"STUDIES IN STABILIZATION OF LEVOTHYROXINE SODIUM TABLETS"

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IN

PHARMACEUTICS

BY

AISHWARYA MISHRA (16MPH101), B. PHARM.

Under the guidance of

Dr. Tejal A. MEHTA – GUIDE
Professor, Department of Pharmaceutics

NIRMA UNIVERSITY
INSTITUTE OF PHARMACY
NAAC ACCREDITED 'A' GRADE

Department of Pharmaceutics
Institute of Pharmacy
Nirma University
Ahmedabad-382481
Gujarat, India.

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CERTIFICATE

*This is to certify that the dissertation work entitled "**Studies in Stabilization of Levothyroxine Sodium Tablets**" submitted by Mr. Aishwarya Mishra with Regn. No. (16MPH101) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutics" is a bonafide research work carried out by the candidate at the Department of Pharmaceutics, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.*

Guide:

Dr. TEJAL A. MEHTA
M. Pharm., Ph.D.,
Professor,
Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University

Director:

14/5/18

PROF. MANJUNATH GHATE
M. Pharm., Ph.D.
Director
Institute of Pharmacy,
Nirma University

Date : 11 / 05 2018

Sun Pharmaceutical Industries Ltd.
Sarhaul, Sector-18,
Gurugram-122 015 (Haryana), INDIA
Phone : (91-124) 4194400
Fax : (91-124) 2342017
www.sunpharma.com
CIN : L24230GJ1993PLC019050

SUN PHARMA

January 23, 2018

TO WHOMSOEVER IT MAY CONCERN

This is to certify that Mr. Aishwarya Mishra from Institute of Pharmacy, Nirma University Ahmedabad has undergone the Summer Internship in Sun Pharmaceutical Industries Ltd in Product Development Research Department and has completed the project report on "Stabilization Of Levothyroxine Sodium Tablet" under the guidance of Dr. Ravindra Agarwal and Mohd Amir from June 04, 2017 to January 23, 2018. The internship was completed to the satisfaction of the company guide and the organization.

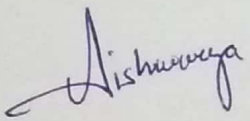
We wish him all the best for all future endeavors.

for Sun Pharmaceutical Industries Ltd.

Vijay Taneja
General Manager
HR Operations

DECLARATION

*I hereby declare that the dissertation entitled "**Studies in Stabilization of Levothyroxine Sodium Tablets**", is based on the original work carried out by me under the guidance of Dr. Ravindra Agarwal, General Manager, Production, Sun Pharmaceuticals Industries Ltd. and Dr. Tejal A. Mehta, Professor, Department of Pharmaceutics, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.*



Mr. Aishwarya Mishra (16MPH101)
Department of Pharmaceutics,
Institute of Pharmacy,
Nirma University,
Sarkhej - Gandhinagar Highway,
Ahmedabad-382481,
Gujarat, India

Date: 11 /05/ 2018

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List of Abbreviations

LTX	Levothyroxine Sodium
T4	Thyroxine
T3	Triiodothyronine
CDSCO	Central Drugs Standards Control Organization
USFDA	United States Food and Drug Administration
ICH	International Conference on Harmonisation
API	Active Pharmaceutical ingredient
MIT	Mono-iodotyrosine
DIT	Di-iodotyrosine
TRH	Thyrotropin Releasing Hormone
TBG	Thyroxine-binding Globulin
TBPA	Thyroxine-binding prealbumin
USP	United States Pharmacopeia
HPLC	High Performance Liquid Chromatography
AR	Adverse Reactions
SMCC	Silicified Microcrystalline cellulose
HDPE	High Density Polyethylene

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STUDIES IN STABILIZATION OF LEVOTHYROXINE SODIUM TABLETS

ABSTRACT

Levothyroxine is a salient medication that is used for the treatment of hypothyroidism. Levothyroxine have been recalled many times since its introduction to US market. For a quality product, stability over a shelf life is a critical parameter. Various parameter affects the stability of API like excipient used, process of manufacturing, various storage condition (temperature, moisture, humidity) as well as packaging of product, all these increases the degradation of levothyroxine and thus Potency.

Experiments were performed to study and demonstrate the effect of formulation and various processing variable to study the stability of levothyroxine. At 60^{o}C and 40^{o}C/75% RH excipient and Levothyroxine are studies under ICH stability condition. Also tablet manufactured by wet granulation, dry granulation and direct compression are evaluated.

For Six months, Levothyroxine found to be stable at 40^{o}C/75% Relative Humidity both in open as well as closed containers. Tablets were manufactured by different excipients, SMCC, LHPC, Olive oil, Carrageenan, MCC. Tablets composed with SMCC (Silicified Microcrystalline Cellulose) failed to meet USP requirement at One and three months. 5% loss in potency after three and six months of accelerated stability study with pH modifier. Thus it is observed that use of different excipient in the manufacturing of levothyroxine affected the stability of Levothyroxine. Desiccant also help in maintaining the stability of Levothyroxine sodium tablet.

Tablets manufactured by Wet granulation as well as direct compression, after 3 months of storage (ICH accelerated stability condition) there is not much difference in in stability/ Potency. Thus Manufacturing method/ process also affect the stability of levothyroxine sodium tablets. Thus it is observed that formulation as well as process affects the stability of levothyroxine tablets.

KEY WORDS- Levothyroxine Sodium, Formulation, Stability, Excipients, Desiccant, Moisture, Humidity, shelf life, pH modifier, wet Granulation.

CHAPTER-1

Introduction

1. INTRODUCTION

1.1 Stability

Drug substance / Drug product capacity to meet the specification that will ensure the identity, purity, quality throughout the expiry date is defined as Stability.

To determine the suitability, stability (active components) is the main criteria in design/ evaluation of dosage form of drug. Instability can occur in various forms.

Chemical Degradation occur which lower the therapeutic index quantity of dosage form. It is having a major significance in narrow therapeutic index, careful titration is done so that the serum level is neither high (Toxic) nor low (ineffective). Since this may not be so extensive thus during decomposition process toxic degradant can be formed [1]

Instability affects the bioavailability, which lead to lowering of therapeutic efficacy.

It can be due to physical as well as chemical changes in excipient used in dosage form.

Forth, physical appearance change in dosage form also affects the stability.

The major principles which need to be considered is, that in presence of water, oxygen, light most of the pharmaceutical reaction occur [2]. Therefore, Photolysis, decarboxylation, hydrolysis, and racemization cause decomposition.

1.2 Stability testing of Tablets

Safety, quality, efficacy are the major concern thus regulatory authorities require stability testing of drug product. ICH having a draft guideline, i.e.- stability testing of drug product and drug substance. [14]

Storage conditions:

- Long-term testing: 25^{o}C / 60% RH for 12 months
- Intermediate-testing: 30^{o}C / 65% RH for 6 months
- Accelerated-testing: 40^{o}C / 75% RH for 6 months

Tablets are evaluated on the basis of-

Odor, assay, degradation, moisture, Dissolution Friability, appearance and color

Change which is significant should not occur. E.g.-

i. Product which is exceeding its pH limit.
ii. Any degradant which is exceeding its limit.
iii. Exceeding the dissolution limit.
iv. Not meeting the appearance or physical properties specification.

1.3 Study of Degradation

Stress Study also called as forced degradation is important in pre-formulation. It is done in more severe condition than the accelerated study, and used in development phase.

Conditions in which stress study is carried out are-

- Burgeoning/ validation of stability methodology.
- Establishment of constitute stability of drug molecule.
- To determine the degradation pathway of drug product as well as drug substance.

FDA conducted various degradation studies.
Drug substance is subjected to being stressed in solution/ suspension under acidic as well as alkaline pH. It is done under high Oxygen environment. Drug is stressed under high temperature and also under excess humidity.
By the use of NMR, MS, UV etc. characterization of degradation product. Also Drug photolytically stressing in solution state and also at solid state.

Thus when drug s studies under degradation studies it is subjected to exposure under acid, base oxidative, light thermal and humidity.

1.4 Microenvironment and pH of Tablet

Drug substance stability is affected by the excipient water quantity. Since some of the moisture is always present in tablet microenvironment thus considered as pH of microenvironment of tablet prospective technique performed to improve the stability of drug product by formulating at pH of maximum stability. [4]

Citric acid, Tartaric acid are acidic additives while Sodium bicarbonate, Sodium carbonate, Magnesium and Calcium oxides are basic additives used to control microenvironment pH of tablet

Badaway et al (1999) studies solid –state stability effect on solid in ester prodrug. pH4 is the max. stability and it is found in solution. [5] Different acid are used for the adjustment of microenvironment pH of tablets. Increases in hydrolysis by the use of strong acid.

1.5 Levothyroxine: Consistency problems, regulatory and background

Levo is orally administered and used as a replacement therapy in various condition in which there is absence or diminished function of thyroid for e.g. myxedema, cretinism and hypothyroidism.

It is common condition in united states of America one in every 5 thousand babies is born with hypothyroidism. Prevalence of hypothyroidism is 0.5% to 1.3% in adults, while in men it increases 2.7% and in women it increases. Mental retardation occurs due to congenital hypothyroidism and it can be avoided by early diagnosis and early Therapy

Screening of newborn is necessary in America, Europe and also in japan. Earlier people with hypothyroidism is treated with tablets that are containing animal thyroid gland. These tablets are containing both T3 (triiodothyronine) and T4 (thyroxine) [6] First synthetic levothyroxine is marketed in 1958. Before FDA laws are placed thyroid hormones are already in the market, thus these hormones manufacturers were not required testing. [21] Thus this replacement therapy is “grandfather” in this system.

After 40 years of market authorization and introduction FDA issued notice “drug that are orally administered and containing levothyroxine sodium is new drug”. The information was showing serious stability as well as potency problem. [9] The potency of the product is not through the expiration date and thus there is the difference in the tablet strength from lot to lot. Thus it causes serious health consequences in public health.

FDA said that orally administered levothyroxine sodium is important medically and allow manufacturers 3yr to get NDA approval.

In the vicinity of 1987 and 1994, the FDA got 58 antagonistic medication encounter reports related with the strength of orally managed levothyroxine sodium items. 47 reports recommended that the items were sub potent, while 9 proposed super potency. 2 of the reports included irregularity in thyroid hormone blood levels. 4 hospitalizations were incorporated into the reports; two were credited to item sub-potency and two were ascribed to item super-potency. [7] The greater part of the fifty-eight reports were bolstered by thyroid capacity blood tests. A portion of the detailed issues were the consequence of exchanging brand. Be that as it may, other unfavorable occasions happened when patients got a refill of an item on which they had beforehand been steady, showing an absence of consistency in soundness, strength, and between various loads of tablets from a similar producer. Since levothyroxine sodium items were physician recommended drugs promoted without affirmed NDA's, makers are explicitly required, under 21 CFR 310.305, to report antagonistic medication encounters that are unforeseen and genuine; notwithstanding they are not required, concerning the items with endorsed applications (21 CFR 314.80) to intermittently report all unfavorable medication encounters, including expected or less genuine occasions.[8] Therefore, some antagonistic medication encounters identified with irregularities in strength of orally regulated levothyroxine sodium items may not be viewed as genuine or surprising and, subsequently, may go unreported. Reports got by the FDA, in this way, may not mirror the aggregate number of unfriendly occasions related with irregularities in item intensity.

1.6 Issue in stability

FDA with USP convention organized a workshop in 1982 set use of HPLC assay as the stability indicting method of the drug. Earlier assay is done based on iodine content method but it does not indicate the stability. By use of HPLC, there were various reporting of stability of levo. products. [10] Every market leader as well as manufacturer of Levo has reported the stability and potency of the drug and thus the recalls were made.

From 1991, levothyroxine recalls are from more than 10 firms. In one major case the recalls are due the sub- potency as the potency is not maintained through the expiration date. [22] While the other recalls are being based on the super-potency of the drug. FDA also warn the manufacturer stating the stability issue of orally administered Levo sodium Product.

1990-1992, 46 lots of Levo Na product were destroyed as they not meet the potency and uniformity specification. 1989, 21 lots were recalled because of sub-potency. 26 lots were recalled in February and 15 lots in June due to sub-potency. [12]

FDA found that the 14% lot that were manufactured during the period of 1991 to 1993 were rejected as well as destroyed as they failed to meet the assay and uniformity specification (assay specification were 95-105%)

During march 1993, FDA issued a warning letter to a firm that levo tablets are adulterated as they were unable to maintain its potency throughout the expiration period. Warning letter also state the objection on storing condition of drug that is 15 to 20^{o}C. [11] It is objected as it is not conforming in the USP about the storing condition. Thus the storing condition labelling is changed to 8 - 15^{o}C. [14]

With addition to these issues which is about the potency/ assay of levo sodium product, thus it is recommended that two year of shelf of is not suitable as it vulnerable to accelerated degradation due to various factors.

Factors affecting the stability of Levothyroxine are

- Light
- Temperature
- Air
- Moisture
- Humidity
- Excipients

1.6.1. Humidity/Moisture and Oxygen

- Some tablets decompose by approximately 1% per month, but rate increases up to 40% in 30 days once the bottles containing the tablets are opened.
- Ideal Moisture condition- RH 40-60.

1.6.2. Sunlight

- Exposure to direct sunlight for 80 min: - 60% decomposition.
- Formulation is made under Sodium Light.

1.6.3. Temperature

- Stable in dry air.
- Thyroxin tablets were found unstable even at room temperature, and storage temperatures of 8°C to 15°C were required to maintain potency.
- Ideal Temperature condition is 23–25°C
- API is stored in cold room in 2°-3°C

1.6.4. Compatibility with Pharmaceutical Excipients

- The compatibility of drug and its excipients is extremely critical
- Requires careful selection of excipients.
- Used to facilitate administration, promote consistent release and bioavailability of the drug.

1.7 Change in formulation: -

Marketing of oral administered levothyroxine sodium was done without approval, no FDA approval by manufacturers while the formulate their product. In 1982, it is reformulated by omitting 2 inactive ingredients and also by changing the dye and colors. This reformulated batch enhance the potency of the product. Also found that it has 100% of the label claim as compared to other formulation.

In 1990 (Gupta,1990) one batch of levothyroxine shoes chromatographic variation that suggest the different excipients.

1.8 Regulatory status

Levothyroxine sodium is utilized as substitution treatment when endogenous thyroid hormone generation is inadequate. [23] The support dose must be resolved on a patient-by-quiet premise. Levothyroxine sodium items are showcased in various dose qualities, that may fluctuate by just 12 µg, subsequently allowing watchful titration of measurements. In view of levothyroxine sodium's thin restorative file, it is especially essential that the measure of accessible dynamic medication be predictable for a given tablet quality. Varieties in the measure of accessible dynamic medication can influence both security and viability. [13] Sub-potent tablets won't be compelling in controlling hypothyroid indications or sequelae. The medication substance, levothyroxine sodium, is insecure within the sight of light, temperature, air, and moistness. Unless the assembling procedure can be deliberately and reliably controlled, orally regulated levothyroxine sodium items may not be completely powerful through the named termination date, or be of predictable intensity from part to parcel. [15] There is confirm from reviews, antagonistic medication encounter reports, and assessment reports that notwithstanding when a doctor reliably recommends a similar brand of orally directed levothyroxine sodium, patients may get results of variable strength at a [24] given measurement. Such varieties in item power exhibit genuine security and viability concerns (Government Enlist, 1997).

As per the FDA, levothyroxine sodium is protected and successful in treating hypothyroidism, when it is deliberately and reliably made and put away, and it is recommended in the right sum. However, starting at 1997, none of the industrially accessible oral levothyroxine sodium items had exhibited predictable power and strength and, in this way, were not for the most part perceived as sheltered and successful (Government Enroll, 1997).

Levothyroxine sodium items are restoratively vital on the grounds that they are utilized to treat hypothyroidism and no elective medication is depended upon by the medicinal group as a sufficient substitute. [16] As needs be, FDA allowed orally directed levothyroxine sodium items to be promoted without endorsed NDA's until August 14, 2000, keeping in mind the end goal to give makers time to lead the required investigations and to get ready and submit applications, and to permit time for audit of and activity on these applications. This date was reached out to August 14, 2001 at which time just two (Unithyroid and Levoxyl) items were endorsed, with a termination date of only year and a half. [25] At present, there are 8 applications documented with the FDA as recorded in Table 1. All the rest of the makers have until December 2003 to progressively pull back their item

Table 1.1- Approved Marketed Formulation: -

Name of Product	**Manufacturer**	**Date of Approval**
Unithroid	Jerome Stevens Pharmaceuticals	Approved: 8/21/2000 Approval Type: Original (NDA)
Levo-T	Mova Pharmaceutical	Approved: 3/1/2002 Approval Type: Original (NDA)
Levothroid	Forest Laboratories	Application Submitted
Levothyroxine Sodium	Mylan	Approved: 6/5/2002 ApprovalType: Original (ANDA)
Levoxyl	Jones Pharma (Parent: King Pharmaceuticals)	Approved: 5/25/2001 Approval Type: Original (NDA)
Novothyrox	GenPharm International (Parent: Medarex)	Approved: 5/31/2002 Approval Type: Original (NDA)
Synthroid	Knoll Pharmaceutical (Parent: Abbott Laboratories)	Approved: 7/24/2002 Approval Type: Original (NDA)
Thyro-Tabs	Lloyd Inc.	Approved: 10/24/2002 Approval Type: Original (NDA)

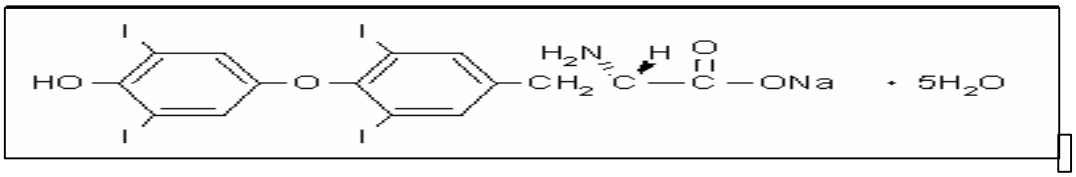
1.9 INTRODUCTION TO DRUG

1.9.1 Description

Levothyroxine sodium is synthetic crystalline L-3, 3', 5, 5'-tetraiodothyronine sodium salt. Synthetic It is identical to that produced in the human thyroid gland (Clarke's 1986).

Empirical formula of levothyroxine sodium penta-hydrate

Fig 1.1: Chemical structure of levothyroxine pentahydrate



a) Hydrated sodium salt - $C_{15}H_{10}I_4NNaO_4 . 5 H_2O$
b) Anhydrous sodium salt- C15H10I4NNaO4
c) Acid - $C_{15}H_{11}I_4NO$

Characteristic-

- pale yellow powder
- tasteless
- amorphous/crystalline form

Solubility:

Very slightly soluble in water (1 in 700), slightly soluble in alcohol (1 in 300), and dissolves in aqueous solutions of alkaline hydroxides (Moffat, 1986). The aqueous solubility of levothyroxine is dependent on the pH of the surrounding media. [26] The solubility reduces from pH 1 to 3, remains constant from 3 – 7, and significantly increases above pH ~7 (Won, 1992, Post and Warren, 1976)

Levothyroxine sodium has three ionizable moieties and it can exist as cation, zwitterion, anion, or di-ion depending on the pH of the solution. For the carboxyl group, pka1 = 2.40; for the phenolic group, pka2 =6.87 and for the amino group, pka3 = 10.1 (Won, 1992. Post, 1976).

