Assay of tylosin using sodium metaperiodate as an oxidant

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Simple and sensitive spectrophotometric methods (M 1 and M 2) based on the oxidation of tylosin (TS) with excess of sodium metaperiodate and estimating either the amount of periodate consumed (with celestine blue (CB) in the presence of Te(IV), M 1) or product formed (iodate with p-N-methylaminophenol sulphate–sulphanilamide (PMAP-SA, M 2) have been described. 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. The recovery range is 99.02 to 100.96%.

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Tylosin\textsuperscript{1-3} 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-\beta-D-allopyranosyl] oxy]methyl]-6-[[3, 6-dideoxy-oxy-4-O-(2, 6-dideoxy-3-C-methyl-\alpha-L-ribo-hexopyranosyl)-3-(dimethyl amino)-\beta-D-glucopyranosyl] oxy]-16-ethyl-4-hydroxy-5, 9, 13-trimethyl-2, 10-dioxooxacyclo hexa deca-11, 13 diene-7-acetaldehyde]. Little attention was paid in developing visible spectrophotometric methods\textsuperscript{4-6}. Under appropriately controlled conditions, sodium metaperiodate will selectively oxidise 1,2-diols, 1,2-amino alcohols, 1,2-hydroxy aldehydes and ketones, 1,2-diketones and 1,2-amino aldehydes at different rates. The presence of 1, 2-diol in the molecule of TS renders it vulnerable to stoichiometric attack by periodate to produce iodate besides the unreacted periodate. The present communication describes the development of two indirect two-step procedures\textsuperscript{7}, each utilizing the one of the above without interfering each other. In both the methods, first step is the oxidation of TS with excess periodate, giving the oxidation products of TS besides its reduced form iodate. In method M 1, the iodate is masked with tellurite\textsuperscript{8} (tellurite forms ion – pair with IO\textsubscript{3} –) and the unreacted periodate is estimated with CB. It has been observed that tellurite selectively allows the oxidation of CB (dye to its leuco form) with periodate (and not with iodate) under acidic conditions, in fixed time intervals thereby minimizing the interference of iodate in periodate determination. In method M 2, the unreacted periodate is masked\textsuperscript{9} with molybdenum(VI) as 6-molybdo periodate [I(MoO\textsubscript{4})\textsubscript{6}] and the iodate formed is estimated with PMAP-SA. The results for both the methods were statistically validated.

Experimental Procedure

Instruments

A Milton Roy Spectronic 1201 and Systronics 106 digital spectrophotometer with 1 cm matched quartz cells were used for the spectral and absorbance measurements. An Elico LI-120 digital pH meter was used for measurements.

Reagents

All the chemicals and reagents used were of analytical grade and solutions were prepared in double distilled water. Aqueous solutions of sodium metaperiodate (BDH, 9.35×10\textsuperscript{-4} M), sodium tellurite (BDH, 4.51×10\textsuperscript{-3} M), CB (Chroma, 5.49×10\textsuperscript{-4} M) and HCl (E-Merck, 5.0 M) were prepared for method M 1. Aqueous solutions of sodium metaperiodate (BDH, 9.35×10\textsuperscript{-4} M), sodium molybdate (Qualigens, 8.26×10\textsuperscript{-2} M), PMAP (Loba, 8.71×10\textsuperscript{-3} M), SA (Sd-Fine Chemicals, Mumbai, 1.16×10\textsuperscript{-3} M) and potassium acid phthalate buffer (pH 3.0) were prepared for method M 2.

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 the stock solution was diluted step wise with 0.1 M HCl to obtain the working standard solution having 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 (4 × 15 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 stepwise with 0.1 M HCl to get working standard solution of concentration, 50 μg/mL. These solutions were analyzed as under procedures described for bulk samples.

Method M₁

Into a series of 25 mL calibrated tubes containing aliquots of standard TS solution (0.5-2.5 mL, 50 μg/mL), 3.0 mL of 5M HCl and 1.0 mL of NaIO₄ solutions were added. The volume was brought to 18 mL with distilled water and kept in a boiling water bath for 10 min. After cooling to room temperature, 2.0 mL of Te(IV) solution and 5.0 mL of CB solution were added successively. The absorbance was measured after 5 min at 540 nm against a similar reagent blank (Fig. 1). The amount of TS was deduced from its calibration curve.

Method M₂

Into a series of 25 mL calibrated tubes containing aliquots of standard TS solution (1.0-4.0 mL, 50 μg/mL), 1.0 mL of NaIO₄ solution was added. The volume was brought to 5.0 mL with distilled water and kept on a boiling water bath for 10 min. After cooling to room temperature, 15 mL of buffer solution and 1 mL of sodium molybdate solution were added to each flask. After 5 min, 1.5 mL of PMAP solution was added and kept aside for 2 min. Then 2.0 mL of SA solution was added and the volume was made up to the mark with distilled water. The absorbance was measured after 10 min at 520 nm against a similar reagent blank (Fig. 2). The amount of TS was deduced from its calibration curve.

