2.7.1.4. Appendix to the Summary of Biopharmaceutic Studies and Associated Analytical Methods

Tirzepatide (LY3298176)

Lilly Research Laboratories
Eli Lilly and Company
Indianapolis, Indiana, USA

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List of Tables

2.7.1.4.1. Formulations

Table APP.2.7.1.1. Summary of Formulations Used in Clinical Studies

Product Used	Study Identifier
Tirzepatide 5 mg via Fixed, multi-dose, single-patient-use prefilled pen (multi-dose PFP) with preservatives in the formulation	I8F-MC-GPIP
Tirzepatide 5 mg via Single-dose pen (SDP) without preservatives in the formulation	I8F-MC-GPIP

2.7.1.4.1.1. Clinical Study Codes and Corresponding Clinical Trial Material Lot Numbers

Refer to Module 3, Section 3.2.P.2.2 Drug Product, Table 3.2.P.2.2.4-1, for a summary list of clinical trial material information associated with the study trials.

2.7.1.4.1.2. Manufacturing Summary of Clinical Trial Lots

Information summarizing manufacturing of clinical trial materials used in the clinical studies is provided within Module 3, Section 3.2.P, Drug Product as follows:

Table APP.2.7.1.2. Manufacturing Summary of Clinical Trial Batches

Section	Reference Tables	Information Provided
3.2.P.2, Pharmaceutical Development	Table 3.2.P.2.2.4-1 Clinical Study Codes and Corresponding Clinical Trial Material Batch Numbers	The clinical study code, the package batch number, the dose form batch number, the drug substance batch number, the package item, and the drug product strength.
3.2.P.5.4.2, Batch Analysis of Clinical Trial Lots of Multiple-Dose Tirzepatide Injection	Table P.5.4.2-1 Batch Analysis Data of a Clinical Trial Batch Manufactured by the Commercial Process for Multiple-Dose Drug Product (Tirzepatide Injection)	The batch number, the drug substance batch number, the dosage, the batch size, the manufacturing site, date of manufacture, and the batch use. Also, test results for each batch are included in this table.

2.7.1.4.2. Bioanalytical Methodology

Table APP.2.7.1.3. Summary of Method Performance

Bioanalytical method validation report name, amendments, and hyperlinks	Report 191444PVDJS_EII_R2		
Method description	Partial Method Validation for the Quantitation of Tirzepatide (LY3298176) in Human Plasma by HRAM LC/MS		
Materials used for standard calibration curve and concentration	Tirzepatide lot RS1058 Internal standard (IS): LSN3316897 lot BCA-BE03935-132 Citrate buffer solution		
Validated assay range	2.00 to 500 ng/mL		
Material used for quality controls (QCs) and concentration	Tirzepatide lot RS1058 Internal standard (IS): LSN3316897 lot BCA-BE03935-132 Dry powder		
Minimum required dilutions (MRDs)	Not applicable		
Source and lot of reagents (LBA)	Not applicable		
Regression model and weighting	Weighted $1/x^2$ least squares linear regression		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	8	191444PVDJS_EII_R2 Section 5.3.1.4
	Cumulative accuracy (%bias) from LLOQ to ULOQ Tirzepatide	-2.8% to 4.8%	191444PVDJS_EII_R2 Section 5.3.1.4
	Cumulative precision (%CV) from LLOQ to ULOQ Tirzepatide	≤4.8%	191444PVDJS_EII_R2 Section 5.3.1.4
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 3 QCs QCs: Tirzepatide	-2.1% to 2.8%	191444PVDJS_EII_R2 Section 5.3.1.4
	Inter-batch %CV QCs: Tirzepatide	≤12.9%	191444PVDJS_EII_R2 Section 5.3.1.4
	Total error (TE) QCs:	Not applicable	
Selectivity & matrix effect	Number of total lots tested. Range of observed bias. State any issue		Six lots of blank plasma were tested. Response was ≤12.3% of LLOQ.
Interference & specificity	Number of total lots tested. Range of observed bias. State any issue		Not applicable
Hemolysis effect	Number of total lots tested. Range of observed bias. State any issue		One lot of 2% hemolytic plasma was tested. Response was 6.9% of LLOQ.

