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RESEARCH ARTICLE

PREPARATION, CHARACTERIZATION, EVALUATION AND SOLUBILITY ENHANCENMENT STUDY OF DRY POWDER FOR INJECTION OF LAMOTRIGINE USING MIXED SOLVENCY TECHNIQUE

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ABSTRACT

Lamotrigine is an anti-epileptic drug used in the treatment of epilepsy and bipolar disorder. An effort was made to formulate the dry powder for injection of lamotrigine with the help of mixed solvency technique. In this technique for poorly soluble drugs to reduce concentration of individual solubilizers and to minimize the toxic effect of solubilizers. Formulation SB-6 shows maximum solubility while other shows less solubility. Formulation SB-1 shows maximum stability as compared to other formulations.

Key words: Epilepsy, lamotrigine, solublizers.

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1. INTRODUCTION

Parenteral administration of drugs involves the injection of therapeutic agents, in the form of solutions, suspensions or emulsions, into the body. In so doing, one of the major barriers to drug entry (the skin) is breeched. There are various means by which drugs are delivered to the body for therapy such as tablets, capsules etc. Disadvantages of this kind of therapy are peak and trough profile leading to greater chances of adverse effects. Therapy is inefficient since large amount of drug is lost in the vicinity of the target organ. To overcome this problem Parenterals are administered by injection under or through one or more layers of skin or mucous membrane into body tissues and directly into blood stream[1].

Lamotrigine is a synthetic phenyltriazine with anti-epileptic and analgesic properties. Lamotrigine enhances the action of gamma amino butyric acid, an inhibitory neurotransmitter, which may result in a reduction of pain related transmission of signals along nerve fibers. This agent may also inhibit voltage gated sodium channels, suppress glutamate release and inhibit serotonin reuptake. It is white to pale cream colored powder and its half life 29 hours[2]. Marketed preparations of lamotrigine are- Lamitor- OD, lemetec, lamidus, lamepil etc[3].

2. MATERIALS AND METHODS

Preformulation studies

Preformulation studies are the first step in the rational development of dosage form of a drug substance. The objective of Preformulation studies is to develop a portfolio of information about the drug substance, so that this information useful to develop different dosage forms. Preformulation can be defined as investigation of physical and chemical properties of drug substance alone and when combined with excipients.

Preformulation investigations are designed to identify those physicochemical properties and excipients that may influence the formulation design, method of manufacture, and pharmacokinetic-biopharmaceutical properties of the resulting product.

Melting point determination

Melting point was determined by open capillary method. Drug sample was filled in a capillary which was previously sealed at one end. The capillary was then placed into Thiel's tube, filled with liquid paraffin, along with a thermometer. The tube was heated and melting point was recorded.

SPECTROPHOTOMETRIC ANALYSIS OF DRUG SAMPLE

UV spectrum of lamotrigine in demineralized water

Fifty mg of lamotrigine was accurately weighed and transferred to 100 ml volumetric flask. The drug was dissolved by addition of 50 ml methanol and volume was made upto 100 ml with methanol so as to obtain solution of 500 mcg/ml. Then 10 ml of this solution was taken in another 100 ml volumetric flask, and the volume was made upto 100 ml with demineralized water. The concentration of this resulting solution (stock solution) was 50 mcg/ml. Then 4 ml aliquot of the stock solution was taken in 10 ml volumetric flask and volume was made up with demineralized water to obtain the solution of 20 mcg/ml. The sample was scanned between 200 nm to 400 nm on a double beam UV/Visible spectrophotometer (Shimadzu 1700) against demineralized water.

UV spectrum of lamotrigine in 0.1 N Hydrochloric Acid (pH 1.2)

Fifty mg of lamotrigine was accurately weighed and transferred to 100 ml volumetric flask. The drug was dissolved by addition of

50 ml methanol and volume was made upto 100 ml with methanol so as to obtain solution of 500 mcg/ml. Then 10 ml of this solution was taken in another 100 ml volumetric flask, and volume was made upto 100 ml with 0.1N hydrochloric acid. The concentration of this resulting solution (stock solution) was 50 mcg/ml. Then 4 ml aliquot of the stock solution was taken in 10 ml volumetric flask and volume was made up with 0.1N hydrochloric acid to obtain the solution of 20 mcg/ml. The sample was scanned between 200 nm to 400 nm on a double beam UV/Visible spectrophotometer.

