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Applying DMAIC Principles For Improving Method Performance Of Quantitative Determination Of Levothyroxine Sodium In Tablet Dosage Form Using High Performance Liquid Chromatography Technique.

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ABSTRACT

A simple and improved reverse phase liquid chromatographic method has been developed for the estimation of Levothyroxine Sodium in tablet formulation. The drug is official with Indian Pharmacopeia, United States Pharmacopeia British Pharmacopoeia. The separation was carried out using Nucleosil CN, 250*4.6mm, 5µ column and the mobile phase consisting of water, acetonitrile(ACN) and o-phosphoric acid in the ratio of 650 : 350 : 10 (v/v) in isocratic mode. The flow rate was 1.00 mL/min and effluent was monitored at 225 nm. The new method for the assay of Levothyroxine Sodium tablet was found statistically superior as compared to the official IP 2010 method. Define, Measure, Analysis, Improve and Control (DMAIC) principles were used for problem solving, root cause investigation, risk management to improve method performance. Statistical process control evaluation of the method before and after modification revealed that modified method is under statistical process control. Levothyroxine sodium in tablets formulation under acidic condition was found stable up to 42h; whereas the drug was found stable only up to 8h under alkaline condition. The marked difference was found for assay values by both the methods. The methods were found statistical different. The advantage of modified method is better extraction capacity and solution stability of Levothyroxine Sodium in tablet formulation.This study was applied in analytical quality control laboratory to improve the quality of the method, reduce method defects and increase right first time of assay test method by applying the Lean Six Sigma (LSS) methodology. LLS is considered one of the successful approaches in the field of quality improvement and cost reduction. The case study laboratory working environment was analyzed to isolate the root causes for the defects generation. Remedies and counter measures were suggested and some were implemented. The study compares the performance of the test method before and after implementation of the proposed solutions for defects reduction. We subjected a high performance liquid chromatographic (HPLC) analytical procedure used for screening drugs on authenticity to a Failure Mode and Effects Analysis (FMEA), including technical risks as well as risks related to human failure. An FMEA tool broke down the HPLC analytical method into process steps and identified possible failure modes for each step. Each failure mode was ranked on estimated frequency of occurrence (O), probability that the failure would remain undetected later in the process (D) and severity (S), each on a scale of 1–10. Failure risks were calculated by Risk Priority Numbers (RPNs) =O×D×S. Failure modes with the highest RPN scores were subjected to corrective actions and the FMEA was repeated, showing reductions in RPN scores and resulting in improvement indices up to 5.0. The present method was successfully used for quantitative determination of Levothyroxine sodium in tablet dosage form. The investigations showed that it was feasible to define an HPLC method with an improved quality compared to IP2010 assay method.

Keywords: Levothyroxine Sodium; Thyroxine Sodium; HPLC method; Lean Six Sigma; DMAIC; FMEA; Indian Pharmacopeia.

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INTRODUCTION

The fast changing economic conditions such as the severe global competition, declining profit margin, customer demand for high quality product, product variety and the need to reduce lead–time have major impact on manufacturing industries. To respond to these needs various industrial engineering and quality management strategies such as ISO 9000, Total Quality Management, Kaizen engineering, Just–in–time manufacturing, Enterprise Resource Planning, Business Process Reengineering and Lean Management have been developed. A new paradigm in this area of manufacturing strategies is Six Sigma. The Six Sigma approach has been increasingly adopted worldwide in the manufacturing sector in order to enhance productivity and quality performance and to make the process robust to quality variations [1]. Quality is defined as the fitness for use or purpose at the most economical level [2]. It is an integral part of the process of design, manufacture and assembly. It can be assured by having effective procedures and controls at various stages. In manufacturing industries, to overcome the competition problem and to retain the share of the market, it is necessary to constantly improve the quality of the product without the increase in the price. The price is influenced by the cost of production, which in turn is influenced by waste, rework, rejection and downgrading rates. Attention to quality assurance can reduce the process waste, which results in a quality production and company's growth and profitability.

Six sigma methodologywas introduced for quality improvement[3]. The six sigma concept was introduced in the early 80's by Motorola due to two reasons. First reason was the nature of mass production and second reason was the threat of the Japanese products in the American market. It is known that a process working at 3 sigma level introduces 2600 defect per million which is not acceptable in many situations like the production of the printed circuit boards. The implementation of six sigma is always done using DMAIC approach **[3]**which is simply explained as follows:

- **D**: Define; what problem needs to be solved?
- **M**: Measure, What is the capability of the process?
- **A**: Analysis, When and where do defects occur?
- **I**: Improve, How the process capability can be improved?
- **C**: Control, What control can be put in place to sustain the gain?

An implementation model for six sigma was applied. The modelimplies a top down approach were strategic decisions based on the market/customer analysis must be taken by the management [4]. The model calls also for tactical decisions implying bottom up approach, where engineers or technicians are primarily involved in the decision making process in terms of the design of detailed plans to form low-level improvement teams, and the implementation, documentation, and revision of the plans' executions.Both lean and six sigma are key business process strategies which may be employed by companies to enhance their manufacturing performance [1-4]. Integrated Lean Six Sigma (LSS) model for manufacturing industry is illustrated in **Figure 1**, which is similar to the regular six sigma train of thoughts.

