ANALYTICAL STUDIES IN THE DETECTION
AND ESTIMATION OF COMPOUNDS
IN DRUG FORMULATIONS

DISSERTATION
SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF
Master of Philosophy
IN
CHEMISTRY

BY
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1994
Dedicated
to my
Mummy & Papa
TO WHOM IT MAY CONCERN

This is to certify that the dissertation entitled, "Analytical Studies in the Detection and Estimation of Compounds in Drug Formulations" is the original work of Miss. Rumana Faruqui and suitable for submission for the award of Degree of Master of Philosophy in Chemistry.

(Dr. (Mrs.) Iqbal Akhtar)  (Prof. Saidul Zafar Qureshi)
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I pay my thanks to almighty Allah who led me to this path of success.

( RUMANA FARUQUI )
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CHAPTER I

GENERAL INTRODUCTION
**Analytical Chemistry** is concerned with the theory and practice of methods used to determine the composition of matter. The Science of Analysis can be divided into areas called qualitative analysis and quantitative analysis. It is concerned with what elements or compounds are present in the sample. Quantitative analysis is concerned with the determination of how much of particular substance is present in the sample. The substance determined, often referred to as the desired constituents or analyte, may constitute small or large part of the sample analyzed. A complete analysis actually consists of five main steps: (1) sampling, that is, selection of a representative sample of the material to be analyzed; (2) dissolution of the sample; (3) conversion of analyte into a form suitable for measurements; (4) measurement; and (5) calculation and interpretation of the measurement.

The importance of Analytical Chemistry can be illustrated by considering its impact on clinical analysis, in pharmaceutical research and quality control, and in environmental analysis.

In the past, medical profession used clinical results qualitatively and many of their diagnoses were based on symptoms and/or X-ray examination. Currently, over one billion laboratory tests are performed in clinical laboratories per year. The major portion of
these tests deal with blood and urine samples and include the determination of glucose, urea nitrogen, protein nitrogen, sodium potassium, calcium HCO3/H2CO3, uric acid and pH.

In the pharmaceutical industry the quality of the manufactured drugs in tablet, solution and emulsion form must be carefully controlled. Slight changes in composition or in the purity of drug itself can affect the therapeutic values. In other pharmaceutical studies, it is necessary to establish the properties and therapeutic value of a drug before it is approved and made available to the public. Establishment of the permissible level of dosage of a drug requires the determination of its decomposition product, its toxicity, and its metabolite products at various stages.

It is an important task for the practicing analytical chemist to choose an analytical method which provides the best solution to the problem. Methods of quantitative testing and quality control of pharmaceuticals are controlled by FDA, Federal Drug Administration.

Pharmaceutical analysis, today, is on the frontiers of Analytical Chemistry. Analysis in both bulk and dosage forms is important. The matrix in many cases may be extremely complex and therefore,
detection, determination and some form of separation is usually required. Usually methods should be comparable to or improvements over methods published from standard (B.P., U.S.P., I.P.) pharmacopeia laboratory.

All the drugs in their nature are divided into inorganic and organic. Both can be prepared from natural sources, or artificially i.e. synthetically. The enormous number of drugs used in medicine can be classified into two ways.

1. **PHARMACOLOGICAL CLASSIFICATION**: In this section, drugs are divided on the bases of their action on an organism (the heart, brain, lymphatic system, stomach, intestine etc.) Accordingly, drugs are divided into such groups as narcotics, soporifics, analgesics diuertics and local anaesthetics.

2. **CHEMICAL CLASSIFICATION**: Here, the drugs are divided according to their chemical structure and properties regardless of their pharmacological action. It is beyond the scope of this work to discuss all the types of drugs, however, few important class of compound used as drugs in medicine are discussed below.

**ALKALOIDS**: Alkaloids are substances mostly of plants and rarely of animal origin. In medicine alkaloids are sucessfully used as drugs in the treatment of
cardiovascular, nervous, alimentary tract and many other diseases. They are organic substances of basic nature because their molecule always contain nitrogen. The alkaloids are chiefly tertiary amines, and only some of them are secondary amines or derivatives of tetrasubstituted ammonium bases. Such class of drugs can be grouped into the following heads.

