CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

215809Orig1s000

CLINICAL PHARMACOLOGY REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number	215809
Link to EDR	\\CDSESUB1\evsprod\NDA215809\0001
Submission Date	June 30, 2021
Submission Type	Standard
Brand Name	To be determined
Generic Name	Levothyroxine sodium solution
Dosage Form and Strength	30 mcg/mL
Route of Administration	Oral
Proposed Indication	Hypothyroidism and suppression of thyroid-stimulating hormone
Applicant	Mylan Pharmaceuticals Inc.
OCP Review Team	S. W. Johnny Lau, R.Ph., Ph.D., Amal Ayyoub, Ph.D.

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1. EXECUTIVE SUMMARY

This original New Drug Application (NDA 215809) was submitted to support the bioequivalence of levothyroxine sodium oral solution referring SYNTHROID (NDA 21210) dated June 30, 2021. The applicant established the clinical bridge to SYNTHROID for their levothyroxine sodium oral solution via the relative bioavailability study (LVOS-1-19094).

1.1 Recommendations

The Office of Clinical Pharmacology/Division of Cardiometabolic and Endocrine Pharmacology (OCP/DCEP) has reviewed the clinical pharmacology data submitted under NDA 215809 and recommends approval.

1.2 Post-Marketing Requirements and Commitments

None.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 What is the regulatory background for the submission?

The applicant submitted NDA 215809 on June 30, 2021 to seek approval of levothyroxine sodium oral solution to treat hypothyroidism and suppression of thyroid-stimulating hormones.

2.2 Is the levothyroxine sodium oral solution (test formulation) bioequivalent to the SYNTHROID (reference formulation)?

Yes, the levothyroxine pharmacokinetics (PK) following levothyroxine sodium oral solution (test formulation) met the bioequivalence (BE) criteria referencing that of SYNTHROID formulation (reference formulation).

BE between the 2 products was assessed in an open-label, single dose, randomized, 2-period, 2-sequence, crossover study in healthy volunteers (Study LVOS-1-19094, see the study synopsis in Attachment). Oral doses of 600 mcg (20 mL of 150 mcg/5 mL levothyroxine sodium oral solution (test product), or 2 of 300 mcg SYNTHROID (levothyroxine sodium) tablets (reference product)) were administered under overnight fasting condition (see information on the investigational products in Table 1). A washout period of 35 days separated each dose.

Table 1 Information on Test and Reference Products

Product	Test	Reference
Formulation	Levothyroxine Sodium Oral Solution 150 mcg/5mL	SYNTHROID 300 mcg
Batch No.	PDS-1C	1109288
Manufacturing	04JUN2019	NA
Expiry Date	NA	30APR2020

The test and reference products were acceptable as valid investigational products (e.g., batch size and stability) to support the pivotal BE study according to CMC information.

During each study period, serial blood samples (1 × 10 mL) were collected in plain red top serum collection tubes from each participant at -0.5, -0.25, and pre-dose (0-hour, within 5 minutes before dosing) and post-dose at study hours 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10.0, 12.0, 18.0, 24.0, and 48.0 hours.

The post-dose concentration-time profiles were corrected by the baseline concentrations. If a negative serum concentration value resulted after baseline correction, this value was to be set to 0 prior to calculating the baseline-adjusted AUC. The primary PK endpoints (i.e., CPEAK and pAUC48) were estimated using conventional non-compartmental analysis.

A total of 36 subjects were enrolled, and 4 subjects. Four subjects were dismissed due to violations of the protocol during Period 2 (3 were due to positive drug test and 1 was due to potential positive pregnancy test) (Table 2).

