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Importance of the Oxidation Reaction of Sodium Metaperiodate for Spectrophotometric Assay of Tylosin

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A simple and sensitive spectrophotometric method by exploiting the importance of the oxidation reaction of sodium metaperiodate for the assay of tylosin has been described. This method is based on the oxidation of tylosin with excess of periodate and estimating the dialdehyde formed with 3-methyl-2-benzothiazolinone hydrazone (MBTH). The recoveries range from 99.06-100.86%.

Tylosin (TS)[1-3] is a macrolide antibiotic used in veterinary medicine in the prophylaxis and treatment of various infections caused by susceptible organisms. Literature survey has revealed that little attention was paid in developing visible spectrophotometric methods[4-6]. The presence of vicinal diol group in TS render it vulnerable to stoichiometric attack by periodate giving a dialdehyde. The present communication describes a method based on the oxidative coupling of the dialdehyde (from TS) with MBTH to yield a blue cationic dye[7,8].

A Milton Roy Spectronic 1201, UV/Vis spectrophotometer with 1 cm matched quartz cells was used for the absorbance measurements. All the chemicals used were of analytical grade and all the solutions were prepared with double distilled water. Aqueous solutions of sodium metaperiodate (BDH, 9.35×10^{-3} M), MBTH (Fluka, 8.56×10^{-3} M) and acetic acid (BDH, 3.49 M) were prepared.

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A 1 mg/ml solution was prepared by dissolving 100 mg of pure TS in 100 ml of 0.1 M HCl and this stock solution was further diluted with distilled water to obtain the working standard solution of concentration of 50 µg/ml. An accurately weighed amount of tablet powder or measured volume of injection equivalent to 100 mg of TS was extracted with isopropanol (4x15 ml) and filtered. The combined filtrate was evaporated to dryness and the residue was dissolved in 100 ml of 0.1 M HCl to achieve a concentration of 1 mg/ml. The solution was further diluted with distilled water to get formulation solution of concentration 50 µg/ml.

Into a series of 25 ml calibrated tubes containing aliquots of standard TS solution (1.0-6.0 ml, 50 µg/ml solution), 1.0 ml of $NaIO_4$ and 0.5 ml of acetic acid were added. The volume was brought upto 10 ml with distilled water and kept in a boiling water bath for 10 min. After that, 1.0 ml of MBTH solution was added and heated further for 2 min. The solutions were cooled to room temperature and the volume was made upto the mark with distilled water. The absorbance

was measured at 620 nm against a similar reagent blank. The amount of TS was deduced from its calibration curve.

The parameters involved in the method (the effect of reagent concentration, volume of acid, temperature and time of heating) were studied by varying one parameter at a time and keeping the others fixed. The optimised parameters thus determined were incorporated into the procedure.

The Beer's law was found to be valid over the concentration range given in Table 1. Sandell's sensitivity, molar absorptivity, slope, intercept and correlation coefficient data for the determination of TS by the proposed method is given in Table 1. The precision of the method was tested by analysing six replicate samples, (using about 2/3 of the upper Beer's law limit of pure TS) by the procedure. The percent S.D. and percent range of error at the 95% confidence level for the method are shown in Table 1.

The effect of a wide range of excipients and additives on the analysis of TS by the proposed procedure was investigated. Starch, lactose, propylene glycol, gum arabic and other additives containing vicinal diol groupings seriously interfere in the assay since they consume periodate. It was therefore found necessary to separate TS from these ingredients by extracting the drug from the formulation with isopropanol in a preliminary step. The results of analysis of the

TABLE 1: OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHOD FOR TS.

Parameters	Proposed Method
λmax (nm)	620
Beer's law limits (μg/ml)	2-12
Detection limit (μg/ml)	0.213
Molar absorptivity ($1l.mol^{-1}.cm^{-1}$)	4.62×10^4
Sandell's sensitivity	0.019
(μg cm^{-2} / 0.001 absorbance unit)	
Optimum photometric range (μg/ml)	2.6-10.8
Regression equation (y)*	
Slope (b)	5.02×10^{-2}
Intercept (a)	2.26×10^{-3}
Correlation coefficient (r)	0.9998
Relative standard deviation (%)**	0.459
% range of error (confidence limits)	0.482

* y = a + bc, where c is the concentration in μg/ml and y is the absorbance unit. ** six replicate samples.

TABLE 2: ASSAY OF TS IN PHARMACEUTICAL FORMULATIONS.

Pharmaceutical formulations*	Labelled amount	% Recovery by proposed method (mg)**	Reference method (mg)
Tablets - T_1	200 mg	99.06 ± 0.41 t = 0.86 F = 2.68	99.51 ± 0.25
Tablets - T_2	200 mg	99.13 ± 0.38 t = 1.00 F = 3.61	99.92 ± 0.20
Injections - I_1	50 mg/ml	100.86 ± 0.38 t = 1.00 F = 1.40	99.56 ± 0.45
Injections - I_2	50 mg/ml	99.97 ± 0.39 t = 1.01 F = 1.64	99.83 ± 0.50

* Two different batches each of tablets and injections from a pharmaceutical company. ** Average (± RSD) of six determinations; the t- and F- values refer to comparison of the proposed method with the reference method. Theoretical values at 95% confidence limits, t = 2.57, F = 5.05.

formulation by proposed procedures and the chosen reference[1] procedure are given in Table 2. The application of t and F-tests to these results show that they do not differ significantly. The results are summarised in Table 2.

The proposed method exploits the oxidation reaction of TS with sodium metaperiodate due to the presence of vicinol diol in TS. This method does not involve any critical reaction conditions and has distinct edge over the reported methods. Thus the proposed method is simple and sensitive with good precision and accuracy for the assay of TS in the pure form and pharmaceutical formulations.

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Spectrophotometric Methods for the Determination of Flutamide in Tablets

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Simple and sensitive spectrophotometric methods for the determination of flutamide in either pure form or in its pharmaceutical preparations are described. The first method is based on the reaction of reduced flutamide with Ehrlich reagent in alcohol medium to produce a yellow Schiff base with a λ_{max} of 430 nm. In the second method, the diazotisation of reduced flutamide followed by complexation with molybdate ions and pyrocatechol in sulphuric acid medium to give a pink coloured complex with a λ_{max} of 540 nm. Both the methods are highly reproducible and results of the assay of flutamide in tablets compare favourably with the reported method.

Flutamide (FLA), chemically known as 2-methyl-N[4-nitro-3-(trifluoromethyl)phenyl]propanamide is widely used as antineoplastic and antiandrogen drug[1]. This new drug is recently included in the USP, which involves a chromatographic method for the analysis of the pure drug and FLA capsules[2]. The reported methods for the determination of FLA include polarography[3], gas-chromatography[4], HPLC[5,6] and spectrophotometric methods[7-12]. In continuation of our work on the spectrophotometric determination of organic compounds of pharmaceutical importance[13-15], we have succeeded in developing two visible spectrophotometric methods (A and B) for the determination of FLA. Method A is based on the reaction of 4-dimethylaminobenzaldehyde (DAB) with the reduced flutamide. Method B is based on the reaction between the diazotisation product of reduced FLA with molybdate ions and pyrocatechol. The methods offer the advantages of sensitivity, selectivity and rapidity without the need for extraction or heating.

A JASCO model UVIDEC-610 UV/VIS spectrophotometer with 1 cm matched cells was used for absorbance mea-

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