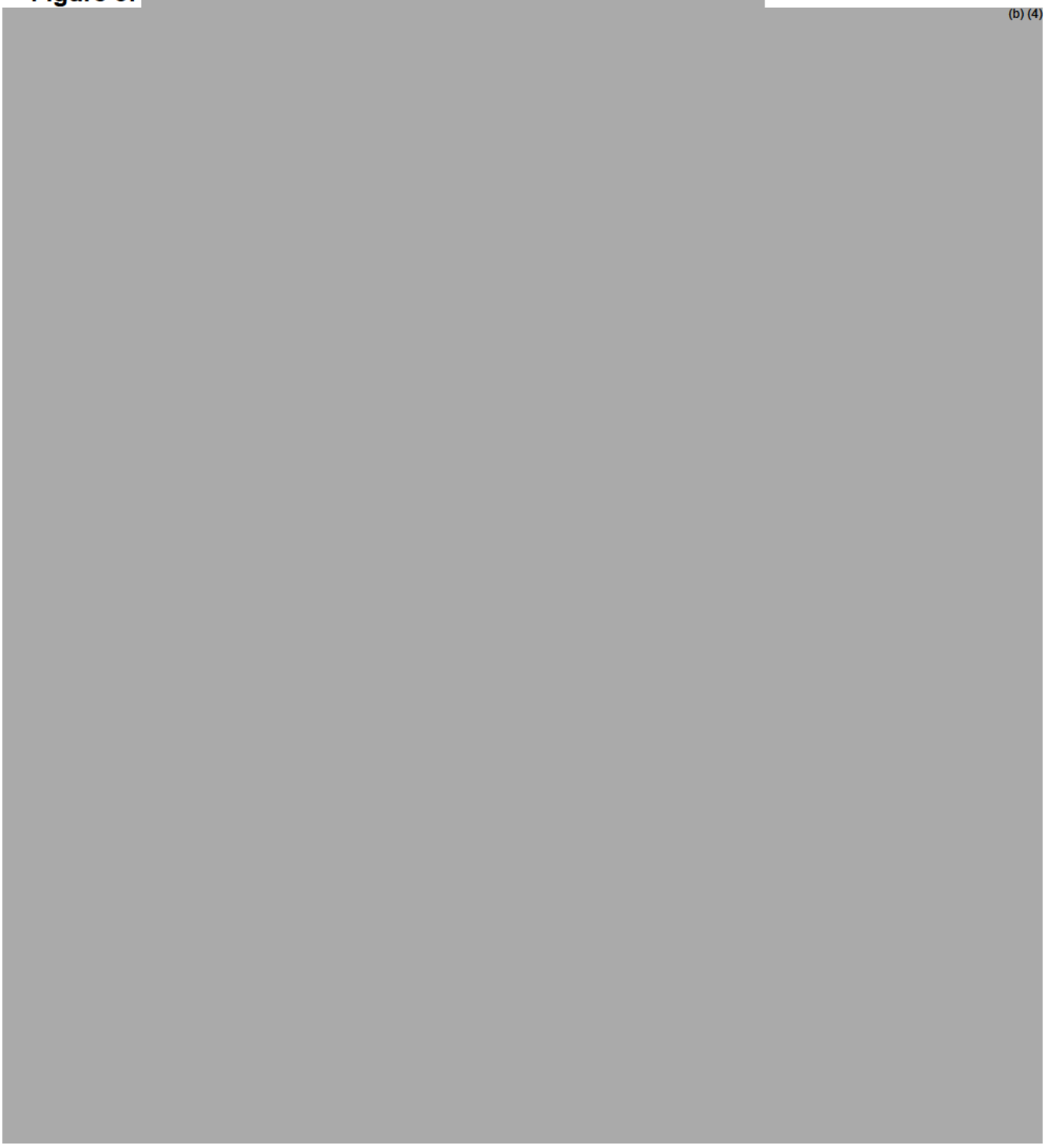
Table 14: Compounds for Risk Assessment

	Compound	Highest Detected Level		Reviewer's PDE (mcg/day)	Trend Analysis	Adequacy
		ppm	mcg/day			
[redacted]	[redacted]	[redacted]	[redacted]	(b) (4) [redacted]	Lower than PDE outlined in ICH Q3C	Adequate
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	(b) (4) [redacted] Maximum amounts observed at 12 months. Trend analysis shows compounds (b) (4) [redacted]	Adequate

