Simple, Sensitive and Rapid Spectrophotometric Determination of Albendazole
Based on Redox and Complex Formation Using N-Bromosuccinimide

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Two simple, sensitive and rapid methods are described for the determination of albendazole (ABZ) in bulk drug and in formulations using N-bromosuccinimide as the oxidimetric reagent. The methods involve the addition of a known excess of NBS to ABZ in HCl medium followed by estimation of the unreacted oxidant by two reaction schemes involving the use of iron(II) and thiocyanate (method A) or tiron (method B). In both methods, the absorbance is found to decrease linearly with ABZ concentration. Beer’s law is obeyed over the ranges 0.5-5.0 and 1-21 μg mL⁻¹ for method A and method B, respectively. The calculated molar absorptivity values are 4.8×10⁴ and 1.1×10⁴ L mol⁻¹ cm⁻¹ for method A and method B, respectively. The limit of detection (LOD) and quantification (LOQ) are also reported for both methods. The RSD values for intra-day and inter-day precision studies were less than 2.0 and 3.0%, respectively. Both the methods were applied to the determination of ABZ in tablet preparations and the results were satisfactory, and were comparable with those obtained by the reference method. The accuracy and reliability of the proposed methods were further ascertained by recoveries studies, and the recoveries of the spiked drug ranged between 98.5 and 103.2%.

Key Words: Albendazole Determination; Spectrophotometry; N-Bromosuccinimide; Pharmaceuticals

Introduction

Albendazole (ABZ, Fig. 1) is a benzimidazole carbamate anthelmintic active against most nematodes and some cestodes. It is used in the treatment of intestinal and tissue nematode infections and in higher doses in the treatment of echinococcosis (Extra Pharmacopoeia [1]). Many methods have been described for the determination of ABZ using different techniques, the most widely used technique being high performance liquid chromatography (HPLC). ABZ in tablets and oral suspensions has been assayed by HPLC with uv-detection [2-11]. There is also a report on the assay of ABZ by packed column supercritical fluid chromatography [12]. Most of the chromatographic methods reported for pharmaceuticals are tedious, time-consuming and relatively less sensitive, besides requiring special and expensive apparatus.

Amongst the non-chromatographic methods, the methods based on voltammetric technique [13,14,15] are also tedious and require complicated and expensive instrumentation. The uv-spectrophotometric method reported by Mandal [16] is applicable over 1-60 μg mL⁻¹ range, and the first derivative uv-spectrophotometric method of Wu [17] is also less sensitive with the linear range being 5-30 μg mL⁻¹. The three titrimetric procedures developed by our research group, although sensitive, use unstable reagents [18,19,20] or require scrupulously anhydrous medium [21]. Visible spectrophotometric methods based on several reaction schemes have also been suggested for the assay of ABZ in pharmaceuticals. Ion-pair extractive spectrophotometric methods proposed by Sane [8] and using four dyes, although sensitive (2-12 μg mL⁻¹) involve a liquid–liquid extraction step and rigid pH control. A similar method but using picric acid as the ion-pair reagent [22] also suffers from the same disadvantages. Two procedures proposed by Sastry et al. [23] using N-bromosuccinimide as the oxidimetric reagent are fairly sensitive (ε=3.56 x 10³ and 3.66 x 10⁴ L mol⁻¹ cm⁻¹). A few spectrophotometric methods reported recently by our research group [24,25] although employ a stable reagent, chloramine-T, involve rigid control of experimental variables. Even the method Zarapakar and Deshpande [26] involves the use of a non-specific reagent (Table 1).

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The objective of this investigation was to devise simple, rapid, sensitive and economically viable procedures that could be used to determine ABZ in bulk drug and pharmaceutical dosage forms. The methods rely on the use of NBS as the oxidimetric reagent, and iron(II) and thiocyanate or tiron as the subsidiary reagents. The proposed methods have been demonstrated to be superior to the reported methods with respect to speed, simplicity, sensitivity, cost-effectiveness and eco-friendliness.

Experimental

Apparatus

A Systronics Model 106 digital spectrophotometer provided with 1-cm matched quartz cells were used for all absorbance measurements.

Reagents and Materials

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

N-bromosuccinimide (NBS): An approximately 0.01 mol L\(^{-1}\) NBS solution was prepared by dissolving about 1.8 g of chemical (SRL Research Chemicals, India) in water with the aid of heat and diluted to one liter with water and standardized\(^{[27]}\). The solution was stored in an amber coloured bottle and was diluted appropriately to get 180 and 650 \(\mu\)g mL\(^{-1}\) NBS for use in spectrophotometric method A and method B, respectively. The NBS solution was kept in a refrigerator when not in use.

