Assay of Tylosin in Pharmaceutical Formulations by Visible Spectrophotometry

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Simple spectrophotometric methods (M_1 and M_2) for the assay of tylosin (TS) in pharmaceuticals are described. Method M_1 is based on the formation of oxidative coupling product of tylosin with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in the presence of ferric chloride (Fe(III) (λ_{max} 640 nm). Method M_2 is based on the formation of a coloured charge transfer complex between TS and chloranilic acid (CA) (λ_{max} 520 nm). Beer's law limits, precision and accuracy of the methods are checked by the UV reference method. The methods are found to be suitable for the assay of tylosin in pharmaceutical formulations. The per cent recoveries range from 99.02 to 100.96.

INTRODUCTION

Tylosin[1–3] (TS) is a macrolide antibiotic used in veterinary medicine in the pro- phylaxis 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] methyl]-6-[[3,6-dideoxy- 4-O-(2,6-dideoxy-3-C-methyl-α-L-ribo-hexopyranosyl)-3-(dimethyl amino)-β-D-glucopyranosyl] oxy]-16-ethyl-4-hydroxy-5,9,13-trimethyl-2,10-dioxooxa- cyclo-hexadeca-11,13-diene-7-acetaldehyde)]. Literature survey revealed that the analytically useful functional groups (aldehyde and tetriary amine) in TS have not been properly exploited for designing suitable visible spectrophotometric methods[4–6] and so still offer a scope to develop the present methods by exploiting different structural features of TS such as the presence of an aldehyde [oxidative coupling product with 3-methyl-2-benzothiazolinone hydrazone (MBTH) M_1] and a tetriary amino group [change-transfer complex with chloranilic acid (CA) M_2]. These methods can be applied for the determination of TS in pharmaceutical formulations. The results are statistically validated.

EXPERIMENTAL

A Milton Roy Spectronic 1201 and Systronics 106 digital spectrophotometer

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with 1 cm matched quartz cells were used for the spectral and absorbance measurements in the UV and visible regions respectively.

All the reagents and chemicals used were of analytical or pharmacopoeial grade purity and double distilled water was used throughout. Aqueous solutions of MBTH (Fluka, 8.55×10^{-3} M) and Fe(III) (BDH, 2.96×10^{-2} M) were prepared by dissolving the required amount in doubly distilled water. A solution of CA (Sd-fine, 4.78×10^{-3} M) was prepared by dissolving 100 mg of *p*-chloranilic acid initially in 20 mL of isopropanol followed by dilution to 100 mL with chloroform.

Preparation of Standard Drug Solutions

A 1 mg/mL solution was prepared by dissolving 100 mg of pure tylosin in 100 mL of 0.1 M HCl and this stock solution was diluted further with 0.1 M HCl to obtain the working standard solutions of concentration of 50 μg/mL (for method M_1). For method M_2, 500 μg/mL solution was prepared by dissolving 50 mg of pure tylosin in 100 mL of chloroform.

For Pharmaceutical Formulations

An accurately weighed amount of tablet powder or measured volume of injection equivalent to 50 mg of TS was extracted with isopropanol (4 × 15 mL) and filtered. For method M_1, 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 500 μg/mL. The solution was further diluted to 100 mL with 0.1 M HCl to get working standard solution (50 μg/mL). For method M_2, the residue obtained by evaporating isopropanol extract in separate bulk was dissolved in chloroform and brought to 100 mL with same solvent to get 500 μg/mL solution. These solutions were analysed as under procedures described for bulk samples.

The UV spectrophotometric method which was suggested for the identification of TS in BP (Vet)[1] has been moulded for its assay and chosen as reference method for ascertaining the accuracy of the proposed methods.

Recommended Procedures

Method M_1: Into a series of 25 mL calibrated tubes containing aliquots of standard TS solution (1.0–6.0 mL, 50 μg/mL), 2.0 mL of MBTH solution was added and kept aside for 5 min. After that, 1.0 mL of Fe(III) solution was added and kept aside for 15 min. The volume was made up to the mark with distilled water. The absorbance was measured at 640 nm against a similar reagent blank. The amount of TS was deduced from its calibration curve.