Lipemic effect	Number of total lots tested. Range of observed bias. State any issue	One lot of lipemic plasma was tested. Response was 7.3% of LLOQ.
Dilution linearity & hook effect	100-fold dilution validated. Hook effect not applicable.	
Bench-top/process stability[a]	Plasma: 24 hours at room temperature Extracted plasma: 177 hours at room temperature	
Freeze-thaw stability[a]	5 freeze-thaw cycles at -20°C and -70°C	
Long-term storage[a,b]	680 days at -20°C and -70°C	
Parallelism	Not applicable	
Carry over	There was no significant carryover.	
Method performance in Studies		
Assay passing rate	GPIP: 21 out of 22 runs passed (95%)	GPIP
Standard curve performance	• Cumulative bias range: GPIP: -1.8% to 5.3% • Cumulative precision: GPIP: ≤7.4% CV	GPIP
QC performance	• Cumulative bias range: GPIP: -0.2% to 2.0% • Cumulative precision: GPIP: ≤6.5% CV	GPIP
Method reproducibility	GPIP: 9% of samples were run in ISR and 97% passed criteria.	GPIP
Study sample analysis/ stability	Samples were kept at -70°C for up to 145 days. Stability was established for 680 days at -70°C.	GPIP

Abbreviations: CV = coefficient of variation; GPIP = I8F-MC-GPIP; HRAM = high-resolution accurate mass monitoring; ISR = incurred sample reanalysis; LBA = ligand-binding assay; LC/MS = liquid chromatographic-mass spectrometry; LLOQ = lower limit of quantitation; QC = quality control; ULOQ = upper limit of quantitation.

[a] Determined in method 151682VKM_EII_R2.

[b] Determined in Run 19 in Study I8F-MC-GPGB.

2.7.1.4.3. Clinical Study Results

Table APP.2.7.1.4. Summary of Bioequivalence Study

Study Identifier	Study Objective	Study Design	Treatments (Dose, Dosage Form, Route) [Product ID]	Subjects (No. (M/F) Type Mean Age (Range)	Geometric Mean (CV [%]) of Parameter Estimates						Study Report Location
					C_{max} (ng/mL)	t_{max}[a] (hr)	$AUC_{(0\text{-}tlast)}$ (ng*hr/mL)	$AUC_{(0\text{-}\infty)}$ (ng*hr/mL)	$t_{1/2}$[b] (hr)	Kel (hr^{-1})	
I8F-MC-GPIP	To evaluate the bioequivalence between the multi-dose PFP (test) and the SDP (reference), as assessed using tirzepatide PK in healthy participants	Open-label, randomized, 2-period, 2-sequence, crossover study	Test product: 5 mg SC injection (Batch# D577651A)	62 completing (28M/34F) Healthy participants mean age 40.5 (22 – 69)	524 (27)	36.0 (8.00 – 144)	118000 (22)	119000[e] (22)	126[e] (81.5 – 186)	0.00552[e] (15)	Module 5.3.1.2
			Ref. product: 5 mg SC injection (Batch# D529855C)	65 completing (30M/35F) Healthy participants mean age 41.1 (22 – 70)	647 (31)	12.0 (7.97 – 168)	124000 (23)	126000 (22)	122[d] (39.0 – 178)	0.00567[d] (20)	

Abbreviations: $AUC_{(0\text{-}tlast)}$ = area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration; $AUC_{(0\text{-}\infty)}$ = area under the concentration versus time curve from zero to infinity; C_{max} = maximum observed drug concentration; CSR = clinical study report; CV = coefficient of variation; F = female; Kel = elimination rate constant (also known as λ_z in most calculation software); M = male; PFP = prefilled pen (test); PK = pharmacokinetics; SC = subcutaneous; SDP = single-dose prefilled pen (reference); $t_{1/2}$ = half-life associated with the terminal rate constant in noncompartmental analysis; t_{max} = time of maximum observed drug concentration.

[a] Median (minimum – maximum).

[b] Geometric mean (minimum – maximum).

[c] N = 61. One subject was excluded due to the $AUC_{(0\text{-}\infty)}$ calculated with ≥20% extrapolation.

[d] N = 64. One subject was excluded due to the half-life estimated over a time window less than 2 half-lives.

[e] N = 60. Two subjects were excluded due to the half-life estimated over a time window less than 2 half-lives.

Source: GPIP CSR, Table GPIP.5.6.