Hydrochloric acid: Concentrated hydrochloric acid (S.D. Fine Chem, Mumbai, India; sp. gr.1.18) was diluted appropriately with water to get 5 mol L\(^{-1}\) for method A and 1 mol L\(^{-1}\) for use in method B.

Ferrous ammonium sulphate: FAS (400 and 1400 \(\mu\)g mL\(^{-1}\)): A stock solution equivalent to 0.01 mol L\(^{-1}\) FAS was prepared by dissolving about 400 mg of the salt (S.D. Fine Chem, Mumbai, India) in 50 mL of water containing 1 mL of dil H\(_2\)SO\(_4\) and diluted to 100 mL with water, and standardized \(^{[28]}\) using pure potassium dichromate. The stock solution was then diluted appropriately with water to get 400 and 1400 \(\mu\)g mL\(^{-1}\) FAS for method A and method B, respectively.

Tiron (1.0\%): About 1.0 g of tiron (Loba Chemie, Mumbai, India) was dissolved in 100 mL of water.

Ammonium thiocyanate (3 mol L\(^{-1}\)): Prepared by dissolving 23 g of the chemical (S.d. Fine Chem. Ltd., Mumbai, India) in 100 mL water.

Sodium acetate tri hydrate (1.5 mol L\(^{-1}\)): Prepared by dissolving 24.5 g of the chemical (S.d. Fine Chem. Ltd., Mumbai, India) in 100 mL water.

Buffer of pH 1.09: Prepared by mixing of 50 mL of 1 mol L\(^{-1}\) sodium acetate and 70 mL of 1mol L\(^{-1}\) HCl and diluting to 250 mL with distilled water.

Standard drug solution: Pharmaceutical grade ABZ was kindly provided by Glaxo Smithkline Pharmaceuticals, India and was used as received. The purity of ABZ as reported by the supplier was 99.6%. A stock standard solution equivalent to 500 \(\mu\)g mL\(^{-1}\) ABZ was prepared by dissolving 50 mg of pure drug in 50 mL glacial acetic acid and diluted to volume in a 100 ml calibrated flask with water. For spectrophotometric investigation, the stock solution was diluted appropriately with water to get 20 \(\mu\)g mL\(^{-1}\) and 70 \(\mu\)g mL\(^{-1}\) for method A and method B, respectively.

Procedures

Method A

Different aliquots (0-2.5 mL) of standard 20 \(\mu\)g mL\(^{-1}\) ABZ solution were accurately measured and transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was adjusted to 3.0 mL by adding water. To each flask was added 1 mL each of 5 mol L\(^{-1}\) HCl and NBS (180 \(\mu\)g mL\(^{-1}\)), the last being added using microburette. The content was mixed and the flasks were let stand for 5 min. Then, 1 mL of 400 \(\mu\)g mL\(^{-1}\) FAS was added to each flask (micro burette),
and again the flasks were let stand for 5 min followed by 1 mL of 3 mol L⁻¹ thiocyanate. The volume was diluted to the mark with water, mixed well and absorbance of each solution was measured at 470 nm against water blank.

**Method B**

Varying aliquots (0-3.0 mL) of standard ABZ solution (70 μg mL⁻¹) were accurately measured into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 3 mL by adding water. The solution in each flask was acidified by adding 1 mL of 1 mol L⁻¹ HCl before adding 1 mL of NBS (650 μg mL⁻¹) by means of micro burette. The content was mixed well and allowed to stand for 15 min with occasional shaking. To each flask was then added 1 mL of 1400 μg mL⁻¹ FAS, and after 5 min, 1 mL each of 1.5 mol L⁻¹ sodium acetate, buffer of pH 1.09 and 1% Tiron were added and diluted to the mark with water. The absorbance of each solution was measured at 670 nm against water blank.

In either spectrophotometric method, a standard graph was prepared by plotting the decreasing absorbance values versus concentration of ABZ. The concentration of the unknown was read from the standard graph or computed from the respective regression equation derived using the Beer’s law data.

**Procedure for Tablets**

Tablets containing ABZ were purchased from local commercial sources for investigation. Twenty tablets were finely powdered and an appropriate portion equivalent to 100 μg of ABZ was weighed into a 100 mL calibrated flask, 50 mL of glacial acetic acid added and shaken for 20 min. Then, diluted to volume with water, mixed well and filtered using a Whatman No 42 filter paper. The tablet extract (1 μg mL⁻¹) was diluted suitably with water to get working concentrations of 20 and 70 μg mL⁻¹ for method A and method B, respectively before subjecting to analysis by spectrophotometric methods.

