TWO PHASE TITRATION AND BROMATOMETRIC ASSAY OF PENA VAIRIUM BROMIDE

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ABSTRACT
Two titrimetric methods for the determination of penavarium bromide (PNB) have been proposed. Based on the quaternary ammonium moiety present in PNB, a titrimetric method (method A) based on ion association complex formation with sodium lauryl sulphate (SLS) as titrant and dimethyl yellow as indicator has been proposed. In another method (method B), a stoichiometric reaction between penavarium bromide and in situ generated bromine has been used in a back titrimetric approach. The methods are applicable over the concentration range 2.0–15 mg and 1.0–10.0 mg for method A and method B, respectively. Calculations are based on 1:1 molar ratio, i.e., PNB: SLS for method A and PNB: Br₂ for method B. Method A is applicable to the determination of PNB in bulk drug and tablet, whereas method B is only applicable to bulk drug due to the interferences from tablet excipients because of non-specific reaction of bromine. The methods are validated for accuracy and precision.

Key Words: Two-Phase Titration, Bromination, Bulk Drug

INTRODUCTION
Pinvarerium bromide is chemically known as 4-(2-Bromo-4, 5-dimethoxy-benzyl)-4-\{2-[2-(6, 6-dimethyl-bicyclo [3.1.1]hept-2-yl)-ethoxy]-ethyl\}-morpholin-4-ium (Figure 1). Pinaverium bromide is a locally acting spasmolytic agent of the digestive tract (Guslandi, 1994). It acts upon inhibition of calcium ion entrance into smooth muscle cells (calcium-antagonist effect). In humans, pinaverium facilitates gastric emptying and decreases intestinal transit time in patients with constipation (Guslandi, 1994). Based on our literature survey, there were only two analytical methods reported for the determination of PNB (De-Weerdt et al., 1983; Ren et al., 2011). The method involves extraction, use of internal standard and reduction of analyte/internal standard using Raney-Nickel (De-Weerdt et al., 1983). Thus for this complicated and time consuming method, an alternative method with faster analysis time and less complicated steps were proposed (Ren et al., 2011). Although both the methods are equally sensitive and applicable to blood sample, there lack a macro-quantitative method for the determination PNB in routine quality control laboratories.

In this regard, we proposed two selective titrimetric methods for microgram determination of PNB in bulk drug and tablet formulation. Method A is based on the diphasic ion association complex formation with SLS using dimethyl yellow as indicator. Method B is based on the stoichiometric reaction between in situ generated bromine and PNB, wherein unreacted bromine corresponds to the concentration of the PNB reacted. The methods have been validated in accordance with the ICH guidelines.

MATERIALS AND METHODS
All chemicals used were analytical grade purchased from Merck or Loba Chemie (Mumbai, India). Distilled water was used throughout. A stock standard solution of 2.0 and 1.0 mg/mL of pharmaceutical grade PNB (Jubilant Life Sciences, India) solution was prepared in water for method A and method B. A 0.0035 M SLS, 0.01N KBrO₃ and 0.01N Na₂S₂O₃ was prepared in water. Chloroform (spec. gra. 1.48) and absolute ethanol were used as such. A 0.01% w/v dimethyl yellow was prepared in absolute ethanol. 5 N hydrochloric acid and 2 M sulfuric acid was prepared by appropriately diluting concentrated acids with water.
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Assay Procedure

Method A

Varying aliquots (2-15 mL) of 1 mg/mL standard PNB solution were accurately measured into a 100 mL beaker and the volume adjusted to 10 mL with water. A 2.0 mL of 2 N H₂SO₄ and 0.2 mL of 0.01% dimethyl yellow were added and mixed well.

Further, 10 mL of chloroform was added and the solution was vigorously stirred on a magnetic stirrer till the organic phase turned yellow. Then the solution was titrated against 0.0035 M sodium lauryl sulfate upon stirring continuously, until the organic layer turned pink at the end point. The amount of PNB in the aliquot was computed from the following formula:

\[
\text{Amount} = \frac{(S - B) \times M_w \times C}{n}
\]

Where, \( S \) = volume of titrant consumed for sample, mL, \( B \) = volume of titrant consumed for blank, mL, \( M_w \) = relative molecular mass of drug, \( C \) = strength of titrant, \( M \), \( n \) = number of moles of titrant reacting with one mole of PNB.

Method B

A 10 mL of 0.01 N KBrO₃, 5 mL of 10% KBr, and 5 mL of 5N HCl was taken into a Erlenmeyer flask and the flask was kept closed for 5 minutes. Then, varying aliquots (1–10 mL) of 1 mg/mL standard PNB solution were accurately measured into it and the flasks were kept closed for 30 minutes. The unreacted bromine was titrated against standard thiosulfate using starch as indicator towards the end point.

The assay of PNB was calculated using the formula-

\[
\text{Amount} = \frac{(S - B) \times M_w \times C}{n}
\]

Where, \( S \) = volume of Na₂S₂O₃ consumed for sample, mL, \( B \) = volume of Na₂S₂O₃ consumed for blank, mL, \( M_w \) = relative molecular mass of drug, \( C \) = strength of titrant, \( M \), \( n \) = number of moles of titrant reacting with one mole of PNB.