2.7.1.4.4. Additional Summary Tables for Bioequivalence Studies Per FDA CDER Guidelines

Table APP.2.7.1.5. Product Information

Product	Test	Reference
Treatment ID	Tirzepatide 5 mg via fixed, multi-dose, single-patient-use prefilled pen	Tirzepatide 5 mg via SDP
Product name	Tirzepatide	Tirzepatide
Manufacturer	Eli Lilly and Company	Eli Lilly and Company
Batch/Lot No.	D577651A	D529855C
Manufacture Date	January 2023	July 2022
Strength	5 mg/0.6 mL	5 mg/0.5 mL
Dosage Form	Solution	Solution
Dose Administered	5 mg	5 mg
Route of Administration	Subcutaneous	Subcutaneous

Abbreviations: ID = identification number; SDP = single-dose prefilled pen.

Table APP.2.7.1.6. Bioanalytical Method Validation

Analytical Validation Report Location	191444PVDJS_EII_R2 Module 5.3.1.4
This analytical method was used in the following studies:	GPIP
Short description of the method	Method validation for the quantitation of tirzepatide in human plasma using HRAM LC-MS
Biological matrix	Plasma
Analyte Location of product certificate	Tirzepatide 191444PVDJS_EII_R2
Internal standard (IS)[a] Location of product certificate	LSN3316897 191444PVDJS_EII_R2
Calibration concentrations (units)	2.00, 4.00, 10.0, 50.0, 100, 250, 400, and 500 ng/mL
Average recovery of the drug (%)	Not applicable
Average recovery of the IS (%)	Not applicable
Lower limit of quantification (units)	2.00 ng/mL
QC concentrations (units)	2.00, 6.00, 200, and 375 ng/mL
Between-run accuracy	-2.1% to 2.8%
Between-run precision	8.1% to 12.9%
Within-run accuracy	-10.2% to 12.2%
Within-run precision	3.3% to 11.0%
Matrix factor (MF) (all QC)[a]	Not applicable
IS normalized MF (all QC)[a]	
CV (%) of IS normalized MF (all QC)[a]	
% of QCs with >85% and <115% n.v.[a]	
% matrix lots with mean <80% or>120% n.v.[a]	
Long-term stability of the stock solution and working solutions[b]	24 hours at room temperature
Short-term stability in biological matrix at room temperature or at sample processing temperature	24 hours at room temperature
Long-term stability in biological matrix	680 days at -20°C and -70°C
Autosampler storage stability	Not evaluated
Postpreparative stability	165 hours at room temperature
Freeze and thaw stability (observed change %)	5 cycles at -20°C and -70°C
Dilution integrity	100-fold
Selectivity	No interfering peaks noted in blank plasma samples
Partial validation	This partial validation was performed to verify a shorter LC gradient than that used in validation 151682VKM_EII.
Cross-validation	A cross-validation was performed to establish equivalent performance between the 2 methods.

Abbreviations: CV = coefficient of variation; HRAM LC-MS = high-resolution accurate mass liquid chromatography mass spectrometry; n.v. = nominal value; QC = quality control.

[a] Might not be applicable for the given analytical method.

[b] Report short-term stability results if no long-term stability on stock and working solution are available.

Table APP.2.7.1.7. Summary of Standard Curve and QC Data for Bioequivalence Sample Analyses

Bioequivalence Study Identifier: I8F-MC-GPIP								
Analyte Name: Tirzepatide								
Parameter	**Standard Curve Samples**							
Concentration (ng/mL)	2.00	4.00	10.0	50.0	100	250	400	500
Inter-day precision (CV [%])	6.40	7.12	5.57	5.08	5.43	4.43	7.38	3.24
Inter-day accuracy (% actual)	1.50	-1.75	-1.20	-1.24	0.09	-0.62	5.26	-1.00
Linearity	(range of R^2 values) 0.9881 to 0.9997							
Linearity range (ng/mL)	2.00 to 500.00							
Sensitivity/LOQ (ng/mL)	2.00							
Parameter	**QC Samples**							
Concentration (ng, mcg/mL)	6.00		25.00		200.00		375.00	
Inter-day precision (CV [%])	6.54		5.81		5.17		4.51	
Inter-day accuracy (%Actual)	2.00		-0.16		0.78		1.14	

Abbreviations: CV = coefficient of variation; LOQ = lower limit of quantitation; QC = quality control.

Table APP.2.7.1.8. SOPs Dealing with Bioanalytical Repeats of Study Samples

SOP Identifier	Effective Date of SOP	SOP Title
ADV_OP_TX0006 Revision 14	01 April 2023	Bioanalytical Repeat Sample Analysis

Abbreviation: SOP = standard operating procedure.