(b) (4) [redacted] *were both detected at 12-month timepoints at* (b) (4) [redacted] *respectively). A trendline for each compound was calculated as follows:*

Figure 2: (b) (4) [redacted]

Figure 3: (b) (4)



2.7 Regulatory Background

This is a 505(b)(2) application referencing the Agency's previous findings of safety and efficacy of BLOXIVERZ (NDA 204078) and ROBINUL (NDA 017558). Several meetings

with the Applicant occurred under PIND 139866. This is a first NDA submission for this combination product.

3 Studies Submitted

Study Title	Study #	Study Type	Location
In Vitro Blood Compatibility (Hemolysis) Study of Neostigmine Methylsulfate and Glycopyrrolate Injection 10 mg and 0.2 mg per mL in Human Blood	(b) (4) 001	Hemolysis	EDR
Intravenous and Paravenous Local Tolerance Study of Neostigmine Methylsulfate and Glycopyrrolate Injection 1.0 mg and 0.2 mg per mL in New Zealand White Rabbits	(b) (4) 3497	IV/PV, Local Tolerance	EDR (See also Declaration Letter: EDR)

4 Pharmacology

No new primary pharmacology studies or data with neostigmine or glycopyrrolate were submitted. The Applicant is relying upon the data in the referenced product label.

5 Pharmacokinetics/ADME/Toxicokinetics

No new pharmacokinetic, ADME, or toxicokinetic studies with neostigmine or glycopyrrolate were submitted. The Applicant is relying on the labeling based on the RDs.

6 General Toxicology

No new single- or repeat-dose toxicity studies with neostigmine or glycopyrrolate were submitted, and the Applicant also indicated that no relevant studies were identified in a literature search. The Applicant is relying upon the Agency previous findings of safety and efficacy of the referenced products.

7 Genetic Toxicology

No new genetic toxicity studies with neostigmine or glycopyrrolate were submitted, and no relevant studies were identified in a literature search. The Applicant is relying upon the Agency previous findings of safety and efficacy of the referenced products.

8 Carcinogenicity

No new carcinogenicity studies with neostigmine or glycopyrrolate were submitted, and no relevant studies were identified in a literature search. As the proposed drug product

is for acute use, a carcinogenicity evaluation for neostigmine and glycopyrrolate is not needed.

9 Reproductive and Developmental Toxicology

No new reproductive and developmental toxicology studies with neostigmine or glycopyrrolate were submitted, and no relevant studies were identified in a literature search. The Applicant is relying upon the Agency previous findings of safety and efficacy of the referenced products.

10 Special Toxicology Studies

Local Tolerance Studies

10.1 Study Title+: In Vitro Blood Compatibility (Hemolysis) Study of Neostigmine Methylsulfate and Glycopyrrolate Injection 1.0 mg and 0.2 mg per mL in Human Blood

Study no.:	(b) (4) 001
Study report (Electronic) location:	EDR
Type of Study:	Other
If "Other":	In Vitro Blood Compatibility (Hemolysis)
Study initiation date:	July 13, 2021
Conducting laboratory and location:	(b) (4)
Duration:	1
Duration Units:	days
GLP compliance:	Y
Drug, lot #, and % purity:	Neostigmine Methylsulfate and Glycopyrrolate Injection IH, 1.0 mg/0.2mg per mL, ATY101, 101.1% Neostigmine Methylsulfate, 99.9% Glycopyrrolate