Results and Discussion

Optimum conditions fixation

The optimum conditions for the colour 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. The following experiments were conducted for this purpose and the conditions so obtained were incorporated in recommended procedures.

Method M₁

In the first step, an optimum range of 2.5 to 3.5 mL of 5M HCl and 0.8 to 1.2 mL of 9.35×10⁻⁴ M periodate and a heating for 5 to 15 min on a boiling water bath were found to be necessary for getting constant and reproducible absorbance values. So list volumes of HCl (3.0 mL, 5 M) and periodate (1.0 mL, 9.35×10⁻⁴ M) and a heating time of 10 min on a boiling water bath were preferred for further investigations. In the second step, 2.0 mL of 4.51×10⁻³ M sodium tellurite was necessary for suppressing the reactivity of iodate in the reaction between CB and periodate. Five mL of 5.49×10⁻⁴ M CB and a waiting period of 5 min for getting consistency in absorbance after the gradual decrease in colour of the dye were found to be optimal. The colour was stable for 20 min.

Method M₂

A volume range of 0.8 to 1.2 mL of 9.35×10⁻³ M periodate and heating time of 10 min on a boiling water bath were found to be optimal. Addition of periodate less than 0.8 mL resulted in low absorbances particularly with higher concentrations within Beer’s law limits. Alteration of heating time beyond 10 min led to erratic results. So 1.0 mL of 9.35×10⁻³ M periodate was chosen for further investigations. Fifteen mL of 5.49×10⁻⁴ M CB and a waiting period of 5 min for getting consistency in absorbance after the gradual decrease in colour of the dye were found to be optimal. The colour was stable for 20 min.

Analytical data

In order to test whether the coloured species formed in above methods adhere to Beer’s law, the absorbances at appropriate lengths of a set of solutions containing varying amounts of TS and specified amounts of reagents (as given in the recommended procedures) were recorded against the corresponding reagent blanks. Beer’s law limits, molar absorptivity, the sensitivity and working photometric range for TS in each method developed with mentioned reagents were calculated. Least square regression analysis was carried out for getting the slope, intercept and the correlation coefficient values. The precision of the proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of TS in total solution (3 μg/mL for M₁ and 6 μg/mL for M₂). The per cent relative standard deviation and per cent range of error (95% confidence limits) were calculated for the proposed methods (Table 1). The results of analysis of the formulation by each of the proposed procedures and reference procedure¹ are given in Table 2. The application of t and F-tests to these results shows that they do not differ significantly. The results are summarized in Table 2.

Conclusions

The proposed methods evaluate the oxidation reactions of TS with sodium metaperiodate due to presence of 1,2-diol in it. These methods do not involve any critical reaction conditions and, thus, have distinct edge over reported methods. Hence, the proposed methods are simple and sensitive with good precision and accuracy for the assay of TS in pure form and pharmaceutical formulations.
# Table 2—Assay of TS in pharmaceutical formulations

<table>
<thead>
<tr>
<th>Pharmaceutical formulations*</th>
<th>Labelled amount</th>
<th>% Recovery by proposed methods (mg) **</th>
<th>UV Reference method¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M₁</td>
<td>M₂</td>
</tr>
<tr>
<td>Tablets – T₁</td>
<td>200 mg</td>
<td>99.02 ± 0.22</td>
<td>100.96 ± 0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.00</td>
<td>t = 0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.29</td>
<td>F = 1.08</td>
</tr>
<tr>
<td>Tablets – T₂</td>
<td>200 mg</td>
<td>99.97 ± 0.22</td>
<td>99.06 ± 0.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 0.38</td>
<td>t = 0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.21</td>
<td>F = 2.72</td>
</tr>
<tr>
<td>Injections – I₁</td>
<td>50 mg/mL</td>
<td>99.95 ± 0.44</td>
<td>99.02 ± 0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>t = 1.00</td>
<td>t = 1.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.04</td>
<td>F = 1.38</td>
</tr>
<tr>
<td>Injections – I₂</td>
<td>50 mg/mL</td>
<td>100.10 ± 0.36</td>
<td>100.03 ± 0.26</td>
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<tr>
<td></td>
<td></td>
<td>t = 0.42</td>
<td>t = 1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F = 1.92</td>
<td>F = 3.69</td>
</tr>
</tbody>
</table>

*Two different batches each of tablets and injections from a pharmaceutical company

**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

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**References**