Asian Journal of Chemistry; Vol. 27, No. 7 (2015), 2518-2522

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2015.17967

Formulation and Evaluation of Hyoscine Butylbromide Parenteral Dosage Form

BENCY MOL[1], UDAY B. KAMATH[2] and N. ANUSHA[1,*]

[1]School of Chemical & Biotechnology, SASTRA University, Thanjavur-613 401, India
[2]Karnataka Antibiotics & Pharmaceutical Ltd., Bangalore-560 058, India

*Corresponding author: Fax: +91 4362 264120; E-mail: anusha@scbt.sastra.edu

Received: 8 May 2014; *Accepted*: 25 July 2014; *Published online*: 30 March 2015; AJC-17070

A cost-effective, stable and efficacious parenteral formulation of hyoscine butylbromide, a quaternary ammonium derivative with antimuscarinic and anticholinergic activity used to relieve smooth-muscle spasm in gastro-intestinal and genito-urinary disorders, has been developed in the present study. This expensive drug is unstable in the presence of moisture, light and heat. We have investigated effect of temperature, light, pH and oxygen on the stability of drug. The drug was made into single dose-small volume injectable formulation for administration *via* intravenous. Formulation trails were carried out in different combinations of different buffers and excipients. Out of all the trials, the best optimized formulations which showed better stability as well as efficacy and which passed all tests satisfactorily when subjected to accelerated stability testing were finalized as commercially viable formulation.

Keywords: Hyoscine butylbromide, Parenteral, Acetate and citrate buffers, Preservatives.

INTRODUCTION

Hyoscine is an alkaloidal derivative present in the plant genus *Duboisia* grown in South America and Australia[1]. 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[2]. 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[3-6].

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[7].

Determination of melting point: Melting point of hyoscine butyl bromide 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 ºC 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: 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, water and sealed. 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: 1 % hyoscine butylbromide solution was filled in 50 mL glass containing 0.1 N NaOH, 0.1 N HCl and water. These vials were placed at room temperature. Before sealing the vials, one of the groups was purged to deprive oxygen while the other was sealed with air trapped inside. The groups were observed for colour change, level of impurities and drug content.

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 ºC/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 procedures[8,9].

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 mL, 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 Pharmacopoiea. They were sterilized and used as the

TABLE-1
COMPOSITION OF FORMULATIONS F01 TO F10

Ingredients	Quantity for 25 mL									
	F01	F02	F03	F04	F05	F06	F07	F08	F09	F10
Hyoscine butylbromide (mg)	500	500	500	500	500	500	500	500	500	500
Sodium chloride (mg)	225	225	225	225	225	225	225	225	225	225
Glacial acetic acid (mL)	–	0.1	0.05	0.05	0.05	–	–	–	–	–
Sodium acetate (mg)	–	30	125	125	125	–	–	–	–	–
Benzyl alcohol (mL)	–	–	–	0.5	–	–	–	–	–	–
Phenol (mg)	–	–	–	–	125	–	–	–	–	–
Sodium metabisulfite (mg)	–	–	50	50	50	–	–	–	–	–
Citric acid (mg)	–	–	–	–	–	480	25	25	25	12.5
Sodium citrate (mg)	–	–	–	–	–	735	125	75	50	50
Water for injection	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs	Qs

TABLE-2
COMPOSITION OF FORMULATIONS F11 TO F19

Ingredients	Quantity for 25 mL								
	F11	F12	F13	F14	F15	F16	F17	F18	F19
Hyoscine butylbromide (mg)	500	500	500	500	500	500	500	500	500
Sodium chloride (mg)	225	225	225	225	225	225	225	225	225
Citric acid (mg)	25	25	25	25	25	25	25	25	25
Sodium citrate (mg)	50	50	50	50	50	50	50	50	50
Sodium methyl paraben (mg)	45	–	45	–	–	–	–	–	–
Sodium propyl paraben (mg)	–	5	5	–	–	–	–	–	–
Sodium meta bisulfite (mg)	50	50	50	50	–	50	–	50	–
Cresol (mg)	75	75	–	75	75	–	–	–	–
Phenol (mg)	–	–	125	–	–	125	125	–	–
Benzyl alcohol (mL)	–	–	–	–	–	–	–	0.5	0.5
Ascorbic acid	–	–	–	–	25	–	25	–	25
Water for injection	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 ºC and soyabean-casein digest medium at 22 ºC) 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[10].

RESULTS AND DISCUSSION

The melting point of hyoscine butylbromide was found to be 140.05 ± 0.05 ºC, within the range (139-141 ºC) 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 water for injection and in slightly acidic medium as compared to that in basic medium.

TABLE-3
SOLUBILITY PROFILE OF HYOSCINE BUTYLBROMIDE IN VARIOUS SOLVENTS

Solvent	Solubility (mg/mL)
Water for injection (WFI)	500
0.1 N NaOH (pH-8.5)	c375
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 kept in cold condition and at room temperature (Table-4). While no precipitation was observed in case of drug in WFI, there was slight crystal growth on the 4th week in drug solution in 0.1 N NaOH and 0.1 N HCl at 50 ºC.

Effect of light on drug stability: The drug solutions in 0.1 N NaOH , 0.1 N HCl and WFI were found to be 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.

