Chemical Methodologies 3(2019) 166-182

Chemical Methodologies

Journal homepage: http://chemmethod.com

Original Research article

# Quantification of a Neuroprotective Agent Edaravone by Stability Indicating TLC Method and UV–Visible Spectroscopic Method in Bulk and Pharmaceutical Dosage Form

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**ARTICLE INFORMATION**

Received: 24 August 2018
Received in revised:07 September 2018
Accepted: 08 October 2018
Available online: 19 October 2018

DOI: 10.22034/chemm.2018.145347.1077

**KEYWORDS**

Validation
Edaravone
Forced degradation study
TLC
UV-Visible Spectroscopy
Neuroprotective
ALS

**ABSTRACT**

An accurate, sensitive, reproducible and precise stability indicating thin layer chromatographic method and UV-Visible spectroscopic method was established for the amelioration and validation of neuroprotective agent edaravone in bulk and its pharmaceutical dosage form. Edaravone injection is used to treat amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease; a condition in which the nerves that control muscle movement slowly die, causing the muscles to shrink and weaken). Edaravone injection is in a class of medications called antioxidants. The high Performance thin layer chromatographic method was developed with proposed chromatographic condition with mobile phase containing n-Hexane: ethyl acetate (7:3, v/v). Accomplishment of UV–Visible spectroscopic determination was done at wavelength maxima of 245 nm using methanol as a solvent. The linearities were in the range of 1-9 µg/ml for UV-Visible spectroscopic method and 100-1400 ng/band for TLC method, respectively. Validation of proposed method has been accomplished with respect to linearity, accuracy, precision, specificity and robustness. For acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photolytic degradation conditions were performed using stock solution of EDA, and quantification which has been achieved by proposed TLC method. Owing to sensitivity, promptness and accuracy of methods, we rely on both intended methods which will be useful for the regular quality control analysis and quantification of drug in bulk and pharmaceutical dosage form.

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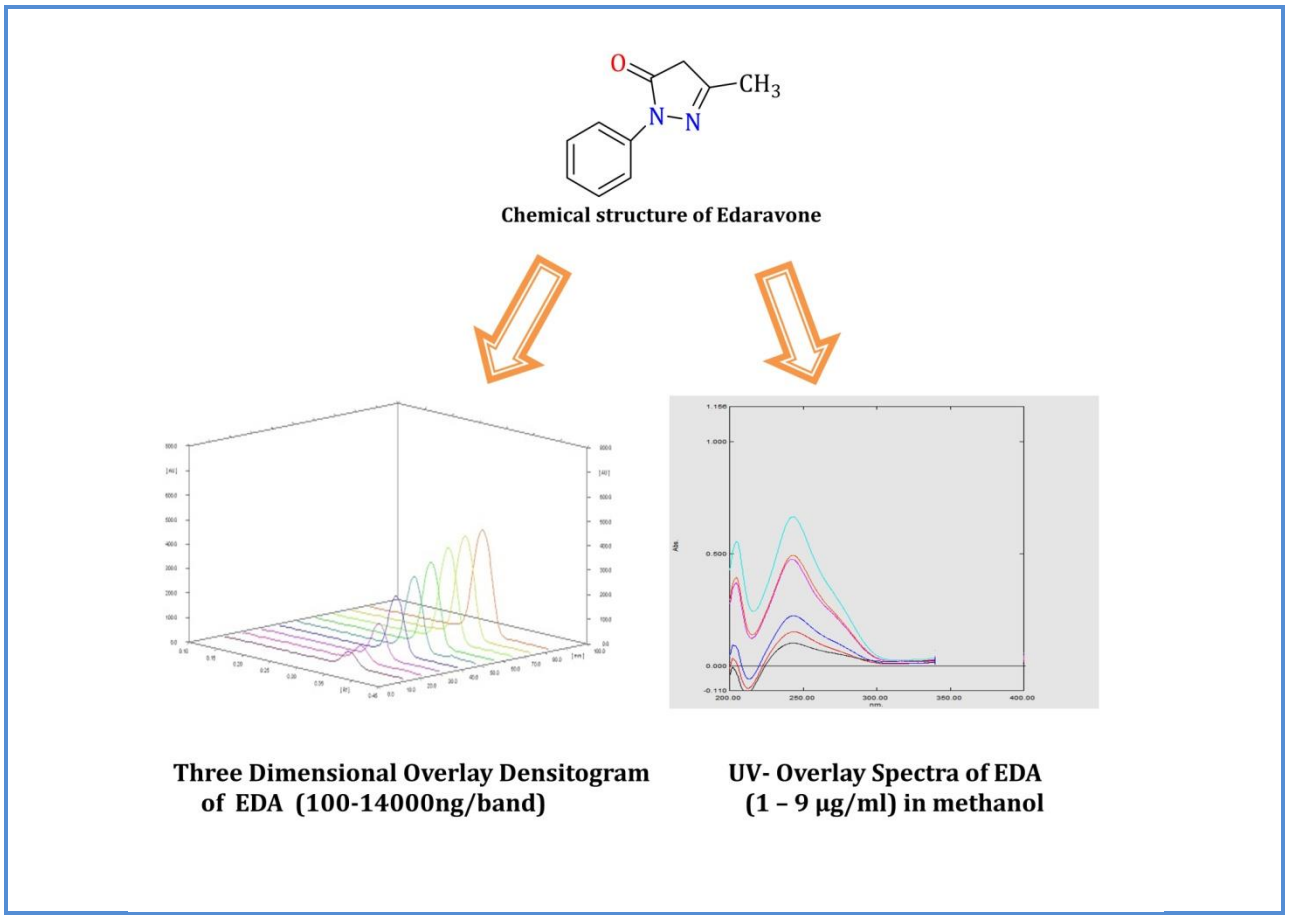
## Graphical Abstract




