CHAPTER VII

Novel spectrophotometric methods for the assay of buzepide metiodide in formulations

Abstract

Three simple, sensitive and accurate spectrophotometric methods for the determination of buzepide metiodide (BZP) in pure and pharmaceutical preparations have been proposed. The methods are based on the measurement of absorbances of tris (o-phenanthroline) iron (II) [Method A] and tris (bipyridyl) iron (II) [Method B], and oxidative coupling of the BZP with 3-methylbenzothiazolin-2-one hydrazone in presence of iron (III) chloride as an oxidant [Method C] at 510, 522 nm and 610 nm, respectively. Reaction conditions have been optimized to obtain colored complexes of higher sensitivity and longer stability. The absorbances were found to increase linearly with increase in concentrations of BZP, which were corroborated by correlation coefficient values (0.9956, 0.9948 and 0.9969 in methods A, B and C, respectively). The complexes obeyed Beer's law over the concentration range of 1.8-27, 2.9-34 and 0.7-14.8 μg/ml in methods A, B and C, respectively. The developed methods have been successfully applied for the assay of BZP in bulk samples and pharmaceutical formulations. The common excipients and additives did not interfere in their determinations. The results obtained by the proposed methods were statistically compared by means of F- and t-tests.

The results of this Chapter have been communicated to Analytical Sciences, Japan (2007).
GENERAL DRUG PROFILE

Buzepide metiodide (BZP)

Chemical name
1-(4-amino-4-oxo-3,3-diphenylbutyl)hexahydro-1-methyl-1H-azepinium iodide

Molecular formula
C_{23}H_{31}ON_{2}I

Molecular weight
478.42

Description
Colorless or slightly yellow amorphous powder

Solubility
Soluble in ethanol

Category
Antidepressant

INTRODUCTION

BZP is an antidepressant. In a meta analysis of randomized Clinical Trial (RCT) it was concluded that antidepressants may be effective in reducing Irritable Bowel Syndrome (IBS) symptoms in about one-third of patients. Since BZP is an antidepressant, attempts have been made to assess its effect to treat the IBS. The mechanism of action of these compounds (in the treatment of IBS, mostly tricyclic antidepressants) is probably related to their central effect and the anticholinergic properties. It is also used in the treatment of functional intestinal disorders in combination with haloperidol.
Critical literature survey revealed only HPLC methods\textsuperscript{6,7} for the assay of BZP. No spectrophotometric method is reported so far for its determination. In view of this, it was felt worthwhile to develop simple and sensitive spectrophotometric methods for the determination of BZP in bulk samples and pharmaceutical preparations using the analytical reagents \textit{viz.}, Fe(III)-1,10-phenanthroline (FPL), Fe(III)-2,2'-bipyridyl (FBL) and 3-methylbenzothiazolin-2-one hydrazone (MBTH).

**EXPERIMENTAL**

**Reagents**

MBTH (0.2\%, \textit{w/v}) solution was prepared in distilled water.

FPL and FBL were prepared\textsuperscript{8} as follows:

1) FPL was prepared by mixing 0.198 g of 1,10-phenanthroline (PNL) with 2 ml of 1 M HCl and 0.16 g of ferric ammonium sulphate dodecahydrate and diluting with distilled water to 100 ml.

2) FBL was prepared by mixing 0.16 g of 2,2'-bipyridyl in 2 ml of 1 M HCl with 0.16 g of ferric ammonium sulphate dodecahydrate and diluting with distilled water to 100 ml.

**Standard drug solution**

A stock solution of standard BZP (100 \textmu g/ml) was prepared in ethanol and stored in an amber colored bottle in a refrigerator. The solution was diluted as and when required.
RECOMMENDED PROCEDURES

The following procedures were recommended for the assay of BZP after a systematic study on various parameters involved in the formation of colored products (as described under results and discussion).

Analysis of pure drug

Methods A and B

Aliquots of standard drug solutions of BZP were transferred separately into a series of 10 ml calibrated flasks. To these were added 6 ml of FPL for method A and 7 ml of FBL for method B. The solutions were heated on a water bath to 85 °C for 5 min in method A and for 10 min in method B. The solutions were cooled, diluted up to the mark with distilled water and mixed well. The absorbances of complexes were recorded at 510 nm and 522 nm in methods A and B, respectively against the corresponding reagent blank. Calibration graphs were plotted.