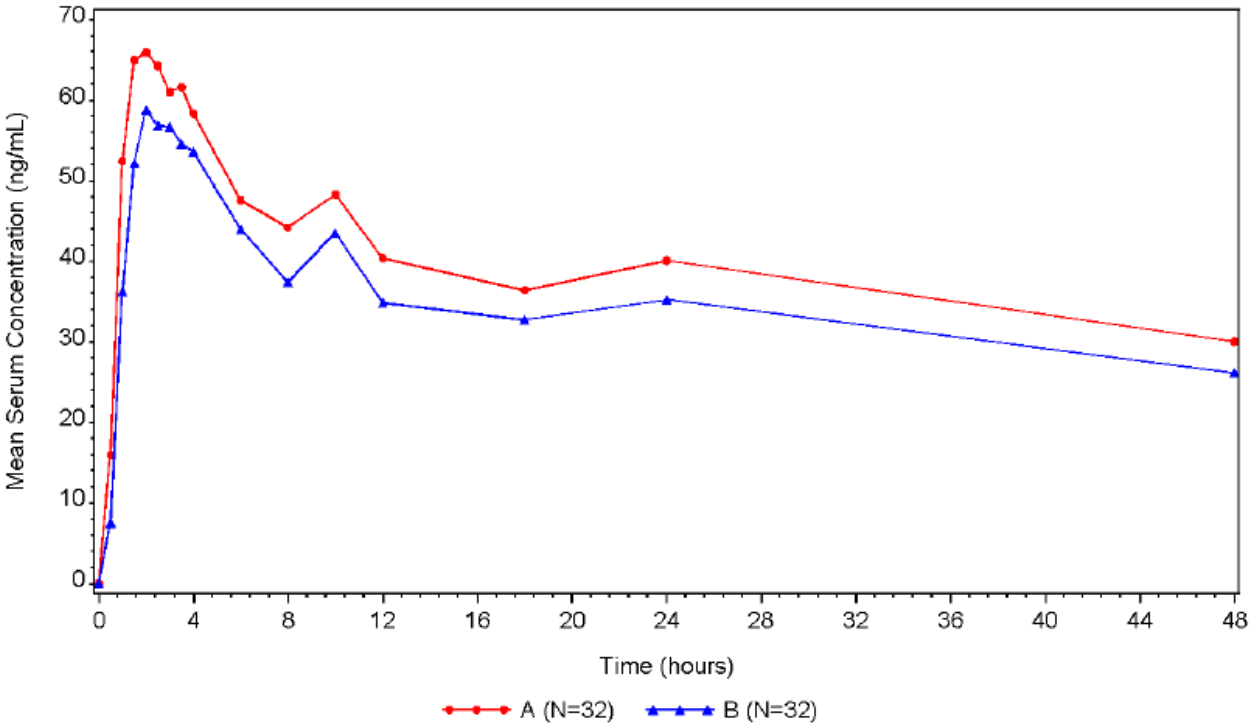
Table 2. Summary of Subject Disposition (Table 10.2 of Clinical Study Report)

	Sequence		
	AB	**BA**	**Total**
Subjects Randomized	**18**	**18**	**36**
Subjects Who Successfully Completed the Study	**17**	**15**	**32**
Subjects Who Withdrew Consent	**0**	**0**	**0**
Subjects Dropped by the Investigator	**1**	**3**	**4**
Subjects Dropped by the Applicant	**0**	**0**	**0**
Subjects Analyzed	**17**	**15**	**32**

Treatment A: Levothyroxine Sodium Oral Solution, 150 mcg/5mL

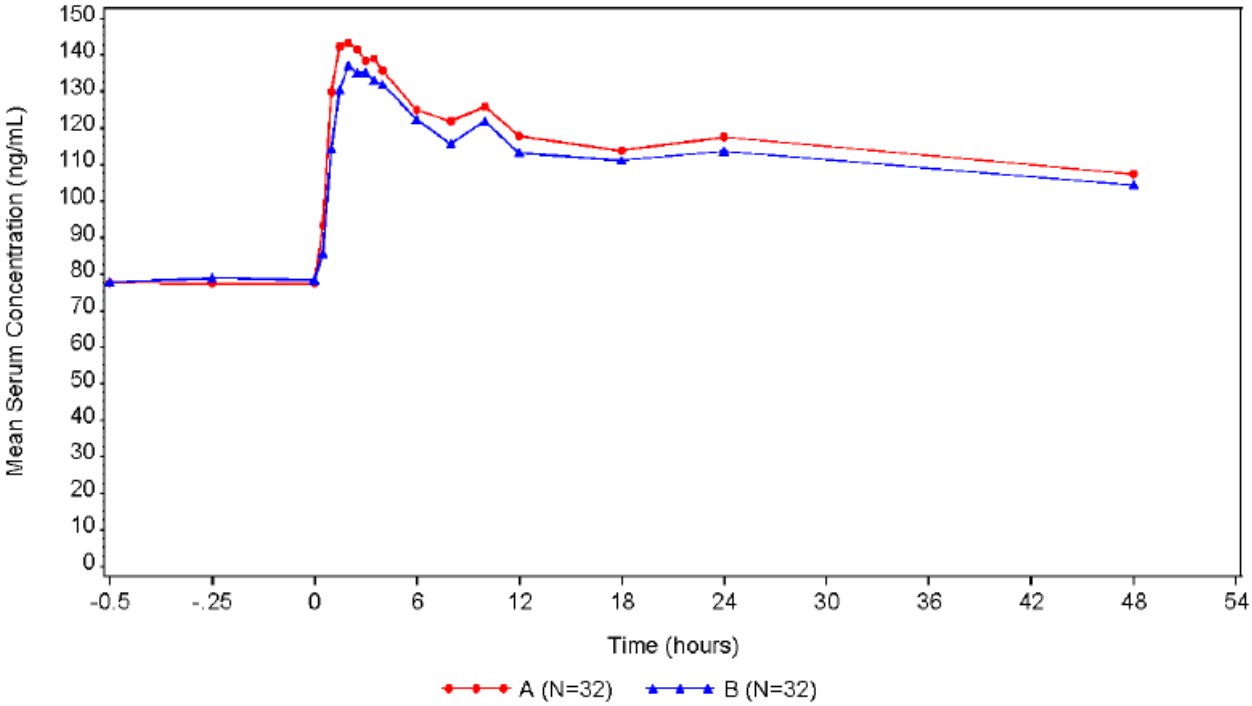
Treatment B: Synthroid® Tablets, 300 mcg