## Introduction

Edaravone is a neuroprotective agent and use for the purpose of aiding neurological recovery following acute brain ischemia and subsequent cerebral infarction. It acts as a free radicals scavenger, protecting against oxidative stress and neuronal apoptosis and potent anti-oxidant [1-3]. Chemically it is 5-methyl-2-phenyl-4*H*-pyrazol-3-one, the structure of edaravone shown in Figure 1.

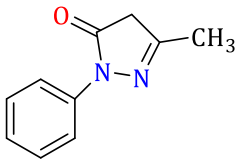


**Figure 1.** Structure of edaravone

An extensive literature survey revealed that there are several procedures which have been published such as HPTLC method for the *in vitro* estimation of edaravone in human plasma [4].

Concurrent evaluation of edaravone and citicoline sodium by ratio derivative spectroscopic method in synthetic mixture [5], concurrent estimation of edaravone and citicoline sodium by ratio derivative spectroscopic technique in synthetic mixture [6]. Confirmation of content and related substance of edaravone injection by HPLC [7] and confirmation of concentration of edaravone in human serum [8], thus shows that none of the stability indicating TLC method and UV–Visible spectroscopic technique has been published for quantification of edaravone in active pharmaceutical ingredient and its marketed formulation, hence our thought of interest is the development and validation of method for evaluation of edaravone in bulk and pharmaceutical dosage.

## Experimental

### Development and validation of HPTLC method

#### Selection of solvent

Initially water, methanol, ethyl acetate, *n*-hexane, toluene and acetonitrile (ACN) were used to ascertain the solubility of edaravone, where the drug was freely soluble in ethyl acetate, ACN and methanol, sparingly soluble in water, insoluble in, n-hexane. Therefore, ethyl acetate was selected as a common solvent for analysis.

#### Preparation of standard stock solution (100 µg/mL)

Accurately weighed EDA (5 mg) was transferred into 10.0 mL volumetric flask having few mL of methanol and swirling to dissolve and diluted up to scale with methanol to acquire solution having concentration of EDA (500 µg/mL). From the above solution, pipette out 1.0 mL of aliquot and transfer into another 10 mL volumetric flask and diluted up to scale with methanol to acquire standard stock solution of 50 µg/mL (stock A). Similarly withdraw 2 mL of aliquot and pipetted it into another 10 mL volumetric flask and diluted up to the mark with methanol to acquire standard stock solution of 100 µg/mL (stock B).

#### Selection of analytical wavelength

The solution of EDA was processed in methanol at a concentration of 10 µg/mL. It was examined in the wavelength range of 400–200 nm. Analytical wavelength of 245 nm was preferable for perseverance of EDA.

**Calibration curve**

Pertinent aliquots of stock solution A and B were taken of working standard solution through Hamilton syringe and applied on pre coated TLC plate with the help of camag applicator to acquire final concentration of 100, 200, 400, 600, 800, 1000, 1200, 1400 ng/band.

**Selection of chromatographic condition**

Stationary phase: Pre-coated silica gel G60-$F_{254}$ aluminum sheet (Merck, Germany) (10×10 cm). Thickness layer 0.2 mm prewashed with methanol and dried at room temperature
Mobile phase: *n*-hexane: ethyl acetate (7:3, v/v)
Chamber saturation time: 30 min
Migration distance of mobile phase: 80 millimeter
Common wavelength for detection: 245 nm
Slit dimension: 6×0.45 millimeters
Scanning speed: 20 mm/sec.
Spotting parameters:

1. Band width: 8 millimeters
2. Syringe size: 100 µL

**Validation of HPTLC method**

Validation of the proposed developed TLC method was performed out as per the International Conference on Harmonization (ICH) guidelines Q2 (R1) [9].

**Linearity and range**

Calibration curve were contrived with scheming peak area versus concentration of EDA and the regression equation were computed. The calibration curves were plotted over 8 different concentration range in the range of 100–1400 ng/band the calibration curve were established by marking peak area contra to concentration (n=6).

**Accuracy**

The accuracy of the method was ascertained by computing recovery of EDA by procedure of standard additions. Pre-noted amounts of EDA (0, 150, 300 and 450 ng/band) were drawn from the working standard solution (150 µg/mL of EDA). It was combined to pre-quantified samples to obtain 300, 450, 600, 750 ng/band. The quantification of EDA was evaluated by measuring area and by appropriately placing these numerals to the straight line equation of the calibration curve.

