ROLE OF ALKALOIDAL PRECIPITANTS FOR THE ASSAY OF IMIPRAMINE HYDROCHLORIDE IN BULK AND PHARMACEUTICAL FORMULATIONS

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ABSTRACT

Simple spectrophotometric methods (A-C) for the assay of Imipramine hydrochloride (IMP) based on the formation of its complexes with alkaloidal precipitants are described. IMP undergo quantitative precipitation in the form of molecular complexes with iodine (I₂ method A), ammonium molybdate (AM, method B) or phosphomolybdic acid (PMA, method C) when used in excess. In addition to precipitation reactions, color reactions have also been combined to estimate IMP. They are based on the color formation with either un-reacted precipitant of the filtrate (in I₂) or released precipitant from the molecular complex (in AM or PMA) with chromogenic reagent such as P-N-methyl amino phenol sulphate (PMAP)-sulphanilic acid (SAc) (for I₂), potassium thiocyanate (PTC) (for AM), cobalt nitrate (Co(II))-disodium salt of ethylene diamine tetra acetic acid (EDTA) complex (for PMA).

Keywords: Imipramine hydrochloride, Spectrophotometer, alkaloids, molecular complexes, precipitants, pharmaceutical formulations

INTRODUCTION

Alkaloids are detected with the aid of group of reactions due to their chemical properties, structure and presence of functional groups. These reactions are based on the ability of the alkaloid to yield insoluble complexes mainly with AM, I₂ and PMA and hence these reagents are named as alkaloidal precipitants¹. The precipitate is ascribed due to the formation of a molecular complex resulting from the interaction of the unshared electron on nitrogen in amine with an unoccupied molecular orbital of the alkaloidal precipitant molecule. Imipramine hydrochloride (IMP) is an antidepressant agent and chemically it is 5-3-(Dimethylamino) propyl-10, 11-dihydro-5H-dibenzazepine hydrochloride. Literature survey reveals that Spectrophotometric¹-¹⁰ HPLC¹¹-³² and LC-MS ³³, ³⁴ methods were reported for the determination of IMP in its formulation and biological fluids. The aim of the present work is to provide simple and sensitive spectrophotometric method for the estimation of IMP in bulk and formulations. The effects in this accord resulted to develop the present methods. IMP furnishes precipitates with alkaloidal precipitants given above, since it contains the nitrogen containing groups (tertiary amino groups). In addition to precipitation reactions color reactions have also been combined to estimate IMP. They are based on the chemical reaction with either released alkaloidal precipitant from the precipitate with acetone ( AM) or un-reacted precipitant in the filtrate (I₂) with chromogenic reagents such as potassium thiocyanate ³⁵ (for AM) PMAP-SAc³⁶ (for I₂) or EDTA- Co (II) ³⁷ (for PMA). The results are statistically validated.
EXPERIMENTAL

Instruments
Spectral and absorbance measurements were made on Systronics UV-Visible Spectrophotometer 117 with 10mm matched quartz cells.

Reagents
All the chemicals & reagents used were analytical grade and the solutions were freshly prepared. Aqueous solution of \( I_2 \) (0.089%) in 0.83% of potassium iodide (KI), PMAP (2%), SAc (0.4%), hydrochloric acid (HCl) (1M) for method A; AM (2%), PTC (10%), conc.HCl (used as it is) for method B; PMA (4%) Co (II) (3%), EDTA (4%) for method C, 0.01 M HCl for methods B and C were prepared in triple distilled water. A one mg/ml solution of IMP was prepared by dissolving 100 mg of pure IMP in 100 ml of distilled water and this stock solution was diluted stepwise with distilled water to obtain the working standard solution of concentrations of 200 \( \mu g / ml \) for method A and C, 400 \( \mu g / ml \) for method B respectively.

Method A
Aliquots of working standard solution (0.5-1.5 ml, 200 \( \mu g / ml \)) were delivered into a series of centrifuge tubes and the volume in each tube was adjusted to 3.0 ml with distilled water. Then 2.0 ml each of 1M HCl and \( I_2 \) were added successively and centrifuged for 5 min. The precipitate was collected by filtration and subsequently washed with 2 ml of distilled water. The filtrate and washings were collected in 25 ml graduated test tubes, then 3.0 ml of PMAP solution and 2.0 ml SAc solution were added successively and the volume was made up to the mark with distilled water. The absorbance was measured during next 30 min. at 520 nm against distilled water. A blank experiment was also carried out omitting the drug. The decrease in absorbance and intern drug concentration was obtained by subtracting the absorbance of the test solution from blank. The amount of drug was calculated from calibration graph.

Method B
Aliquots of working standard solution (0.5-1.5 ml, 400 \( \mu g / ml \)) were delivered into a series of centrifuge tubes and the volume in each tube was adjusted to 3.0 ml with 0.01M HCl. Then 1.0 ml of AM was added and centrifuged for 5 min. The precipitate was collected by filtration followed by washing with 50% alcohol until it is free from the reagent. The precipitate in each tube was dissolved in 5.0 ml of acetone and transferred into 25.0 ml graduated tube. The 5 ml of conc. HCl and 3 ml PTC solution were successively added and kept aside for 30 min and then volume in each tube was made up to the mark with distilled water. The absorbance was measured at 480 nm against a same blank reagent. The amount of drug IMP was calculated form the calibration graph.

