Assay of Rifampicin in Bulk and its Dosage Forms by Visible Spectrophotometry using Chloranilic Acid

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ABSTRACT
The electron donor-acceptor complex of the proposed method is simple, rapid and sensitive with reasonable precision and accuracy. The precision of the method was found by analyzing a set of eight solutions, each containing a final concentration value approximately in the middle of the Beer’s law range. The percent relative standard deviation in this method is presented in table-2. The accuracy of the method was determined by taking different known amounts (within Beer’s law limits) of the drug and analyzing them by proposed method. The results are given in table – 3. In the determination of Rifampicin the excipients usually present in formulations (glucose, starch, sodium hexa phosphate and some vitamins) and the other antibiotics such as cyslcerine, streptomycin, lidocaine or penicillins did not interfere.

Keywords: Rifampicin, Spectrophotometer, Chloranilic acid, Electron donor-acceptor complex.

INTRODUCTION
The formation of outer complexes or electron donor acceptor (EDA) complexes between quinones and amines is well known. These complexes are usually characterized by an intermolecular charge – transfer absorption band which often appears in the visible region. The energy of the band for a given complex agrees well with the electron donating and accepting properties of the two components [1]. In some cases more than one intermolecular charge-transfer transition is observed [2]. These could correspond to transitions from the highest filled and the penultimate filled levels in the donor to the lowest empty level in the acceptor. The complexes with tertiary amines persist for a long time without further chemical reaction, although such subsequent reactions are dependant to a great extent, on the nature of the solvent[3,4] with most aromatic amines, the EDA complex fades with time because of other reactions which lead eventually to substitution (usually disubstitution in the 2nd and 5th positions).

Chloranilic acid is an yellow crystalline substance only slightly soluble in water, but soluble in many common organic solvents. It has a fairly high electron affinity[5] (1.4.e.v) and it is known to be a strong electron acceptor forming complexes with various Lewis bases. These reactions have been interpreted in terms of charge – transfer theory as proposed by Mulliken[6]. Although complexes of chloranilic acid with polycyclic aromatic hydrocarbons have been studied in detail[7,8]. little attention has been given to the n – Π charge – transfer complexes of chloranilic acid [9]. Chloranilic acid has been largely used in the determination of aromatic amines[10,11] amino acid [12,13] carboxylic acids and their salts[14]. The present work describes the spectrophotometric determination of rifampicin in bulk samples and dosage forms at pH 7.0.

MATERIALS AND METHODS
Preparation of reagents
Chloranilic acid
It was prepared by dissolving 100mg of analytical grade compound in 100ml methanol.
Rifampicin
It was prepared by dissolving 100 mg of Rifampicin (USP grade) in 100 ml of methanol.
Buffer solution (pH=7.0)
It was prepared by mixing 38.8 ml of potassium dihydrogen phosphate (0.06M) and 61.2 ml of disodium hydrogen phosphate (0.06M). Other reagents and solvents were of analytical grade.
**Instrumentation**

Spectral and absorbance measurements were made on shimadzu double beam spectrophotometer UV-140 with matched 1cm quartz cells and pH measurements were carried out using Systronics pH meter 335.

**Absorption spectra**

The Absorption Spectrum of the colored species was scanned over the wavelength region 400 – 650 nm against a reagent blank and the data is graphically represented (Fig.-2). The absorption curves show a maximum at 510nm against reagent blank.

**Procedure**

Aliquots of standard solutions (0.2 – 2.5 ml) were transferred into different 10 ml stoppered test tubes containing 6.0 ml of buffer and 1.0 ml of chloranilic acid solutions and made up to the mark with distilled water. After 5 min, the absorbance of developed purple-red color was measured at 510nm. The amount of rifampicin present in sample solution were computed from the standard curve.

**For dosage forms**

Sample powder equivalent to 50 mg of rifampicin was extracted with ethyl acetate. The residue from ethyl acetate extract was dissolved in methanol to produce a solution containing 200 µg/ml.

**RESULTS AND DISCUSSION**

The data given in Tables-2 and 3 suggest that the proposed method has reasonable precision and accuracy. The suitability of the method for analysis of pharmaceutical preparations was established by the data represented in Table-4. The proposed method was simple as it makes use of aqueous solutions, complete color development takes with in 2min and stable for considerable period.

**Chemistry involved**

Semi polar and polar solvent media facilitate the formation of radical ions and substitution usually at 2\textsuperscript{nd} or 5\textsuperscript{th} positions [15] Based on this fact the reaction of rifampicin with chloranilic acid at pH 7.0 may be formulated as given in Scheme-1.

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**Table-1: Optical Characteristics**

<table>
<thead>
<tr>
<th>Concentration range (µg/ml) (C)</th>
<th>Regression equation</th>
<th>Correlation Co-efficient</th>
<th>Molar Absorptivity (l.mole(^{-1}). cm(^{-1}))</th>
<th>Sandell’s sensitivity (µg / cm(^2) / 0.001 absorbance unit)</th>
<th>Optimum photometric range (µg / ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-50</td>
<td>A=0.0009+0.0119C</td>
<td>0.9999</td>
<td>9.83 X10(^4)</td>
<td>0.084</td>
<td>7.9-39.1</td>
</tr>
</tbody>
</table>

*Found in this work; It must be determined independently by users of the method.*
Table-2: Precision of the Method

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>% RSD</th>
<th>Percent range of error confidence limit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.05 level</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>1.90</td>
<td>± 2.00</td>
</tr>
</tbody>
</table>

Scheme-1

Fig.-2: Absorption Spectra of Rifampicin with Chloranilic Acid

Table-3: Accuracy of the Method

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Amount of antibiotic (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Taken</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>500</td>
</tr>
</tbody>
</table>
Table-4: Assay of formulations and % recovery data*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Labelled amount (mg)</th>
<th>Amount found (mg) in method</th>
<th>% Recovery (proposed method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin (Capsule)</td>
<td>150</td>
<td>148.0</td>
<td>98.66</td>
</tr>
<tr>
<td>(Tablet)</td>
<td>150</td>
<td>148.1</td>
<td>98.70</td>
</tr>
</tbody>
</table>

* Each result is an average of three determinations  ** After adding 5 mg of drug.

CONCLUSION
The proposed method is simple, rapid and sensitive with reasonable precision and accuracy and it is useful for the determination of rifampicin in bulk samples, pharmaceutical preparations and biological fluids.

REFERENCES

Scope and Coverage:
Water: Research & Development [Water R&D] is an international Research Journal, dedicated to ‘Water’. It is a truly interdisciplinary journal on water science and technology. It'll showcase the latest research related to Water in the field of chemistry, physics, biology, agricultural, food, pharmaceutical science, and environmental, oceanographic, and atmospheric science. It includes publication of reviews, regular research papers, case studies, communications and short notes.

Its Coverage area is:
Water Pollution; Ecology of water resources, including groundwater; Monitoring, remediation and conservation of water resources; Rain Water Harvesting; Absorption and Remediation; Aquatic and Marine life ; Corrosion ; Industrial Effluent treatments; Physics, Chemistry and Biology of water; Water, as a Green solvent/ Reaction Medium; Management of water resources and water provision; Wastewater and water treatment; Water related Rules, Policies, Laws; Dyes and Pigments; Water and Health; Sustainable use of water; Policies and Regulations about water; Degradation of aquatic ecosystem; Water Footprints and Virtual water calculations.

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