Open Access Journal of Chemistry
Volume 2, Issue 3, 2018, PP 9-13
ISSN 2637-5834

LC Method for the Estimation of Edaravone in Bulk and its Pharmaceutical Dosage Form

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ABSTRACT

An accurate, sensitive, selective and precise HPLC method was developed for the development and validation of Edaravone in bulk and its pharmaceutical dosage form. The HPLC was carried out with mobile phase containing methanol. UV - Visible Spectroscopic determination was carried out at an absorption maximum of 243 nm using methanol as a solvent. The linearity werefound to be in the range of 0.1-30 µg/ml. Validation of proposed method has been carried out with respect to linearity, accuracy, precision, specificity, selectivity and robustness. Due to sensitivity, reproducibility and accuracy of methods, our thought of interest that the proposed methods will be useful for the routine quality control analysis and quantification of drug in bulk and pharmaceutical dosage form.

Keywords: *Edaravone; HPLC; Validation*

INTRODUCTION

Ischemic stroke occurs when an artery to the brain is blocked. The brain depends on its arteries to bring fresh blood from the heart and lungs. The blood carries oxygen and nutrients to the brain, and takes away carbon dioxide and cellular waste. If an artery is blocked, the brain cells (neurons) cannot make enough energy and will eventually stop working. If the artery remains blocked for more than a few minutes, the brain cells may die. This is why immediate medical treatment is critical. ischemic stroke can be caused by several different kinds of diseases [(1)]

ALS:

ALS, or amyotrophic lateral sclerosis, is a progressive neurodegenerative disease that affects nerve cells in the brain and the spinal cord. Motor neurons reach from the brain to the spinal cord and from the spinal cord to the muscles throughout the body. The progressive degeneration of the motor neurons in ALS eventually leads to their demise. When the motor neurons die, the ability of the brain to initiate and control muscle movement is lost. With voluntary muscle action progressively affected, people may lose the ability to speak, eat, move and breathe. The motor nerves that are affected when you have ALS are the motor neurons that provide voluntary movements and muscle control. Examples of voluntary movements are making the effort to reach for a smart phone or step off a curb. These actions are controlled by the muscles in the arms and legs.[(1-2)]

Edaravone is a neuroprotective agent used for aiding neurological recovery following acute brain ischemia and subsequent cerebral infarction. It acts as a potent antioxidant and strongly scavenges free radicals, protecting against oxidative stress and neuronal apoptosis**.** [(3)]

An extensive literature survey revealed that there are several methods which has been published such as HPTLC Method for the in Vitro Estimation of Edaravone in Human Plasma [(4)] , Simultaneous Estimation of Edaravone and Citicoline sodium by Ratio derivative spectroscopic method in synthetic mixture[(5)] , Simultaneous Estimation of Edaravone and Citicoline sodium by Ratio derivative spectroscopic method in synthetic mixture[(6)] , Determination of content and related substance of edaravone injection by HPLC[(7)] and Determination of concentration of edaravone in human serum[(8)], thus shows that none of the LC

method has been published for quantification of edaravone in bulk and its pharmaceutical dosage form by RP – LC method, hence our thought of interest is the development and validation of method for estimation of EDARAVONE in bulk and its pharmaceutical dosage

MATERIALS AND METHODS

Instruments

The liquid chromatographic system consists of Waters series 2998 (Shelton, USA) equipped with a PDA detector, series 515 quaternary isocratic pump and manual injector rhysodine valve with 20 µL fixed loop. The analytes were monitored at 243 nm. Chromatographic analysis was performed on Sun fire C18 column having 250 mm× 4.6 mm i.d. and 5 µm particle sizes. All the drugs and chemicals were weighed on Mettler Toledo electronic balance (ME204/A04, METTLER TOLEDO Group).

Chemicals and Reagents

Analytically pure EDA from Sun pharmaceutical industry ltd., Vadodara, India were obtained as gift samples. Methanol (HPLC grade) of SRL Private Ltd. Marketed formulation Edstar (Lupin Pharmaceutical Pvt. Ltd., Gujarat, India) containing 1.5 mg/ml of EDA, was procured from local pharmacy.

EXPERIMENTAL WORK

Selection of Detection Wavelength

The analytical wavelength 243 nm was selected for determination of EDA.

Optimization of Mobile Phase

The mobile phase containing methanol showed satisfactory results at a flow rate of 1 ml/min. EDA shows 2.5 min retention time where total time of analysis was 5 min.

Preparation of Mobile Phase

Mobile phase was prepared by taking methanol 100 ml volumetric flask. The mixture was sonicated for 20 min for degassing the mixture prior to use. This solution was used as mobile phase.

Preparation of Standard Stock Solution

Accurately weigh 10mg of EDA and transfer to 10ml volumetric flask containing few ml of methanol and makeup the volume upto the mark with methanol which gives the solution having concentration of 1000µg/ml of EDA. Pipette out an aliquot from the stock solution and dilute with mobile phase to obtain working standard of 100µg/ml of EDA.

