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Development and Validation of UV Spectrophotometric Methods for Quantification of Edaravone and Application of Greenness and Blueness Study

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Abstract: *This work presents a simple, rapid, accurate and precise UV-spectrophotometric methods for estimation of Edaravone in bulk and its pharmaceutical preparation using an Acetonitrile and Water 5:5 mixture as solvent. Method A was performed using maximum absorbance zero order derivative method with 242nm and Method B was based on Zero order Area under Curve method with wavelength from 230nm to 244nm. The development of the method was validated as per ICH Q2 (R1) guidelines. The methods showed good linear response across a concentration range 2-12µg/ml with r^2 value of 0.999 respectively. The percentage Relative Standard Deviation was found to be less than 2, indicating the method was precise. Additionally, the methods greenness were assessed by means of ecological tools which were up-to-date, namely, AGREE (Analytical Greenness Metric Approach), MoGAPI (Modified Greenness Analytical Procedure Index) for greenness. The methods were also assessed for blueness using BAGI (Blue Applicability Grade Index) a recently developed metric for assessing practicality of procedure.*

Keywords: *Edaravone, UV-spectrophotometry, Zero order derivative, Area Under Curve, Quantitative analysis, Greenness evaluation.*

INTRODUCTION:

Edaravone (EDA) (3-Methyl-1-phenyl-2-pyrazoline-5-one) is an antioxidant, free radical deactivator and has neuroprotectant properties. It exists in three tautomeric forms. Edaravone deactivates reactive oxygen species, of which finds applications in neurological disorders, like amyotrophic lateral sclerosis (ALS) and cerebral ischemia[1,2].Structurally, Edaravone is $C_{10}H_{10}N_2O$ with Molecular weight of 174.20 g/mol and exhibits moderate lipophilicity (LogP: 1.12) and a pKa of 7.0 which is around the physiological pH[3]. The structure of EDA is shown in Fig.1.

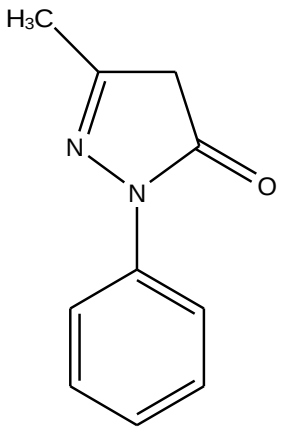

Fig. 1. Structure of Edaravone

The Quantification and Determination of Edaravone in Bulk and its Formulation is critical for ensuring therapeutic use, efficacy and safety. There have been several methods for analysis of Edaravone including HPTLC[4,5], HPLC[6,7,8], LC-MS/MS[9], Fluorescent assay[10], Spectrophotometry[11,12,13,14].

Through the use of the maximum absorbance zero order derivative method and the zero order Area Under Curve method, this study seeks to develop and validate a new UV-Spectrophotometric method that is liner, precise, accurate, sensitive, robust and rugged for the estimation of Edaravone in bulk and its formulation using acetonitrile and water (5:5) as a solvent. The method is evaluated and validation is performed in accordance to ICH Q2(R1) Guidelines[15]. In addition, greenness of the methods were evaluated using green metric software tools[16].

MATERIALS AND METHODS:

Apparatus and Software:

For measurement of absorbance double beam spectrophotometer with model Shimadzu UV-1800 in connectivity with computer loaded having software Shimadzu UV Probe 2.70 was utilized. Sample cell made of quartz of 1 cm was employed to measure the absorbance spectra of the blank and sample solution within the range of 200-400nm.

Materials:

Edaravone sample was received as gift sample from BDR Pharmaceuticals Pvt Ltd. EDASTAR injection manufactured by Lupin Pharmaceuticals was purchased from retail pharmacy. (Label claim was 1.5mg/ml).

