Eco-friendly based stability-indicating RP-HPLC technique for the determination of escitalopram and etizolam by employing QbD approach

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ABSTRACT
ESC (Escitalopram) and ETZ (Etizolam) are essential anti-anxiety medications. This research suggests employing a Green Analytical Quality by Design (AQbD) based HPLC technique to determine ESC and ETZ are environmentally friendly. According to literature, there is currently no published article by this method. Using Phenomenex column C18 containing ethanol and phosphate buffer (60:40 percent v/v) and the flow rate is fixed to be 1 mL/ min, and the detection was carried out at 254 nm the proposed approach can separate ESC and ETZ and their degradation products. Retention times of ESC and ETZ were found to be 3.5 and 6.5 min respectively. This approach was verified and demonstrated linearity ranges from 5–30 μg/mL for ESC, 2–12 μg/mL for ETZ, accuracy, repeatability, and selectivity, with R² values were 0.9975 and 0.9987 for ESC and ETZ, respectively. The novel method was also evaluated using four different approaches, including other assessment methods: NEMI, GAPI, AGMS, AGREE and it was found to been environment friendly. Based on our results, HPLC techniques with the Green Analytical Performances by the QbD technique for evaluating stability of the drug might be benefit in the development of novel pharmaceutical drugs such as ESC and ETZ.

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1. Introduction
Green chemistry involves the development of synthetic processes and products in which the use of hazardous compounds is reduced or eliminated. Environmentally friendly chemistry refers to every aspect of the chemical life cycle, including production, use, and disposal. Green chemicals either break down into harmless byproducts or are collected and reused. Plants and animals are affected by toxic substances in the environment owing to global climate change, ozone layer depletion, and smog generation (1, 2). Green analytical chemistry (GAC) focuses on creating analytical procedures that are both environmentally and analytist-friendly (3, 4).

The GAC method has numerous benefits, such as reducing the use of dangerous chemicals and reagents, using equipment that uses less energy, and producing less waste.

High-performance liquid chromatography (HPLC) is one of the most effective and quick chromatographic techniques. HPLC machines feature more delicate columns and smaller sizes, which might be a key factor in laboratory environments (5, 6). Any soluble substance, regardless of its volatility, can be handled by HPLC. Selection of suitable eco-friendly solvents could be achieved with the help of several solvent selection guidelines proposed by different companies. Solvent
selection guidelines used in various companies such as Pfizer guide, Vapourtec, Gsk, ACS GCI Pharmaceutical Roundtable (7–9). The majority of the guidelines classify solvents according to their environmental hazard safety (EHS) and net cumulative energy demand (NED) (CED). Only a few solvents that are both ecologically friendly and have low CED values are available. However, these solvents cannot be used effectively as an organic phase in drug analysis, owing to limitations such as high equipment noise and poor drug compatibility. This study concluded that ethanol is an excellent alternative to methanol and propylene carbonate, which are hazardous solvents. To make it acidic, orthophosphoric, glacial acetic, or formic acids were used. For the C₁₈ column, the pH should be adjusted to 3–6.5, or the queue should be destroyed. It is possible to adjust the pH from 1 to 11 if an HPLC column is pH stable (10). Therefore, we conclude that ethanol is a greener solvent that can be used instead of dangerous methanol and polypropylene carbonate.

Analytical quality by design (AQbD) has promoted the advancement of GAC principles in analytical procedures. The use of an AQbD to create a method has numerous benefits: (i) It reduces costs and saves time; (ii) it eliminates the need for revalidation; (iii) it can also be applied to other systems immediately; and (iv) it ensures regulatory compliance. It is both innovative and vital to the long-term success of combining these three concepts into a dependable method. Revalidation was eliminated when AQbD was used, and the GAC principles were strengthened. Finally, using an AQbD-based HPLC technique with GAC principles is always safe. Therefore, this trio is believed to go well with each other, demonstrating the importance of growing analytically.