Methods

Test System:	Fresh Human Whole Blood
Frequency of dosing:	Once
Number/Sex/Group:	Mixture blood from 3 human volunteers
Dose volume:	10 mcL
Formulation/Vehicle:	Ready-to-use from Applicant
Comment on Study Design and Conduct:	Blood collected from three human volunteers was pooled and subjected to plasma-free hemoglobin concentration determination. Plasma-free hemoglobin concentration was

determined at 0.157 mg/mL, whole blood hemoglobin concentration was 152.30 mg/mL and was diluted to 10 mg/mL with CMF-PBS. Test item ratios of neat, 1:2, 1:4, and 1:8 were utilized. Blank, normal saline (vehicle control), test item at concentrations, plasma (negative control), Saponin 1% (positive control) were added to different tubes. Following incubation, samples were mixed gently and centrifuged. Absorbance of reactions were spectrophotometrically measured at wavelength 540 nm.

Sample-ID	Test Item Quantity in mL	Diluent Quantity in mL	Final Volume in mL	Concentration
T-1	2	-	2	Neat
T-2	1	2	3	1:2 (1 Part of test Item +2 parts of 0.9% of Sodium chloride)
T-3	1	4	5	1:4 (1 Part of test Item +4 parts of 0.9% of Sodium chloride)
T-4	1	8	9	1:8 (1 Part of test Item +8 parts of 0.9% of Sodium chloride)

Dosing Solution Analysis: Applicant provided finished drug product certificate of analysis (CoA)

Key Study Findings

- Percentage of hemolysis of test article against negative control were -0.188% (Neat), -0.108% (1:2), 0.081% (1:4), and -0.161% (1:8), respectively
- No evidence of hemolysis in any of the concentrations tested of the test article

Observations and Results

The concentration of hemoglobin for the blank, negative, and positive controls were reported at 0.381±0.005, 0.585±0.005, 1.538±0.009 mg/mL respectively. The percentage of hemolysis for the negative and positive controls were 2.1% and 12.0%.

The concentrations of hemoglobin of the test item formulations for the Neat, 1:2, 1:4, and 1:8 were 0.363±0.009, 0.371±0.005, 0.389±0.030, and 0.3650±005 mg/mL, respectively. The percentage hemolysis against that of the negative control were calculated at -0.188%, -0.108%, 0.081% and -0.161%, respectively.

Table 15: Plasma Free Hemoglobin, Total Blood Hemoglobin, and Total Diluted Blood Hemoglobin Concentration

Sample	Absorbance (OD Values)			Average Absorbance	Standard Deviation	Hemoglobin Concentration mg/mL
	R1	R2	R3			
Plasma Free Hemoglobin (PFH)	0.065	0.066	-	0.066	0.001	0.517
Total Blood Hemoglobin (C)	0.155	0.154	-	0.155	0.001	152.30
Total Diluted Blood Hemoglobin (T)	0.157	0.163	0.162	0.161	0.003	10.08

Plasma free hemoglobin concentration (mg/mL) = A^{PFH} x F x 2
Total blood hemoglobin concentration (mg/mL) = A^{C} x F x 251
Total diluted blood hemoglobin concentration (mg/mL) = A^{T} x F x16
A^{PFH} – Average absorbance of plasma free hemoglobin
A^{C} – Average absorbance of total blood hemoglobin
A^{T} – Average absorbance of total diluted blood hemoglobin
Calibration coefficient (F) = 3.915

From 4.2.3.6 Invitro Blood Compatibility study report, pg. 18/30.

