CHAPTER VI

TITRIMETRIC, SPECTROPHOTOMETRIC, KINETIC AND HPLC METHODS FOR THE DETERMINATION OF ALBENDAZOLE
SECTION 6.0
DRUG PROFILE AND LITERATURE SURVEY

6.0.1. DRUG PROFILE

Albendazole (ALB) is an anthelmintic drug having the chemical name [5-(propylthio)-1H-benzimidazole -2-yl] carbamic acid methyl ester. It has the empirical formula C_{12}H_{15}N_{3}O_{2}S and has the molecular weight 265.33.

It was synthesised by R. J. Gyurik and V. J. Theodorides [1]
[U. S. Pat. 3,915,986 (1975 to SmithKline)]

ALB has the following structure

\[
\begin{align*}
\text{H} & \\
\text{NHCOOCH}_3 & \\
\text{H}_3\text{CH}_2\text{CH}_2\text{CS} & \\
\end{align*}
\]

It is a white, fluffy, odourless powder and soluble in glacial acetic acid, methanol, sparingly soluble in ethanol and practically insoluble in water.

Albendazole is an anthelmentic benzimidazole drug widely used in veterinary and human medicine for the treatment of infections caused by helminates [2]. In higher doses it is also used in the treatment of echinococcosis [3,4].
6.0.2. LITERATURE SURVEY OF TITRIMETRIC AND SPECTROPHOTOMETRIC METHODS FOR ALBENDAZOLE

The following paragraphs contain a brief description of the titrimetric, spectrophotometric and a high performance liquid chromatographic methods reported so far for the determination of ALB in pharmaceuticals.

Titrimetric methods

Literature survey revealed that ALB has been assayed titrimetrically based on neutralisation reaction in non-aqueous medium and on a redox reaction. The non-aqueous titration method [5] involved use of perchloric acid as the titrant and crystal violet as the indicator in acetic acid medium. The method was applied to determine 2-20 mg of ALB in bulk drug and tablets.

In two redox titrimetric procedures reported by the same authors [6] N-chlorosuccinimide (NCS) was employed as the oxidimetric reagent. In one method 2-13 mg of ALB was titrated directly in hydrochloric acid medium with NCS using indigo carmine indicator. In another method, applicable to 4 to 14 mg of ALB, the acidified solution of ALB was treated with measured excess of NCS followed by iodometric back titration of the unreacted oxidant. Both methods were applied successfully to determine ALB in tablets with quantitative recoveries.

Visible spectrophotometric methods

The reported visible spectrophotometric methods are based on the neutralisation, redox and complex formation reactions. The method based on the
neutralization reaction [5] consisted of treating acetus solution of ALB with perchloric acid crystal violet mixture followed by measurement of change in absorbance at 570 nm. A linear correlation was found between the absorbance and the concentration of ALB over 5-60 μg ml⁻¹ ALB with a ε value of 2.2×10³ l mol⁻¹ cm⁻¹ and a detection limit of 1.02 μg ml⁻¹. The method when applied to tablets gave recoveries in the range 97.63 to 102.4 % and RSD of 0.85 – 1.54%.

In an indirect method reported by the same authors [6] ALB was reacted with known excess of NCS in acid medium and the excess of oxidant was determined through a complex formation reaction involving iron (II) and thiocyanate and measuring the absorbance of the resulting complex iron (III) cyanate at 480 nm. Beer’s law was obeyed over 0.6 to 10 μg ml⁻¹ with an ε value of 1.41 ×10⁴ l mol⁻¹ cm⁻¹ and detection limit of 0.61 μg ml⁻¹. The method was applied to two brands of tablets with quantitative recoveries.

In two similar procedures Sastry et al. [7] have used NBS as the oxidimetric reagent. In one method, ALB was treated with NBS in acetic acid medium and the unreacted oxidant was determined through a charge-transfer complex formation reaction involving metol and sulphanamide. The absorbance of the complex was measured at 520 nm. The method was found to be applicable over 4-40 μg ml⁻¹ ALB and the ε value was 3.58×10³ l mol⁻¹ cm⁻¹. But the measured coloured species was less stable (15 min). In the second approach, the unreacted NBS was treated with celestine blue dye and the change in absorbance was measured at 540 nm and related to the drug concentration. The absorbance-concentration plot was linear for 0.4 to 2 μg ml⁻¹
ALB with an $\varepsilon$ value of $3.66 \times 10^4$ l mol$^{-1}$ cm$^{-1}$. Both methods were applied to ALB containing formulations.

Zarapkar and Deshpande [8] have described a method based on complex formation and involving the use of Folin-Ciocalteu (F-C) reagent, the molybdenum blue complex resulting from the reduction of F-C reagent by ALB was measured at 760 nm. The calibration graph was rectilinear from 2 to 16 $\mu$g ml$^{-1}$. The method was applied to tablets and syrups with CV of 0.6 and 0.7.

There are two reports on the use of ion-pair complex formation reaction for the extractive spectrophotometric determination of ALB. The method described by Kumar et al. [9] involved the reaction of ALB with picric acid, extraction of the complex into chloroform and measurement at 410 nm. Beer's law was obeyed over 1 to 40 $\mu$g ml$^{-1}$. On applying the method to dosage forms, the results were quantitative with a recovery of 99.41 to 99.66%.

In a similar procedure employed by Sane et al. [10], four dyes-bromocresol green (BCG), bromophenol red (BPR), bromophenol blue (BPB) and bromothymol blue (BTB) were used as ion-pair agents. The ion pair complex formed between ALB and the dye was extracted into chloroform and measured at 420 nm. All the four methods were applicable for 2 to 12 $\mu$g ml$^{-1}$ ALB. When the methods were applied to pharmaceuticals, the recoveries were quantitative and no interference from coformulated substances was observed. But the complex formation requires a rigid pH control.
High performance liquid chromatographic methods

A good number of methods have been proposed for the assay of ALB in pharmaceuticals using HPLC.

A reverse phase HPLC method was reported by Krishnaiah et al. [11] for the determination of ALB. The analysis was performed on a RP C18 column. The mobile phase consisted of acetonitrile and water (containing 0.4% triethylamine adjusted to pH 3.6 with 5% phosphoric acid) (46:54) with UV detection at 254 nm. Mebendazole was used as an internal standard. The linearity of the method was from 0.1 to 40 μg ml⁻¹. The method was applied to dosage forms.

An HPLC method [11] for the quantitation of ALB on a Lichrosorb 10 RP-18 column at ambient temperature with a mobile phase of tetrahydrofuran-water (55:45) and 0.5% acetic acid has been reported. The column effluent was monitored at 296 nm. The calibration graph was linear over a range 50 to 300 μg ml⁻¹ ALB. The limit of detection was reported was 0.2 μg ml⁻¹. The method was applied to the determination of tablets and the recovery ranged from 99.5 to 100.1 % with a RSD of 0.09%.

Gomes and Nagaraju [12] have reported a reverse phase HPLC method for the determination and separation of potential impurities of ALB in bulk drug.

Nary et al. [13] have reported a method for the determination of ALB in oral suspension. The analysis was performed on a Nucleosil C18 (5 cm×150 mm) Phenomenex column and mobile phase consisting of methanol 0.05 M phosphoric acid buffer (70:30) and UV-detection at 254 nm.
In a method reported by Sane et al. [14] for the determination of ALB in pharmaceuticals, the separation and determination were achieved on μBondapak C\textsubscript{18} (30 cm × 3.9 mm) column with methanol-water-acetonitrile (55:44:1) as mobile phase (1.5 ml min\textsuperscript{-1}) and detection at 256 nm. Tinidazole was used as an internal standard. The calibration graph was rectilinear from 100 to 400 µg ml\textsuperscript{-1}. The method was applied to tablets and suspension. Recoveries were quantitative and coefficient of variation were 0.7 and 0.5 % for tablets and suspension, respectively.

Malan et al. [15] have described HPLC analysis of ALB in a mixture, on a column of 4 µm Nova pak C\textsubscript{18} cartridge (25 cm×3.9 mm id) and mobile phase of 0.5 M dibasic ammonium phosphate : methanol (3:1) of pH 3.6 at a flow rate of 1 to 1.5 ml min\textsuperscript{-1}, detection at 250 nm. Calibration graph was linear for 12 to 60 µmol ml\textsuperscript{-1}. The compatibility of the method was correlated with differential scanning colorimetry (DSC).

Isocratic separation of ALB [16] was achieved by HPLC on a column (30 cm × 4 mm) of ODS Micropak (10 µm), with 17 mM phosphoric acid – acetonitrile (3:2) as mobile phase (at 35° to 60°) and photo-diode-array detection at 290 nm. Calibration graph was rectilinear from 0.06 to 2 µg ml\textsuperscript{-1}. The detection limit was 1.25 ng.

Other methods.

The other methods reported for the determination of ALB in pharmaceuticals are UV-spectrophotometry [13, 17], derivative UV-spectrophotometry [18, 19], voltammetry [20, 21], differential pulse voltammetry [22] and superficial fluid chromatography [23].
A close examination of the reactions employed for the titrimetric and spectrophotometric assay methods reported for ALB revealed that ALB is prone to oxidation, and there is scope for applying other oxidimetric reagents for the determination of ALB. Keeping this point in view, new titrimetric, spectrophotometric and kinetic methods have been developed for the assay of ALB in pharmaceuticals. The new methods use bromate-bromide mixture, iodate and chloramine-T as the oxidimetric reagents.

Besides, a new HPLC method with higher sensitivity, long dynamic range of linear response and shorter retention time has also been developed for ALB. The details of the method development and method validation are presented in this Chapter.
SECTION 6.1
TITRIMETRIC, SPECTROPHOTOMETRIC AND KINETIC
METHODS FOR THE DETERMINATION OF ALBENDAZOLE
USING BROMATE BROMIDE AND METHYL ORANGE AS
REAGENT

6.1.1. INTRODUCTION

Bromate-bromide mixture in combination with methyl orange has in the past few years been applied for the titrimetric and spectrophotometric determination of numerous pharmaceuticals and were reviewed in the previous chapters. From the review of the literature on the assay methods for ALB using various techniques presented in the preceding Section (6.0.2), it is clear that ALB has not been determined bromatometrically using titrimetric, spectrophotometric techniques and kinetic methods. In this work, this combination (bromate-bromide/methyl orange) has been evaluated as a reagent for the titrimetric, spectrophotometric and kinetic assay of ALB. The details of the assay methods are presented in this section.

6.1.2. EXPERIMENTAL

6.1.2.1. Apparatus

A Systronics model 107 digital spectrophotometer with 1 cm quartz cells was used for the absorbance measurement.