**Precision**

Precision was assessed concerning intra-day and inter-day precisions. Intra-day precision was driven by analyzing sample solutions of EDA (400, 800, 1200 ng/band) at three levels taking low, medium, and high concentrations of the calibration curve three times on the same day (n=3). Inter day precision was evaluated by analyzing sample solutions of EDA (400, 800, 1200 ng/band) at three levels taking low, medium, and high concentrations over a period of 3 days (n=3). The peak areas acquired were used to compute mean and relative standard deviation (%RSD) values.

To examine scanner repeatability, the same spot was scanned six times and peak area was determined. The percentage RSD was calculated. To check injection repeatability, measurement of peak area was evaluated by pertaining EDA sample (800 ng/band) six times on the same plate.

**Sensitivity**

The limit of detection (LOD) is construed as the lowest concentration of an analyte that can reliably be distinguished from background levels. The limit of quantification (LOQ) of a discrete analytical procedure is the lowest quantity of analyte that can be quantitatively examined with suitable precision and accuracy.

As per ICH guidelines, LOD and LOQ were calculated using the following equation:

$LOD=3.3\times\sigma/S$

$LOQ= 10\times\sigma/S$

Where $\sigma$ is the standard deviation of $y$-intercepts of regression lines and S is the slope of the calibration curve.

**Robustness**

Minor deviations in the chamber saturation time, composition of mobile phase and detection wavelength were acquainted, and the output on the results was assessed. Robustness of the method was directed in triplicate at concentration levels of 400 ng/band of EDA. The average value for area and %RSD of the $R_f$ were computed.

**Solution stability**

The stock solution of EDA (1000 ng/mL) was prepared and store in room temperature analyzed by HPTLC at regular intervals of 0, 4, 8, and 24 hrs.

**Specificity**

Specificity is the capability to assessing equivocally the analyte in the dubiety of components, which may be expected to be present. Often impurities, degradants, preservatives etc., might be present. Preservative like sodium chloride was added to the drug and was prepared. The drug was analyzed from prepared injection using developed method.

The system suitability was performed and peak purity was found to be 0.999 (Figure 4) with asymmetric factor 1.0.

**Analysis of marketed formulation**

The injection comprised strength of 1.5 mg/mL solution. So from this solution 1 mL was transferred to 25 mL volumetric flak and the volume was adjusted up to the mark with methanol to obtain 60 µg/mL EDA. 10 µL (600 ng/band) solutions of EDA were applied on precoated $F_{254}$ TLC plate and develop according to the proposed chromatographic conditions and peak was recorded. The quantitation was accomplished by putting the numerals of peak area to the straight line equation by calibration curve.

**Forced degradation study**

Force degradation study was performed with acid and alkali hydrolysis, oxidation by use of chemical, photolytic degradation and dry heat degradation were accomplished and interruption of the degradation products was examined. EDA was weighed (10 mg) and transferred to 10 mL volumetric flasks and expose to various stress conditions.

**Heat induced alkali hydrolysis**

To the 10 mL volumetric flask, 10 mg of EDA was taken and 2 mL of 0.1 N NaOH was incorporated to achieve heat induced base hydrolysis. The flask was heated at 80 °C for 2 hours and let it to cool at room temperature. Solution was neutralized with 0.1 N HCl and volume was made up to scale with methanol. 1 mL of aliquot was withdrawn from the above solution and diluted with mobile phase to acquire final concentration of 100 $\mu g/mL^{-1}$ of EDA.

**Heat induced acid hydrolysis**

To the 10 mL volumetric flask, 10 mg of EDA was taken and 2 mL of 0.5 N HCl was incorporated to perform heat induced acid hydrolysis. The flask was heated at 80 °C for 2 hrs and let it to cool at room temperature. Solution was neutralized with 0.5 N NaOH and volume was made up to scale

with methanol. 1 mL of aliquot was withdrawn from the above solution and diluted with mobile phase to acquire final concentration of 100 μg/mL$^{-1}$ of EDA.

**Heat induced oxidative stress degradation**

To heat induced perform oxidative stress degradation, 10 mg of EDA was taken in 10 mL volumetric flask and 2 mL of 3% hydrogen peroxide was added. The mixture was heated in a water bath at 80 °C for 2 hrs and let it to cool at room temperature and volume was made up to scale with methanol. 1.0 mL of aliquot was withdrawn from above solution and diluted with mobile phase to acquire final concentration of 100 μg/mL$^{-1}$ of EDA.

**Dry heat degradation**

Analytically pure 10 mg sample of EDA was exposed in oven at 80 °C for 4 hrs. The solids were let to cool and transferred to volumetric flasks (10 mL) and dissolved in few mL of methanol. Volume was made up to scale with the methanol. Solution was further diluted by mobile phase to acquire final concentration of 100 μg/mL$^{-1}$ of EDA.