1.10 CLINICAL PHARMACOLOGY

Thyroid is a gland of 2 inches that present under the skin which is below the Adam's apple in the neck. The two parts (flaps) of the organ are associated in the center (called the isthmus), so the thyroid organ looks like the letter H or a tie (Williams, 1989., Merck manual).

Inanimate iodide is effectively moved in the thyroid epithelial cells to a level roughly 30 times its fixation in plasma. Inside minutes subsequent to entering the thyroid, inorganic iodide is oxidized, in a peroxidase-subordinate response, to a natural frame. This oxidation is not really recognizable from the following response, in which natural iodine is consolidated into tyrosine buildups inside thyroglobulin, a vast glycoprotein particle. The resultant MIT and DIT are brought into nearness and are coupled through an ether linkage to shape thyroxine (likewise called tetra-iodothyronine) and triiodothyronine, the main thyroid hormones. [27] Evidently, just iodinated tyrosine’s are coupled on the grounds that non-iodinated thyronine isn't found inside the thyroid organ. Both coupling and iodination requires oxidative conditions, both may include a similar peroxidase, and both are hindered by thiourea subsidiaries.

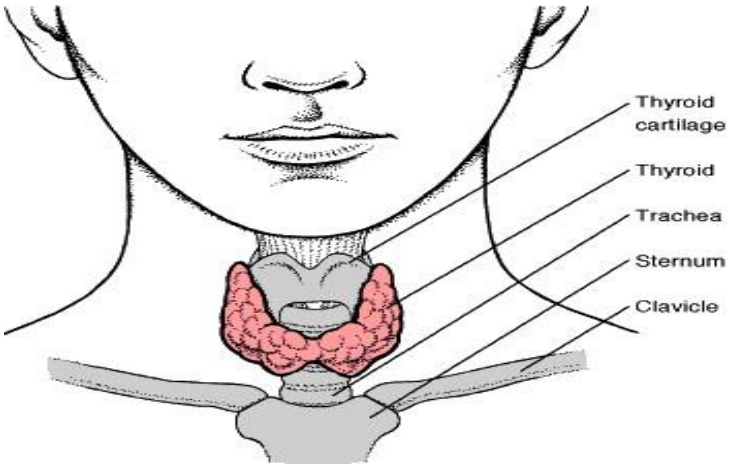


Fig 1.2: Anatomy of Thyroid Gland

In thyroid an endocrine organ and in its expansive stockpiling limit are generally moderate with the arrival of the hormone: and the organ typically hold around 8000 micrograms of iodine, a hold adequate is no less than hundred days.

Thyroxine and Triiodothyronine are mainly put away from the colloid. Thyroid additionally, contains a substantially littler measure of some thyralbumin and indoprotein that is fundamentally the same as egg whites. An organic substance is developed in number of hyper-functioning thyroids and some in a neoplasm as well. [29]

Arrival of hormone from the thyroid includes re-entry of thyroglobulin by endocytosis from the colloid into the apical segments of the thyroid follicular cell. The inundated beads intertwine with lysosomes and are hydrolyzed, discharging thyroxine and triiodothyronine into the flow. Hydrolysis of thyroglobulin additionally creates a few iodotyrosine inside the follicular cells; these mixes are de-iodinated in a response catalyzed by iodotyrosine deiodinase, reestablishing iodide to an intracellular pool from which reincorporation into hormone can happen. The intracellular pool is an imperative wellspring of iodine; hypothyroidism what's more, goiter create in patients lacking in iodotyrosine deiodinase.

The proteolytic advance is empowered by thyroid stimulating hormone (likewise called thyrotropin, or TSH) and restrained by iodine; this activity of iodine is most likely its central anti-thyroid impact. The proteolytic advance is additionally hindered by lithium yet maybe at a site unique in relation to that of hindrance by iodine; the anti-thyroid impacts of iodine and lithium are some of the time added substance.

In the circling thyroid the dynamic hormones are thyroxine and triiodothyronine. Circling triiodothyronine is created inside the thyroid from the coupling of MIT and DIT and from the peripheral mono-deiodination of thyroxine. The last procedure likewise works inside the tissues to process extra triiodothyronine. Around 15-20 percent of coursing triiodothyronine emerges from thyroid emission, and the rest emerges incidentally. In both hyperthyroidism and hypothyroidism, be that as it may, a fundamentally bigger part of the circling triiodothyronine than the normal.

Around 30 µg of triiodothyronine is created every day by the fringe de-iodination of thyroxine. This procedure is critical in a general direction of thyroid hormone. There are 2 de-iodinases, Sort I de-iodinase, establish in the kidney and the liver, follows up on coursing thyroxine to create triiodothyronine for fringe tissues. [33] Hypothyroidism can happen because of under action of sort I De-iodinase initiated by deficiency of selenium. Sort two de-iodinase is discovered basically in the placenta, pituitary and cerebrum. It is in charge of the Mono-deiodination of thyroxine in the pituitary; the subsequent triiodothyronine has coordinate administrative consequences for TSH amalgamation. The thyroid hormones have physical impact that is delivered by triiodothyronine. Around 70-90 percent of fringe triiodothyronine is created by mono-deoidination of thyroxine at the fifth position (external ring). [17] Fringe mono-deiodination of thyroxine at the fifth positions (inward ring) brings about the arrangement of turn around triiodothyronine, which is calorigenically idle.

In the emission of the actual thyroid hormones, Levothyroxine [T4] and l-Triiodothyronine [T3], from the typically working thyroid organ are controlled by complex criticism systems of the hypothalamic-pituitary-thyroid pivot. The thyroid organ is invigorated to emit thyroid hormones by the activity of thyrotropin that is created in the

front pituitary organ. The Thyroid stimulating hormone discharge is under controlled by TRH (thyrotropin - discharging -hormone) delivered in the hypothalamus flowing thyroid hormones, and perhaps different instruments. Thyroid hormones moving in the blood perform as a response mechanism of both Thyroid-Stimulating-Hormone and Thyrotropin-Discharging-Hormone excretion. In this way, when serum convergences of triiodothyronine and thyroxine is expanded, emission of Thyroid stimulating hormone and Thyrotropin discharging hormone is diminished.

The instruments by which thyroid hormones apply their physiologic activities have not been totally explained. Triiodothyronine and Thyroxine are transported into cells by uninvolved and dynamic systems. Triiodothyronine in cell cytoplasm and triiodothyronine produced from thyroxine inside the cell diluted into the core and tie to thyroid receptor proteins, which give off an impression of being essentially connected to DNA. [31] Receptor restricting prompts enactment or suppression of DNA interpretation, along these lines changing the measures of messenger-RNA and response proteins.

Changes in protein focuses are in charge of the metabolic changes saw in organs and tissues. Thyroid hormones upgrade oxygen utilization of most body tissues and increment the basal metabolic rate and digestion of sugars, lipids, and proteins. Accordingly, they apply a significant impact on each organ framework and are of specific significance in the improvement of the focal sensory system. Thyroid hormones additionally, seem to effect on tissues, for example, expanded myocardial contractility and diminished foundational vascular protection (Mercksource.com, Jameson, 2003., Gilman, 2001)

➢ Indication

1. As substitution or supplemental treatment in patients of all ages or state (counting pregnancy) with hypothyroidism of any etiology aside from transient hypothyroidism amid the recuperation period of subacute thyroiditis: essential hypothyroidism coming about because of thyroid brokenness, essential decay, or incomplete or add up to nonappearance of the thyroid organ, or from the impacts of surgery, radiation or drugs, with or without the nearness of goiter, including subclinical hypothyroidism; auxiliary (pituitary) hypothyroidism; and tertiary (hypothalamic) hypothyroidism. Levothyroxine sodium infusion can be utilized intravenously when quick repletion is required, and either intravenously or intramuscularly when the oral course is blocked.
2. As a pituitary thyroid stimulating hormone(TSH) suppressant in the treatment or counteractive action of different sorts of euthyroid goiters, including thyroid knobs, subacute or endless lymphocytic thyroiditis, multinodular goiter, and in conjunction with surgery and radioactive iodine treatment in the administration of thyrotropin-subordinate all around separated papillary or follicular carcinoma of the thyroid. [19] The measurements and rate of organization of levothyroxine sodium is dictated by the sign, and should for each situation be individualized by quiet reaction and research center discoveries.

➢ Pharmacokinetics

Retention – Absorption of orally regulated thyroxine from the gastro-intestinal tract ranges from 40-80 percent Most of the levothyroxine dosage is retained from the jejunum and upper ileum. The relative bioavailability of levothyroxine tablets, contrasted with an equivalent ostensible dosage of oral levothyroxine sodium arrangement, is roughly 98 percent. [18] Thyroxine assimilation is expanded by fasting, and diminished in malabsorption disorders and by specific sustenance's, for example, soybean newborn child equation. Dietary fiber diminishes bioavailability of thyroxine. Assimilation may likewise diminish with age. Furthermore, numerous medications and nourishments influence thyroxine retention.

Distribution – Circulating thyroid hormones are more noteworthy than 99 percent bound to plasma proteins, including thyroxine restricting globulin [TBG], thyroxiner estricting prealbumin [TBPA], and egg whites [TBA], whose limits and affinities change for every hormone. The higher fondness of both TBPA and TBG for thyroxine in part clarifies the higher serum levels, slower metabolic endorsement, and longer half-existence of thyroxine contrasted with triiodothyronine. [30] Protein-bound thyroid hormones exist backward harmony with little measures of free hormone. Just unbound hormone is metabolically dynamic. Numerous medications and physiologic conditions influence the authoritative of thyroid hormones to serum proteins. Thyroid hormones don't promptly cross the placental hindrance.

Digestion – Thyroxine is gradually disposed of. The real pathway of thyroid hormone digestion is through successive de-iodination. Around 80 percent of coursing triiodothyronine is gotten from fringe thyroxine by mono-deiodination. [34] The liver is the real site of debasement for both triiodothyronine and thyroxine, with thyroxine de-iodination likewise happening at some of extra destinations, including the kidney and different tissues. Around 80 percent of the everyday dosage of thyroxine is de-iodinated to yield approach measures of thyroxine and reverse-thyroxine. Thyroxine and reverse-thyroxine is further de-iodinated to diiodothyronine. Thyroid hormones are additionally used by means of amalgation with glucuronides and sulfates and discharged straightforwardly into the bile and gut where they experience enterohepatic distribution.

Disposal – Thyroid hormones are fundamentally killed by the kidneys. A bit of the conjoin hormone achieves the colon unaltered and is killed in the dung. Roughly 20 percent of thyroxine is dispose of in the footrest.

1.11 Difference between synthetic and natural Levothyroxine

Both the forms natural as well as synthetic Levo are available commercially. Natural is made from desiccated thyroid gland of animal and patient thinks that natural is better. Synthetic lev is identical to our own body levothyroxine. Natural which is derived from animal contains liothronine & levothyroxine or mixture.

Advantages: -

It is low- cost. Levothyroxine is more stable than liothyronine. Synthetic levo is non-allergic. It is also having long half-life of 7 days. Manufacturing process is more consistent.

Thus synthetic levothyroxine is more preferred than that of natural levo. For the treatment of hypothyroidism

1.12 Increase in stability with formulation:

Various attempts were made to produce stabilize formulation.

US Patent 5,225,204 (Chen et. al. 1993) by dissolving povidone and levothyroxine in any polar solvent and adding cellulose carrier and making a stabilize complex. It is also studied that the geometric mixture (dry) and cellulose carrier make a stable complex.

In another way it is also claimed the form stable levothyroxine by formulating with

i) Carbohydrate with molecular weight 500-150000
ii) Use of inorganic salt
iii) Glycine

CHAPTER-2

Literature Review

2. Literature Review

1. **Al-Omari et al. (2001)** studies Microenvironment and pH effect in rug pattern on enalapril tablets stability, here when the pH was basic, the stability improves. Thus this shows that the degradation of enalapril is dependent on pH.

2. **Chen et al. (2000)** study the fluroropyridinyl degradation in the formulation. It was found that in acidic microenvironment drug is unstable. Also lactose anhydrous affect the stability of the drug profile. Thus basic microenvironment is recommended for its formulation.

3. **Badaway et al (1999)** studied acid effects on stability of an ester pro-drug on solid state. It was also observed that maximum stability of drug is at pH 4 in solution. Different acids were used for adjusting microenvironment pH of tablets. Use of different acids reduces the hydrolysis of drug. Thus by the use of strong acids, hydrolysis increases (microenvironment pH lower than max. stability). Hence by regulating microenvironment pH towards max. stability of drug (solution) led to hydrolysis reduction as well as enhancing the stability of drug

4. **Won (1992)** "studied the kinetics of degradation of levothyroxine sodium in aqueous solutions. Levothyroxine sodium solutions were stored for prolonged periods at various temperatures. TLC, EI-MS and NMR were used to identify the degradation products. It was concluded that levothyroxine sodium in solution degraded by deiodination. The degradation was pH dependent and followed first order kinetics.
   It also studied the solid state stability of levothyroxine sodium at elevated temperatures (open vial at 60^{o}C for 7 weeks). The degraded levothyroxine sodium was separated using TLC and then an EI-MS was done on the major degradation product. In solid state, the degradation of levothyroxine sodium indicated a deamination reaction following bi-phasic degradation pattern.

5. **Won et al. (1993)** studied temperature effect i.e. 50-80^{o}C upon Levo. Na degradation. With increase in temperature the degradation rate become faster as also shows biphasic

degradation kinetic. Till 50 ^{o}C little/ no degradation of drug product is observed but above 50 ^{o}C degradation starts.

6. **Wortsman et al. (1989)** studied the thermal inactivation (HPLC and DSC techniques) of levothyroxine sodium from 50–250^{o}C. They observed that the degradation of levothyroxine sodium started at 80^{o}C or higher.

7. **Kazemiford et al. (2001)** studied the photo-degradation of levothyroxine sodium tablets from three manufacturers." The extracted levothyroxine sodium solution was irradiated with a 500W Xenon lamp at 320 nm for 2 hours. Assay was performed using an HPLC method with electrochemical detection. Also LC/MS/MS was done in order to elucidate the structure of the degradation products. The observed degradation products were Levothyroxine, Diiodothyronine, Iodothyronine, Diiodotyrosine, Iodotyrosine and Tyrosine. It was concluded that levothyroxine sodium is photosensitive.

8. **Garnick et al. (1984)** studied LTX sodium tablets (API) degradation under different temperature and RH condition. It concluded that LTX sodium at 80^{o}C is more sensitive. It was also found that in the presence of oxidizing agent degradation of LTX sodium increases.

9. **Gupta et al. (1990)** studied 2 different manufacturer's LTX sodium and showed that one set of tablets had some excipients (not reported) that hasten the decomposition after extraction of levothyroxine sodium in the presence of light. They concluded that the presence of certain excipients affected the stability of levothyroxine sodium tablets.

10. **Won, 1992, Post and Warren, 1976** Very slightly soluble in water (1 in 700), slightly soluble in alcohol (1 in 300), and dissolves in aqueous solutions of alkaline hydroxides (Moffat, 1986). The aqueous solubility of levothyroxine is dependent on the pH of the surrounding media. The solubility reduces from pH 1 to 3, remains constant from 3 – 7, and significantly increases above pH ~7

11. **Won, 1992. Post, 1976** Levothyroxine sodium has three ionizable moieties and it can exist as cation, zwitterion, anion, or di-ion depending on the pH of the solution. For the carboxyl group, pka1 = 2.40; for the phenolic group, pka2 =6.87 and for the amino group, pka3 = 10.1

12. **Rhodes (1998)** reviewed the regulatory aspects of the formulation and evaluation of levothyroxine tablets. He reported that levothyroxine tablets stability is a complex problem and a scientific study of the degradation process would result in the use of appropriate formulation and processing methods, which would effectively remove current problems. The above statement might shed light to some of the factors that have been associated with the current problems associated with commercial levothyroxine sodium tablets.

13. **Chen et. al. 1993)** described a way of producing a stabilized complex of levothyroxine sodium in tablets by dissolving poloxamer or povidone and levothyroxine sodium in a polar solvent and adding a cellulose carrier in the liquid and drying this. It was also claimed that a dry geometric mixture of levothyroxine sodium and a cellulose carrier (microcrystalline cellulose, hydroxyl-methyl propyl-cellulose, etc.) forms a stable complex.

14. **Mitra, (1998)** argued the claims of the previous patent and showed that such a production procedure resulted in a product with worse stability than the commercially available products. It claimed another way to stabilize levothyroxine sodium products was by formulating with:
    A) A carbohydrate with MW between 500 and 1500000.
    B) An inorganic salt
    C) Glycine

CHAPTER-3

AIM & OBJECTIVE

3.1 Rationale

Currently there is no orally administered levothyroxine sodium product that has shown the consistent potency as well as stability. Therefore, no product (LTX sodium) is considered to be safe and effective.

FDA required to file new NDA's by all the manufacturer. Current formulation is approved with expiration date of 18 months

It is found to that levothyroxine is unstable in light, moisture, pH (acidic), temperature and oxidation. Also the type excipient used affect the stability of levo. According to Rhodes, 1998, it is a complex problem of the stability of levo tablets study of degradation is due to appropriate use formulation as well as processing methods which can remove current issues.

