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Spectrophotometric Methods for the Assay of Tylosin

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Two simple, sensitive and reproducible spectrophotometric methods (**Method A** and **Method B**) are developed for the determination of tylosin (TS) in bulk and dosage forms. These methods are based on the formation of coloured species on treatment of tylosin with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and Fe(III) ions [**Method A**] and oxidation/reduction with Folin-Ciocalteu (FC) reagent under alkaline conditions [**Method B**].

Key Words: Assay, Tylosin, Spectrophotometric methods.

INTRODUCTION

Tylosin[1–3] (TS) is a macrolide antibiotic used in veterinary medicine in the prophylaxis and treatment of various infections caused by susceptible organisms. It is chemically known as [4R-(4R* 5S*, 6S*, 7R*, 9R*, 11E, 13E, 15R*, 16R*)]-15-[[6-deoxy-2,3-di-*o*-methyl-β-D-allopyranosyl) oxy]-6-[[3,6-dideoxy-4-*o*-(2,6-dideoxy-3-*o*-methyl-α-L-ribo-hexo-pyranosyl)-3-(dimethylamino)-β-D-glaucopyranosyl] oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-2,10-dioxooxacylohexa deca-11,13-diene-7-acetaldehyde]. Little attention was paid to developing visible spectrophotometric methods[4–6].

This paper describes two visible spectrophotometric methods for the assay of TS by making use of the reported procedures. Sawicki *et al.*[7] developed a visible spectrophotometric procedure for determining amines and phenols by condensation with MBTH in the presence of an oxidant to form an intensely coloured oxidized product. Reduction of heteropolyacid complexes by organic reagents was utilized as the basis for the determination of several organic compounds. Among the various heteropolyacids, phosphomolybdotungstic acid, the well known Folin-Ciocalteu (FC) reagent[6], was preferred by a number of workers[9, 10] for the determination of drugs containing not only phenolic or amino group but also certain other drugs which contain neither of these groups[11].

EXPERIMENTAL

Systronics model No. 2201 UV-Vis double beam spectrophotometer was used for absorbance measurements. All the chemicals used were of analytical grade and all the solutions were prepared with double distilled water. Freshly prepared solutions were always used. Aqueous solution of MBTH (Loba) 0.2%, $FeCl_3$ (Qualigens) 0.7% in 0.5 M HCl were prepared for **Method A.** Aqueous solution of Na_2CO_3 (Qualigens) 10% and FC reagent (S.D. Fine Chem.) 1 N were prepared for **Method B.**

Standard and Sample Solutions

TS (100 mg) was accurately weighed and dissolved in 100 mL of 0.1 M HCl and the stock solution was diluted stepwise with 0.1 M HCl to obtain the working standard solution having a concentration of 100 μg/mL **(Method A)** or 200 μg/mL **(Method B).**

Assay Procedures

Method A: To 10 mL volumetric flasks containing different aliquots (1.0–6.0 mL, 100 μg/mL) of TS, 1.5 mL MBTH solution was added and allowed to stand for 2 min at room temperature. After that 1.5 mL of Fe(III) solution was added, allowed to react for 5 min and diluted to 10 mL with distilled water. Absorbance was measured during the next 30 min at 520 nm against a reagent blank prepared in a similar manner omitting the drug. The amount of the drug in the sample was computed from Beer-Lambert plot.

Method B: Aliquots of standard TS (1.0–5.0 mL, 200 μg/mL) were taken into a series of 20 mL graduated tubes. Then 2.5 mL of FC reagent and 7.0 mL of Na_2CO_3 were added successively and kept aside for 15 min. The solution was made up to 20 mL with distilled water and the absorbance was measured at 750 nm against a reagent blank. The concentration of the drug was computed from Beer-Lambert plot.

RESULTS AND DISCUSSION

The optimum conditions were established by varying one parameter at a time and keeping the others fixed and observing the effect produced on the absorbance of the coloured species. The effect of MBTH and $FeCl_3$ concentrations, keeping time for **Method A** and FC reagent and Na_2CO_3 concentration for **Method B**, with respect to maximum sensitivity, minimum blank, adherence to Beer's law and stability were studied for both the methods through control experiments and the optimum conditions were incorporated in procedures of **Methods A** and **B**. The optical characteristics and figures of merit are given in Table-1, together with the regression equations (obtained by linear least square treatment) for the calibration plots for both the methods. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in Table-1.

The accuracy of the methods was ascertained by comparing the results obtained with the proposed and reference methods in the case of formulations

and are presented in Table-2. As an additional check on the accuracy of the methods, recovery experiments were performed by adding known amounts of pure drug to pre-analyzed formulations and per cent recovery values obtained are listed in Table-2. Recovery experiments indicated the absence of interferences from the commonly encountered pharmaceutical additives and excipients.

TABLE-1
OPTICAL CHARACTERISTICS, PRECISION AND ACCURACY OF THE PROPOSED METHODS

Parameters	Method A	Method B
λ_{max} (nm)	520	750
Beer's law limits (μg/mL)	10–60	10–50
Sandell' s sensitivity (μg/cm^2/0.001 abs. unit)	0.007	0.016
Molar absorptivity (L mol^{-1}, cm^{-1})	1.20×10^5	5.51×104
Regression equation (y)†:		
Slope (b)	1.31×10^{-1}	6.21×10^{-2}
Intercept (a)	2.40×10	-6.21×10^{-3}
Correlation coefficient (r)	0.9999	0.9999
Relative standard deviation (%)†	0.4810	0.3160
% Range of error (95% confidence limits)	0.5070	0.3330

* Y = a + bx, where x is the concentration in μg/mL and y is the absorbance unit.
† For six replicate samples.

TABLE-2
ASSAY OF TYLOSIN IN PHARMACEUTICAL FORMULATIONS

Formulations	Labelled amount	% Recovery by proposed method*		UV reference method
		Method A	Method B	
Tablet-I	200 mg	99.03 ± 0.22 t = 1.20 F = 1.29	100.86 ± 0.27 t = 0.70 F = 1.09	99.51 ± 0.25
Tablet-II	200 mg	99.97 ± 0.23 t = 0.38 F = 1.52	99.07 ± 0.31 t = 0.40 F = 2.75	99.92 ± 0.19
Injection-I	50 mg/mL	99.94 ± 0.45 t = 1.22 F = 1.05	99.02 ± 0.54 t = 1.35 F = 1.39	99.56 ± 0.52
Injection-II	50 mg/mL	100.10 ± 0.37 t = 0.42 F = 1.99	100.00 ± 0.26 t = 1.00 F = 3.69	99.83 ± 0.50

*Average 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.

Thus the proposed methods are simple and selective with reasonable precision and accuracy. They can be used for the routine determination of tylosin in quality control analysis.

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