6.1.2.2. Reagents

All chemicals used were of analytical reagent grade. Double-distilled water was used throughout the experiment.
Potassium bromate (0.02 M). Prepared as described in section 2.1.2. and was stepwise diluted to get a working concentration of 0.002 M and 15 µg ml⁻¹ solutions for direct titrimetry and spectrophotometry, respectively.

Potassium bromate (0.01 M)–potassium bromide mixture (0.05 M). Preparation is described in section 2.1.2

Potassium bromide (10%). Prepared as described in section 2.1.2.

Sodium thiosulphate (0.01M). Sodium thiosulphate (2.5 g, SISCO Chem., India) was dissolved in water in one litre volumetric flask and the solution was standardized using pure potassium iodate [38].

Potassium iodide (10%). Prepared as described in section 2.2.2.

Starch (1%). Prepared as described in section 2.2.2.

Methyl orange (1000 µg ml⁻¹). Prepared and diluted to 50 and 40 µg ml⁻¹ as described in section 2.1.2.

Methyl orange (0.1%). Prepared as described in section 2.1.2

Hydrochloric acid (5 M). Concentrated acid (442.5 ml, Sd. Fine Chem., India Ltd., Sp. gr. 1.18) was diluted to one litre with water.

Standard solution of ALB (2 mg ml⁻¹). Accurately weighed 0.5 g of ALB (Cipla India Ltd., Mumbai) was dissolved in a minimum quantity of glacial acetic acid and diluted to 250 ml with water in a 250 ml volumetric flask, and was used for titrimetric and kinetic work. This (2000 µg ml⁻¹) was diluted stepwise to obtain a 20 µg ml⁻¹ working standard solution for spectrophotometric work.
6.1.2.3. Procedures

Direct Titrimetry (Method A)

A 10 ml aliquot of pure drug solution containing 3 to 20 mg of ALB was accurately measured into a 100 ml titration flask followed by the addition of 5.0 ml of hydrochloric acid and 5.0 ml of 10% potassium bromide. The contents were mixed well and titrated with bromate (0.002 M) solution using methyl orange as indicator till the discharge of indicator colour. A blank determination was run, and the volume was subtracted from the volume required for sample titration.

\[
\text{Amount (mg)} = \frac{VM_\text{w}R}{n}
\]

where

- \( V \) = volume bromate consumed by ALB, ml
- \( M_\text{w} \) = relative molecular weight of ALB
- \( R \) = strength of bromate solution, mol l\(^{-1}\)
- \( n \) = number of moles of bromate required to react with each mole of ALB.

Indirect Titrimetry (Method B)

Transferred a 10 ml aliquot of drug solution containing 1-10 mg of ALB to a glass stoppered volumetric flask. Added 5.0 ml 5 M hydrochloric acid followed by 5.0 ml of 0.02 M potassium bromate and 5.0 ml of 10% potassium bromide solutions. The flask was kept aside for 40 min with occasional swirling. Then, 5.0 ml of 10% potassium iodide solution were added and the liberated iodine was titrated with 0.01 M sodium thiosulphate \((V_1)\) using starch indicator. A blank experiment was carried out in the same manner \((V_2)\). The amount of the drug was calculated from the equation.
\[
\text{Amount (mg)} = \frac{(V_2 - V_1) M_w R}{n}
\]

where

\(V_1\) = volume of sodium thiosulphate solution consumed in the sample titration, ml

\(V_2\) = volume of sodium thiosulphate solution consumed in the blank experiment, ml

\(M_w\) = relative molecular mass of drug

\(R\) = molarity of KBrO_3 solution and

\(n\) = number of moles of KBrO_3 required per mole of ALB

**Kinetic method (Method C)**

In kinetic method, 5, 10, 15, 20 and 25 ml aliquots of drug solution (100 \(\mu g\) ml\(^{-1}\)) were transferred into separate 50 ml volumetric flasks containing 25 ml of methyl orange solution (40 \(\mu g/ml\)) and diluted to volume with water. Into separate test tubes of similar dimensions, 5.0 ml of the above prepared solution and 5.0 ml 0.01 M KBrO_3 – 0.05 M KBr solution were accurately measured. Both the tubes were immersed in an ice bath until they reached 4 to 5°. The stop clock (accurate to 0.2 s) was started and the two solutions were thoroughly mixed noting the time of addition (initial time \(T_i\)). The time required for methyl orange colour to discharge was noted (final time, \(T_f\)). The actual time \((T_c)\) was computed, where, \(T_c = T_f - T_i\). A blank experiment was carried out simultaneously by mixing bromate–bromide solution with methyl orange solution, omitting addition of drug. Bleaching time \((T_b)\) for blank experiment was recorded. The corrected \((T_{c'})\) time was computed using \(T_{c'} = T_c - T_b\). A calibration curve was prepared by plotting \(T_{c'}\) as a function of concentration of drug or the regression equation was calculated using the bleaching time and concentration.
data. The concentration of the unknown was read from the calibration curve or computed using the regression equation.

**Spectrophotometry (Method D)**

Different aliquots (0.25- 2.0 ml) of 20 $\mu$g ml$^{-1}$ of ALB solution were transferred into a series of 10 ml volumetric flasks. To each flask were added 1.0 ml of 5 M hydrochloric acid, 1.0 ml of 15 $\mu$g ml$^{-1}$ of potassium bromate and 2.0 ml 10% potassium bromide solutions. The contents were mixed well and kept aside for 20 min with occasional shaking. Then, 1.0 ml of 50 $\mu$g ml$^{-1}$ methyl orange solution was added and diluted to mark with water. The absorbance was measured after 5 min at 510 nm. A calibration curve was prepared by plotting increasing values of absorbance versus concentration of drug. The concentration of the unknown was read from the calibration graph or computed from the regression equation.

**Procedure for dosage forms.**

Twenty to 40 tablets depending on the content per tablet were weighed and finely powdered. An accurately weighed portion equivalent to 200 mg of ALB was transferred into 100 ml volumetric flask, 20 ml glacial acetic acid and 40 ml of water were added and shaken thoroughly for about 20 min. Then, the volume was made up to the mark, mixed well and filtered using a quantitative filter paper. First 10 ml portion of the filtrate was rejected and a suitable aliquot of the filtrate was treated as described under the procedure for titrimetric and kinetic methods determination. The formulation was diluted to 20 $\mu$g ml$^{-1}$ for spectrophotometric assay.
<table>
<thead>
<tr>
<th>Method**</th>
<th>Amount taken</th>
<th>Amount Found*</th>
<th>Range</th>
<th>RE, %</th>
<th>SD</th>
<th>RSD, %</th>
<th>ROE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method A</td>
<td>7.00</td>
<td>7.06</td>
<td>0.09</td>
<td>0.86</td>
<td>0.07</td>
<td>1.00</td>
<td>± 0.99</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.10</td>
<td>0.05</td>
<td>1.0</td>
<td>0.03</td>
<td>2.97</td>
<td>± 2.96</td>
</tr>
<tr>
<td></td>
<td>15.00</td>
<td>15.10</td>
<td>0.60</td>
<td>0.66</td>
<td>0.02</td>
<td>1.33</td>
<td>± 1.32</td>
</tr>
<tr>
<td>Method B</td>
<td>2.0</td>
<td>2.03</td>
<td>0.26</td>
<td>1.50</td>
<td>0.05</td>
<td>2.46</td>
<td>± 2.45</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>4.02</td>
<td>0.23</td>
<td>6.50</td>
<td>0.08</td>
<td>1.99</td>
<td>± 1.98</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>8.03</td>
<td>0.32</td>
<td>0.38</td>
<td>0.02</td>
<td>0.24</td>
<td>± 0.23</td>
</tr>
<tr>
<td>Method C</td>
<td>50</td>
<td>50.23</td>
<td>0.32</td>
<td>0.46</td>
<td>0.22</td>
<td>0.43</td>
<td>± 0.42</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>98.90</td>
<td>0.24</td>
<td>1.10</td>
<td>0.48</td>
<td>0.48</td>
<td>± 0.47</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>148.79</td>
<td>0.85</td>
<td>0.81</td>
<td>0.89</td>
<td>0.60</td>
<td>± 0.59</td>
</tr>
<tr>
<td>Method D</td>
<td>15.0</td>
<td>15.12</td>
<td>0.58</td>
<td>0.80</td>
<td>0.05</td>
<td>0.33</td>
<td>± 0.32</td>
</tr>
<tr>
<td></td>
<td>25.0</td>
<td>25.17</td>
<td>0.78</td>
<td>0.68</td>
<td>0.15</td>
<td>0.59</td>
<td>± 0.58</td>
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<td>35.0</td>
<td>35.49</td>
<td>0.2</td>
<td>1.40</td>
<td>0.42</td>
<td>1.18</td>
<td>± 1.17</td>
</tr>
</tbody>
</table>

* Average of seven replicate analyses

** In methods A and B amount taken/found, range, SD are in mg, and in μg for methods C and D.

SD. Standard deviation, RE relative error, RSD. Standard deviation, ROE. Range of error.
Table 6.1.2. Comparison of results of ALB determination by the proposed methods and reference method

<table>
<thead>
<tr>
<th>Tablets*</th>
<th>Label claim mg / tablet</th>
<th>Found** (%recovery ± SD)</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
<th>Method D</th>
<th>Reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alminth(^a)</td>
<td>200</td>
<td></td>
<td>99.15 ± 0.31</td>
<td>99.88 ± 0.50</td>
<td>98.39 ± 0.81</td>
<td>99.01 ± 0.42</td>
<td>99.74 ± 0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t= 2.14</td>
<td>t= 2.27</td>
<td>t= 2.19</td>
<td>t= 2.35</td>
<td></td>
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<tr>
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<td></td>
<td>F= 3.26</td>
<td>F= 1.25</td>
<td>F= 2.09</td>
<td>F= 1.77</td>
<td></td>
</tr>
<tr>
<td>Albental(^b)</td>
<td>400</td>
<td></td>
<td>99.81 ± 1.15</td>
<td>101.05 ± 0.98</td>
<td>102.59 ± 2.12</td>
<td>99.89 ± 1.02</td>
<td>100.96 ± 0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>t= 1.96</td>
<td>t= 0.15</td>
<td>t= 1.69</td>
<td>t= 1.74</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F= 1.56</td>
<td>F= 1.13</td>
<td>F= 5.30</td>
<td>F= 1.23</td>
<td></td>
</tr>
<tr>
<td>Zoleban(^d)</td>
<td>500</td>
<td></td>
<td>99.15 ± 1.33</td>
<td>98.98 ± 1.31</td>
<td>101.01 ± 1.21</td>
<td>99.58 ± 0.85</td>
<td>100.84 ± 0.86</td>
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<td>t= 2.44</td>
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<td></td>
<td></td>
<td>F= 2.39</td>
<td>F= 2.32</td>
<td>F= 1.98</td>
<td>F= 1.02</td>
<td></td>
</tr>
</tbody>
</table>