(i) pyridine and piperidine derivatives (labeline and its companions).
(ii) tropane derivatives (atropine, hyoscyamines, cocaine etc)
(iii) quinoline derivatives (quinoline, quidine etc).
(iv) isoquinoline derivatives (opium, alkaloids).
(v) indole derivatives (physostigmine, reserpine etc).
(vi) imidazol derivatives (pilocarbine, omeperazole).
(vii) purine derivatives (caffeine, theobromine, theophyllin etc.)

**ANTIBIOTICS**: Antibiotics are very important class of pharmaceuticals consisting of antibacterials, anti-infectives, antifungals, antiparasitics and antimicrobials. Antibiotics are organic compounds in their chemical nature. Antibiotics are chemical compounds derived from or produced by living organisms, which are capable, in small concentrations, of inhibiting the life processes of microorganism. (2).
The chemistry of antibiotics is so varied that a chemical classification is of little value. However, some antibiotics are the products of similar mechanisms in different organisms and that these structurally similar products may exert their activities in a similar manner. A number of antibiotics have in common a macrolide structure, that is, a large lactone ring. Erythromycin, oleandomycin are included in this family. Tetracyclin family represents a group of compounds very closely related chemically. Streptomycins, Kanamycins, neomycins, paramomycins and gentamycins are included into a family of compounds containing closely related amino sugar moieties. The antifungal antibiotics nystatins and amphotericins are examples of a group of conjugated polyenes. The pencillins and cephalosporins are antibiotics derived from amino acids. A large number of polypeptide compounds that exhibit antibiotic action include bacitracins, tyrothricins and polymixins.

**ANALGESICS**: A drug which brings about insensibility to pain without loss of consiousness is known as analgesic. There are numerous drugs which, in addition to possessing distinctive pharmacologic activities in other area, may also posses analgetic properties (3). The analgetic property may have direct or indirect effect.
Such class of drugs are grouped as sedatives (eg. barbiturates); muscle relaxants (eg. mephenesin, methocarbamol); tranquilizers (eg. meprobamate); morphine and related compounds (morphine, hydromorphone and codeine phosphate/sulphate etc); synthetic analgesics (pentazocaine, methotrimeprazine, methadone hydrochloride, nefopam etc.); antipyretic analgesics (salicylic acid derivatives, arylacetic acid derivatives, aniline and p-aminophenol derivatives, pyrazolone and pyrazolidinedione derivatives).

**CENTRAL NERVOUS SYSTEM DEPRESSANTS** : The agents that produce depressant effect on the central nervous system (4) as their principle pharmacological action, include the general anesthetics, hypnotic sedatives, skeletal muscle relaxants, tranquilizing agents and anticonvulsants. The general anesthetics (eg. ether) and hypnotic sedatives (eg. phenobarbital) produce generalized or non-selective depression on C.N.S. function. Many sedatives if given in large doses, produce anesthesia. Certain analgesics also produce depression on central nervous system.

**CENTRAL NERVOUS SYSTEM STIMULANTS** : Central nervous system is a complex network of subunits which act as conducting pathways between peripheral receptors and effectors, enabling man to respond to his environment. Drugs which have in common the property of
increasing the activities of various portions of the central nervous system are called (5,6) central nervous system stimulants (respiratory stimulants and analeptics). Carbonic acid is a most effective respiratory stimulant.

Analeptics, agents used to lessen narcosis brought about by excess of depressant drugs often stimulate a variety of nerve centres as well as many analeptic drugs are also pressor drugs because their stimulation of the vasomotor centre produce an increase in vasoconstriction.

There are many drugs (cocaine, atropine, amphetamines, ephedrine etc.) of varying pharmacologic classes, in addition to, their desired effect elicit a pronounced stimulatory effect on central nervous system.

**CARDIOVASCULAR AGENTS**: Agent used for their action on the heart or on other parts of the vascular system so that they modify the total output of the heart or the distribution of blood to certain parts of the circulatory system, are called cardiovascular agents (7). This class include cardiotonic drugs vasodilators, hypotensive drugs, antihyper cholesterolemic drugs and sclerosing agents (8). Certain drugs have a direct
action on cardiovascular system, and in addition, this class of drug that affect certain constituents of blood. The later include anticoagulant drugs, the hypoglycemic agents the thyroid hormones and the antithyroid drugs.