Table 16: Hemoglobin Concentration and % Hemolysis

Sample	Replicates	Absorbance at 540 nm	Hemoglobin Concentration (mg/mL)*	% Hemolysis
Blank control	R1	0.049	0.384	-
	R2	0.048	0.376	
	R3	0.049	0.384	
	Mean	**0.049**	**0.381**	
	±SD	**0.001**	**0.005**	
Negative Control (Plasma)	R1	0.075	0.587	**2.098**
	R2	0.074	0.579	
	R3	0.075	0.587	
	Mean	**0.075**	**0.585**	
	±SD	**0.001**	**0.005**	
Vehicle Control (0.9% sodium chloride Injection)	R1	0.047	0.368	**-0.188**
	R2	0.046	0.360	
	R3	0.046	0.360	
	Mean	**0.046**	**0.363**	
	±SD	**0.001**	**0.005**	
Positive control (1% Saponin)	R1	0.197	1.542	**12.023**
	R2	0.199	1.558	
	R3	0.197	1.542	
	Mean	**0.198**	**1.548**	
	±SD	**0.001**	**0.009**	

*Average absorbance × F × 2, F = Calibration Coefficient (Slope); SD: Standard Deviation. Blank correction was not considered

Sample	Replicates	Absorbance at 540 nm	Hemoglobin Concentration (mg/mL)*	% Hemolysis
Test Item (100%) (Neat)	R1	0.047	0.368	-0.188
	R2	0.045	0.352	
	R3	0.047	0.368	
	Mean	**0.046**	**0.363**	
	±SD	**0.001**	**0.009**	
Test Item (1:2)	R1	0.048	0.376	-0.108
	R2	0.047	0.368	
	R3	0.047	0.368	
	Mean	**0.047**	**0.371**	
	±SD	**0.001**	**0.005**	
Test Item (1:4)	R1	0.048	0.376	0.081
	R2	0.054	0.423	
	R3	0.047	0.368	
	Mean	**0.050**	**0.389**	
	±SD	**0.004**	**0.030**	
Test Item (1:8)	R1	0.047	0.368	-0.161
	R2	0.046	0.360	
	R3	0.047	0.368	
	Mean	**0.047**	**0.365**	
	±SD	**0.001**	**0.005**	

*Average absorbance × F × 2, F = Calibration Coefficient (Slope); SD: Standard Deviation. Blank correction was not considered.

From 4.2.3.6 Invitro Blood Compatibility study report, pg. 19-20/30.

Overall, no hemolysis was found at any of the concentrations under the experimental conditions tested.

Reviewer Comment: *It is noted that, while a hemolysis study was submitted, the Applicant did not provide protein flocculation or platelet activation data with the combination product. It is recognized that there is clinical experience for use of these products together under current clinical practice. Given the absence of the findings in the provided nonclinical studies and the lack of safety signals raised from clinical experience of both products together and individually, we do not have any concerns regarding the blood compatibility of this combination product. See the clinical review memo for additional information.*

10.2 Study Title+: Intravenous and Paravenous Local Tolerance Study of Neostigmine Methylsulfate and Glycopyrrolate Injection 1.0 mg and 0.2 mg per mL in New Zealand White Rabbits

Study no.:	(b) (4) 3497
Study report (Electronic) location:	EDR
Type of Study:	Other
If "Other":	Local Tolerance Study
Study initiation date:	June 23 2021
Conducting laboratory and location:	(b) (4)
Duration:	1
Duration Units:	days
GLP compliance:	N
Drug, lot #, and % purity:	Neostigmine Methylsulfate and Glycopyrrolate Injection 1.0 mg and 0.2 mg per mL, ATY101, Neostigmine Methylsulfate (101.1%) and Glycopyrrolate (99.9%)

Methods

Doses:	0.2 mg Neostigmine Methylsulfate/0.04 mg Glycopyrrolate Vehicle Control (G1), Intravenous (G2), Paravenous (G3)
Frequency of dosing:	Single dose
Number/Sex/Group:	3 (Males only)
Dose volume:	0.2 mL
Formulation/Vehicle:	Ready-to use from Applicant
Route of administration:	INTRAVENOUS
Species:	RABBIT
Strain:	NEW ZEALAND
Age / Sexual Maturity:	4-5 months
Comment on Study Design and Conduct:	Non-GLP study utilizing only males. Groups were treated with the vehicle control or test article in the right ear and the placebo on the left ear.

