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RESEARCH ARTICLE

Formulation and Evaluation of Parenteral Drug Edaravone

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ABSTRACT

Edaravone have best property of neurological recovery and it is a kind of cerebral protective agent (free redical scavenger) also it acts as an antioxidant. The present study was under taken with an intension to develop a stable and effective parenteral formulation containing the drug Edaravone. Solubility analysis of the drug Edaravone performs soluble in CCl_4 and insoluble in water. So, various effects of various co-solvents in the solubility of edaravone have been evaluated. Edaravone was tried with co solvents such as CCl_4, ethanol, methanol active ethyl acetate and water. The drug was made in to injection formulation for administering as an infusion. Various batches of Edaravone injection formulation were prepared in order to assess the influence of heat, light, atmospheric oxygen and antioxidant on the stability of the drug. The formulation were also subjected to accelerated stability test out of all trials. Formulation containing all the ingredients like L-cystein hydrochloride monohydrate, NaCl, sodium bi sulphate, phosphoric acid, NaOH pellets and water for injection was found to be more stable and passed test C2 satisfactorily.

KEYWORDS

Edaravone, Formulation, Antioxidant, Evaluation, Stability.

INTRODUCTION

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a potent free radical scavenger which has been shown to provide neuroprotection against cerebral ischemia-reperfusion injuries in experimental animal models[7]. Moreover, the clinical efficacy of edaravone has been demonstrated in patients with acute brain infarction and it was approved in 2001 by the health authorities in Japan as a neuroprotective agent for the treatment of acute cerebral infarction. Recently, several studies also demonstrated the in vivo efficacy of edaravone in amyotrophic lateral sclerosis, traumatic brain injury and Parkinson's disease.

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Although the neuroprotective effects of edaravone on different models of neurotoxicity have been described, to the best of our knowledge, there is no report on the protective effect of edaravone against induced neurotoxicity up to now.

Parenteral formulations are widely used especially when an immediate psychological response is needed in life threatening emergency conditions and for administering those drug that are destroyed by digestive secretions[8].

The aim of the present study is to formulate and evaluate the parenteral dosage form containing Edaravone. The objectives of the study are, to study the solubility behavior of the drug in different solvents, to develop an analytical method for assay of Edaravone, to design and formulate a stable parenteral formulation of

Edaravone, to evaluate prepared parenteral formulations of Edaravone.

MATERIALS AND METHODS

Edaravone is gift sample taken from UCB India Pvt. Ltd Mumbai and other all excipients provided from the Akums Pharmaceuticals Ltd Plant 3rd Sidkul, Haridwar.

List of ingredients used is given in Table 1.

Preformulation Studies[2,3,4,5,6]

Solubility Studies of Edaravone in Different Solvents (Saturation Solubility Method)

Excess of drug was added to different solvents in 10 ml Stoppard volumetric flasks. Then Drug was made to dissolve in the solvent by placing the volumetric flask in the shaker bath at 25° C for 6 hours. The volumetric flasks were then placed at room temperature for 24 hours. The solutions were filtered and appropriate dilutions were made to measure absorbance at 244nm using UV visible spectrophotometer, and CCl_4 as blank. The data are given in Table 2.

Effect of Temperature on Stability of Drug

1% Edaravone solution in 0.0.1N NaOH is filled into vials. The ampoule were sealed and placed at refrigeration, room-temperature, 50°C, 75°C and 95°C for 1 week and observed for colour change and crystal growth. The samples placed at refrigeration and room temperature served as controls. The data are given in Table 3.

Light Stability of Drug

1% of Edaravone solution in 0.0.1N NaOH is filled in to 20ml glass vials (amber and clear). Also samples of drug substance are placed in an open Petri dish to expose a large surface. Drug and dilutions placed in a light-resistant amber colored glass vials, foil wrapped and in a cardboard box as controls. This is carried out for 4 weeks with weekly examinations for visible colour change or precipitation in solution in clear vials, the compound can be considered as potentially light sensitive and should be handled accordingly. The data are given in Table 4.

