INTRODUCTION

Hyoscine is an alkaloidal derivative present in the plant genus *Duboisia* grown in South America and Australia. It is chemically modified by adding a butyl group to get a quaternary ammonium structure so that the molecule still preserves the anticholinergic activities as that of hyoscine. Butyl moiety has also significantly enhanced the water solubility, which prevents the passage across the blood brain barrier. Oral hyoscine butylbromide is poorly absorbed (8 %) and has a low systemic bioavailability (1 %). However, it remains available at the site of action and gives pain relief locally. Plasma protein binding (albumin) of the drug is also low (4.4 %). The half-life of the drug is 5 h and 50 % of the drug is excreted via renal and faecal route.

It is important to design a parenteral dosage form which would reduce the amount of drug in the formulation and provide the required amount needed for the therapeutic action alone. Since the drug is expensive, the main aim is to formulate a stable and effective parenteral dosage form which would be cost effective as well. The main problem involved with formulating a parenteral is its instability with other excipients and buffers. The objective of the study is to select the correct combination of ingredients in the formulation to make it effective and stable.

EXPERIMENTAL

Hyoscine butylbromide was obtained from Vital Laboratories Pvt. Ltd., Gujarat, India. Sodium chloride, sodium citrate, citric acid, glacial acetic acid, sodium acetate, benzyl alcohol, cresol, phenol, sodium methyl paraben, sodium propyl paraben, sodium metabisulfite and ascorbic acid were obtained from KAPL, Bangalore. The analytical grade reagents and solvents and freshly prepared water were used throughout the study.

Methods: The preformulation studies were carried out in accordance with procedures reported in the literature. Determination of melting point: Melting point of hyoscine butylbromide was determined by capillary method. Small quantity of the drug was filled into the capillary tubes, whose one end was sealed by using Bunsen burner. The tubes were then kept into the melting point apparatus where the temperature was raised slowly. The temperature at which the drug started melting was noted down.

Fourier transform infrared spectroscopy: The pure drug was subjected to FT-IR studies by mixing with 100 mg of potassium bromide and analyzed.

Solubility studies: The solubility studies of hyoscine butylbromide were carried out in solvents of different pH and water for injection by saturation solubility method.

General procedure for drug stability studies

Heat stability of drug: 1 % hyoscine butylbromide solution was filled in 50 mL glass vials containing 0.1 N NaOH, 0.1 N HCl, Water and sealed. The sealed vials were maintained under refrigeration, at room temperature and at 45 °C for a month to observe for possible colour change, crystal growth and level of
impurities. The refrigerated vial and the room temperature vial were taken as control.

**Light stability of drug:** A 1% hyoscine butylbromide solution was filled in 50 mL glass vials (separate amber and clear vials) containing 0.1 N NaOH, 0.1 N HCl, and water. The samples were placed in an open petri dish exposing larger surface to light. The drug solution placed in amber coloured glass vials was used as control. The samples were placed into a well-vented temperature monitored light cabinet and exposed for 4 weeks with weekly examination for colour change, presence of precipitates and impurities.

**Effect of oxygen on drug:** A 1% hyoscine butylbromide solution was filled in 50 mL glass vials and maintained at 40 °C /75% RH in stability chamber and were observed for possible physical changes for over a month.

**Drug-excipient compatibility:** To understand the physical compatibility of the drug with various excipients, binary mixture of drug with various excipients in 1:1 ratio were prepared, placed in glass vials and maintained at 40 °C/75% RH in stability chamber and were observed for possible physical changes for over a month.

**Formulation development:** Buffering agents and excipients were taken as major dependent variables in formulation dependent. Various formulations (F001 to F019) were prepared to achieve stable parenteral formulation, as shown in Tables 1 and 2.

**Post formulation evaluation:** The following post formulation evaluations were carried out in accordance with established procedures8,9.

**Test for particulate matter:** Injections that are solutions are visually inspected by the unaided eye, under suitable conditions of visibility for particle free clear solution.

