the Me2 extract possessed antibacterial activity. The extracts showed no antifungal activity against the microorganisms tested and the results are shown in Table 1. From these results it can be concluded that the alkaloids, flavonoids, cardiac glycosides, cyanogenetic glycosides of the extracts have no antimicrobial activity. This equally proves that the antibacterial agents of the plant are polar.

On further fractionation of the alkaloid-free methanol extract, eight bands were obtained. The Rf values, chemical classes and percentage yield of these bands are reported in Table 2. Out of the eight bands only three showed antibacterial activity and they belong to steroid glycosides. Fractions belonging to the same class of compounds in a plant are assumed to have the same biosynthetic origin hence the same basic structural unit responsible for antibacterial and antifungal activities in a plant[2]. These fractions may have the same basic structural unit. As some of the extracts show activity against some bacteria, the antibacterial potential has to be further investigated.

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Visible Spectrophotometric Methods for Estimation of Clarithromycin from Tablet Formulation

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Three simple visible spectrophotometric methods have been developed for the estimation of clarithromycin from tablet formulation. The first developed method is based on formation of orange-red coloured complex of drug with concentrated hydrochloric acid and acetone. The coloured complex shows absorbance maxima at 485 nm and obeys Beer’s law in concentration range of 50-500 µg/ml. Second and third developed methods are based on formation of chloroform extractable complex of drug with bromocresol green and bromophenol blue, respectively, both of which show absoorxince maxima at 414 nm and linearity in concentration range of 0-60 µg/ml of drug. Results of analysis for all the methods were validated statistically and by recovery studies.

Clarithromycin, chemically 6-O-methyl erythromycin is a semi-synthetic macrolide antibiotic used in the treatment of respiratory tract infection and in skin and soft tissue infections[1]. The drug is not yet official in any of the pharmaco-
poemia. For the estimation of clarithromycin from biological fluids, four HPLC methods have been reported. However, no spectrophotometric method is reported for estimating clarithromycin formulations. An attempt has been made in the present study to develop three simple visible spectrophotometric method for the analysis of clarithromycin from tablet formulation.

A Jasco UV/Vis recording spectrophotometer (model-7800) with 1 cm matched quartz cells was used in the present study. All reagents used were of analytical grade. Acid phthalate buffer of pH 2.4 and 3.0 was prepared. Bromocresol green reagent (0.1%) was prepared in buffer of pH 2.4 and bromophenol blue reagent (0.1%) was prepared in buffer of pH 3.0. Both the reagents were extracted several times with chloroform to remove chloroform soluble impurities. Standard drug solution of clarithromycin (1 mg/ml and 100 µg/ml) were prepared in chloroform.

For method I, standard drug solution in chloroform (1 mg/ml) was diluted with chloroform to give several dilutions in concentration range of 50-500 µg/ml of Clarithromycin. To 10 ml of each dilution taken in a separating funnel, 5 ml concentrated hydrochloric acid was added followed by 10 ml of acetone. Reaction mixture was shaken gently for 5 min for formation of stable coloured complex. The upper orange-red coloured layer was separated out and absorbance measured at 485 nm against reagent blank. A calibration curve was prepared.

For the analysis of sample, twenty tablets were accurately weighed and average weight of the tablet was determined. The tablets were powdered and powder equivalent to 40 mg of clarithromycin was accurately weighed and transferred to 100 ml volumetric flask. Chloroform (75 ml) was added and shaken for 5 min to dissolve the drug. The solution was filtered through a Whatman filter paper No. 41 into another 100 ml volumetric flask. The filter paper was washed with chloroform. The washings were added to the filtrate and the final volume was made with chloroform. Ten milliliters of filtrate was taken in a separating funnel and treated as per procedure described for calibration curve. Absorbance was measured at 485 nm and the concentration of drug in sample solution was determined from calibration curve. Results of analysis are presented in Table 1.