In this dissertation we have developed a new method for the determination of thyroxine sodium in drug formulations, therefore, a more detailed study has been done on the drug.

Thyroxine (9) is an active substance which is isolated from thyroid gland (usually the sheep). It is the tetra-iod-derivatives of amino acid.

\[ \text{THYROXINE SODIUM} \]

Thyroxine sodium is a crude salt. It has the several thousand times the activity of a fresh gland. Defatted thyroid substance has been used for many years as a replacement therapy in thyroid gland deficiencies (10-12). The efficacy of the thyroid gland is known to depend on its thyroglobulin content which is an iodine containing globulin. Kendall in 1916 obtained thyroxine as a crystalline derivative and found that it showed as much the same action as the
whole thyroid substance. Recent studies show that even more potent iodine containing hormone exist, which is known as triiodothyronine. Thyroxine is supposed to be the storage form of the hormone, while the triiodothyronine is the circulatory form. In the blood thyroxine is more firmly bound to the globulin fraction than is triiodothyronine, which can then enter the tissue cells.

**LEVOTHYROXINE SODIUM U.S.P.** : sodium L-3-[4-(4-hydroxy-3, 5 diodophenoxy) -3, 5-diodophenyl] alanine, sodium L-3-, 3, 5 5, -tetraiodothyronine. This compound is the sodium salt of levoisomer of thyroxine, which is an active physiological principle obtained from the thyroid gland of domesticated animals use for food by man. It is also prepared synthetically. The salt is light yellow tasteless odourless powder. It is hygroscopic but stable in dry air at room temperature. It is soluble in alkali hydroxides, 1:275 in alcohol and 1:500 in water to give a pH of ≈ 8.9.

Levothyroxine sodium is used in replacement therapy of
deceased thyroid function (hypothyroidism). In general, 100 μg of levothyroxine sodium is clinically equivalent to thyroxine 30-60 mg of thyroid U.S.P.

**LIOTHYRONINE SODIUM U.S.P. CYTOMEL**: Sodium L-3 [4-(4-hydroxy-3-iodophenoxy)-3,5-diodophenyl] alanine is the sodium salt of L-3,3,5, triiodo thyronine. It occurs as a light tan, odourless, crystalline powder slightly soluble in water or alcohol and has a specific rotation of +18° to +22° in acid (HCl) alcohol.

![Chemical Structure of Liothyronine](image)

**LIO THYRONINE SODIUM**

Liothyronine occurs in vivo together with levothyroxine it has the some qualitative activities as thyroxine but is more active. It is absorbed readily from the gastrointestinal tract, is cleared rapidly from the bloodstream and is bound more closely to the plasma proteins than the thyroxine, probably due to the less acidic phenolic hydroxyl group.

It is used same as that of levothyroxine, including treatment of metabolic insufficiency, male infertility and certain gynaecological disorder.
ANALYSIS OF DRUG: The methods for analyzing medicinal agents are divided into physical, chemical, physico-chemical and biological once.

Physical methods of analysis involve the studying of the physical properties of a substance. They include the determination of solubility, transparency and the degree of turbidity, colour, density or specific gravity, melting, boiling and freezing points.

Chemical methods used for the analysis of drugs are based on chemical reactions. They include the determination of ash content, the hydrogen ion concentration (pH) of medium and characteristic numerical indices of oils and fats. Quantitative and qualitative analysis of drugs are generally carried out with respect to functional groups.

Physicochemical methods are used to study the physical phenomenon that occur as a result of chemical reaction. Among the physicochemical method of analysis, photometry including photocolormetry and spectrophotometry, electrochemical and chromatographic methods. Recently various kinds of chromatography (column, paper, thin layer, gas, gas-liquid) and of photometric methods based on the absorption of light by the analyte have found there use in pharmaceutical analysis. NMR, PMR, and mass spectrometry with gas chromatography
are the most important powerful analytical methods used for the analysis of drug.