**pH:** The pH of the formulations were checked every week to observe any pH fluctuations.

**Assay:** The drug content was determined using liquid chromatography. A volume of injection containing about 40 mg of hyoscine butylbromide was diluted in 100 mL of 0.001 M hydrochloric acid to prepare ‘test solution’. 100 mL of 0.04% w/v solution of hyoscine butylbromide RS was made in 0.001 M hydrochloric acid to form ‘reference solution’.

A stainless steel column 25 cm × 4.6 mm, packed with octylsilane bonded to porous silica (C8) was used as stationary phase. The mobile phase was prepared by dissolving 2 g of sodium lauryl sulfate in mixture of 370 mL of 0.001 M hydrochloric acid and 680 mL of methanol. The mobile phase was run at 1.5 mL/min with detection carried out at 210 nm. While the injection volume was confined to 20 μL, the run time was limited to ten minutes. Vials containing blank solution (0.001 M HCl), reference standard solution and each of the test solution were kept in the auto-sampler tray of the HPLC unit. The chromatograms and the peak response were then measured and the content of hyoscine butylbromide estimated.

**Test for sterility:** Fluid thioglycollate medium and Soyabean-Casein Digest medium were prepared according to Indian Pharmacopoia. They were sterilized and used as the

| Ingredients                          | Quantity for 25 mL |
|--------------------------------------|--------------------|
| Hyoscine butylbromide (mg)           | F01 F02 F03 F04 F05 F06 F07 F08 F09 F10 |
| Sodium chloride (mg)                 | 500 500 500 500 500 500 500 500 500 500  |
| Glacial acetic acid (mL)             | 225 225 225 225 225 225 225 225 225 225  |
| Sodium acetate (mg)                  | 20 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05 0.05  |
| Benzyl alcohol (mL)                  | 30 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5  |
| Phenol (mg)                          | 125 125 125 125 125 125 125 125 125 125  |
| Sodium metabisulfite (mg)            | 125 125 125 125 125 125 125 125 125 125  |
| Citric acid (mg)                     | 50 50 50 50 50 50 50 50 50 50  |
| Sodium citrate (mg)                  | 50 50 50 50 50 50 50 50 50 50  |
| Water for injection                  | 75 75 75 75 75 75 75 75 75 75  |

| Ingredients                          | Quantity for 25 mL |
|--------------------------------------|--------------------|
| Hyoscine butylbromide (mg)           | F11 F12 F13 F14 F15 F16 F17 F18 F19 |
| Sodium chloride (mg)                 | 500 500 500 500 500 500 500 500 500  |
| Glacial acetic acid (mL)             | 225 225 225 225 225 225 225 225 225 225  |
| Sodium acetate (mg)                  | 25 25 25 25 25 25 25 25 25 25  |
| Benzyl alcohol (mL)                  | 25 25 25 25 25 25 25 25 25 25  |
| Sodium metabisulfite (mg)            | 25 25 25 25 25 25 25 25 25 25  |
| Citric acid (mg)                     | 50 50 50 50 50 50 50 50 50 50  |
| Sodium citrate (mg)                  | 50 50 50 50 50 50 50 50 50 50  |
| Water for injection                  | 75 75 75 75 75 75 75 75 75 75  |

| Ingredients                          | Quantity for 25 mL |
|--------------------------------------|--------------------|
| Hyoscine butylbromide (mg)           | Qs Qs Qs Qs Qs Qs Qs Qs Qs Qs  |
| Sodium chloride (mg)                 | Qs Qs Qs Qs Qs Qs Qs Qs Qs Qs  |
| Glacial acetic acid (mL)             | Qs Qs Qs Qs Qs Qs Qs Qs Qs Qs  |
| Sodium acetate (mg)                  | Qs Qs Qs Qs Qs Qs Qs Qs Qs Qs  |
| Benzyl alcohol (mL)                  | Qs Qs Qs Qs Qs Qs Qs Qs Qs Qs  |
| Sodium metabisulfite (mg)            | Qs Qs Qs Qs Qs Qs Qs Qs Qs Qs  |
| Citric acid (mg)                     | Qs Qs Qs Qs Qs Qs Qs Qs Qs Qs  |
| Sodium citrate (mg)                  | Qs Qs Qs Qs Qs Qs Qs Qs Qs Qs  |
| Water for injection                  | Qs Qs Qs Qs Qs Qs Qs Qs Qs Qs  |
medium for inoculation of the test formulation. Since the quantity of the liquid formulation in the ampoules were 1 mL or more but less than 40 mL, the minimum quantity used for each culture medium was half of the container. The liquid from the test ampoule was removed with a sterile pipette and transferred into the culture medium. The inoculated medium were then incubated (thioglycollate medium at 32 °C and soyabean-casein digest medium at 22 °C) for 2 weeks and periodically observed for any microbial growth during the 2 weeks.