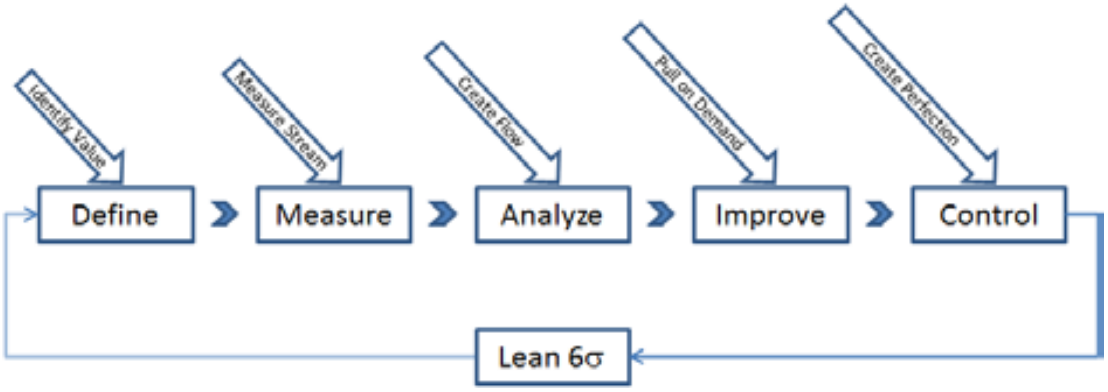

Figure 1.Integrated Lean Six Sigma (LSS) model approach.

Levothyroxine sodium is official in Indian Pharmacopeia [5], United States Pharmacopeia [6]and British Pharmacopeia [6]. Levothyroxine sodium is a synthetic thyroid hormone that is chemically identical to thyroxine (T4), which is naturally secreted by the follicular cells of the thyroid gland. It is used to treat thyroid

hormone deficiency, and occasionally to prevent the recurrence of thyroid cancer. The drug is very slightly soluble in water; slightly soluble in ethanol (~750 g/L) TS; practically insoluble in acetone and ether. It dissolves in solutions of alkali hydroxides. Levothyroxine sodium is labile to the heat, moisture, and oxidative conditions, chemical reaction these conditions typically occur during levothyroxine formulation, tableting, packaging, and storage [7].To optimize the method for improved performance with respect to better extraction and solution stability DMAIC principles to be used for problem solving, root cause investigation, risk management. In this context the present work reports a development of modified quantitative method for the determination of Levothyroxine sodium from tablet formulations [1-8].With the implementation of six sigma, it is possible to determine the key factors affecting an analysis process, identify the optimum levels or tolerances and improvement opportunities. The objective of this research work was to improve Levothyroxine sodium assay chromatographic method from tablet formulation using operational excellence tools such as Supplier, Input, Process, Output, and Customer*(*SIPOC), 5 why's, Cause and Effect, and FMEA etc. The assay of a drug substance (DS) in solid dosage form is one of the tests required to confirm the Active Pharmaceutical Ingredient (API) quality at release. Nowadays specific chromatographic assay procedures are preferred. However, the usually executed high performance liquid chromatographic (HPLC) methods tend to be less precise and have a larger total method variation. In this context the present work reports an improved quantitative HPLC determination of Levothyroxine sodium in the tablet formulation.

EXPERIMENTAL

Reagents

All the reagents like methanol, acetonitrile (ACN), sodium hydroxide, o-phosphoric acid, hydrochloric acid, used were of analytical reagent grade. Purified water was used from Millipore's Milli-Q water purification system.

Reference standards

Levothyroxine Sodium was used as a reference standard.

Instrumentation

Mettler Toledo XP 105 Delta range analytical balance was used to weigh the required materials, Mettler Toledo Seven Multi pH meter was used to adjust the pH of solutions. Waters Alliance chromatographic system with 2690 separation module, and 2487 dual wavelength detector was used.

Preparation of Diluent (A)

A mixture of 600 volumes of water, 400 volumes of ACN and 0.5 volumes of o-phosphoric acid.

Preparation of Diluent (B)

Dissolve 400 mg of sodium hydroxide in 500 mL of water, cool, and add 500 mL of methanol.

Preparation of Reference solution

Reference solution was prepared by dissolving 20 mg of Levothyroxine sodium RS with 30 mL of diluent B, sonicate and dilute to 50.0 mL with diluent B. Dilute 5.0 mL of this solution to 200 mL with diluent A.

Preparation of Test solution

Test solution was prepared by weighing the powder of 20 or more tablets. Weigh accurately the powder containing about 1 mg of thyroxine Sodium, add 40 mL of diluent A (a mixture of 600 volumes of water, 400 volumes of acetonitrile and 0.5 volumes of o-phosphoric acid) and shake for about 30 minutes and dilute with diluent A to produce 100 mL, mix well and filter.

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HPLC technique

Waters HPLC coupled with 2690 separation module and 2487 dual wavelength detector was used for the experiment. Thyroxine sodium was eluted on stainless steel column with 25 cm length, 4.4 mm internal diameter, packed with Cyno bonded to porous silica with 5µm particle size. The chromatographic conditions are listed under **Table1**.

Table 1:Chromatographic Conditions.

Sr.No.	Parameter	Condition
1	Mobile phase	650 : 350 : 10 v/v (Water :ACN : o-phosphoric acid) pH adjusted to 2.5
2	Column Temperature	Ambient
3	Flow rate	1 mL per minute
4	Detection Wavelength	225 nm
5	Injection volume	20 µL

RESULTS AND DISCUSSION

In the first phase –the Define phase- of the current six sigma (DMAIC) process there were four main steps implemented as follows: (1) collecting preliminary data, (2) writing problem definition statement (3) Supplier, Input, Process, Output, and Customer (SIPOC) diagram (4) process mapping. Levothyroxine Sodium assay determination process in the tablet dosage form as per IP 2010 monograph is composed mainly of the following steps. Tablets are ground to fine powder from which sufficient amount of fine powder is weighed then the diluent is added to dissolve and extract the drug from the sample matrix. This solution is shaked for 30 minutes vigorously. The solution is diluted with diluent to produce 100mL solution. Further mixed and filtered through 0.45µm nylon membrane and subjected to HPLC. Standard solution is prepared with the diluent to produce 0.001% (w/v) solution of levothyroxine sodium. SIPOC is an acronym standing for supplier, input, process, output, and customer. It refers to the technique of analyzing a process relative to these parameters to fully understand their impacts. A SIPOC diagram for the Levothyroxine Sodium assay determination process is given in **Figure 2**.