Method M_2: Into a series of 10 mL calibrated tubes containing aliquots of standard TS solution (1.0–5.0 mL, 500 μg/mL in chloroform), 1.0 mL of chloranilic acid was added and the volume made up to the mark with chloroform. The absorbance was measured after 5 min at 520 nm against a similar reagent blank. The amount of TS was deduced from its calibration curve.

RESULTS AND DISCUSSION

The optimum conditions for the color development of methods were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the coloured species[7]. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.

Method M_1: The selection of oxidant was based on the applicability of MBTH in combination with various oxidising agents such as Fe(III), Ce(IV) and IO_4^-. The λ_{max} (nm) and ε_{max} (1 mol^{-1} cm^{-1}) values were found to be 640, 4.44×10^4; 620, 3.06×10^4 and 540, 1.48×10^4 for Fe(III), Ce(IV) and IO_4^- respectively. Since Fe(III) produces higher λ_{max} and ε_{max} values than the other two, it was used for further investigations. Volume ranges of 1.5 to 2.5 mL of 8.55×10^{-3} M MBTH and 0.8 to 1.2 mL of 2.96×10^{-2} M Fe(III) were found to be optimal. So list volumes of MBTH (2.0 mL, 8.55×10^{-3} M) and Fe(III) (1.0 mL, 2.96×10^{-2} M) were preferred for further investigations. The order of addition of reagents has no significant effect. Maximum colour intensity was attained within 15 min and remained stable for further 30 min. The colored product was measured at 640 nm (Fig. 1).

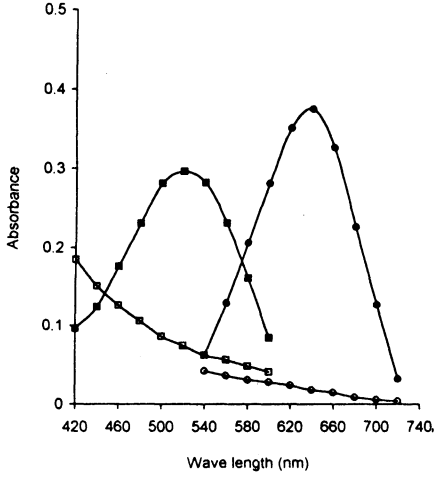


Fig. 1. Absorption spectrum of TS-MBTH-Fe(III) system (●–●) *vs.* reagent blank (○–○) *vs.* distilled water (M_1): [TS] = 8.73×10^{-6} M, [MBTH] = 6.84×10^{-4} M, [Fe(III)] = 1.18×10^{-3} M. Absorption spectrum of TS-CA system (■–■) *vs.* reagent blank (□–□) *vs.* chloroform (M_2): [TS] = 1.63×10^{-4} M, [CA] = 4.78×10^{-4} M.

Method M_2: An optimum range 0.8 to 1.2 mL of 4.78×10^{-3} M chloranilic acid was found to be necessary to produce constant and reproducible absorbance

values. If the volume of the reagent is less than 0.8 mL, complete color development within the Beer's law limits is not possible and increase in the volume of the reagent above 1.2 mL leads to high blank values. So 1.0 mL of 4.78×10^{-3} M chloranilic acid was chosen. Chloroform was preferred for dilutions due to its high sensitivity over the other solvents tried. The color product was obtained within 5 min and remained stable for a further 20 min. The coloured product was measured at 520 nm (Fig. 1).

Mole ratio method for TS : CA complex[8]: The method was applied to determine the mole ratio of TS and CA complex. The absorbances were measured for a series of solutions which contain varying volumes of drug with a constant volume of reagent (equimolar solutions of both). The absorbance was plotted against mole ratio of drug to reagent. When the constant formation of complex was favourable, two straight lines of different slopes were obtained. The intersection of these two lines occurs as a mole ratio corresponding to the existing ratio in the complex. The mole ratio of TS and CA in EDA complex has been found to be 1 : 1 by mole ratio method (Fig. 2).