Effect of Oxygen on Drug

1% of Edaravone in 0.0.1N NaOH is filled into vials and placed at 30°C and 40°C. One group is purged and another group is sealed with air. Solutions are observed for colour change and drug content. The data are given in Table 5.

Formulation Development

In these process general all ingredients mix in the tank step by step and after it injection prepared the methodology is given bellow:

Table 1: Concentration of different ingredients used in various trial formulations

Name of ingredients	F1 (gm)	F2 (gm)	F3 (gm)
Edaravone	1.553	1.553	1.553
L-Cystein Hydrochloride	0.5	0.5	0.5
NaCl	6.75	6.75	6.75
Sodium bisulphate	1.0	1.0	1.0
Phosphoric Acid	-	39.00	39.00
NaOH pellets	16.00	16.00	-
Water For Injection	q.s	q.s	q.s

Active material has been taken equivalent to its 100%. Assay on as is basis considering the minimum.

Assay – NLT 99% (ODB) and maximum LOD NMT 0.5%w/w # this quantity is taken only for pH adjustment.

Calculation

Required Quantity of Edaravone

$$\frac{\text{lable claim}\left(\frac{\text{mg}}{\text{ml}}\right)\times 100\times 100\times \text{Batch Size}}{P\times(100-Q)\times 1000}$$

Averages: 2%

"P" indicates assay NLT 99% (ODB)

"Q" indicates the % LOD NMT 0.5%w/w

Note: **-** During formulation required quantity of active shall be taken equivalent to100%

Assay on as is basic considering the estimated assay and %LOD% water contents

The data are given in Table 6.

Post Formulation Evaluations

Assay Content of Edaravone

*Instrument Required***:**

- Electronic weighing balance
- HPLC
- pH meter

Apparatus Required:

- Volumetric flask -100ml,1000ml
- Measuring cylinder
- Clean and dry beaker
- Bulb pipette – 2ml,10ml

Reagent Required:

- Methanol HPLC Grade
- Sodium Acetate

Edaravone Working Standard

Sodium Acetate Buffer Preparation

Dissolve 13.61g of Sodium acetate in sufficient H_2O to produce 1000 ml, adjust the pH 5.0 with HCl solution or NaOH solution

Mobile Phase Preparation

Mix sodium acetate buffer and methanol (HPLC grade) in the ratio of 30:70 filter and degas.

Standard Preparation

Dissolve accurately weighed 30 mg of edaravone working standard in 50 ml of mobile phase, mix and make the volume 100ml further dilute the 10ml of this solution to 100ml with the same.

Sample Preparation

Dilute 2ml of sample to 100ml with the mobile phase.

Chromatographic System

Column : 50min *

Detection : 254nm

Flow Rate : 1.0ml/min

Injection Volume **:** 20µl

Procedure

Separately inject equal volume (20µl) of diluents, six replicate standard preparations and two injection of sample preparation in to the chromatograph, record the chromatogram and measure the responses for major peaks.

System suitability parameter

Tailing factor: NMT 2.0%

Relative standard deviation: NMT 2.0%

Calculation

$$\frac{\text{Sample area}}{\text{standard area}} \times \frac{\text{Standard weight}}{100} \times \frac{10}{100} \times \frac{100}{2} \times \frac{\text{Potency}}{100}$$

= mg/ml

Acceptance Criteria

For finished products- 90% to 110%

For bulk products -98% to 105%

Sterilization Studies

The injection samples were taken in glass syringe, the membrane filter holder was attached to the syringe. A prefilter of 1.5 micrometers was placed in this holder, after which filters of 0.22, 0.45, 1.2 and 1.5 micrometers were placed successively and tested whether the injection sample could pass through these membrane or not. The data are given in Table 7.