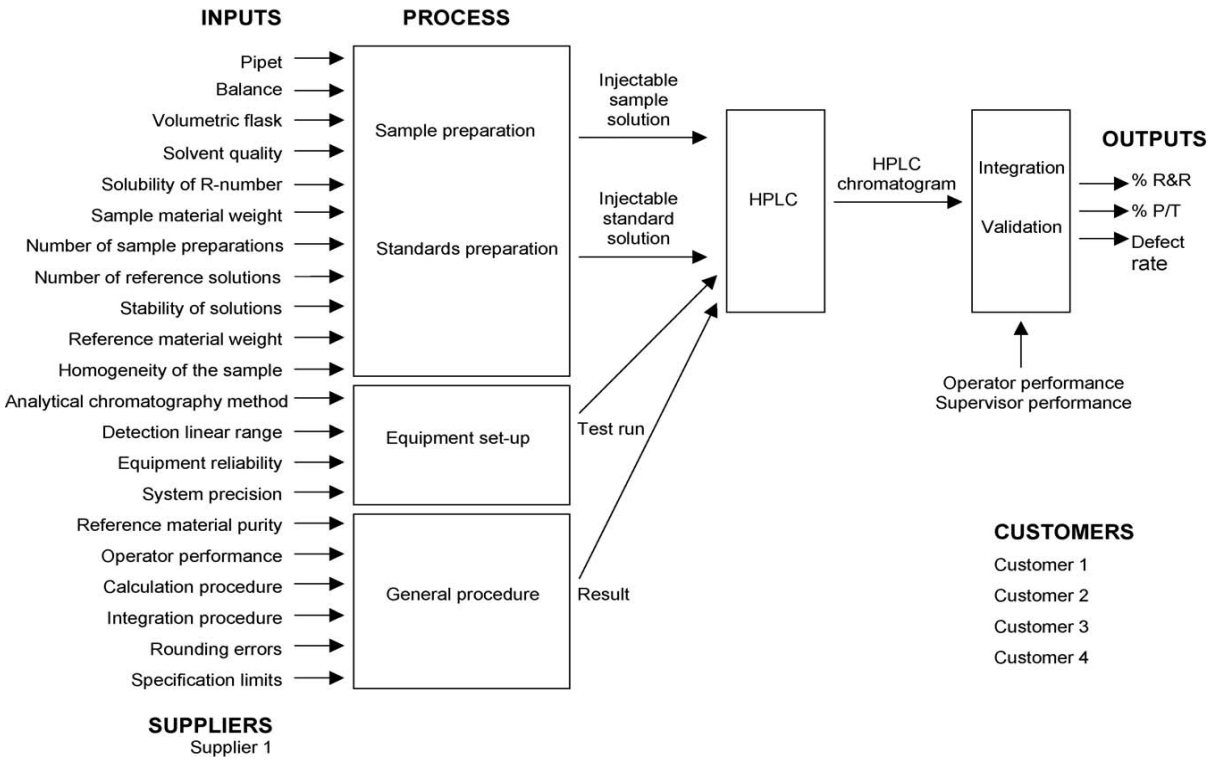

Figure 2: SIPOC diagram for Levothyroxine Sodium assay determination process.

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Collection of Preliminary Data

Preliminary data was collected wherein Levothyroxine sodium assay determined as per IP2010 monograph. Assay values were found lower than the expected. The average percentage assay was found about 101% against the input of 105% of Levothyroxine sodium. Levothyroxine sodium tablets are manufactured in different strength such as 25µg, 50µg, 75µg, 100µg and 125µg. Which is very low compared to other drug substances. Then a powerful methodical technique such as lean six sigma was needed to be implemented to improve the Levothyroxine sodium assay against the input.

Problem Definition Statement

To investigate and identify the root cause the lowering of Levothyroxine sodium assay values determined from Levothyroxine sodium tablets IP 2010 monograph.

Process Mapping

In order to have a detailed understanding of the sample preparation processes in the assay method of analysis as per IP2010 Thyroxine sodium tablets monograph process and their relationships, the process map **(Figure 3)**as one of the tools of LSS was used. The process map highlights the different areas where the variation may be generated. Studying the process elements revealed that there are three main types of waste namely; inadequate tablet grinding, incorrect weighing of tablet grind, inadequate extraction of Thyroxin sodium from sample matrix and incorrect filtration.