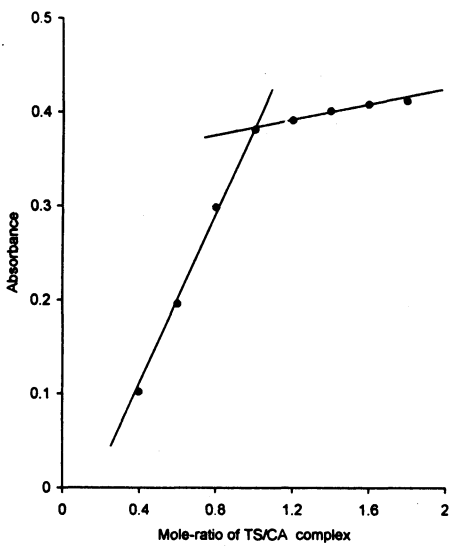


Fig. 2. Mole-ratio method for TS/CA complex (M_2).

Analytical Data: The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's senstitivity for the methods are given in Table-1. The precision of the method was found by measuring absorbances of six separate samples containing known amounts of drug and the results obtained are incorporated in Table-1. Regression analysis using the method of least squares was made to evaluate the slope (b), intercept (a), correlation coefficient (r) and standard error of estimation (S_e) for each system and are presented in Table-1.

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

Parameters	M_1	M_2
λ_{max} (nm)	640	520
Beer's law limits (μg/mL)	2–12	50–250
Detection limit (μg/mL)	0.175	5.620
Molar absorptivity (1 mol^{-1} cm^{-1})	4.44×10^4	1.74×10^3
Sandell's sensitivity (μg cm^{-2}/0.001 absorbance unit)	0.020	0.205
Optimum photometric range (μg/mL)	2.8–10.9	56.2–199.5
Regression equation (y)*		
Slope (b)	4.64×10^{-2}	1.90×10^{-3}
Intercept (a)	5.33×10^{-3}	4.60×10^{-3}
Correlation coefficient (r)	0.9998	0.9998
Relative standard deviation (%)†	0.364	0.726
% range of error (95% confidence limits)	0.383	0.762

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

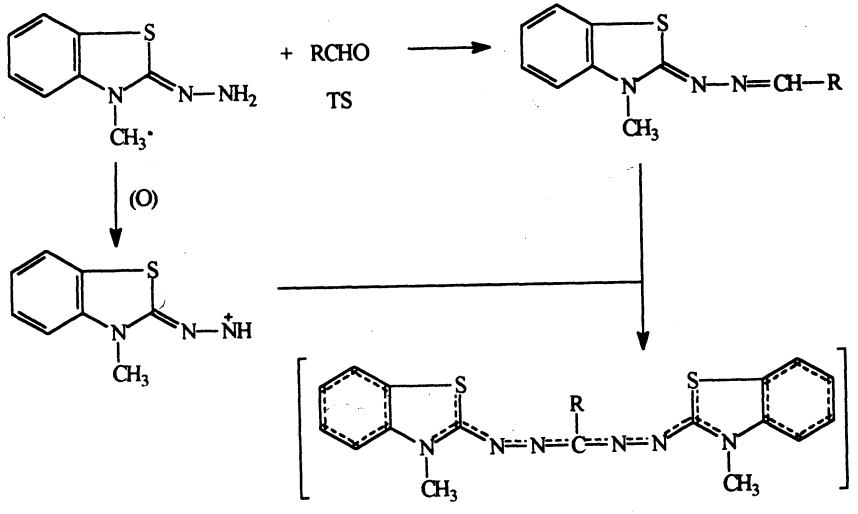
Interference studies: The effects of a wide range of concomitants and other additives usually present in the formulations in the assay of TS under optimum conditions were investigated separately in both the methods. The commonly used concomitants and additives in the preparation of formulations such as talc, starch, boric acid, stearic acid, magnesium stearate, kaolin, sodium lauryl sulfate and gelatin even when added in larger quantity than anticipated in pharmaceutical formulations did not interefere with the assay of TS by proposed methods. Commerical formulations (tablets and injections) containing TS were successfully analysed by the proposed methods. The values obtained by the proposed and reference methods for formulations were compared statistically with t- and F-tests and found not to differ significantly. The results are summarised in Table-2.