Escitalopram + etizolam (ESC) (ETZ) is a blend of two medications, ESC and ETZ, which have anti-anxiety and anti-depressant properties. C₂₀H₂₁FN₂O is the chemical formula for ESC. It has the chemistry (1S)-1-(3-dimethylamino propyl)−1-(4-fluorophenyl)−1, 3-dihydro-2-benzofuran-5-carbonitrile. C₁₇H₁₅ClN₄S is the chemical formula of ETZ. It has the chemistry 4-(2-chlorophenyl)−2-ethyl-9-methyl-6H-thieno[3,2-f] [1,2,4] triazolo [4,3 - a][1,4] diazepine. The ESC and ETZ structures are shown in Figure 1 (11). ESC is a medicine known as a selective serotonin reuptake inhibitor. It works by increasing serotonin levels in the brain. Serotonin is a chemical substance in the brain that aids in the relief of depressive symptoms, including mood and physical symptoms. It also helps with anxiety, panic attacks, and symptoms of obsessive – compulsive disorder. ETZ is a benzodiazepine that induces sleep by boosting GABA activity (a chemical messenger in the brain that serves as a natural nerve-calming agent). Consequently, ESC + ETZ helps relax muscles, reduce anxiety, and fall asleep. This combination has also been approved by the US Food and Drug Administration for use in African – American patients (12). In IP’10, ESC is official, whereas in JP XV, ETI is official (13, 14).

A comprehensive study was conducted on the analytical procedures used to determine ESC and ETZ. Because the presented HPLC procedure employs only one variable at a time, it is inefficient as a single component. Single ESC UV-spectrophotometry (15), HPLC (16–19), and single ETZ UV-spectrophotometry (20–22) The combined study of ESC and ETZ of UV-spectrophotometry (23), HPLC (24–26), and UPLC (27). This generates false results that should be avoided. A systematic and quantifiable method to improve the reaction parameters is required to acquire crucial and accurate findings in fewer trials. The design of experiments used to evaluate and optimize the effects of the selected parameters on the reaction by measuring the influence of different variables on the response is one of the most common and valuable chemometric optimization procedures. Response surface methodology (RSM) is a statistical and mathematical technique for evaluating and improving the development of different processes in the design space. RSM is used after factorial designs have been used to determine the parameters that seem to have a significant effect on the response.

Furthermore, no study on ESC and ETZ utilizing this triple combination has been reported (GAC, AQbD, and HPLC). This study proposes a technique for detecting stability that is quick, easy, sensitive, accurate, and repeatable. The current approach was employed to determine the presence of ESC and ETZ in the formulation, which was verified according to International conference of Harmonization (ICH) recommendations (28).

Figure 1. Structure of ESC and ETZ.
2. Experimental

2.1. Reagents and chemicals

The bulk drugs ESC oxalate and ETZ were obtained as gift samples from Chandra labs in Hyderabad, Andhra Pradesh, India, and the marketed formulation Etizola plus (ESC 10mg and ETZ 0.5mg) produced by Macleods pharmaceutical Pvt. Ltd. was obtained from the local market in Hyderabad. HPLC grade ethanol and AR grade potassium dihydrogen phosphate were procured from the local market.

2.2. Software installation and equipment

The HPLC (Agilent) equipment used in the study included an 1100 class HPLC pumping, an auto sample with a 20μL sample loop, the dual absorption detector, and empower software to monitor and integrate the output signal. The pH of the solutions was determined using a System that automatically performs Electronic pH Meter 802 (Gujarat-India). An ultrasonicator was used for degassing the solvents. For AQbD, Design-Expert® version 12 was utilized as a trial. (Minneapolis-based Stat-Ease Inc.)

2.3. Condition of chromatography

A phase comprising mobile phase ethanol and phosphate buffer in a 60:40% v/v proportion was injected at a flow rate of 1 mL/min for chromatographic purposes. The wavelength of the drug’s maximum absorption in a 10 μg/mL dilution in mobile phase ethanol and phosphate buffer in a 60:40% v/v was scanned using a UV-Visible spectrophotometer in the wavelength range of 200–400 nm against a blank mobile phase ethanol and phosphate buffer in a 60:40% v/v. ESC had a max of 244 nm, ETZ had a max of 284 nm, and the combination had a max of 254 nm. The approach was validated per ICH recommendations.