* - Marketed by: \(^a\) - Torrent, \(^b\) - Micro labs, \(^d\) - Combat Drugs, India
** - Average of five measurements
\# - Tabulated value at 95% confidence level in 2.77
\$ - Tabulated value at 95% confidence level in 6.39
Table 6.1.3. Results of recovery studies by standard – addition method

<table>
<thead>
<tr>
<th>Method</th>
<th>Alminth (200 mg)</th>
<th></th>
<th></th>
<th>Albental (400 mg)</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Amount of drug in formulation</td>
<td>Amount pure drug added</td>
<td>Total found*</td>
<td>% recovery of pure drug</td>
<td>Amount of drug in formulation</td>
<td>Amount pure drug added</td>
</tr>
<tr>
<td>Method A</td>
<td>2.97</td>
<td>5.00</td>
<td>7.77</td>
<td>97.50</td>
<td>2.96</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>2.97</td>
<td>10.00</td>
<td>12.74</td>
<td>98.21</td>
<td>2.96</td>
<td>10.00</td>
</tr>
<tr>
<td></td>
<td>2.97</td>
<td>15.00</td>
<td>17.71</td>
<td>98.55</td>
<td>2.96</td>
<td>15.00</td>
</tr>
<tr>
<td>Method B</td>
<td>0.99</td>
<td>2.50</td>
<td>3.43</td>
<td>97.60</td>
<td>1.11</td>
<td>2.50</td>
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<tr>
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<td>0.99</td>
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<td>5.45</td>
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<td>4.50</td>
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<td>0.99</td>
<td>6.50</td>
<td>7.37</td>
<td>98.15</td>
<td>1.11</td>
<td>6.50</td>
</tr>
<tr>
<td>Method C</td>
<td>0.41</td>
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<td>0.89</td>
<td>97.41</td>
<td>0.49</td>
<td>0.50</td>
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<td>0.41</td>
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<td>1.39</td>
<td>98.88</td>
<td>0.49</td>
<td>1.00</td>
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<td>0.41</td>
<td>1.50</td>
<td>1.91</td>
<td>97.99</td>
<td>0.49</td>
<td>1.50</td>
</tr>
<tr>
<td>Method D</td>
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<td>10.00</td>
<td>12.64</td>
<td>101.60</td>
<td>2.49</td>
<td>10.00</td>
</tr>
<tr>
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<td>2.48</td>
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<td>22.98</td>
<td>102.50</td>
<td>2.49</td>
<td>20.00</td>
</tr>
<tr>
<td></td>
<td>2.48</td>
<td>30.00</td>
<td>32.14</td>
<td>98.87</td>
<td>2.49</td>
<td>30.00</td>
</tr>
</tbody>
</table>

* Average of three determinations

** In Methods A and C amount of drug in formulations/pure drug added/total found are in mg, and in Method B and C all are in μg.
Fig. 6.1.1. Calibration graph (Method C)

Fig. 6.1.2. Beer's law plot
6.1.3. RESULTS AND DISCUSSION

The bromimetric determination of ALB is based on oxidation as well as bromination of the drug molecule. The bromate-bromide reagent in acid medium serves a source of bromine, which effects oxidation / bromination of the drug.

6.1.3.1. METHOD DEVELOPMENT

Direct titrimetry (Method A)

The titration was carried out in hydrochloric acid medium, 5 ml of 5M acid being optimum in a total volume of about 25 to 30 ml. Methyl orange served better compared to other dyes tried. The reaction stoichiometry was found to be 1:1 (ALB:KBrO₃)

The relationship between the titration end-point obtained by this method and the drug amount was examined. The linearity between the amount of the drug and titrimetric endpoint is apparent from the correlation coefficient, r obtained by determining the best fit line via linear least squares treatment. The correlation coefficient of 0.9992 shows that the reaction proceeds stiochiometrically in a molar ratio 1:1 (ALB:KBrO₃). Under the optimum conditions, the method was applicable for assay in 3-20 mg range.

Indirect Titrimetry (method B).

The quantitative nature of the reaction between ALB and bromate-bromide reagent was checked by treating 1-10 mg of drug with a known excess of reagent and back titrating the surplus reagent iodometrically. The stoichiometry was calculated to
be 1:1 (ALB:KBrO₃). This reveals that the reaction between ALB and bromate bromide agent can be represented by the following scheme:

\[
\text{BrO}_3^- + 5\text{Br}^- + 6\text{H}^+ \rightarrow 3\text{Br}_2 + 3\text{H}_2\text{O}
\]

The reaction was carried out in hydrochloric acid medium. The stoichiometry was found to be unaffected when 2 to 5 ml of 5 M HCl was used in a total volume of about 30 ml. The reaction was found to be slow taking 40 min for completion. Contact times up to 60 min had no effect on the stoichiometry of the reaction.

The method was found to be applicable in the range 1-10 mg of ALB. The relationship between titration end points and the drug amounts was examined. The linearity between the amount of the drug and titration end point is apparent from the correlation coefficient, \(r\) obtained by determining by the best fit line via linear least squares treatment. The correlation coefficient of -0.9983 shows that the reaction proceeds stiochiometrically in a molar ratio of 1:1 (ALB: KBrO₃).
Kinetic method (Method C)

Oxidation / bromination was found to be much faster than the rate of bromine production following addition of acid to bromate–bromide solution at room temperature (32 ± 2°). However, by employing small concentration of drug and decreasing the reaction temperature to 4 to 5°, a small steady state concentration of bromine, which acts as a chemical clock, is setup; a rate that approximately the reaction rate. The sharp increase in bromine production after the reaction is sufficient to discharge the acid colour of methyl orange, and the time of bleaching is directly proportional to the concentration of the drug.

The calibration curve of time (\(T_c\) in s) Vs concentration (C) (Fig. 6.1.1) in the range of 5 to 25 \(\mu\)g ml\(^{-1}\) calculated from the final dilution after methyl orange addition can be described by the following regression equation derived by the method of least squares. \(T_c = 0.4290 - 0.2372 C\). The regression coefficient for the above relationship was calculated to be 0.9951. Extrapolation of the linear relationship intercepts the time axis at a point almost equal to the blank reading. The experimental reading can therefore be corrected.

Spectrophotometry (Method D)

The ability of bromine to bleach methyl orange has been used to develop an indirect spectrophotometric method for ALB. When increasing amounts of ALB were treated with a fixed amount of bromate-bromide mixture in HCl medium, there will be a proportional decrease in the concentration of \textit{insitu} generated bromine after the specified time. On reacting with a fixed amount of methyl orange there will be
concomitant increase on the concentration of dye as shown by the linear increase in the absorbance at 510 nm, with increase in concentration of ALB.

The various parameters involved in the determination were optimized. It was found that 1.0 ml of 50 μg ml\(^{-1}\) methyl orange in a total volume of 10 ml was required to obtain a convenient maximum absorbance. The colour due to this was found to be completely bleached by 1.0 ml of 15 μg ml\(^{-1}\) of KBrO\(_3\) in the presence of excess of bromide. Hence, different amounts (5-40 μg) were reacted with a fixed amount of (150 μg) of bromate and excess of bromide in HCl medium to determine the working concentration range. Hydrochloric acid medium was found to be suitable for both oxidation - bromination of the drug and bleaching of methyl orange by bromine. A 1.0 ml of 5 M HCl was found adequate for both reactions. Although a 15 min standing time was found adequate for oxidation – bromination reaction, 20 min was allowed for the reaction. The bleaching of the dye was found to be complete in 5 min.

The increasing absorbance values at 510 nm were plotted against the increasing concentration of ALB to obtain a calibration graph (Fig. 6.1.2). Beer’s law was obeyed over the concentration range 5.0 - 40 μg of ALB in a total aqueous volume of 10 ml, the equation of the line being,

\[
Y = 0.0 + 0.105X
\]

where \(Y\) is the absorbance and \(X\) is the amount of ALB (μg). The correlation coefficient of the calibration plot was calculated as 0.9990 (\(n = 8\)) confirming a linear increase in absorbance with increasing concentration of ALB. The calculated molar absorptivity was found to be \(2.90\times10^4\) \(\text{lmol}^{-1}\ \text{cm}^{-1}\) at 510 nm and the Sandell
sensitivity was 9.7 ng cm\(^{-2}\). The limit of detection and limit of quantification were 0.054 and 0.181 µg ml\(^{-1}\), respectively, and indicate the high sensitivity and of the method.

### 6.1.3.2. METHOD VALIDATION

#### Accuracy and precision

To establish the accuracy and precision of the proposed methods, pure drug at three different levels (within the working limits) was determined, each determination being repeated seven times. The relative error (%) which is a measure of accuracy and RSD (%) a measure of precision are summarised in Table 6.1.1 and reveal the high accuracy and precision of the methods. For a better picture of reproducibility on a day-to-day basis a series was run in which the standard drug solution at three levels was analyzed each day for five days. The day-to-day RSD values were in the range of 1.5 to 3.5% and represented the best appraisal of the methods in routine use.

#### Determination of ALB in Tablets

The proposed methods were applied to the determination of ALB in some representative tablets, which were commercially available in the local market. The drug content of same batch tablets and injections was also determined by the reference method [13] and the results are presented in Table 6.1.2. It is clear from the results that there is close agreement between the results obtained by the proposed methods and those of the reference method [13]. The results were also compared statistically by Student’s t-test for accuracy and a variance ratio F-test for precision with those of the reference method at 95% confidence level. The calculated t- and F-values (Table...
6.1.2) did not exceed the tabulated values ($t=2.77$, $F=6.39$) for four degrees of freedom indicating that there was no significant difference between the proposed methods and the reference method in respect of accuracy and precision.

**Recovery studies**

To establish the reliability and validity of the proposed methods a standard – addition method was followed. To a fixed amount of ALB in tablet preparation (pre-analyzed), pure ALB at three different levels were added. Each level was repeated three times. The total amount of the drug was determined by the proposed procedures and the results of the recovery study complied in Table 6.1.3 show that tablet excipients like starch, lactose, talc, stearate, alginate etc. did not interfere in the determination.
SECTION 6.2.

TITRIMETRIC AND SPECTROPHOTOMETRIC
DETERMINATION OF ALBENDAZOLE USING POTASSIUM IODATE AS REAGENT

6.2.1. INTRODUCTION

The use of potassium iodate as the reagent for the determination of many bioactive compounds was reviewed in Chapter 5, Section 5.2.1. From the literature survey on ALB presented in section 6.0.2 it is clear that no method has been developed using potassium iodate for the determination of ALB in bulk drug and in formulations. The author has investigated the reaction of ALB with iodate and has developed titrimetric and spectrophotometric methods based on this reaction and the details are described in this Section.