Reagents and chemicals:

Solvents incorporated in UV Spectrophotometric method were acetonitrile (ACN) with high purity (Research-labs Fine Chem Industries, Mumbai, India) and distilled water (5:5). These solvents were utilized as diluents in UV spectrophotometric method.

Stock Solution Development:

For spectrophotometric method, 25 mg of EDA was precisely measured and placed into a 25 ml volumetric flask filled with a small volume of a mixture of acetonitrile and water (5:5) as the diluent. The same ACN and water (5:5) mixture was used to make up the volume to mark to produce 1000µg/ml of EDA.

Working Standard Solution Development:

From stock solution, 1 ml was pipetted out and placed in 10 ml volumetric flask, using mixture of ACN and water volume was made up to the mark with 10 ml to produce working standard solution of EDA (100µg/ml).

Selection of Analytical wavelength:

Method A: Maximum Absorption Zero order derivative:

Standard solution of EDA was subjected to scanning in the range of 200-400nm wavelength in UV-Vis Spectrophotometer and the solution depicted λmax at 242nm as shown in Figure 2 which was selected as the analytical wavelength. Prepared calibration standard was analysed at 242nm wavelength.

Method B: Area under Curve (AUC) Zero order derivative:

This method is based on selection of wavelengths λ1 and λ2 and absorbance values are integrated between wavelengths λ1 and λ2. The solution of drug was scanned in the range of 200-400nm. For area under curve method, the wavelength ranges selected for estimation of the Edaravone were from 230nm to 244nm.

Construction of Calibration Curve of Standard EDA:

From the Working Standard solution of EDA (100µg/ml), solutions of 2 ml, 4 ml, 6 ml, 8 ml,10 ml, 12ml were withdrawn from the working standard and transferred to 10ml of volumetric flask. Mixture of ACN and Water was added to make up the volume upto the mark to produce 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, 12 µg/ml solution containing EDA respectively, absorbance and Area under Curve was noted, and graph was prepared between Absorbance and concentration, Area and Concentration and regression equation, regression coefficient was obtained.

Formulation analysis by the developed method:

The developed method was utilized for analyse of Edaravone (EDA) formulation available in market. “EDASTAR Injection” of which the manufacturer is Lupin Pharmaceuticals was procured from retail pharmacy. 1ml of solution was taken from the marketed formulation of 1.5mg/ml in 10ml of volumetric flask and diluted with ACN and Water (5:5) upto mark so as to produce concentration of solution of 150 µg/ml. For clear solution filtration was performed using Whatman filter paper no. 45. 0.2ml of this solution was pipetted out and placed in a 10ml volumetric flask to produce solution of concentration of 6 µg/ml. The resulting solution was analysed at 242nm, and absorbance was recorded by both methods.

Validation of the developed method:

Both the developed methods that is **Method A and Method B** were validated according to ICH Q2 (R1) guidelines [15] and data in accordance with the standards were produced.

Linearity and Beer's Range:

Method A: The linearity of method was calculated thrice by analysing prepared concentrations of EDA ranging from 2-12 µg/mL for UV. The linear regression formula was derived within the concentration range (y = mx + c). Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated from standard deviation of response and slope of calibration curve.

Method B: Area was noted for concentration range from 2-12 µg/ml, graph plot was obtained between area and concentration, regression was obtained.

Precision:

Reproducibility of the methods was confirmed by conducting intra-day precision (three times a day) and inter-day precision (repeated triplicates for three consecutive days). of Standard deviation and %Relative standard Deviation (%RSD) are utilized for expression of results of precision.

Accuracy:

Accuracy of the developed method was checked using recovery studies using pre-analysed sample with three deferent level of standard addition 50%, 100%, 150%. Percentage Recovery was calculated and determined from the average of three determinations at each standard addition level.

Ruggedness and Robustness:

Robustness was evaluated using effect of variation in solvent ACN and water in ratio of 45%-55% and 55%-45% as solvent was recorded. Ruggedness of both the methods was confirmed by measuring absorbance for Method A and area for Method B of solution by two different analysts.