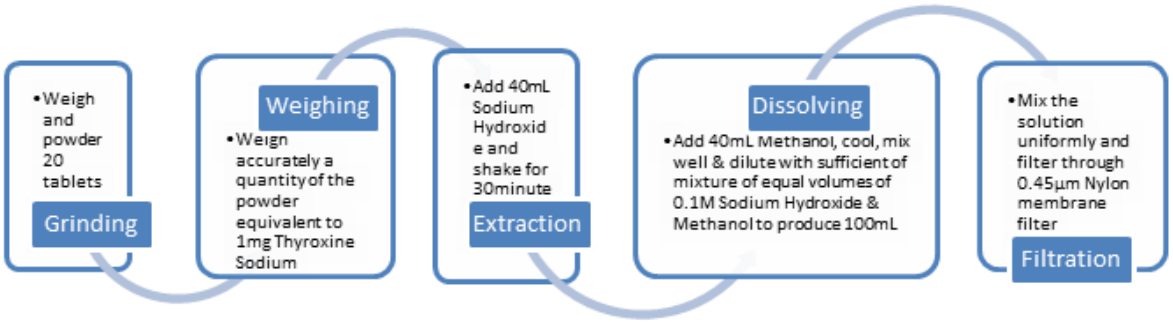

Figure 3: Process Map - Sample preparation as per IP 2010 Thyroxin sodium tablets assay method.

Measure phase

The use of statistical techniques to analyze a process in order to monitor, control, and improve it. The objective is to have a stable, consistent process that produces the fewest defects possible. The central idea of SPC is to control variation so as to avoid product defects. There are two kinds of variation in any process: common causes and special causes. Common causes refer to occurrences that contribute to the natural variation in any process. Special causes are unusual occurrences that are not normally (or intentionally) part of the process. While some degree of common cause variation will naturally occur in any process, it's important to identify and attempt to eliminate special causes of variation. During measure phase percentage assay values were subjected for statistical evaluation wherein control chart was utilised to evaluate whether IP2010 test method within statistical process control.

Statistical Process Control

The individual control chart shows no trend of the data with variability. The moving range chart shows one point i.e. sample no 7 is outside the control limits and other 8 points inside the control limits exhibiting a non-random pattern, suggesting the presence of special causes shown in **Figure 4**. Analysis process as per IP2010 IP 2010 Thyroxin sodium tablets monograph is observed to be variable with special causes. The statistical evaluation from the control chart revealed one point more than 3.00 standard deviations from centre line hence the test failed. The control chart is in not under statistical control and thus cannot be used for monitoring and controlling the future production batches.

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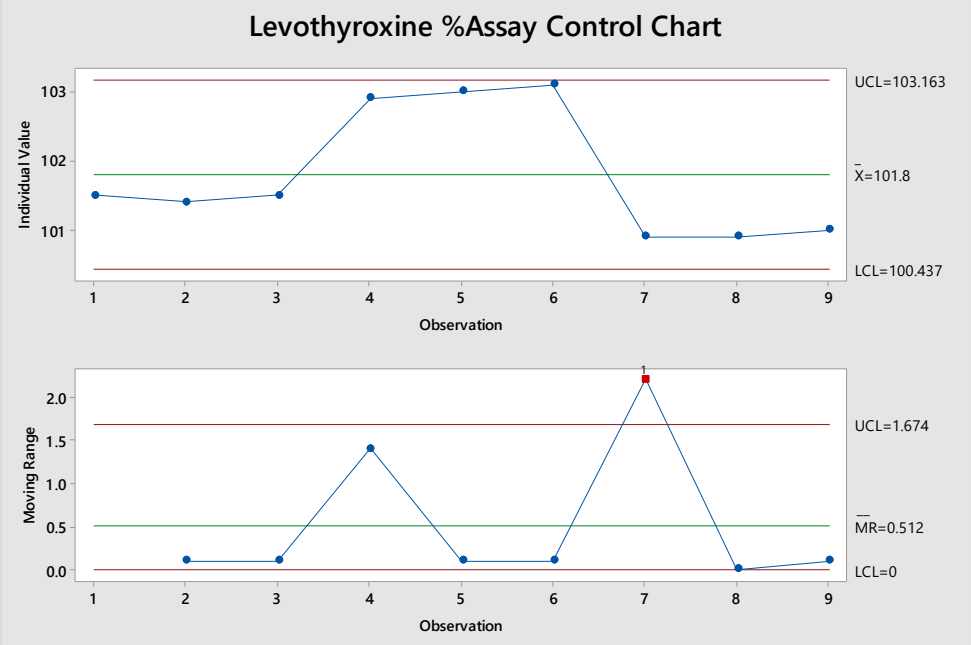

Figure 4:Control Chart –percentage assay as per IP 2010 Thyroxin sodium tablets monograph.

Solution Stability IP2010 method

From the literature it is found that Thyroxine alkaline solutions are unstable. During measure phase, pH of sample diluent was checked which was found about pH=12. The solution stability of sample prepared as per IP2010 monograph was evaluated. The solution stability study revealed that Thyroxine sodium in sample solution as per IP2010 method degrades rapidly in alkaline solution. Thyroxine sodium was found stable up to 8h at pH=12, refer **Figure 5**. The plot of percentage difference in assay from initial to 42h found increasing on time which concluded that the Thyroxine sodium alkaline solution is stable till 16h however the drug started degrading after 16h with 16.4% degradation at 42h.