Procedure for Tablets

Twenty tablets were weighed accurately and ground into a fine powder. Four portions of the powder equivalent to 50 mg of PNB were accurately weighed into a 100 mL volumetric flask and extraction was done by shaking for half an hour with 40 mL of water specified under each method; then, the volume was diluted to the mark with the water, mixed well and filtered using a Whatman no. 42 filter paper. First 10 mL portion was discarded and subsequent portion of the tablet extract was subjected to titration by following the procedure mentioned under each method. A severe interferences from tablet excipient was found in method B, therefore, the tablet analysis with method B was not persuaded.

RESULTS AND DISCUSSION

The anionic surfactant, sodium lauryl sulphate, forms ion association complex with cationic amino group of PNB. As per Tharpa et al., (2012), the relatively neutral complex in the form of an emulsion in aqueous phase gradually dissolved in chloroform layer. The end point is detected by pH indicator containing tertiary amino group which can participate in diphasic movement. In this regard, dimethyl yellow is widely used as a two phase indicator. The overall reaction is pH dependent, which determines the indicator color change at the end-point as shown as-

\[
\begin{align*}
\text{Yellow (organic phase)} & \quad \text{Pink (organic phase)} \\
\end{align*}
\]

In method B, a well known in-situ generation of bromine from following reaction as been used-
2BrO₃⁻ + 10Br⁻ + 12H⁺ → 6Br₂ + 3H₂O

The known excess of liberated bromine was allowed to react with PNB for constant time of 30 minutes. Thereby, unreacted bromine was quantitatively titrated against standardized sodium thiosulfate. A constant stoichiometric ration of 1.0 was observed within a PNB concentration range of 1-10 mg.

Method Validation
Methods were validated in accordance to the ICH guidelines (ICH Harmonised Tripartite Guideline, 1996).

Range
The concentration range of PNB was established based on the stoichiometric relation exist with that of the titrant, i.e., with SLS and in situ generated bromine in case of method A and method B, respectively. The concentration range for PNB in method A is 1.0 – 15.0 mg and in method B is 1.0 – 10.0 mg.

Accuracy and Precision
The accuracy of the proposed methods was determined by performing replicate determinations. The intra-day and inter-day variation in the analysis of PNB was measured at three different levels. The accuracy of an analytical method expresses the closeness between the reference and the found value. Accuracy was evaluated as percentage relative error between the measured and taken amounts/concentrations. The precision of the methods was evaluated in terms of intermediate precision (intra-day and inter-day). Three different amounts of PNB within the range in each method were analysed in three replicates during the same day (intra-day precision) and two consecutive days (inter-day precision). The results of these studies are tabulated (Table 1).

Application to Tablet
The tablet solution was analyzed based on preparation shown as above (under preparation of tablet solution).
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Table 1: Intra-day and Inter-day accuracy and precision

<table>
<thead>
<tr>
<th>Method</th>
<th>PNB Taken Mg</th>
<th>Intra-day (n=3)</th>
<th></th>
<th></th>
<th>Inter-day (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PNB found</td>
<td>Precision</td>
<td>Accuracy</td>
<td>PNB found</td>
</tr>
<tr>
<td>Method A</td>
<td>15</td>
<td>15.68</td>
<td>0.66</td>
<td>4.93</td>
<td>15.62</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10.25</td>
<td>0.20</td>
<td>2.5</td>
<td>10.13</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5.12</td>
<td>0.10</td>
<td>2.0</td>
<td>5.06</td>
</tr>
<tr>
<td>Method B</td>
<td>3</td>
<td>2.86</td>
<td>0.09</td>
<td>4.67</td>
<td>2.92</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4.72</td>
<td>0.10</td>
<td>5.60</td>
<td>4.67</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.59</td>
<td>0.05</td>
<td>7.38</td>
<td>8.82</td>
</tr>
</tbody>
</table>

Table 2: Recovery study

<table>
<thead>
<tr>
<th>Method</th>
<th>PNB tablet solution taken (Eldicit-50 mg)</th>
<th>Pure drug solution added found</th>
<th>Amount found in mg</th>
<th>Precision</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method A</td>
<td>5</td>
<td>2.5</td>
<td>7.63</td>
<td>0.10</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>9.90</td>
<td>0.10</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7.5</td>
<td>12.64</td>
<td>0.10</td>
<td>1.12</td>
</tr>
</tbody>
</table>

Recovery Study
The accuracy of the developed method was studied by standard addition technique. Since method B suffers from matrix interference, the accuracy of the method was studied by method A. The precision of the recovery study along with %RE data are presented in Table 2.

Conclusion
Two microgram determination of pinavarium bromide was developed and validated. The methods are found to be sensitive, accurate and precise beside method B which has its limitations in its application to formulations. The method A which is highly selective gave accurate result while application to tablet solutions.
REFERENCES