Biological method of analysis characterizes the pharmaceutical effect of the drug. Biological tests are conducted on animals and less frequently separate isolated organs or part of organs of animals. The effect of drug is expressed in A.U. (activity units) and is determined by the comparison with the activity of a reference substance. The biological methods are very sensitive. Alkaloids, vitamins and antibiotics, still reveal activity when diluted millions of times.

Photocolorimetric methods of analysis are based on measuring the absorption of non-monochromatic light by coloured compounds, in the visible part of the spectrum. If the analytes are colourless, they are converted into coloured compounds by reaction with suitable reagent. In the visual method, called colorimetry, the intensity of the colour of analyte solutions is compared to that of standard solutions if the concentration of substance is known.

In the recent years, pharmaceutical analysis has been characterized by the use of methods such as flame photometry and differential spectrophotometry in the qualitative analysis of drugs. These methods enable one to determine the large amount of individual components of a mixture because the absorbance of the
analyte solution is measured not relative to the pure solvent (or a solution of reagent), but relative to a reference solution containing a known amount of the analyte.

For the determination of specific penicillin in pharmaceutical products spectrophotometric methods have been developed. Spectrophotometry has been used for the determination of amoxycillin (13, 14, 15). Chromatographic procedure have appeared for ampicillin, amoxycillin and pencillin G (16). A diazotization spectrophotometric method has been used to characterize amoxycillin by reaction with aromatic amines and amino acids in bulk drugs and also in capsules, tablets and syrups. A polymer immobilized enzyme optrode for penicillin has been introduced for determination of penicillin (17).

\[ ^1H \text{ NMR has been applied most frequently to the analysis of common analgesics (18,19) } ^{19} \text{F- androgenic blockers (20), and the antineoplastic melchlorothamine hydro chloride (21) and carbachol (22). Near IR analysis has been used to assay tablets containing acetaminophen in combination with caffeine (23). The procedure was adopted to cough cold syrups. Sulpurar based cardiovascular drugs, antiallergy or anti inflammatory drugs and phenothizine have been analyzed by } ^1H \text{ and } ^{13}C \text{ NMR spectroscopy (24, 25).} \]
A variety of cololimetric procedures have been developed for analgesics, antipyretics and antiinflammatorics. Acetaminophen has been measured by itself (26, 27) and in combination with other compounds such as salicylamide and oxyphenbutazone (28, 29). Methods have also been published for aspirin (30, 31) Novalgin (32, 33) dipyrone (34), indomethacin (35), naproxen (36), and oxyphenbutazone (37).

Iron reagents have been used to assay the chlorpheniramine and pheniramine (38), meperidine (39), nifedipine (40), nitrazepam (41), and nitroxazepine HCl (42). A simple and sensitive ion-exchange method has also been published for the detection of chlorpromazine in human urine (43). Other types of colorimetric procedures have also been developed for the analysis of pharmaceuticals. Qureshi and co-workers have recently developed spectrophotometric methods for the determination of paracetamol (44) and acetaminophen (45).

During the last 5 years a variety of electroanalytic, chromatographic and spectroscopic assay have appeared for the selective determination of vitamin C. Spectroscopic methods are based on the oxidation of ascorbic acid. A method based on
inhibition of peroxides activity has been described (46). An extraction spectrophotometric procedure have also been developed for the determination of vitamin C (47) in drug formulations, urine and fruit guices with potassium iodate.

Colorimetric procedures have been found based on either the formation of charge transfer complex between the active and iodine (48, 49), nitrosation of active (50, 51) or reaction of or, active with a diazotized reagent (52, 53). Recently a review on the analysis of pharmaceuticals and related drugs have been appared (54), representing a survey of pharmaceutical analysis and related methodology.