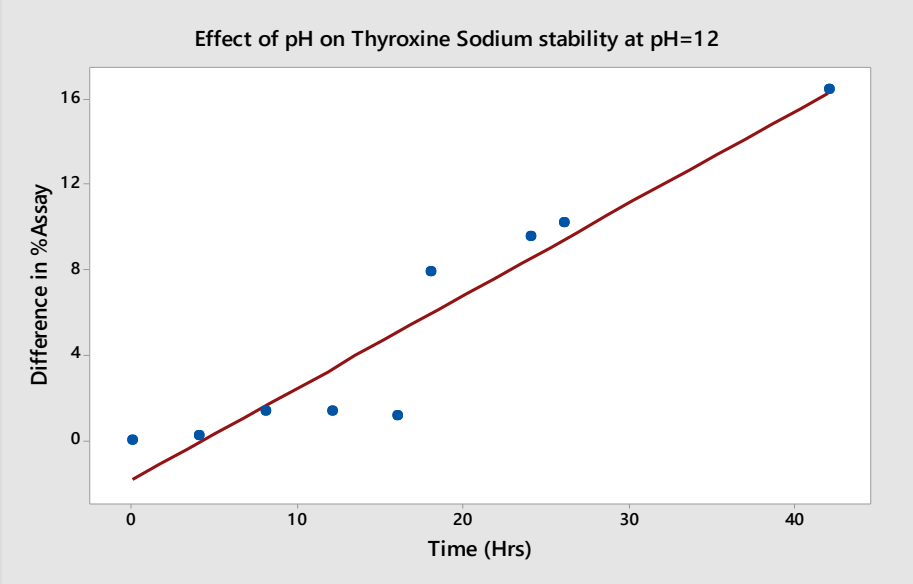

Figure 5: Regression plot effect of pH on Thyroxine sodium stability at pH=12.

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Analyse phase

A cause and effect illustrating the main parameters controlling the results of analysis is presented in **Figure 6** wherein factors affecting lowering of assay value were identified.

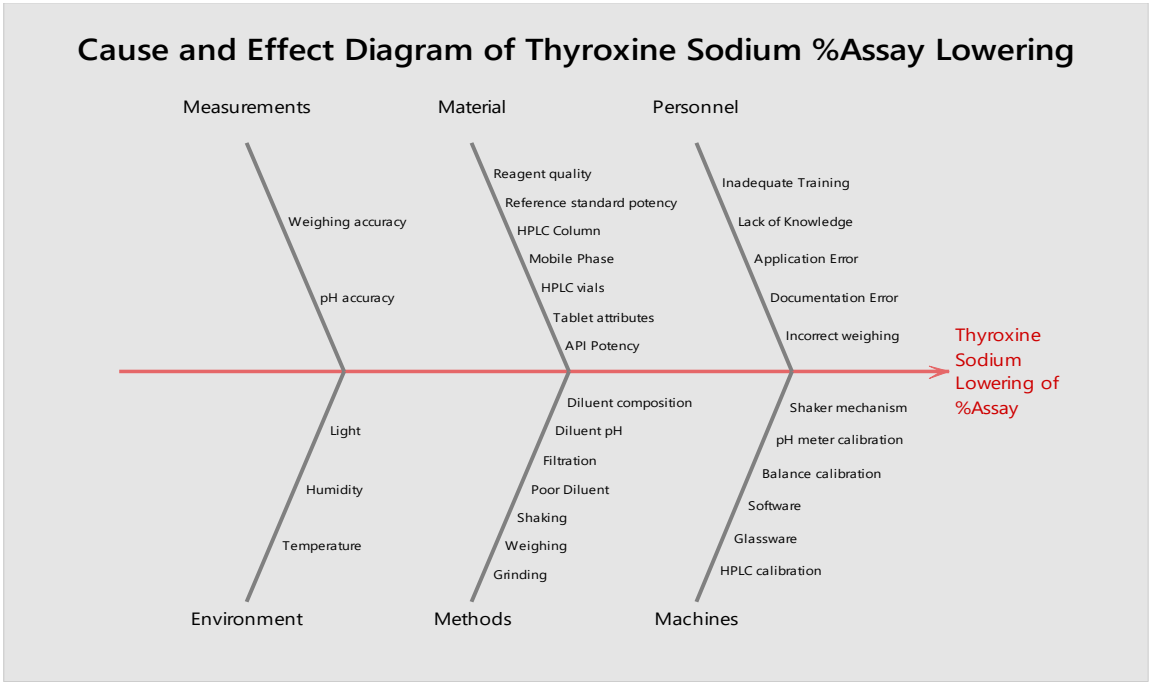

Figure 6:Cause and effect diagram of factors affecting percentage assay lowering.

A process flow cause- effect analysis is drawn for the total process to identify exact location of occurrence in complete process. Sample preparation of levothyroxine as per IP 2010 **[5]**, the process is started from the grinding and completed after filtration. The intermediate stages are weighing, extraction and dissolving. The process flow cause- effect diagram is developed to identify the causes, sub causes and exact location of causes for the defects in lowering of assay values. As defects in product may occur anywhere in the total process, so it is very necessary to identify and eliminate the causes which are responsible for defects in the process. The process flow cause- effect diagram is shown in **Figure7.**

