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Research Article

TWO PHASE TITRATION AND BROMATOMETRIC ASSAY OF PENAVARIUM 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 stiochiometric 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, respecttively. Calculations are based on 1:1 molar ratio, i.e., PNB: SLS for method A and PNB: Br_2 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-specifiic 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 stiochiometric 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 $KBrO_3$ and 0.01N NaS_2O_3 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.

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 2.0mL of 2 N H_2SO_4 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:

$$Amount = \frac{(S - B) \times M_w \times C}{n}$$

Where, *S* = volume of titrant consumed for sample, mL, *B* = volume of titrant consumed for blank, mL, *Mw* = 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 $KBrO_3$, 5 mL of 10% KBr, and 5 mL of 5N HCl was taken into a Erlynmer 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-

$$Amount = \frac{(S - B) \times M_w \times C}{n}$$

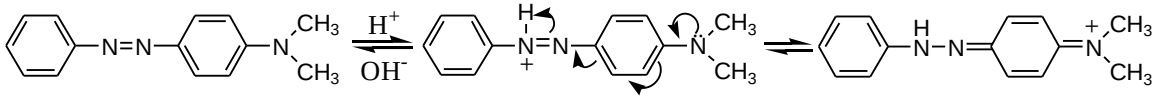
Where, *S* = volume of $Na_2S_2O_3$ consumed for sample, mL, *B* = volume of $Na_2S_2O_3$ consumed for blank, mL, *Mw* = relative molecular mass of drug, *C* = strength of titrant, *M*, *n* = number of moles of titrant reacting with one mole of PNB.

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-



Yellow (organic phase) **Pink (organic phase)**

In method B, a well known in-situ generation of bromine from following reaction as been used-

$$2BrO_3^- + 10Br^- + 12H^+ \longrightarrow 6Br_2 + 3H_2O$$

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 stiochiometric 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 stiochiometric 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).

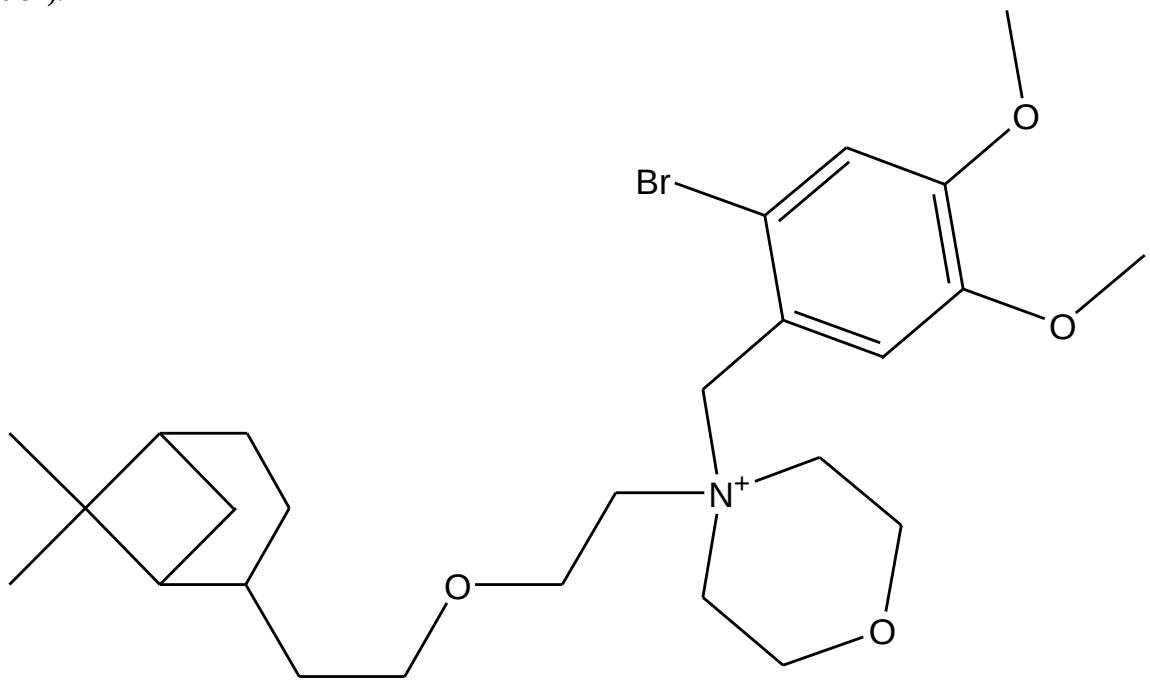


Figure 1: Pinvarium bromide

Table 1: Intra-day and Inter-day accuracy and precision

Method	PNB Taken Mg	Intra-day (n=3)			Inter-day (n=3)		
		PNB found	Precision	Accuracy	PNB found	Precision	Accuracy
	15	15.68	0.66	4.93	15.62	0.10	4.13
Method A	10	10.25	0.20	2.5	10.13	0.20	1.3
	5	5.12	0.10	2.0	5.06	0.10	1.2
	3	2.86	0.09	4.67	2.92	0.05	2.66
Method B	5	4.72	0.10	5.60	4.67	0.10	6.6
	8	8.59	0.05	7.38	8.82	0.05	7.75

Table 2: Recovery study

Method	PNB tablet solution taken (Eldicet-50 mg)	Pure drug solution added found	Amount found in mg	Precison	Accuracy
	5	2.5	7.63	0.10	1.73
Method A	5	5	9.90	0.10	1.0
	5	7.5	12.64	0.10	1.12

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.

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