Chemistry of the coloured species: In method M_1, under the reaction conditions MBTH on oxidation with Fe(III) loses two electrons and one proton forming an electrophilic intermediate which is the active coupling species, which then condenses with aldehyde to give a brilliant blue coloured cationic dye[9] (as shown in scheme).

In method M_2. TS behaves as a good electron donor (since it possesses aliphatic tertiary amino group) and CA as a good electron acceptor. The colour formation between these two is believed due to the charge transfer complex[10]. Chloranilic acid exists in three forms, the natural yellow H_2A at very low pH, the dark violet HA^- which is most unstable at pH 2.0 and pale violet A^{2-} stable at high pH. As the reaction products in 20% isopropanol in chloroform are dark violet (purple) while the colour of blank is golden yellow, it appears that HA^- is

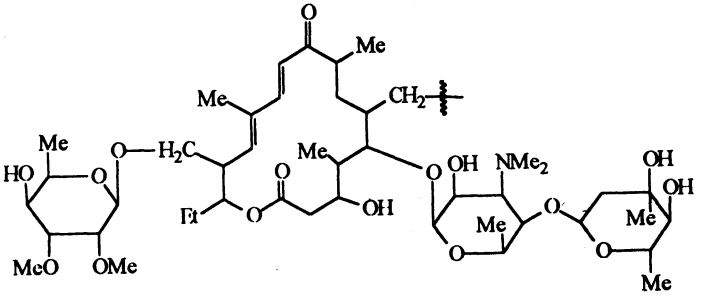
the form of chloranilic acid involved in the complex formation (as shown in scheme).

Method M_1:

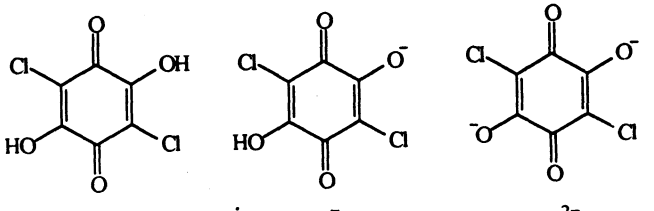


Brilliant blue cationic dye

Where R =



Method M_2:



H_2A HA^- A^{2-}

Scheme

TABLE-2
ASSAY OF TS IN PHARMACEUTICAL FORMULATIONS

Pharmaceutical formulations*	Labelled amount	% Recovery by proposed methods (mg)†		Reference method (mg)
		M_1	M_2	
Tablets—T_1	200 mg	99.97 ± 0.30	100.96 ± 0.21	99.51 ± 0.25
		t = 0.67	t = 0.68	
		F = 1.44	F = 1.41	
Tablets—T_2	200 mg	99.02 ± 0.30	99.13 ± 0.38	99.92 ± 0.20
		t = 1.33	t = 1.00	
		F = 2.25	F = 3.61	
Injections—I_1	50 mg/mL	99.86 ± 0.48	99.07 ± 0.47	99.56 ± 0.45
		t = 1.50	t = 0.27	
		F = 1.13	F = 1.09	
Injections—I_2	50 mg/mL	99.93 ± 0.44	99.10 ± 0.36	99.83 ± 0.50
		t = 1.23	t = 0.42	
		F = 1.29	F = 1.29	

*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.

Conclusions

The proposed methods exploit the various functional groups in TS molecule. The concomitants which do not contain the functional groups chosen in the present investigation do not interfere in the color development by proposed methods. Thus the proposed methods are simple, accurate and constitute better alternatives to the reported ones in the assay of TS in bulk form and pharmaceutical formulations.

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