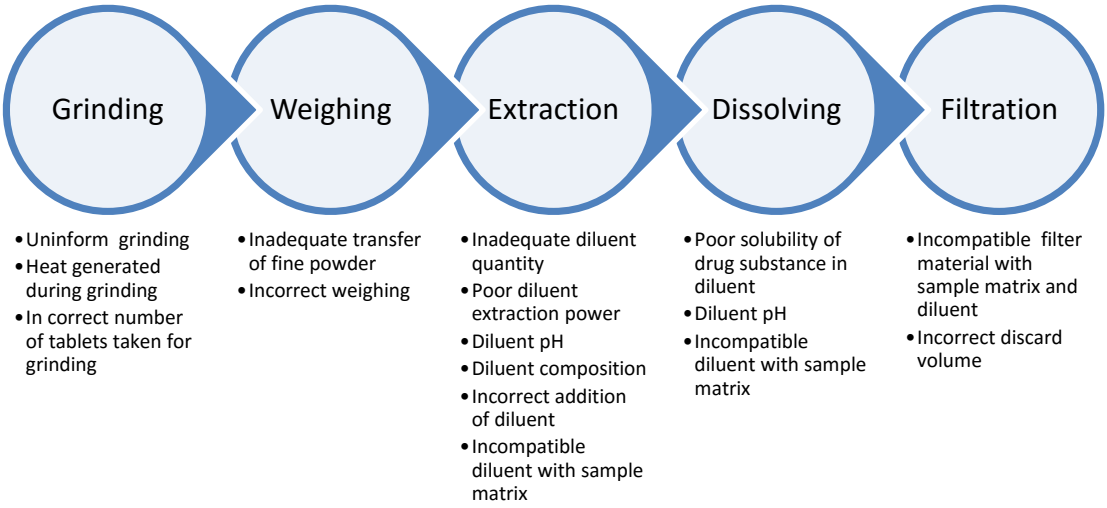

Figure 7:Process flow cause-effect diagram for sample preparation as per IP 2010.

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Levothyroxine sodium sample preparation method as per IP2010 [5] method was broken down to single process steps these steps are listed under **(Table 2)**. Subsequently, failure modes were identified for each of their meaning steps. Each failure mode was then ranked by its estimated frequency of occurrence (O), its probability that the failure would remain undetected (D) and its severity (S), each on a scale of 1–10. A high number represents a high risk. Ranking was performed by a consensus decision of the team.

Table 2: Levothyroxine sodium sample preparation single process steps IP 2010.

Step No.	Process
1	Grinding of tablets to fine powder
2	Weighing of fine powder accurately equivalent to 1mg Levothyroxine
3	Extracting drug substance from sample matrix to sodium hydroxide solution
4	Dissolving drug substance in diluent
5	Filtering diluted solution using 0.45µm nylon membrane filter and subjecting to HPLC system

For each identified failure mode, the RPN was calculated by multiplying the rankings for O, D and S. Consequently, the highest RPN that was theoretically possible became 1000 (10×10×10) and the lowest theoretically possible RPN became 1. FMEA results were reviewed with respect to the four failure modes with the highest RPN scores. The calculated RPN score and corrective actions are listed under **(Table 3)**. It was revealed that the inadequate extraction was scored high with RPN 400. The investigation was targeted to the factors affecting inadequate extraction.

Table 3:Levothyroxine sodium sample preparation FMEA IP 2010.

Step	Failure mode	Possible effect	Possible cause	Estimated frequency of occurrence	Estimated frequency of detection	Estimated severity	RPN	Corrective action
1	Inadequate grinding	Incorrect assay value	Human error	3	10	5	150	Training
2	Inadequate weighing	Incorrect assay value	Human error	3	10	5	150	Training
3	Inadequate extraction	Lowering of Assay values	Diluent pH, composition, extraction capacity	8	10	5	400	Change in sample preparation method and diluent
4	Inadequate filtration	Incorrect assay value	Incompatible filter material, incorrect discard volume	4	10	5	200	Change in filter type with accurate discard volume

The literature survey revealed that Levothyroxine alkaline solution is unstable. pH of diluent was measured and found to be 12 [9]. This suggests that lowering of assay value could be due to alkalinity of sample solution obtained from diluents. These causes were considered in the improve phase of the lean six sigma process to be addressed for possible improvement according to the available resources [10].

Improve phase

Based on previous studies and process knowledge, the most important controllable factors are composition and pH of diluent used for sample preparation. IP 2010 Levothyroxine method was modified for diluent composition used for standard and sample preparation only with rest method parameters unaltered. Method parameters compared and listed under **Table 4** for method parameters comparison.

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Table 4: Method comparison IP 2010 Thyroxin sodium tablets and modified IP 2010.

Parameters	IP 2010 Method	Modified IP 2010 method
Chromatography	RP-HPLC	RP-HPLC
Column	Nucleosil CN, 250*4.6mm, 5µ	Nucleosil CN, 250*4.6mm, 5µ
Wavelength	225nm	225nm
Column Load	20µL	20µL
Mobile phase	650 : 350 : 10 v/v (Water :ACN : o-phosphoric acid)	650 : 350 : 10 v/v (Water :ACN : o-phosphoric acid)
Mobile phase pH	2.5	2.5
Standard diluent	0.1N NaOH :Methanol (1:1 v/v)	0.2N NaOH :Methanol (1:1 v/v)
Sample diluent	0.1N NaOH :Methanol (1:1 v/v)	600 : 400 : 0.5 v/v (Water : ACN : o-phosphoric acid)
Sample diluent pH	12.2	2.5
Sample weight	1.68gm (equivalent to 1000µg Thyroxine sodium)	1.68gm (equivalent to 1000µg Thyroxine sodium)
Sample volume	100mL	100mL
Acquisition time	15 minutes	15 minutes
Retention time	7.4 minutes	7.4 minutes

Samples were analysed as per IP2010 and modified IP2010 method using parameters indicated in **Table 4**. Assay results obtained from IP 2010 and modified IP2010 method were compared using time series plot refer **Figure 8**. The comparison revealed that the assay values obtained from modified IP2010 method were higher compared to IP2010 method.