Selectivity:

Selectivity of the method was confirmed by the absences of any absorbing or interfering excipients in UV-spectrophotometric method.

Statistical Comparison:

Statistical Comparison were conducted using t-test and F-test to compare the significance between accuracy of the methods developed with the reported method. The t-test is a statistical test used to compare the means of two groups (or in this case two methods) and determine if they are significantly different or not. The t-value is ratio of the departure of the estimated value of a parameter from its hypothesized value to its standard error. Comparison of variance is performed by F-test. The f-test tells us if two population have the same variance. The null hypothesis in an F-test is that the variances are equal. The F-test gives us the F-value which is ratio between-group variability and within-group variability [16].

Greenness Assessment:

In line with the principles of Green Analytical Chemistry (GAC), the environmental impact of analytical methods can be critically assessed using modern greenness assessment tools such as AGREE and MoGAPI. AGREE (Analytical Greenness Metric) is an Automated, holistic tool built upon twelve GAC principles, offering a circular, color-coded diagram alongside a quantitative score ranging from 0-1, thereby providing a comprehensive overview of the method's sustainability. It facilitates rapid comparison and optimization by highlighting areas of environmental concern. In contrast MoGAPI (Modified Green Analytical Procedure Index) integrated the pictographic clarity of GAPI with the numeric strength of the Analytical Eco-scale, representing the analytical workflow through a pentagram-based visual and delivering a cumulative greenness score. Unlike its predecessor, MoGAPI provides detailed stepwise evaluation across sample preparation, reagent use, and instrumentation. It has three colour levels, green, yellow and red. These tools are especially significant in the context of pharmaceutical analysis, where method development not only demands precision and robustness but must also align with sustainable regulatory expectations. Incorporating such metrics during method validation and quality by design (QbD) processes encourages the adoption of greener practices without compromising analytical performance [16].

Blueness Assessment:

In the evolution of landscape of sustainable analytical chemistry, the Blue Application Grade Index (BAGI) has emerged as a novel metric for assessing the practicality and applicability of analytical methods. Rooted in the principles of White Analytical Chemistry, BAGI complements traditional green metrics by focusing on attributes, including the type if analysis, including the type of analysis, number of analytes determined simultaneously, sample throughput, reagent and materials requirements, instrumentation complexity, automation level, sample preparation needs, and sample volume. These parameters are integrated into a visual "asteroid" pictogram and a cumulative score, offering both qualitative and quantitative insights. The blue color, inspired by the RGB model, symbolizes the method's readiness for real-worlds applications. BAGI is particularly

significant in pharmaceutical analysis, where methods must not only be environmentally sound but also efficient, scalable, and user-friendly. By identifying strengths and limitations in the method design, BAGI supports informed decision-making during method development and validation, especially under Quality by Design (Qbd) frameworks. Its open-source software further enhances accessibility, making it a valuable addition to the analytical chemist's toolkit[16].

RESULTS AND DISCUSSIONS:

242 nm was found to be suitable for selection of analytical wavelength for standard EDA solution , when solution of EDA with concentrations 2-12 µg/ml were scanned between 200nm-400nm in UV-Spectrophotometer.