**Study photolytic degradation**

Analytically pure 10 mg of drug were exposed to UV light for 24 hrs. The solids were let to transfer to volumetric flask (10 mL) and dissolve in few mL of methanol. Volume was made up to scale with the methanol. Solution was further diluted with the mobile phase to acquire final concentration of 100 μg/mL$^{-1}$ of EDA. The resulting concentration of all the solutions 400 ng/band was applied on the HPTLC plates and chromatograms were noted.

**Result and Discussion**

**Optimization of the mobile phase**

To burgeon the HPTLC method for the appraisal of EDA in dosage form, selection of the mobile phase was conducted on the basis of polarity. A mobile phase that would give a close and firm band with convenient $R_f$ value for EDA was desired. The mobile phase *n*-hexane: ethyl acetate (7:3, *v/v*) was found to be gratified and gave result (Figure 2). It was also ascertained that chamber saturation time and solvent migration distance were pivotal in the chromatographic separation. A chamber saturation time 30 min and solvent migration distances greater than 80 mm ensued in diffusion of the analyte band. So, *n*-hexane: ethyl acetate (7:3, *v/v*) mobile phase with a chamber saturation time of 30 min at circling condition and solvent progression distance of 80 mm was chosen as an optimal

condition. These chromatographic conditions created an unambiguous, compact band of EDA with $R_f$ 0.4 ± 0.05.

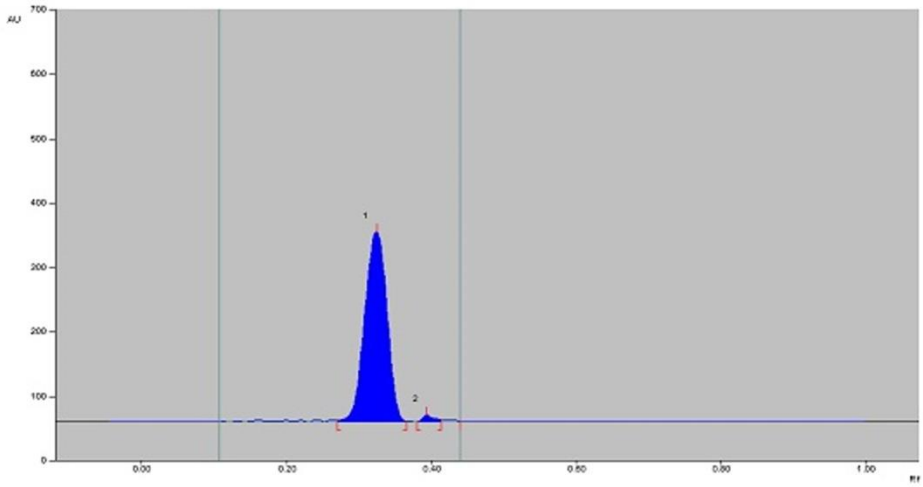


**Figure 2.** Densitogram of EDA with mobile phase *n*-hexane: ethyl acetate 7:3 (800 ng/band)

## Selection of detection wavelength

The solution was applied in the form of a band in concentration range of 100-1200 ng/band of EDA which was prepared in methanol and regression data of analysis is shown in Table 1. The plate was made using hexane: ethyl acetate (7:3, *v/v*) and dried in air. It was levied to densitometric measurements in scanning mode in the UV region of 200–400 nm, and the overlay spectrum was chronicled using CAMAG TLC Scanner 4. The three dimensional overlay spectra revealed that the drug absorb substantially at 245 nm (Figure 3). Therefore, it was selected as detection wavelength.

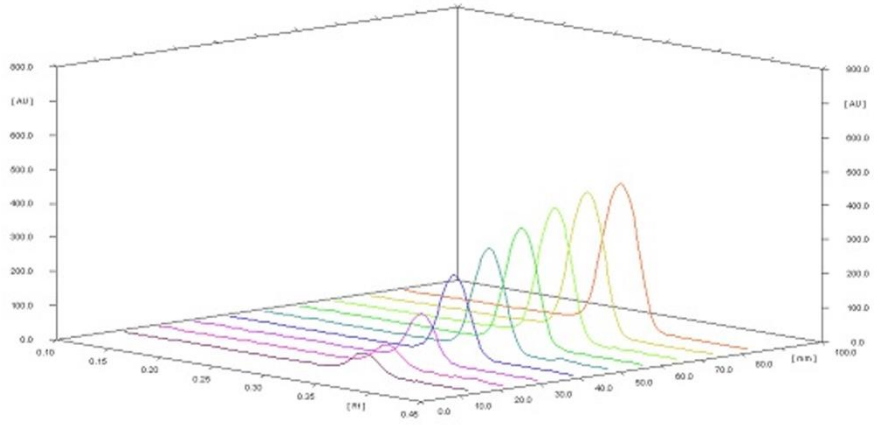


**Figure 3.** Three-dimensional overlay of HPTLC densitogram of calibration bands (100 ng, 1400 ng/band)

**Table 1.** Regression alaysis of calibration curve

| Parameters | Edaravone |
|---|---|
| Range (ng/band) | 100-1400 |
| Regression coefficient( r2) | 0.9958 |
| Slope of regression equation | 7444.4 |
| Standard deviation of slope | 827.18 |
| Intercept of regression | 12873 |
| Standard deviation of Intercept | 1390.8 |

### Accuracy

Accuracy was determined by calculating the recovery. The method was found to be accurate with % recovery 98.5-100.9 of EDA (Table 2). The value near to 100 percentages indicates that method is accurate.