Table APP.2.7.1.9. Reanalysis of Study Samples

Study Identifier: I8F-MC-GPIP								
Study Report Location	**Number of Samples Reanalyzed**				**Number of Recalculated Values Used after Reanalysis**			
	Actual Number		**% of Total Assays**		**Actual Number**		**% of Total Assays**	
Reason why assay was repeated	**Test**	**Reference**	**Test**	**Reference**	**Test**	**Reference**	**Test**	**Reference**
Raised LLOQ, samples below LOQ repeated	4	4	0.23	0.23	4	4	100	100
Internal standard outlier	9	8	0.51	0.45	9	8	100	100
Original concentration >ULOQ	0	7	0	0.40	NA	7	NA	100
Original diluted concentration <LLOQ	1	0	0.06	0	1	NA	100	NA
Total	14	19	0.79	1.07	14	19	100	100

Abbreviations: LLOQ = lower limit of quantitation; LOQ = limit of quantitation; NA = not applicable; ULOQ = upper limit of quantitation.

Table APP.2.7.1.10. Study Information

Study Identifier: I8F-MC-GPIP				
Study Title: A Bioequivalence Study to Compare the Pharmacokinetics of Tirzepatide Administered Subcutaneously by a Fixed-Dose Multi-use Prefilled Pen Versus Single-Dose Pen in Healthy Participants				
Study Type	☒ In Vivo BE	☐ In Vitro BE	☐ Permeability	☐ Other (Specify)
Submission Location:				
Study Report	Section 5.3.1.2			
Validation Report	191444PVDJS_EII_R2, Section 5.3.1.4			
Bioanalytical Report	GPIP, Section 5.3.1.2			
Clinical site (Name, Address, Phone #)	[redacted]			
Principal Clinical Investigator	[redacted]			
Dosing Dates	21 April 2023 through 10 June 2023			
Principal Analytical Director	[redacted]			
Storage Period of Biostudy Samples (# days from the first day of sample collection to analysis)	145			

Redacted under Section 41 and Section 43 of the FOI Act.

Redacted under Section 40 FOI Act.

Redacted under Section 40 of the FOI Act.

Abbreviation: BE = bioequivalence.

Table APP.2.7.1.11. Statistical Summary of the Bioequivalence Data

Tirzepatide Dose (5 mg) Least Squares Geometric Means, Ratio of Means, and 90% Confidence Intervals Fasted Bioequivalence Study (I8F-MC-GPIP)				
Parameter (units)	**Test**	**Reference**	**Ratio**	**90% CI**
$AUC_{(0\text{-}tlast)}$ (ng*hr/mL)	116984	124019	0.943	0.927 – 0.960
$AUC_{(0\text{-}\infty)}$ (ng*hr/mL)	119618	126177	0.948	0.931 – 0.965
C_{max} (ng/mL)	523.0	646.7	0.809	0.780 – 0.838

Abbreviations: $AUC_{(0\text{-}tlast)}$ = area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration; $AUC_{(0\text{-}\infty)}$ = area under the concentration versus time curve from time zero to infinity; CI = confidence interval; C_{max} = maximum observed drug concentration; CSR = clinical study report.

Source: GPIP CSR, Table GPIP.5.7

Table APP.2.7.1.12. Dropout Information

Study Identifier: I8F-MC-GPIP

Subject ID	Reason for Dropout/Replacement	Period	Replaced (Y/N)	Replacement's Subject ID
1104	Positive drug screen	1	N	NA
1304	Adverse event: Assessment anxiety	1	N	NA
1313	Adverse event: Covid-19	1	N	NA

Abbreviations: CSR = clinical study report; ID = identification number; N = no; NA = not applicable; Y = yes.
Source: GPIP CSR Section 4.1; 17 CSR APP - list disposition disco-cp

Table APP.2.7.1.13. Demographic Profile of Subjects Completing the Crossover Bioequivalence Study I8F-MC-GPIP

Study Identifier: I8F-MC-GPIP		Treatment Groups[a]	
		Test Product N=62	Reference Product N=62
Age (years)	Mean (SD)	40.5 (13.3)	40.5 (13.3)
	Range	(22 – 69)	(22 – 69)
Sex	Male	28 (45.2%)	28 (45.2%)
	Female	34 (54.8%)	34 (54.8%)
Race	Asian	1 (1.6%)	1 (1.6%)
	Black or African American	6 (9.7%)	6 (9.7%)
	White	55 (88.7%)	55 (88.7%)
BMI (kg/m^2)	Mean (SD)	25.4 (2.5)	25.4 (2.5)
	Range	(19 – 30)	(19 – 30)
Weight (kg)	Mean (SD)	70.9 (11.4)	70.9 (11.4)
	Range	(46 – 98)	(46 – 98)

Abbreviations: BMI = body mass index; N = number of participants; SD = standard deviation.

