RESEARCH PAPER

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New Spectrophotometric Methods for the Determination of Pyrithioxine

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Three simple and sensitive spectrophotometric methods for the determination of Pyrithioxine (PYT) are described. The first method (Method A, λ_{max} : 470 nm) is based on the oxidative coupling reaction of PYT with 4-Aminophenazone (4AP). The second one (Method B, λ_{max} : 495 nm) depends on the formation of ion-association complex of PYT with Tropaeolin OOO (TPOOO) which is extracted into chloroform. The third one (Method C, λ_{max}: 600 nm) is based on the reduction of fe^{3+} to fe^{2+} by the PYT, which forms the coloured complex with 2,4,6-tripyridyl-s-triazine (TPTZ). These methods are extended to the analysis of pharmaceutical formulations and results compared with the U.V. reference method.

PYRITHIOXINE[1] is a neurotropic agent, reduce permeability of blood brain barrier to phosphate ions. The reported methods in the literature for the determination of PYT are HPTLC[2], polarography[3,4], HPLC[5,6] and spectrophotometry.[7-11] Most of the spectrophotometric methods possess one or more deficiencies such as a low λ^{max} value, time consuming, less sensitive and the necessity of preliminary treatment (reduction). It is, therefore, of interest to develop fast and simple procedures for the determination of PYT in pharmaceutical formulations.

This paper describes the spectrophotimetric methods for the determination of PYT based on the oxidative coupling reaction with 4AP in the presence of potassium ferricyanide or formation of ion-association complex with TPOOO (C.I. No.: 14600) or reduction of ferric ion to ferrous ion yields a coloured complex with TPTZ.

EXPERIMENTAL

A Systronics model 106 digital spectrophotometer equipped with 1cm matched glass cells and an Elico LI-120 digital pH meter were used for all absorbance and pH measurements.

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All solutions were prepared in doubly distilled water and all Chemicals of analytical grade were used. Aqueous solutions of 4AP (1%, Ferak), potassium ferricyanide (4%, Loba), TPOOO (0.2%, Fluka) and 0.1M HCl and Methanolic solutions of TPTZ (0.312%, Loba) and $FeCl_3$ (0.27%, Loba) were used. A buffer solution (pH : 10) was prepared by dissolving 0.34g of ammonium chloride with distilled water and made up to 100ml with water after addition of 2.9ml of ammonia solution.[12]

PYT pure drug solutions 100 μg/ml and 50 μg/ml in water and 0.1M HCl were used.

Procedure

Method A : Aliquots of the standard drug solution containing 50- 500 μg of PYT, 1 ml of 4AP solution, 1 ml of buffer solution (pH δ 10) and 1 ml of potassium ferricyanide were successively added to 25 ml volumetric flasks. The contents of each flask were mixed well and made up to volume with distilled water. The absorbance was measured against a reagent blank at 470 nm with in the stability period (up to 15 min.). The amount of PYT present was calculated from calibration graph.

Method B: A portion of standard drug solutions containing 10-150 μg of PYT were transferred into a 125 ml separating funnel. Then 6.0 ml of 0.1M HCl and 2.0 ml of TPOOO were added and total volume of the aqueous phase was adjusted to 15 ml with distilled water. The 10 ml of chloroform was added to each and the contents were shaken for 2 minutes. The absorbance of the separated cloroform layer was measured at 495 nm against a reagent blank with in the stability period (1min. - 10hrs.). The amount of PYT present was calculated from calibration graph.

Method C: In to a 10ml volumetric flasks containing 20-200 μg pf PYT, 0.5 ml of $FeCl_3$, 1.0 ml of TPTZ were added and volume was brought to 8 ml with distilled water and kept in boiling water bath for 40 minutes. The flasks were removed from the bath, cooled and made up to the mark with distilled water. The absorbance of the coloured complex was measured at 600 nm against a reagent blank with the stability period (1min. - 60min.).The amount of PYT present was computed from the calibration graph.