Method C

3 ml of MBTH was transferred into a series of 10 ml volumetric flasks. An aliquot solution of BZP was added so that the final concentration of drug was in the range of 1-14.8 μg/ml. Then 2.5 ml of ferric chloride solution was added, mixed well and allowed to stand for 20 min. The volume was adjusted upto the mark with water. The absorbance was measured against a reagent blank at 610 nm. The absorbance versus the concentration was plotted to get the calibration curve.
Analysis of pharmaceutical preparations

Ten tablets of the selected drugs were finely powdered. An amount equivalent to 25 mg of the drug was weighed accurately and transferred into a 100 ml beaker. Using a mechanical stirrer, the powder was completely disintegrated in ethanol. The solution was diluted to the mark in a 100 ml volumetric flask and filtered. An aliquot of the drug solution was taken and analyzed as described earlier.

RESULTS AND DISCUSSION

Ferric salts play a prominent role in the spectrophotometric determination of many pharmaceutical drugs.\(^9\)\(^{14}\) Acting as an oxidant, a ferric salt gets reduced to ferrous salt and this amount corresponds to drug concentration. The amount of Fe(II) so formed could be determined using analytical reagents such as 1,10-phenanthroline (PNL) and 2,2'-bipyridyl (BPL). These properties have been utilized to develop spectrophotometric methods for the determination of BZP.

The reaction of BZP with MBTH in presence of an oxidant, iron(III) chloride produced blue colored species having maximum absorption at 610 nm. Under reaction conditions, MBTH on oxidation with Fe\(^{3+}\) ions loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling species.\(^{15}\) The absorption spectra of all the colored species of BZP are shown in Fig 1.
**Spectral characteristics**

In order to determine the wavelength of maximum absorption ($\lambda_{\text{max}}$) of colored products formed in all the proposed methods, fixed amount of BZP was taken separately and the reaction product was developed following the procedure given above. The absorption spectrum was scanned on a spectrophotometer in the wavelength region of 400-700 nm against the corresponding reagent blank. BZP undergoes oxidation by Fe(III) present in FPL and FBL. The Fe(II) so formed readily combined with PNL of FPL or BPL of FBL to form a red colored complex, $[\text{Fe(phen)}_3]^{2+}$ having absorption maximum at 510 nm or $[\text{Fe(bipy)}_3]^{2+}$ exhibiting absorption maximum at 522 nm. Under the experimental conditions each reagent blank showed a negligible absorbance at the corresponding $\lambda_{\text{max}}$, there by permitting good analytical conditions for the assay of BZP.

BZP reacted with MBTH in presence of Fe(III) and yielded a blue colored complex. The absorption spectra are shown in Fig. 1.

**Fixation of optimum reaction conditions**

**For methods A and B**

The optimum reaction conditions for quantitative determination of BZP were established via a number of preliminary experiments. The effects of the reagents were studied by measuring the absorbances of solutions containing a fixed concentration of BZP and varied amounts of the reagent separately. These optimum conditions were incorporated in the procedures.
Effect of FPL and FBL on sensitivity of the colored products

Experimental results indicated that a volume of 5 ml of FPL/FBL in method A/B was necessary for obtaining maximum and stable absorbance readings (Fig 2). Low absorbances were observed with the volume of the reagent which was less than or more than 5 ml.

Effect of temperature on colored products

The formation of colored complex was slow at room temperature and required longer time for completion of the reaction. Hence, efforts were made to accelerate the reaction by carrying out the reaction at higher temperatures. It was noticed that the intensity of the colored complexes attained maximum only after heating the reaction mixture to 85 °C (Fig. 3) for 5 min in method A and for 10 min in method B. The absorbances of the complexes remained constant at room temperature for more than 24 h.