[a] 65 subjects participated in this crossover study. Three subjects discontinued from the study before receiving the test product.

Table APP.2.7.1.14. Incidence of Adverse Events in Study I8F-MC-GPIP

Summary of Treatment Emergent Adverse Events
Preferred Term by Decreasing Frequency within System Organ Class
Safety Analysis Set
I8F-MC-GPIP

System Organ Class MedDRA Preferred Term	5 mg tirzepatide SC (SDP) (N=65) Number of Subjects (%)	 Number of Occurrences	5 mg tirzepatide SC (MUPFP) (N=62) Number of Subjects (%)	 Number of Occurrences	Total (N=65) Number of Subjects (%)	 Number of Occurrences
Subjects with >= 1 TEAE	41(63.1)	91	35(56.5)	82	51(78.5)	173
Gastrointestinal disorders	29(44.6)	45	23(37.1)	39	39(60.0)	84
Nausea	16(24.6)	17	14(22.6)	15	24(36.9)	32
Dyspepsia	10(15.4)	10	7(11.3)	7	16(24.6)	17
Vomiting	9(13.8)	9	7(11.3)	7	14(21.5)	16
Diarrhoea	3(4.6)	3	4(6.5)	4	7(10.8)	7
Gastrooesophageal reflux disease	1(1.5)	2	3(4.8)	3	3(4.6)	5
Abdominal distension	3(4.6)	3	1(1.6)	1	4(6.2)	4
Abdominal pain	0	0	1(1.6)	1	1(1.5)	1
Abdominal tenderness	1(1.5)	1	0	0	1(1.5)	1
Eructation	0	0	1(1.6)	1	1(1.5)	1
Metabolism and nutrition disorders	17(26.2)	18	14(22.6)	14	23(35.4)	32
Decreased appetite	17(26.2)	18	14(22.6)	14	23(35.4)	32
Nervous system disorders	12(18.5)	15	7(11.3)	8	17(26.2)	23
Headache	10(15.4)	11	5(8.1)	6	14(21.5)	17
Dizziness	3(4.6)	3	0	0	3(4.6)	3
Dysgeusia	0	0	1(1.6)	1	1(1.5)	1
Hyperaesthesia	0	0	1(1.6)	1	1(1.5)	1
Tremor	1(1.5)	1	0	0	1(1.5)	1

Abbreviations: MUPFP = Multi-use prefilled pen; N = Number of subjects in safety population; SC = Subcutaneous;
SDP = Single-dose pen; TEAE = Treatment-emergent adverse events.

MedDRA Version 26.0

Program Location: /lillyce/prd/ly3298176/i8f_mc_gpip/final/programs/stat/tfl/sld/smteae01.sas
Output Location: /lillyce/prd/ly3298176/i8f_mc_gpip/final/output/shared/smteae01.rtf
Data Set Location: /lillyce/prd/ly3298176/i8f_mc_gpip/final/data/analysis/shared

Summary of Treatment Emergent Adverse Events Page 2 of 3
Preferred Term by Decreasing Frequency within System Organ Class 11:50 11AUG2023
Safety Analysis Set PDPM
I8F-MC-GPIP