Solubility study of the drug

Determination of solubility comprises of preparing a saturated solution of the given substance and finding out the amount present in a definite quantity of the solution. Rapid solution can be obtained by constant agitation of the solvent and an excess amount of the drug substance. After a given period of stirring, the clear solution is analyzed. The result is taken as the solubility at that particular temperature.[4]

3. METHOD OF PREPARATION

Formulation development of dry powder of lamotrigine for injection

To develop lamotrigine dry powder for injection such blend were SB-1, SB-2, SB-3, SB-5 and SB-6 selected. The mixed blends in which solubility of lamotrigine was more than 5 mg/ml and much different in their compositions.

Initially all the required ingredients of formulation were dried in oven at temperature 40-50 ^{0}C. After drying all the ingredients were passed through sieve number 80 to reduce the particle size separately. Then the required quantity of all excipients and drug was weighed and mixed by geometric dilution method with the help of mortar and pestle aseptically. The mixed blend was again passed through sieve and mixed manually in plastic bag of suitable size. The mixed powder was then analysed for the uniformity of mixing of drug by taking four samples from the four corners of powder heap. The prepared formulation was then transferred to vials and vials were stoppered and sealed immediately.

Table 1-Ingredient Table [5]

S.No.	Ingredients	SB-1 (6mg/3ml)	SB-2 (9mg/3ml)	SB-3 (9mg/3ml)	SB-5 (9mg/3ml)	SB-6 (13.5mg/3ml)
1	Lamotrigine	6.0 mg	9.0 mg	9.0 mg	9.0 mg	13.5 mg
2	Lignocain hydrochloride	0.15 gm	0.15 gm	0.15 gm	0.15 gm	0.18 gm
3	PEG-4000	0.21 gm	0.15 gm	0.15 gm	0.21 gm	0.18 gm
4	Niacinamide	0.21 gm	0.15 gm	0.15 gm	0.21 gm	0.18 gm
5	PVP-40000	-----	0.15 gm	0.15 gm	0.18 gm	-----
6	Sodium benzoate	0.18 gm	0.15 gm	-----	-----	0.18 gm
7	PVP-k_{90}	-----	-----	0.15 gm	-----	-----
8	Sterile water for injection required for reconstitution	q.s. to 3 ml	q.s. to 3 ml	q.s. to 3 ml	q.s. to 3 ml	q.s. to 3 ml

EVALUATION OF DRY POWDER FOR INJECTION [6]

Determination of pH of reconstituted injection

The developed formulations were reconstituted by water for injection and the pH was determined by using digital pH meter.

Determination of reconstitution time

For reconstitution of developed dry powder injection, 2.5 ml of water for injection was injected into the vial through the rubber closure. The vial was then vigorously shaken for proper mixing of the contents. The reconstitution times so obtained were recorded.

Clarity testing of reconstituted injection

Clarity test of reconstituted product was performed by visually inspecting the externally clean vial under a good light, baffled against reflection into the eyes, and viewed against black and white background, with the content set in swirling motion.

Stability of lamotrigine in reconstituted product

The stability of lamotrigine in the bulk solution after reconstitution was studied up to 10 hrs under room temperature and refrigerated (2 to 8 ˚C) conditions. Seven hundred and fifty mg of dry powder injection was reconstituted with 2.5 ml of water for injection in twelve vials separately. Vials were subjected to refrigerate and room temperature conditions (5 vials at each condition). At interval of 2 hrs one vial from each condition were withdrawn and diluted up to 250 ml with demineralized and analysed under UV/Visible spectrophotometer at 306 nm against respective reagent blanks and absorbances were noted. The % drug remaining was calculated and recorded[7].

4. RESULT AND DISCUSION

Preformulation studies

Organoleptic characteristics

Organoleptic Characteristics was visually determined which was compliance with the standard

Table 2: Organoleptic characteristic of lamotrigine

S. No	Properties	Standard	Observed
1	Appearance	White to pale cream colored powder	White to pale cream colored powder
2	Odor	Odorless	Odorless
3	Taste	Bitter	Bitter

Melting point of lamotrigine

Melting point was determined by Thiele's tube method. Melting point of lamotrigine was found to be in the range of 220 ˚C which was in compliance with the official value.

UV SPECTROPHOTOMETRIC ANALYSIS OF DRUG SAMPLE [8]

UV spectrum of lamotrigine in demineralized water

The UV spectrum of lamotrigine showed peak at 306 nm which is same as reported in literature.

