CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214047Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology Review

NDA Number	214047
Link to EDR	\\cdsesub1\evsprod\NDA214047
Submission Date	January 31, 2020
Submission Type	Standard
Brand Name	THYQUIDITY
Generic Name	Levothyroxine sodium
Dosage Form and Strength	Solution; 100 µg/5 mL
Route of Administration	Oral
Proposed Indications	To treat hypothyroidism and suppress pituitary thyrotropin-stimulating hormone
Applicant	EMP Levo US B.V.
Associated preIND	127792
OCP Review Team	S.W. Johnny Lau, RPh, PhD
OCP Final Signatory	Jayabharathi Vaidyanathan, PhD (Team Leader)

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1. EXECUTIVE SUMMARY

The applicant is developing levothyroxine sodium solution (THYQUIDITY) to treat hypothyroidism and suppress pituitary thyrotropin-stimulating hormone (TSH). The applicant submitted NDA 214047 via the 505(b)(2) regulatory pathway for the 100 µg/5 mL strength of THYQUIDITY for once daily oral administration.

The innovator product (reference) for levothyroxine sodium solution is the 300 µg SYNTHROID oral tablet. SYNTHROID has the approved indication to treat hypothyroidism and suppress TSH.

NDA 214047 is primarily supported by one relative bioavailability study, EMP-P2-777 titled "Single Dose Crossover Comparative Bioavailability Study of Levothyroxine Oral Solution and Tablets in Healthy Male and Female Volunteers Following The Administration of a 600 µg Dose / Fasting State."

1.1 Recommendations

The Office of Clinical Pharmacology/Division of Cardiometabolic and Endocrine Pharmacology has reviewed NDA 214047's clinical pharmacology data submitted on January 31, 2020 and found the data acceptable and recommend approval of the NDA 214047.

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	The relative bioavailability study (EMP-P2-777) provides a bridge to reference the effectiveness and safety of the THYQUIDITY oral solution to the Food and Drug Administration's findings of the innovator's effectiveness and safety for levothyroxine sodium oral tablets (SYNTHROID).
General dosing instructions	The proposed dosing is acceptable. See section 2.2.
Dosing in patient subgroups (intrinsic and extrinsic factors)	The proposed dosing is acceptable. See section 2.2.
Labeling	See section 2.4 for labeling recommendations.
Bridge between the to-be-marketed and clinical trial formulations	The tested formulation of levothyroxine sodium oral solution in the bridging study (EMP-P2-777) is identical to the to-be-marketed formulation of levothyroxine sodium oral solution.

1.2 Post-Marketing Requirements and Commitments

None

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Findings of the Pivotal Relative Bioavailability Study

Study EMP-P2-777 shows that the 90% confidence interval of the baseline-adjusted levothyroxine C_{max} geometric mean ratio (GMR) and AUC_{0-72} GMR for 30 mL of 100 µg/5 mL levothyroxine sodium oral solution versus two 300 µg SYNTHROID oral tablets are all within the 80 – 125% bioequivalence goalpost under fasting.

Baseline-adjusted Levothyroxine Parameter	LS Geometric Means		Geometric Mean Ratio (GMR, %)	90% Confidence Interval	
	Test	Reference		Lower	Upper
C_{max} (ng/mL)	71.7	66.4	107.93	101.65	114.60
AUC_{0-72} (ng•h/mL)	2527.1	2317.3	109.05	100.60	118.21

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

- Administer once daily, on an empty stomach, one-half to one hour before breakfast.
- Administer at least 4 hours before or after drugs that are known to interfere with THYQUIDITY absorption.
- Administer orally with syringe provided by the pharmacy.
- Evaluate the need for dose adjustments when regularly administering within one hour of certain foods that may affect THYQUIDITY absorption.
- Starting dose depends on a variety of factors, including age, body weight, cardiovascular status, concomitant medical conditions (including pregnancy), and concomitant medications, co-administered food, and the specific nature of the condition being treated. Peak therapeutic effect may not be attained for 4-6 weeks.
- See dosing information in the "Therapeutic individualization" section (2.2.2 below).
- Adequacy of therapy determined with periodic monitoring of TSH and/or levothyroxine (T4) as well as clinical status.