Group	Group Description	Route of Administration	Dose* (mg/rabbit)	Conc.* (mg/mL)	Dose Volume (mL/rabbit)	No. of Animals/ Group	Animal No. Male
G1	Vehicle Control# (right-IV and left ear-PV)	IV and PV	0	0	0.2	3 M	Nb7970 to Nb7972
G2	Test (right ear) and Placebo (left ear)	IV	0.2/0.04	1.0/0.2	0.2	3 M	Nb7973 to Nb7975
G3	Test (right ear) and Placebo (left ear)	PV	0.2/0.04	1.0/0.2	0.2	3 M	Nb7976 to Nb7978

IV: Intravenous (marginal ear vein); PV: Paravenous (Adjacent to marginal ear vein); M: Male; Conc.: Concentration; *: Neostigmine Methylsulfate / Glycopyrolate; #: sterile water for injection.

Dosing Solution Analysis: CoA presented of drug product. Neostigmine Methylsulfate characterized at 101.1%, and Glycopyrrolate characterized at 99.9%

Key Findings

- Study was non-GLP with only males evaluated.
- No adverse clinical signs or local skin reactions were reported.
- Gross pathology and histopathology showed no adverse test article-related findings.

Observations and Results

Mortality

No mortality was reported in this study.

Clinical Signs

Tremors, ventral recumbency, and lethargy were reported approximately 2 to 10 minutes in all animals of the IV treatment group and one rabbit in the PV treatment group. Animals were normal after 96-hour post-dose. No other clinical signs were reported.

Local Skin Reaction

No erythema or edema was reported at the site of the injection in either the Control or Test animals.

Table 17: Summary of Local Skin Reactions Scoring Record

Refer Appendix 2

Group, Sex & Dose (mL/rabbit)	No. of Animals	Local Skin Reactions	
		Erythema/ No. of Animals	Oedema/ No. of Animals
G1, M & 0.2	3	0/3	0/3
G2, M & 0.2	3	0/3	0/3
G3, M & 0.2	3	0/3	0/3

M: Male; 0: Absent; 1: Present.

From 4.2.3.6 InVitro PV local Tolerance Study-report, pg. 21/45.

Body Weights

No statistically significant ($p<0.05$) changes in mean body weights, percent body weight change was reported in the treated groups compared to controls.

Table 18: Summary of Body Weights (Kg) Record

Refer Appendix 3

Group, Sex & Dose (mL/rabbit)		Body Weight (kg) on Days		
		1	3	5
G1, M & 0.2	Mean	2.119	2.169	2.225
	±SD	0.090	0.060	0.054
	n	3	3	3
G2, M & 0.2	Mean	2.121	2.157	2.206
	±SD	0.091	0.097	0.095
	n	3	3	3
G3, M & 0.2	Mean	2.127	2.158	2.207
	±SD	0.101	0.102	0.103
	n	3	3	3

M: Male; SD: Standard Deviation; n: Number of animals.

From 4.2.3.6 InVitro PV local Tolerance Study-report, pg. 23/45.

Table 19:Summary of Percentage Change in Body Weight (%) With Respect to Day 1 Record

Refer Appendix 4

Group, Sex & Dose (mL/rabbit)		Percent Change in Body Weight (%) during Days	
		1 to 3	1 to 5
G1, M & 0.2	Mean	2.39	5.05
	±SD	1.54	1.94
	n	3	3
G2, M & 0.2	Mean	1.68	4.01
	±SD	0.24	0.20
	n	3	3
G3, M & 0.2	Mean	1.48	3.77
	±SD	0.04	0.08
	n	3	3

M: Male; ; SD: Standard Deviation; n: Number of animals.

From 4.2.3.6 InVitro PV local Tolerance Study-report, pg. 24/45.

Hematology and Clinical Chemistry

No hematology or clinical chemistry was performed.

Gross Pathology

No gross pathological changes were reported in either the Control or Test groups.