**Test for extractable volume:** Since the nominal volume of the formulation is not exceeding more than 5 mL, the container complies with the requirements of the method reported earlier in the literature.

**RESULTS AND DISCUSSION**

The melting point of hyoscine butylbromide was found to be 140.05 ± 0.05 °C, within the range (139-141 °C) reported in the literature.

The solubility of drug in water, 0.1 N NaOH and 0.1 N HCl is given in Table-3. Though the drug was freely soluble in water, the solubility of hyoscine butylbromide was more in slightly acidic medium as compared to that in basic medium.

### TABLE-3

| Solvent                          | Solubility (mg/mL) |
|---------------------------------|--------------------|
| Water for injection (WFI)        | 500                |
| 0.1 N NaOH (pH-8.5)             | 375                |
| 0.1 N HCl (pH-2.0)              | 425                |

**Effect of heat on drug stability:** The drug solutions in 0.1 N NaOH, 0.1 N HCl and WFI were stable for a month when filled in amber coloured vials (Table-5). However, there was slight turbidity on the 4th week in drug solutions in 0.1 N NaOH, 0.1 N HCl and WFI kept in clear glass vials.

**Effect of oxygen on drug stability:** The drug solutions in 0.1 N NaOH, 0.1 N HCl and WFI were stable for 2 weeks in purged vials kept at room temperature (Table-6). No precipitation was observed in any of the three purged vials. However, little precipitation was observed seen after 2 weeks in the air-sealed vial containing drug solution in 0.1 N NaOH.

### TABLE-4

| Condition       | Hyoscine butylbromide in 0.1 NaOH (Duration in Weeks) | Hyoscine butylbromide in 0.1 HCl (Duration in Weeks) | Hyoscine butylbromide in water for injection (Duration in Weeks) |
|-----------------|------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------|
| Refrigeration   | 1 2 3 4                                               | 1 2 3 4                                             | 1 2 3 4                                                      |
| Room temperature| - - - +                                               | - - - +                                             | - - - +                                                      |
| At 50 °C        | - - - +                                               | - - - +                                             | - - - +                                                      |

### TABLE-5

| Condition       | Hyoscine butylbromide in 0.1 NaOH (Duration in Weeks) | Hyoscine butylbromide in 0.1 HCl (Duration in Weeks) | Hyoscine butylbromide in water for injection (Duration in Weeks) |
|-----------------|------------------------------------------------------|-----------------------------------------------------|---------------------------------------------------------------|
| Clear glass     | 1 2 3 4                                               | 1 2 3 4                                             | 1 2 3 4                                                      |
| Amber coloured glass | - - - +                                           | - - - +                                             | - - - +                                                      |

**Compatibility with excipients:** The results of compatibility studies are shown in Table-7, from which it is evident that there were no chemical interactions between the drug and excipients.

**Formulation trials:** Table-8 shows the initial and final values of pH, drug content and clarity for the 19 formulations listed in Tables 1-2. Six formulations (F014 to F019) containing sodium chloride as tonicity agent, citrate buffer, basic preservative along with sodium meta bisulfite as antioxidant were found to be more stable. Hence, these 6 formulations were chosen for one-month stability study at accelerated condition.