2.2.2 Therapeutic individualization

Primary Hypothyroidism in Adults and in Adolescents in Whom Growth and Puberty are Complete

Start THYQUIDITY at the full replacement dose in otherwise healthy, non-elderly individuals who have been hypothyroid for only a short time (such as a few months). The average full replacement dose of THYQUIDITY is approximately 1.6 µg per kg per day (for example: 100 to 125 µg per day for a 70 kg adult).

Adjust the dose by 12.5 to 25 µg increments every 4 to 6 weeks until the patient is clinically euthyroid and the serum TSH returns to normal. Doses greater than 200 µg per day are seldom

required. An inadequate response to daily doses of greater than 300 µg per day is rare and may indicate poor compliance, malabsorption, drug interactions, or a combination of these factors.

For elderly patients or patients with underlying cardiac disease, start with a dose of 12.5 to 25 µg per day. Increase the dose every 6 to 8 weeks, as needed, until the patient is clinically euthyroid and the serum TSH returns to normal. The full replacement dose of THYQUIDITY may be less than 1 µg per kg per day in elderly patients.

In patients with severe longstanding hypothyroidism, start with a dose of 12.5 to 25 µg per day. Adjust the dose in 12.5 to 25 µg increments every 2 to 4 weeks until the patient is clinically euthyroid and the serum TSH level is normalized.

Secondary or Tertiary Hypothyroidism

Start THYQUIDITY at the full replacement dose in otherwise healthy, non-elderly individuals. Start with a lower dose in elderly patients, patients with underlying cardiovascular disease or patients with severe longstanding hypothyroidism as described above. Serum TSH is not a reliable measure of THYQUIDITY dose adequacy in patients with secondary or tertiary hypothyroidism and should not be used to monitor therapy. Use the serum free-T4 level to monitor adequacy of therapy in this patient population. Titrate THYQUIDITY dosing per above instructions until the patient is clinically euthyroid and the serum free-T4 level is restored to the upper half of the normal range.

Pediatric Dosage - Congenital or Acquired Hypothyroidism

The recommended daily dose of THYQUIDITY in pediatric patients with hypothyroidism is based on body weight and changes with age as described in Table 1. Start THYQUIDITY at the full daily dose in most pediatric patients. Start at a lower starting dose in newborns (0-3 months) at risk for cardiac failure and in children at risk for hyperactivity (see below). Monitor for clinical and laboratory response.

Table 1. THYQUIDITY Dosing Guidelines for Pediatric Hypothyroidism

AGE	**Daily Dose Per Kg Body Weight[a]**
0-3 months	10-15 µg/kg/day
3-6 months	8-10 µg/kg/day
6-12 months	6-8 µg/kg/day
1-5 years	5-6 µg/kg/day
6-12 years	4-5 µg/kg/day
Greater than 12 years but growth and puberty incomplete	2-3 µg/kg/day
Growth and puberty complete	1.6 µg/kg/day
a. The dose should be adjusted based on clinical response and laboratory parameters.	

Newborns (0-3 months) at risk for cardiac failure: Consider a lower starting dose in newborns at risk for cardiac failure. Increase the dose every 4 to 6 weeks as needed based on clinical and laboratory response.

Children at risk for hyperactivity: To minimize the risk of hyperactivity in children, start at one-fourth the recommended full replacement dose, and increase on a weekly basis by one-fourth the full recommended replacement dose until the full recommended replacement dose is reached.

Pregnancy

Pre-existing Hypothyroidism: THYQUIDITY dose requirements may increase during pregnancy. Measure serum TSH and free-T4 as soon as pregnancy is confirmed and, at a minimum, during each trimester of pregnancy. In patients with primary hypothyroidism, maintain serum TSH in the trimester-specific reference range. For patients with serum TSH above the normal trimester-specific range, increase the dose of THYQUIDITY by 12.5 to 25 µg/day and measure TSH every 4 weeks until a stable THYQUIDITY dose is reached and serum TSH is within the normal trimester-specific range. Reduce THYQUIDITY dosage to pre-pregnancy levels immediately after delivery and measure serum TSH levels 4 to 8 weeks postpartum to ensure THYQUIDITY dose is appropriate.