Concentration-time profiles after levothyroxine 600 mcg were shown in Figures 1 and 2 as well as the PK parameters were summarized in Tables 3 and 4.



Source: Figure 14.1 of report for Study LVOS-1-19094

Figure 1. Mean (SD) Levothyroxine Concentration-Time Profiles with Baseline Correction by Treatments



Source: Figure 14.2 of report for Study LVOS-1-19094

Figure 2. Mean (SD) Levothyroxine Concentration-Time Profiles without Baseline Correction by Treatments

Table 2. Mean Baseline Corrected Levothyroxine Pharmacokinetic Parameters

Mean (%CV) Baseline Corrected Pharmacokinetic Parameters in Thirty-two Healthy Adult Subjects Following a Single Oral 600 µg Dose of Levothyroxine Oral Solution or Tablets under Fasting Conditions PROTOCOL NUMBER: LVSOS-1-19094							
	Treatment A = Levothyroxine Sodium Oral Solution, 150 µg/5 mL (Mylan; PDS-1C) (Levothyroxine)		Treatment B = Synthroid® Tablet USP, 300 µg (AbbVie; Lot 1109288) (Levothyroxine)		LSMEANS Ratio (A/B)‡		
Parameter	N	Geometric LS Mean Arithmetic Mean (%CV)	N	Geometric LS Mean Arithmetic Mean (%CV)	N		90% Confidence Interval†
pAUC48 (ng•hr/mL)	32	1858.62 1885.26 (17.54%)	32	1601.27 1655.53 (24.39%)	32	1.16	110.51% – 121.91%
CPEAK (ng/mL)	32	70.69 72.21 (21.33%)	32	64.88 66.76 (23.25%)	32	1.09	103.12% –115.11%
Tpeak (hr)*	32	2.00 (1.00 – 3.52)	32	2.00 (1.02 – 4.00)	--		--

‡ Ratio (A/B) = e [LSMEAN of (LNA – LNB)]; †Used Natural Log Transformed Parameter;
**Arithmetic Mean (%CV); *median (min-max);
Source: Study LVSOS-1-19094's report Table 14.6

Table 4. Mean Baseline Uncorrected Levothyroxine Pharmacokinetic Parameters

Mean (%CV) Baseline Uncorrected Pharmacokinetic Parameters in Thirty-two Healthy Adult Subjects Following a Single Oral 600 µg Dose of Levothyroxine Oral Solution or Tablets under Fasting Conditions PROTOCOL NUMBER: LVSOS-1-19094							
	Treatment A = Levothyroxine Sodium Oral Solution, 150 µg/5 mL (Mylan; PDS-1C) (Levothyroxine)		Treatment B = Synthroid® Tablet USP, 300 µg (AbbVie; Lot 1109288) (Levothyroxine)		LSMEANS Ratio (A/B)‡		
Parameter	N	Geometric LS Mean Arithmetic Mean (%CV)	N	Geometric LS Mean Arithmetic Mean (%CV)	N		90% Confidence Interval†
pAUC48 (ng•hr/mL)	32	5541.39 5599.55 (14.49%)	32	5353.28 5413.27 (14.95%)	32	1.04	101.43% – 105.64%
CPEAK (ng/mL)	32	147.84 149.55 (15.50%)	32	143.30 145.04 (15.34%)	32	1.03	100.25% –106.18%
Tpeak (hr)*	32	2.00 (1.00 – 3.52)	32	2.00 (1.02 – 4.00)	--		--