**Table 2.** Result of proposed accuracy study

| Quantity of drug taken from sample(ng/band) | Quantity of standard drug spiked (ng/band) | Quantity of drug found (ng/band) (n=3) | % Recovery ± SD |
|---|---|---|---|
| 300 | 0 | 302.83 | 100.9 ± 1.1 |
| 300 | 150 | 449.43 | 99.8 ± 1.9 |
| 300 | 300 | 597.86 | 99.2 ± 0.2 |
| 300 | 450 | 745.60 | 98.5 ± 1.4 |

### Precision

### Repeatability

Repeatability of the scanning device and injection was studied by applying and analyzing EDA sample (800 ng/band) six times. The %RSD values obtained were less than 2% which means the results indicates that the method is repeatable.

### Intra and inter day precision

Variation of results within the same day (intra-day), variation of results between days (inter-day) were analyzed. The method was found to be precise with %RSD 0.82-4.12 for inter-day study (n=3) and %RSD 0.75-3.56 for inter-day study (n=3) (Table 6). The %RSD <2 for EDA is indicating that the method is precise.

### Limit of detection and limit of quantification

Under the experimental conditions used, the lowest amount of drug that could be detected (LOD) for EDA was found to be 0.039 µg/mL. The limit of quantification (LOQ) for EDA was found to be 0.12 µg/mL. This indicates that the nano gram quantity of drug can be estimated accurately and

precisely which means the method is sensitive. Summary of validation parameters are shown in Table 3. The peak purity spectra are shown in Figure 4.

**Table 3.** Summary of validation parameters of HPTLC

| Parameters | EDARAVONE |
|---|---|
| Range (ng/band) | 100 – 1400 |
| Retention factor (n=5) | 3.4 |
| Detection limit (µg/mL) | 0.039 |
| Quantitation limit (µg/mL) | 0.12 |
| Accuracy (%) | 98.5-100.9 |
| **Precision (%RSD)** | |
| Intra-day (n=3) | 0.75 - 3.56 |
| Inter-day (n=3) | 0.82 - 4.12 |
| **Instrument precision (%RSD)** | |
| Repeatability (n=6) | 1.07 |
| Scanner (n=6) | 0.27 |

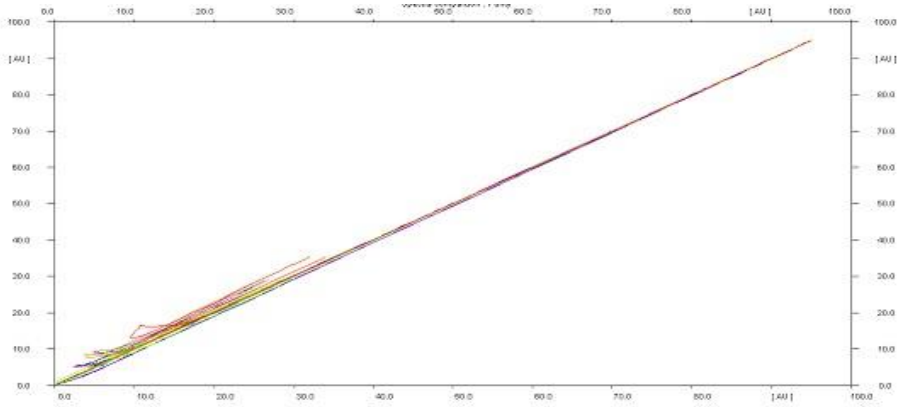


**Figure 4.** Peak purity spectra of EDA (100–1400 ng/band)

### Robustness

The %RSD values not more than 2% were acquired after acquainting small, preconceived deviations in parameters of the developed HPTLC method, affirming its robustness.

### Solution stability

The stock solution was prepared and store in room temperature. The stock solution was appropriate diluted up to 400 ng/mL and analyzed by developed method. The % amount of drug was found to be 99.8-100.93% which indicated that the solution was stable for 24 hrs.

### Analysis of formulation

The marketed formulation was analyzed by developed method. The percentage amount of drug was found to be 100.2-102.02% for EDA (Table 4).

**Table 4.** Assay of marketed formulation (800 ng/band)

| Marketed formulation A* | Quantity of drug taken (ng/band) | Quantity of drug found (µg/ml) | % quantity of drug found ± SD (n=3)* |
|---|---|---|---|
| | 800 | 814.75 | 101.8 ± 1.30 |

* Mean value ± standard deviation of three determinations; Each vial formulation A is edastar (Lupin Pharmaceutical Industries Ltd, Gujarat, India) containing labeled amount of 1.5 mg/mL of edaravone

## Forced degradation study

Forced degradation study was carried out by subjecting the drug to acid and alkali hydrolysis, chemical oxidation, dry heat degradation and photolytic conditions. The EDA was found to be susceptible to degraded in acid and alkali hydrolysis (Figure 5 and 6), and chemical oxidative (Figure 7) conditions. The EDA was found to be stable dry heat degradation and photolytic (UV–Visible light) condition. Summary of data derived from forced degradation study by proposed TLC Method is shown Table 5.