Table 20: Summary of Gross Pathology Findings

Sex	Male		
Group description	Vehicle Control	Test Item	Test Item
Route of administration	IV and PV	IV	PV
Group	G1	G2	G3
Dose (mL/rabbit)	0.2	0.2	0.2
Number of animals	3	3	3
No. of dead rabbits during treatment	-	-	-
No. of moribund sacrificed rabbits	-	-	-
No. of terminally sacrificed rabbits	3	3	3
No of rabbits showing gross pathology	-	-	-

-: No incidence; IV: Intravenous (marginal ear vein); PV: Paravenous (Adjacent to marginal ear vein).

From 4.2.3.6 InVitro PV local Tolerance Study-report, pg. 25/45

Histopathology

Adequate Battery: Y (Local Tolerance Only)

Peer Review: N

At the injection site, a single incidence of focal perivascular fibrosis (minimal, 1 Control animal) and a single incidence of focal perivascular infiltration of mononuclear ells (minimal, 1 Paravascular animal) were reported. These were considered injection or procedure-related and non-adverse changes. No other incidences were reported.

Table 21: Summary of Histopathology Findings

Route of administration				IV and PV	IV	PV
Group description				Vehicle Control	Test Item/ Placebo	Test Item/ Placebo
Dose (mL/rabbit)				0.2	0.2	0.2
Group				G1	G2	G3
Sex				M	M	M
Number of Animals				3	3	3
Injection Site	Left Ear	Number examined		3	3	3
		Within normal limits		2	3	2
		Fibrosis, Perivascular	Minimal	1	-	-
		Infiltrate mononuclear cells, Perivascular	Minimal	-	-	1
	Right Ear	Number examined		3	3	3
		Within normal limits		3	3	3

M: Male; ; -: No incidence; X: Not applicable; IV: Intravenous (marginal ear vein); PV: Paravenous (Adjacent to marginal ear vein).

From 4.2.3.6 InVitro PV local Tolerance Study-report, pg. 26/45.

Reviewer Comment: *It is noted that the submitted study was non-GLP and conducted in males only. In the Day 74 letter, the Applicant was requested to provide additional information including how the local tolerance study deviated from GLP, how these deviations did not affect the integrity of the study, along with clinical and safety data for how both the neostigmine and glycopyrrolate combination are commonly utilized in a clinical setting.*

The following response was received for Information Request (IR) was sent to the Applicant on July 7, 2022 in the Day 74 Letter:

> *We acknowledge that your submitted blood compatibility study (Study No: (b) (4) 001) was conducted under Good Laboratory Practices (GLP), however, the intravenous and paravenous local tolerance study (Study (b) (4) 3497) was not conducted under GLP. Note all nonclinical studies to support human safety should be GLP compliant. Provide a detailed list of how your local tolerance study deviated from GLP along with a justification as to why these deviations do not affect the integrity and/or conclusions of your study. In addition, submit additional justification, such as clinical use and safety data that this combination is commonly utilized in a clinical setting. If an adequate justification is not provided, you may need to repeat the local tolerance study under GLP conditions during this review cycle.*

The Applicant submitted a report on July 29, 2022 (see 4.2.3. InVitro PV local Tolerance Study-report) comparing the study to the study plan, raw data, and in-house SOPs. Overall, the report states that instruments used in the study were calibrated as per in-house SOPs, and all personnel involved in the conduct of the study were trained as per principles of GLP.

Reviewer Comment*: While the applicant had provided further details on how their study conforms to the principles of GLP, issues regarding using only a single sex (i.e., only males) and not both males and females in the study were not addressed.*

The following information request was sent to the Applicant:

> *We acknowledge the information sent on July 29, 2022, regarding study number (b) (4) 3497 and how aspects of the study followed the principles of GLP. However, we note that your study report did not account for sex differences, given that the study only consisted of 3 males per group. Provide a justification as to how your study adequately addresses the local safety for both sexes given the use of only females in your study.*

The Applicant responded on September 16, 2022. The response cited the study was run in accordance with the European Medicines Agency (EMA) “Guideline on non-clinical local tolerance testing of medicinal products” [2] which recommends that evaluation of one species should be sufficient. The applicant also cites an article by

Jochims et al., 2003 [3], which noted that local irritating effects of test formulations are unlikely sex-dependent in either humans or in animals.