System Organ Class MedDRA Preferred Term	5 mg tirzepatide SC (SDP) (N=65) Number of Subjects (%)	 Number of Occurrences	5 mg tirzepatide SC (MUPFP) (N=62) Number of Subjects (%)	 Number of Occurrences	Total (N=65) Number of Subjects (%)	 Number of Occurrences
General disorders and administration site conditions	6(9.2)	7	12(19.4)	12	15(23.1)	19
Pain	4(6.2)	4	6(9.7)	6	7(10.8)	10
Fatigue	0	0	3(4.8)	3	3(4.6)	3
Injection site haemorrhage	1(1.5)	1	2(3.2)	2	3(4.6)	3
Early satiety	1(1.5)	1	1(1.6)	1	2(3.1)	2
Injection site haematoma	1(1.5)	1	0	0	1(1.5)	1
Investigations	0	0	2(3.2)	4	2(3.1)	4
Blood pressure increased	0	0	2(3.2)	2	2(3.1)	2
Alanine aminotransferase increased	0	0	1(1.6)	1	1(1.5)	1
Aspartate aminotransferase increased	0	0	1(1.6)	1	1(1.5)	1
Injury, poisoning and procedural complications	1(1.5)	1	1(1.6)	2	2(3.1)	3
Burns second degree	1(1.5)	1	0	0	1(1.5)	1
Fall	0	0	1(1.6)	1	1(1.5)	1
Radius fracture	0	0	1(1.6)	1	1(1.5)	1
Musculoskeletal and connective tissue disorders	2(3.1)	2	1(1.6)	1	3(4.6)	3
Myalgia	1(1.5)	1	1(1.6)	1	2(3.1)	2
Back pain	1(1.5)	1	0	0	1(1.5)	1

Abbreviations: MUPFP = Multi-use prefilled pen; N = Number of subjects in safety population; SC = Subcutaneous;
SDP = Single-dose pen; TEAE = Treatment-emergent adverse events.

MedDRA Version 26.0

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Data Set Location: /lillyce/prd/ly3298176/i8f_mc_gpip/final/data/analysis/shared

Summary of Treatment Emergent Adverse Events
Preferred Term by Decreasing Frequency within System Organ Class
Safety Analysis Set
I8F-MC-GPIP

System Organ Class MedDRA Preferred Term	5 mg tirzepatide SC (SDP) (N=65) Number of Subjects (%)	 Number of Occurrences	5 mg tirzepatide SC (MUPFP) (N=62) Number of Subjects (%)	 Number of Occurrences	Total (N=65) Number of Subjects (%)	 Number of Occurrences
Infections and infestations	2(3.1)	2	0	0	2(3.1)	2
COVID-19	1(1.5)	1	0	0	1(1.5)	1
Gastroenteritis	1(1.5)	1	0	0	1(1.5)	1
Skin and subcutaneous tissue disorders	1(1.5)	1	1(1.6)	1	1(1.5)	2
Skin burning sensation	1(1.5)	1	1(1.6)	1	1(1.5)	2
Psychiatric disorders	0	0	1(1.6)	1	1(1.5)	1
Insomnia	0	0	1(1.6)	1	1(1.5)	1

Abbreviations: MUPFP = Multi-use prefilled pen; N = Number of subjects in safety population; SC = Subcutaneous;
SDP = Single-dose pen; TEAE = Treatment-emergent adverse events.

MedDRA Version 26.0

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Data Set Location: /lillyce/prd/ly3298176/i8f_mc_gpip/final/data/analysis/shared

2.7.1.4.5. Additional Summary Tables for Bioequivalence Studies per EMA Guidelines

Table APP.2.7.1.15. Test and Reference Product Information

Product Characteristics	Test Product	Reference Product
Name	Tirzepatide 5 mg via fixed, multi-dose, single-patient-use prefilled pen	Tirzepatide 5 mg via SDP
Strength	5 mg/0.6 mL	5 mg/0.5 mL
Dosage form	Solution	Solution
Manufacturer	Eli Lilly and Company	Eli Lilly and Company
Batch number	D577651A	D529855C
Location of certificate of analysis	Section 2.7.1.4.6	Section 2.7.1.4.6
Member state where the reference is purchased from:	Not applicable	Not applicable
This product was used in the following studies:	I8F-MC-GPIP	I8F-MC-GPIP

[a] List for each active substance for fixed combinations.

Abbreviation: SDP = single-dose prefilled pen.