**Results and Discussion**

**Method Development**

The methods are based on the oxidation of ABZ by a known excess of NBS in hydrochloric acid medium, reducing the unreacted oxidant by iron (II) and subsequent determination of iron (III) by thiocyanate method [29] or by tiron method of Vector Potter and Armstrong [30] and modified by Keshavayya et al. [31]. The possible reaction scheme is given in Fig. 2. When a fixed concentration of NBS is made to react with increasing concentration of ABZ, there occurs a concomitant fall in the former’s concentration. When the unreacted NBS is reduced by a fixed concentration of iron (II), there will be a proportional decrease in the concentration of iron (III). This is observed as a proportional decrease in the absorbance of iron (III) – thiocyanate complex and iron (III)-tiron complex on increasing the concentration of ABZ (Fig. 4), which formed the basis for the determination of drug.

Various parameters associated with the oxidation of ABZ by NBS and subsequent reduction of the residual oxidant by iron (II) was optimized. Considering 5.5 μg mL⁻¹ as the upper limit of iron that could be determined by thiocyanate method, 18 μg mL⁻¹ NBS was found to produce it from 38.7 μg mL⁻¹ FAS. However, slightly higher concentration (40 μg mL⁻¹) FAS was used to ensure a quantitative reaction in method A. Similarly in method B, fixing 18 μg mL⁻¹ as the upper limit of iron that could be determined by tiron method Keshavayya et al. [31], 140 μg mL⁻¹ FAS and 65 μg mL⁻¹ NBS were used. One mL of 5 mol L⁻¹ HCl in a total volume of 6 mL was used for the oxidation step and the same quantity of acid was used for the reduction step and the same quantity of acid was used for the reduction of NBS and complexation of iron (III) with thiocyanate. However,

![Fig. 2: Tentative Reaction Scheme](image-url)
Table 2. Analytical and regression parameters of spectrophotometric methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_{\text{max}}$, nm</td>
<td>470</td>
<td>670</td>
</tr>
<tr>
<td>Color Stability, min</td>
<td>90</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Beer’s Law Limits, $\mu$ g mL$^{-1}$</td>
<td>0.5-5.0</td>
<td>1-21</td>
</tr>
<tr>
<td>Molar absorptivity, L mol$^{-1}$ cm$^{-1}$</td>
<td>4.8$\times$10$^4$</td>
<td>1.1$\times$10$^4$</td>
</tr>
<tr>
<td>Sandell sensitivity, $\mu$ cm$^{-2}$</td>
<td>0.006</td>
<td>0.023</td>
</tr>
<tr>
<td>Limit of detection, $\mu$ g mL$^{-1}$</td>
<td>0.06</td>
<td>0.25</td>
</tr>
<tr>
<td>Limit of quantification, $\mu$ g mL$^{-1}$</td>
<td>0.20</td>
<td>0.75</td>
</tr>
<tr>
<td>Regression equation, $Y^*$</td>
<td>$0.6280$</td>
<td>$0.5947$</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>-0.1060</td>
<td>-0.0268</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>-0.9981</td>
<td>-0.9973</td>
</tr>
<tr>
<td>Correlation coefficient, ($r$)</td>
<td>0.012</td>
<td>0.015</td>
</tr>
<tr>
<td>$S_a$</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td>$S_b$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$Y = a + bX$, where $Y$ is the absorbance and $X$ concentration in $\mu$ g mL$^{-1}$.

The formation of iron(III)-tiron complex(1:1) is pH dependent and 1 mL of 1 mol L$^{-1}$ HCl in a total volume of ~5 mL was used to cause oxidation of drug by NBS and the latter’s reduction by iron(II), and later the pH was raised to ~1.0 by adding 1.0 mL of 1.5 mol L$^{-1}$ sodium acetate solution. To ensure an optimum pH for the complex formation reaction, 1 mL of buffer of pH 1.09 was also added. The oxidation of ABZ was complete in 5-15 min but the reduction of NBS by iron(II) and subsequent complexation of iron(III) with thiocyanate or tiron was instantaneous.

**Analytical Parameters**

A linear relation is found between absorbance and concentration in the ranges given in Table 2. In both methods, Beer’s law is obeyed in the inverse manner. The calibration graphs are described by the equation:

$$Y = a + b X$$

(where $Y =$ absorbance, $a =$ intercept, $b =$ slope and $X =$ concentration in $\mu$ g mL$^{-1}$) obtained by the method of least squares. Correlation coefficients, intercepts and slopes for the calibration data are also presented in Table 2. Sensitivity parameters such as molar absorptivity and Sandell sensitivity values, and the limits of detection and quantification calculated according to ICH guidelines [32] are also compiled in Table 2, and demonstrate the high sensitivity of the methods.

**Method Validation**

**Evaluation of Accuracy and Precision**

Intra-day and inter-day precision were assessed from the results of seven replicate analyses on pure drug solution. The mean values and relative standard deviation (RSD) values for seven replicate analyses at three different concentration levels were calculated. The accuracy of the methods was determined by calculating the percentage deviation observed in the analysis of pure drug solution and expressed as the relative error (RE).