Sterility Testing

Direct Transfer Method

Aliquots of the samples are transferred aseptically into fluid thioglycolate medium and soybean casein digest medium. The inoculated thioglycolate medium is incubated at 32°C and soybean casein digest samples at 22°C for 7 days. Likewise negative and positive controls are prepared. The data are given in Table 13.

Stability Studies

For any pharmaceutical dosage form stability of the prepared formulation is a very basic and important factor, from point of view of safety of the patient being treated with and to get a safe and maximum therapeutic response of the drug. The provision of rapid means of quality control, which ensures that no unexpected changes in the stored product are occurred like: Crystal growth, pH changes, Clarity and % Drug content.

Crystal Growth

10 ml of the each prepared formulations C2 were placed at refrigeration, room temperature, 37°C, 40°C and 45°C respectively for six weeks and observed for crystal growth. The data are given in Table 15.

pH Changes

10 ml of the each prepared formulations C2 were kept at different temperatures/ conditions such as refrigeration, room temperature, 37°C, 40°C, 45°C and under light. At regular time intervals the samples were examined for pH changes for six weeks using a digital pH meter. The data are given in Table 14.

Clarity

10 ml of the formulations were placed at refrigeration, room temperature, 37°C, 40°C and 45°C for six weeks and observed for colour change or turbidity. The data are given in Table 16.

% Drug Content

The drug content of the formulations C2 were determined by following the same procedures as mentioned in assay. The estimates were done at intervals of one week upto six weeks. The data are given in Table 17.

RESULTS AND DISCUSSION

FT-IR Spectrum of Pure Edaravone

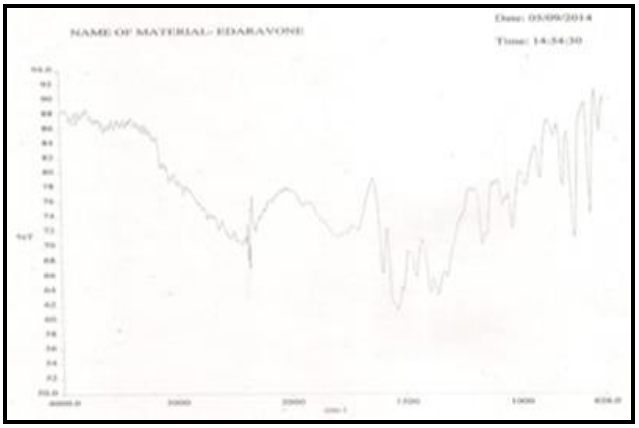

Figure 1: FT-IR spectrum of pure Edaravone

Table 2: Solubility profile of Edaravone in different solvents

Descriptive Term	Parts of solvent required for part of solute
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10000
Insoluble or practically insoluble	10000 or more

Soluble in CCl_4, freely soluble in methanol, ethanol, slightly soluble in acetone or in ethyl acetate and in soluble in water.

Stability Evaluation

Various stress tests are performed on solid and solution sample to establish the effect of heat, light and oxygen on the drug substance stability.

Heat Stability

Table 3: Heat stability profile of Edaravone

Temperature (C)	Duration (weeks)			
	1	2	3	4
Refrigeration	-	-	-	-
Room Temperature	-	-	-	-
40	-	-	-	-
50	+	+	+	+
75	+	+	+	+

+ Color change – No color change

Light Stability

Table 4: Light stability study of Edaravone

Withdrawal week	Observation	
	Clear	Amber
1	-	-
2	-	-
3	-	-
4	+	-

-Clear, -Turbidity

Effect of Oxygen

Table 5: Test for color change after a week

Temperature (^{0}C)	Air sealed vials	Perged vials
25	+	-
35	+	+

+ Color change – No color change

Formulation Development

A stable parenteral formulation of water insoluble drug edaravone was formulated after performing trials with various solvent. Thus prepared formulations were subjected for various tests and results are discussed in the following section.