Also study of process and formulation variables on the stability of levo sodium tablets, will help in studying the issue which lead to potency issue, and thus help in formulating a stable levothyroxine Na tablets

3.2 Objective, Aim and hypothesis

i. Objective

To study the effect of process and formulation on the stability and potency of LTX Sodium tablet.

ii.Hypothesis

The stability of LTX sodium is affected by various process variable, formulation and environmental factors.

iii.AIM

To study the effect of process variable, effect of different excipient in formulation of LTX sodium tablets as well as environmental factor on stability of LTX Sodium tablet

Formulation variables

a. Excipient type-
   - Lubricants
   - Disintegrants
   - Binder
   - Stabilizer
   - pH adjuster

b. Manufacturing variables
   - Direct Compression
   - Wet Granulations

CHAPTER-4

EXPERIMENTAL WORK

4. Experimental Work

4.1 Materials

NAME	COMPANY
Acetonitrile - HPLC	Fisher Scientific, Fair Lawn NJ
Aluminum lake blue # 2	Colorcon
ATLAS® mannitol	ICI Americas, Inc
Citric acid	Fisher Scientific
Croscarmellose sodium	AcDiSol®, FMC Corporation
Crospovidone	BASF, Ludwigshafen
Dibasic calcium phosphate	Emcompress®, Penwest
D-Thyroxine	ACROS Organics, Fair Lawn NJ
Diiodo L thyronine	Sigma Chemical Co
Diiodothyroacetic acid	Sigma Chemical Co
High-density polyethylene bottles (HDPE)	SETCO Inc., Anaheim
Hydrochloric acid	Fisher Scientific,
Lactose anhydrous	Quest International
Levothyroxine sodium pentahydrate	ACROS Organics, Fair Lawn NJ
Magnesium stearate	Mallinckrodt Chemical Inc
Microcrystalline cellulose (MCC)	Emcocel® 90M, Penwest
Starch® 1500	Colorcon
Phosphoric acid	Fisher Scientific
Potassium chloride	Fisher Scientific
Potassium hydrogen phthalate	Fisher Scientific
Potassium Phosphate monobasic	Fisher Scientific

NAME	COMPANY
Povidone	PVP, BASF, Ludwigshafen
Hydroxypropyl methycellulose	HPMC, Methocel® K100LV, Dow Chemical Co. Midland
Sodium bicarbonate	Fisher Scientific
Sodium carbonate	Fisher Scientific
Sodium hydroxide	Fisher Scientific
Sodium starch glycolate	Explotab®, Penwest
Stearic acid	Mallinckrodt Chemical Inc
Tartaric acid	Fisher Scientific
Tetraiodothyroacetic acid	Sigma Chemical Co.
Triiodothyroacetic acid	Sigma Chemical Co
Water – HPLC	Fisher Scientific

4.2 Equipment

NAME	COMPANY
Accumet 1002 pH meter	Fisher Scientific
Balances PB1502, AB104	Mettler Toledo
Beckman System Gold HPLC system	Beckman Coulter
Carver Laboratory Press	Fred S. Carver Inc
Cyano - spherisorb (5µm, 25cm x 2mm I.D.) HPLC Column	Water Corp., Milford
Computrac Moisture Analyzer MAX 50	Arizona Instrum
Dissolution Tester VK7000	VanKel Technology Group
Espec Humidity Cabinet LHL112	Tabai Espec Corp
Hardness Tester	Key International Inc
Isotemp Incubator 655D	Fisher Scientific
Micromass mass spectrophotometer with electro-spray ionization	Micromass ltd
Moisture sorption balance	VTI Corporation
Planetary Mixer	Kitchen Aid
Rotary tablet press Manesty D3B	Manesty Machines Ltd.
Spectrophotometer DU 640	Beckman Coulter
Starrett Micrometer	Starrett Athol MA, USA
Turbula Mixer T2G	Glen Mills Inc.

4.3 Methodology

Different approaches for the formulation of LTX sodium tablet were performed, i.e.

4.3 (A) Change in Formulation & Manufacturing Process:

- Formulation with SMCC (Silicified Microcrystalline Cellulose)
- Granulation with olive oil in following combination
  - Granulation of whole blend.
  - Granulation of excipient, then add API.
  - Granulation of excipients by API + olive oil. (1%, 3%, 10%)
- Formulation with Carrageenan

4.3 (B) Compatibility of Dye:

- Formulation with MCC + FeO Yellow
- Formulation with MCC + Lake blend Yellow

Packing	HDPE Bottles
Desiccant	One Oxygen Absorber and cotton
Stress study condition	60°C and 40°C/75% RH
Analytical method of Assay	H.P.L.C Assay

4.3.1. Batch -1

AIM- Preparation of levothyroxine tablet with use of Silicified Microcrystalline cellulose (for direct compression).

Table 4.1: levothyroxine tablet with use of Silicified Microcrystalline cellulose

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Silicified Microcrystalline cellulose	69.390
Pregelatinised startch (starch 1500)	55.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
Total (mg)	**130**

PROCEDURE-

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were mixed and sieved through #60 ASTM and collected in separate polybag. Levothyroxine eas passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient are added and mixed uniformly. Blending is done for 40 minutes

Direct compression was done with punch 6.80mm and with a target weight of 130mg. The process was carried under sodium light.

INFERENCE:

Table 4.2: IPQC of Batch 1

PARAMETER	OBSERVATION
Disintegration Time	2.24 minutes
Hardness	6.5Kpa
Weight	130 mg
Hausner Ratio	1.12
Compressibility Index	6.57%

4.3.2. Batch-2

AIM- Preparation of levothyroxine sodium tablet with 10% Olive oil (whole blend granulation).

Table 4.3: levothyroxine tablet with use of Silicified Microcrystalline cellulose

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (avicel-101)	54.390
Pregelatinised startch (starch 1500)	47.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
olive oil	13
neusilin	10
Total	**130**

PROCEDURE: -

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending was done for 10 minutes.

All the remaining excipient were added and mixed uniformly. Blending is done for 40 minutes. Weigh olive oil Olive oil and mixed it with acetone. Granulation is performed in a granulator with olive oil. Wait till acetone gets evaporate. Neusilin is added

Blending was done for 10 minutes in V-cone blender. Compression is done with punch 6.80mm and with a target weight of 130mg.

The process was carried out under sodium light

INFERENCE:

Table 4.4: IPQC of Batch 2

PARAMETER	OBSERVATION
Disintegration Time	2 minutes
Hardness	6.5Kpa
Weight	130 MG
Hausner Ratio	1.14

4.3.3 Batch-3

AIM- Preparation of levothyroxine sodium tablet with 3% Olive oil (whole blend granulation).

Table 4.5: formulation of levothyroxine sodium tablet with 3% Olive oil (whole blend granulation).

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (avicel-101)	55.490
Pregelatinised startch (starch 1500)	55.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
olive oil	3.9
neusilin	10
Total	**130**

PROCEDURE: -

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine was uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient are added and mixed uniformly. Blending was done for 40 minutes. Weigh olive oil Olive oil and mixed it with acetone. Granulation was performed in a granulator with olive oil. Wait till acetone gets evaporate.

Neusilin was added. Blending is done for 10 minutes in V-cone blender. Compression was done with punch 6.80mm and with a target weight of 130mg. The process was carried out under sodium light.

INFERENCE:

Table 4.6 IPQC of Batch 3

PARAMETER	OBSERVATION
Disintegration Time	2 minutes
Hardness	6.3Kpa
Weight	130 MG
Hausner Ratio	1.12

4.3.4 Batch-4

AIM- Preparation of levothyroxine sodium tablet with 1% Olive oil (whole blend granulation).

Table 4.7: formulation of levothyroxine sodium tablet with 3% Olive oil (whole blend granulation).

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (avicel-101)	58.09
Pregelatinised startch (starch 1500)	55.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
olive oil	1.3
neusilin	10
Total	**130**

PROCEDURE: -

1. Excipient and API were weighed accurately and collected in separate polybag. All the excipients were sieved through #60 ASTM and collected in separate polybag. Levothyroxine is passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient are added and mixed uniformly. Blending is done for 40 minutes. Weigh olive oil Olive oil and mixed it with acetone.Granulation is performed in a granulator with olive oil. Wait till acetone gets evaporate. Neusilin was added. Blending

was done for 10 minutes in V-cone blender. Compression is done with punch 6.80mm and with a target weight of 130mg. The process was carried out under sodium light.

INFERENCE:

Table 4.8 IPQC of Batch 4

PARAMETER	OBSERVATION
Disintegration Time	1.50 minutes
Hardness	6.3Kpa
Weight	130 MG
Hausner Ratio	1.12

4.3.5 Batch-5

AIM- Preparation of levothyroxine sodium tablet with 1% Olive oil (whole blend granulation)

Table 4.9: Formulation of levothyroxine sodium tablet with 1% Olive oil (whole blend granulation).

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (avicel-101)	55.490
Pregelatinised startch (starch 1500)	55.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
olive oil	3.9
neusilin	10
Total	**130**

PROCEDURE: -

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient are added and mixed uniformly. Blending was done for 40 minutes. Weigh olive oil Olive oil and mixed it with acetone.

Granulation was performed in a granulator with olive oil. Wait till acetone gets evaporate. Neusilin is added. Blending is done for 10 minutes in V-cone blender. Compression is done with punch 6.80mm and with a target weight of 130mg. The process was carried out under sodium light.

INFERENCE:

Table 4.10: IPQC of batch 5

PARAMETER	OBSERVATION
Disintegration Time	1.40 minutes
Hardness	6.7 Kpa
Weight	130 MG
Hausner Ratio	1.12

4.3.6 Batch-6

AIM- Preparation of levothyroxine sodium tablet with 1% Olive oil (excipient granulation).

Table 4.11 Formulation of levothyroxine sodium tablet with 1% Olive oil (excipient granulation).

INGREDIENTS	***QUANTITY (MG/TABLET)***
Levothyroxine	0.100
Microcrystalline cellulose (avicel-101)	55.490
Pregelatinised startch (starch 1500)	55.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
olive oil	3.9
neusilin	10
Total	**130**

PROCEDURE: -

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were sieved through #60 ASTM and collected in separate polybag. Levothyroxine is passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending was done for 10 minutes. All the remaining excipient are added and mixed uniformly. Blending was done for 40 minutes. Weigh olive oil Olive oil and mixed it with acetone.

Granulation was performed in a granulator with olive oil. Wait till acetone gets evaporate. Neusilin is added. Blending is done for 10 minutes in V-cone blender. Compression is done

with punch 6.80mm and with a target weight of 130mg. The process was carried out under sodium light.

INFERENCE:

Table 4.12: IPQC of Batch 6

PARAMETER	OBSERVATION
Disintegration Time	1.35 minutes
Hardness	6.3Kpa
Weight	130 MG
Hausner Ratio	1.14

4.3.7 Batch-7

AIM- Preparation of levothyroxine sodium tablet with MCC (Avicel-101) and lake blend yellow dye.

Figure 4.13: Formulation of levothyroxine sodium tablet with MCC (Avicel-101) and lake blend yellow dye.

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (AVICEL-101)	69.390
Pregelatinised startch (starch 1500)	55.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
Total	**130**

PROCEDURE-

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were mixed and sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine was uniformly mixed with Pregelatinised starch (starch1500). Blending was done for 10 minutes. All the remaining excipient were added and mixed uniformly. Blending was done for 40 minutes Direct compression is done with punch 6.80mm and with a target weight of 130 mg. The process was carried under sodium light.

INFERENCE:

Table 4.14: IPQC of Batch 7

PARAMETER	OBSERVATION
Disintegration Time	10 seconds
Hardness	6.3Kpa
Weight	130 MG
Hausner Ratio	1.07

4.3.8 Batch-8

AIM- Preparation of levothyroxine sodium tablet with MCC (Avicel-101) and FeO yellow.

Table 4.15: Formulation of LTX sodium tablet with MCC (Avicel-101) and FeO yellow.

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (AVICEL-101)	69.390
Pregelatinised startch (starch 1500)	55.900
LHPC (LH-21)	3.900
Iron Oxide (FeO) yellow	0.260
Magnesium Sterate	0.450
Total	**130**

PROCEDURE-

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were mixed and sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes All the remaining excipient are added and mixed uniformly. Blending is done for 40 minutes. Direct compression is done with punch 6.80mm and with a target weight of 130mg.
The process was carried under sodium light.

INFERENCE:

Table 4.16: IPQC of Batch 8

PARAMETER	OBSERVATION
Disintegration Time	12 seconds
Hardness	6.8Kpa
Weight	130 MG
Hausner Ratio	1.08

4.3.9 Batch-9

AIM- Preparation of levothyroxine sodium tablet with MCC (Avicel-101) and Carrageenan (PH-812) as a stabilizer.

Table 4.17: Formulation of LTX with Carrageenan (PH-812)

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (AVICEL-101)	69.390
Pregelatinised startch (starch 1500)	50.900
Carrageenan (PH-812)	10
LHPC (LH-21)	3.900
Lake blend Lb-5200006 yellow	0.260
Magnesium Sterate	0.450
Total	**130**

PROCEDURE-

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were mixed and sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient are added and mixed uniformly. Blending is done for 40 minutes

Direct compression was done with punch 6.80 mm and with a target weight of 130mg. The process was carried under sodium light.

INFERENCE:

Table 4.18: IPQC of Batch 9

PARAMETER	OBSERVATION
Disintegration Time	9.10 minutes
Hardness	7 Kpa
Weight	130 MG
Hausner Ratio	1.10

4.3.10 Batch-10

AIM- Preparation of levothyroxine sodium tablet with MCC (Avicel-101) and Carrageenan (PH-911) as a stabilizer.

Table 4.19: Formulation of LTX with Carrageenan (PH911)

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (AVICEL-101)	69.390
Pregelatinised startch (starch 1500)	50.900
Carrageenan (PH-911)	10
LHPC (LH-21)	3.900
Lake blend Lb-5200006 yellow	0.260
Magnesium Sterate	0.450
Total	**130**

PROCEDURE-

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were mixed and sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient are added and mixed uniformly. Blending is done for 40 minutes.
Direct compression done with punch 6.80 mm and with a target weight of 130mg. The process was carried under sodium light.

INFERENCE:

Table 4.20: IPQC of Batch 10

PARAMETER	OBSERVATION
Disintegration Time	10.15 minutes
Hardness	7.1 Kpa
Weight	130 MG
Hausner Ratio	1.09

4.3.11 Batch-11

AIM- Preparation of levothyroxine with 1% olive oil as antioxidant (drug to be dissolved in acetone)

Table 4.21: Formulation of LTX with 1% olive oil (drug dissolved)

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (avicel-101)	58.09
Pregelatinised startch (starch 1500)	55.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
olive oil	1.3
neusilin	10
Total	**130**

PROCEDURE-

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient are added and mixed uniformly. Blending is done for 40 minutes.
Weigh olive oil Olive oil and mixed it with acetone. Granulation was performed in a granulator with olive oil. Wait till acetone gets evaporate. Neusilin is added. Blending is done for 10 minutes in V-cone blender. Compression was done with punch 6.80mm and with a target weight of 130mg. The process was carried out under sodium light.

INFERENCE:

Table 4.22: IPQC of Batch 11

PARAMETER	OBSERVATION
Disintegration Time	40 seconds
Hardness	6.3Kpa
Weight	130 MG
Hausner Ratio	1.11

4.3.12 Batch-12

AIM- Preparation of Levothyroxine Sodium Tablet with Silicified Microcrystalline cellulose and niacin (as an antioxidant).

Table 4.23: formulation of LTX SMCC and Niacin

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Niacin	10
Microcrystalline cellulose (avicel-101)	64.390
Pregelatinised startch (starch 1500)	50.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
Total	**130**

PROCEDURE:

Excipient and API were weighed accurately and collected in separate polybag. All the excipients are sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes.

All the remaining excipient are added and mixed uniformly. Blending is done for 40 minutes. Compression was done with punch 6.80mm and with a target weight of 130mg. The whole process was carried out under sodium light.

INFERENCE:

Table 4.24: IPQC of Batch 12

PARAMETER	OBSERVATION
Disintegration Time	50 seconds
Hardness	6.5 Kpa
Weight	130 MG
Hausner Ratio	1.10

4.3.13 Batch-13

AIM- Preparation of Levothyroxine Sodium Tablet with Microcrystalline cellulose, niacin and 3% olive oil (Excipient Granulation).

Table 4.25: Formulation of LTX with MCC and 3% olive oil (excipient Granulation)

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Microcrystalline cellulose (avicel-101)	58.490
Pregelatinised startch (starch 1500)	46.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
Niacin	10
olive oil	3.9
neusilin	6
Total	**130**

PROCEDURE:

Excipient and API were weighed accurately and collected in separate polybag. All the excipients are sieved through #60 ASTM and collected in separate polybag. Levothyroxine is passed and sieved through #60ASTM. All the remaining excipient (except olive oil) are added and mixed uniformly. Blending was done for 40 minutes. Olive oil is mixed with acetone and stirred properly. Granulation of excipient was done with olive oil and acetone mixture in RMG.

Neusilin is added to the granulated part. Blending was done for 5 minutes Levothyroxine is then added uniformly. Blending was done for 20 min. Compression is done with punch 6.80mm and with a target weight of 130mg. The whole process is carried out under sodium light.

INFERENCE:

Table 4.26: IPQC of Batch 13

PARAMETER	OBSERVATION
Disintegration Time	1.30 minutes
Hardness	6 Kpa
Weight	130 MG
Hausner Ratio	1.14

4.3.14 Batch-14

AIM- Preparation of Levothyroxine Sodium Tablet with Microcrystalline cellulose, niacin and 1% olive oil (Excipient Granulation).

Table 4.27: Formulation of LTX with niacin and 1% olive oil (excipient granulation)

INGREDIENTS	***QUANTITY (MG/TABLET)***
Levothyroxine	0.100
Microcrystalline cellulose (avicel-101)	58.490
Pregelatinised startch (starch 1500)	49.500
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
Niacin	10
olive oil	1.3
neusilin	6
Total	**130**

PROCEDURE:

Excipient and API were weighed accurately and collected in separate polybag. All the excipients are sieved through #60 ASTM and collected in separate polybag. Levothyroxine is passed and sieved through #60ASTM. All the remaining excipient (except olive oil) are added and mixed uniformly. Blending is done for 40 minutes. Olive oil is mixed with acetone and stirred properly. Granulation of excipient is done with olive oil and acetone mixture in RMG. Neusilin is added to the granulated part. Blending is done for 5 minutes

Levothyroxine is then added uniformly. Blending was done for 20 min. Compression is done with punch 6.80mm and with a target weight of 130mg. The whole process was carried out under sodium light.

INFERENCE:

Table 4.28: IPQC of Batch 14

PARAMETER	OBSERVATION
Disintegration Time	1.42 seconds
Hardness	6 Kpa
Weight	130 MG
Hausner Ratio	1.15

4.3.15 Batch-15

AIM- Preparation of Levothyroxine Sodium Tablet with Lake blend yellow and niacin (as an antioxidant).

Table 4.29: Formulation of LTX with Lake blend yellow and Niacin

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Niacin	10
Microcrystalline cellulose (avicel-101)	64.390
Pregelatinised startch (starch 1500)	50.900
LHPC (LH-21)	3.900
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
Total	**130**

PROCEDURE:

Excipient and API were weighed accurately and collected in separate polybag. All the excipients are sieved through #60 ASTM and collected in separate polybag.. Levothyroxine is passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient were added and mixed uniformly. Blending was done for 40 minutes. Compression was done with punch 6.80mm and with a target weight of 130mg.

The whole process was carried out under sodium light.

INFERENCE:

Table 4.30 IPQC of Batch 15

Parameter	Observation
Disintegration Time	10 seconds
Hardness	6.5Kpa
Weight	130 MG
Hausner Ratio	1.10

4.3.16 Batch-16

AIM- Preparation of Levothyroxine Sodium Tablet with Iron Oxide (FeO) yellow and niacin (as an antioxidant).

Table 4.31 Formulation of LTX with FeO & niacin

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Niacin	10
Microcrystalline cellulose (avicel-101)	64.390
Pregelatinised startch (starch 1500)	50.900
LHPC (LH-21)	3.900
Iron Oxide (FeO) yellow	0.260
Magnesium Sterate	0.450
Total	**130**

PROCEDURE:

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were sieved through #60 ASTM and collected in separate polybag. Levothyroxine is passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending was done for 10 minutes. All the remaining excipient are added and mixed uniformly. Blending was done for 40 minutes. Compression was done with punch 6.80mm and with a target weight of 130mg. The whole process was carried out under sodium light.