6.2.2. EXPERIMENTAL

6.2.2.1. Apparatus

A Systronics model 106 digital spectrophotometer provided with 1-cm quartz cells was used for absorbance measurements.

6.2.2.2. Reagents and solutions

All used chemicals were analytical reagent grade and solutions were prepared in distilled water.

Potassium iodate (0.002 M). Prepared as described in Section 5.2.2.

Potassium iodate (0.4 %). Prepared by dissolving 0.4 g of the chemical in 100 ml water and used for spectrophotometric method.
**Dichlorofluorescein (0.01 %)** Prepared as described in Section 5.2.2.

**Monochloroacetic and reagent** Prepared as described in Section 5.2.2.

**Sodium thiosulphate (0.012 M).** Prepared as described in Section 5.2.2.

**Starch indicator (1%).** Prepared as described in Section 5.2.2.

**Sulphuric acid (1N).** Prepared as described in Section 5.2.2.

**Sodium chloride (6%).** Prepared as described in section 5.2.2.

**Sulphuric acid (10 M).** Prepared as described in Section 2.4.2.

**Preparation of standard ALB solution**

A stock standard solution containing 1 mg ml\(^{-1}\) of the drug was prepared dissolving accurately weighed 100 mg of the pure ALB in minimum quantity of glacial acetic acid and diluting to 100 ml with water in a volumetric flask, and used in titrimetric work. Working solutions of 100 µg ml\(^{-1}\) for spectrophotometric work was prepared by appropriate dilution of the stock solution whenever required.

**6.2.2.3. Procedures**

**Titrimetry**

To a 10 ml aliquot of pure drug solution containing 1 – 10 mg of ALB in a 100 ml titration flask, 4 ml of 10 M sulphuric acid were added followed by 10 ml of 0.002 M potassium iodate solution. The contents were mixed well and set aside for 30 min with occasional swirling. The mixture was diluted to 60 ml with water and 5 ml of 10% potassium iodate solution were added and let stand for 5 min. and the liberated iodine was titrated with standard 0.012 M thiosulphate solution using starch indicator.
A blank was run in the same way with 10 ml of distilled water. The drug content was calculated from:

\[
\text{Amount (mg)} = \frac{(B-S)M_wR}{n}
\]

where
- \( B \) = volume of thiosulphate consumed in the blank titration, ml
- \( S \) = volume of thiosulphate consumed in the sample titration, ml
- \( M_w \) = relative molecular mass of drug
- \( R \) = molarity of potassium iodate solution, mol l\(^{-1}\)
- \( n \) = number of moles of iodate reacting with one mole of drug

**Spectrophotometry**

Different volumes (0.5 – 3.0 ml) of 100 µg ml\(^{-1}\) ALB solution were transferred into a series of 10 ml standard flasks by means of a micro burette. One ml each of 0.5 M sulphuric acid, 0.4 % potassium iodate solutions were added to each flask followed by 0.5 ml of 6% sodium chloride. The contents were mixed and kept aside for 5 min with occasional shaking. Then, 2.0 ml each of 0.01% dye solution and chloroacetic acid buffer were added and the volume was diluted to the mark with distilled water. The absorbance of the solution was measured at 520 nm against the reagent blank after 20 min. The concentration of the unknown was read from the calibration graph or computed from the regression equation derived using the Beer’s law plot.

**Procedure for tablets**

Twenty tablets containing ALB were weighed and powdered. A quantity of the powder equivalent to 250 mg of the active component was transferred into a 250 ml standard flask, 20 ml of glacial acetic acid and about 100 ml distilled water were
added and shaken thoroughly for about 20 min; then, the volume was made up to the mark, mixed well and filtered using a quantitative filter paper. First 20 ml of the filtrate was rejected and a suitable aliquot was subjected to analysis by titrimetry. For spectrophotometric work, 10 ml of the filtrate was diluted to 100 ml with water, to provide a 100 µg ml⁻¹ solution, and a convenient aliquot was subjected to analysis as described under the general procedure.
Table 6.2.1 Determination of accuracy and precision

<table>
<thead>
<tr>
<th>Method**</th>
<th>ALB taken</th>
<th>ALB found*</th>
<th>RE, %</th>
<th>Range</th>
<th>SD</th>
<th>RSD, %</th>
<th>ROE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titrimetry</td>
<td>4.0</td>
<td>4.01</td>
<td>0.25</td>
<td>0.02</td>
<td>0.04</td>
<td>0.99</td>
<td>± 0.98</td>
</tr>
<tr>
<td></td>
<td>6.0</td>
<td>5.96</td>
<td>0.66</td>
<td>0.11</td>
<td>0.02</td>
<td>0.33</td>
<td>± 0.32</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.91</td>
<td>1.12</td>
<td>0.60</td>
<td>0.04</td>
<td>0.50</td>
<td>± 0.49</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>100.00</td>
<td>101.50</td>
<td>1.50</td>
<td>0.25</td>
<td>0.89</td>
<td>0.88</td>
<td>± 0.87</td>
</tr>
<tr>
<td></td>
<td>200.00</td>
<td>199.70</td>
<td>0.25</td>
<td>0.36</td>
<td>1.25</td>
<td>0.63</td>
<td>± 0.62</td>
</tr>
<tr>
<td></td>
<td>300.00</td>
<td>298.50</td>
<td>0.50</td>
<td>0.34</td>
<td>2.32</td>
<td>0.78</td>
<td>± 0.77</td>
</tr>
</tbody>
</table>

* Average of seven determinations
** In titrimetry, taken/found, range, SD are in mg, and in μg for spectrophotometry.
RE. Relative error, SD. Standard deviation, RSD. Relative standard deviation, ROE. Range of error.

Table 6.2.2. Results of determination of albendazole tablets by the proposed method

<table>
<thead>
<tr>
<th>Tablets**</th>
<th>Label claim, mg/tablet</th>
<th>Found*(% of label claim ± SD)</th>
<th>Reference Method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Titrimetry (T)</td>
<td>Spectrophotometry (S)</td>
</tr>
<tr>
<td>Almintha</td>
<td>200</td>
<td>97.28 ± 1.06, t= 2.44, F= 2.58</td>
<td>98.43 ± 0.85, t= 0.39, F= 1.65</td>
</tr>
<tr>
<td>Albendolb</td>
<td>400</td>
<td>96.74 ± 0.86, t= 1.24, F= 1.42</td>
<td>97.58 ± 1.34, t= 0.33, F= 3.46</td>
</tr>
<tr>
<td>Zentlec</td>
<td>400</td>
<td>100.84 ± 0.58, t= 0.87, F= 4.00</td>
<td>99.86 ± 0.94, t= 0.75, F= 1.52</td>
</tr>
</tbody>
</table>

* Average of five determinations
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39
**Marketed by: a. Torrent; b. Micro-Labs; c. SmithKline Beecham; India.
Table 6.2.3. Results of recovery study by the standard-addition method

| Formulation | Titrimetry | | | Spectrophotometry | | |
|-------------|------------|-------|-------|------------------|-------|
|             | Amount of drug in formulation, mg | Amount of pure drug added, mg | Total found*, mg | % recovery of pure drug | Amount of drug in formulation, µg | Amount of pure drug added, µg | Total found*, µg | % recovery of pure drug |
| Alminth (200 mg) | 1.95 | 3.00 | 4.91 | 98.62 | 49.22 | 100 | 150.00 | 100.78 |
|             | 1.95 | 4.00 | 5.92 | 99.16 | 49.22 | 150 | 201.23 | 101.34 |
|             | 1.95 | 5.00 | 6.84 | 97.83 | 49.22 | 200 | 252.34 | 101.56 |
| Albendol (400 mg) | 1.93 | 3.00 | 4.97 | 101.34 | 48.79 | 100 | 147.54 | 98.75 |
|             | 1.93 | 4.00 | 5.96 | 100.85 | 48.79 | 150 | 197.53 | 99.16 |
|             | 1.93 | 5.00 | 6.97 | 100.78 | 48.79 | 200 | 249.67 | 100.44 |

* Average of three trials
Fig. 6.2.1. Beer’s law curve
6.2.3. RESULTS AND DISCUSSION

Both methods are based on the oxidation of sulphur atom of the drug molecule by potassium iodate in acid medium. In titrimetry, the reaction was followed by back titration of the unreacted iodate while the determination of iodine released in the redox process through an auxiliary reaction involving the use of dichlorofluorescein formed the basis of quantification in spectrophotometry.

6.2.3.1. METHOD DEVELOPMENT

Titrimetry

Potassium iodate was found to react quantitatively with ALB in sulphuric acid medium. A study of the stoichiometry of the reaction in different acids with different concentrations revealed that 4 ml of 10 M sulphuric acid in a total volume of 25 ml gave the best results. Constant reaction ratio was obtained within 3 – 5 ml of 10 M sulphuric acid in a total volume of 25 ml were used. Below the lower limit, the reaction was slow and above the upper limit no discernible starch end point was obtained.

To study the stoichiometry of the reaction, different known amounts of the drug (within the working range of 1-10 mg) were reacted with measured excess of potassium iodate solution in sulphuric acid medium, and the excess of iodate was determined iodometrically after 30 min. The study revealed that 3 moles of the drug reacted with 1 mole of potassium iodate in conformity with the possible reaction scheme shown below:
The stoichiometry indicates that only S-atom of the drug was oxidized to sulphoxide and other sites of the molecule were unaffected. For the 1-10 mg range studied, a 10 ml volume of 0.002 M potassium iodate solution was sufficient for complete oxidation of the drug. Though the oxidation of the drug was complete in 30 min, contact times upto 2h had no effect on either the stoichiometry of the reaction or results of the study.

Titrimetry was found to be applicable in the range 1-10 mg outside which the results were deviant besides taking longer reaction times for more than 10 mg of drug. The relationship between the titration end point and the drug amount was examined by calculating the correlation coefficient value, r via linear least square treatment and was found to be -0.9876 indicating that the reaction between the drug and iodate proceeds stoichiometrically in the ratio 3:1.

