# Optimized and Validated RP-HPLC Method for the Estimation of Ethosuximide in Pure and Pharmaceutical Formulation: Evaluation of Green and White Analytical Chemistry Principles

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## ABSTRACT

**The aim was to develop and validate a coherent and sustainable reverse-phase high-performance liquid chromatographic method for the quantification of Ethosuximide in oral solution. The partitioning was achieved using a stationary phase Shimpak solar $C_{18}$ column (4.6 × 250 mm, 5 µm) with the mobile phase (60% acetonitrile and 40% water), flow rate (1 mL/min) and wavelength (210 nm). The developed method was validated as per ICH $Q_2R_1$ guidelines. The optimized method was evaluated for greenness and whiteness using analytical tools: AES, AGREE, MoGAPI, BAGI, RAPI, RGB fast models and compared to the reported methods. Ethosuximide was eluted at 3.445 min. The method was linear at 10-70 µg/mL with $r^2$ = 0.9995. All the validation parameters were within the accepted values. The projected HPLC method met the requirements for effectiveness, greenness, efficiency, analytical performance, and productivity in terms of green and white analytical chemistry principles. Moreover, the optimized and validated analytical method was significantly better compared to the two reported methods on the basis of analytical performance, eco-friendliness and throughput. The results of the developed analytical method are reproducible, efficient with excellent chromatographic properties and effectively employed for regular quality assessment of Ethosuximide in oral solution.**

## KEYWORDS

**Green and White analytical chemistry; Ethosuximide; Validation; Sustainability; ICH $Q_2R_1$**

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## INTRODUCTION

Epilepsy is associated with serious physical, mental health and cognitive comorbidities. Anti-epileptic drugs (AEDs) are used as single or combination drug therapy in the management of several forms of epilepsy.[1] Among the existing AEDs, Ethosuximide (Figure 1) is the leading choice for the handling of petit mal and myoclonic seizures due to its efficiency and a typically acceptable side effect profile.[1] Ethosuximide exhibits its pharmacological actions by affecting neuronal excitability through blocking the T-type calcium channel present in thalamic neurons, leading to a reduction in the abnormal rhythmic activity and thus normalize absence seizures.[1] In a recent study, Ethosuximide has found to be associated with an increased mood improvement and reduction of anxiety compared to other drugs employed for treating epilepsy.[2]

RP-HPLC is a well-developed and widely used analytical technique for separating and analysing various compounds, including pharmaceuticals, biomolecules and environmental samples.[3] The solvents used for routine analytical processes in large quantities may cause hazardous, toxic effects and are costly to use. These can be minimized by applying Green analytical chemistry approaches. Green Analytical Chemistry progressed as an exploration for practical replacements to the off-line waste and residues treatment, by replacing polluting approaches with clean ones. It incorporates several approaches to reduce or exclude the use of lethal substances and the waste generation.[4] On the other hand, white analytical chemistry (WAC) principles are an improvised version of GAC principles. White Analytical Chemistry takes into account of analytical performance (red) and practical throughput (blue) apart from regular evaluation of environmental friendliness (green). Various algorithmic tools are used to determine the ecological greenness and overall whiteness of the developed analytical methods.[5] Tools like, Analytical Eco-Scale (AES), Modified Green Analytical Procedure Index (MoGAPI), Analytical Greenness calculator (AGREE), Blue Applicability Grade Index (BAGI), Red Analytical Performance Index (RAPI) and Red-Green-Blue (RGB) fast models were used.