For method C

Effect of MBTH concentration

The effect of the concentration of MBTH was studied by measuring the absorbances at the specified wavelength for solutions containing a fixed concentration of BZP and varying amounts of MBTH. A volume of 3 ml of 1% MBTH in a total volume of 10 ml was found to be sufficient to give maximum color intensity. The absorbances were found to be low or unstable when less than or more than 3 ml of the reagent was added (Fig. 2).
Effect of Fe\textsuperscript{+3} concentration

The optimum volume of iron (III) chloride solution necessary for getting the colored species of maximum absorbance and stability was noticed to be 2.5 ml of 1% solution in a total volume of 10 ml of the reaction mixture. Further, higher concentration of Fe (III) yielded less intense colored chromophore (Fig. 2). The optimal HCl concentration used for the preparation of iron (III) chloride was observed to be 1.0 M.

Effect of reaction time

The colored product was formed within 15 min at 30 °C in the proposed method and remained stable for more than 10 h.

Optical characteristics of the colored products (Methods A, B and C)

To examine whether the colored products formed in the proposed methods adhere to Beer’s law or not, the absorbances of a series of solutions containing increased amounts of the drug were measured against the corresponding reagent blank at respective \( \lambda_{\text{max}} \) values. A linear relationship was found between the absorbance at \( \lambda_{\text{max}} \) and the concentration of colored species in the concentration range of 1.8-27, 2.9-34 and 0.7-14.8 in methods A, B and C, respectively (Fig. 4). The molar absorptivity and Sandell’s sensitivity values have been evaluated and the results are tabulated in Table 1. Regression analyses of Beer’s law plots at \( \lambda_{\text{max}} \) values revealed a good correlation. Graphs of absorbances versus concentration showed zero intercept and are described by regression equation \( Y = aX + b \) (where \( Y \) is the
absorbance of a 1 cm layer, 'a' is the slope, b is the intercept and X is the concentration of the selected drug in μg/ml) obtained by least-squares method. The results are summarized in **Table 1**.

**Detection and quantification limits**

The LOD and LOQ values for methods A, B and C were calculated and the same are given in **Table 1**.

**Precision and accuracy of the proposed methods**

The precision of the proposed methods was ascertained from absorbance values obtained by actual determination of five replicates of fixed amount of the bulk sample of each drug. The low values of percent relative standard deviation recorded in **Table 1** indicated good precision of the methods.

In order to determine the accuracy of the proposed methods, known amounts of bulk samples of BZP were taken separately within the Beer's law limits and analyzed. The percentage errors were found to be low indicating thereby that the proposed methods were reasonably accurate. The results are shown in **Table 1**.

**Interference studies**

The effects of common excipients and additives were tested for their possible interferences in the assay of BZP. It was evident from the results (**Table 2**) that the talc, glucose, starch, lactose, sodium alginate, gumacacia, dextrose and magnesium stearate did not interfere in the determination at the levels found in dosage forms. The corresponding results are given in **Table 2** for a representative method, **Method A**.
Recovery studies

The recovery technique was applied to judge the suitability of the proposed methods. For this, known quantities of pure BZP solutions were mixed separately with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined using the proposed methods and the amount of the added drug was calculated by difference. The results were found to be in the range of 99.12 – 101.67%.

Analysis of practical samples

The proposed methods were applied to the assay of studied drugs in formulations. Table 3 showed the percentage recoveries obtained by the proposed methods. The low percent RSD values indicated high reproducibility of the results.

CONCLUSIONS

The reagents provide fairly high sensitivity for the assay of BZP. The proposed methods are simple and accurate. The results of the proposed methods are reproducible and reasonably accurate. Method C was found to be more sensitive compared to methods A and B for the assay of BZP. The colored species are quite stable which make the methods more practicable. The validity of the proposed methods is well demonstrated by analyzing tablets containing BZP. In view of these, the proposed methods could be adopted for routine quality control.
REFERENCES