Table APP.2.7.1.16. Bioanalytical Method Validation

Analytical Validation Report Location	191444PVDJS_EII_R2 Module 5.3.1.4
This analytical method was used in the following studies:	GPIP
Short description of the method	Method validation for the quantitation of tirzepatide in human plasma using HRAM LC-MS
Biological matrix	Plasma
Analyte Location of product certificate	Tirzepatide 191444PVDJS_EII_R2
Internal standard (IS)[a] Location of product certificate	LSN3316897 191444PVDJS_EII_R2
Calibration concentrations (units)	2.00, 4.00, 10.0, 50.0, 100, 250, 400, and 500 ng/mL
Average recovery of the drug (%)	Not applicable
Average recovery of the IS (%)	Not applicable
Lower limit of quantification (units)	2.00 ng/mL
QC concentrations (units)	2.00, 6.00, 200, and 375 ng/mL
Between-run accuracy	-2.1% to 2.8%
Between-run precision	8.1% to 12.9%
Within-run accuracy	-10.2% to 12.2%
Within-run precision	3.3% to 11.0%
Matrix factor (MF) (all QC)[a]	Not applicable
IS normalized MF (all QC)[a]	
CV (%) of IS normalized MF (all QC)[a]	
% of QCs with >85% and <115% n.v.[a]	
% matrix lots with mean <80% or>120% n.v.[a]	
Long-term stability of the stock solution and working solutions[b]	24 hours at room temperature
Short-term stability in biological matrix at room temperature or at sample processing temperature	24 hours at room temperature
Long-term stability in biological matrix	680 days at -20°C and -70°C
Autosampler storage stability	Not evaluated
Postpreparative stability	165 hours at room temperature
Freeze and thaw stability (observed change %)	5 cycles at -20°C and -70°C
Dilution integrity	100-fold
Selectivity	No interfering peaks noted in blank plasma samples
Partial validation	This partial validation was performed to verify a shorter LC gradient than that used in validation 151682VKM_EII.
Cross-validation	A cross-validation was performed to establish equivalent performance between the 2 methods.

Abbreviations: CV = coefficient of variation; HRAM LC-MS = high-resolution accurate mass liquid chromatography mass spectrometry; n.v. = nominal value; QC = quality control.

[a] Might not be applicable for the given analytical method.

[b] Report short-term stability results if no long-term stability on stock and working solution are available.

Table APP.2.7.1.17. Storage Period of Study Samples

Study alias and analyte	Longest storage period
GPIP tirzepatide	145 days at temperature -70°C

Table APP.2.7.1.18. Sample Analysis of Study I8F-MC-GPIP

Study alias	GPIP
Analyte	Tirzepatide
Longest storage period	145 days at temperature -70°C
Total numbers of collected samples	1772
Total number of samples with valid results	1771
Total number of reassayed samples[a,b]	33
Total number of analytical runs[a]	22
Total number of valid analytical runs[a]	21
Incurred sample reanalysis	
Number of samples	152
Percentage of samples where the difference between the 2 values was less than 20% of the mean for chromatographic assays or less than 30% for ligand binding assays	97

[a] Without incurred samples.

[b] Due to other reasons than not valid run.

Table APP.2.7.1.19. Study Description of Study I8F-MC-GPIP

Study Title: A Bioequivalence Study to Compare the Pharmacokinetics of Tirzepatide Administered Subcutaneously by a Fixed-Dose Multi-use Prefilled Pen Versus Single-Dose Pen in Healthy Participants

Report location:		CSR GPIP	
Study Periods		2	
Clinical:		3 April 2023	- 17 July 2023
Bioanalytical:		10 July 2023	- 28 July 2023
Design	Dose:		5 mg
	Single/Multiple dose:		Single dose in each study period.
	Number of periods:		2
	Two-stage design:		No
	Fasting/Fed:		Fasting
	Number of subjects		
	-dosed:		65
	-completed the study:		62
	-included in the final statistical analysis of AUC:		62
	-included in the final statistical analysis of C_{max}:		62

Abbreviations: AUC = area under the concentration versus time curve; C_{max} = maximum observed drug concentration; CSR = clinical study report.

Source: GPIP CSR

Table APP.2.7.1.20. Study Site(s) of Study I8F-MC-GPIP

	Name	Address	EU Authority Inspection	
			Year	Authority
Clinical study site	[redacted]		NA	NA
			NA	NA
			NA	NA
Bioanalytical study site			NA	NA
Statistics (including programming deliverables)			NA	NA
Sponsor of the study	Eli Lilly and Company	Indianapolis, IN, USA		

Redacted under Section 41 and Section 43 of the FOI Act.

Abbreviations: NA = not applicable; EU = European Union.

Table APP.2.7.1.21. Pharmacokinetic Data for Tirzepatide in Study I8F-MC-GPIP—All Subjects

Pharmacokinetic parameter	Geometric Mean Parameters (CV [%])	
	Test product	Reference product
N	62	65
$AUC_{(0\text{-}tlast)}$ (ng*hr/mL)	118000 (22)	124000 (23)
$AUC_{(0\text{-}\infty)}$ (ng*hr/mL)	119000[a] (22)	126000 (22)
C_{max} (ng/mL)	524 (27)	647 (31)
t_{max}[b] (hr)	36.0 (8.00 – 144)	12.0 (7.97 – 168)

Abbreviations: $AUC_{(0\text{-}tlast)}$ = area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration; $AUC_{(0\text{-}\infty)}$ = area under the concentration versus time curve from time zero to infinity; C_{max} = maximum observed drug concentration; CSR = clinical study report; CV = coefficient of variation; N = number of participants; t_{max} = time of maximum observed drug concentration.