New Onset Hypothyroidism: Normalize thyroid function as rapidly as possible. (b) (4) (b) (4) (b) (4) Evaluate serum TSH every 4 weeks and adjust THYQUIDITY dosage until a serum TSH is within the normal trimester specific range.

TSH Suppression in Well-differentiated Thyroid Cancer

Generally, TSH is suppressed to below 0.1 mIU per liter, and this usually requires a THYQUIDITY dose of greater than 2 µg per kg per day. However, in patients with high-risk tumors, the target level for TSH suppression may be lower.

2.3 Outstanding Issues

None

2.4 Summary of Labeling Recommendations

Summary of key labeling recommendation for different sections are listed below:

- Section 2: The proposed dosing recommendations are acceptable.

2.3 Dosing in Specific Patient Populations

- *New Onset Hypothyroidism*: Normalize thyroid function as rapidly as possible. (b) (4) (b) (4)

(b) (4) Evaluate serum TSH every 4 weeks and adjust THYQUIDITY dosage until a serum TSH is within the normal trimester specific range.

- *TSH Suppression in Well-differentiated Thyroid Cancer*
- Generally, TSH is suppressed to below 0.1 mIU per liter, and this usually requires a THYQUIDITY dose of greater than 2 µg per kg per day. However, in patients with high-risk tumors, the target level for TSH suppression may be lower.
- Section 7: The proposed labeling for Drug Interactions is acceptable.
- Section 8: The proposed labeling for Use in Specific Populations is acceptable.
- Section 12.3: Delete the following statement under Section 12.3 Pharmacokinetics of THYQUIDITY's proposed label because the Guidance for the Clinical Pharmacology Labeling Section recommends the term "bioequivalence" or the comparative PK data not be included in the labeling.

(b) (4)

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

The applicant's levothyroxine sodium solution is for oral administration. The innovator's 300 µg levothyroxine sodium oral tablets (SYNTHROID) were approved on July 24, 2002 (NDA 021402).

The Division of Metabolism and Endocrinology Products (DMEP) sent written responses to the applicant's meeting requests on December 18, 2015 (DARRTS Reference ID: 3863164) and October 6, 2017 (DARRTS Reference ID: 4164027) that the reliance on 300 µg SYNTHROID oral tablets as reference for the proposed levothyroxine sodium oral solution is acceptable. DMEP sent written responses to the applicant's request for meeting that the proposed bioequivalence study design is acceptable and the sponsor does not need to conduct additional clinical studies to support this submission on January 24, 2019 (DARRTS Reference ID: 4380573).

3.2 General Pharmacology and Pharmacokinetic Characteristics

Mechanism of Action of Levothyroxine

Thyroid hormones exert their physiologic actions through control of DNA transcription and protein synthesis. Triiodothyronine(T3) and T4 diffuse into the cell nucleus and bind to thyroid receptor proteins attached to DNA. This hormone nuclear receptor complex activates gene transcription and synthesis of messenger RNA and cytoplasmic proteins.

The physiological actions of thyroid hormones are produced predominantly by T3, the majority of which (approximately 80%) is derived from T4 by deiodination in peripheral tissues.

Summary of General Clinical Pharmacology and Pharmacokinetics

Pharmacokinetics

Absorption

Absorption of orally administered T4 from the gastrointestinal tract ranges from 40% to 80%. The majority of the levothyroxine dose is absorbed from the jejunum and upper ileum. T4 absorption is increased by fasting, and decreased in malabsorption syndromes and by certain foods such as soybeans. Dietary fiber decreases bioavailability of T4. Absorption may also decrease with age. In addition, many drugs and foods affect T4 absorption.