The determination of iodinated thyronines is of great importance. As the concentration of iodinated thyronines in plasma are very low; only trace method can be applied for the determination. Radioimmunoassay (54, 55), fluorescence immunoassay (57), enzyme immunoassay (58) and competitive protein binding assay (59), are most frequently used for the determination of thyroxine sodium. A selective determination of enatiometric thyromines is not possible with these techniques. The reaction of L-thyroxine antibodies with D-thyroxine for a radioimmunoassay was found to be 100% (60).
Recently the determination of D.L-triiodothyronine and D.L-thyroxine by derivatization liquid chromatography has been described (61). The D.L-thyronines are derivatized with tertiarybutyloxy-L-leucine-N-hydroxysuccinimide ester and the resulting diasteromeric peptides are separated by ion pair chromatography on reversed phase columns. This method was successfully applied to the control of purity of pharmaceuticals and its applications in human plasma seemed to be promising. Chromatographic separations of thyroid hormones can be carried out using gel chromatography (62, 63), ion-exchange chromatography (64, 65), gas chromatography (64), high performance liquid chromatography HPLC (65).

Van der shiji and I, verms et al (66) have reported the effect of temperature on free thyroxine measurements and its analytical, and clinical consequences. Thyroxine and 3,5,3 - triiodothyroxine has been determined by sub and super equivalence isotope dilution analysis (67) at trace level (160 and 20 mg/ml) and radioimmunoassay (68), for measurement of free thyroxine in serum. New homogenous enzyme immunoassay for the determination of thyroxine and thyroxine uptake have been developed recently (69). A multicentre evaluation of a two step enzyme immunoassay (70) of free thyroxine have also been developed.
In this dissertation, we describe the determination of thyroxine sodium in pure and dosage forms using well known diazotization reaction. The method is based on the reaction of amino group with nitrous acid in an ice-cold medium.
REFERENCES


7. R.F. Doerge, ibid, pp. 594.


CHAPTER II

SPECTROPHOTOMETRIC DETERMINATION
OF
THYROID SODIUM
INTRODUCTION

The human thyroid gland produces L-isomers of thyroxine sodium and D-isomer is being produced synthetically. D-thyroxine has been found to reduce the cholestrol level significantly, by increasing the rate of degradation and oxidation of cholestrol (1-4). D-thyroxine has been described to cause the supression of thyrotropine.

Levothyroxine sodium i.e. thyroxine sodium (L-3-3', 5-5'-tetralochothyronine), which is an active physiologic principle obtained from the thyroid gland of domesticated animals used for food by man (5). It is used in replacement therapy of decreased thyroxine function (hypothyroidism).

The British pharmacopoeia 1980 estimated (6) thyroxine sodium by applying the oxygen-flask combustion for iodine. Several other analytical procedures have been reported for the determination of thyroxine sodium, include polarography (7), gas chromatography (8,9), thin layer chromatography (10,6) and high performance liquid chromatography (11, 12) and isotope dilution analysis (13). Radioimmunoassay (14, 15, 16), enzyme immunoassay (17,18), colourimetric (19) and fluorometric (20) procedure for the determination of thyroxine sodium have also been developed. An spectrophotometric method (21) for the determination of
thyroxine sodium with p-benzoquinone have been appeared recently.

In this investigation a spectrophotometric method for the determination of thyroxine sodium based on the reaction of nitrous acid with functional group of thyroxine sodium, in an ice-cold medium (0-5°C) has been described.
**EXPERIMENTAL**

Reagents and Apparatus: A 1 mg/ml solution of thyroxine sodium (Glaxo India) was prepared in a alcohol-dimethyl sulfoxide mixture (50:50, v/v). A 0.2 M sodium nitrite (freshly prepared daily) and 1.5 M hydrochloric acid solutions were prepared in high purity conductivity water. All other chemicals used were of analytical grade.

A Bausch & Lomb Spectronic-20 spectrophotometer was used for absorptiometric measurements.

Procedure: To a precooled solutions of 3.5 ml sodium nitrite and 1.5 ml HCl in a 10 ml volumetric flasks, added accurately measured volume of thyroxine sodium (0.1-1.6 ml) and mixture was then diluted upto the mark with 50:50 alcohol : DMSO mixture (v/v). The flask were then kept for 15 minutes in ice-cold bath for the complete development of yellow colour and absorbances were read at 410 nm against a reagent blank. Calibration graph is shown in figure. 3
RESULTS AND DISCUSSION

Nitrous acid has been proved to be useful reagent for the determination of amines and amino acids. The reaction is carried out by adding aqueous solution of sodium nitrite to a cold aqueous solution of amine or amino acids in dilute mineral acids (22).