Several analytical methods were reported for the determination of Ethosuximide either alone or with other antiepileptics such as UV-spectrophotometry, HPLC, LC/MS, and GC/MS.[6-9] The literature study revealed that only two RP-HPLC methods have been published and are mainly concentrated on forced degradation, profiling of related substances, and impurities.[6-7] These reports possess some drawbacks such as, the use of more than two solvents, higher retention time, and higher consumption of solvents. Therefore, a simple, quick, efficient, eco-friendly and validated RP-HPLC method for estimating Ethosuximide in pure and oral solution to improve the drawbacks of previously published methods. The Greenness and Whiteness of the optimized analytical method were evaluated using AES, AGREE, MoGAPI, BAGI, RAPI and RGB models. Further, comparison of proposed and two reported analytical methods was also carried out in these lines. To the best of our knowledge, this is the first report to combine green and white analytical assessment tools for the quantification of Ethosuximide, offering an environmentally responsible and practically feasible alternative to previously published methods.

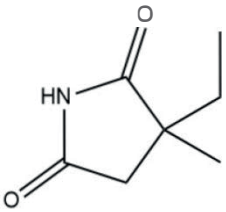



**Figure 1:** Structure of Ethosuximide.

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## EXPERIMENTAL

### Instrumentation

The study was carried out using a Shimadzu prominence LC-20 AD with PDA connected to Lab solution software, spectrophotometer (LAB India- 3000+), ultrasonic water bath (Power Sonic 405), digital weighing balance (Shimadzu ATX224R) and micro-pipette (10-1000 µL from Eppendorf) based on the requirements of analytical work.

### Chemicals and dosage form

Ethosuximide was obtained as a gift sample for research purposes from industry. Absenz (Ethosuximide oral solution IP) was purchased from Sun Pharma Laboratories Pvt. Ltd. High performance liquid chromatography-grade water and acetonitrile (purchased from Thermo Fisher Scientific, Mumbai, India), methanol (purchased from S D Fine-Chem Limited, Mumbai, India) were used throughout this study.

### Standard solution preparation

Ethosuximide (10 mg) was weighed and transferred into a 10 mL volumetric flask, dissolved using diluent and sonicated for 10 min, and the volume was made up to the mark with 60% acetonitrile: 40% water (1000 µg/mL). The sub-stock was prepared by pipetting 1 mL of the above solution into a 10 mL volumetric flask with diluent. The final concentration of 30 µg/mL was prepared by taking 3 mL from the sub-stock into a 10 mL volumetric flask, sonicated for 10 min and made up to the mark using diluent.

### Sample solution preparation

The Absenz oral solution contains 250 mg of Ethosuximide in 5 mL. For the preparation of stock solution, 2 mL of Ethosuximide oral solution was pipetted into a 10 mL volumetric flask, and diluted with mobile phase, sonicated and filtered with a PES filter membrane media syringe. For sub-stock, 1 mL of the above stock solution was transferred to a 10 mL volumetric flask and made up with diluent and sonicated. Then, 3 mL of sub-stock solution was transferred to a 10 mL volumetric flask, diluted and made up to the mark with mobile phase to give a concentration of 30 µg/mL.

### Method development

The selection of the mobile phase for Ethosuximide depended on its physical and chemical properties. Various solvents such as methanol, acetonitrile, water and glacial acetic acid were used with varying ratios to get a suitable, stable, mobile phase composition. To develop and optimize the chromatographic conditions, fine-tuning of flow rate and mobile phase composition was attempted.

### Method validation

HPLC method validation involved finding and demonstrating that a method consistently produces expected, reliable and accurate results under specified conditions, while meeting all of the validation parameters outlined in the ICH $Q_2R_1$ guidelines including system suitability, specificity, linearity, instrumental precision, method precision, accuracy, robustness, LOQ, LOD and ruggedness.