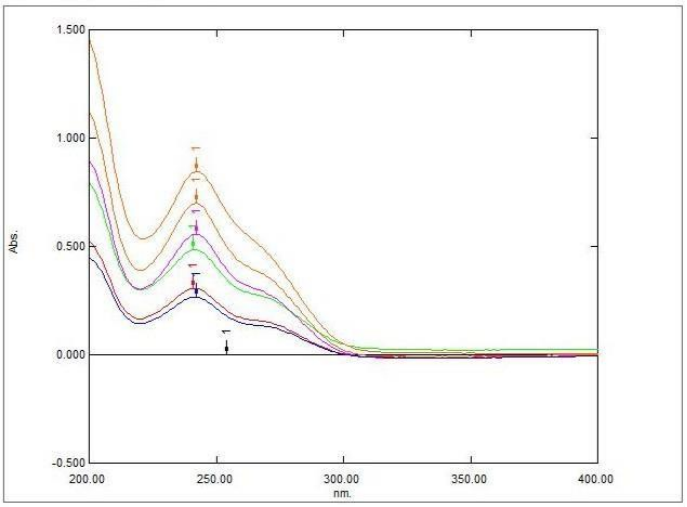

Figure 2: Overlay spectra of EDA analytical wavelength at 242nm

10 µg/ml solution of EDA was scanned for determination of wavelength range in Area under Curve method and was found from 230nm to 240nm. Fig 3 shows the resulting Spectra with AUC for 10 µg/ml.

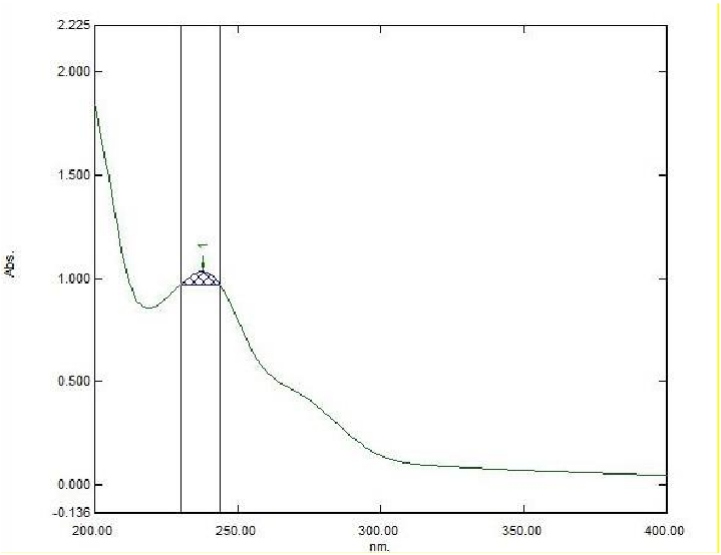

Fig 3: UV Spectra showing AUC of EDA 10 µg/ml

In Method A, correlation coefficient r^2 0.999 was observed with regression equation of y=0.058x+0.1425 which indicates ther is good correlation between concentration and absorbance, graph of which is shown in Fig 4.

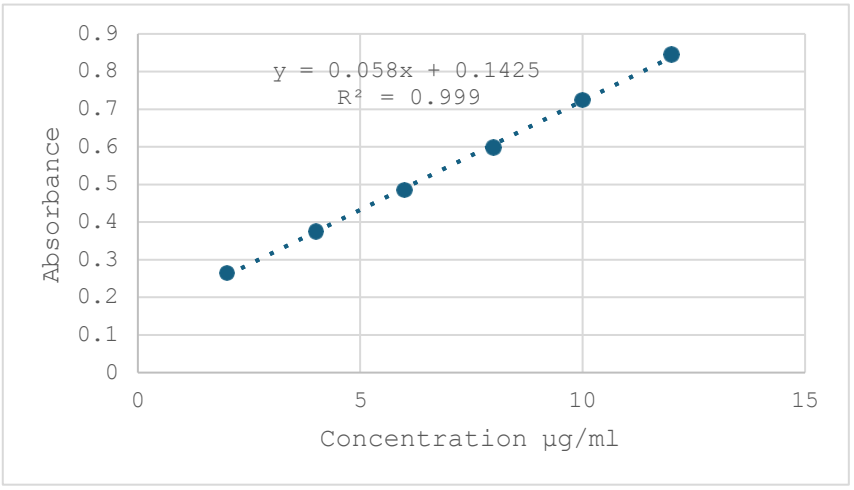

Fig 4: (Method A)Calibration graph of Edaravone in ACN and Water (5:5) (2-12µg/ml)

In Method B, correlation coefficient r^2 0.9991 is obtained with regression equation of y=0.0501x+0.2076, indicating good correlation, which is shown in Fig 5.