**Table 5.** Summary of data derived from forced degradation study by proposed TLC method

| Conditions | Time (h) | % Recovery EDA | $R_f$ value of Degradants of EDA |
|---|---|---|---|
| Base 1 N NaOH* | 2 | 90.3 | 0.14 |
| Acid 1 N HCl* | 2 | 84.5 | 0.15, .0.21 |
| 3% Hydrogen Peroxide* | 2 | 84.2 | 0.13, 0.22 |
| Dry Heat* | 4 | 98.3 | -- |
| UV – Visible Light | 24 | 99.3 | -- |

*samples were heated at 80° for specified period of time.

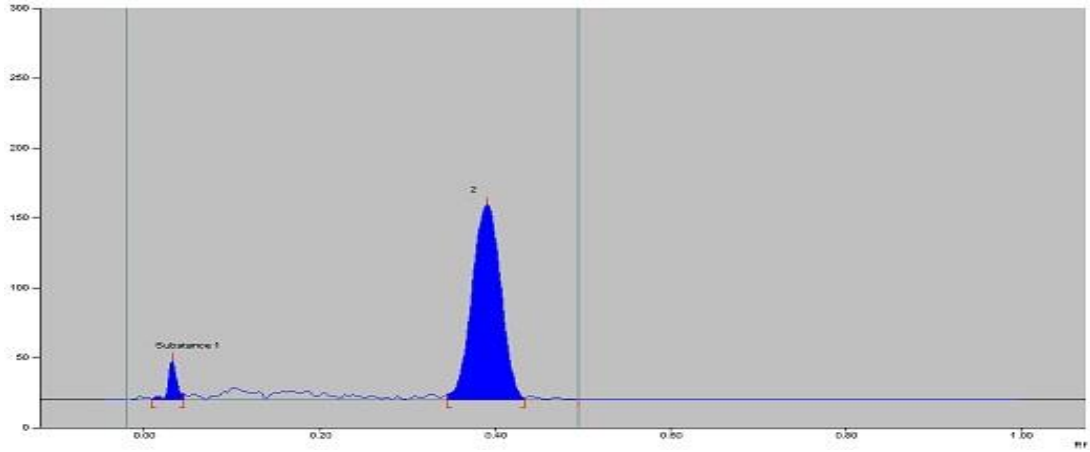


**Figure 5.** Densitogram of alkali (1 N NaOH) degraded EDA (500 ng/band) reflux at 80 °C for 2 hrs

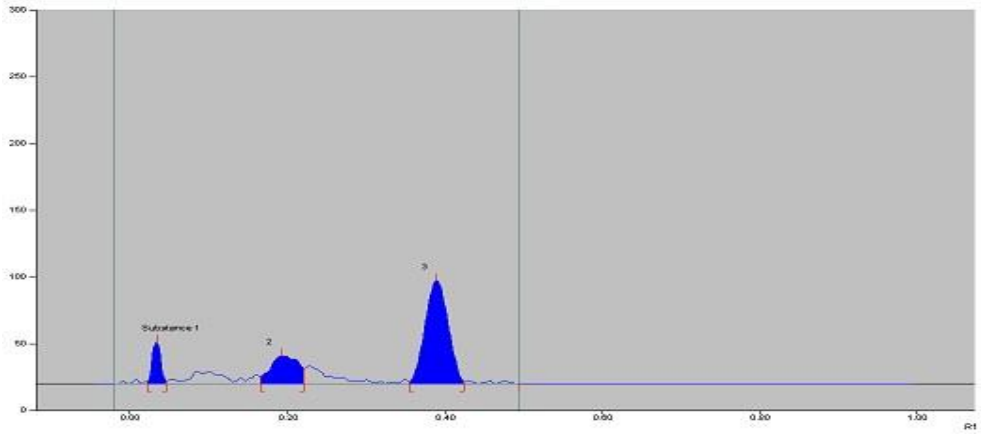



**Figure 6.** Densitogram of acid (1 N HCl) degraded EDA (500 ng/band) reflux at 80 °C for 2 hrs

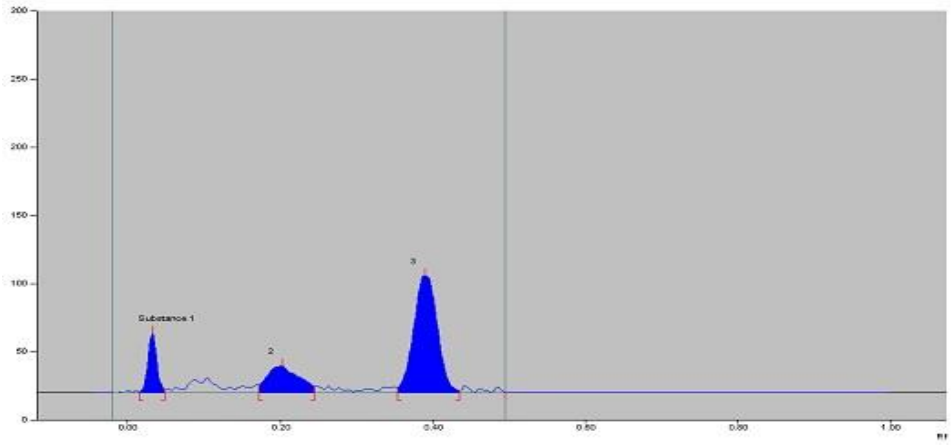



**Figure 7.** Densitogram of chemixal oxidation (3% $H_2O_2$) degraded EDA (500 ng/band) reflux at 80 °C for 2 hrs

## Development and validation with result and discussion of UV-Visible spectroscopic method

### selection of solvent

EDA is soluble in organic solvent and is slightly soluble in aqueous solvent. It is freely soluble in methanol, Therefore methanol was chosen as a solvent for stock solution.

### Preparation of standard stock solution (100 µg/mL)

Meticulously weighed EDA (25 mg) was pipette out into 25 mL volumetric flask having few mL (10 mL) of methanol and swirling to dissolve and diluted up to scale with methanol to acquire solution having concentration of EDA (1000 µg/mL). To acquire standard stock solution of 100 µg/mL, pipette out 1 mL of aliquot and transfer into another 10 mL volumetric flask and diluted up to scale with methanol from the above solution.

### Selection of analytical wavelength

The solution of EDA was prepared in methanol at a concentration of 10 µg/mL. It was examined in the wavelength range of 400–200 nm. For determination of EDA, analytical wavelength selected was 245 nm.

### Calibration curve

Appropriate aliquots of stock solution were pipetted out and transferred into in six different 10 mL volumetric flask. To acquire resulting concentration of 1, 2, 3, 5, 7, and 9 µg/mL, volume was adjust up to scale with methanol.