Spectrophotometry

The formation of 2', 7'-dichloro-4', 5'-diiodo fluorescein from 2', 7'-dichlorofluorescein under the experimental conditions described is responsible for the observed colour. The formation of the diiodo compound can be explained on the basis
of the iodination of the dichloro compound as a result of the reaction of potassium iodate with ALB in acidic medium, to generate iodine in accordance with equation (1)

$$2 \text{IO}_3^- + 6 \text{ALB} + 12 \text{H}^+ \rightarrow \text{I}_2 + 6 \text{ALB sulphoxide} + 6 \text{H}_2\text{O} \quad (1)$$

When experiments were carried out to iodinate 2', 7'-dichlorofluoroscein using stoichiometric amount of iodine solution (prepared by equilibrating solid iodine with water in the absence of iodide) in acidic medium very low absorbance was obtained indicating that iodination reaction was not taking place as expected. However, when the same reaction was carried out in the presence of potassium iodate and a stoichiometric amount of iodine, a high absorbance was observed. This observation can be explained by the fact that the reaction of iodine with iodate is acidic medium leads to the formation of ICl which is stabilized to $\text{ICl}_2^-$ in the presence of chloride ions in accordance with equation (2) and (3)

$$2 \text{I}_2 + \text{IO}_3^- + 6 \text{H}^+ + 5 \text{Cl}^- \rightarrow 5 \text{ICl} + 3\text{H}_2\text{O} \quad (2)$$

$$5 \text{ICl} + 5 \text{Cl}^- \rightleftharpoons 5 \text{ICl}_2^- \quad (3)$$

Iodine monochloride is widely used as an iodinating agent in aromatic substitution reaction and the formation of ICl under the experimental conditions used here is highly favoured. The formation of the species responsible for the colour system is shown in fig below
A 1 ml volume of 0.5 M sulphuric acid in a total volume of about 5 ml was found to be optimum for the oxidation of the drug to its sulphoxide and will be complete in 5 min. since, subsequent iodination reaction requires lower acidity, it was achieved by adding 2 ml of chloroacetic acid buffer, which produced an effective pH of 3.30 ± 0.10. Though the formation of 2', 7'-dichloro-4', 5'-diiodo fluorescein is reported to be almost instantaneous, maximum colour development was fund to be complete in 20 min and was stable for 3 h thereafter.

Beers law is obeyed in the range 5-30 µg ml⁻¹. The apparent molar absorptivity and Sandell sensitivity values were 3.02×10³ l mol⁻¹ cm⁻¹ and 87.86 ng cm⁻², respectively. The linear plot gave the regression equation:

\[ A = 0.0019 + 0.0111C \]

where, A is the absorbance and C concentration in µg ml⁻¹, and with a correlation coefficient of 0.9985 (n = 7). The limit of detection and the limit of quantification were 0.45 µg ml⁻¹ and 1.49 µg ml⁻¹, respectively.
6.2.3.2. METHOD VALIDATION

Accuracy and precision

To find out the accuracy and precision of the methods, seven replicate determinations on the same solution containing three different levels of ALB were performed. The percent error, the RSD and the range of error values at 95% confidence level presented in Table indicate the high accuracy and precision of the method.

To ascertain the repeatability of the methods, four replicate determinations at three different concentration levels of the drug were carried out over a period of 5 days by preparing all solution afresh. The values of between-day RSD for three different concentrations of the drug were below 4%.

Application

The proposed methods were applied to the determination of ALB in tablets and the results were statistically compared with those obtained by spectrophotometric method [13]. When the Student’s t- and F- tests at the 95% confidence level were applied, the calculated values of t and F- did not exceed the tabulated values (Table 6.2.2), implying no significant difference in the mean recoveries and precision between the proposed and the reference methods.

Recovery studies

To establish the reliability and accuracy of the proposed methods, a standard-addition method was followed. To a fixed amount of ALB in tablet preparations (pre-analyzed), pure ALB at three different levels was added. Each level was repeated three
times. The total amount of the drug was determined by the proposed procedures and the results of the recovery study compiled in Table 6.2.3 show that tablet excipients like talc, starch stearate, alginate etc do not interfere in the determination.
SECTION 6.3.

TITRIMETRIC AND SPECTROPHOTOMETRIC
DETERMINATION OF ALBENDAZOLE USING
CHLORAMINE-T AS REAGENT

6.3.1. INTRODUCTION

Important features of chloramine-T (CAT) as an oxidimetric reagent were mentioned in Section 5.3.1 and applications of CAT in the titrimetric and the spectrophotometric determination of a variety of pharmaceuticals were also reviewed in Section 5.3.1. From this review and from the survey of literature on the analytical methods for the assay of ALB in pharmaceuticals presented in Section 6.0.2, it is clear that CAT has not been applied for the assay of ALB either by titrimetry or by spectrophotometry. The author used CAT successfully for the titrimetric and the spectrophotometric determination of ALB. In titrimetry, an acidified solution of ALB was titrated directly with CAT in the presence of bromide using methyl orange as indicator. Iodometric back titration procedure was employed in indirect titrimetry. In spectrophotometric methods unreacted CAT was determined using methyl orange and indigo carmine. And in one of the spectrophotometric methods the reaction involving CAT, metol and a primary amine such as sulphanilic acid was also used. The details of these methods are presented in this Section 6.3.
6.3.2. EXPERIMENTAL

6.3.2.1. Apparatus

All absorbance measurements were made with a Systronics model 106 digital spectrophotometer provided with 1cm quartz cells.

6.3.2.2. Reagents

All chemicals used were of analytical reagent grade and distilled water was used to prepare all sample and reagent solutions.

**Chloramine-T.** A 0.02 M solution was prepared by dissolving about 5.7 g of the chemical in water and diluting one litre with water. The solution was stored in amber coloured bottle and standardised iodometrically [30] and used for titrimetric work. It was diluted appropriately with water to obtain a concentration of 0.01 M for titrimetry and 500, 150 and 25 µg ml⁻¹ for assay by spectrophotometric methods.

**Indigo carmine (100 µg ml⁻¹).** A 1000 µg ml⁻¹ solution was prepared by dissolving exactly weighed 111 mg of the dye (S.d. Fine Chem. India, 90% purity) in water and diluting to 100 ml with water in a calibrated flask. This solution was diluted 10 times with water.

**Methyl orange (50 µg ml⁻¹).** Prepared as described under section 2.1.2.

**Hydrochloric acid (5 M).** A 440 ml volume of concentrated acid (S.d. fine Chem India, Sp. Gr. 1.18) was diluted to 1 liter with water.

**Methyl orange indicator.** A 0.05% solution of dye was prepared in water.
Sodium thiosulphate An approximately 0.04 M solution was prepared by dissolving 4.88 g of the salt in water and diluting to one litre with water and standardized using pure potassium iodate [38].

Starch indicator (1%) A paste of 1.0 g of soluble starch with a little water was poured, with constant stirring into 100 ml of boiling water, boiled for a min and cooled.

Buffer solution A mixture of 50 ml of 1 M sodium acetate and 50 ml of 1 M hydrochloric acid was diluted to 250 ml with water (pH 2.71 ± 0.10).

Metol solution Prepared as described under section 5.3.2.

Sulphanilic acid solution (0.1%) Prepared as described under section 5.3.2.

Potassium iodide (10%) Prepared by dissolving 10 g of the chemical in 100 ml water.

ALB solution. A stock standard solution containing 2 mg ml\(^{-1}\) of the drug was prepared by dissolving 500 mg (accurately weighed) of the pure drug in 25 ml of glacial acetic acid and diluting 250 ml with water is a calibrated flask and used in titrimetry. For spectrophotometric work, the solution was diluted stepwise to get the working concentration of 100 and 10 µg ml\(^{-1}\).

6.3.2.3. Procedures

Direct titrimetry (Method A).

A 10 ml aliquot of the sample solution equivalent to 1-14 mg of ALB was transferred into a 100 ml titration flask, 5 ml of 5 M hydrochloric acid and 2 drops of methyl orange indicator were added and titrated with 0.01 M chloramine-T solution to
a colourless end-point. An indicator blank was performed under identical conditions and a correction for it was applied. From the volume of chloramine-T consumed by ALB, the amount of the drug in the measured aliquot was calculated from:

\[
ALB \text{ (mg)} = \frac{VM_{w}R}{n}
\]

where

- \( V \) = volume chloramine-T consumed by drug, ml
- \( M_{w} \) = relative molecular weight of drug
- \( R \) = strength of chloramine-T solution, M
- \( n \) = number of moles of chloramine-T required to react with each mole of drug.

**Indirect titrimetry (method B)**

A 10 ml aliquot of the standard solution containing 1-15 mg of ALB was accurately measured into a glass-stoppered Erlenmeyer flask and acidified by adding 5 ml of 5 M hydrochloric acid. Ten ml of 0.02 M chloramine-T solution were added to the flask by means of a pipette and the contents were mixed well and set aside for 5 min with occasional swirling. Then, 10 ml of 10% potassium iodide solution were added, and the liberated iodine was titrated with 0.04 M sodium thiosulphate solution using starch indicator towards the end-point. The experiment was repeated without ALB solution. The amount of ALB in the measured aliquot was calculated from:

\[
(\text{B-S})M_{w}R
\]

where,

- \( B \) = volume of thiosulphate solution consumed in the blank titration, ml,
S = volume thiosulphate solution consumed in the test sample solution, ml,

R = molarity of chloramine-T solution, M

n = number of moles of chloramine-T required to react with each mole of drug.

**Spectrophotometry using methyl orange (method C)**

Accurately measured volumes of 0.25 - 1.5 ml of 10 μg ml⁻¹ standard drug solution were transferred into a series of 10 ml calibrated flasks. To each flask was added 1 ml of 5 M hydrochloric acid followed by 2 ml of 25 μg ml⁻¹ chloramine-T solution. The contents were mixed well and allowed to stand for 5 min. Finally, 1 ml of 50 μg ml⁻¹ methyl orange dye solution was added to each flask, diluted to volume with water. The absorbance of each solution was measured at 510 nm against a water blank after 10 min.

**Spectrophotometry using indigo carmine (method D)**

In each of a series of 10 ml calibrated flasks were placed different aliquots in the range, 0.5 – 4.0 ml of 10 μg ml⁻¹ standard drug solution. The volume was adjusted to 4 ml by adding requisite volume of water. Then to each flask were added 1 ml each of 5 M hydrochloric acid and 150 μg ml⁻¹ chloramine-T solutions. The contents were mixed well and let stand for 5 min before adding 2 ml of 100 μg ml⁻¹ indigo carmine dye solution and diluting to the mark with water. The absorbance of the solutions was measured at 610 nm against water blank.

**Spectrophotometry (Method E)**

In each of a series of 10 ml calibrated flask were placed 0.25 – 2.5 ml of 100 μg ml⁻¹ ALB solution by means of a micro burette. Then, 2 ml of the buffer solution
were added to each flask followed by 1.0 ml of 500 µg ml\(^{-1}\) chloramine-T solution. The contents were mixed well and the flasks were let stand for 10 min with occasional shaking. Finally, 1 ml each of 0.2% metol and 0.1 % sulphanilic acid solutions were added to each flask and the volume was diluted to the mark with water. The absorbance of each solution was measured at 520 nm against water blank after 10 min.

In all the spectrophotometric methods, a calibration graph was prepared by plotting the absorbance versus concentration of drug. The concentration of the unknown was read from the calibration graph or deduced from the regression equation, derived from the Beer’s law data.