UV spectrum of lamotrigine in 0.1 N Hydrochloric Acid (pH 1.2)

The UV spectrum of lamotrigine in 0.1 N Hydrochloric acid showed peak at 267 nm which is same as reported in literature.

Table 3: Solubility studies

S.No	Solvent	Solubility
1.	Methanol	Soluble
2.	Chloroform	Soluble
3.	0.1N HCL	Slightly Soluble
4.	Water	Very Slightly soluble

Preparation of Calibration Curve of lamotrigine [9]

- **In demineralized water**

In this study Calibration curve was plotted Concentration Vs Absorbance by preparing dilution between the ranges of 10 mcg/ml to 50 mcg/ml. Absorbance was determined range "between" 0.241 to 1.271.

- **In presence of solubilizers**

PEG-4000 at 306nm

In this study Calibration curve was plotted Concentration Vs Absorbance by preparing dilution between the ranges of 10 mcg/ml to 50 mcg/ml. Absorbance was determined range "between" 0.240 to 1.257.

Niacinamide at 306nm

In this study Calibration curve was plotted Concentration Vs Absorbance by preparing dilution between the ranges of 10 mcg/ml to 50 mcg/ml. Absorbance was determined range "between" 0.239 to 1.284.

Sodium benzoate at 306nm

In this study Calibration curve was plotted Concentration Vs Absorbance by

dilution between the ranges of 10 mcg/ml to 50 mcg/ml. Absorbance was determined range "between" 0.239 to 1.312.

Chromatographic study of solubilized drug product[10]

In order to predict the possible interaction and/or complexation between drug and solubilizers the TLC studies were performed. A plate of silica gel GF 254 (Merck) was activated at 110 for 1 hour and used. The methanolic solution of lamotrigine alone and the methanolic solution of solubilizers along with lamotrigine was prepared (amount of drug and solubilizers as in blend B-19, B-21 and B-22). Methanolic solution was spotted on the base line with the aid of microdropper. Then, the plate was left in air for 10 min to dry and transferred to a solvent jar saturated with solvent system composed of mixture of Ethyl acetate, Methanol and Liquid Ammonia (35%) solution (17: 2: 1 v/v/v).

The solvent system was allowed to run for about 4.2 cm. Finally, the plate was transferred to an oven maintained at temperature 80 for 2 min and observed in UV chamber at short wavelength for visualization of spots. The respective R_F values were determined and recorded.

Table 4: R_F values of lamotrigine

Solvent system	Adsorbent	R_F value			
Ethyl Acetate: Methanol: Ammonia	Silica gel GF 254	LTG	B-19	B-21	B-22
17: 2: 1 (v/v)		0.74	0.74	0.73	0.74

Drug-excipient interaction studies[11]

The compatibility of the drug with the excipients was assessed by drug-excipient interaction studies. The drug was mixed with the excipients in 1:1 ratio (50 mg) in separate glass vials which were then properly sealed and kept undisturbed at different storage conditions at room temperature, at 40ºC, and in refrigerator for a period of one month. After every week, vials were withdrawn and contents were observed for any change in their physical appearance (compared with initial appearance) and observations were recorded.

Table 5: Observations of drug-excipient interaction study

S. No.	Drug-Excipients Mixture	Initial Appearance	Storage conditions											
			Refrigera				Room				40°C			
			Weeks				Weeks				Weeks			
			1	2	3	4	1	2	3	4	1	2	3	4
1	LTG	White powder	N	N	N	N	N	N	N	N	N	N	N	N
2	LTG+PEG 4000	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
3	LTG+LH	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
4	LTG+SB	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
5	LTG+PVP K_{90}	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
6	LTG+PVP-FT	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
7	LTG+NM	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N
8	LTG+SC	Colorless solid powder	N	N	N	N	N	N	N	N	N	N	N	N

Key words - LTG = Lamotrigine; N = No Change; LH = Lignocaine hydrochloride; NM= Niacinamide; SB = Sodium benzoate; SC = Sodium citrate; PVP-FT = PVP-40000.