***Reviewer Comment**: The Applicant provided a citation indicating that sex-dependent differences with respect to local toxicity from parenteral drug effects are unlikely.*

In discussions with the Clinical Review Team, it is also noted that the combination of drug products neostigmine methylsulfate and glycopyrrolate are commonly used together, and that there are no signals indicating sex-dependent differences.

Given the totality of evidence presented (i.e., that both the local toxicity effects of the drug product are unlikely to be sex-dependent and that this combination of drug products has been often given in both sexes), there are no outstanding safety concerns with the proposed combination product.

11 Integrated Summary and Safety Evaluation

The Applicant submitted NDA 21903 for a combination product that consists of neostigmine methylsulfate (1.0 mg/mL) and glycopyrrolate (0.2 mg/mL) via the 505(b)(2) regulatory pathway. The Applicant has cited NDA 204078 (BLOXIVERZ) and NDA 017558 (ROBINUL) as the Reference Drugs (RDs) for neostigmine methylsulfate and glycopyrrolate, respectively. The Maximum Daily Dosage (MDD) for this product is 5 mg/day neostigmine, 1 mg/day glycopyrrolate.

In the NDA, the Applicant submitted a comparison of physiochemical properties between their proposed product, BLOXIVERZ (Neostigmine Methylsulfate), Glycopyrrolate Injection, USP (0.2 mg/mL), and a combined mixture between these products in a 1:1 ratio. The proposed drug product is isotonic and within the pH range of the RDs.

To support the nonclinical safety of their drug product, the Applicant had submitted literature from the time of approval of the RD to the date of submission regarding pharmacology, pharmacokinetic, ADME, toxicokinetic, and toxicology data/information.

The Applicant's proposed specifications for drug substance and drug product impurities are within the levels outlined in ICH Q3A(R2) and Q3B(R2). (b) (4) Elemental impurities are below the control threshold of 30% as per ICH Q3D.

To support the safety of the container closure system, the Applicant provided extractables and leachables studies. No leachables above the SCT of (b) (4) were identified, and therefore there are no concerns with the safety of the container closure system.

To support the safety for local toxicity, the Applicant provided an in vitro hemolysis study and a local tolerance study (IV/PV). It is noted that while the hemolysis study was GLP,

the local tolerance was non-GLP and utilized rabbits of one sex. In a response to an information request, the Applicant communicated that the local tolerance study was conducted in the spirit of GLP. The Applicant also provided some literature indicating that sex-dependent differences regarding local toxicity by parenteral drug are unlikely. In discussions with the Clinical Review Team, it is also noted that the combination of drug products neostigmine methylsulfate and glycopyrrolate are often used in combination in both sexes. Based on the totality of evidence, there are no outstanding safety concerns regarding combination product.

Taken together, NDA 216903 for neostigmine methylsulfate and glycopyrrolate is recommended for approval from a pharmacology/toxicology perspective.

12 Appendix/Attachments

12.1 References

1. Roethlisberger, D., et al., *If Euhydric and Isotonic Do Not Work, What Are Acceptable pH and Osmolality for Parenteral Drug Dosage Forms?* J Pharm Sci, 2017. **106**(2): p. 446-456.
2. Agency, E.M., *Guideline on non-clinical local tolerance testing of medicinal products*. 2015, European Medicines Agency.
3. Jochims, K., et al., *Local tolerance testing of parenteral drugs: how to put into practice.* Regul Toxicol Pharmacol, 2003. **38**(2): p. 166-82.

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/s/

ALEXANDER I SON
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