[a] N=61. One subject excluded due to the $AUC_{(0\text{-}\infty)}$ calculated with ≥20% extrapolation.

[b] Median (range).

Source: GPIP CSR, Table GPIP.5.6

Table APP.2.7.1.22. Pharmacokinetic Data for Tirzepatide in Study I8F-MC-GPIP—Completers Only

Pharmacokinetic parameter	Geometric Mean Parameters (CV [%])	
	Test product	Reference product
N	62	62
$AUC_{(0\text{-}tlast)}$ (ng*hr/mL)	118000 (22)	125000 (23)
$AUC_{(0\text{-}\infty)}$ (ng*hr/mL)	119000[a] (22)	127000 (22)
C_{max} (ng/mL)	524 (27)	649 (31)
t_{axa}[b] (hr)	36.0 (8.00 – 144)	12.0 (7.97 – 168)

Abbreviations: $AUC_{(0\text{-}tlast)}$ = area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration; $AUC_{(0\text{-}\infty)}$ = area under the concentration versus time curve from time zero to infinity; C_{max} = maximum observed drug concentration; CV = coefficient of variation; N = number of participants; PK = pharmacokinetics; t_{max} = time of maximum observed drug concentration.

[a] N=61. One subject was excluded due to the $AUC_{(0\text{-}\infty)}$ calculated with ≥20% extrapolation.

[b] Median (range).

Table APP.2.7.1.23. Additional Pharmacokinetic Data for Tirzepatide in Study I8F-MC-GPIP Redacted under Section 40 of the FOI Act.

Plasma concentration curves where	Related information
$AUC_{(0\text{-}tlast)}$ / $AUC_{(0\text{-}\infty)}$ < 0.8	Subject ID=[redacted][a], Period 2, F=T[b]
C_{max} is the first point postdose	Subject ID=[redacted] Period 1, F=R[b]
	Subject ID=[redacted] Period 1, F=R[b]
	Subject ID=[redacted] Period 1, F=T[b]
	Subject ID=[redacted] Period 2, F=R[b]
	Subject ID=[redacted] Period 1, F=R[b]
	Subject ID=[redacted] Period 1, F=R[b]
	Subject ID=[redacted] Period 1, F=R[b]
	Subject ID=[redacted] Period 1, F=R[b]
	Subject ID=[redacted] Period 2, F=T[b]
	Subject ID=[redacted], Period 1, F=R[b]
	Subject ID=[redacted] Period 1, F=R[b]
Pre-dose sample >5% C_{max}	NA

Abbreviations: $AUC_{(0\text{-}tlast)}$ = area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration; $AUC_{(0\text{-}\infty)}$ = area under the concentration versus time curve from time zero to infinity; C_{max} = maximum observed drug concentration; F = formulation; ID = identification number; NA = not applicable; R = single dose pen (reference); T = multi-use prefilled pen (test).

[a] Last sampling point of $AUC_{(0\text{-}tlast)}$ was 816 hours.

[b] F = T for the test formulation or F = R for the reference formulation.

Table APP.2.7.1.24. Bioequivalence Evaluation of Tirzepatide in Study I8F-MC-GPIP (Completers Only)

Pharmacokinetic parameter	Geometric Mean Ratio Test/Reference	90% Confidence Intervals	CV (%)[a]
$AUC_{(0\text{-}tlast)}$ (ng*hr/mL)	0.943	0.926 – 0.960	5.9
C_{max} (ng/mL)	0.808	0.780 – 0.838	11.9

Abbreviations: $AUC_{(0\text{-}tlast)}$ = area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration; C_{max} = maximum observed drug concentration; CSR = clinical study report; CV = coefficient of variation.

[a] Estimated from the residual mean squares.

Source: GPIP CSR, Table GPIP.5.9 and Table GPIP.5.10

2.7.1.4.6. Certificates of Analysis

The certificate of analysis effective at the time of assay validation is provided below.

The following pages have been redacted under Section 41 and Section 43 of the FOI Act.