Distribution

Circulating thyroid hormones are greater than 99% bound to plasma proteins, including thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA), and albumin (TBA), whose capacities and affinities vary for each hormone. The higher affinity of both TBG and TBPA for T4 partially explains the higher serum concentrations, slower metabolic clearance, and longer half-life of T4 compared to T3. Protein-bound thyroid hormones exist in reverse equilibrium with small amounts of free hormone. Only unbound hormone is metabolically active. Many drugs and physiologic conditions affect the binding of thyroid hormones to serum proteins. Thyroid hormones do not readily cross the placental barrier.

Elimination

Metabolism

T4 is slowly eliminated. The major pathway of thyroid hormone metabolism is through sequential deiodination. About 80% of circulating T3 is derived from peripheral T4 via monodeiodination. The liver is the major site of degradation for both T4 and T3, with T4 deiodination also occurring at a number of additional sites, including the kidney and other tissues. About 80% of the daily dose of T4 is deiodinated to yield equal amounts of T3 and reverse T3 (rT3). T3 and rT3 are further deiodinated to diiodothyronine. Thyroid hormones are also metabolized via conjugation with glucuronides and sulfates and excreted directly into the bile and gut where they undergo enterohepatic recirculation.

Excretion

Thyroid hormones are primarily eliminated by the kidneys. A portion of the conjugated hormone reaches the colon unchanged and is eliminated in the feces. About 20% of T4 is eliminated in the stool. Urinary excretion of T4 decreases with age.

Table 2. Pharmacokinetic Parameters of Thyroid Hormones in Euthyroid Patients

Hormone	**Ratio in Thyroglobulin**	**Biologic Potency**	**$t_{1/2}$ (days)**	**Protein Binding (%)[a]**
Levothyroxine (T4)	10 - 20	1	6-7[b]	99.96
Liothyronine (T3)	1	4	≤ 2	99.5

a. Includes TBG, TBPA, and TBA
b. 3 to 4 days in hyperthyroidism, 9 to 10 days in hypothyroidism

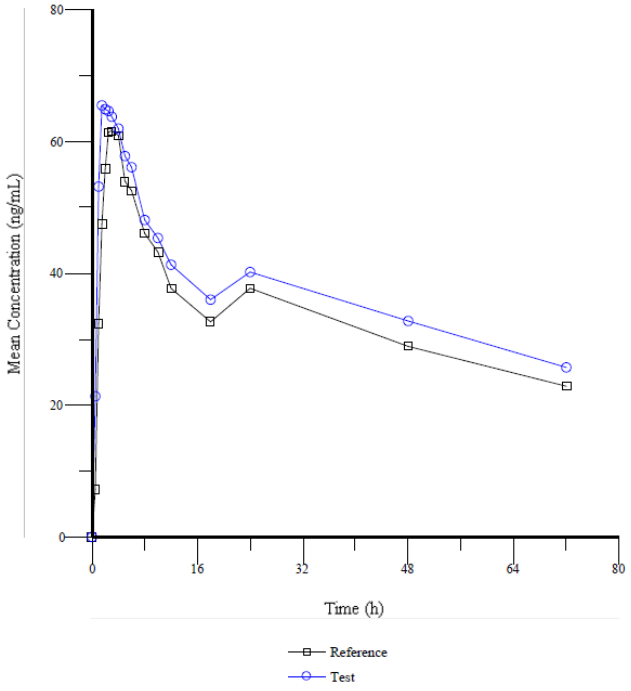
3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

The applicant conducted 2 relative bioavailability studies to support NDA 214047, namely Studies EMP-P2-777 and PAT-P0-890. Study EMP-P2-777 is the pivotal bridging bioequivalence study to reference the effectiveness and safety of the reference listed product SYNTHROID. Study PAT-P0-890 is a pilot study and the focus of this review will be on the pivotal study.