To determine the inter-day precision, analysis was performed over a period of five days preparing all solutions afresh each day. Table 3 summarizes the intra-day precision and accuracy data for the determination of ABZ by the proposed methods. The inter-day RSD values were less than 3.0%.

**Application**

Table 4 gives the results of assay and reveals that there is close agreement between the results obtained by the proposed methods and the label claim. The results were also compared statistically with those obtained by a reference method[16] by applying Student’s t-test for accuracy and F-test for precision. At the 95% confidence level, the calculated t- and F-values did not exceed the tabulated values ($t = 2.77$ and $F = 6.39$), suggesting that...
the proposed methods are as accurate and precise as the reference method.

Accuracy and validity of the methods were further ascertained by performing recovery experiments via standard addition technique. To a fixed and known amount of ABZ in tablet powder (pre-analysed), pure drug was added at three levels and the total was found by the proposed methods. Each test was repeated three times. The percent recovery of pure ABZ added to tablet powder was (97.7-103.2) for method A and (98.9–102.5) for method B, indicating that commonly encountered tablet excipients and additives such as talc, starch, lactose, sodium alginate, magnesium stearate, calcium gluconate and calcium dihydrogen orthophosphate did not interfere in the assay procedures.

Conclusions

Two new methods have been developed and appropriately validated for the assay of ABZ. Both spectrophotometric methods are based on well-characterised complexation reactions and the thiocyanate method is the most sensitive ever reported for ABZ in terms of wide linear dynamic concentration range and molar absorptivity (Table 1). An additional advantage of the methods is that the absorbance is measured at longer wavelengths where the interference from excipients is far less than at shorter wavelengths. The stability of the coloured species and sensitivity of the reactions used are not critically dependent on any experimental variable unlike many reported methods. These advantages coupled with a fairly degree of accuracy and precision qualify the methods for use in quality control laboratories.

Acknowledgements

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References

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Table 3. Evaluation of accuracy and precision

<table>
<thead>
<tr>
<th>Method</th>
<th>ABZ taken μg mL⁻¹</th>
<th>ABZ found** μg mL⁻¹</th>
<th>Recovery (Percent±SD)</th>
<th>Range, μg mL⁻¹</th>
<th>RE%</th>
<th>SDM μg mL⁻¹</th>
<th>CI, μg mL⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.5</td>
<td>1.48</td>
<td>98.67 ± 0.81</td>
<td>0.08</td>
<td>1.33</td>
<td>0.005</td>
<td>±0.011</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>2.95</td>
<td>98.33 ± 0.71</td>
<td>0.09</td>
<td>1.67</td>
<td>0.008</td>
<td>±0.019</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>4.46</td>
<td>99.11 ± 0.74</td>
<td>0.12</td>
<td>0.89</td>
<td>0.012</td>
<td>±0.031</td>
</tr>
<tr>
<td>B</td>
<td>5.0</td>
<td>4.93</td>
<td>98.60 ± 1.03</td>
<td>0.12</td>
<td>1.40</td>
<td>0.019</td>
<td>±0.047</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.95</td>
<td>99.50 ± 0.78</td>
<td>0.11</td>
<td>0.50</td>
<td>0.029</td>
<td>±0.072</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>14.8</td>
<td>98.67 ± 0.83</td>
<td>0.15</td>
<td>1.33</td>
<td>0.046</td>
<td>±0.114</td>
</tr>
</tbody>
</table>

** Mean value of seven replicate determinations
RE: Relative error; SDM: Standard deviation of mean; CI: Confidence interval at the 95% confidence level and six degrees of freedom.

Table 4. Results of determination of albendazole in tablets and statistical comparison with the reference method

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Nominal amount, mg</th>
<th>Reference method</th>
<th>% of label claim* ± SD</th>
<th>Method A</th>
<th>Method B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gekarea</td>
<td>400</td>
<td>102.3±0.62</td>
<td>101.2±1.39±1.73F=5.03</td>
<td>101.9±1.18=0.70F=3.62</td>
<td></td>
</tr>
<tr>
<td>Alminth</td>
<td>400</td>
<td>97.2±1.06</td>
<td>98.3±1.48±1.37F=1.95</td>
<td>99.1±1.92±2.01F=3.28</td>
<td></td>
</tr>
<tr>
<td>Vermitel</td>
<td>400</td>
<td>101.3±0.62</td>
<td>99.8±1.32±2.44F=4.53</td>
<td>100.1±1.33±1.94F=4.60</td>
<td></td>
</tr>
</tbody>
</table>

*Mean value of five determinations
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39