Thyroxine sodium with structural formula sodium-L-3 \([4-(4\text{-}\text{hydroxy-3,5-diiodophenoxy})\text{-}3,5\text{-diiodophenyl}]\) alanine, or sodium L-3, 3', 5,5'-tertraiodothyroxine is the levoisomer of thyroxine.

![Structural formula of thyroxine sodium](image)

LEVOTHYROXINE SODIUM

The reaction of thyroxine sodium with nitrous acid involves the possible kinetic mechanism of the following functional groups.

1. Involvement of aliphatic primary amine \(-\text{NH}_2\) group.
2. Carboxylic group
3. Hydroxy group
4. Iodide group

In the above groups the most effective activation involves the action of nitrous acid on \(-\text{NH}_2\) group. The rest of the groups do not have the condusive conditions to be activated. We therefore decided to discuss a tentative mechanism of aliphatic primary amines with nitrous acid. However, the product formed is extremely unstable and there-
fore the possibility of such reaction is ruled out. In another approach the formation of nitrosamine which is characteristically pale yellow appears to be more positive. Although it is also unstable but the possibility of making it stable under experimental conditions can not be ruled out.

The reaction is quantitative one, involving the measurement of intensity of yellow colour using alcohol: DMSO (1:1); DMSO has a boosting effect on increasing colour intensity. The effect of solvent on equilibrium constant (K), the heat of formation, and the molar extinction coefficient (ε) has been studied on the formation of complexes of nitro compounds. Dimethyl sulphoxide plays an important role to enhance the colour intensity (23) of these compounds and the most versatile example of the use of DMSO, is the detection of nitro compounds, where the sensitivity of the identification is increased tremendously (24).

Nitrous acid is unstable and is usually prepared by mixing a freshly prepared solution of NaNO₂ (sodium nitrite) with appropriate concentration of hydrochloric acid at 0–5°C. These conditions provide a source of NO which is transferred readily to the nucleophilic nitrogen of amine.

\[
\text{HONO} + \text{H} \rightarrow \text{H}_2\text{O} + :\text{N}=\text{O}
\]

\[
\text{N}^+ + :\text{N}=\text{O} \rightarrow \text{N}^+\text{N}=\text{O}
\]

In the reaction of primary aliphatic amine present in throxine sodium, formation of N-nitrosamine takes place as follows.
STUDY OF THE PROPOSED EXPERIMENTAL CONDITIONS

Under the proposed experimental conditions 0.8 ml of 1.5 M HCl and 2.0 ml of 0.2M sodium nitrite (Table 1 and 2) was found sufficient to produce high intensity coloured product for 0.5 ml of drug. The results are shown in Fig. 2 and 3. Beer's law is obeyed in the concentration range of 10 µg/ml- 160 µg/ml of thyroxine sodium with apparent molar absorptivity of $3.56 \times 10^3$ L mol$^{-1}$ cm$^{-1}$.

To test the reproducibility of the procedure 10 replicate determinations of 0.5 ml and 1.0 ml thyroxine sodium were done. The relative standard deviation was found to be 0.65% and 0.85% for 0.5 ml and 1.0 ml of thyroxine sodium respectively. Statistical analysis (Table 3) of the result obtained by proposed method and by other spectrophotometric method (21) showed no significant difference between the performance of the two methods.

Interferences of various ingredients used for the preparation of thyroxine sodium tablets were checked. The results are shown in table 4 and no interference was found for these ingredients.

The work is under progress and its application for the analysis of commercial thyroxine sodium tablets is under study.
TABLE 1. Effect of varying volume of HCl on the formation of thyroxine sodium complex.

