

Spectrophotometric determination of thyroxine sodium with Folin Ciocalteu reagent in bulk and pharmaceutical formulations

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ABSTRACT

A simple, sensitive and reproducible spectrophotometric method was developed for the determination of thyroxine sodium in bulk and in pharmaceutical formulations. This method is based on the measurement of blue coloured species formed when phosphomolybdic acid present in Folin Ciocalteu reagent is reduced by the thyroxine sodium in the presence of sodium carbonate, having maximum absorption at 730nm. Beer's law is obeyed in the range of 5-25 µg/mL. Results of analysis were validated statistically and by recovery studies. This method was successfully employed for the determination of thyroxine sodium in various pharmaceutical preparations and biological samples.

Key words: Thyroxine sodium, Visible Spectrophotometric determination, Beer's Law, Sandell's sensitivity.

INTRODUCTION

Thyroxine hormones (3,5,3',5'-tetraiodothyronine, T₄, and 3,5,3'-triiodothyronine, T₃) secreted by the pituitary gland are compounds having major biological roles since they are critically important for normal development of the central nervous system in infants, skeletal growth and maturation in children, as well as for the normal function of multiple organ systems in adults. These important hormones are synthesized from L-tyrosine residues in thyroglobulin, a dimeric glycoprotein that constitutes the bulk of the thyroid follicles¹. Metabolically, these hormones increase the oxygen uptake by mitochondria and heat production; in physiological concentrations both hormones increase synthesis of RNA and protein; in higher doses they act catabolically, causing negative nitrogen balance and mobilization of fat deposits².

Numerous methods, such as immunoassays³⁻⁶, electrochemical^{7,8}, HPLC^{9,1}, GC-MS¹⁰, fluorescence¹¹ and electrochemiluminescence¹² have been reported for the determination of thyroxine in body fluids and pharmaceutical preparations. Beside these, flow injection methods have also been reported for the determination of thyroxine based on various detection systems^{13,14}. The flow injection-spectrophotometric method has been reported for the determination of thyroxine to be an inhibitor of immobilized glutamate dehydrogenase¹³. The change in NADH absorbance at 340 nm in the presence of an enzyme and thyroxine is measured online related to the percent inhibition. Another flow injection-chemiluminescence (FI-CL) method based on quenching of the emission intensity by thyroxine has been reported¹⁴. Exploiting the various functional groups present in the above compounds, the authors have made attempts in this direction and succeeded

in developing a spectrophotometric method for the determination of thyroxine sodium in bulk and pharmaceutical formulations.

EXPERIMENTAL

Apparatus

Systronics UV – Visible Double beam spectrophotometer model 2201.

Materials and Reagents

All the chemicals used were of analytical grade. All the solutions were freshly prepared in distilled water.

- Folin ciocalteau reagent: This reagent is commercially available. The original stock reagent was diluted to 1:2 ratio with water.
- 20 % sodium carbonate (w/v): Prepared by dissolving 20gm of sodium carbonate in 100ml of distilled water.

Preparation of standard and sample solution

Accurately weighed 100mg of thyroxine sodium was dissolved in 100mL of distilled water to give a concentration of 1mg/mL. The final concentration was brought to 100 µg/mL.

Assay procedure

To a series of 10 mL volumetric flasks containing different samples of Thyroxine sodium ranging from 0.5-2.5 mL (1mL = 100 µg), 1:2 diluted Folin Ciocalteau reagent and (1.5 mL) and 20% sodium carbonate (1mL) were added. The solution was made up to the mark with distilled water and kept aside for 20 min. The absorbance of the blue colored solution was measured at 730 nm against the corresponding reagent blank. The amount of Thyroxine sodium was computed from the corresponding calibration curve.

RESULTS AND DISCUSSION

The proposed method was based on the reduction of phosphomolybdic acid present in Folin Ciocalteau reagent by thyroxine sodium in the presence of sodium carbonate to give blue color. The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity for these methods are

presented in Table-1. The regression analysis using the method of least squares was made for the slope (a) and intercept (b) obtained from different concentrations are summarized in Table-1. The precision and accuracy were found by analyzing five replicate samples containing known amounts of the drug and the results are summarized in Table-1. The accuracy of the method was ascertained by comparing the results obtained with the proposed and reference methods in the case of formulation are presented in Table-2. As an additional check on the accuracy of these methods, recovery experiments were performed by adding known amounts of pure drug to pre-analyzed formulation and percent recovery values obtained are listed in Table-2. Recovery experiments indicated the absence of interferences from the commonly encountered pharmaceutical additives and excipients.

Thus the proposed method was simple and sensitive with reasonable precision and accuracy. These can be used for the routine determination of thyroxine sodium in quality control analysis.

Table 1: Optical characteristics, precision and accuracy of proposed method

Parameters	Method
λ_{\max} (nm)	730
Beer's law limit (µg/ mL)	5 - 25
Sandell's Sensitivity (µg/cm ² /0.001 abs. unit)	0.0375
Molar absorptivity(Litre.mole ⁻¹ .cm ⁻¹)	2.364 ×10 ⁴
Stability of Color (hours)	20
Regression equation (Y)*	
Intercept (a)	0.0074
Slope(b)	0.00297
% RSD [§]	0.97
% Range of errors (95% confidence limits):	
0.05 significance level	0.81
0.01 significance level	1.199

* Y= a + bx, where Y is the absorbance and x is the concentration of thyroxine sodium in µg/ mL

§ for five replicates

Table 2: Assay and recovery of thyroxine sodium in pharmaceutical formulations

Formulations	Labelled amount(μg)	Recovery by reference method*(%)	Recovery by proposed methods (%) **
Tablet I	100	99.9	99.8
Tablet II	100	99.8	98.6
Tablet III	100	98.7	99.2

* Reference method was UV method developed in the laboratory.

** Recovery amount was the average of five determinants

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