INFERENCE:

Table 4.32: IPQC of batch 16

PARAMETER	OBSERVATION
Disintegration Time	12 seconds
Hardness	6.7Kpa
Weight	130 MG
Hausner Ratio	1.09

4.3.17 Batch-17

AIM- Preparation of Levothyroxine Sodium Tablet with niacin (as an antioxidant), Lake blend yellow and Carrageenan Ph812 (stabilizer).

Table 4.33: Formulation of LTX with Niacin and carrageenan (pH 812)

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Niacin	10
Microcrystalline cellulose (avicel-101)	58.390
Pregelatinised startch (starch 1500)	50.900
LHPC (LH-21)	3.900
Carrageenan pH-812	6
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
Total	**130**

PROCEDURE:

Excipient and API were weighed accurately and collected in separate polybag. All the excipients were sieved through #60 ASTM and collected in separate polybag. Levothyroxine was passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient was added and mixed uniformly. Carrageenan is added and mixed uniformly. Blending was done for 40 minutes. Blending is done in V-Cone blender
Compression was done with punch 6.80mm in Compression Machine. Target weight of tablet is 130mg. The whole process was carried out under sodium light.

INFERENCE:

Table 4.34: IPQC of Batch 17

PARAMETER	OBSERVATION
Disintegration Time	10 minutes
Hardness	7.1Kpa
Weight	130 MG
Hausner Ratio	1.08

4.3.18 Batch-18

AIM- Preparation of Levothyroxine Sodium Tablet with niacin (as an antioxidant), Lake blend yellow and Carrageenan Ph812 (stabilizer).

Table 4.35: Formulation of LTX with lake blend yellow and niacin

INGREDIENTS	***QUANTITY (mg/tablet)***
Levothyroxine	0.100
Niacin	10
Microcrystalline cellulose (avicel-101)	58.390
Pregelatinised startch (starch 1500)	50.900
LHPC (LH-21)	3.900
Carrageenan pH-911	6
Lake Blend LB-520006 yellow	0.260
Magnesium Sterate	0.450
Total	**130**

PROCEDURE:

Excipient and API was weighed accurately and collected in separate polybag. All the excipients were sieved through #60 ASTM and collected in separate polybag. Levothyroxine is passed and sieved through #60ASTM. Levothyroxine is uniformly mixed with Pregelatinised starch (starch1500). Blending is done for 10 minutes. All the remaining excipient are added and mixed uniformly. Carrageenan was added and mixed uniformly. Blending was done for 40 minutes. Blending is done in V-Cone blender. Compression was done with punch 6.80mm in Compression Machine. Target weight of tablet is 130mg.

The whole process was carried out under sodium light.

INFERENCE:

Table 4.36: IPQC of Batch 18

PARAMETER	OBSERVATION
Disintegration Time	10.12 minutes
Hardness	6.9 Kpa
Weight	130 MG
Hausner Ratio	1.12

4.4. Tablet Evaluation

4.4.1 Physical Properties

Tablet wt. variation- 10 tablets from every batch are weighed and Avg. weight, Relative S.D (standard Deviation) and S.D (Standard Deviation) reported

Thickness – It is determined for ten tablets which were done pre weighed for every batch using Micro-meter and also S.D, Relative S.D as well as Avg. Thickness is reported

Hardness – Estimated for 10 Tablets having wt. and known thickness of every batch, S.D, Relative S.D as well as Avg. Thickness is reported

4.4.2. Content uniformity

This is performed acc. to USP, "uniformity of Dosage form units", using assay method which is described below. The batch will be according to USP criteria if the amount of API in each of the ten tablets lie under the specification range of 95% to 105% of label claim as well as S.D is less than 6%.

If any of these condition is not met, then additional twenty tablets are tested. NMT 1 of 30 tablets should be outside the range of 95%-105% of the label claim. And relative S.D should not be more than 7.8%. Thus content-uniformity of all the batches are tested according to these specifications by the Assay procedure given below.

4.4.3. Assay

An HPLC system equipped with an auto sampler and a UV detector set at 225 nm was used for the analysis of all samples (Garnick et al, 1984). The reversed phase HPLC assay method used a Waters® Spherisorb Cyano, 25 cm x 2 mm column (particle size 5 µm) and an acetonitrile: water mixture (40 : 60) with 0.5 ml/l phosphoric acid as mobile phase at a flow rate of 0.3 ml/min.

For the analysis of samples of tablets manufactured with pH modifiers, the phosphoric acid in the mobile phase was replaced with trifluoroacetic acid (0.3 ml/l) as this enhanced the recovery of levothyroxine sodium. Spiked levothyroxine sodium samples with various degradation products, namely, triiodo L thyronine, diiodo L thyronine, tetraiodothyroacetic acid, triiodothyroacetic acid and diiodothyroacetic acid were injected to test the methods" (Garnick et al, 1984).

4.4.4. Moisture Determination

Moisture content in samples (approximately 100 mg powdered sample) was determined by Karl Fisher titration with Hydranal® Composite 2, according to the USP titrimetric method for water determination. Three determinations were performed for the water titer value and relative standard deviation.

4.4.5. Stability

The tablets were evaluated for stability using ICH accelerated stability conditions. The tablets were packed in a HDPE bottle with / without desiccant and stored at 40^{o}C / 75% relative humidity for 6 months. A stability-indicating assay was performed at 0, 3, 6 months.

The tablets were tested for:

- Percentage loss in potency (Assay value of levothyroxine)
- Percentage moisture content
- identification of possible tablet degradation products.

4.4.6. Dissolution

Levothyroxine Sodium dissolution is performed by USP apparatus 2 (paddle): 50RPM; 1000ml 0.1 N HCl.

Withdrawing of sample at 5, 10, 15, 30, 45, 60, 120, 180, 240 minutes.

100 µl of above withdrawn sample are then assayed for Levo Na by HPLC assay method

Chapter – 5

RESULT AND INFERENCE

5. RESULT AND INFERENCE

5.1. Stability of Levothyroxine Sodium

Drug Substance

Levo Na is found to be stable when it is stored for six months (Accelerated Stability) i.e. 40ºC / 75% RH in open container as well as closed container with Assay potency of 95% to 105%

Solution

The pH of the surrounding medium affected the stability of levothyroxine sodium confirming results by Won (1992). [20] As the pH of the surrounding aqueous medium was increased, the stability of levothyroxine in solution improved or less degradation was observed over time. This was further confirmed by testing the stability of levothyroxine sodium at pH 2 and 10, for 10 days at room temperature with sampling at different time intervals in which case similar phenomena were observed.

In the presence of H_2O_2, more oxidation was observed at pH 10 (complete loss of drug in 1 hour) than at pH 2 (6.7% degradation in 24 hours). Thus, it was concluded that levothyroxine sodium was more sensitive to degradation by oxidation at basic pH.

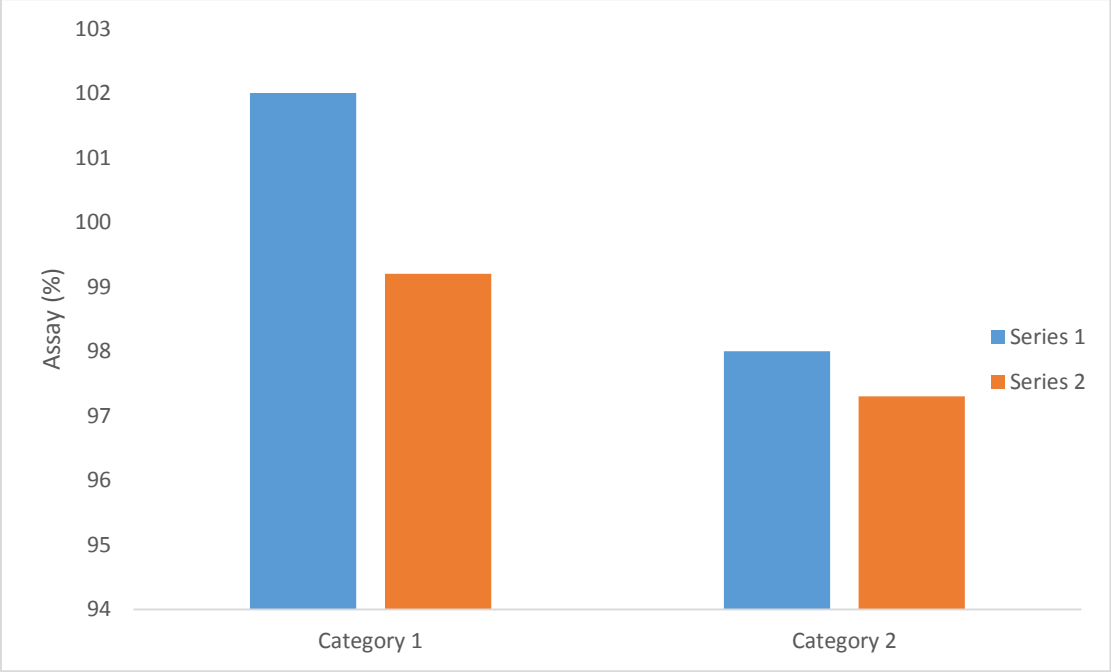


Figure 5.1: Stability of levothyroxine sodium drug substance at 40ºC/75 % RH in closed and open containers for six months

5.2. Assay Report

5.2.1. Assay Result of Batch 1

Table 5.1

	Packing	***Stability Condition***	***Assay***
Levothyroxine + SMCC (Silicified Microcrystalline Cellulose)	HDPE+ 1 Oxygen absorber	Initial	78.3
		60°/ 15 Days Closed	75.4
		60°/ 30 Days Closed	69.3
		40°/75% 1 Month Closed	81.0

5.2.2. Assay Result of Batch 2

Table 5.2

	Packing	***Stability Condition***	***Assay***
Levothyroxine+ olive oil 10% (granulation of whole blend)	HDPE+ 1 Oxygen absorber	Initial	66.7
		60°/ 15 Days Closed	54.6
		60°/ 30 Days Closed	38.1
		40°/75% 1 Month Closed	67.5

5.2.3. Assay Result of Batch 3

Table 5.3

	Packing	***Stability Condition***	***Assay***
Levothyroxine+ olive oil 3% (granulation of whole blend)	HDPE+ 1 Oxygen absorber	Initial	80.1
		60°/ 15 Days Closed	71.6
		60°/ 30 Days Closed	65
		40°/75% 1 Month Closed	83.3

5.2.4. Assay Result of Batch 4

Table 5.4

	Packing	***Stability Condition***	***Assay***
Levothyroxine+ olive oil 1% (granulation of whole blend)	HDPE+ 1 Oxygen absorber	Initial	84
		60°/ 15 Days Closed	80.1
		60°/ 30 Days Closed	74.1
		40°/75% 1 Month Closed	85.3

Figure 5.2. Assay of Batch 1, Batch 2, Batch 3, Batch 4 at 60ºC

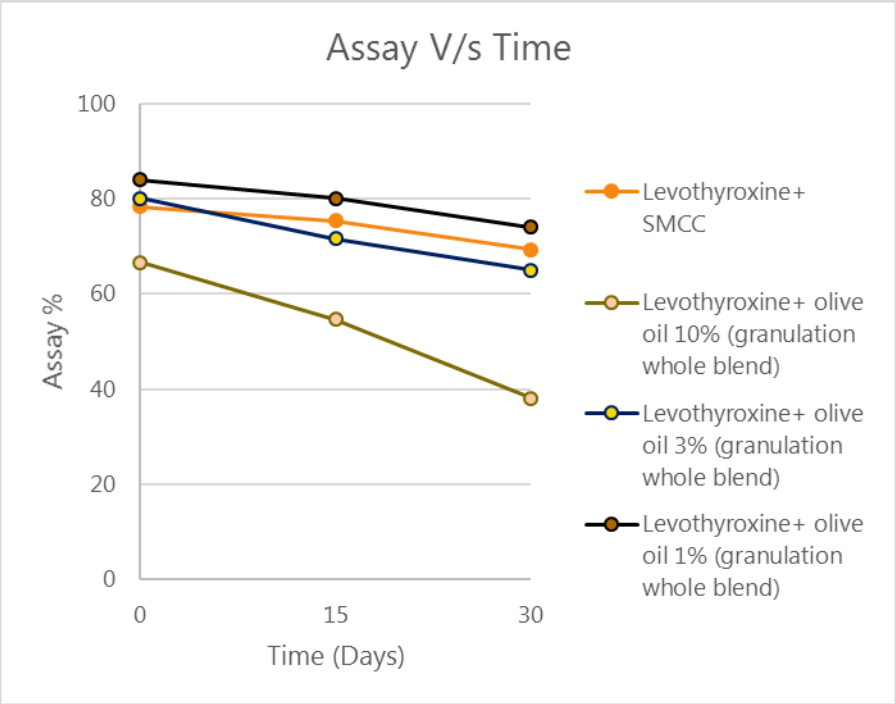
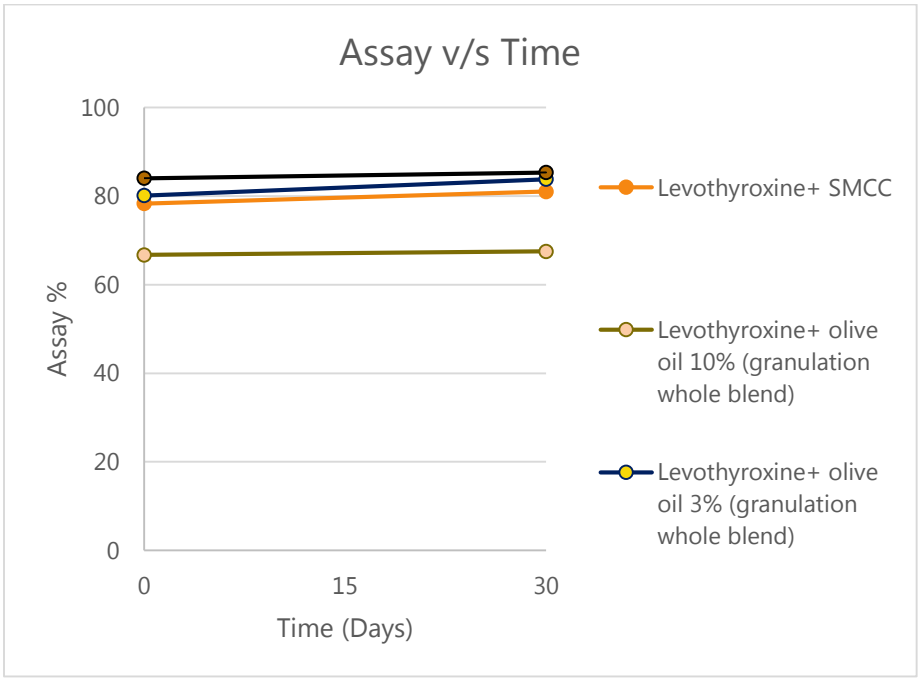


Figure 5.3. Assay of Batch 1, Batch 2, Batch 3, Batch 4 at 40ºC/75%RH



INFERENCE:

1. There is not much assay decrease in Silicified microcrystalline cellulose (SMCC) batch on initial and 40°/75% RH stability condition, while in 60°/ 30 Days there is 10% assay drop.
2. With 10% olive oil there is decrease in assay after keeping the tablet HDPE bottles in 60° for 15 and 30 days.
3. It is Concluded that with increase percentage of olive oil in a formulation, the Assay of the batch decreases especially in 60° for 30 Days.

5.2.5. Assay Result of Batch 5

Table 5.5

	Packing	***Stability Condition***	***Assay***
Levothyroxine+ olive oil 1% (granulation of Excipient blend)	HDPE+ 1 Oxygen absorber	Initial	102.3
		60°/7 days open	100.1
		60°/7 days Closed	103.5
		60°/ 15 Days open	70.2
		60°/15 days Closed	100.5
		60°/ 30 Days open	93.8
		60°/ 30 Days Closed	94.5
		40°/75% 7 Days open	104.8
		40°/75% 7 Days Closed	104.9
		40°/75% 15 Days open	104.0
		40°/75% 15 Days Closed	103.9
		40°/75% 1 Month Open	102.6
		40°/75% 1 Month Closed	103.7

5.2.6. Assay Result of Batch 6

Table 5.6

	Packing	***Stability Condition***	***Assay***
Levothyroxine+ olive oil 1% (granulation of whole blend)	HDPE+ 1 Oxygen absorber	Initial	93.9
		60°/ 15 Days Closed	72.4
		60°/ 30 Days Closed	82.4
		40°/75% 15 Days Closed	98.3
		40°/75% 1 Month Closed	97.9

Figure 5.4: Assay of batch 6 at 60º closed

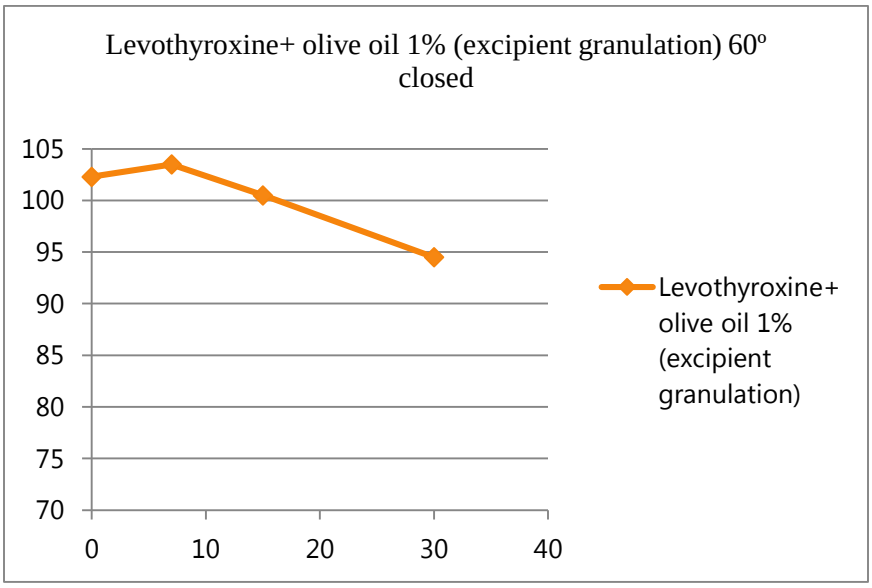


Figure 5.5: Assay of batch 6 at 40º/75% RH open

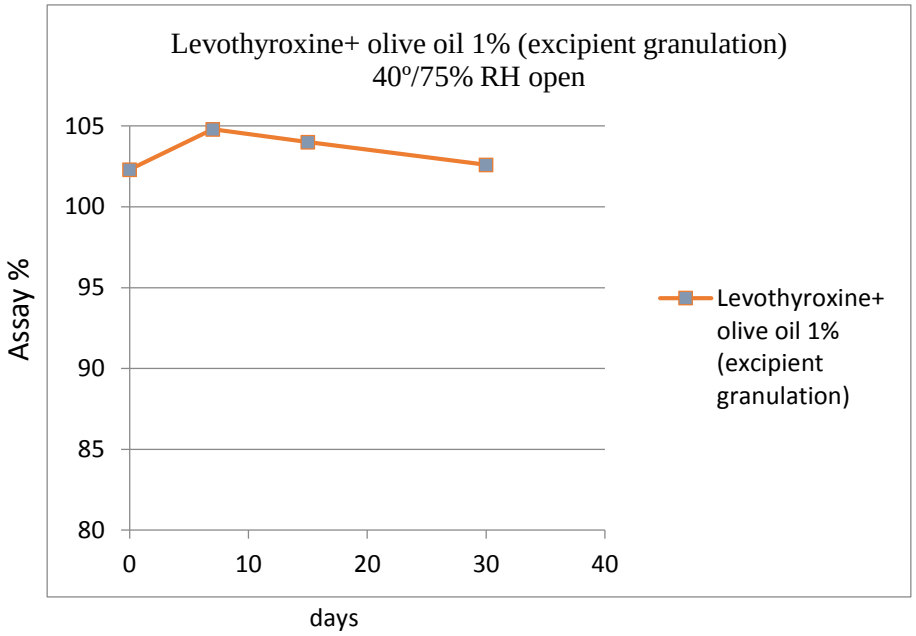


Figure 5.6: Levothyroxine & olive oil 1% (excipient granulation) 40º/75% RH open of batch 6