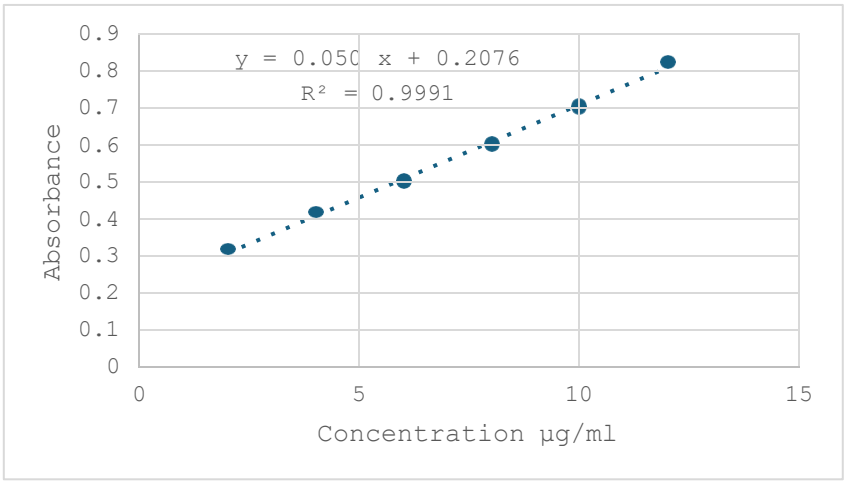

Fig 5: (Method B) Calibration graph of Edaravone in ACN and Water (5:5) (2-12µg/ml)

The results of the parameters of validation for both methods are mentioned in Table 1.

Table 1: Summary of validation parameters of EDA by the developed method.

Parameters	**(Method A) Result**	**(Method B) Result**
Analytical Wavelength	242nm	230-244nm
Linearity Range	2-12 µg/ml	2-12 µg/ml
Slope	0.057971	0.050128
Intercept	0.142533	0.2076
Correlation Coefficient (r^2)	00.999	00.9991
Limit of Detection (LOD)	0.361 µg/ml	0.455 µg/ml
Limit of Quantification (LOQ)	1.095 µg/ml	1.38 µg/ml

The results of assay of the EDASTAR injection for both the Methods A and B are expressed with respect to %RSD, Standard Deviation and standard error. The %RSD, Standard Deviation, and Standard are shown in Table 2.

Table 2: Result of analysis of EDASTAR Injection (EDA) (*n= three determinations)

Method	Actual conc. Of EDA (mg)	Amount of EDA found*(mg)	%Label claim	Standard Deviation	%RSD	Standard Error
Method A	1.5	1.517	101.1667	0.004243	0.0082%	0.002
Method B	1.5	1.502	100.1	0.707107	1.162049%	0.353554

In the developed methods the lowest limit of analyte that can be detected accurately and precisely (LOD) was found to be 0.361 µg/ml by Method A and 0.455 µg/ml by Method B. This indicates the method was sensitive. The limit of Quantification was reported to be 1.095 µg/ml by Method A and 1.38 µg/ml by Method B.

The %RSD of intra-day and inter-day precision by Method A was found to be 0.081% and 0.138% and by Method B was found to be 0.53177% and 0.00287% respectively. The results are mentioned in the Table 3. Thus, the developed method is precise.

Table 3: Results of Intra-day and Inter-day precision. (*n=three determinations)

Method	Precision*	Standard Deviation	%RSD	Standard Error
Method A	Intra-day	0.00058	0.081%	0.00058
	Inter-day	0.0010	0.138%	0.00058
Method B	Intra-day	0.449885	0.53177%	0.0886
	Inter-day	0.003215	0.00287%	0.00095

% Recovery was found between 99-102% as shown in Table 4. It shows high percentage recovery hence in the developed methods there were absence of interference of the excipients used in the formulation.