**Assay procedure for dosage forms**

Dosage forms containing ALB were purchased from local commercial sources. Twenty tablets were weighed accurately and ground into a fine powder. The amount of the powder equivalent to 200 mg of active component was accurately weighed into a 100 ml calibrated flask, 10 ml of glacial acetic acid and 50 ml of water were added, and shaken thoroughly for about 20 min. Then, the volume was diluted to the mark with water, mixed well and filtered using a Whatman No 41 filter paper. First 10 ml portion of the filtrate was rejected and a convenient aliquot was assayed by titrimetric methods (Method A and B). The filtrate (2 mg ml\(^{-1}\)) was appropriately diluted to 100 and 10 µg ml\(^{-1}\) solution and the steps described under spectrophotometric methods (methods C, D and E) were applied on a convenient aliquot.
Table 6.3.1. Analytical Parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method C</th>
<th>Method D</th>
<th>Method E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s law limits ((\mu g \text{ ml}^{-1}))</td>
<td>0.25 - 1.5</td>
<td>0.50 - 4.0</td>
<td>2.5-25</td>
</tr>
<tr>
<td>Detection limit ((\mu g \text{ ml}^{-1}))</td>
<td>0.0108</td>
<td>0.0742</td>
<td>1.15</td>
</tr>
<tr>
<td>Quantification limit ((\mu g \text{ ml}^{-1}))</td>
<td>0.0362</td>
<td>0.2473</td>
<td>3.83</td>
</tr>
<tr>
<td>Molar absorptivity ((1 \text{ mol}^{-1} \text{ cm}^{-1}))</td>
<td>(1.08 \times 10^5)</td>
<td>(2.59 \times 10^4)</td>
<td>(6.24 \times 10^3)</td>
</tr>
<tr>
<td>Sandell sensitivity ((\text{ng cm}^{-2} \text{ per 0.001 A unit}))</td>
<td>2.46</td>
<td>10.26</td>
<td>42.54</td>
</tr>
<tr>
<td>Regression Coefficient</td>
<td>0.9989</td>
<td>0.9997</td>
<td>-0.9998</td>
</tr>
<tr>
<td>Regression equation*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept, a</td>
<td>0.0213</td>
<td>0.0043</td>
<td>0.6562</td>
</tr>
<tr>
<td>Slope, b</td>
<td>0.3737</td>
<td>0.095</td>
<td>-0.023</td>
</tr>
</tbody>
</table>

*\(?A = a + bC\), where A is the absorbance, and C, concentration in \(\mu \text{g ml}^{-1}\)
Table 6.3.2. Evaluation of accuracy and precision.

<table>
<thead>
<tr>
<th>Method**</th>
<th>Amount of drug taken</th>
<th>Amount of drug found*</th>
<th>Range</th>
<th>Error, %</th>
<th>SD</th>
<th>RSD, %</th>
<th>ROE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.00</td>
<td>2.98</td>
<td>0.05</td>
<td>0.67</td>
<td>0.04</td>
<td>1.68</td>
<td>±1.67</td>
</tr>
<tr>
<td></td>
<td>9.00</td>
<td>8.90</td>
<td>0.02</td>
<td>1.1</td>
<td>0.02</td>
<td>0.90</td>
<td>±0.89</td>
</tr>
<tr>
<td></td>
<td>12.00</td>
<td>12.13</td>
<td>0.04</td>
<td>1.08</td>
<td>0.12</td>
<td>0.91</td>
<td>±0.90</td>
</tr>
<tr>
<td>B</td>
<td>4.00</td>
<td>4.01</td>
<td>0.10</td>
<td>0.25</td>
<td>0.02</td>
<td>0.50</td>
<td>±0.49</td>
</tr>
<tr>
<td></td>
<td>8.00</td>
<td>7.93</td>
<td>0.20</td>
<td>0.88</td>
<td>0.17</td>
<td>2.10</td>
<td>±2.00</td>
</tr>
<tr>
<td></td>
<td>12.00</td>
<td>12.21</td>
<td>0.22</td>
<td>1.33</td>
<td>0.07</td>
<td>0.58</td>
<td>±0.57</td>
</tr>
<tr>
<td>C</td>
<td>5.00</td>
<td>5.04</td>
<td>0.23</td>
<td>0.80</td>
<td>0.02</td>
<td>0.99</td>
<td>±0.98</td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>7.55</td>
<td>0.42</td>
<td>0.67</td>
<td>0.03</td>
<td>1.19</td>
<td>±1.18</td>
</tr>
<tr>
<td></td>
<td>10.00</td>
<td>9.89</td>
<td>0.33</td>
<td>1.10</td>
<td>0.05</td>
<td>1.11</td>
<td>±1.10</td>
</tr>
<tr>
<td>D</td>
<td>10.00</td>
<td>10.09</td>
<td>0.20</td>
<td>0.90</td>
<td>0.08</td>
<td>1.19</td>
<td>±1.18</td>
</tr>
<tr>
<td></td>
<td>20.00</td>
<td>20.21</td>
<td>0.15</td>
<td>1.05</td>
<td>0.06</td>
<td>1.53</td>
<td>±1.52</td>
</tr>
<tr>
<td></td>
<td>30.00</td>
<td>29.82</td>
<td>0.16</td>
<td>0.60</td>
<td>0.02</td>
<td>1.41</td>
<td>±1.40</td>
</tr>
<tr>
<td>E</td>
<td>50.00</td>
<td>50.77</td>
<td>0.16</td>
<td>1.54</td>
<td>0.31</td>
<td>0.63</td>
<td>±0.62</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>99.43</td>
<td>0.21</td>
<td>0.57</td>
<td>1.24</td>
<td>1.25</td>
<td>±1.24</td>
</tr>
<tr>
<td></td>
<td>150.00</td>
<td>149.76</td>
<td>0.11</td>
<td>0.16</td>
<td>0.53</td>
<td>0.36</td>
<td>±0.35</td>
</tr>
</tbody>
</table>

* Average of seven determinations
** In Methods A and B amount taken/found, Range, SD are in mg, and in μg for Methods C, D and E
RE. Relative error, SD. Standard deviation, RSD. Relative standard deviation, ROE.
Range of error.
Table 6.3.3. Results of assay of albendazole in pharmaceutical formulations

<table>
<thead>
<tr>
<th>Preparation*</th>
<th>Label claim mg/tablet</th>
<th>Found ** (% of label claim ± SD)</th>
<th>Reference method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Method A</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Method B</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Method C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Method D</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Method E</td>
<td></td>
</tr>
<tr>
<td>Alminth(^a)</td>
<td>200</td>
<td>98.65 ± 1.21 (t= 0.34)</td>
<td>99.88 ± 1.90 (t= 1.56)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(F= 0.18)</td>
<td>(F= 2.88)</td>
</tr>
<tr>
<td>Albazole(^b)</td>
<td>400</td>
<td>99.10 ± 1.45 (t=2.15)</td>
<td>101.10 ± 1.89 (t= 0.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(F=1.39)</td>
<td>(F= 2.36)</td>
</tr>
</tbody>
</table>

* Marketed by: a. Torrent; b. Wings Pharma; India

** Mean value of five determinations.

*** Tabulated value at 95% confidence level is 2.77

**** Tabulated value at 95% confidence level is 6.39
Table 6.3.4 Results of recovery studies by standard-addition technique.

<table>
<thead>
<tr>
<th>Method</th>
<th>Formulations</th>
<th>Amount of drug in formulation</th>
<th>Amount of drug added</th>
<th>Total found</th>
<th>% Recovery of pure drug *</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Alminth (200 mg)</td>
<td>2.96</td>
<td>3.00</td>
<td>6.00</td>
<td>101.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.96</td>
<td>7.00</td>
<td>9.75</td>
<td>97.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.96</td>
<td>10.00</td>
<td>13.10</td>
<td>101.40</td>
</tr>
<tr>
<td>B</td>
<td>Alminth (200 mg)</td>
<td>0.99</td>
<td>4.00</td>
<td>4.97</td>
<td>99.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.99</td>
<td>8.00</td>
<td>9.07</td>
<td>101.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.99</td>
<td>12.00</td>
<td>13.14</td>
<td>101.58</td>
</tr>
<tr>
<td>C</td>
<td>Alminth (200 mg)</td>
<td>4.90</td>
<td>5.00</td>
<td>10.03</td>
<td>102.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.90</td>
<td>7.50</td>
<td>12.51</td>
<td>101.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.90</td>
<td>10.00</td>
<td>15.19</td>
<td>102.90</td>
</tr>
<tr>
<td>D</td>
<td>Alminth (200 mg)</td>
<td>4.96</td>
<td>10.00</td>
<td>15.15</td>
<td>101.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.96</td>
<td>20.00</td>
<td>24.52</td>
<td>97.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.96</td>
<td>30.00</td>
<td>35.32</td>
<td>101.20</td>
</tr>
<tr>
<td>E</td>
<td>Alminth (200 mg)</td>
<td>49.84</td>
<td>50.00</td>
<td>100.22</td>
<td>100.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>49.84</td>
<td>100.00</td>
<td>149.22</td>
<td>99.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>49.84</td>
<td>150.00</td>
<td>200.09</td>
<td>100.17</td>
</tr>
</tbody>
</table>

* Average of three determinations, ** in Methods A and B amount taken/added/found are in mg, and in μg for other methods
Fig. 6.3.1. Beer's law plot (Method C)

Fig. 6.3.2. Beer's law plot (Method D)
Fig. 6.3.3. Beer's law plot (Method E)
6.3.3. RESULTS AND DISCUSSION

The present work is based on the oxidation of the sulphur atom of the propylthio group of the ALB molecule by chloramine-T. Using different reaction schemes, following this reaction, two titrimetric and three spectrophotometric methods have been developed.

6.3.3.1. METHOD DEVELOPMENT

Direct titrimetry (Method A).

The direct titration of the propylthio group of the drug with standard chloramine-T solution was successful in hydrochloric acid medium using methyl orange as the indicator. With methyl red or indigo carmine as indicator, the end-point was not very sharp and the results obtained were very high. It is clear from Table 2 that the proposed titrimetric method could be used for the determination of 1-14 mg ALB using 0.01 M solution of chloramine-T. Below 1 mg and above 14 mg, higher and lower recoveries were obtained. The oxidation reaction was found to be quantitative and instantaneous in hydrochloric acid to facilitate a direction titration. The reaction followed a 1:2 molar ratio (ALB: chloramine-T). There was no significant change in the reaction stoichiometry when 2 to 10 ml of 5 M hydrochloric acid were used in a total volume of 20-30 ml at the end point. Below 2 ml, n values less than 2 were obtained resulting in lower recoveries. The relationship between the drug amount and the titration end-point was checked by calculating the correlation coefficient via the method of least squares and the value was found to be 0.9991 indicating a definite stoichiometric reaction in the range (1-14 mg) investigated.
Indirect titrimetry (method B)

Chloramine-T reacts readily and quantitatively with ALB in hydrochloric acid medium. The reaction proceeds at room temperature and the stoichiometry of the reaction is 1:2 with respect to ALB and chloramine-T based on which a possible reaction scheme shown below is suggested.