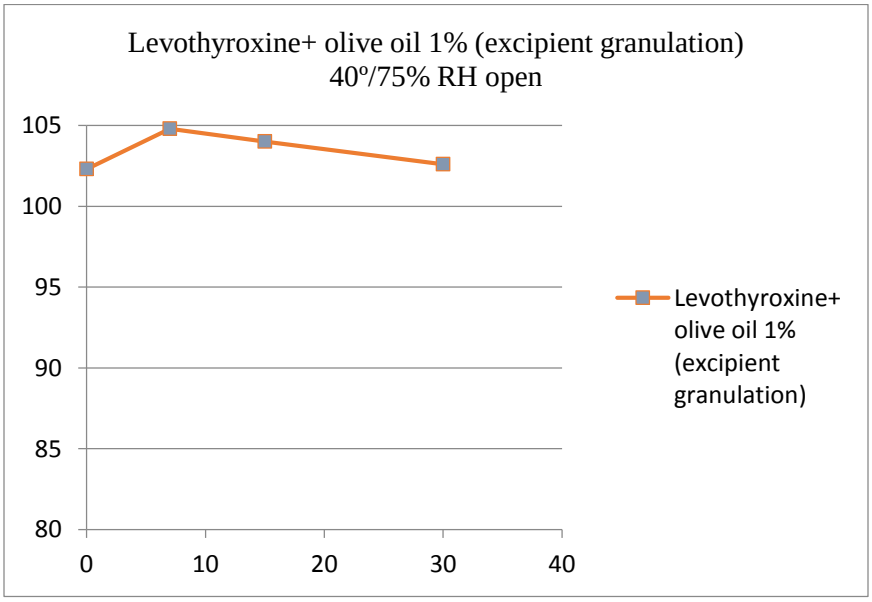


Figure 5.7: Assay of Batch 6 at 40º/75%RH closed

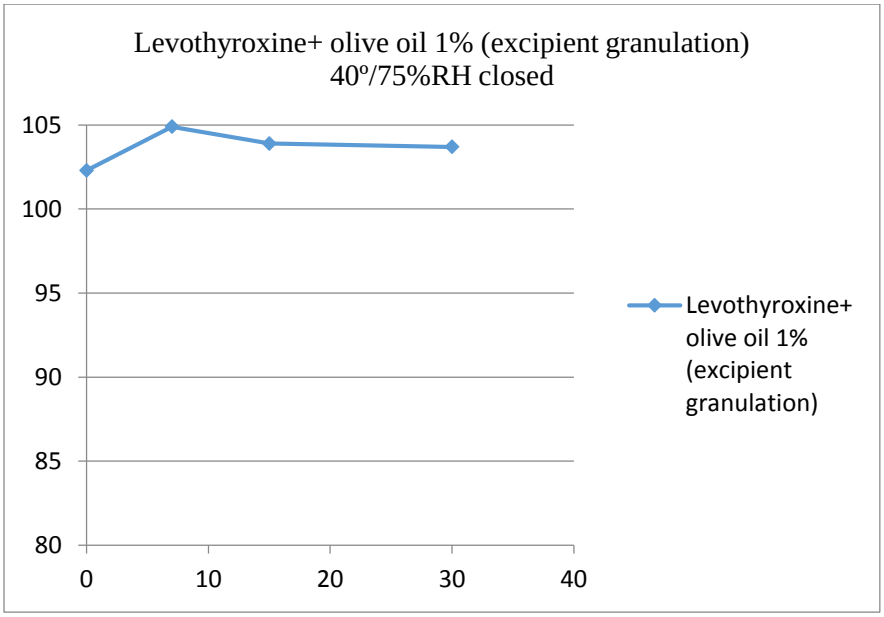
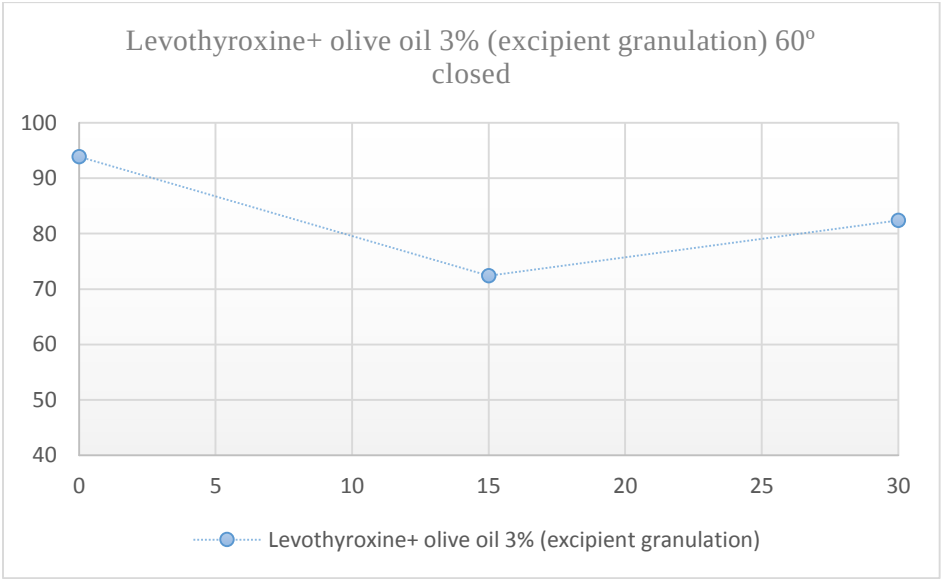


Figure 5.8: Assay of batch 6 at 60º closed



INFERENCE:

- In 3% olive oil (granulation of Excipient) batch, it is found to be stable in 40°/75% Rh Condition
- While studies at 60°C condition, it is observed that there is 10% to 20% assay decrease in 15 and 30 Days.
- In 1% olive oil, it is observed to be the best batch,
- It is more stable and potency of the batch is within the specification range of 95% to 105%
- Its potency/ assay is maintained in all the condition
- Major decrease in assay of above 1% Excipient batch granulation is observed in 60° for 15 days in which the HDPE Bottles are kept open
- Thus the Assay Percentage of 1% olive oil excipient granulation batch is within the specification range of 95% - 105%

5.2.7. Assay Result of Batch 7

Table 5.7

	Packing	***Stability Condition***	***Assay***
Levothyroxine+ MCC (AVICEL-102) + Lake Blend yellow	HDPE+ 1 Oxygen absorber	Initial	97
		60°/ 15 Days open	91.3
		40°/75% 15 Days open	96.1
		40°/75% 1 Month open	95.6
		40°/75% 1 Month Closed	96.7

Figure 5.9: Assay of batch 7 at 40º/75%RH Open

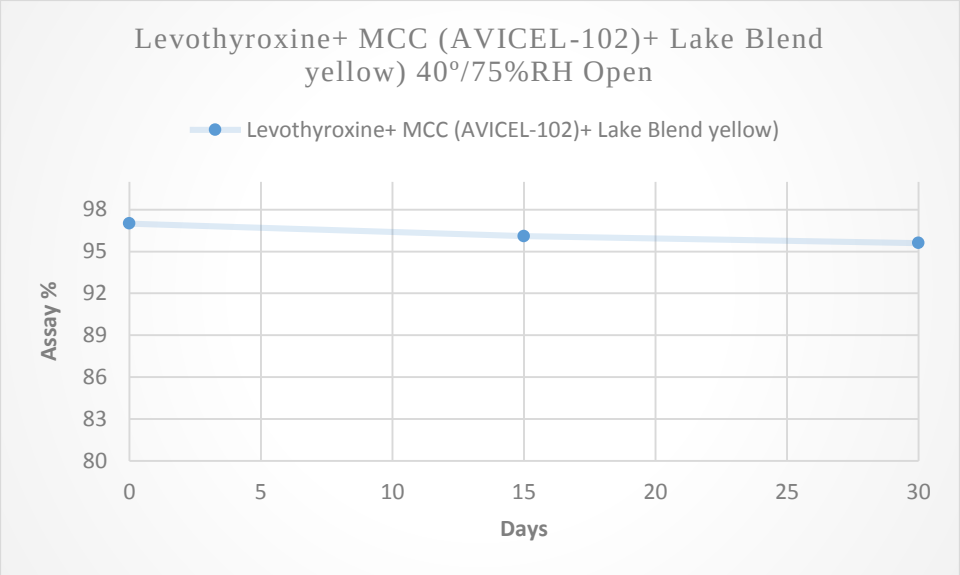


Figure 5.10: Levothyroxine, MCC (AVICEL-102) & FeO Yellow) 60º open of batch 7

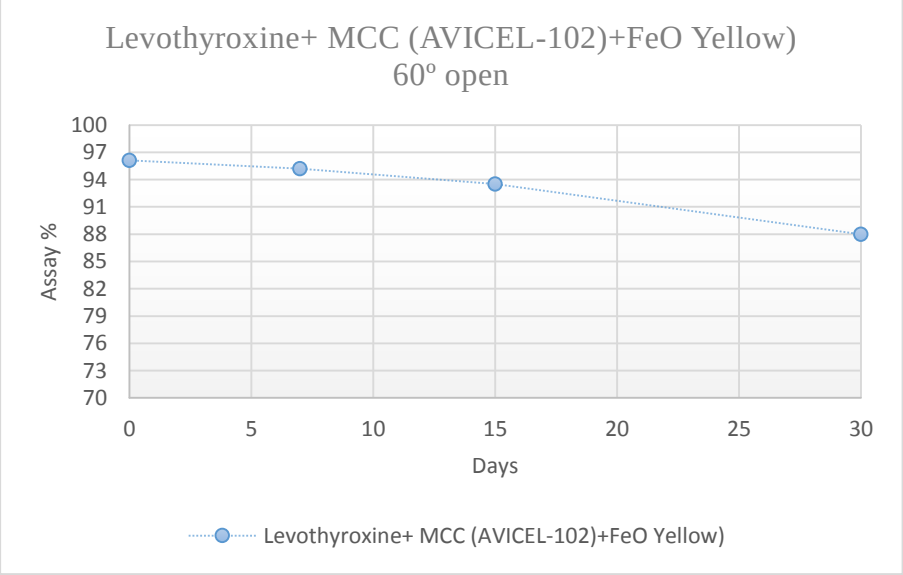
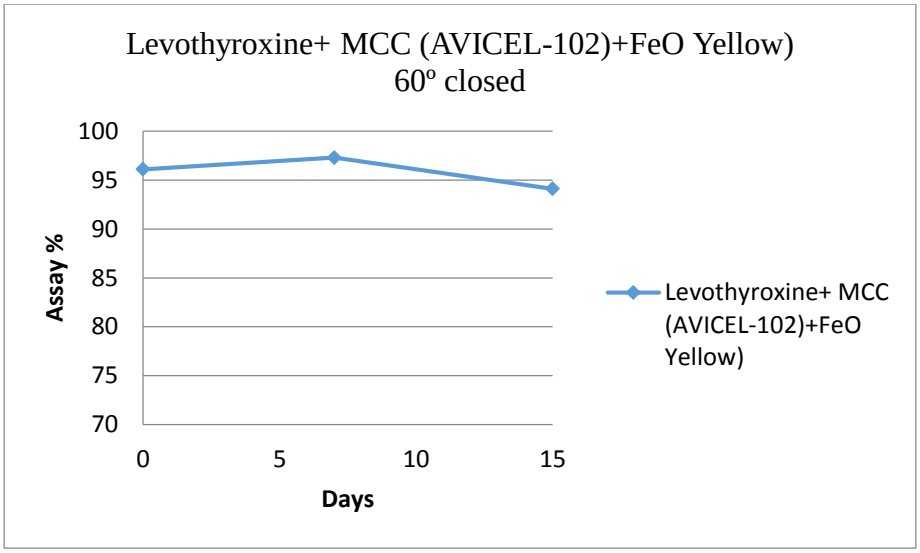


Figure 5.11: Assay of batch 7 at 60º closed



5.2.8. Assay Result of Batch 8:

Table 5.8

	Packing	***Stability Condition***	***Assay***
Levothyroxine+ MCC (AVICEL-102) + FeO Yellow)	HDPE+ 1 Oxygen absorber	Initial	96.1
		60°/7 days open	95.2
		60°/7 days Closed	97.3
		60°/ 15 Days open	93.5
		60°/15 days Closed	94.1
		60°/ 30 Days open	88
		40°/75% 7 Days open	98.1
		40°/75% 7 Days Closed	98.8
		40°/75% 15 Days open	96.3
		40°/75% 1 Month Closed	96.9

Figure 5.12: Assay of Batch 8 at 40º/75% RH Open

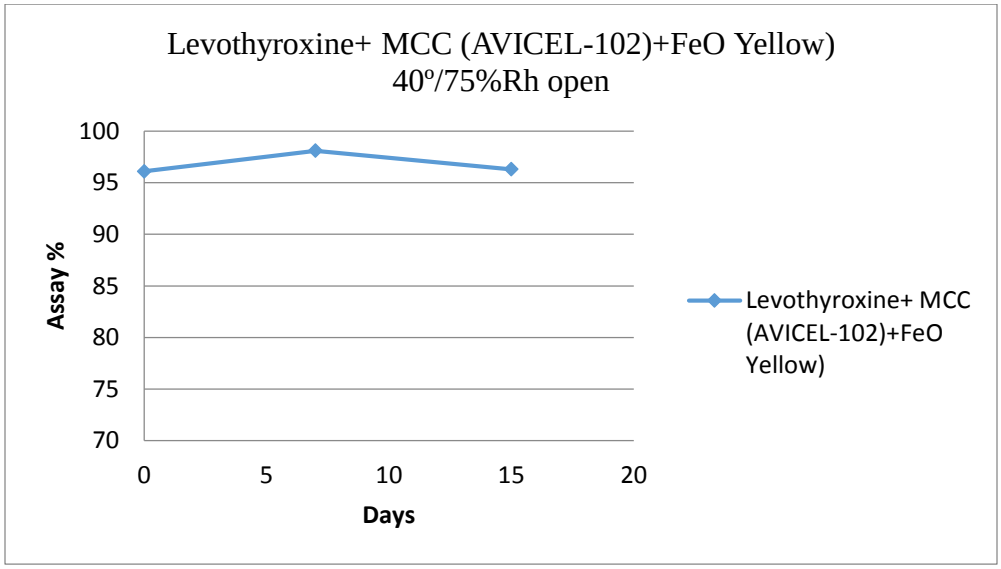
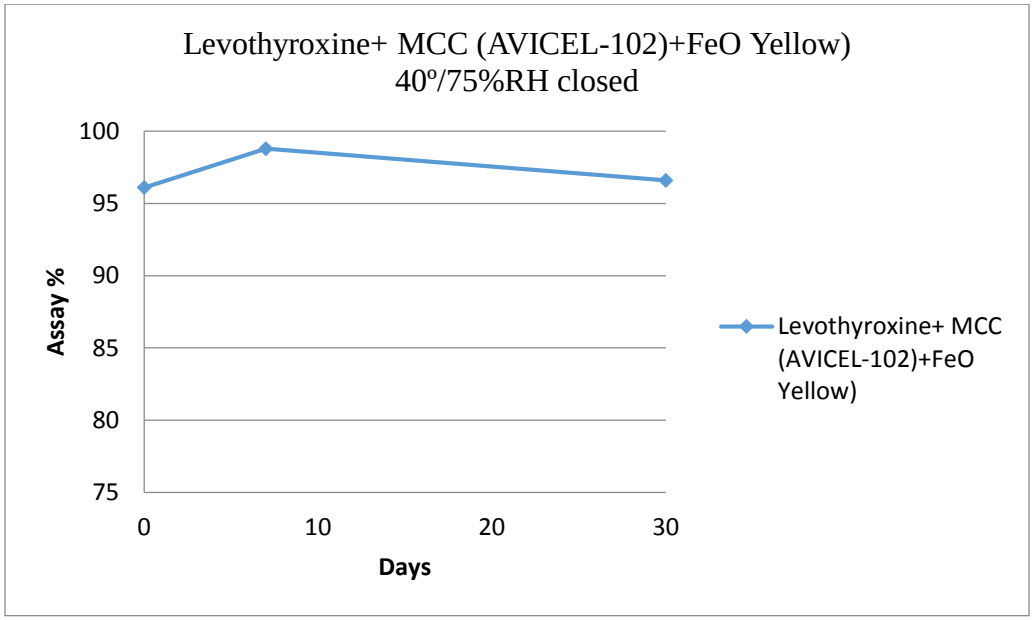


Figure 5.13: Assay of Batch 8 at 40º/75% RH Open



INFERENCE:

Formulation with Lake Blend Yellow-

- When batch is kept under 60°C for 15 days in open condition, assay came out from the specification range
- While, it is found to be stable when it is kept at 40°C/75% RH.