### Validation of UV- Visible method

Validation of the developed UV-Visible method was accomplished according to the international conference on harmonization (ICH) guidelines Q2 (R1) [9].

### Linearity and Range

By constructing calibration curves at six concentration levels over a range of 1-9 µg/mL for EDA, linearity of method was evaluated. The calibration curve was established by scheming absorbance versus concentration (n=6). The overlay spectra are shown in Figure 5. Statistical data for EDA by spectrophotometry method is shown in Table 6.

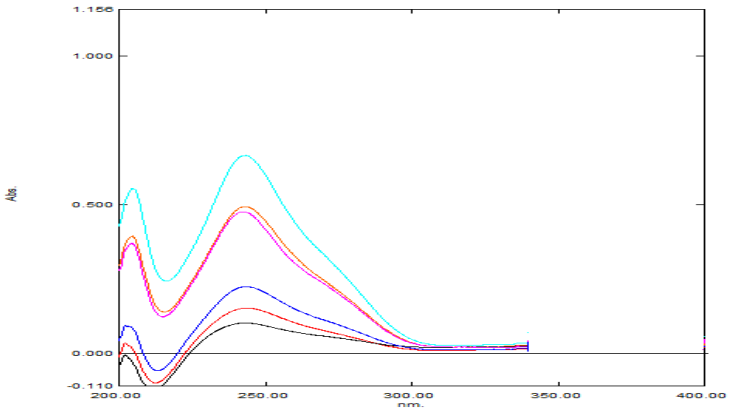



**Figure 8.** Overlay spectra of EDA (1–9 µg/mL) in methanol

**Table 6.** Statistical data for EDA by spectrophotometry method

| Parameters | Edaravone |
|---|---|
| Range (µg/mL) | 1-9 |
| Regression coefficient( $r^2$) | 0.994 |
| Slope of regression equation | 0.112 |
| Standard deviation of slope | 0.008 |
| Intercept of regression | 0.046 |

| | |
|---|---|
| Standard deviation of Intercept | 0.005 |

**Accuracy**

Evaluation of accuracy of the procedure was accomplished by computing recoveries of EDA by method of standard additions. Avowed quantity of EDA (0%, 50%, 100%, 150%) were incorporated to a pre quantified sample solution, and the quantity of EDA was evaluated by measuring the absorbance and by appropriately placing these values to the straight line equation of calibration curve (Table 7).

**Table 7.** Accuracy data for EDA by the proposed UV–Visible spectroscopic method

| Quantity of drug taken from drug sample (µg/mL) | Quantity of standard drug spiked (µg/mL) | Quantity of drug found (µg/ml) (n=3) | % Recovery ± SD (n = 3) |
|---|---|---|---|
| 3 | 0 | 3.02 | 100.9 ± 1.71 |
| 3 | 1.5 | 4.44 | 98.14 ± 1.84 |
| 3 | 3 | 5.96 | 99.5 ± 0.62 |
| 3 | 4.5 | 7.48 | 99.6 ± 1.72 |

**Precision**

**Repeatability**

Preparation of standard solution of EDA (5 µg/mL) was done and spectra were noted. Absorbance was measured at 245 nm using methanol as a blank. The absorbance of the similar concentration solution was measured six times and %RSD was calculated.