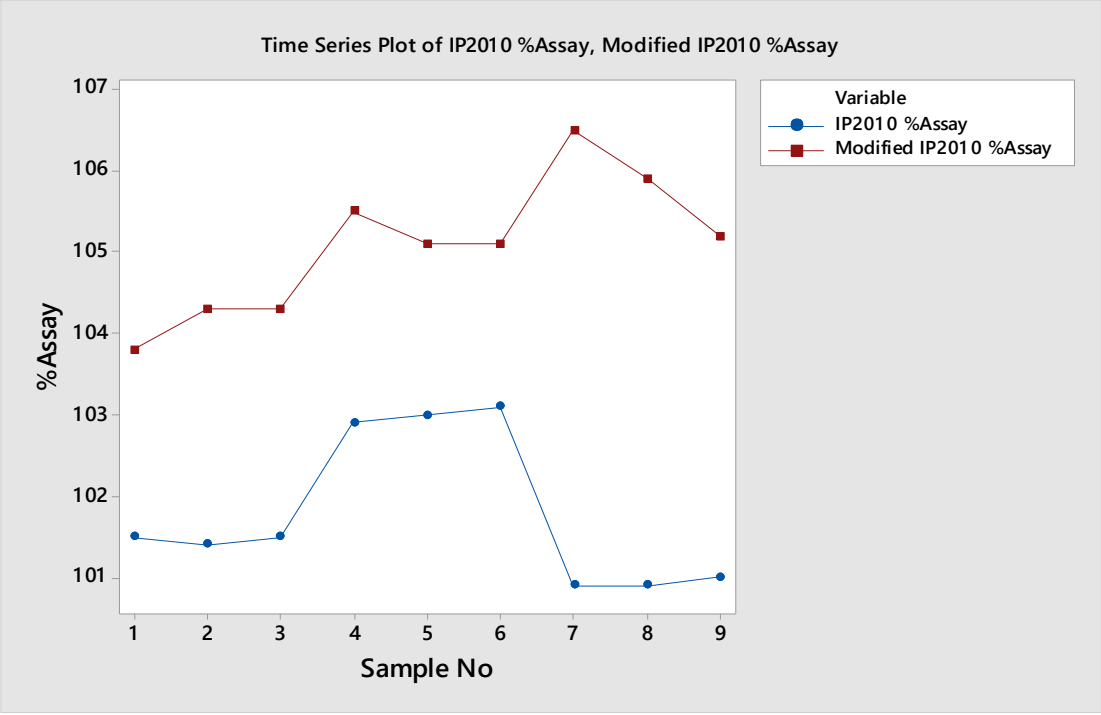

Figure 8: Time series plotIP 2010 and modified IP2010 method.

Statistical equivalency of Levothyroxine sodium IP2010 and modified IP method was evaluated and found that the compared methods are not statistically equivalent. Null hypothesis was failed hence the equivalency could not be claimed **(Figure 9)**.

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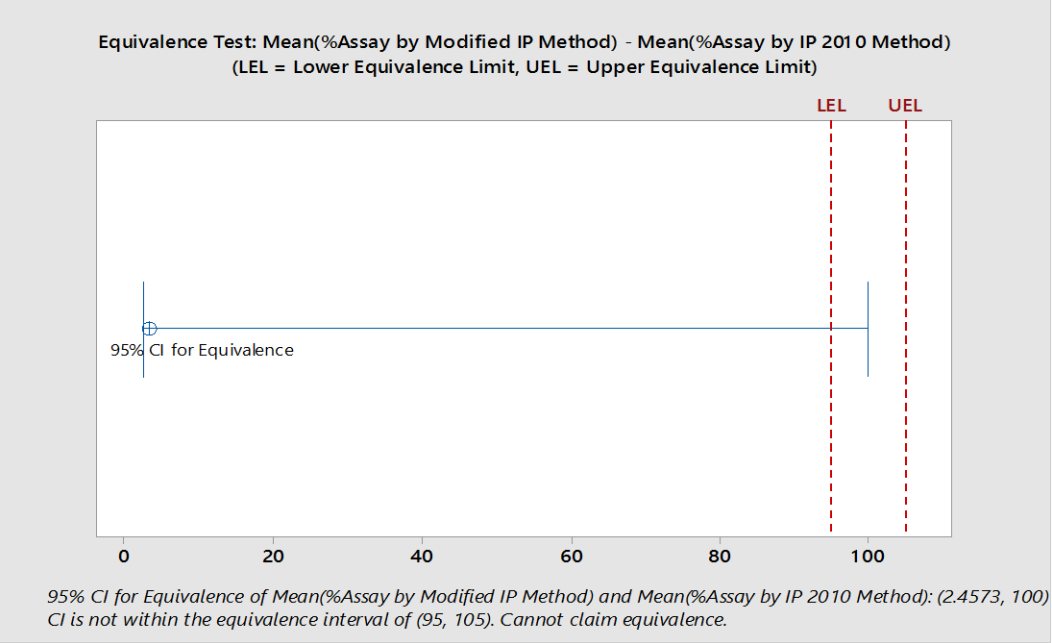

Figure 9:Equivalence testIP 2010 and modified IP2010 method.

Solution Stability after improvement

During improve phase, pH of sample diluent was checked which was found about pH=2.5. The solution stability of sample prepared as per modified IP2010 monograph was evaluated. The solution stability study revealed that Levothyroxine sodium in sample solution as per IP2010 method degrades rapidly in alkaline solution compared to modified IP 2010 method. Considerable increase in % difference of assay values were found from initial to different time points; however found increasing considerably of Thyroxine sodium was found stable up to 8h at pH=12whereas,the drug was found stable up to 42h**(Figure 10)**.