‡ Ratio (A/B) = e [LSMEAN of (LNA – LNB)]; †Used Natural Log Transformed Parameter;
**Arithmetic Mean (%CV); *median (min-max);
Source: Study LVSOS-1-19094's report Table 14.7

Reviewer's Comment

Dr. Yaning Sun's reanalyses of the baseline corrected and baseline uncorrected are consistent with the applicant's findings that the 20 mL of 150 mcg/5 mL levothyroxine sodium solution (test product) is bioequivalent to the 2 of 300 mcg SYNTHROID tablets (reference product).

2.3 Is the bioanalytical assay validation acceptable?

The serum total levothyroxine (T4) concentrations were measured via the high-performance liquid chromatography with mass spectrometry detection (LC-MS/MS) (Bioanalytical Laboratory Project #13-023; Validation Report) with a range of 20 – 250 ng/mL. The assay validation was acceptable (Table in Appendix 4.2).

Reviewer's Comment

Inspection request to the Office of Study Integrity and Surveillance (OSIS) was submitted for the original submission. The OSIS declined to inspect the sites with recommendation dated October 15, 2021 in DARRTS Reference ID: 4873317 that the Office of Regulatory Affairs (ORA) inspected the clinical site in February 2019, which falls within the surveillance interval. The OSIS inspected the bioanalytical site in November 2018, which falls within the surveillance interval. The final classification for both inspections was No Action Indicated.

4. APPENDICES

4.1 Study Synopsis

Name of Sponsor/Company: Mylan Pharmaceuticals, Inc.	Individual Study Table Referring to Part of the Dossier Volume: Page:	*(For National Authority Use Only)*
Name of Finished Product: Levothyroxine Sodium Oral Solution, 150 µg/5mL		
Name of Active Moiety: levothyroxine		

Objectives:
The primary objective of this study was to evaluate the relative bioavailability of a test formulation of levothyroxine sodium oral solution, 150 µg/5 mL (DPT Laboratories, Ltd.) compared to Synthroid® Tablets USP, 300 µg (AbbVie Inc.) following a single 600 µg oral dose to healthy adult male and female subjects under fasting conditions. The secondary objective was to assess the safety and tolerability of a single 600 µg oral dose of levothyroxine sodium oral solution when administered to healthy adult subjects under fasting conditions.

Main Criteria for Inclusion:
Main criteria for inclusion from protocol, e.g.: Healthy, non-tobacco/nicotine using, volunteers between the ages of 18 and 50 years, weighing at least 50 kg (110 lbs) for men and women, with a Body Mass Index (BMI) less than or equal to 18.0 kg/m^2 but greater than or equal to 30.0 kg/m^2, who were judged to be healthy based on a pre-study physical examination and clinical laboratory tests.

Name of Test Drug/Investigational Product:
Levothyroxine Sodium Oral Solution 150 µg/5mL
Mode of Administration: Oral
Manufactured for Mylan Pharmaceuticals Inc.
Manufacturing Location: DPT Laboratories, Ltd.
Lot No.: PDS-1C, Manufacturing Date: 04JUN2019

Name of Reference Drug:
Synthroid® (Levothyroxine Sodium) Tablets, USP 300 µg (0.3 mg)
Mode of Administration: Oral
Manufactured by AbbVie Inc.
Lot No.: 1109288, Expiration Date: 30APR2020

Treatment Periods:
Period 1: August 15, 2019 – August 17, 2019
Period 2: September 19, 2019 –September 21, 2019

Number of Subjects (planned and analyzed):
A total of 36 subjects were enrolled in the study. Subjects [redacted] (b) (6) were discontinued for positive urine drug screen results and Subject [redacted] (b) (6) was discontinued for indeterminate pregnancy test results. Therefore, 32 subjects completed the clinical portion of the study and 32 subjects were included in the pharmacokinetic and statistical analysis for levothyroxine.