Formulation with FeO yellow-

- The only decrease in assay which is observed is when the HDPE bottle are kept at 60°C for 30 Days. Potency decreases.
- At 60°C in closed condition as well as at 40°C/75% RH (open /close) it is under the specification range and not much fall in assay is seen with the initial one

5.2.9. Assay Result of Batch 9:

Table 5.9

	Packing	***Stability Condition***	***Assay***
Levothyroxine (Carrageenan PH 812 +Lake Blend Yellow)	HDPE+ 1 Oxygen absorber	Initial	98.4
		60°/ 7 Days open	96.7
		60°/ 7 Days Closed	94.2
		60°/ 15 Days Open	98.5
		40°/75% 7 Days Open	98.8
		40°/75% 15 Days Open	97.3

5.2.10. Assay Result of Batch 10:

Table 5.10

	Packing	***Stability Condition***	***Assay***
Levothyroxine (Carrageenan PH 812 +Lake Blend Yellow)	HDPE+ 1 Oxygen absorber	Initial	97.7
		60°/ 7 Days open	91.4
		60°/ 7 Days Closed	93.4
		60°/ 15 Days Open	97.1
		40°/75% 7 Days Open	96.5
		40°/75% 15 Days Open	96.6

Figure 5.14: Assay of batch 9 and batch 10 at 60º Open

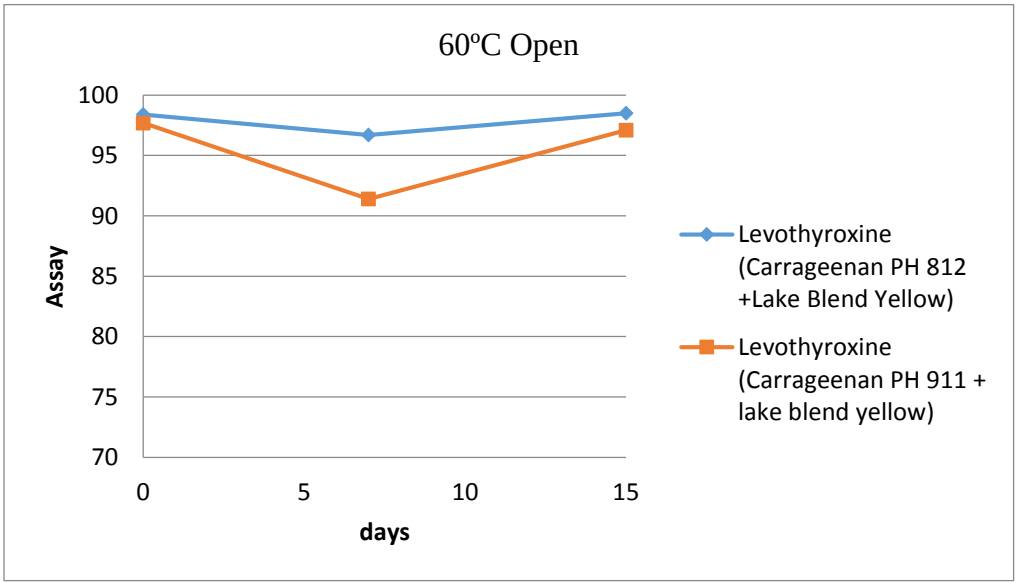
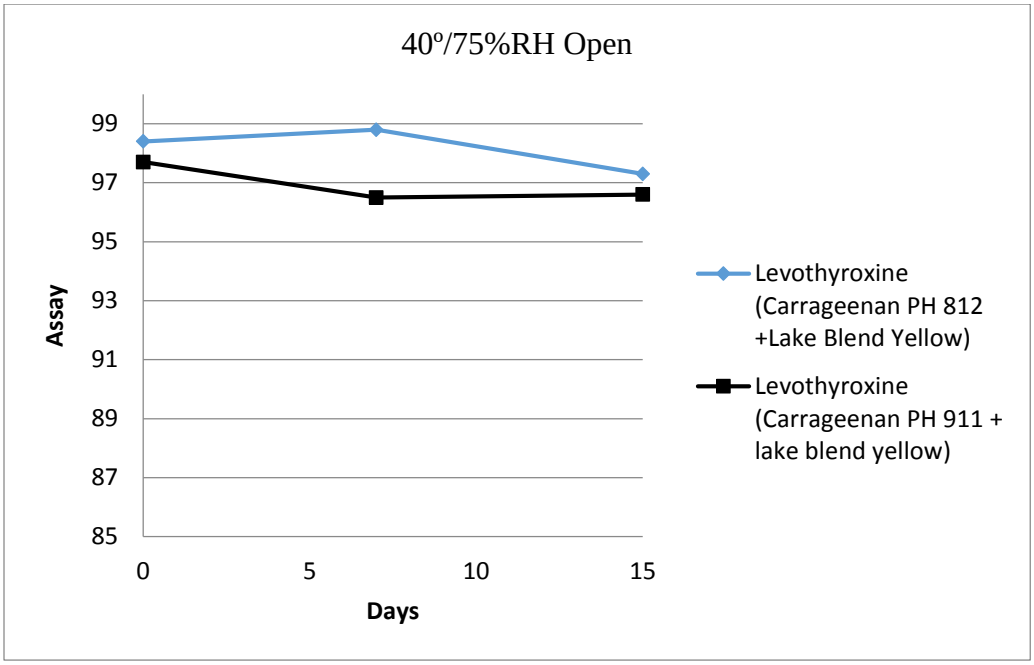


Figure 5.15: Assay of batch 9 & batch 10 at 40º/75% RH Open



INFERENCE:

- Hardness-6.5Kpa
- Disintegration time: 11-12 min
- There is not much Assay difference between initial and 60°C & 40°C/75% RH.
- Carrageenan help in stabilization of levothyroxine same as that of Lake blend yellow and FeO yellow batch
- The Issue which is observed with this batch is the increase in disintegration time from 3 min to 12 min

5.2.11. Assay Result of Batch 11

Table 5.11

	Packing	***Stability Condition***	***Assay***
Levothyroxine+ olive oil 1% (API dissolve in Olive oil)	HDPE+ 1 Oxygen absorber	Initial	103.8
		60°/ 7 Days open	95.6
		60°/ 7 Days Closed	97.9
		60°/ 15 Days open	93.4
		40°/75% 7 Days Open	90.6
		40°/75% 15 Days Open	93.5

Figure 5.16: Batch 11, Levothyroxine & olive oil 1%(Drug dissolve) 60º Open

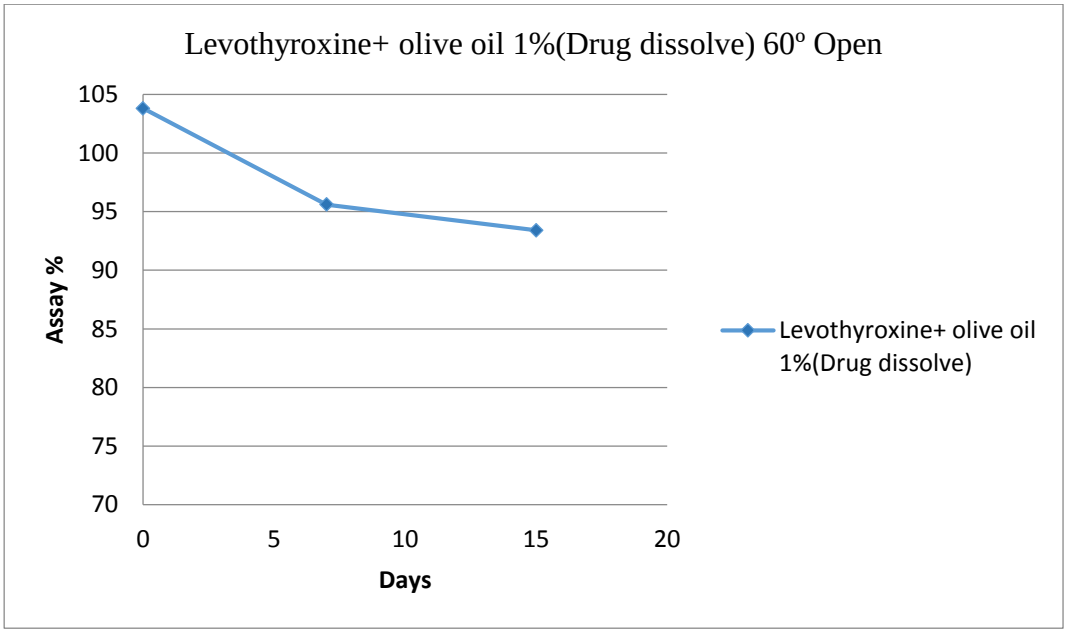
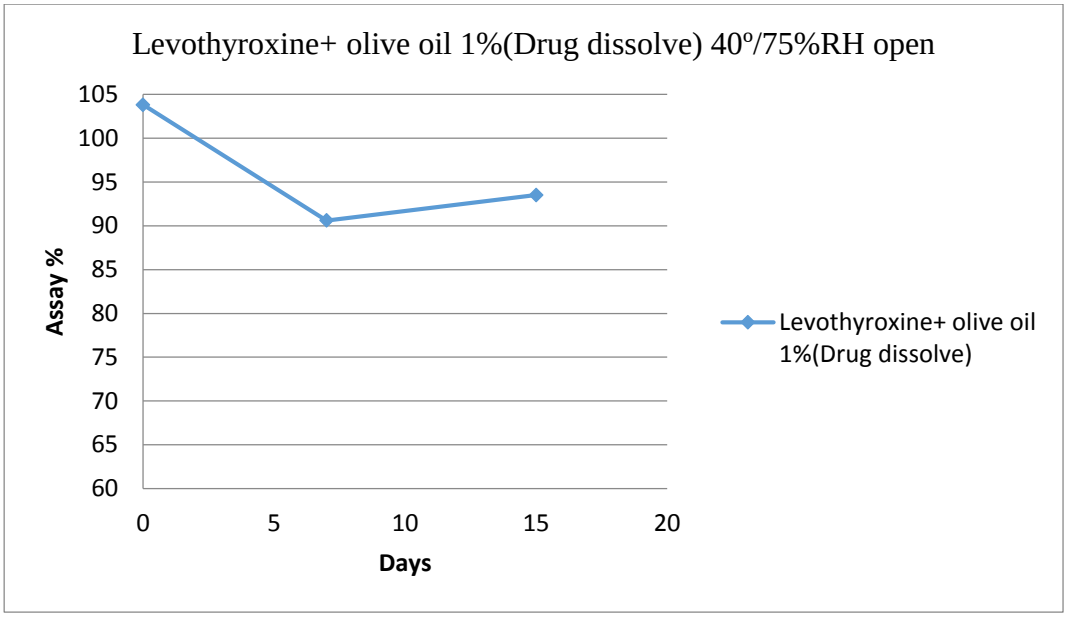


Figure 5.17: Batch 11, Levothyroxine & olive oil 1%(Drug dissolve) 40º/75%RH open



INFERENCE:

- It is under the specification range when HDPE bottles are kept in closed condition at 60°C for 7 Days.
- As compared to whole blend granulation, it is more stable though it is below the specification range (95%-105%)
- Direct contact of API with olive oil (dissolved form) shows the decrease in assay.
- At 40°/75% RH some decrease in assay was observed till 7 Days.

5.2.12. Assay Result of Batch 12

Table 5.12

	Packing	***Stability Condition***	***Assay***
Levothyroxine+ MCC (AVICEL-102) + Niacin	HDPE+ 1 Oxygen absorber	Initial	101.1
		60°/7 days open	99.2
		60°/7 days Closed	100.5
		60°/ 15 Days open	97.4
		60°/15 days Closed	99.1
		40°/75% 7 Days open	101.3
		40°/75% 7 Days Closed	102.9
		40°/75% 15 Days open	99.2
		40°/75% 15 days Closed	100.1

Figure 5.18: Assay of batch 12 at 60°C Open

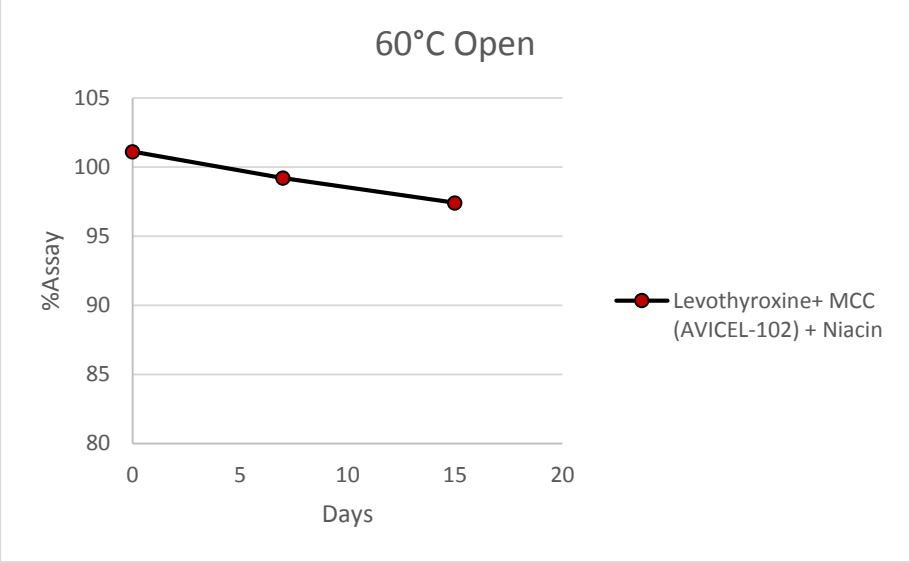


Figure 5.19: Assay of batch 12 at 60°C Closed

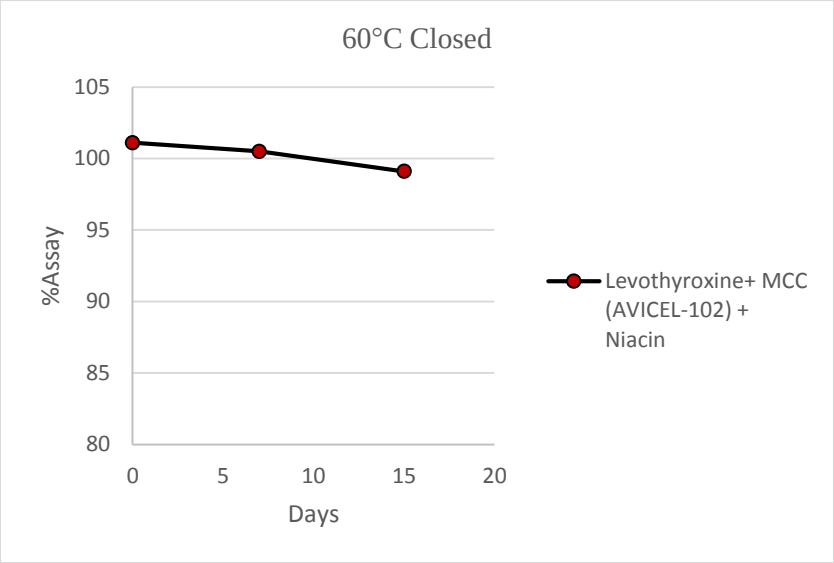


Figure 5.20: Assay of Batch 12 at 40°C/75% RH Closed

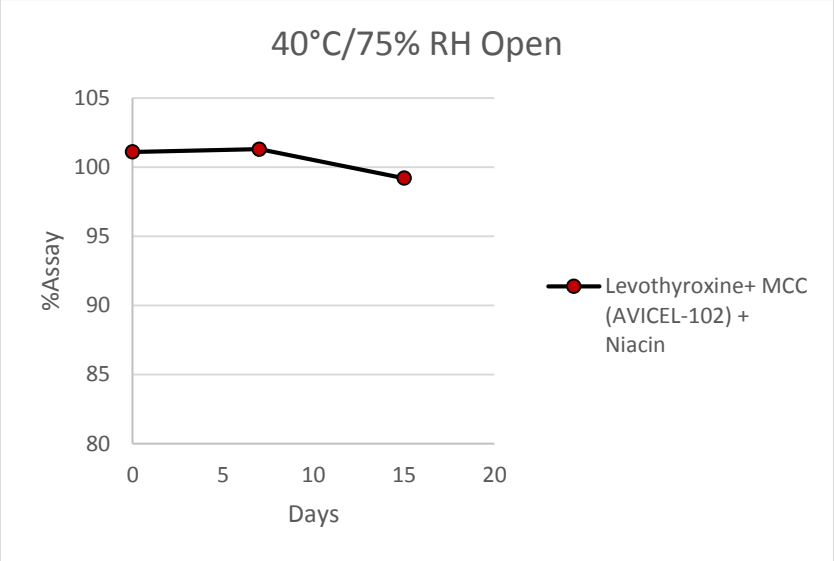
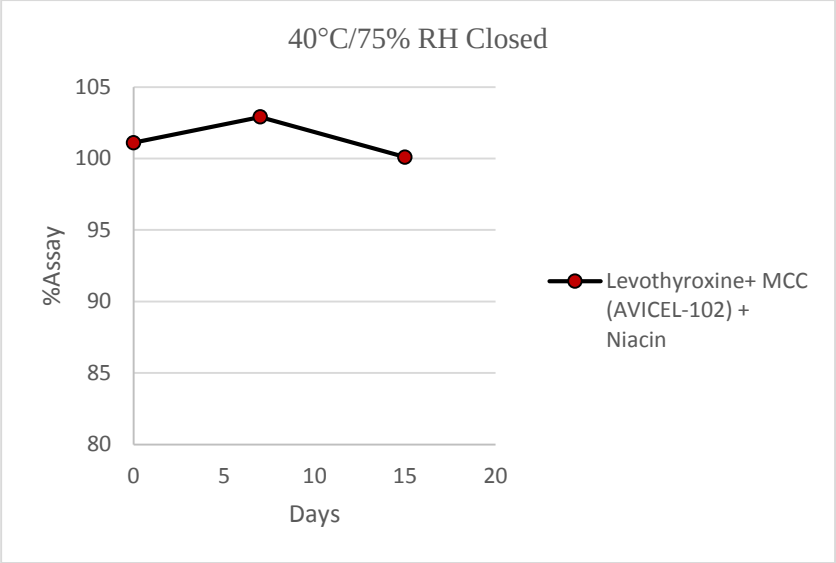


Figure 5.21: Assay of Batch 12 at 40°C/75% RH Closed



5.2.13. Assay Result of Batch 13

Table 5.13

	Packing	***Stability Condition***	***Assay***
Levothyroxine + Niacin + Olive oil 3% (Excipient Granulation)	HDPE+ Oxygen absorber 1	Initial	98.7
		60°/7 days open	94.5
		60°/7 days Closed	96.2
		60°/ 15 Days open	91.2
		60°/15 days Closed	93.6
		40°/75% 7 Days open	97.2
		40°/75% 7 Days Closed	99.6
		40°/75% 15 Days open	96.2
		40°/75% 15 days Closed	98.1