2.4. AQbD study methodology and sample techniques were developed

2.4.1. Buffer and solvent system preparation

1.360 grams of potassium dihydrogen orthophosphate (KH$_2$PO$_4$) were weighed and dissolved in 100 mL of water, and the amount was increased to 500 mL with water. Using triethylamine, adjust the pH to 3.5. To eliminate all tiny particles and gases, the buffer was filtered using 0.45 filters. The 40mL of KH$_2$PO$_4$ Buffer and 60mL of Ethanol (HPLC grade) were taken to prepare a mobile phase ethanol and phosphate buffer in a 60:40% v/v. To remove gases, the liquid mobile phase ethanol and phosphate buffer in a 60:40% v/v was degassed for 10 min.

2.4.2. Preparation of ESC and ETZ standard stock solution

A standard stock solution was prepared by weighing 10 mg of ESC and ETZ, which were weighed and transferred to the 10 mL standard flask and diluted with ethanol. From that solution of ESC and ETZ, take 0.1 mL of each solution and make it up with 10 mL of ethanol and the concentration of the solution is 10μg/mL. The chromatogram obtained for 10μg/mL ESC and ETZ is depicted in Figure 2.

2.4.3. Preparation of an ESC and ETZ sample solution

Twenty tablets (10 mg ESC, 0.5 mg ETZ) were weighed accurately in a mortar, where they were crushed to a fine powder and evenly mixed. Dissolving weight corresponding to 10 mg of ESC and 0.5 mg of ETZ in 10 mL of ethanol yielded tablets standard solution of ESC and ETZ (mg/mL). The solution was then filtered through a 0.45-micron syringe filter, Sonicated for 5 min, then diluted to 10mL with a solvent system.

2.5. Forced degradation study

The stock solutions of ESC and ETZ were subjected to degradation studies. The conditions of stress studies include acid (1 M HCl, room temperature for 1 h), alkali (1 M NaOH, room temperature for 12 h), oxidative (10% H$_2$O$_2$ at room temperature for 12 h), photolytic degradation (UV chamber at 27°C for 48 h) and thermal degradation (70°C for 48 h)

Acid Degradation: In order to create up to 10 mL for acid studies, 1 mL of stock solutions and 1 mL of 1M HCl was combined and maintained at room temperature for 1 h.

Alkali Degradation: Combine 1 mL of stock solutions and 1 mL of 1NaOH in a standard flask. Let the mixture sit at room temperature for 1 h before emulsifying to form 10 mL.

Oxidative Degradation: Prepare 10 percent H$_2$O$_2$ by adding 10 mL of H$_2$O$_2$ to a 100 mL standard flask for oxidative studies. Add 1 mL of stock solution and 1 mL of 10% H$_2$O$_2$ to create up to 10 mL in a standard flask held at room temperature for 12 h.

Photolytic Degradation: 1 mL of stock solution in a 10 mL standard flask stored in a UV chamber at 27 °C for 48 h is used for photolytic studies.
Thermal Degradation: 1 mL of the stock solution in a 10 mL standard flask was heated to 70 °C for 48 h.

3. Results and discussion

Developing an analytical approach while sticking to GAC concepts was a game-changing concept for a long-term, eco-friendly strategy. The development of a greener analytical approach that does not rely on the AQbD methodology, on the other hand, may result in poor method performance and need revalidation. Combining these concepts in the HPLC technique improves the system’s stability and long-term viability. As a result, we have integrated all three ways to create an environmentally beneficial and reliable design. This is how the entire process of creating this revolutionary approach went.