**Intra and inter day precision**

Dissimilarity in results of three different concentrations (1, 7 and 9 µg/mL) within the same day (intra-day) and difference in results between different days (inter-day) were analyzed. Intra-day precision was evaluated by analyzing EDA thrice on the same day. Inter-day precision was evaluated by analyzing EDA daily for three days.

**Limit of detection**

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOD was measured by using mathematical expressions given in section.

Calculation of limit of detection (LOD) of the drug by using the following equations adopted by International Conference on Harmonization (ICH) guideline:

$$LOD = 3.3 X\sigma/S$$

Where, σ = the standard deviation of the response at y–intercept, S = slope of the calibration curve.

**Limit of quantification**

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. Then LOQ was measured by using mathematical expressions given in section. Calculation of limit of quantification (LOQ) by using the following equations adopted by international conference on harmonization (ICH) guideline:

$$LOQ = 10 X\sigma/S$$

Where, σ = the standard deviation of the response at y-intercept

S = slope of the calibration curve

**Ruggedness**

It is the degree of reproducibility of test results acquired by analyzing the same sample under variety of normal test conditions such as different analysts, instruments, days, reagents and columns. Ruggedness study was accomplished by analyzing drug using different analysts and different instruments.

**Solution stability**

The stock solution of EDA (100 µg/mL) was made and store in room temperature which was analyzed by UV at regular at intervals 0, 2, 4 and 8 hrs.

Summary of validation parameters are reported in Table 8.

**Table 8.** Summary of validation parameters of spectrophotometry

| **Parameters** | |
|---|---|
| Range ( µg/mL) | 1 – 9 |
| Detection limit (µg/mL) | 0.017 |
| Quantitation limit (µg/mL) | 0.051 |
| Accuracy (%) (n=3) | 98.1-100.9 |
| **Precision (%RSD)** | |
| Intra-day (n=3) | 0.15-1.49 |
| Inter-day (n=3) | 0.20-1.49 |
| Repeatability(n=6) | 0.32 |

### Analysis of marketed formulation

The injection holds strength of 1.5 mg/mL solution. 1 mL was pipette out from the solution is then shifted to 25 mL volumetric flask and adjustment of volume is done up to scale with methanol to acquire 60 µg/mL EDA. 1 mL was pipette out from the above solution and shifted to 10 mL volumetric flask. To acquire 6 µg/mL solution, volume with methanol was adjusted to the mark. The emanate solution was analyzed by proposed spectroscopic methanol. The quantization was held out by putting those values to the straight line equation by calibration curve (Table 9).

**Table 9.** Assay results of marketed formulation

| Marketed formulation A* | Quantity of drug taken (µg/ml) | Quantity of drug found (µg/ml) | % Quantity of drug found ± SD* (n=3) |
|---|---|---|---|
| | 6 | 5.96 | 99.4 ± 2.11 |

* Mean value ± standard deviation of three determinations; each vial formulation A is edastar (Lupin pharmaceutical industries Ltd, Gujarat, India) comprising labeled amount of 1.5 mg/mL of edaravone.

### Comparison between TLC and UV-Visible spectroscopic method

The urged analytical methods were correlated using statistical analysis and student's F–test was exercised which does not divulge noteworthy difference between the experimental values acquired in the assay of sample analysis by the two methods. The calculated F-value ($F_{cal}$ =1.6) was found to be not more than the critical F-value ($t_{cri}$ = .91) at 95% remarkable level hence it was concluded that both the methods do not differ appreciably. Therefore, both these methods can be conveniently used for routine quality control analysis of EDA in bulk and its pharmaceutical dosage form.

### Conclusion

Acquainting TLC and UV–Visible spectroscophotometric method in pharmaceutical analysis serve as a crucial step in quality assurance. Urged study construe stability revealing TLC and UV–Visible spectroscophotometric method for the appraisal of neuroprotective agent EDA in bulk and its pharmaceutical dosage form. The methods were validated and found to be sensitive, accurate and precise. Statistical analysis concluded that method was repeatable and fussy for the analysis of EDA deprived of any intrusion from the excipients. EDA was found to be naive under alkali and acid hydrolysis, chemical oxidative conditions, and it is stable in dry heat and photolytic conditions. As the thin layer chromatographic method disunite the drugs from its degradant products, can be applied for the analysis of sample acquired during accelerated stability experiments to anticipate expiration dates of pharmaceuticals.

**Acknowledgement**

The wordsmiths are very contented to SICART and Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, for providing necessary facilities to carry out research work.

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**How to cite this manuscript:** Jaineel V. Desai, Usmangani K. Chhalotiya*, Himani B. Dalwadi, Anjali M. Thakkar, Dimal A. Shah. Quantification of a Neuroprotective Agent Edaravone by Stability Indicating TLC Method and UV–Visible Spectroscopic Method in Bulk and Pharmaceutical Dosage Form. Chemical Methodologies 3(2), 2019, 166-182. DOI:10.22034/chemm.2018.145347.1077.