Figure 5.22: Assay of batch 13 at 60°C Open

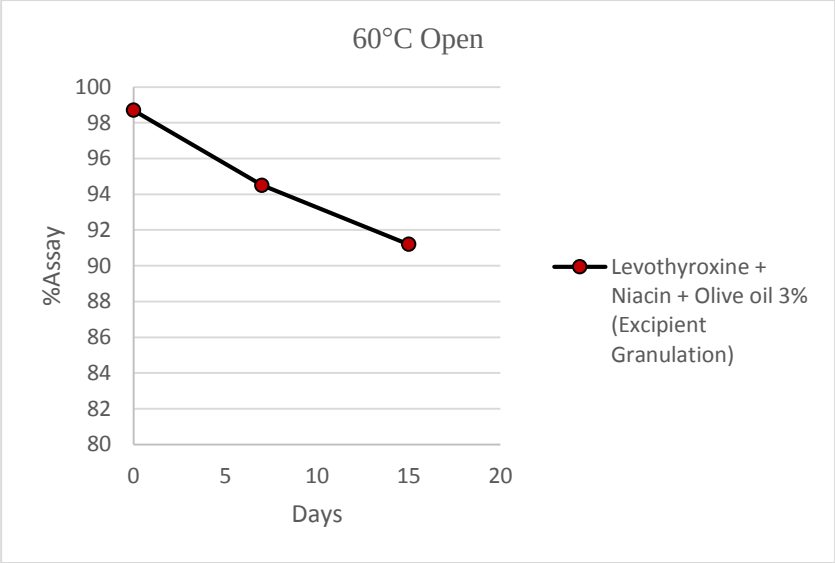


Figure 5.23: Assay of batch 13 at 60°C Closed

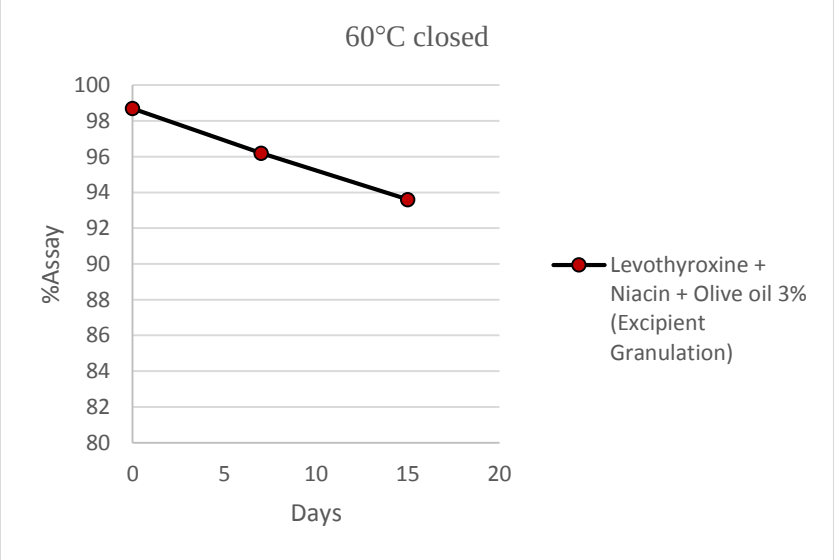


Figure 5.24: Assay of Batch 13 at 40°C/75% RH Open

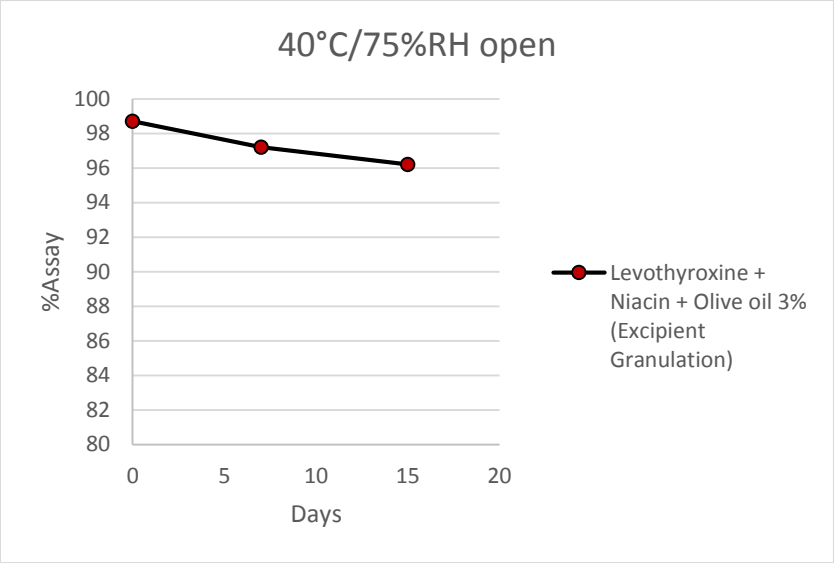
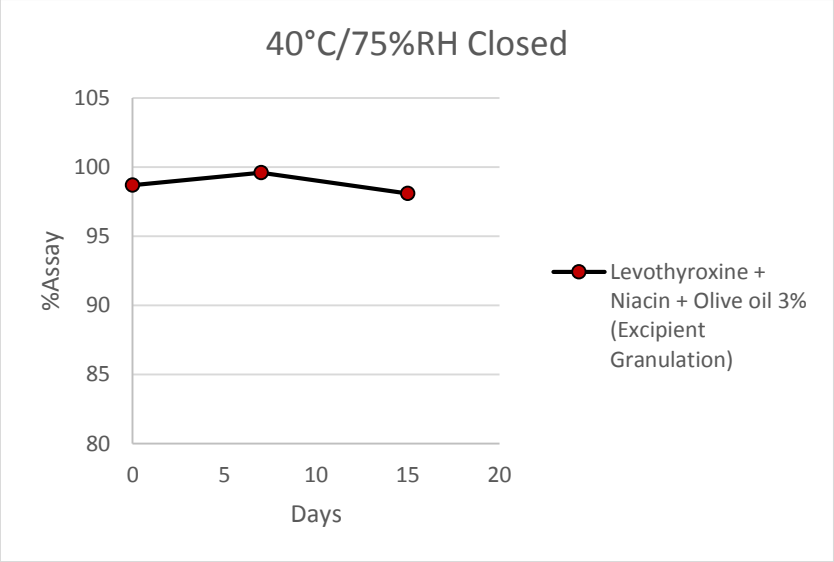


Figure 5.25: Assay of Batch 13 at 40°C/75% RH Closed



5.2.14. Assay Result of Batch 14

Table 5.14

	Packing	***Stability Condition***	***Assay***
Levothyroxine + Niacin + Olive oil 1% (Excipient Granulation)	HDPE+ Oxygen absorber 1	Initial	102.3
		60°/7 days open	100.1
		60°/7 days Closed	103.5
		60°/ 15 Days open	97.2
		60°/15 days Closed	100.5
		40°/75% 7 Days open	104.8
		40°/75% 7 Days Closed	104.9
		40°/75% 15 Days open	104
		40°/75% 15 days Closed	103.9

Figure 5.26: Assay of batch 14 at 60°C Open

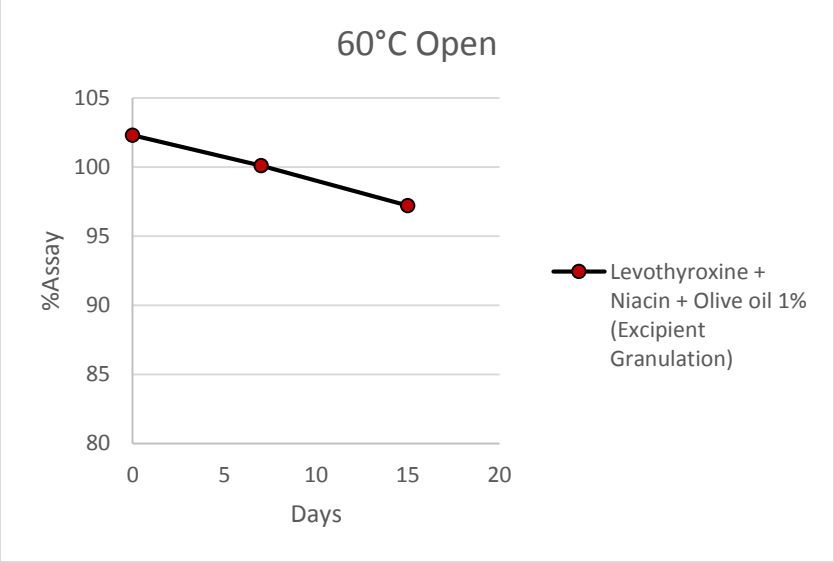


Figure 5.27: Assay of batch 15 at 60°C Closed

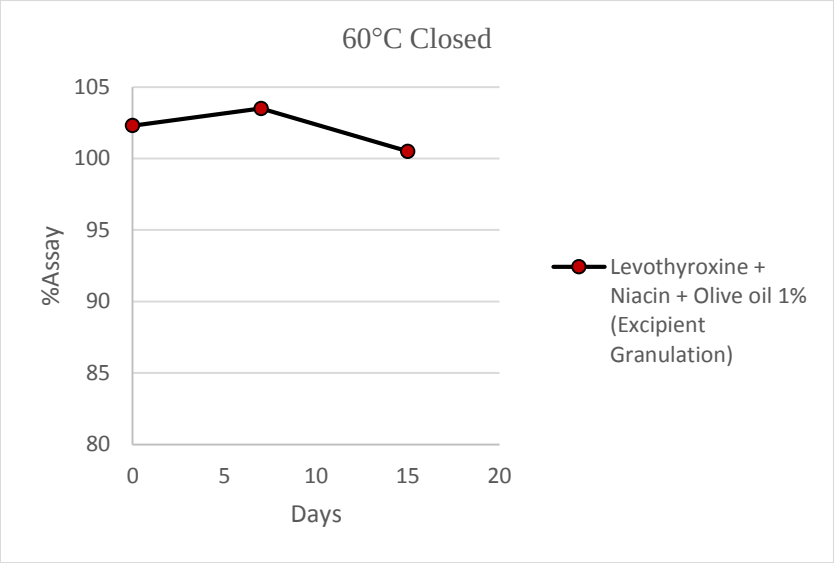


Figure 5.28: Assay of Batch 14 at 40°C/75% RH Open

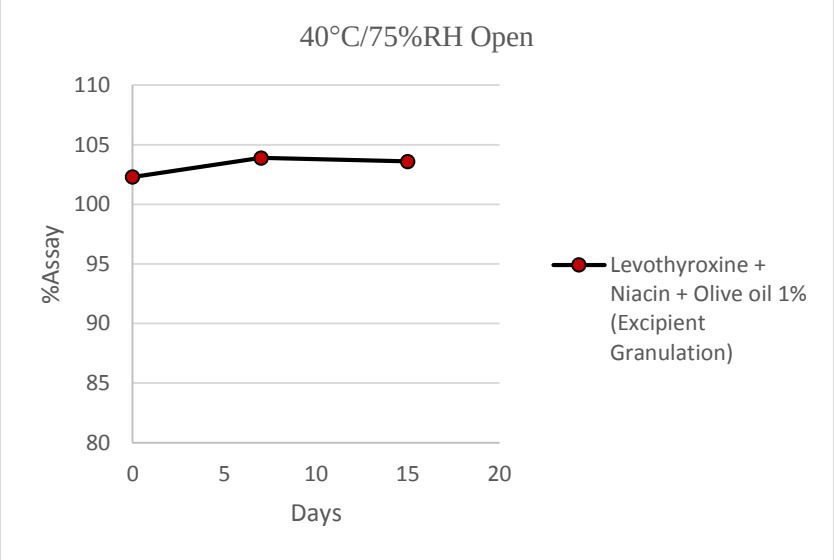
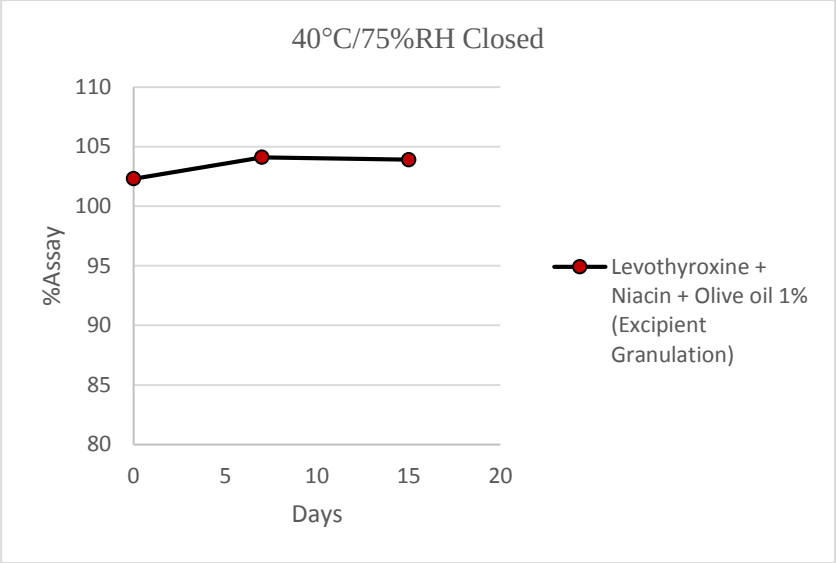


Figure 5.29: Assay of Batch 14 at 40°C/75% RH Closed



5.2.15. Assay Result of Batch 15

Table 5.15

	Packing	***Stability Condition***	***Assay***
Levothyroxine + Niacin + Lake Blend Yellow	HDPE+ 1 Oxygen absorber	Initial	97.5
		60°/7 days open	97.2
		60°/7 days Closed	98.1
		60°/ 15 Days open	97.8
		60°/15 days Closed	96.4
		40°/75% 7 Days open	97.8
		40°/75% 7 Days Closed	98.4
		40°/75% 15 Days open	97.1
		40°/75% 15 days Closed	97.6

Figure 5.30: Assay of batch 15 at 60°C Open

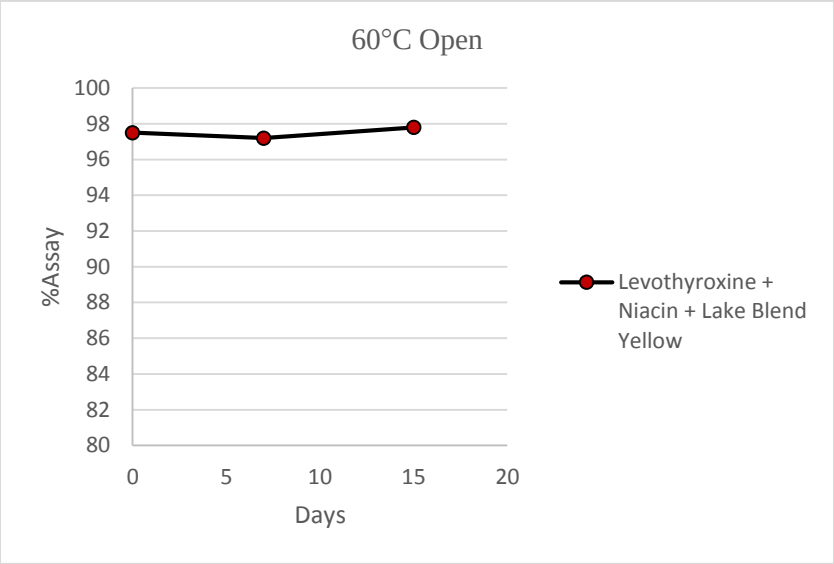


Figure 5.31: Assay of batch 15 at 60°C Closed

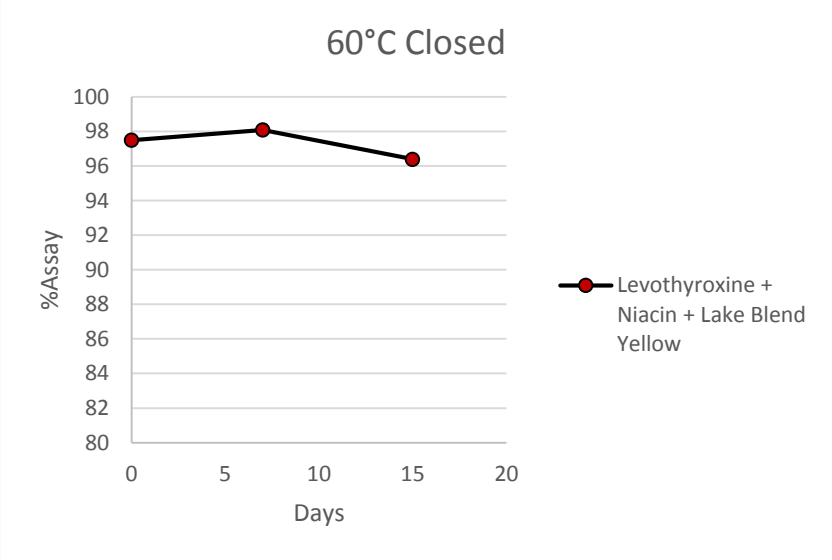


Figure 5.32: Assay of Batch 15 at 40°C/75% RH open

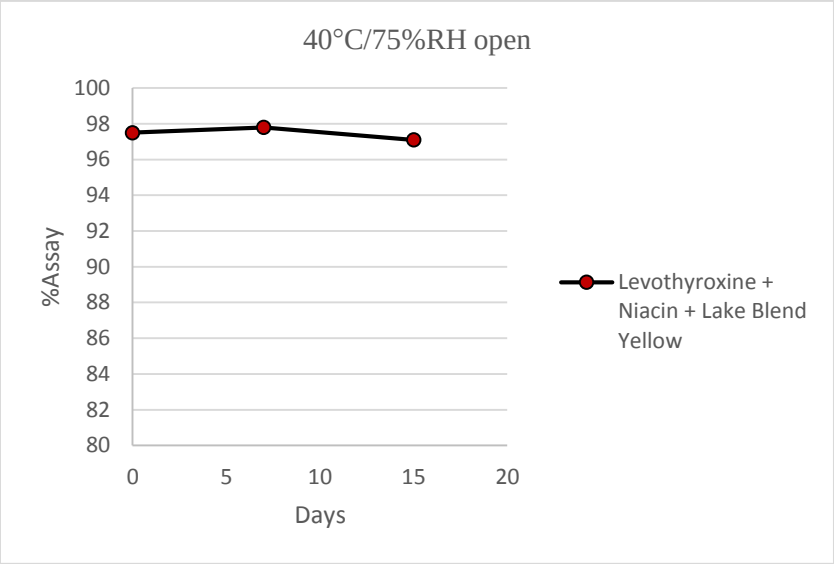
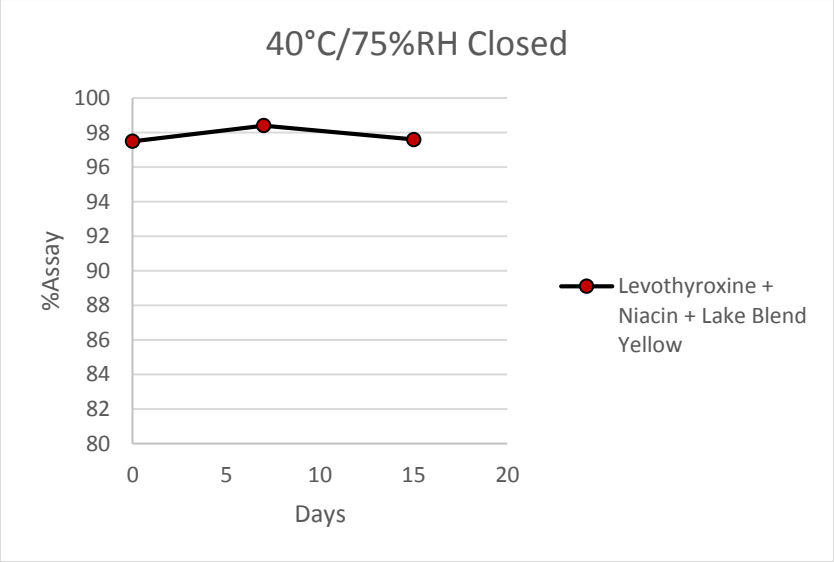