Name of Sponsor/Company: Mylan Pharmaceuticals, Inc.	Individual Study Table Referring to Part of the Dossier Volume: Page:	*(For National Authority Use Only)*
Name of Finished Product: Levothyroxine Sodium Oral Solution, 150 µg/5mL		
Name of Active Moiety: levothyroxine		

Clinical Procedures:
This was an open-label, single-dose, randomized, two-treatment, two-period, crossover study. Subjects were housed from the evening prior to dosing (at least 16 hours before dosing) and until at least 24 hours after dosing and returned to the clinical facility for a scheduled blood sample collection 48 hours post-dose. Subjects received dinner the evening prior to dosing. Following a supervised overnight fast of at least 10 hours, each subject received a total dose of 600 µg of the test product, 20 mL of 150 µg/5 mL Levothyroxine sodium oral solution, or a total dose of 600 µg of the reference product, 2 × 300 µg Synthroid® (levothyroxine sodium) tablets, with 240 mL of room temperature water. Standard low-fat meals (lunch and dinner) were provided at least 4 and approximately 10 hours after dosing. Additional meals and snacks were provided at appropriate times thereafter during confinement to the clinic. Subjects were required to consume 240 mL ± 10 mL of ambient temperature water 1.25 hours before dosing and no earlier than 1 hour after dosing. Water was restricted from one hour before dosing until one hour after dosing, except for that given at dosing. A washout period of 35 days separated each dose. During each study period, blood samples (1 × 10 mL) were collected in plain red top serum collection tubes from each subject at -0.5, -0.25, and pre-dose (0-hour, within 5 minutes before dosing) and post-dose at study hours 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10.0, 12.0, 18.0, 24.0 and 48.0 hours. Serum samples were frozen in an upright position in a -70°C ± 15°C freezer unit.

Analytical Methods:
Samples were assayed for levothyroxine at Mylan Pharmaceuticals Inc. from the period of September 26, 2019 through October 2, 2019. The method developed for the analysis of levothyroxine in 100 µL human serum involved solid phase extraction followed by LC-MS/MS analysis, which had a limit of quantification of 20 ng/mL for levothyroxine. The assay was linear from 20 ng/mL to 250 ng/mL for levothyroxine.

Statistical Methods:
Single-dose pharmacokinetic parameters for levothyroxine baseline uncorrected as well as baseline corrected were calculated using non-compartmental techniques. Statistical analyses were performed on the pharmacokinetic parameters using the General Linear Models Procedure (PROC GLM) of SAS Software (SAS Institute, Cary, NC).

The model tested for treatment effects in the parameter means at an alpha level of 0.05. The TMAX, was analyzed statistically using the non-transformed data. The natural log transformed parameters pAUC48 and CPEAK were also statistically analyzed. The tests were performed to analyze for statistically significant differences in the pharmacokinetic parameters using Least Squares Means. Ninety (90%) percent confidence intervals were constructed using the two one-sided tests procedure to assess average bioequivalence between the two products.

Criteria for Bioequivalence Evaluation:
The 90% confidence interval for the geometric LSMeans ratio of CPEAK and pAUC48 for the test and reference product should be between 80.00% and 125.00% for the baseline-corrected natural log-transformed data for levothyroxine. The 90% confidence interval for the geometric LSMeans ratio of CPEAK and pAUC48 for the test and reference product should be between 80.00% and 125.00% for the baseline-uncorrected natural log-transformed data for levothyroxine is presented for supportive information.

Name of Sponsor/Company: Mylan Pharmaceuticals, Inc.	Individual Study Table Referring to Part of the Dossier	*(For National Authority Use Only)*
Name of Finished Product: Levothyroxine Sodium Oral Solution, 150 µg/5mL	Volume:	
Name of Active Moiety: levothyroxine	Page:	

Summary Conclusions:

Pharmacokinetic Results:

Levothyroxine Baseline Corrected Pharmacokinetic Results: (n = 32)

Results from a randomized, two-way, cross-over comparative bioavailability study in which the rate and extent of absorption of levothyroxine were determined and compared following the oral administration of a 600 µg dose (20 mL of 150 µg/5 mL solution) of Mylan's Levothyroxine Sodium Oral Solution, 150 µg/5 mL or 600 µg dose (2 × 300 µg/tablet) of AbbVie's Synthroid® Tablets USP, 300 µg to healthy adult volunteers under fasting conditions.