Table 6: Drug content of various formulation trials containing Edaravone

Formulation	Drug content (mg/ml)	% Drug content
C1	1.55234	103.4893
C2	1.50133	100.0865
C3	1.49532	99.688

*Each value is an average of three determinants

Table 7: Filter pore size and filterability of the formulation of Edaravone

Formulation	Filter pore size (µm)	Observation
C1	0.22	+
	0.45	+
	1.2	+
	1.5	+
C2	0.22	+
	0.45	+
	1.2	+
	1.5	+
C3	0.22	+
	0.45	+
	1.2	+
	1.5	+

+Injection passes though.-Injection does not pass through

All the formulation was found to be easily passing through the entire pore size filter and hence 0.22$^{\mu}$m pore size filter was selected to filter all the prepared formulations separately.

None of the formulations showed turbidity or signs of microbial growth (except the positive control) at the end of incubation period, indicating all the formulations were sterile and thus all the formulations are subjected to further evaluations.

Post Formulation Studies

Effect of Different Temperature on Crystal Growth

Table 8: Effect of different temperature on crystal growth

Formulation	RT	40°C	Light
C1	-	-	+
C2	-	-	-
C3	-	+	+

+crystal growth,- crystal growth

Effect of Different Temperature on Clarity

Table 9: Effect of different temperature on clarity

Formulation	RT	40°C	Light
C1	-	-	+
C2	-	-	-
C3	-	+	+

+Turbidity, -Clear.

Effect of Different Temperature on Color Change

Table 10: Effect of different temperature on color change

Formulation	5°C	RT	40°C
C1	-	-	+
C2	-	-	-
C3	-	-	+

+ Color change – No color change

Scale up Studies

Assay of the formulations

$$= \frac{2134567}{2124135} \times \frac{30}{100} \times \frac{10}{100} \times \frac{100}{2} \times \frac{99.6}{100}$$

= 1.50133 mg/ml

= 100.088%

Acceptance Criteria

For finished products- 90% to 110%

For bulk products -98% to 105%

Table 11: Drug content of C2

Formulation	Drug content	%Drug content
C2	1.50133	100.088

Table 12: filter pore size and filterability of the formulations of Edaravone

Formulation	Filter pore size(µm)	Observation
C2	0.22	+
	0.45	+
	1.2	+
	1.5	+

+Injection passes through,-Injection does not pass through

The results of filterability show that both the formulation of Edaravone passes through all the four membrane filters. Hence they can be sterilized by filtration

Direct Transfer Method

Table 13: The growth of bacteria in soya bean casein digest medium and fluid thioglycollate medium after seven days

Formulation	Soyabean-casein digest medium (SCDM)	Thioglycollate medium
C2	-	-

-clear, +Turbid

Accelerated Stability Studies

pH changes

Table 14: pH changes of formulation C2 at different temperatures

Formulation	Withdrawal week	37ºC	40ºC	RT
C2	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
	6	-	-	-

Crystal growth

Table 15: Crystal growth of formulation C2 at different temperatures

Formulation	Withdrawal week	37ºC	40ºC	45ºC
C2	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
	6	-	-	-

+ Crystal growth,-no crystal growth

No crystal growth was observed in the formulations at different temperatures.

Clarity Studies

Table16: Clarity of formulation C2at different temperatures

Formulation	Withdrawal week	37ºC	40ºC	45ºC
C2	0	-	-	-
	1	-	-	-
	2	-	-	-
	3	-	-	-
	4	-	-	-
	5	-	-	-
	6	-	-	-

+Turbid,-clear

All the formulations were clear at different temperatures.

Drug Content

Table17: Percent drug content of formulation C2 at different temperatures

Sample withdrawal (week)	% Drug content		
	37 ºC	40 ºC	Light
1	100.003	101.23	101.13
2	100.023	100.59	101.34
3	100.018	100.23	100.67
4	100.011	100.01	99.92
5	099.998	99.98	99.67
6	100.006	99.67	98.20

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