5. EVALUATION

Table 6: pH values of reconstituted injection formulations

Formulation code	pH
SB-1	6.8
SB-2	6.2
SB-3	5.4
SB-5	6.7
SB-6	7.2

Table 7: Reconstitution time of various formulations

Formulation code	Reconstitution time (minutes)
SB-1	4.2
SB-2	4.8
SB-3	6.5
SB-5	5
SB-6	4.5

Table 8: Clarity of various reconstituted injections

Formulation code	Clarity
SB-1	Clear
SB-2	Clear
SB-3	Clear
SB-5	Clear
SB-6	Clear

Table 9: Kinetic data for various batches of lamotrigine dry injection formulations

Temperature	k (day^{-1})				
	SB-1	SB-2	SB-3	SB-5	SB-6
2-8 ^{0}C	0.0004	0.0007	0.0005	0.0004	4E-04
25 ^{0}C	0.0005	0.0014	0.0009	0.0008	6E-04
40 ^{0}C	0.0009	0.0022	0.0015	0.002	9E-04

Table 10: Shelf lives of various lamotrigine dry powder injection

Formulation	Shelf life (days)
SB-1	210
SB-2	75
SB-3	116
SB-5	131
SB-6	175

The developed formulations of lamotrigine, dry powder injection showed good stability. The formulation SB-1 shows the maximum shelf life of 210 days.

Dilution Profile of Reconstituted Injection

Table 11: Dilution profile of reconstituted solution of formulation (SB-1)

Dilution	Time (hrs.)											
	Normal saline solution						5% dextrose solution					
	1	2	4	6	8	#	1	2	4	6	8	24
01:01	-	-	-	-	-	-	-	-	-	-	-	-
01:05	-	-	-	-	-	-	-	-	-	-	-	-
01:10	-	-	-	-	-	-	-	-	-	-	-	-
01:20	-	-	-	-	-	-	-	-	-	-	+	+
01:30	-	-	-	-	-	-	-	-	-	-	+	+
01:40	-	-	-	-	-	+	-	-	-	-	+	+
01:50	-	-	-	-	-	+	-	-	-	-	+	+
0.11111	-	-	-	-	-	+	-	-	-	-	+	+
0.38889	-	-	-	-	-	-	-	-	-	-	-	-

(-) No precipitation, (+) Precipitation

Table 12: Dilution profile of reconstituted solution of formulation (SB-6)

Dilution	Time (hrs.)											
	Normal saline solution						5% dextrose solution					
	1	2	4	6	8	#	1	2	4	6	8	#
01:01	-	-	-	-	-	-	-	-	-	-	-	-
01:05	-	-	-	-	-	-	-	-	-	-	-	-
01:10	-	-	-	-	-	-	-	-	-	-	-	-
01:20	-	-	-	-	-	-	-	-	-	-	-	-
01:30	-	-	-	-	-	-	-	-	-	-	-	-
01:40	-	-	-	-	-	-	-	-	-	-	+	+
01:50	-	-	-	-	+	+	-	-	-	-	+	+
0.111111111	-	-	-	-	+	+	-	-	-	-	+	-
0.388888889	-	-	-	-	-	-	-	-	-	-	-	-

(-) No precipitation, (+) Precipitation

The above results indicate that the formulations (SB-1 and SB-6) were observed to have stability (up to 6 hours) towards precipitate formation in normal saline solution and 5% dextrose solution. As the dilution ratio was increased, the appearance of precipitate was faster, but after much higher dilution (e.g. 1:100), the precipitate partly disappeared. This might be due to the redissolution of precipitate formed.

6. DISCUSION

Pre formulation study

The lamotrigine identified by white to pale cream colored powder, and bitter in test which is compliance with standard value of lamotrigine, its show starting melting at 220°C which was between the range, Solubility of lamotrigine in different solvents was performed, the study indicate the affinity of lamotrigine toward non-aqueous solvents. The solubility of lamotrigine was show in solvent like methanol, chloroform, 0.1N hydrochloric acid and very slightly soluble in Distilled water.

Preparation of dry powder

Out of many method of preparation of dry powder "formation of dry powder from dry powder filling" was selected. Different concentration of solubilzers like PEG-4000, Niacinamide, PVP-40000, Sodium benzoate, PVP k_{90} and Lignocain

hydrochloride was used for preparation of lamotrigine dry powder formulation. Temperature was maintained room temperature.

7. CONCLUSION

Aim of the present study was to formulate and characterization of dry powder for injection of lamotrigine by using mixed solvency technique. Preformulation study of the drug (lamotrigine) was done. Lamotrigine was identified by solubility, melting point, absorption maxima (lambda max) etc. Drug-excipients interaction study was carried out to check possible interaction between the drug component and excipients used for dry powder for injection of lamotrigine formulation, which confirmed that, no interaction was found between them. Dry powder for injection of lamotrigine was prepared by "Dry powder filling "technique..

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