Study EMP-P2-777 is a study of 30 mL of the to-be-marketed 100 µg/5 mL levothyroxine sodium oral solution (Test) versus two US commercial 300 µg SYNTHROID oral tablets (Reference). The biobatch size of 2000 L for the levothyroxine sodium oral solution is identical to that of the commercial batch size. The design was an open label, randomized 2-treatment, 2-period, 2-sequence, crossover, single-dose study. A 35-day washout separated the 2 single-dose oral administrations. Forty two healthy volunteers completed both treatments of the levothyroxine sodium solution and the SYNTHROID tablets under fasting conditions. Serial serum samples were collected predose and 72 hours postdose to determine the total (bound and unbound) levothyroxine concentrations via validated high performance liquid chromatography coupled to tandem mass spectrometry assay. Baseline serum levothyroxine concentrations were measured about 0.5 hour, 15 minutes, and 5 minutes prior to drug administration. The mean of these 3 predose concentrations was used for baseline adjustment.

Figure 1. Mean baseline-adjusted serum levothyroxine concentration-time profile upon dosing of the levothyroxine sodium oral solution (Test) versus SYNTHROID oral tablets (Reference) under fasting conditions.



Source: Figure 1 of Study EMP-P2-777's report, Page 41 of 107

Table 3 shows the mean and CV for the C_{max}, ln (C_{max}), T_{max}, AUC_{0-72}, and ln (AUC_{0-72}) of serum baseline-adjusted levothyroxine pharmacokinetic parameters for Study EMP-P2-777.

Table 3. Serum baseline-adjusted levothyroxine pharmacokinetic parameters for Study EMP-P2-777.

PARAMETER	**TEST (n=42)**		**REFERENCE (n=42)**	
	MEAN	**C.V. (%)**	**MEAN**	**C.V. (%)**
C_{max} (ng/mL)	73.9	(23.8)	68.2	(21.6)
ln (C_{max})	4.2749	(5.6)	4.1994	(5.2)
T_{max} (hours)	2.00	(1.00-6.10)	2.50	(1.50-10.00)
AUC_{0-72} (ng·h/mL)	2650.0	(28.2)	2419.7	(25.3)
ln (AUC_{0-72})	7.8367	(4.2)	7.7594	(3.3)

Test = 30 mL of the to-be-marketed 100 µg/5 mL levothyroxine sodium oral solution; Reference = 2 US commercial 300 µg SYNTHROID oral tablets
Source: Modified from Table 7 in Study EMP-P2-777's report, Page 40 of 107.

The sponsor did not present the baseline-unadjusted levothyroxine pharmacokinetic parameters in the study report of Study EMP-P2-777.

Table 4. Statistical comparisons of baseline-adjusted levothyroxine pharmacokinetics upon the administration of 30 mL of 100 µg/5 mL levothyroxine sodium oral solution versus the administration of two 300 µg SYNTHROID oral tablets under fasting.

Baseline-adjusted Levothyroxine Parameter	LS Geometric Means		Geometric Mean Ratio (GMR, %)	90% Confidence Interval	
	Test	Reference		Lower	Upper
C_{max} (ng/mL)	71.7	66.4	107.93	101.65	114.60
AUC_{0-72} (ng•h/mL)	2527.1	2317.3	109.05	100.60	118.21

Source: Modified from Table 8 in Study EMP-P2-777's report, Page 40 of 107.

The 90% confidence intervals of baseline-adjusted levothyroxine C_{max} GMR and AUC_{0-72} GMR show that 30 mL of 100 µg/5 mL levothyroxine sodium oral solution is bioequivalent to the administration of two 300 µg SYNTHOID oral tablets under fasting because the 90% confidence intervals are within the 80 – 125% bioequivalence goalposts.

To note, the sponsor used the 0 – 72 hours sampling time interval for AUC determination. As longer sampling time may potentially be confounded by baseline and circadian rhythm leading to greater PK variability, the FDA has accepted AUC_{0-48}. However, as the Study EMP-P2-777 met BE criteria for both AUC_{0-72} and C_{max}, the AUC_{0-48} is not necessary.

For NDA 214047, the Office of Study Integrity and Surveillance (OSIS) declined to inspect Study EMP-P2-777's clinical and bioanalytical sites because:

- Inspection of the clinical site was conducted in June 2019.
- Inspection of the bioanalytical site was conducted in September 2019.