Geometric LS mean and arithmetic mean (%CV) baseline corrected levothyroxine pharmacokinetic parameters in thirty-two (32) healthy adult subjects following a single oral 600 µg dose of levothyroxine under fasting conditions are presented in the following table:

Statistical Summary of the Comparative Bioavailability Data for Baseline Corrected Levothyroxine

Parameter	Treatment A = Levothyroxine Sodium Oral Solution, 150 µg/5 mL (Mylan; PDS-1C) (Levothyroxine)		Treatment B = Synthroid® Tablet USP, 300 µg (AbbVie; Lot 1109288) (Levothyroxine)		LSMEANS Ratio (A/B)‡		90% Confidence Interval†
	N	Geometric LS Mean Arithmetic Mean (%CV)	N	Geometric LS Mean Arithmetic Mean (%CV)	N		
pAUC48 (ng•hr/mL)	32	1858.62 1885.26 (17.54%)	32	1601.27 1655.53 (24.39%)	32	1.16	110.51% – 121.91%
CPEAK (ng/mL)	32	70.69 72.21 (21.33%)	32	64.88 66.76 (23.25%)	32	1.09	103.12% –115.11%
Tpeak (hr)*	32	2.00 (1.00 – 3.52)	32	2.00 (1.02 – 4.00)	--		--

‡ Ratio (T/R) = $e^{[LSMEAN\ of\ (LNA - LNR)]}$;
†Used Natural Log Transformed Parameter; **Arithmetic Mean (%CV)
*median (min-max);
Source: Sections 16.2.6.1 and 16.2.6.2 of Appendix 16.2.6

Name of Sponsor/Company: Mylan Pharmaceuticals, Inc.	Individual Study Table Referring to Part of the Dossier Volume: Page:	*(For National Authority Use Only)*
Name of Finished Product: Levothyroxine Sodium Oral Solution, 150 µg/5mL		
Name of Active Moiety: levothyroxine		

Levothyroxine Uncorrected Pharmacokinetic Results: (n = 32)

Results from a randomized, two-way, cross-over comparative bioavailability study in which the rate and extent of absorption of levothyroxine were determined and compared following the oral administration of a 600 µg dose (4 × 150 µg/5 mL) of Mylan's Levothyroxine Sodium Oral Solution, 150 µg/5 mL or 600 µg dose (2 × 300 µg/tablet) of AbbVie's Synthroid® Tablets USP, 300 µg to healthy adult volunteers under fasting conditions.

Geometric LS mean and arithmetic mean (%CV) baseline uncorrected levothyroxine pharmacokinetic parameters in thirty-two (32) healthy adult subjects following a single oral 600 µg dose of levothyroxine under fasting conditions are presented in the following table:

Statistical Summary of the Comparative Bioavailability Data for Baseline Uncorrected Levothyroxine

Parameter	Treatment A = Levothyroxine Sodium Oral Solution, 150 µg/5 mL (Mylan; PDS-1C) (Levothyroxine)		Treatment B = Synthroid® Tablet USP, 300 µg (AbbVie; Lot 1109288) (Levothyroxine)		LSMEANS Ratio (A/B)‡		90% Confidence Interval†
	N	Geometric LS Mean Arithmetic Mean (%CV)	N	Geometric LS Mean Arithmetic Mean (%CV)	N		
pAUC48 (ng•hr/mL)	32	5541.39 5599.55 (14.49%)	32	5353.28 5413.27 (14.95%)	32	1.04	101.43% – 105.64%
CPEAK (ng/mL)	32	147.84 149.55 (15.50%)	32	143.30 145.04 (15.34%)	32	1.03	100.25% –106.18%
Tpeak (hr)*	32	2.00 (1.00 – 3.52)	32	2.00 (1.02 – 4.00)	--		--

‡ Ratio (T/R) = $e^{[LSMEAN\ of\ (LNA - LNB)]}$;
†Used Natural Log Transformed Parameter; **Arithmetic Mean (%CV)
*median (min-max);
Source: Sections 16.2.6.3 and 16.2.6.4 of Appendix 16.2.6

Safety Results:

No serious adverse events (SAEs) were reported. Eleven (11) subjects experienced a total of 17 treatment emergent adverse events (AEs) over the course of the study. All reported AEs were mild in severity. There were 7 adverse events (diarrhea × 2, abdominal discomfort, lethargy, nausea × 2, somnolence) that were considered probably related, 1 AE (dizziness) that was considered possibly related, and 4 AEs (epistaxis, throat irritation × 3) that were considered unlikely to be related to the oral administration of Mylan's Levothyroxine Sodium Oral Solution, 150 µg/5 mL. There were 4 adverse events (headache, feeling jittery, heart rate increased, abdominal pain upper) that were considered probably related to the oral administration of AbbVie Inc.'s Synthroid® (levothyroxine sodium) tablets, USP, 300 µg. A single adverse event (blood iron decreased) occurred at study exit and was considered possibly related to the treatments received during the study; however, this AE cannot be definitely attributed to any specific treatment (A or B) as clinical laboratory tests were performed only at screening and study exit and the affected subject had received both treatments.