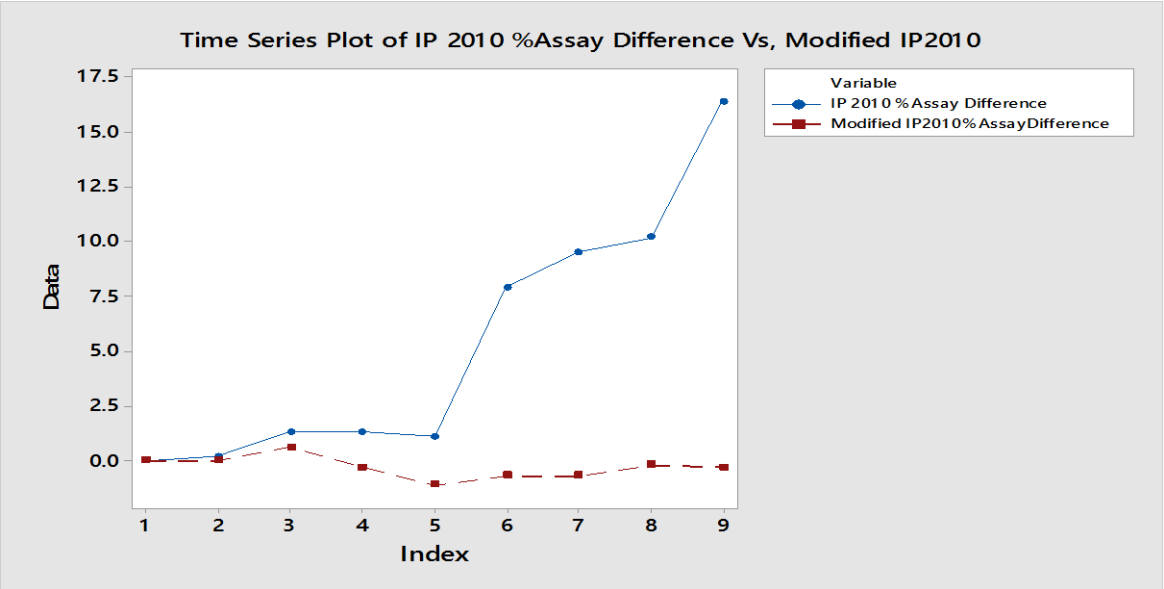

Figure 10. Time series plot of solution stability by IP 2010 and modified IP2010 method.

Control phase

Confirmation experiments were conducted to check the achieved improvement. Individual and moving range charts were created on the assay values determined by modified method. Individual value and

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moving range plots are depicted under **Figure 11**. The statistical evaluation from the control chart revealed no point more than 3.00 standard deviations from centre line was observed hence the test passed. The control chart depicted under **Figure 11**shows that the modified method is in statistical process control and thus can be used for monitoring and controlling the future production batches.

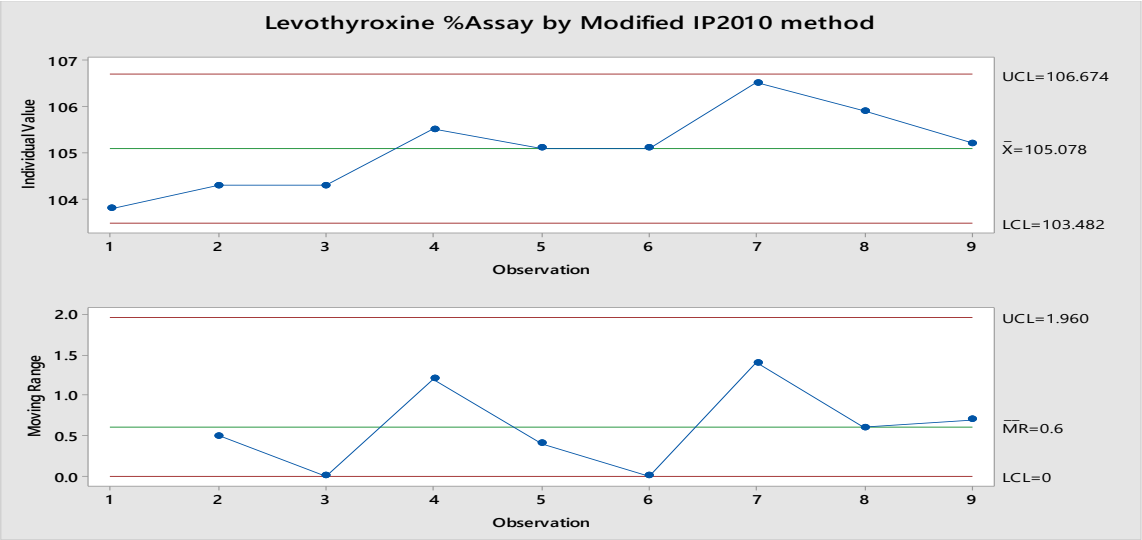

Figure 11:Control chart of percentage assay by Modified IP2010 method.

CONCLUSION

Levothyroxine sodium in Thyroxine tablets under acidic conditions is stable up to 42h, whereas the drug alkaline condition was found stable only up to 8h. There is marked difference in assay values determined by both methods. The statistical comparison revealed that these methods are not statically similar. Assay results by modified method were found closer to input value of the drug. Levothyroxine sodium assay method was improved in such a way that better assay values are obtained by changing diluent composition for sample preparation keeping all other method parameters constant. The main recommendation from this study is to apply suggested diluent composition for sample preparations. It is evident from the study that the diluent plays an important role for extraction and solution stability of Levothyroxine sodium from the tablets matrix. The advantage of using proposed method is better extraction and solution stability of Levothyroxine sodium from the tablets matrix. The proposed method doesn't negatively impact the product quality and hence the modified method can be considered as alternative quantitative determination of Levothyroxine sodium from the tablets dosage form.

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