These OSIS inspections occurred within their surveillance interval (Memorandum in DARRTS Reference ID: 4583413 dated March 30, 2020).

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimens are consistent with the dosing regimens for the US approved reference product, SYNTHROID.

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic factors?

The effect of intrinsic factors on THYQUIDITY has not been studied. Relevant information from the reference SYNTHROID product label was incorporated into the THYQUIDITY product label. See Section 2.2.2 Therapeutic individualization for details.

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

The applicant did not conduct food-drug or drug-drug interaction studies to support NDA 214047. Relevant food-drug or drug-drug interaction information from the reference SYNTHROID product label was incorporated into the THYQUIDITY product label.

3.3.5 What is the oral bioavailability of glycerol content in the levothyroxine sodium solution? What are the metabolizing enzymes for glycerol in humans and the ontogeny of these enzymes?

The Division of General Endocrinology consulted the Division of Pediatrics and Maternal Health (DPMH) to assist with the Pregnancy and Lactation subsections of the proposed THYQUIDITY labeling. DPMH had concern for the (b) (4) glycerol contained in the maximum clinical dose of 300 µg/daily levothyroxine sodium oral solution and its potential impact on dosing for neonates and small infants. A teleconference occurred on August 12, 2020 between DPMH staff and NDA 214047's review team to discuss the glycerol issue. Per the discussion of this teleconference, this reviewer focused on obtaining the following information for glycerol in humans:

- oral bioavailability
- metabolizing enzymes
- ontogeny of these metabolizing enzymes

The applicant used glycerin in the formulation of levothyroxine sodium solution (Table 5), whereas the DPMH staff has concerns for glycerol. According to the CMC Review Lead, Dr. Muthukumar Ramaswamy, glycerol is the same as glycerin. This reviewer used PubMed and Google Scholar to search for relevant articles for this task.

Table 5. Formulation of levothyroxine sodium solution used in Study EMP-P2-777.

		Batch no. 80569
Composition Quantity Per 1 mL	Levothyroxine Sodium	20 mcg*
	Glycerin	(b) (4)
	Citric Acid Monohydrate	
	Methylparaben Sodium	
	Sodium Hydroxide	
	Purified Water	
DP batch size		
Number of filled bottles		
DP batch date of manufacture		
DP manufacturer		
DS manufacturer		
Batch no. of DS	DP manufacturer	180588
	DS manufacturer	21024
DP Container closure system		Amber Type III glass bottles with 100 mL nominal (deliverable) capacity screwed with plastic child-resistant and tamper-evident cap.
Use of batch		US exhibit batch & biobatch of BE study no. EMP-P2-777

Source: Modified from Table 61 in Module 3.2.P.2, Page 72 of 76.

The absolute oral bioavailability of glycerol from the levothyroxine sodium solution is not determinable because the applicant did not study the intravenous administration of levothyroxine sodium solution. The absorption of glycerol occurs rapidly with t_{max} of about 1 hour after oral administration in adults according to the following 2 articles:

- Pelkonen R, Nikkila EA, Kekki M. Metabolism of glycerol in diabetes mellitus. *Diabetologia* 1967;3:1-8.
- Sommer S, Nau R, Wieland E, and Prange HW. Pharmacokinetics of glycerol administered orally in healthy volunteers. *Arzneim-Forsch/Drug Res* 1993;43:744-7.

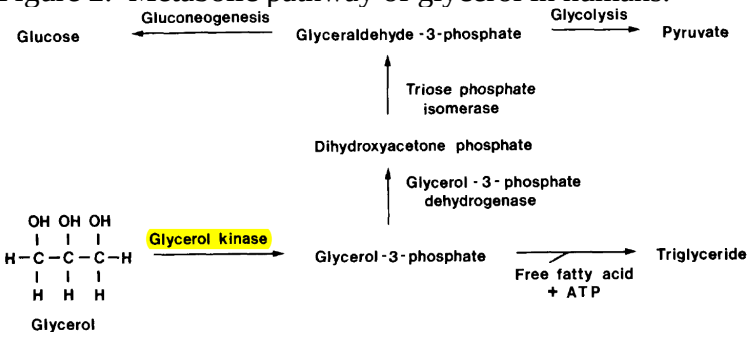
However, this reviewer could not find glycerol absorption data in neonates and small infants.