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{CH}_2\text{S} & + 7\text{H}^+ + 2\text{H}_2\text{O} \\
\text{CH}_2\text{CH}_2\text{CH}_2\text{S} & + 2\text{NaCl} + 2\text{Na}_2\text{SO}_4
\end{align*}
\]

In order to establish a back-titration method, it was necessary to consider several factors. Therefore, the effect of reaction time, the excess of chloramine-T required and the hydrochloric acid concentration was investigated. The oxidation reaction was found to be complete in 5 min and standing times up to 30 min had no effect on the stoichiometry of the reaction. Three-fold excess of chloramine-T had no significant effect on the stoichiometry of the reaction and the results were reproducible for reaction times of 5 – 30 min. Even the hydrochloric acid concentration was not

285
critical. Identical molar ratio was obtained when 2 to 10 ml of 5 M hydrochloric acid in a total volume of 25 was used. The relationship between the drug amount and the end-point was ascertained by calculating the correlation coefficient via the method of least squares and the value was found to be -0.9998 suggesting a definite stoichiometric reaction in the range (1-15 mg) investigated.

**Method C.** Chloramine-T has been reported to destroy methyl orange to a colourless product in acid medium, and this property has been successfully exploited for the direct titration of many pharmaceuticals with chloramine-T using methyl orange as the indicator [24-29]. Taking advantage of this observation, a highly sensitive indirect spectrophotometric method has been developed for the assay of ALB.

Methyl orange is an azo dye with a characteristic maximum at 520 nm in acid medium. Chloramine-T destroys the dye to a colourless product, and as a result, there will be a decrease in absorbance at 510 nm with increasing concentration of chloramine-T. When a fixed amount of chloramine-T is reacted with increasing amounts of ALB in hydrochloric acid medium, proportionally increasing amounts of the oxidant are used up by the drug. As a result, there will be a decrease in the chloramine-T concentration for reacting with the fixed amount of methyl orange dye added. This causes a concomitant increase in the absorbance of the solutions, which is proportional to ALB concentration (Fig. 6.3.1).

The various parameters involved in the assay method were optimized. In a preliminary experiment, it was found that 1 ml of 50 μg ml⁻¹ methyl orange in 10 ml produced a convenient maximum absorbance. This was completely destroyed to near
zero absorbance by 50 µg ml\(^{-1}\) of chloramine-T. Hence, different amounts of ALB were reacted with 50 µg of chloramine-T and the unreacted oxidant were reacted with 1 ml of 50 µg ml\(^{-1}\) methyl orange in a total volume of 10 ml, to determine the Beer's law range. The increasing absorbance values at 510 nm were plotted against the increasing concentration of ALB to obtain the calibration graph.

The redox reaction between ALB and chloramine-T was found to be rapid in hydrochloric acid medium, and for the range studied it was complete in less than 5 min. However, contact times upto 20 min had no effect on the results. Similarly, 0.5 to 2.5 ml of 5 M hydrochloric acid in a total volume of 10 ml (0.25 - 1.25 M overall) produced identical results. At higher concentrations of chloramine-T, discharge of methyl orange colour was instantaneous but at lower concentrations it took about 5 min, and hence, a standing time of 10 min was fixed as optimum after the addition of the dye and before measuring the absorbance.

**Method D.** Chloramine-T is known to convert the blue indigo carmine dye to a green product in acid medium [30], this colour change being taken as the end point in the titration of several types of organic substances using chloramine-T as the oxidant. This observation has been used in the present study to determine microquantities of ALB. The method is based on the oxidation of the drug by a known excess of chloramine-T in hydrochloric acid medium and subsequent determination of the residual oxidant by reacting it with a fixed amount of indigo carmine dye, and measuring the change in absorbance of the latter at 610 nm.
ALB, when added in increasing amounts to a fixed amount of chloramine-T, consumes the latter, and there will be a concomitant decrease in its concentration. When a fixed amount of indigo carmine is added to decreasing concentrations of chloramine-T, a concomitant increase in concentration of the blue form of the dye is obtained. This is observed as a proportional increase in the absorbance of the dye at 610 nm with increasing concentration of the drug (Fig. 6.2.2).

The first step in the assay procedure is the determination of the upper limit indigo carmine that can be measured spectrophotometrically at 610 nm; and this was found to be 20 μg ml⁻¹ in a preliminary experiment. This was completely converted to the green product, as indicated by nearly zero absorbance at 610 nm, by 150 μg ml⁻¹ of chloramine-T. Hence, different amounts of ALB were reacted with 1 ml of 150 μg ml⁻¹ of chloramine-T and the unreacted oxidant was treated with 2 ml of 100 μg ml⁻¹ dye solution in a total volume of 10 ml, before measuring the absorbance at 610 nm.

Hydrochloric acid was the medium of choice for the oxidation of ALB by chloramine-T as well as the latter's determination with indigo carmine. The absorbance of indigo carmine was not affected in 0.5 to 1.5 M hydrochloric acid concentration. A 0.5 M was found optimal for the oxidation of ALB in less than 5 min, and hence the same concentration was employed for the determination of chloramine-T with indigo carmine.

The contact times are not critical. Any delay up to 30 min in the determination of unreacted chloramine-T has no effect on the absorbance. A contact time of 5 min
after the addition of indigo carmine is necessary for its reaction with chloramine-T before measuring the absorbance. Even this contact time is not critical.

**Method E**

In mildly acidic conditions, when compounds containing primary aromatic amino groups are made to react with 4-N-methylamino phenol (metol) and chloramine-T, a purple red colour is produced. In the present work, the above observation was used to determine micro quantities of chloramine-T which formed the basis for the indirect spectrophotometric determination of ALB. In the proposed method, ALB was treated with a known excess of chloramine-T and the unreacted chloramine-T was determined by metol-sulphanilic acid (primary arylamine) as a chromogenic agent.

ALB, when added in increasing amounts to a fixed amount of chloramine-T, consumes the oxidant proportionately, and consequently there will be concomitant fall in the chloramine-T concentration and this is indicated by the proportional decrease in the absorbance of the chromogen when fixed amounts of metol and sulphanilic acid are used. This decrease in absorbance was found to be proportional to ALB concentration (Fig. 6.2.3).

In a preliminary experiment, different amounts of chloramine-T were reacted with specified amounts (as indicated in the procedure) of metol and sulphanilic acid in acetate – hydrochloric acid buffer, and colour developed was measured at 520 nm, to determine the upper limit of chloramine-T that could be measured using the proposed colour reaction. The system was found to obey Beer’s law upto 500 µg of
chloramine-T. Hence, in the determination of ALB, varying amounts of the drug were reacted with fixed amounts chloramine-T (500 μg), metol (1 ml of 0.2%) and sulphanilic acid (1 ml of 0.1%) throughout at pH 2.71 ± 0.10 in a total volume of 10 ml.

The reaction between ALB and chloramine-T was fast and complete in 5 min, and standing times up to 20 min before the adding of metol and sulphanilic acid had no effect on the absorbance of the coloured species. Full colour development took 10 min and was stable for 30 min.

Two blanks were prepared for this study. The reagent blank, which contained optimum concentration of all the reactants except ALB, gave maximum absorbance. The other blank was prepared in the absence of the drug and chloramine-T to determine the contribution of other reagent to the absorbance of the system. Since the second blank showed negligible absorbance, the absorbance of the developed colour measured against water blank. The decreasing absorbance values at 520 nm were plotted against increasing concentration of ALB.

The chemistry of the colour reaction may be suggested on the basis of a previously reported mechanism [32-37]. It is believed that the p-N-methyl benzoinone monoimine formed insitu from the metol-chloramine-T combination, being a good electron acceptor, forms charge-transfer complex with the electron donor, sulphanilic acid. The reaction scheme is similar to the one shown in Chapter 5, Section 5.3.
Analytical parameters (Methods C, D and E)

A linear correlation was found between the absorbance at $\lambda_{\text{max}}$ and concentration in the range given in Table 6.3.1. Correlation coefficients, intercepts and slopes for the calibration data for the two methods are also presented in Table 6.3.1. The graphs show zero or negligible intercept as described by the regression equation, $A = a + bC$ except in method E. The apparent molar absorptivity and the Sandell sensitivity values, and the limit of detection as well as the limit of quantification are also compiled in Table 6.3.1. and demonstrate the very high sensitivity of the methods.

6.3.3.2. METHOD VALIDATION

Accuracy and precision of the methods

The accuracy and precision of the proposed methods were evaluated by performing seven replicate analyses on the standard solution at three different levels. The accuracy defined in terms of % deviation of the calculated amount from the actual amount is listed in Table 6.3.2. Within-day precision expressed in terms of % relative standard deviation is also given in Table 6.3.2. The percent error which is a measure of accuracy is less than 1.5% demonstrating the reasonably high accuracy of the methods. The RSD varied from 0.36 to 2.10 % as can be seen from Table 6.3.2. The repeatability of the method was evaluated by calculating the between-day precision. Analyses of the standard drug solution at three different levels over a period of 5-days were performed and the RSD values were less than 4%.
Application to tablets

All the methods were applied to the assay of ALB in some commercial formulations (tablets) and the results are contained in Table 6.3.3. The same batch tablets were determined by an established reference method [13] for comparison. As can be seen from the results in Table 6.3.3, there is a close agreement between the nominal amount and the results obtained. The reliability of the results were ascertained by calculating the Student’s t-value for accuracy and F-value for precision. From the Table 6.3.3 it is evident that the calculated t- and F-values did not exceed the tabulated values inferring that the proposed methods and the reference method have comparable accuracy and precision.

Recovery studies

The reliability and accuracy of the methods were further confirmed by recovery studies through standard-addition method. To a fixed and known amount of the drug in tablet solution (pre-analyzed) pure ALB was added at three different levels and the total amount was found by the proposed methods. Each levels was repeated three times using three different market formulations. The percent recoveries of the added pure drug given in Table 6.3.4 indicate that the commonly encountered tablet excipients such as talc, starch, gum acacia, lactose, sodium alginate and magnesium stearate did not interfere in the determination by the proposed methods.
SECTION 6.4

HPLC DETERMINATION OF ALBENDAZOLE IN

PHARMACEUTICALS

6.4.1. INTRODUCTION

The review of the literature on the HPLC methods for the assay of ALB in pharmaceuticals is given in Section 6.0.2. By employing column material and mobile phase different from those used in earlier methods, the author has developed a sensitive HPLC procedure which has a long dynamic linear range of response and which can be applied for the selective determination of ALB in pharmaceuticals. The details are contained in the Section 6.4.