3.1. Computer-aided optimization

DOE (Design of experiments) is a technique for determining the best composition parameters. It has been used to determine the significant impacts and how they interact. CCD (Central composite model) is an RSM component that shows a quadratic response surface without a three-level factorial design. Based on early empirical investigations of liquid chromatography technique development, the critical parameters and empirical levels are currently being investigated for optimization. Table 1 shows the results of twenty tests with five center points and three parameters for ESC and ETZ. The factor was boosted in this area of Concentrations ranging from 55–65 percent v/v, pH 3–4, and flow rate fluctuations of 0.8-1.2 mL/min. The ESC generation capacity (k1), the retention length of the highest maximum ETZ (tR2), and the ESC and ETZ resolutions (Rs1, 2) were used as replies. In the early investigation, the resolutions between peaks (Rs1, 2) were more than 1.5. As a result, these two peaks were chosen as crucial peaks and used in response tests to help with optimization.

3.2. Methods using CCD for optimization of chromatographic condition

CCD is adaptable and may be used to optimize HPLC separations utilizing various variables. A 3 factorial experimental design was carried out using 15 empirical and 5 center points. Ethanol concentration (A), flow rate (B), and pH were among the factors chosen (C) 15 experimental trial runs with optimum results were carried out. Table 1 shows a summary of the replies.

To make the model more straightforward and application-focused, the answers were examined, and inconsequential variables from the model were removed using a backward elimination technique. Table 2 shows the ANOVA and other descriptive statistics for the answers. The statistically significant difference of a model term is indicated by a P – values less than 0.05. Less than 0.5 p-values were obtained for the polynomial terms, suggesting that they substantially impacted the outcomes. Retention times of ESC and ETZ were found to be 3.5 and 6.5 min respectively.

Figure 2. Standard ESC and ETZ chromatograms.
optimization. All of the replies had an accuracy of at least 4 points. Following optimization, the percentage CV determines whether a model can be repeated. A CV% low is usually a plus when generating consistent outcomes with minor variance. Table 2 shows that the replies have a lower % CV. The ANOVA calculation aids in constructing a polynomial equation using the model to anticipate responses at a particular factor level. For the replies, similar equations were created to predict the outcomes. The response perturbation graphs allow for a comparison of all factors with their relative responses simultaneously. Figure 3 shows the relevant perturbations charts for the replies.

Optimization techniques were used to produce a maximum separation, while retaining the component values at desired levels. The optimization was carried out using Derringer’s desirability function. The solution with the highest level of attractiveness was chosen and tested experimentally from the software’s findings. Figure 4 illustrates the most astounding computation of Derringer’s desirability function for the response surface. Table 3 shows the results of evaluating experimental and software projected values to determine the model’s predictability. The formula was used to compute the percent predicted error. Errors that were predicted proved to be relatively small. This demonstrates that the model’s repeatability and dependability can be used in an industrial setting to separate ESC and ETZ effectively.

### 3.3. Validation of the methodology

The devised HPLC technique for determining ESC and ETZ simultaneously was verified according to the ICH guidelines. The following criteria are investigated as part of process validation per ICH recommendations. Under findings and comments, each parameter was detailed in its section.

1. Suitability and Precision of the System 2. Studies of Specificity a) Blank b) Forced deterioration experiments under various stress settings to demonstrate the established method’s stability indicator 3. Method Accuracy 4. Studies on precision 5. Linearity studies, including the calculation of LOD/LOQ 6. Toughness 7. Stability 8. Using the described procedure, analyze marketed samples.

#### 3.3.1. Linearity

The purpose of the linearity method is to provide findings that are accurate proportionate to sample