Appears this way on original

Name of Sponsor/Company: Mylan Pharmaceuticals, Inc.	Individual Study Table Referring to Part of the Dossier Volume: Page:	*(For National Authority Use Only)*
Name of Finished Product: Levothyroxine Sodium Oral Solution, 150 µg/5mL		
Name of Active Moiety: levothyroxine		

Conclusions:
There were no SAEs or deaths reported during this study. The most frequently reported AE following administration of Levothyroxine Sodium Oral Solution was throat irritation which was reported by 3 of 33 (9.1%) subjects. No single adverse event (headache, feeling jittery, heart rate increased, abdominal pain upper) was reported more frequently than any other adverse event by subjects following administration of Synthroid® Tablets.

All statistical analyses of the baseline-corrected and -uncorrected data reveals that the 90% confidence intervals are within the acceptable bioequivalent range of 80.00% and 125.00% for LNpAUC48 and LNCPEAK for levothyroxine.

Overall, Levothyroxine Sodium Oral Solution, 150 µg/5 mL was well tolerated as a single oral dose of 20 mL (600 µg total dose) under fasting conditions. This study demonstrates that Mylan's Levothyroxine Sodium Oral Solution 150 µg/5mL is bioequivalent to AbbVie's Synthroid® Tablets following a single, oral 600 µg (20 mL of solution or 2 × 300 µg tablets) dose administered under fasting conditions.

4.2 Summary of Bioanalytical Method Validation

Table 5A - Summary of Method Performance

Bioanalytical Method Validation Report Name and Amendments	Levothyroxine Validation Levothyroxine Validation Addendum 1 Levothyroxine Validation Addendum 2		
Method Description	Solid phase extraction; LC-MS/MS-ESI analysis		
Materials Used for Standard Calibration Curve and Concentration	Stripped serum: 20, 25, 30, 40, 50, 90, 130, 170, 210, 250 ng/mL		
Validated Assay Range	20-250 ng/mL		
Material Used for Quality Controls (QCs) and Concentration	Stripped serum: 30, 50, 100, 200 ng/mL Unstripped serum: 50, 100, 150, 200 ng/mL		
Minimum Required Dilutions (MRDs)	N/A		
Source and Lot of Reagents	N/A		
Regression Model and Weighting	$1/concentration^2$		
Validation Parameters	**Method Validation Summary**		**Source Location**
Standard Calibration Curve Performance During Accuracy and Precision Runs	Number of standard calibrators from LLOQ to ULOQ	10	Table 4 of Levothyroxine Validation Report
	Cumulative Accuracy (% bias) from LLOQ to ULOQ levothyroxine	-0.97 to 1.70 %	Table 4 of Levothyroxine Validation Report
	Cumulative Precision (% CV) from LLOQ to ULOQ levothyroxine	≤ 2.83 %	Table 4 of Levothyroxine Validation Report
Performance of QCs During Accuracy and Precision Runs	**Cumulative Accuracy (% bias) in 5 QCs** QCs for Product: stripped serum unstripped serum	0.10 to 0.80 % -6.94% to -5.07%	Table 5 of Levothyroxine Validation Report (stripped serum) Table 6 of Levothyroxine Validation Report (unstripped serum)
	Inter-batch % CV QCs for Product: stripped serum unstripped serum	≤ 4.12 % ≤ 2.71 %	Table 5 of Levothyroxine Validation Report (stripped serum) Table 6 of Levothyroxine