For the other two methods, standard drug solution of clarithromycin (100 µg/ml) was diluted with chloroform to give several dilutions in concentration range of 0-60 µg/ml of clarithromycin. To 10 ml of each dilution taken in a separating funnel, 5 ml of bromocresol green reagent (method II) or bromophenol blue reagent (method III) was added. Reaction mixture was shaken gently for 5 min and allowed to stand for 5 min so as to separate aqueous and chloroform layer. The chloroform layer was separated out and absorbance measured at 414 nm against reagent blank. Respective calibration curves were prepared.

For analysis of sample solution, tablet powder equivalent to 25 mg of clarithromycin was accurately weighed and transferred to 100 ml volumetric flask. Chloroform (75 ml) was added and shaken for 5 min to dissolve the drug. The solution was filtered through Whatman filter paper No. 41 into another 100 ml volumetric flask. The filter paper was washed with chloroform. The washings were added to the filtrate and the final volume was made with chloroform. Ten milliliters of filtrate was further diluted to 100 ml with chloro-

<table>
<thead>
<tr>
<th>Method</th>
<th>Batch</th>
<th>Lable Claim (mg/tab)</th>
<th>% of Lable Claim Estimated*</th>
<th>S.D.</th>
<th>% Recovery**</th>
</tr>
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<tbody>
<tr>
<td>I</td>
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<td>0.453</td>
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<tr>
<td></td>
<td>B</td>
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<tr>
<td></td>
<td>C</td>
<td>250</td>
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<td>0.574</td>
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<tr>
<td>II (BCG)</td>
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<td>250</td>
<td>98.71</td>
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<td></td>
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<td>III (BPB)</td>
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<td>250</td>
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<td>0.532</td>
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</tr>
</tbody>
</table>

BCG stands for bromocresol green and BPB represents bromophenol blue. *Denotes average of three determinations while **denotes average of recovery studies at three different concentration levels.

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form. Ten milliliters of final dilution was taken in a separating funnel and treated as per procedure described for the preparation of calibration curve. Absorbance was measured at 414 nm and the concentration of drug in sample solution was determined from calibration curve. Results of analysis are presented in Table 1.

Recovery studies were carried out by addition of known quantities of standard drug solution to pre-analysed sample at three different concentration levels and the determination was repeated for all the three methods. Results of recovery studies are presented in Table 1.

The proposed methods are colorimetric methods for determination of clarithromycin from tablet dosage form. The methods are very simple and accurate. Reproducibility of each method was checked by recovery studies and results of which were found to be close to 100% and values of standard deviation were satisfactorily low. Since no spectrophotometric method is reported for the estimation of clarithromycin from pharmaceutical formulations, the methods developed in the present investigation may perhaps be used for the analysis of clarithromycin from tablets.

REFERENCES:

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Spectrophotometric Determination of Amiodarone and Ondansetron in Pharmaceutical Dosage Forms with Citric Acid - Acetic Anhydride Reagent

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A sensitive spectrophotometric method is presented for determining amiodarone and ondansetron. The drugs are extracted from formulations with chloroform from an alkaline medium and reacted with citric acid-acetic anhydride reagent to produce a bluish-violet colour having absorption maximum at 580 nm. Beer's law is obeyed between 2-12 µg/ml for amiodarone and ondansetron. The results agree within ± 1.0% with official method.

Amiodarone (as hydrochloride, AD) is a class III antiarrhythmic drug and is chemically known as 2-butyl-3-benzofuranyl-4[2-(diethylamino)ethoxy]-3,5-diiodophenyl ketone hydrochloride. Ondansetron (as hydrochloride, OST) is an antiemetic and is chemically known as 1, 2, 3, 9-tetrahydro-9-methyl-3-(2-methyl-1H-imidazol-1-yl)4H-carbazol-4-one monohydrochloride. AD is official in IP¹ and BP², while OST is official in USP³. A number of methods such as HPLC (OST)⁴⁻⁷, UV (AD)⁸ and visible spectrophotometric (OST)⁹⁻¹¹ are reported in the literature. No visible spec-

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