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Development and Validation of a Spectrophotometric Method for Estimation of Thyroxine Sodium in Bulk and in Tablet Dosage form

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A simple and sensitive spectrophotometric method for the determination of thyroxine sodium was developed. This method was based on condensation of the primary amino group of thyroxine sodium with acetyl acetone and formaldehyde producing a yellow coloured product, which is measured spectrophotometrically at 430nm. The colour was stable for at least 5 hours. Beer's law was valid within a concentration range of 10 - 50 μg/mL. No interference was observed in the presence of common pharmaceutical excipients. The validity of the method was tested by analyzing thyroxine sodium in its pharmaceutical preparations. Good recoveries were obtained. This method was successfully employed for the determination of thyroxine sodium in various pharmaceutical preparations and biological samples.

Key words: Tyroxine sodium; spectrophotometric analysis; acetyl acetone/ formaldehyde reagent; Sandell's sensitivity.

Thyroxine hormones (3,5,3',5'-tetraiodothyronine, T_4, and 3,5,3'-triiodothyronine, T_3) 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 follicles[1]. 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 deposits[2].

Numerous methods, such as immunoassays[3–6], electrochemical[7,8], HPLC[9,1], GC-MS[10], fluorescence[11] and electrochemiluminescence[12] 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

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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 dehydrogenase[13]. 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 reported[14]. 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.

1. Acetylacetone : 8.4% v/v solution was freshly prepared by mixing 2.1 ml of acetylacetone with 10 ml of acetate buffer (pH 5) and diluted to 25 ml with distilled water.
2. Formaldehyde (34 - 40%): Twenty percent solution was prepared by mixing 5 ml of formaldehyde with 10mL of acetate buffer (pH 5) and diluted to 25 ml with distilled water.
3. Acetate buffer (pH 5): Prepared by dissolving 13.6 g of sodium acetate and 6 ml of glacial acetic acid in sufficient water to produce 1000 ml.

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 different aliquots of standard solutions containing 0.1-0.5 mg thyroxine sodium, 1 ml of 8.4% v/v acetylacetone solution and 0.5 ml of 20% formaldehyde reagents were added in a series of 10 ml test tubes. The mixture was heated for 5 min then cooled and diluted to 10 ml with distilled water. The absorbance was

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

Parameters	Method
λ_{max} (nm)	430
Beer's law limit (µg/ mL)	10 - 50
Sandell's Sensitivity (µg/cm^2/0.001 abs. unit)	0.121
Molar absorptivity(Litre.mole^{-1}.cm^{-1})	0.729×10^4
Stability of Color (hours)	5
Regression equation (Y)*	
Intercept (a)	0.0068
Slope(b)	0.00082
% RSD$^{\$}$	0.030
% Range of errors (95% confidence limits):	
0.05 significance level	0.025
0.01 significance level	0.038

* Y= a + bx, where Y is the absorbance and x is the concentration of thyroxine sodium in µg/ mL; $ for six 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	99.9
Tablet II	100	99.8	99.1
Tablet III	100	98.7	98.3

* Reference method was UV method developed in the laboratory.
** Recovery amount was the average of six determinants

measured at 430 nm using the experiment as a blank. The amount of Thyroxine sodium was computed from the corresponding calibration curve.

RESULTS AND DISCUSSION

Hantzsch reaction is a known condensation reaction that was reported in the literatures as a useful pathway for pyrrole and pyridine synthesis[15]. In the same manner, acetylacetone together with formaldehyde react with aliphatic amines by Hantzsch reaction forming a yellow product that can be measured spectrophotometrically. The proposed method for determination of thyroxine sodium was based on Hantzsch condensation reaction using acetylacetone as β-diketone and formaldehyde as an aldehyde to form a yellow colored condensation product. The formed yellow color showed maximum absorption at 430 nm.

The optical characteristics such as absorption maxima, Beer's law limits, molar absorptivity and Sandell's sensitivity for this method was 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 six replicate samples containing known amounts of the thyroxine sodium 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 pharmaceutical dosage forms.

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