Calibration Curve

Pipette out appropriate volume of aliquot from working standard stock solution and transferred to different volumetric flask of 10ml and volume was adjusted with the mark with the mobile phase to give a solution containing 0.1, 0.5, 1,10,20 and 30 µg/ml of EDA. Each solution was analyzed by the proposed method and the chromatogram was recorded. Calibration curve were constructed by plotting concentration v/s peak area and regression equation was computed.

Validation of RP-HPLC Method

Validation of the developed HPLC method was carried out according to the International Conference on Harmonization (ICH) guidelines Q2 (R1) [10]

Linearity

Linearity was studied by preparing standard solution of 6 different concentrations of 0.1, 0.5, 1, 10, 20 and 30µg/ml for EDA. Each concentration was repeated 5 times and linearity was assessed in terms of slope, intercept and regression coefficient of EDA. The calibration curves were developed by plotting concentration v/s peak area (n=5).

Accuracy

The accuracy was determined by calculating recovery of EDA by standard addition method. Known amount of EDA (0%, 50%, 100% and 150%) were added to pre quantified sample solution and the amount of EDA were estimated by putting the value of peak area to the straight line equation of calibration curve.

Precision

Precision was calculated in terms of intraday and interday precisions. Intraday precision was determined by analyzing sample solution of EDA (1, 10 and 20µg/ml) at three levels covering low, medium and high concentration of the calibration curve three times on the same day(n=3).

Now, interday precision was determined by analyzing sample solution of EDA (1, 10 and 20µg/ml) at three levels covering low, medium and high concentration over a period of three days (n=3).The peak areas obtained were used to calculate mean and %RSD values. The repeatability studies were carried out by

estimating the response of 10µg/ml of EDAsix times (n = 6) and results are reported in terms of %RSD.

Limit of Detection and Limit of Quantification

The limit of detection (LOD) is defined as the lowest concentration of analyte that can reliably be differentiated from background levels. The limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte that can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated using the following equation as per ICH guidelines:

$LOD = 3.3 \times \sigma/S$; $LOQ = 10 \times \sigma/S$

Where, σ is the standard deviation of *y*-intercepts of regression lines and *S* is the slope of the calibration curve.

Robustness

Small change in the detection wavelength, flow-rate introduced and the temperature effect on the results were examined. The mean and %RSD of peak were calculated.

Solution stability

Stability of sample solution were studied at room temperature for 24 hrs.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, it is include impurities, degradants, and preservatives etc. Preservative like sodium chloride was added to the drug and was prepared. The drug was analyzed from prepared injection using proposed method.

Analysis of Marketed Formulation

The injection has the strength of 1500µg/ml solution. From this solution 1 ml was pipette out and transferred into 10 ml volumetric flask and then volume was adjusted up to the mark with mobile phase.

From this solution 3 ml was transferred to another 10 ml volumetric flask and Volume was made up to the mark to obtain 18µg/ml EDA. The solution was sonicated for 10 min. Solution were injected as per the above chromatographic condition and the peak areas were recorded. The quantification was carried by keeping this values to straight line equation of calibration curve.

RESULTS

An ideal wavelength is the one that gives good response of detection wavelength. Therefore, analytical wavelength of 243 nm was selected for estimation of EDA.

Optimization of Mobile Phase

The standard solution containing 10 µg/ml of EDA was chromatographed with use of different composition of mobile phases.

As a Mobile Phase Methanol gave sharp symmetric peak with tailing factor (Figure 1) 1.03 therefore it was selected as a mobile phase for determination of EDA. The flow rate was maintained at 1.0 ml/min. Overlay chromatograms of EDA (0.1 – 30µg/ml) are shown in figure 2.