			Validation Report (unstripped serum)
	Total Error (TE) QCs for Product:	N/A	N/A

Selectivity and Matrix Effect	Number of Lots Tested: six in stripped serum (three male and three female) and six in unstripped serum (three male and three female) Range of Observed Bias: for stripped serum: mean internal standard normalized matrix factor: 0.999-1.006 for unstripped serum: mean internal standard normalized matrix factor: 0.999-1.000 Issues: N/A	Table 15A of Levothyroxine Validation Report (stripped serum) Table 15B of Levothyroxine Validation Report (unstripped serum)
Interference and Specificity	Number of Lots Tested: six in stripped serum (three male and three female) and six in unstripped serum (three male and three female) Range of Observed Bias: For levothyroxine in stripped serum- any peak in the blank matrix sources at the retention time of levothyroxine was <20% of the LLOQ of the run for all blank sources For levothyroxine in unstripped serum- the observed levothyroxine peak was sufficiently resolved from any other peak in the chromatogram for all blank sources For the internal standard in stripped and unstripped serum- for all blank sources any peak at the retention time of the internal standard was ≤ 5% of the mean internal standard peak area of the standards used for the analytical run Issues: N/A	Representative Chromatography in Attachment 5A of Levothyroxine Validation Report (stripped and unstripped serum)
Hemolysis Effect	Number of Lots Tested: 1 unstripped serum source at low and high QC concentrations Range of Observed Bias: -5.94 to -1.70% Issues: N/A	Table 16 of Levothyroxine Validation Report
Lipemic Effect	Number of Lots Tested: 1 unstripped serum source at low and high QC concentrations Range of Observed Bias: -3.70 to 6.00 Issues: N/A	Table 17 of Levothyroxine Validation Report
Dilution Linearity and Hook Effect	Highest Concentration Tested: 1000 ng/mL Number of Dilution Factors: one at five-fold dilution factor Range of observed Bias: -2.55%	Table 13 of Levothyroxine Validation Report
Bench-top/Process Stability	22.3 hours at room temperature for stripped serum (-4.53% to -0.05%) 22.5 hours at room temperature for unstripped serum	Table 10A of Levothyroxine

	(-6.37% to -3.98%)	Validation Report (stripped serum) Table 10B of Levothyroxine Validation Report (unstripped serum)
Freeze-Thaw Stability	4 cycles at -15°C and -70°C in unstripped serum (-6.11% to -3.60%)	Table 9 of Levothyroxine Validation Report

Long-Term Storage	1618 days at -15°C and -70°C in unstripped serum (-6.70% to -4.88%)	Table 5 of Levothyroxine Validation Addendum 2 Report
Parallelism	N/A	N/A
Carry Over	No carry over observed For levothyroxine the average area in six blanks (each preceded by a LLOQ and ULOQ sample) did not exceed 20% of the average LLOQ area of the run. For the internal standard the average area in six blanks did not exceed 5% of the average internal standard in the LLOQ and ULOQ samples of the run.	Table 6 of Levothyroxine Validation Addendum 2 Report

Method Performance in Study LVOS-1-19094 (LVOS-1-19094 provided in Section 5.3.1.4)		
Validation Parameters	**Method Validation Summary**	**Source Location**
Assay Passing Rate	Incurred Sample Re-analysis (ISR) Passing Rate: 128 out of 128 samples met acceptance criteria of <20% different from original result, 100% passing rate	Table 5 of LVOS-1-19094 Report
Standard Curve Performance	• Cumulative bias range: -1.44 to 1.38% • Cumulative precision: ≤ 5.21% CV	Table 2 of LVOS-1-19094 Report
QC Performance	• Cumulative bias range: 1.38 to 2.85% • Cumulative precision: ≤ 4.79% CV • TE: N/A (LBA only)	Table 4 of LVOS-1-19094 Report
Method Reproducibility	Incurred sample re-analysis was performed in 11.1% of study samples, and 100% of the samples met the pre-specified criteria.	Table 5 of LVOS-1-19094 Report
Study Sample Analysis/Stability	Study samples were stored for 48 days at -70°C. Frozen QC samples used during the study were stored for 9 days -70°C. Long-term frozen stability in serum has been established for 1618 days at -70°C in Levothyroxine Validation Addendum 2.	Table 5 of Levothyroxine Validation Addendum 2 Report
Standard Calibration Curve Performance	N/A	N/A

Method Performance in Study LVOS-1-19094 (LVOS-1-19094 provided in Section 5.3.1.4)		
Validation Parameters	**Method Validation Summary**	**Source Location**
During Accuracy and Precision Runs		

Bioanalytical Method Validation Report Name	N/A		
Changes In Method	N/A		
New Validated Assay Range (if any)	N/A		
Validation Parameters	**Cross-validation Performance**		**Source Location**
Standard Calibration Curve Performance During Accuracy and Precision Runs	Cumulative accuracy (% bias) in standard calibrators from LLOQ to ULOQ	N/A	N/A
	Cumulative precision (% CV) from LLOQ to ULOQ	N/A	N/A
Performance of QCs During Accuracy and Precision Runs	Cumulative accuracy (% bias) in 5 QCs	N/A	N/A
	Inter-batch % CV	N/A	N/A
	Percent TE	N/A	N/A
Cross-validation	N/A		N/A
List Other Parameters	N/A		N/A

Source: Tables 5A and 5B of the bsummary-biopharm-1.pdf file

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