<table>
<thead>
<tr>
<th>Amount of Drug taken (ml)</th>
<th>Amount of HCl (1.5 M) (ml)</th>
<th>Amount of NaNO&lt;sub&gt;2&lt;/sub&gt; (0.2M) (ml)</th>
<th>Absorbance at 410 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.2</td>
<td>3.5</td>
<td>0.10</td>
</tr>
<tr>
<td>0.5</td>
<td>0.4</td>
<td>3.5</td>
<td>0.18</td>
</tr>
<tr>
<td>0.5</td>
<td>0.6</td>
<td>3.5</td>
<td>0.26</td>
</tr>
<tr>
<td>0.6</td>
<td>0.8</td>
<td>3.5</td>
<td>0.27</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>3.5</td>
<td>0.27</td>
</tr>
<tr>
<td>0.5</td>
<td>1.2</td>
<td>3.5</td>
<td>0.27</td>
</tr>
<tr>
<td>0.5</td>
<td>1.4</td>
<td>3.5</td>
<td>0.27</td>
</tr>
<tr>
<td>0.5</td>
<td>1.5</td>
<td>3.5</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Temperature ice cold bath (0-5°C).
### TABLE 2. Effect of varying volume of NaNO₂ on the formation of thyroxine sodium complex.

<table>
<thead>
<tr>
<th>Amount of drug taken (ml)</th>
<th>Amount of NaNO₂ (0.2M) (ml)</th>
<th>Amount of HCl (1.5M) (ml)</th>
<th>Absorbance at 410 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>1.5</td>
<td>0.22</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>1.5</td>
<td>0.24</td>
</tr>
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<td>0.5</td>
<td>1.5</td>
<td>1.5</td>
<td>0.26</td>
</tr>
<tr>
<td>0.5</td>
<td>2.0</td>
<td>1.5</td>
<td>0.27</td>
</tr>
<tr>
<td>0.5</td>
<td>2.5</td>
<td>1.5</td>
<td>0.27</td>
</tr>
<tr>
<td>0.5</td>
<td>3.0</td>
<td>1.5</td>
<td>0.27</td>
</tr>
<tr>
<td>0.5</td>
<td>3.5</td>
<td>1.5</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Temperature - ice cold bath (0-5°C).
TABLE 3. Statistical analysis of proposed method and other spectrophotometric method.

<table>
<thead>
<tr>
<th>Method</th>
<th>Beer's law obeyed in conc. range</th>
<th>Apparent molar absorptivity</th>
<th>Relative S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed</td>
<td>10 - 160 ug/ml</td>
<td>$3.56 \times 10^3$</td>
<td>$+0.65%$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$1 \text{ mol}^{-1} \text{cm}^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Reference (21)</td>
<td>40 - 200 ug/ml</td>
<td>$2.9 \times 10^3$</td>
<td>$+0.36%$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$1 \text{ mol}^{-1} \text{cm}^{-1}$</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4. Interferences of foreign substances for the estimation of thyroxine sodium (50 \( \mu g/ml \))

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Foreign Substance</th>
<th>Maximum tolerable amount (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Glucose</td>
<td>32.428</td>
</tr>
<tr>
<td>2.</td>
<td>Fructose</td>
<td>36.432</td>
</tr>
<tr>
<td>3.</td>
<td>Lactose</td>
<td>72.464</td>
</tr>
<tr>
<td>4.</td>
<td>Sucrose</td>
<td>68.46</td>
</tr>
<tr>
<td>5.</td>
<td>( \text{Ca}^{2+} )</td>
<td>8.01</td>
</tr>
<tr>
<td>6.</td>
<td>( \text{CO}_3 )</td>
<td>12.0</td>
</tr>
<tr>
<td>7.</td>
<td>Starch</td>
<td>32.48</td>
</tr>
</tbody>
</table>
Figure 1: Absorption spectrum of thyroxine

Wave length (nm)

Absorbance
FIG. 2: Effect of varying volume of HCl (1.5M) on the formation of thyroxine sodium complex, temperature ice-cold bath (0-5°C).
FIG 3: Effect of varying volume of NaNO₂ (0.2M) on the formation of thyroxine sodium complex at temperature ice-cold water bath (0-5°C).
Absorbance

μg/ml of Thyroxine Sodium

FIG. 4: Calibration curve of thyroxine sodium complex at 410 nm. temperature ice-cold bath (0-5°c).

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DS 2573
REFERENCES