### Table 1. CCD experimental design and measured responses.

| Runs | A: Ethanol (% v/v) | B: Flow rate (mL/min) | CpH | Capacity factor (k) | Retention time (Rt₆) | Resolution (RS₁₂) |
|------|-------------------|-----------------------|-----|---------------------|---------------------|------------------|
| 1    | 68.40             | 1.00                  | 3.50| 0.65                | 4.30                | 4.60             |
| 2    | 55                | 0.80                  | 3.00| 1.21                | 7.43                | 11.80            |
| 3    | 60                | 1.00                  | 2.65| 1.01                | 8.34                | 9.30             |
| 4    | 60                | 1.00                  | 3.50| 1.01                | 6.50                | 10.56            |
| 5    | 65                | 0.80                  | 3.00| 0.72                | 4.60                | 6.80             |
| 6    | 60                | 0.66                  | 3.50| 1.02                | 4.70                | 11.80            |
| 7    | 55                | 1.20                  | 3.00| 1.10                | 8.50                | 12.20            |
| 8    | 65                | 0.80                  | 4.00| 0.91                | 4.80                | 6.80             |
| 9    | 60                | 1.00                  | 3.50| 1.01                | 6.58                | 10.82            |
| 10   | 65                | 1.20                  | 4.00| 0.90                | 4.50                | 7.00             |
| 11   | 60                | 1.33                  | 3.50| 0.70                | 4.30                | 8.80             |
| 12   | 55                | 1.20                  | 4.00| 1.23                | 9.70                | 12.60            |
| 13   | 60                | 1.00                  | 3.50| 1.01                | 6.80                | 10.72            |
| 14   | 60                | 1.00                  | 3.43| 1.54                | 8.90                | 13.40            |
| 15   | 60                | 1.00                  | 3.50| 1.01                | 6.58                | 10.24            |
| 16   | 60                | 1.00                  | 3.50| 1.09                | 6.80                | 11.76            |
| 17   | 60                | 1.00                  | 3.50| 0.98                | 6.30                | 10.84            |
| 18   | 65                | 1.20                  | 3.00| 0.89                | 4.40                | 8.60             |
| 19   | 51.59             | 1.00                  | 3.50| 1.69                | 11.00               | 12.60            |
| 20   | 55                | 0.80                  | 4.00| 1.85                | 8.67                | 15.00            |

### Table 2. ANOVA and Regression summary of models.

| Response | Model      | Y (Equation model)                                      | R² (Adjusted) | P-value | % CV | Precision (Adequate) |
|----------|------------|--------------------------------------------------------|---------------|---------|------|----------------------|
| Capacity factor (k) | Quadratic | +1.02-0.27* A-0.081* B+0.14* C+0.11* AB-0.071* AC-0.086* BC +0.052* A²-0.057* B²+0.090* C² | 0.9779 | <0.0001 | 5.87 | 27.11 |
| Retention time (Rt₆) | Quadratic | +6.61-2.00* A+0.067* B+0.26* C-0.33* AB-0.28* AC-0.015* BC-0.31* | 0.9862 | <0.0001 | 4.86 | 29.25 |
| Resolution (RS₁₂) | Quadratic | +10.81-2.64* A+0.64* B+0.50* AB-0.64* AC-0.56* BC-0.76* +A²-0.17* B²+0.20* C² | 0.9385 | <0.0001 | 8.58 | 17.90 |
concentration within a certain range. In the recommended concentration ranges of 5–30 μg/mL for ESC and 2–12 μg/mL for ETZ, the correlation coefficients of ESC and ETZ were \( r^2 = 0.9975 \) for ESC, \( r^2 = 0.9987 \) for ETZ and Table 4 and Figure 5 shows the analytical results.

3.3.2. Precision

Precision was performed twice in one day. For the peak locations of ESC and ETZ, the average percent RSD (Relative Standard Deviation) values were calculated. The precision was more than two, indicating high accuracy Table 4.

3.3.3. Accuracy

The percentage recovery for ESC was 98.93 percent, and for ETZ, it was 99.21 percent, both of which were within the permissible range of 100–2 percent. This was done after the spiking, with three concentrations from the standard of 50%, 100%, and 150 percent.

3.3.4. Limit of quantification and detection

To evaluate the method’s sensitivity, the Limit of Detection and Limit of Quantification were obtained. The LOD and LOQ calculated using the formulas below were in

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Figure 3. (a) Drug effects capacity factors – ESC, ETZ – shown in a perturbation graph. (b) Diagram displaying the Retention time of drugs – ESC, ETZ. (c) Perturbation graph showing the Retention of drugs – ESC, ETZ.