Table 4: Result of Recovery studies of EDA (n=three determinations) by UV Spectrophotometry.

Method	%spiking	Actual Concentration of EDA (µg/ml)	Amount of EDA added (µg/ml)	Amount of EDA recovered (µg/ml)	%Mean Recovery±S.D.	S.D.
Method A	50%	3 µg/ml	1.5 µg/ml	4.474 µg/ml	99.42%±0.010	0.010
	100%	3 µg/ml	3 µg/ml	6.008 µg/ml	100.10%±0.004	0.004
	150%	3 µg/ml	4.5 µg/ml	7.577 µg/ml	101.03%±0.005	0.005
Method B	50%	3 µg/ml	1.5 µg/ml	4.48 µg/ml	99.5%±0.01	0.010
	100%	3 µg/ml	3 µg/ml	6.09 µg/ml	101.5%±0.015	0.015
	150%	3 µg/ml	4.5 µg/ml	7.49 µg/ml	99.86%±0.015	0.015

The developed methods were highly robust. Results for Robustness and Ruggedness are show in Table 5. The %RSD for robustness when concentration of ACN and Water were varied was found to be 0.219%. For ruggedness when measured by different analysts by both the methods the resulting absorbance and area were measured the variation was acceptable of 0.202% RSD and 0.0012% RSD.

Table 5: Result of Robustness and Ruggedness studies of the developed method.

Robustness						
Concentration of EDA	Statistics					
	Absorbance of solution			Mean	Standard Deviation	%RSD
	ACN + Water (5:5)	ACN + Water (5.5:4.5)	ACN + Water (4.5:5.5)			
10 ppM	0.698	0.699	0.696	0.6976	0.001528	0.219%
Ruggedness						
Method A	Statistics					
	Absorbance of solution			Mean		%RSD

Concentration of EDA	Analyst 1	Analyst 2		Standard Deviation	
10 ppM	0.698	0.700	0.699	0.001414	0.202%
Method B	Statistics				
Concentration of EDA	Area		Mean	Standard Deviation	%RSD
	Analyst 1	Analyst 2			
10 ppM	1.119	1.121	1.12	0.001414	0.0012%

Statistical analysis was performed for comparison of %recovery reported method with developed methods. To compare both the methods and the reported [11], values statistically, the t-test and F-test were used. The result of the t-test and F-test between the methods are shown in the Table 6. The calculated t-values for all comparison were lower than the 95% confidence, indicating no significant difference in accuracy between the methods. The F-test showed no significant difference in accuracy between the methods at 95% confidence.

Table 6: Result of t-test and F-test between Method A, B and Reported method (a=Mean of 3 determinations, b= Tabulated value of F=19 and t=4.303).

Comparison	t-test [a,b]	F-test [a,b]
Method A V/s Method B	0.13	1.74
Method A V/s Reported method	1.11	2.64
Method B V/s Reported method	1.51	1.52

The greenness assessment two metrics were used to assess the environmentally friendly the proposed analytical methods are. The first metric used was MoGAPI, the second metric used was AGREE. In MoGAPI the greenness was evaluated by each step and the result was expressed by pictograms. The pictogram revealed 8 yellow, 6 green and 1 red area when evaluated Fig 6 shows the result of MoGAPI. In AGREE metrics are flexible and draws design from 12 principles of green chemistry. The suggested method score is 0.77 which is dependent on multiple numerous variables, For example, solvent type, it's volume, toxicity and impact on environmental factors. The current result shows that the methods have a minimal environmental impact shown in Fig 7.