**Study of stability of formulations F14 to F19 at 40 °C/75 % RH:** The formulations F14 to F19 were maintained at 40 °C/75 % RH condition and were observed for the clarity, pH, assay and impurity profile. It was observed that the formulation ‘F18’ containing drug along with sodium chloride, citrate buffer, benzyl alcohol and sodium metabisulfite was very stable, as evident from its pH of 4.58 even after one month (Table-9). Also the drug content after one month was 101.28 %, comparable to 101.67 % before the accelerated test (Tables 8 and 9). The impurity levels were within the limits specified in the monograph (Table-9). Hence, F18 was chosen as the final formulation and further investigated for accelerated stability studies at 3 different conditions.

### TABLE-6

| Condition       | 0.1 N NaOH 14 days | 0.1 N HCl Initial | WFI Initial 14 days |
|-----------------|-------------------|------------------|---------------------|
| Air seled vials | -                 | +                | -                   |
| Purged vials    | -                 | -                | -                   |
Post-formulation evaluation for the final formulation:
The results of accelerated stability study on the formulation F18 at various conditions are shown in Table-10. The formulation remained stable in terms of pH, for a period of over 3 months under all the conditions. The percentage loss in the drug, over a period of 3 months, were not more than 0.005 % at 40 ± 2 °C/75 % and RH ± 5 %, 0.003 % at 30 ± 2 °C/65 % and RH ± 5 and 0.001 % under refrigeration (2-8 ºC).
Impurity contents were within the limits specified in the monograph.

**Test for extractable volume:** The filled ampoules were tested for the extractable volume and the volumes carried by the ampoules were complying within the specified limits (Table-11).

| Test conditions (monograph limits for impurities) | 40 ± 2 °C/75 % RH ± 5 % | 30 ± 2 °C/65 % RH ± 5 % | Refrigeration (2-8 °C) |
|--------------------------------------------------|------------------------|------------------------|------------------------|
| Period (in months)                                | 1 2 3                  | 1 2 3                  | 1 2 3                  |
| Clarity                                          | Y Y Y                 | Y Y Y                  | Y Y Y                  |
| pH                                               | 4.55 4.56 4.57         | 4.55 4.55 4.55         | 4.55 4.55 4.55         |
| Assay (%)                                        | 101.3 100.9 100.8     | 101.2 101.0 100.9      | 101.3 101.2 101.1      |
| Impurity A Limit < 0.10 %                         | 0.058 0.065 0.078     | 0.043 0.047 0.058      | 0.050 0.060 0.064      |
| Impurity B Limit < 0.20 %                         | 0.114 0.167 0.189     | 0.131 0.147 0.167      | 0.102 0.13 0.124       |
| Impurity C Limit < 0.20 %                         | 0.124 0.142 0.176     | 0.123 0.139 0.148      | 0.114 0.121 0.126      |
| Impurity D Limit < 0.20 %                         | 0.151 0.161 0.177     | 0.147 0.152 0.164      | 0.135 0.141 0.147      |
| Impurity E Limit < 0.10 %                         | 0.067 0.079 0.081     | 0.058 0.062 0.075      | 0.057 0.069 0.082      |
| Impurity F Limit < 0.20 %                         | 0.133 0.156 0.187     | 0.121 0.145 0.168      | 0.121 0.132 0.139      |
| Impurity G Limit < 0.20 %                         | 0.252 0.268 0.285     | 0.185 0.198 0.214      | 0.152 0.168 0.185      |
| Test for sterility                                | NG NG NG              | NG NG NG               | NG NG NG               |

**Conclusion**

A stable and effective formulation of aqueous injection of hyoscine butylbromide has been successfully developed, overcoming the limitations of the drug. The final formulation containing the hyoscine butylbromide along with sodium chloride, citrate buffer, benzyl alcohol and sodium metabisulfite was found to be complying satisfactorily with all the evaluation tests and was stable for long duration. Since the proposed excipients are at its reduced concentration, toxicity or safety related issues may not arise and it suggests that the formulation is economical, safe and convenient for human use.

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