Figure 5.33: Assay of Batch 15 at 40°C/75% RH Closed



5.2.16. Assay Result of Batch 16

Table 5.16

	Packing	***Stability Condition***	***Assay***
Levothyroxine + Niacin + Iron Oxide (FeO) Yellow	HDPE+ 1 Oxygen absorber	Initial	96.1
		60°/7 days open	95.2
		60°/7 days Closed	97.3
		60°/ 15 Days open	93.5
		60°/15 days Closed	94.1
		40°/75% 7 Days open	98.1
		40°/75% 7 Days Closed	98.8
		40°/75% 15 Days open	96.3
		40°/75% 15 days Closed	95.8

Figure 5.34: Assay of Batch 16 at 60°C Open

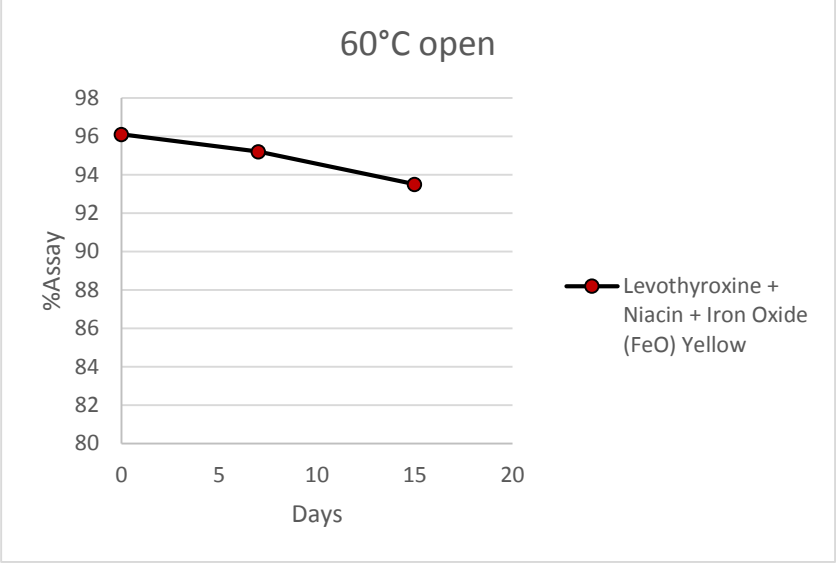


Figure 5.35: Assay of Batch 16 at 60°C closed

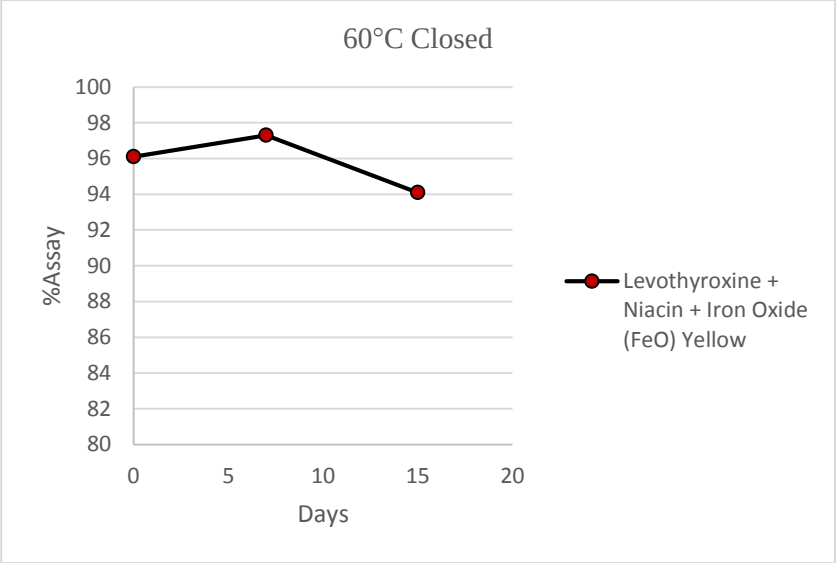


Figure 5.36: Assay of Batch 16 at40°C/75%RH Open

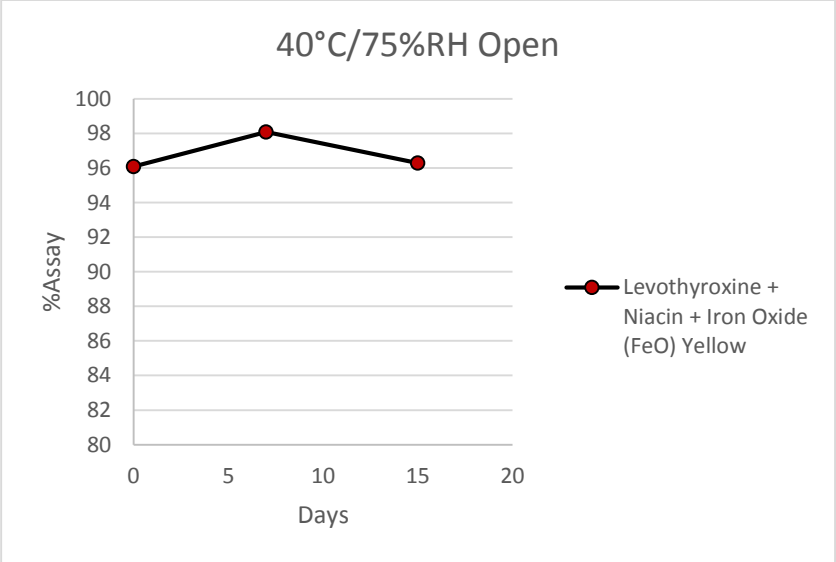
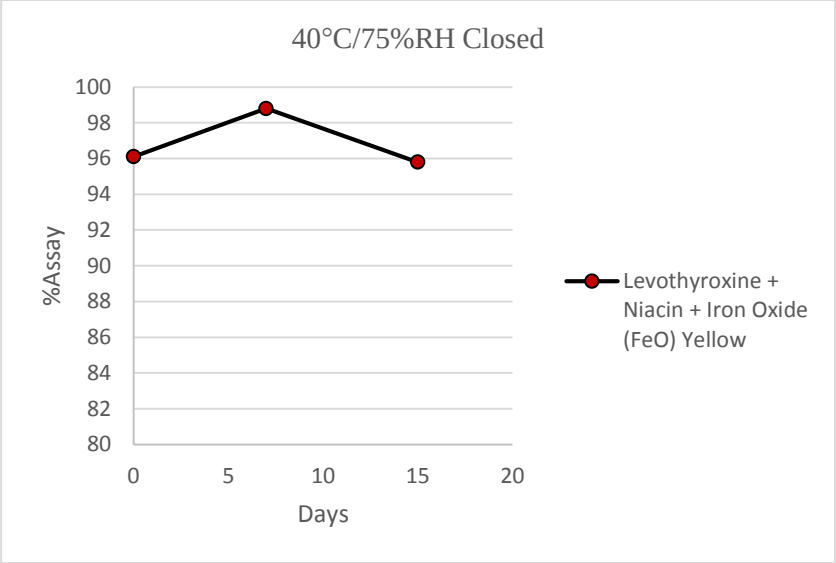


Figure 5.37: Assay of batch 16 at 40°C/75%RH Closed



5.2.17. Assay Result of Batch 17

Table 5.17

	Packing	***Stability Condition***	***Assay***
Levothyroxine + Niacin + Carrageenan pH911	HDPE+ 1 Oxygen absorber	Initial	97.7
		60°/7 days open	91.4
		60°/7 days Closed	93.4
		60°/ 15 Days open	97.1
		60°/15 days Closed	96.3
		40°/75% 7 Days open	96.5
		40°/75% 7 Days Closed	98.1
		40°/75% 15 Days open	96.6
		40°/75% 15 days Closed	97.2

Figure 5.38: Assay of batch 17 at 60°C Closed

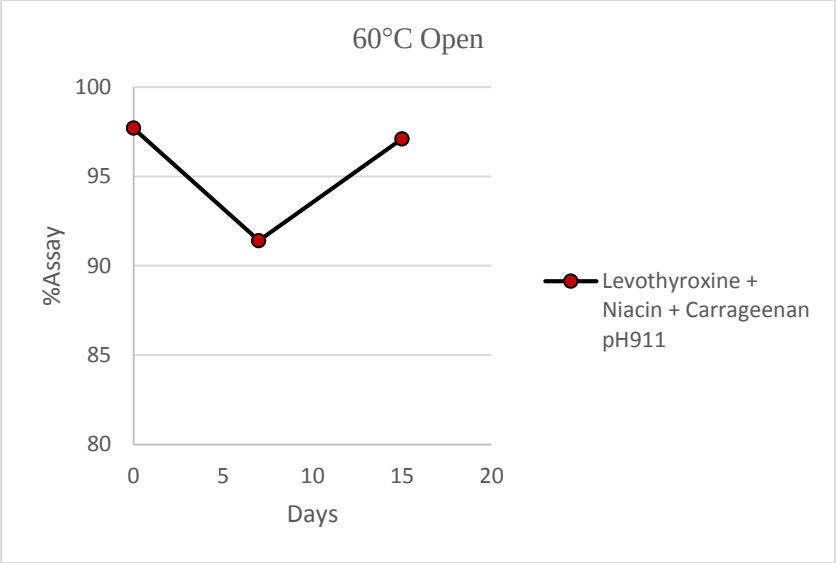


Figure 5.39: assay of Batch 17at 60°C Closed

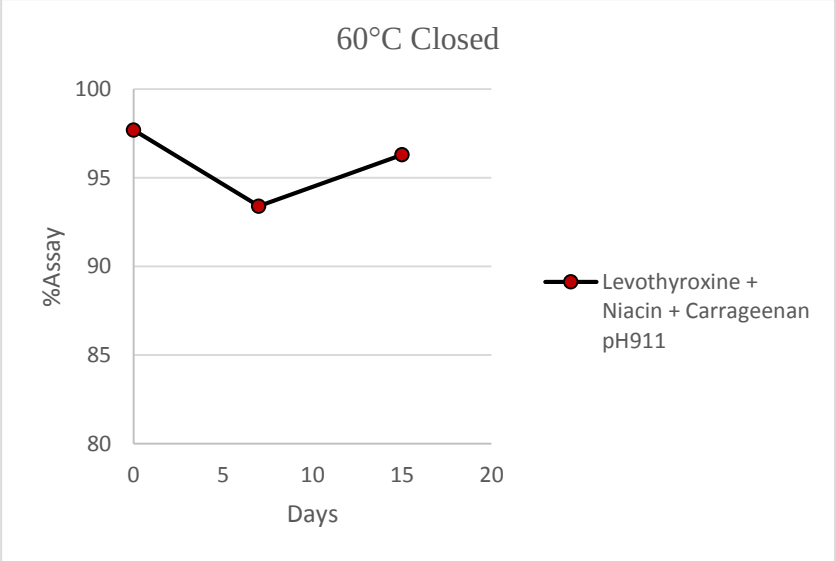


Figure 5.40: Assay of Batch 17 at 40°C/75%RH Open

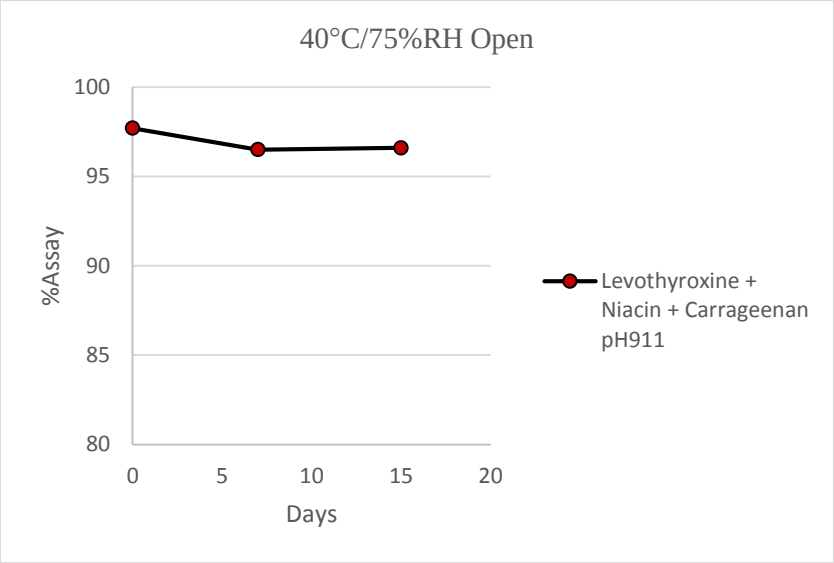
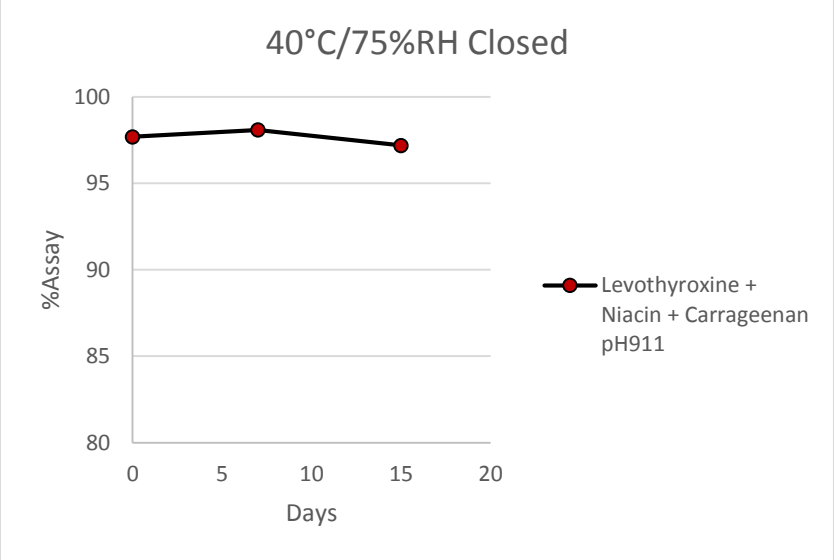


Figure 5.41: Assay of Batch 17 at 40°C/75%RH Closed



CHAPTER-6

SUMMARY & CONCLUSION

6.1 Summary

Levothyroxine Na penta-hydrate is found to be stable, when it is stored for the period of 6 months at ICH Accelerated stability Cond, there is less than 5% loss in potency. Also under normal humidity condition i.e. greater than 30% RH, it is found to be hygroscopic

The pH of surrounding medium also influences the stability of Levothyroxine Na. at basic pH it is found to be more stable (Won, 1992, Post 1976). In addition to this it is also found that Levothyroxine is more susceptible to oxidation at basic pH than Acidic pH.

The excipients which are used in the formulation of levothyroxine Na tablets also influenced degradation (ICH accelerated stability for 6 Months). MCC (microcrystalline cellulose) mostly used for the formulation as diluent, and proved to have degradation of product in study, while Calcium Phosphate found to be the most inert diluent. It is also found that the moisture amount also increases the degradation of product.

Various degradation pathway is: - De-amination, De-Iodination, Decarboxylation. It is also studied that presence of Desiccant did not affect the stability of product.

Saturated solution of levo Na tablets (saturated) has the pH ranged from 5.5 to 7.5. While Saturated solution of Levo Na tablets has the pH of 8.0. “The inherent pH of the microenvironment found in the compressed tablets did not coincide with the pH of maximum stability of levothyroxine sodium solution (basic). This also explains why levothyroxine sodium tablets were less stable than levothyroxine sodium drug substance when stored at ICH accelerated stability conditions. Thus, formulating levothyroxine sodium tablets with the pH of microenvironment coinciding with the pH of maximum stability in solution is a potential technique to improve its stability”.

It is also observed that the formulation made with 1%, 3% olive oil enchases the potency and help in maintain the stability profile of Levothyroxine Sodium Tablets. While increasing the percentage of olive oil in a formulation also affect the stability of product and decreases the potency at ICH accelerated stability Profile.

Formulation made with granulation of excipient blend with olive oil found to have more stability profile of drug product. Thus the potency is maintained within the specification range of 95-

105%. While one more batch in which Levothyroxine API is dissolved in olive oil found to have potency (% Assay) within the specification range.

Study of Dye shows that the Formulation made with lake blend yellow as well as iron oxide Yellow is found to be stable at ICH accelerated stability condition for 6 months. The percentage assay came under the specification range of 95% to 105%.

Study of one more excipient (stabilizer) Carrageenan is done. It is observed that use of both grade of Carrageenan 812 and carrageenan 911 help in maintain the stability profile of Levothyroxine Na tablet and maintain the percentage Assay within the specification range of 95% to 105%. The only drawback of this batch formulation is it increases the Disintegration time of Levothyroxine sodium tablet

"This was further confirmed in levothyroxine sodium tablets formulated with pH modifying additives. The presence of basic additives improved stability of levothyroxine sodium tablets at ICH accelerated stability conditions for six months. Levothyroxine sodium tablets that met USP assay requirements (90-110 %) and did not show significant change in potency (< 5% loss in potency, ICH Q1A(R)), after three and six-month storage at 40^{o}C/75% RH, were manufactured using a basic pH modifier and a low moisture diluent, dibasic calcium phosphate".

The Levothyroxine Sodium tablets which were manufactured by wet Granulation/ or Direct compression, it was studied that initial assay was lower as compared to uncompressed powder or granules of levothyroxine Sodium. Thus is studied that the type of manufacturing process also affects the stability profile of Levothyroxine sodium.

Thus compatible/ optimum formulation of Levothyroxine would include low moisture diluent Di-calcium phosphate, compatible pH modifier (basic), compatible Disintegrant i.e. CMC (Croscarmellose sodium), a compatible lubricant e.g. magnesium Stearate, L-HPC, Olive oil, MCC (microcrystalline cellulose), all these are the excipient used for the stabile levothyroxine drug product profile

6.2 CONCLUSION

Formulation made with granulation of excipient blend with olive oil found to have more stability profile of drug product. Thus the potency is maintained within the specification range of 95-105%. While one more batch in which Levothyroxine API is dissolved in olive oil found to have potency (% Assay) within the specification range.

Study of Dye shows that the Formulation made with lake blend yellow as well as iron oxide Yellow is found to be stable at ICH accelerated stability condition for 6 months. The percentage assay came under the specification range of 95% to 105%.

Study of one more excipient (stabilizer) Carrageenan is done. It is observed that use of both grade of Carrageenan 812 and carrageenan 911 help in maintain the stability profile of Levothyroxine Na tablet and maintain the percentage Assay within the specification range of 95% to 105%. The only drawback of this batch formulation is it increases the Disintegration time of Levothyroxine sodium tablet

The Levothyroxine Sodium tablets which were manufactured by wet Granulation/ or Direct compression, it was studied that initial assay was lower as compared to uncompressed powder or granules of levothyroxine Sodium. Thus is studied that the type of manufacturing process also affects the stability profile of Levothyroxine sodium.

CHAPTER-7

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7. REFERENCES

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