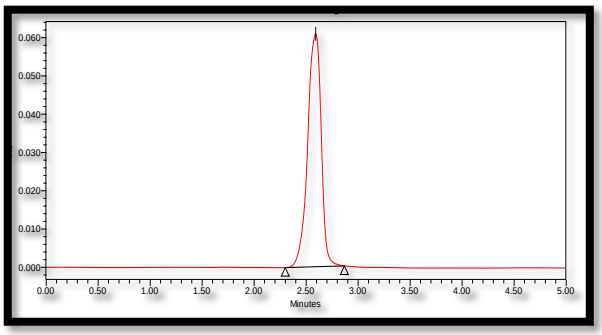

Fig1. *Chromatogram study of EDA (10µg/ml) using Methanol as mobile phase*

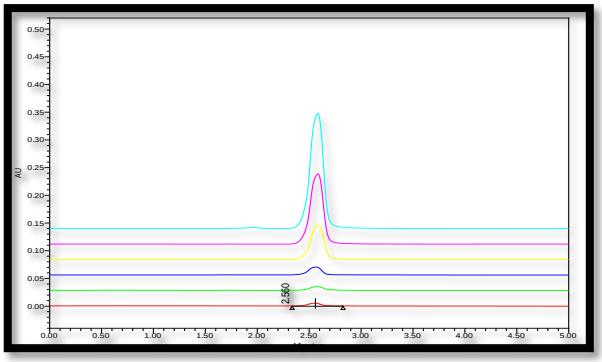

Fig2. *Overlay of Chromatogram of EDA (0.1-30 µg/ml)*

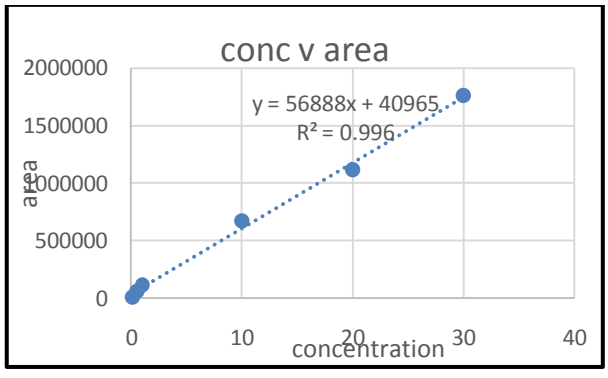

Fig3. *Calibration curve of EDA (0.1 -30 µg/ml)*

Method Validation

Linearity and Calibration curve

The calibration curve of EDA was found to be between 0.1-30 µg/ml having correlation Coefficient of 0.996 (Figure 3). The results of calibration curve and regression analysis of calibration curve are reported in (Table I).

Table1. *Result of Calibration reading at 243 nm for EDA.*

Conc. (µg/ml)	Area(µV. sec) ± SD (n = 5)	%RSD
0.1	1146.8± 57.96	0.50
0.5	62758± 953.78	1.52
1	117345.8± 2282.86	1.95
10	672861.6±11554.8	1.72
20	1120928±7365.12	0.66
30	1764705± 28640.23	1.62

Precision

The intra-day and inter-day precision were carried out and it was found to be 0.97-1.66 and 0.72-1.71 %RSD for EDA Instrumental precision was determined by performing injection repeatability test and the %RSD was found to be 1.94 % . It was found that method is precise. Summary of validation parameters are reported in table II.

Table2. *Result of accuracy study.*

Amount of drug from formulation (µg/ml)	Amount of standard drug spiked (µg/ml)	Amount of drug found (n=3) (µg/ml)	% Recovery± SD
12	0	11.7	98.66± 1.62
12	6	17.6	98.73± 0.32
12	12	23.5	98.04± 1.11
12	18	29.7	98.57± 0.30

Accuracy

The accuracy of the method was determined by calculating recoveries of EDA, where a known amount of standard was spiked into preanalyzed sample solutions. The recoveries were found to be in the range of 99.8 - 99.9 % for EDA (Table III).

Limit of Detection and Limit of Quantification.

The LOD and LOQ were carried out by visual method, were LOD for EDA was found to be 0.04 µg/ml and also LOQ was found to be 0.13 µg/ml.

Robustness

The %RSD was found to be less than 2% after introducing small, deliberate changes in parameters like change in flow rate, temperature and detection wavelength in the developed HPLC method, confirming its robustness.

System suitability

System suitability test was carried out and results are reported in table III.

Table3. *Result of system suitability.*

Parameter	EDA
Retention time (min)	2.5
Capacity factor	2.08
Theoretical plates (N)	5540
Tailing factor	1.05

Table4. *Summary of validation parameters of proposed method y.*

Parameters	EDA
Range(µg/ml)	0.1-30
Retention time	2.5
Detection limit (µg/ml)	0.04

Quantitation limit(µg/ml)	0.13
Accuracy (%)	98.04 - 98.73
Precision (%RSD)	
Intra-day(n =3)	0.97-1.66
Inter-day(n =3)	0.72-1.71
Instrument precision (%RSD)	
Reproducibility (n = 6)	1.94
Specificity	Specific

Analysis of marketed formulation

The proposed method is applied to the determination of EDA in their dosage form. The % amount of drug found to be more than 98 %. The result showed in (Table V)

Table5. *Analysis of marketed formulation*

Formulation (Collar)	**Amount of drug taken (µg/ml)**	**Amount of drug found (µg/ml) (n=3)**	**% of Drug found (n=3) ± SD**
	18	17.7	98.5± 0.11

CONCLUSION

The proposed study, describes LC method was developed for the determination of EDA. The method was validated and found to be simple, sensitive, accurate and precise. Statistical analysis proved that method was repeatable and selective for the analysis of EDA without any interference from the excipients.

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Citation: *Desai Jaineel Vinodrai, Chhalotiya Usmangani K, Thakkar Anjali, Kurvah Omnarayansinh, Dalwadi Himani B, Shah Dimal A.(2018). "Lc Method for the Estimation of Edaravone in Bulk and its Pharmaceutical Dosage Form". Open Access Journal of Chemistry, 2(3), pp.9-13.*