### Table 1. Optical characteristics, precision and accuracy data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method A</th>
<th>Method B</th>
<th>Method C</th>
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<tr>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>510</td>
<td>522</td>
<td>610</td>
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<tr>
<td>Beer's law limits ($\mu$g/ml)</td>
<td>1.8-27</td>
<td>2.9-34</td>
<td>0.7-14.8</td>
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<tr>
<td>Molar absorptivity ($10^4$ l/mol/cm)</td>
<td>1.332</td>
<td>1.006</td>
<td>2.236</td>
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<td>Sandell's sensitivity (ng/cm²)</td>
<td>35.89</td>
<td>49.4</td>
<td>21.39</td>
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<tr>
<td>Correlation coefficient</td>
<td>0.9956</td>
<td>0.9948</td>
<td>0.9969</td>
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<tr>
<td>Regression equation ($Y)^a$</td>
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<td></td>
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<tr>
<td>Slope, a</td>
<td>0.0243</td>
<td>0.019</td>
<td>0.0471</td>
</tr>
<tr>
<td>Intercept, b</td>
<td>0.0418</td>
<td>0.0346</td>
<td>0.0011</td>
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<tr>
<td>Relative standard deviation*, %</td>
<td>0.79</td>
<td>0.68</td>
<td>0.98</td>
</tr>
<tr>
<td>% Error</td>
<td>0.84</td>
<td>0.96</td>
<td>1.18</td>
</tr>
<tr>
<td>Limit of detection ($\mu$g/ml)</td>
<td>0.54</td>
<td>0.87</td>
<td>0.21</td>
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<tr>
<td>Limit of quantification ($\mu$g/ml)</td>
<td>1.82</td>
<td>2.91</td>
<td>0.71</td>
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</table>

*$Y = aX + b$, where $X$ is the concentration of drug in $\mu$g/ml

*Average of five determinations
Table 2. Interference of excipients in the determination of BZP
(3.5 μg/ml; Method A)

<table>
<thead>
<tr>
<th>Excipients added</th>
<th>Amount (mg)</th>
<th>% Recovery ± % RSD</th>
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<tbody>
<tr>
<td>Glucose</td>
<td>30</td>
<td>97.52 ± 0.56</td>
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<tr>
<td>Talc</td>
<td>30</td>
<td>98.32 ± 0.35</td>
</tr>
<tr>
<td>Lactose</td>
<td>30</td>
<td>99.60 ± 0.15</td>
</tr>
<tr>
<td>Starch</td>
<td>30</td>
<td>97.42 ± 0.21</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>30</td>
<td>97.33 ± 0.37</td>
</tr>
<tr>
<td>Sodium alginate</td>
<td>35</td>
<td>97.35 ± 0.62</td>
</tr>
<tr>
<td>Gum acacia</td>
<td>40</td>
<td>97.87 ± 0.57</td>
</tr>
<tr>
<td>Dextrose</td>
<td>30</td>
<td>99.51 ± 0.35</td>
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*Average of five determinations

Table 3. Determination of BZP in pharmaceutical preparations by the proposed methods

<table>
<thead>
<tr>
<th>Drug (Tablet)</th>
<th>Label claim (mg per tablet)</th>
<th>Recovery * ± RSD, % Method A</th>
<th>Method B</th>
<th>Method C</th>
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</thead>
<tbody>
<tr>
<td>Motival(^a)</td>
<td>30</td>
<td>99.28 ± 0.56</td>
<td>99.36 ± 0.86</td>
<td>99.38 ± 0.79</td>
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<tr>
<td></td>
<td>40</td>
<td>99.75 ± 0.68</td>
<td>100.31 ± 0.68</td>
<td>99.45 ± .77</td>
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<tr>
<td></td>
<td>50</td>
<td>100.1 ± 0.80</td>
<td>99.41 ± 0.94</td>
<td>100.42 ± 0.59</td>
</tr>
<tr>
<td>Sensival(^b)</td>
<td>25</td>
<td>100.5 ± 0.79</td>
<td>99.44 ± 0.72</td>
<td>99.14 ± 0.92</td>
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</table>

*Average of five determinations
Fig 1. Absorption spectra of colored species of (a) 22 ppm BZP in Method A (b) 25 ppm BZP in Method B and (c) 14 ppm BZP in Method C. The absorbances of corresponding reagents blank were negligible.

Fig. 2. Effect of volume of reagents on the absorbance of the colored species.
Fig. 3. Effect of temperature (in degree Celsius) on the colored complexes of BZP in Methods A and B

Fig. 4. Beer’s law plots of BZP for methods A, B and C