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 ºC/ 75 % RH: The formulations F14 to F19 were maintained at 40 ºC/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-4
EFFECT OF HEAT ON DRUG STABILITY IN DIFFERENT MEDIA

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

TABLE-5
EFFECT OF HEAT ON DRUG STABILITY IN DIFFERENT MEDIA

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

TABLE-6
EFFECT OF OXYGEN ON SOLUTION OF HYOSCINE BUTYLBROMIDE (+ INDICATES TURBID; – INDICATES CLEAR)

Condition	0.1 N NaOH		0.1 N HCl		WFI	
	Initial	14 days	Initial	14 days	Initial	14 days
Air seled vials	–	+	–	–	–	–
Purged vials	–	–	–	–	–	–

TABLE-7
PHYSICAL OBSERVATIONS OF DRUG EXCIPIENT COMPATIBILITY STUDY (NC REPRESENTS 'NO OBSERVABLE COLOUR CHANGE')

S. No	Physical admixture	Initial Description	25 ºC/60 % RH	50 ºC/75 % RH
			Closed	Open
1	Model drug	White crystalline powder	NC	NC
2	API + Sodium chloride	White colour	NC	NC
3	API + sodium citrate	White colour	NC	NC
4	API + Citric acid	White colour	NC	NC
5	API + Glacial acetic acid	Clear solution	NC	NC
6	API + Benzyl alcohol	Clear solution	NC	NC
7	API + cresol	White colour	NC	NC
8	API + phenol	White colour	NC	NC
9	API + Sodium methyl paraben	White colour	NC	NC
10	API + Sodium propyl paraben	White colour	NC	NC
11	API + Sodium metabisulfite	White colour	NC	NC
12	API + Ascorbic acid	White colour	NC	NC

TABLE-8
COMPARISON OF PH, DRUG CONTENT AND CLARITY OF 19 FORMULATIONS ON DAY-1 AND AT THE END OF WEEK-4 ('Y' REPRESENTS CLARITY; 'N' REPRESENTS TURBIDITY)

Formulation	Initial values			Final values (end of week-4)		
	Clarity	Drug content (%)	pH	Clarity	Drug content (%)	pH
F001	Y	102.08	6.12	Y	101.98	4.67
F002	Y	100.4	3.41	Y	99.98	2.79
F003	Y	102.54	4.53	Y	101.87	4.35
F004	Y	101.67	4.31	Y	101.23	4.41
F005	Y	101.79	4.34	Y	101.14	4.32
F006	Y	101.01	4.27	Y	100.89	3.78
F007	Y	100.98	5.35	Y	102.20	5.67
F008	Y	100.65	5.00	Y	100.98	5.48
F009	Y	102.66	4.50	Y	100.64	4.57
F010	Y	101.10	4.81	Y	99.78	4.32
F011	Y	100.44	5.27	N	100.44	4.71
F012	Y	101.54	4.68	N	101.54	3.34
F013	Y	100.76	5.43	N	100.76	4.54
F014	Y	101.84	4.22	Y	101.35	4.24
F015	Y	101.79	4.27	Y	100.47	4.05
F016	Y	101.44	4.33	Y	100.75	4.33
F017	Y	100.54	4.25	Y	100.01	4.15
F018	Y	101.67	4.55	Y	101.52	4.55
F019	Y	100.45	4.21	Y	99.76	4.03

TABLE-9
VALUES OBSERVED IN TERMS OF CLARITY, pH, ASSAY AND LEVEL OF IMPURITIES PRESENT FOR ALL THE FORMULATION OVER A PERIOD OF ONE MONTH STUDY AT 40 °C/ 75% RH ('Y' REPRESENTS CLARITY; 'NG' REPRESENTS NO MICROBIAL GROWTH)

Parameters	Formulations					
	F14	F15	F16	F17	F18	F19
Clarity	Y	Y	Y	Y	Y	Y
pH	4.07	3.77	4.48	3.9	4.58	4.01
Assay (%)	101.10	100.21	100.66	99.991	101.28	99.52
Impurity A Limit < 0.10 %	0.075	0.083	0.023	0.04	0.058	0.061
Impurity B Limit < 0.20 %	0.16	0.172	0.079	0.093	0.114	0.126
Impurity C Limit < 0.20 %	0.163	0.181	0.067	0.097	0.124	0.143
Impurity D Limit < 0.20 %	0.168	0.188	0.08	0.093	0.151	0.157
Impurity E Limit < 0.20 %	0.084	0.09	0.012	0.014	0.067	0.081
Impurity F Limit < 0.20 %	0.161	0.186	0.093	0.116	0.133	0.157
Impurity G Limit < 0.20 %	0.283	0.291	0.176	0.195	0.252	0.258
Test for sterility	NG	NG	NG	NG	NG	NG

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 ºC/75 % and RH ± 5 %, 0.003 % at 30 ± 2 ºC/65 % and RH ± 5 and 0.001 % under refrigeration (2-8 ºC). The

TABLE-10
POST-FORMULATION EVALUATION AT DIFFERENT CONDITIONS

Test conditions (monograph limits for impurities)	40 ± 2 ºC/75 % RH ± 5 %			30 ± 2 ºC/65 % RH ± 5 %			Refrigeration (2-8 ºC)		
	Period (in months)			Period (in months)			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

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).

TABLE-11

Test parameter	Observed value	Limit specification
Content of each ampoule	1.1 mL	>= 1 mL
Average content of 5 ampoules	1.12 mL	<= 1.15 mL

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.

ACKNOWLEDGEMENTS

The authors thank Karnataka Antibiotics and Pharmaceuticals Limited for providing instrumental facilities and working standards of drugs to carry out this research work.

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