Method C
Aliquots of working standard solution (0.5-1.5 ml, 200 \( \mu g / ml \)) were delivered into a series of centrifuge tubes and volume in each tube was adjusted to 3.0 ml with 0.01M HCl. The 2.0 ml PMA was added and centrifuged for 5 min. the precipitate was collected by filtration followed by washing with distilled water until it is free from the reagent. The precipitate in each tube was dissolved in 5 ml of acetone and transferred into 25 ml graduated tubes. One ml each of Co (II) and EDTA solution was successively added and the tubes were heated for 15 min. at 60°C in water bath. The tubes were cooled and the solution in each tube was made up to the mark with distilled water. The absorbance was measured at 840 nm against a same blank reagent. The amount of drug was calculated from its calibration graph.

RESULTS AND DISCUSSION
The optimum conditions for the color development of methods (A, B and C) were established by varying the parameters one at a time keeping the others fixed and observing the effect produced on the absorbance of the colored species. The optical characteristics such as Beer’s law limits, molar absorptivity and Sandell’s sensitivity for each method (A-C) are given in table1. The precision of each method to the drug was found by measuring the absorbance of six separate samples containing known amounts of the drug and the results obtained are incorporated in table1. 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 (Sₑ) for each system and is presented in table-1. The accuracy of the methods was ascertained by comparing the results by proposed and reference methods (UV) statistically by t- and F-tests (Table 2). The comparison shows that there is no significant difference between the results of studied methods and those of reference ones. The similarity of the results is obvious evidence that during the application of these methods, the excipients that are usually present in pharmaceutical formulations do not interfere in the assay of proposed methods. As an additional check of accuracy of the proposed methods recovery experiments were carried out. The recoveries of the added amounts of standard drug were studied at 3 different levels. Each level was repeated for 6 times and from the amount of drug found, the % recovery was calculated in the usual way.

**CONCLUSIONS**

The higher \( \lambda_{\text{max}} \) values of all the proposed methods have a decisive advantage since the interference from the associated ingredients should be generally less at higher wavelengths than at lower wavelengths. Thus the proposed visible spectrophotometric methods are simple and sensitive with reasonable precision, accuracy and constitute better alternatives to the existing ones to the routine determination of IMP in bulk form and pharmaceutical formulations.

### Table-1: Optical Characteristics, Precision and Accuracy of the Proposed Methods (A, B&C) for IMP

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>520</td>
<td>480</td>
<td>840</td>
</tr>
<tr>
<td>Beer’s Law limits (( \mu g/ml ))</td>
<td>2-18 ( \mu g/ml )</td>
<td>5-35 ( \mu g/ml )</td>
<td>2-20 ( \mu g/ml )</td>
</tr>
<tr>
<td>Molar absorptivity (l mole(^{-1})cm(^{-1}))</td>
<td>1.692(10^4)</td>
<td>6.052 (10^4)</td>
<td>1.451 (10^4)</td>
</tr>
<tr>
<td>Sandell’s sensitivity (( \mu g/cm^2/0.001 ) absorbance unit)</td>
<td>0.019</td>
<td>0.052</td>
<td>0.022</td>
</tr>
<tr>
<td>Regression Equation ( y = a + bc )</td>
<td>0.0541</td>
<td>0.0191</td>
<td>0.0458</td>
</tr>
<tr>
<td>Slope (b), Intercept (a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>0.9998</td>
<td>0.9998</td>
<td>0.9998</td>
</tr>
<tr>
<td>Relative Standard Deviation (%) **</td>
<td>0.2651</td>
<td>0.340</td>
<td>0.364</td>
</tr>
<tr>
<td>% Range of error **</td>
<td>0.2220</td>
<td>0.340</td>
<td>0.305</td>
</tr>
<tr>
<td>(0.05 level confidence limit)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*\( Y = a + bc \), where c is the concentration in \( \mu g/ml \). **From six determinations.

### Table-2: Determination of IMP in Pharmaceutical Formulations

<table>
<thead>
<tr>
<th>Sample (^A) (Tablets)</th>
<th>Labeled method (mg)</th>
<th>UV(^*) Method</th>
<th>Amount obtained (mg)</th>
<th>Proposed method</th>
<th>Recovery(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>T(_1)</td>
<td>50</td>
<td>49.98±0.022</td>
<td>49.97±0.010</td>
<td>50.01±0.026</td>
<td>49.97±0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=2.57</td>
<td>F=1.43</td>
<td>F=2.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t=0.22</td>
<td>t=0.87</td>
<td>t=0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>99.97</td>
<td>100.22</td>
<td>99.97</td>
</tr>
<tr>
<td>T(_2)</td>
<td>50</td>
<td>49.97±0.006</td>
<td>50.01±0.020</td>
<td>49.99±0.024</td>
<td>50.00±0.031</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=1.36</td>
<td>F=1.25</td>
<td>F=2.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t=0.78</td>
<td>t=1.08</td>
<td>t=1.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100.1</td>
<td>99.97</td>
<td>100.13</td>
</tr>
<tr>
<td>T(_3)</td>
<td>50</td>
<td>49.98±0.009</td>
<td>49.98±0.027</td>
<td>50.00±0.032</td>
<td>49.98±0.033</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F=1.54</td>
<td>F=2.07</td>
<td>F=2.09</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>t=0.46</td>
<td>t=1.15</td>
<td>t=1.14</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>99.88</td>
<td>99.87</td>
<td>100.13</td>
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</table>

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Four different batches of tablets from a Pharmaceutical company.

REFERENCES