6.4.2. EXPERIMENTAL

6.4.2.1. Apparatus

The chromatographic system consisted of an Agilent 1100 series chromatograph equipped with an inbuilt solvent degasser, quaternary pump, photodiode assay detector with variable injector and auto analyzer. A steel column (250 cm x 4.6 mm) of Accurasil ODS C\textsubscript{18} (5 \textmu m) was used.

6.4.2.2. Reagents and materials

For HPLC work, distilled water filtered through 0.45 \textmu m filter was used to prepare solutions.

**Orthophosphoric acid (0.1\% (pH 3.0))**: The solution was prepared by diluting 1.0 ml orthophosphoric acid (Spectrochem, Mumbai) to 1 litre of water; the pH was adjusted to 3.0 with triethylamine, and filtered through 0.45 \textmu m filter.
Diluent. The diluent solution was prepared by mixing HPLC grade acetonitrile (Rankem, India) and water in the ratio 60:40.

The solvent system used for chromatography consisted of orthophosphoric acid buffer (pH-3.0): acetonitrile (20+80).

Standard drug solution

A stock standard solution containing 248 µg ml⁻¹ ALB were prepared by dissolving 24.8 mg of pure drug in diluent solution and diluting to the mark in a 100 ml calibrated flask.

6.4.2.3. Procedure

Chromatographic conditions

The chromatographic separation was achieved at ambient temperature on a reversed phase Accurasil ODS 5 µm C₁₈ column using a mobile phase consisting of acetonitrile-0.1% H₃PO₄ of pH 3 (80+20) at a flow rate of 1.0 ml min⁻¹. The detector wavelength was set at 220 nm with a sensitivity of 0.2 a.u.f.s.

Preparation of calibration graph

Working standard solutions containing 2.48 – 148.8 µg ml⁻¹ ALB were prepared by appropriate dilution of the stock standard solution with the diluent solution. Twenty µl aliquot of each solution was injected automatically onto the column in duplicate and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area versus concentration of ALB. The concentration of the unknown was read from the calibration graph or calculated with the help of regression equation derived from the peak area concentration data.
Assay procedure for formulations

An amount of tablet powder equivalent to 25 mg of ALB was accurately transferred into a 100 ml calibrated flask, 60 ml diluent solution added and shaken for 20 min. The mixture was diluted to the mark with the diluent and mixed well. A small portion of this (~10 ml) was withdrawn and filtered through a 0.2 μm filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted to get a convenient working concentration and 20 μl aliquot was then injected to get the chromatogram.
### Table 6.4.1. Evaluation of accuracy and precision

<table>
<thead>
<tr>
<th>ALB taken μg ml⁻¹</th>
<th>ALB found μg ml⁻¹</th>
<th>Range, μg ml⁻¹</th>
<th>RE</th>
<th>RSD*, %</th>
<th>ROE*, %</th>
<th>RSD**, %</th>
<th>ROE**, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.3</td>
<td>20.77</td>
<td>0.35</td>
<td>2.66</td>
<td>± 0.65</td>
<td>± 0.60</td>
<td>4.23</td>
<td>± 3.90</td>
</tr>
<tr>
<td>40.6</td>
<td>42.32</td>
<td>0.37</td>
<td>4.06</td>
<td>± 0.29</td>
<td>± 0.29</td>
<td>3.35</td>
<td>± 3.09</td>
</tr>
<tr>
<td>81.2</td>
<td>82.69</td>
<td>0.62</td>
<td>1.80</td>
<td>± 0.45</td>
<td>± 0.45</td>
<td>4.24</td>
<td>± 3.92</td>
</tr>
</tbody>
</table>

* Mean value of seven determinations
* Based on peak area;
** Based on retention time;
RE – Relative error;
RSD – Relative standard deviation;
ROE – Range of error at 95% confidence level.

### Table 6.4.2. Results of analysis of formulations containing ALB

<table>
<thead>
<tr>
<th>Tablets*</th>
<th>Nominal amount mg/tablet or mg ml⁻¹</th>
<th>Found**(% of nominal amount ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HPLC method</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albazoleᵃ tablets</td>
<td>200</td>
<td>100.65±0.44 t=2.45 F=3.73</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>98.96±0.62 t=2.00 F=3.26</td>
</tr>
<tr>
<td></td>
<td>103.24±0.36 t=2.31 F=4.45</td>
<td>102.58±0.76</td>
</tr>
<tr>
<td>Dispelᵇ tablets</td>
<td>400</td>
<td></td>
</tr>
</tbody>
</table>

* Marketed by: a. Geno Pharm Ltd.; b. Indian Drugs and Pharmaceuticals Ltd.;
** Mean value of five determinations;
Tabulated t-value at 95% confidence level in 2.77;
Tabulated F-value at 95% confidence level in 6.39.
Table 6.4.3. Results of recovery experiments

<table>
<thead>
<tr>
<th>Tablets studied</th>
<th>HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Drug in formulation $\mu$g ml$^{-1}$</td>
</tr>
<tr>
<td>Albazole tablets (200 mg)</td>
<td>5.03</td>
</tr>
<tr>
<td></td>
<td>5.03</td>
</tr>
<tr>
<td></td>
<td>5.03</td>
</tr>
<tr>
<td>Dispel tablets (400 mg)</td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td>5.16</td>
</tr>
<tr>
<td></td>
<td>5.16</td>
</tr>
</tbody>
</table>

* Mean value of three determinations.
Fig. 6.4.1. Calibration graph

Fig. 6.4.2. Typical chromatogram of ALB (100 µg ml\(^{-1}\))
6.4.3. RESULTS AND DISCUSSION

6.4.3.1. METHOD DEVELOPMENT

ALB was determined by HPLC injecting the solution onto an Accurasil ODS 5 μm column with UV detector set at 220 nm. No internal standard was used. The composition and pH of the mobile phase and its flow rate were varied to optimize the chromatographic conditions. A mobile phase consisting of acetonitrile and 0.1% H₃PO₄ of pH 3 was selected after several preliminary experiments with acetonitrile-water and methanol-water. Acetonitrile and phosphoric acid increase the solubility of ALB and prevent its adherence to the packing material in the column. At a flow rate of 1.0 ml min⁻¹, the retention time for ALB was 3.35 min. Under the described experimental conditions the analyte peak was well defined and free from tailing (Fig. 6.4.2).

6.4.3.2. METHOD VALIDATION

The concentration of the unknown was determined by measuring the peak area. A plot of mean peak area versus concentration gave a linear relationship (r = 0.9999) over the concentration range 2.48 – 148.8 μg ml⁻¹ (Fig. 6.4.1). Using the method of least squares, the linear regression equation obtained was

\[ Y = 93.21 + 115.5X \]

where Y is the mean peak area and X concentration in μg ml⁻¹. The limit of detection (LOD) was established at a signal to noise ratio (S/N) of 3 and limit of quantification (LOQ) was established at a S/N ratio of 9. The LOD was calculated to be 0.49 μg ml⁻¹ and the LOQ was calculated to be 1.48 μg ml⁻¹.
**Precision and accuracy of the method**

The within-day precision of the method was determined by replicate analyses of the standard solution containing ALB at three different concentration levels and the results are presented in Table 6.4.1. The relative standard deviation (RSD) which is a measure of precision was evaluated for both retention time and peak area. The inter-day precision was established by performing analysis over a 5 days period with solutions prepared freshly each day. The RSD value were not more than 5%. The peak area based and retention time based inter-day RSD value were 0.8 % and 4.5% respectively. In order to demonstrate the accuracy of the proposed methods, seven replicate analyses were performed on solutions containing ALB at three different levels. The results obtained are compiled in Table 6.4.1 and the accuracy expressed as percent relative error was found satisfactory.

**Application**

Commercially available tablets and injections containing ALB were analysed by the described HPLC method. The results obtained are summarized in Table 6.4.2. As can be seen, the results are in agreement with the labeled amounts. For comparison, reference method [13] was used to analyse the same batch tablets. The results, compiled in Table 6.4.2 were statistically compared by Student’s t-test and F-test. As shown, the calculated t- and F-values were less than the tabulated values inferring that the proposed methods have the same accuracy and precision as that of the reference method at the 95% confidence level. The accuracy and reliability of the methods were further established by performing recovery experiments. The pre-analysed tablets and
injections were spiked with pure ALB at three different levels and the total was found by the proposed methods. Each experiment was repeated three times. The recoveries of the added pure drug were in the range 97.64-101.35% (Table 6.4.3) indicating that co-formulated substances such as talc, starch, gumacacia, lactose, magnesium stearate, sodium alginate, calcium gluconate, and calcium dihydrogenorthophosphate did not interfere in the determination.
CONCLUSIONS ON CHAPTER VI

Albendazole has been assayed in pharmaceutical formulations by four techniques, viz., visual titrimetry, visible spectrophotometry, rate reaction and HPLC. The developed methods are characterized by simplicity, speed, sensitivity besides accuracy and precision. The titrimetric methods are the simplest ever reported for ALB since they do not require any special equipment or scrupulously anhydrous medium unlike the reported methods. The present titrimetric methods are applicable over a micro / semi micro -scale However, the bromatometric methods are not specific. Many substances containing easily substituted hydrogen atoms, unsaturated compounds and compounds which are easily oxidized by bromine would interfere. However, under the described experimental conditions of reaction time/contact time, excipients and diluents present in tablets were found not to interfere.

The developed spectrophotometric methods offer one of the simplest and the most sensitive approaches for the assay of ALB in pharmaceuticals. As can be seen from Table 6.0.1, the bromatometric spectrophotometric using methyl orange is the most sensitive ever developed for albendazole and the sensitivity of all the spectrophotometric methods is comparable to that of many HPLC methods with UV-detection. All the methods are free from heating or extraction step, which is common in most of the presently available spectrophotometric methods. The methods, in addition, don’t entail any stringent experimental variable such as rigid pH control or longer contact time, which would affect the reliability of the results. However, for reasons stated earlier, bromatometric methods lack specificity. However, as shown by
the results of assay, commonly added tablet excipients and injection diluents did not interfere. An additional advantage of the proposed methods lies in the use of bromate, iodate and chloramine-T as the oxidimetric reagent, which keep their strength for several months if properly stored. The methods can be used successfully for the determination of ALB in tablet samples. While retaining the accuracy and precision of the other methods, the proposed methods are superior in terms of simplicity and convenience and are therefore suitable for routine analysis.

The present HPLC method is superior to many reported previously in terms of sensitivity, linear range of response and analysis time. Though the method is intended to determine ALB in single component formulations, it can conveniently be applied for combined dosage forms since, the method is specific for ALB under the described chromatographic conditions.
REFERENCES