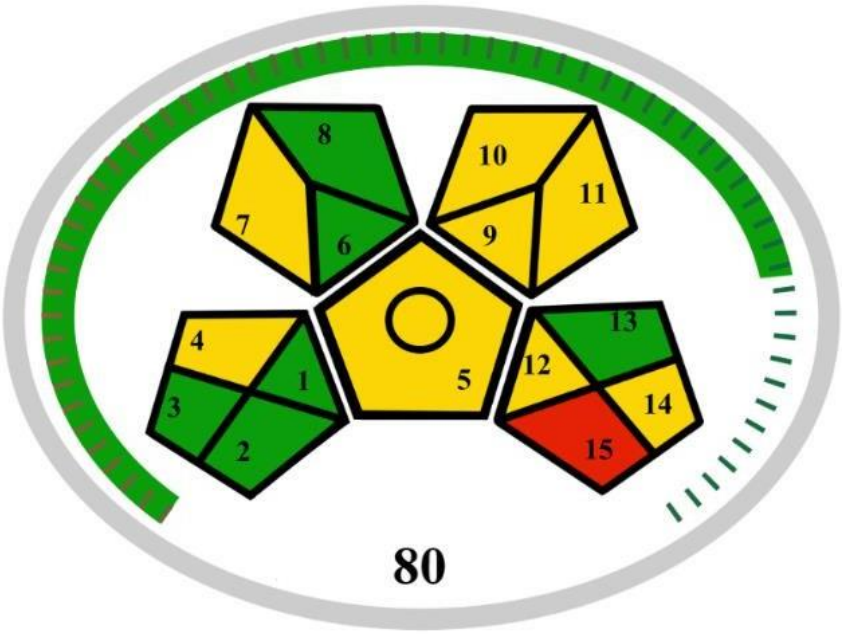

Fig 6: Result of MoGAPI for the developed analytical methods.

Fig 7: Result of AGREE for the developed analytical methods.

In blueness assessment it is advised that final score be greater than 60 for the analytical procedure to be practical. The final score of both the methods Method A and Method B have received an overall score of 80 shown in Fig 8. Hence, the analytical procedure is practical.

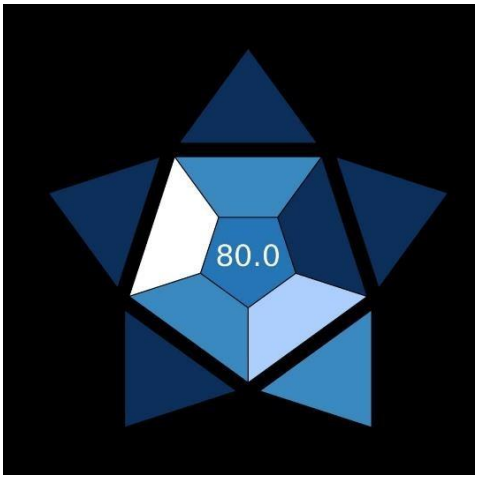

Fig 8: Result of blueness assessment of proposed methods using BAGI.

CONCLUSION:

The proposed validated UV spectrophotometry methods for determination of Edaravone were very sensitive. The method was quick and without involvement of any lengthy sample preparation and extraction steps. The validation method was simple, rapid, accurate, precise and specific. Absence of interference of additive (excipients) in the estimation indicates recovery was in good agreement with the label claim. The method was determined to be green by evaluating it by tools namely, AGREE, MoGAPI. The results indicated that the developed methods have high greenness level and it was confirmed that neither the analyst nor the environment was impacted by the method. The practicality of the method developed was determined by blueness assessment by the tool BAGI which resulted a high overall score which showed that the method developed was highly practical. Therefore, the developed method could be successfully applied for estimating Edaravone in its formulation and bulk.

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Conflicts of Interest: None.

List of abbreviations:

HPLC – High Performance Liquid Chromatography
UV- Ultraviolet
ICH – International Council for Harmonization

AUC – Area under curve
LOD- Limit of detection
LOQ- Limit of Qualification
ACN – acetonitrile
% RSD - % Relative Standard Deviation
AGREE- Analytical Greenness Metric Approach
MoGAPI- Modified Greenness Analytical Procedure Index
BAGI- Blue Applicability Grade Index

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