Glycerol is metabolized via 2 major pathways (Figure 2). After phosphorylation by glycerol kinase, about 70 – 90% is oxidized via glycerol-3-phosphate dehydrogenase to dihydroxyacetone phosphate, which then enters the Embden-Meyerhof pathway at the level of glyceraldehyde-3-phosphate. The remaining 10 – 30% combines with free fatty acids to form triglycerides according to the following article:

- Frank MS, Nahata MC, Hilty MD. Glycerol: a review of its pharmacology, pharmacokinetics, adverse reactions, and clinical use. *Pharmacotherapy* 1981;1:147-60.

About 80% of glycerol metabolism occurs in the liver, whereas 10 – 20% occurs in the kidney; this distribution corresponds to the primary locations of glycerol kinase. Glycerol is filtered and almost completely reabsorbed by the renal tubules until a serum concentration of about 0.15 mg/mL is achieved.

Figure 2. Metabolic pathway of glycerol in humans.



The activities of 3 key enzymes for glycerol metabolism were measured in liver samples from 37 human fetuses ranging in gestational age from 18 weeks to term, neonates (1 – 3 days), and infants to 2 years. Glycerol kinase specific activity was constant throughout the period of fetal development examined and was comparable to that measured in neonates and infants. However, the subcellular distribution of the activity changed markedly, being predominantly particulate in fetal samples and cytoplasmic in postnatal samples. The particulate activity had an elevated K_m for glycerol. Cytoplasmic glycerol-3-phosphate dehydrogenase activity was very low in the fetal period and then rose to adult levels during infancy. There were no kinetic difference between the fetal and postnatal activities. Mitochondrial glycerol-3-phosphate dehydrogenase activity rose somewhat after birth to near adult levels. These data indicate that human fetal, neonatal, and infant liver can metabolize glycerol according to the following article:

- Sadava D, Depper M, Gilbert M, Bernard B, McCabe ERB. Development of enzymes of glycerol metabolism in human fetal liver. *Biol Neonate* 1987;52:26–32.

Overall, according to the DPMH review dated September 2, 2020 with DARRTS Reference ID: 4664627), published animal studies have not shown glycerol to be teratogenic, mutagenic or carcinogenic. Because glycerin is GRAS and the proposed daily maximum dose is within the maximum daily exposure (31g) of inactive ingredient of FDA's approved drug products and the accepted range in food and beverages by ESFU, the amount of glycerol in levothyroxine sodium oral solution is not expected to result in major birth defects, miscarriage, or adverse maternal or fetal outcomes. Thus, there was no safety concern for glycerol at this time with use in lactation.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

Table 6 shows the summary of bioanalytical method validation for levothyroxine in human serum samples.

Table 6 Summary of bioanalytical method validation for levothyroxine in human serum