Analysis of pharmaceutical formulations

Tablets: An amount of the powdered tablets, equivalent to 100 mg of PYT was transferred into a 100ml volumetric flask. About 30 ml distilled water was added and warmed on boiling water bath for 5 minutes with occasional shaking. The solution was cooled to room temperature, made up to 100 ml with distilled water and filtered. Stock solution was further diluted to make working solutions (100 μg/ml and 50 μg/ml) with distilled water.

Injection: five ampoules were pooled and an amount of the freeze dried PYT equivalent to 100 mg of PYT was weighed and dissolved in water. Stock solution was further diluted to make working solutions (100 μg/ml) and 50 μg/ml) with distilled water.

The above working solutions of tablets and injection were analyzed using the procedures of method A, B or C. The amount of PYT present in the sample was computed from the calibration graph.

Suspension: Suspension was thoroughly shaken and volume equivalent to 100 mg of PYT was transferred into a 100 ml volumetric flask. Suspension was dissolved in 10 ml of HCl and made up to 100 ml with water. Stock solution was further diluted to make working solutions (100 μg/ml and 50 μg/ml) with 0.1M HCl and analyzed as according to method A or Method B. The amount of PYT was computed from the calibration graph.

RESULTS AND DISCUSSION

Optimum operating conditions used in the procedures were established adopting variation of one variable at a time (OVAT)[13] method. The optical characteristics such as Beer's law limits (μg/ml), molar absorptivity (1 $mole^{-1}$ cm^{-1}_ and Sandell's sensitivity (μg/cm^2/0.001 absorbance unit) were found to be 2-20, 1.42 x 10^4, 0.032; 1-10, 2.75 x 10^4, 0.017 and 2-20, 1.79 x 10^4, 0.026 for methods A, B and C respectively. The slope, intercept and correlation coefficients obtained by linear least squares treatment of the results were found to be 3.08 x 10^{-2}, 0.062 x 10^{-2}, 0.9999; 6.03 x 10^{-2}, - 0.274 x 10^{-2}, 0.9999 and 3.90 x 10^{-2}, - 0.182 x 10^{-2}, 0.9999 for methods A, B and C respectively. The precision and accuracy of the method was tested by estimating six replicate samples of PYT with in the beer's law limits. The percent standard deviation and the percent range of error at 95% confidence level have been found to be 0.43, 0.45; 0.56, 0.59 and 0.36, 0.37 for methods A, B and C respectively.

These methods were applied for the determination of PYT in tablets, injection and suspension. In case of tablets and injection good results were obtained with all three methods. But in case of suspension, method C gave high values. A serious interference was observed due to the presence of

Table 1: Assay of Pyrithioxine in Pharmaceutical Formulations

Formulations	Labelled amount	Found by proposed method*			Found by** reference method	Recovery of proposed methods, %		
		A	B	C		A	B	C
Tablets	200mg/tab	198.3	199.5	199.5	199.1	99.7	100.3	99.3
Tablets	200mg/tab	199.2	199.9	200.0	200.5	99.6	99.8	99.8
Injection	200mg/amp	199.8	200.6	200.4	199.7	100.3	99.1	100.2
Injection	200mg/amp	201.6	201.2	201.3	201.9	99.8	99.8	99.6
Suspension	100mg/5ml	98.9	99.6	——	99.3	99.3	100.2	——
Suspension	100mg/5ml	99.8	100.3	——	100.2	99.8	100.7	——

*: Average of six determinations
**: U.V. reference method.[10]

excipients which appear to react with $FeCl_3$ slowly. Method C was extended only for the analysis of tablets and injection. Recovery experiments were performed using the standard addition method, results are shown in Table 1. The results obtained for pharmaceutical preparations were compared with U.V. reference method[10] and shown in **Table 1.**

The proposed methods are simple and sensitive with good precision and accuracy and can be used for the routine quality control analysis of PYT in pure from as well as pharmaceutical formulations depending upon the availability of chemicals and nature of other ingredients present in the sample.

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