Figure 4. Graphical representation of the overall desirability function.

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ESC and 2–12 μg/mL for ETZ, the correlation coefficients of ESC and ETZ were \( r^2 = 0.9975 \) for ESC, \( r^2 = 0.9987 \) for ETZ and Table 4 and Figure 5 shows the analytical results.
agreement with ICH guidelines.

\[
LOD = 3.3 \times \frac{\sigma}{S}
\]

\[
LOQ = 10 \times \frac{\sigma}{S}
\]

\(\sigma\) = the standard deviation of the response.
\(S\) = the slope of the calibration curve.

3.4. Analysis of marketed dosage form

The marketed dose version, which included 10 mg ESC and 0.5 mg ETZ, recovered well. The recovery percentages for ESC and ETZ were determined to be 99.55 and 99.94 percent, respectively. The assay of the commercial sample was calculated by comparing the areas of standard and sample peaks. The assay of marketed formulation ETIZOLA PLUS found within limit. This demonstrates that this approach may examine the quality of pharmaceutically prescribed tablet dosage forms. Table 4 and Figure 5 shows the results of the computation.

3.5. Forced degradation studies

According to forced degradation studies, ESC degraded the most under photo degradation stress, followed by significant degradation in an acidic environment. ETZ’s percentage recovery dropped to about 29.28 percent in an alkaline environment, suggesting the drug’s sensitivity to alkali. In other stressful situations like oxidation and dry heat, the medicines were shown to be steady. Figure 6 (A-F) shows the overlapping chromatograms of conventional ESC and ETZ and their stressed samples. The information for stress studies is summarized in Table 5.

4. Greenness assessment for the developed method

4.1. Green assessment for the developed method

For the examination of two medicines, the approach combines tri-combinations. In creating methods, each of these three aspects is equally important. A designed solution cannot merely claim to be environmentally

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Table 4. Validation of analytical parameters using HPLC.

| Parameters                              | ESC     | ETZ     |
|-----------------------------------------|---------|---------|
| Linearity range (µg/mL)                 | 5–30    | 2–12    |
| Correlation coefficient                 | 0.9975  | 0.9987  |
| Slope                                   | 3456.8  | 1564.8  |
| Intercept                               | 567.7   | 678.3   |
| Limit of Detection (µg/mL)              | 0.176   | 0.326   |
| Limit of Quantification (µg/mL)         | 0.456   | 0.987   |
| System precision (% RSD)                | 1.342   | 1.256   |
| Assay of marketed formulation (Etizola plus) | 10mg    | 0.5mg   |
| Label claim                             | 98.93   | 99.21   |
| Amount found                            | 9.88mg  | 0.49mg  |
| % of Assay                              | 99.55   | 99.94   |
| % Recovery (w/w)                        | 98.93   | 99.21   |

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Figure 5. Sample chromatogram of ESC and ETZ.
Figure 6. (a) Blank. (b) Acid Hydrolysis. (c) Alkali Hydrolysis. (d) Oxidation. (e) Photo Degradation. (f) Dry Heat. (a-f) Typical Chromatogram of ESC and ETZ (10 mg/mL) with Stress test.
beneficial without being evaluated using appropriate assessment methods in this competition. The method's greenness was analyzed using four evaluation tools: NEMI (National Environmental Methods Index), GAPI (Green Analytical Procedure Index), AGMS (Analytical Method Greenness Score), and AGREE (Analytical GREEnness Metric). Every instrument seems to have its number of capabilities, disadvantages, and assessment methods. The results of each assessment tool may result in a different impact on which strategy is the most environmentally friendly and which evaluating technique to adopt. Even though numerous tools were employed in this technique evaluation process, all of the outcomes were shown in a single eco-friendly greenway. The following is how the approach was evaluated:

4.2. NEMI

Nemi is a well-known qualitative evaluation technique for assessing green chemistry. At first, it was the only tool available for evaluating GAC approaches. Despite developing new assessment tools for the GAC, NEMI has advantages in looking at the green analytical method. NEMI is shown as a four-quadrant circular symbol with color matching (green and colorless). Quadrant one must deal with the EPA’s TRI (Toxic Regulatory Inventory) list of Persisting Bioaccumulative Toxic (PBT) chemicals (29). In contrast, quadrant two must deal with the Toxic Compounds Regulation Inventory (TRI) list of PBT chemicals. This quadrant is tinted green since the substances used in this method are not listed in PBT. Hazardous compounds, which are controlled mainly through Environmental Protection Agency (EPA) under the RCRA, occupy the second quadrant (Resource Conservation and Recovery Act) (30).

Several substances on the RCRA list were also found in this method; thus, the second quadrant is labeled green. In order to qualify as green, the pH of the analytical solutions in the third quadrant must be below a specific range, and the mobile phase ethanol and phosphate buffer in a 60:40% v/v pH must also be below a specific range; hence, the third quadrant is a green zone. The fourth quadrant focuses on waste, which should be less than 50 g or mL altogether. The third quadrant was given a green tint since the loss in this manner is small due to the recycling process. The primary NEMI picture of a method is shown in Figure 7.

4.3. GAPI

GAPI is a slightly modified version of NEMI with 11 classifications and a color-coded approach signify hazard, tolerance, and environmental friendliness using red, yellow, and green. In his paper, the method is (31, 32) made assessment using GAPI easier by producing free software. The method specifics which need to be evaluated must be entered into the program, which contains 11 straightforward stages to obtain the outcome. Figure 7 depicts the obtained result, demonstrating the method’s viability and future potential.

4.4. AMGS

An alternative method of evaluation for safety, health, and the environment is the HPLC environmental assessment (HPLC-EAT) and SHE (Safety, Health, and Environmental Assessment) are included with AMGS. There were three categories in the AMGS: equipment, solvent energy, and solvent environmental health and safety (33). The method’s overall score is calculated by adding these three scores together, and that should be as minimal as possible to consider making the method as green as possible. The final result procured for the proposed approach was 1242.90 Figure 7, which showed a positive effect of the developed model on the environment after feeding the data required into the calculator made available by only AES Green Computing University for the green evaluation (34).

4.5. AGREE with metrics

The newest green assessment tool, AGREE with metrics (35, 36) encompasses all twelve green analytical concepts. The total outcome, which was represented as 1, the individual principles score obtained from the individual’s rights have been the emphasis. The method’s
greenness is indicated by a rating closer to one. Figure 7 displays the overall outcome after inputting the procedure specifics into the program. The method’s impact on the environment has been described as ‘extremely benign’ and ‘long-term sustainable.’ The primary purpose was to identify the method’s sustainability, although five evaluation tools employed diverse methodologies or processes to analyze the method’s greenness. Regardless of their tactics, all of the methodologies indicated that this technique is environmentally benign and flexible to future green assessment without any problems (37).

5. Conclusion

ESC and ETZ in pharmaceutical formulations dosage forms were identified by developing a GAC-based HPLC method based on stability indicators. In order to do so, AQbD was used to develop a technique that may be applied in the future forever without having to be revalidated. We understood the effects of the technological parameters on the outcomes by proposing a flow rate of 1mL/min and ethanol modification by the mathematical model. The created approach had the quickest duration and the best resolution between two medications when used. The degradation peaks obtained from forced degradation investigations also demonstrate the improved resolution and isolation of ESC and ETZ as from degradation products when utilizing the suggested HPLC approach. The developed analytical technique was evaluated for repeatability, accuracy, specificity, and linearity at the working point was selected and confirmed within the limits. Finally, the most environmentally friendly approach utilized four green assessment methods: NEMI, GAPI, AMGS (1242.90), and AGREE with metrics (0.78). This technique will also help commercial and industrial lab research and testing departments adopt and evaluate the various combination in mass and dosage forms for tablets. AQbD-based approaches for analyzing chemicals in
green solvents might be adopted and improved by the scientific community as a result of the findings of this study.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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