Information Requested	Data
Bioanalytical method validation report location	LVN-V6-628(R1), pp. 1-88
Analyte	Levothyroxine Sodium Salt (Thyroxine Sodium Salt)
Internal Standard (IS)	Levothyroxine-13C9-15N
Method description	Protein precipitation Reversed-phase HPLC with MS/MS detection
Limit of quantitation (ng/mL, %)	10.0 ng/mL Proxy Matrix: Between-run accuracy 95.2% Between-run precision 8.4% Within-run accuracy 85.8% - 104.8% Within-run precision 3.9% - 5.8%
	Within-run accuracy (using LHS) 92.3% Within-run precision (using LHS) 4.2% Human Serum: Between-run accuracy 104.6% Between-run precision 4.5% Within-run accuracy 101.8% - 107.6% Within-run precision 1.8% - 5.4% Within-run accuracy (using LHS) 104.8% Within-run precision (using LHS) 5.4%
Average recovery of drug (Percent extraction yield)	Proxy Matrix: 97.3% - 107.0% Human Serum: 90.0% - 98.3%
Average recovery of IS (Percent extraction yield)	Proxy Matrix: 104.8% Human Serum: 83.9%
Standard curve concentrations (ng/mL)	10.0 ng/mL – 300.0 ng/mL
QC concentrations (ng/mL)	Proxy matrix: 10.0 ng/mL, 30.0 ng/mL, 100.0 ng/mL, 150.0 ng/mL and 225.0 ng/mL Human serum: 59.7 ng/mL, 100.2 ng/mL, 150.2 ng/mL and 225.2 ng/mL
QC Intraday precision range (%)	Proxy Matrix: 1.3% - 7.0% Proxy Matrix (using LHS): 1.3% - 4.2% Human Serum: 1.6% - 5.7% Human Serum (using LHS): 1.6% - 5.4%
QC Intraday accuracy range (%)	Proxy Matrix: 85.8% - 106.0% Proxy Matrix (using LHS): 92.3% - 96.8% Human Serum: 98.1% - 110.5% Human Serum (using LHS): 101.4% - 104.8%

QC Interday precision range (%)	Proxy Matrix: 4.4% - 17.2% Human Serum: 3.8% - 5.9%
QC Interday accuracy range (%)	Proxy Matrix: 95.2% - 101.5% Human Serum: 102.0% - 104.6%
Bench-top stability (23.8 hours)	Confirmed up to 23.8 hours for Levothyroxine in DMSO:MeOH 50:50% v/v at 100.00 µg/mL at 22°C nominal. % deviation: -0.4%. Confirmed up to 23.8 hours for Levothyroxine in DMSO:MeOH 50:50% v/v at 1.00 µg/mL at 22°C nominal. % deviation: 1.1%. Confirmed up to 23.8 hours for Levothyroxine 13C9-15N in DMSO:MeOH 50:50% v/v at 100.00 µg/mL at 22°C nominal. % deviation: -3.9%.
Stock stability (147 days)	DMSO:MeOH 50:50% v/v at 100.00 µg/mL at 4°C nominal. % deviation: -3.7%. Confirmed up to 147 days for Levothyroxine in DMSO:MeOH 50:50% v/v at 1.00 µg/mL at 4°C nominal. % deviation: 0.0%. Confirmed up to 147 days for Levothyroxine-13C9-15N in
	Proxy Matrix: Confirmed up to 25.9 hours at 4°C nominal. Accuracy (% nominal): 93.3% for Low Stability QC and 95.5% for High Stability QC. Human Serum: Confirmed up to 25.9 hours at 4°C nominal. Accuracy (% nominal): 94.3% for Low Stability QC and 99.0% for High Stability QC.
Stability in whole blood (2.0 hours)	Confirmed up to 2.0 hours at Room Temperature. % deviation: 12.2% for Low QCs and 3.7% for High QCs.
Freeze-thaw stability (4 cycles)	Proxy Matrix: 4 cycles. Accuracy (% nominal): 94.2% for Low Stability QC and 95.2% for High Stability QC.
	Human Serum: Confirmed up to 140 days at -80°C nominal. Accuracy (% nominal): 97.6% for Low Stability QC and 99.8% for High Stability QC. Confirmed up to 7 days at -20°C nominal. Accuracy (% nominal): 106.7% for Low Stability QC and 98.6% for High Stability QC.
Dilution integrity	600.1 ng/mL diluted 5-fold. Accuracy (% nominal): 102.4% Precision: 2.8%
Selectivity	No significant interference observed at the retention time of IS in the 6 blank matrix lots screened.

Source: Table 1 of Module 2.7.1

Validations for the high performance liquid chromatography coupled to tandem mass spectrometry assay bioanalytical method to measure levothyroxine in serum samples for Study EMP-P2-777 appear acceptable with reasonable precision and accuracy.

5. REFERNCE

Surks and Sievert. Drugs and thyroid function. *N Engl J Med* 1995 Dec;333:1688-94.

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