### Greenness and Whiteness evaluation of the analytical method

Evaluating the greenness and whiteness of the analytical method was done by assessing the impact of the optimized analytical method on environmental, efficiency and practical performances. These evaluations are critical in modern analytical chemistry to ensure sustainability, safety and effectiveness. Greenness and Whiteness of the developed and reported analytical methods were evaluated using the following tools: Analytical Eco-Scale, AGREE, MoGAPI, BAGI, RAPI and RGB fast model. Analytical Eco-Scale is a semi-measurable tool used to assess the greenness of the analytical method in accordance with the twelve principles of green analytical chemistry. It begins with a perfect score of 100 points, which reflects an ideal green method. Penalty points are distributed based on parameters such as, type and amount of solvents/reagents used and the quantity consumed in proposed and reported methods, energy utilization, occupational hazard, waste production and treatment, and finally safety. The amount of waste generated was calculated by multiplying the run time against the flow rate of a single analysis. Penalty points for waste treatment were given based on the type of treatment adopted for waste management. An eco-scale score above 75 was considered as an excellent green method.[10] AGREE is a simple and easy tool to use to assesses the results of analytical methods. It gives a single score from 0-1 with a score of one considered as perfectly green. AGREE is a circular 12-segment clock shaped pictogram with each segment representing one of the twelve principles of green analytical chemistry. Each segment is colour coded as follows: green (good), yellow (moderate) and red (poor). The AGREE score is presented in the centre of the pictogram. A score close to 1 infers a green method.[11] Modified GAPI is an advanced and realistic tool to overcome the shortcomings of the present GAPI metric. MoGAPI covers a more detailed evaluation of greenness by calculating a total score. A score greater than 75 indicates an excellent green method, 50-75 acceptable green method and less than 50, an inadequate green method.[12]

Blue Applicability Grade Index (BAGI) is considered a well-established tool for green metrics and predominantly concentrates on the practical side of white analytical chemistry. It is a star-shaped pictogram with ten main attributes and a score displayed in the center having a scale of 25-100, that illustrates the functionality and applicability of the optimized method. The inner portion of the pictogram (1 to 5) reflects either the number of steps for sample preparation or the stages for analytical determination, such as the kind of analysis, analytical apparatus, number of analytes analyzed concurrently, and sample preparation steps. The outer portion of the pictogram, from 6 to 10, included the total number of samples investigated per hour, the nature of the materials and reagents used in the analytical method, the level of automation, and the quantity of sample used. Each part of the star-shaped pictogram is assigned a distinct colour based on a score: for high compliance the score is 10 (dark blue), 7.5 for medium compliance (blue), 5.0 for poor compliance (light blue), and 2.5 for no compliance (white). The overall score for analytical method performance is: 100 for outstanding and 25 for the worst. At least 60 points are recommended for any analytical method to be considered as Green and White.[13]

The Red Analytical Performance Index tool is used to deliver multifaceted information about wide-ranging analytical parameters. This tool considers ten parameters for score distribution, which includes intermediate precision, repeatability, reproducibility, recovery and matrix effect, LOQ, working range, trueness, ruggedness/robustness and selectivity. The scoring scale has five levels, which are as follows: a score of 0 is white, scores of 2.5, 5.0, and 7.5 are an intermediate colour, and 10 is dark red. Furthermore, a score of zero means the result is unacceptable. The total of all the scores falls which should be between 0 and 100, is displayed in the central portion of the pictogram.[14] The Red-Green-Blue (RGB) fast model is a well-programmed Excel spreadsheet that facilitates capturing the data for analytical method.[14] This tool is divided into three sections having different colours: red represents efficiency of analytical method, green represents environmental safety and blue signifies analytical productivity. Analytical performance (redness) is evaluated by three parameters including R1-for trueness represented by recovery or relative error, R2-repeatability or precision expressed in %RSD of the analytical results obtained within a day, and R3-for LOD. For greenness assessment, the ChlorTox Scale (G1) is used, which permits the estimation of total risk associated with the

use of reagents or chemicals. The blue parameter B1 indicates the sample throughput. It represents the number of analyses carried out per 24 hours. Another criterion: G2/B2 is related to the cost and energy consumption of an instrument.[15]

## RESULTS AND DISCUSSION

### Chromatographic trails

The total of eight chromatographic trails were carried out. In the first five trails, various solvent combinations consisting of methanol: water (40:60 v/v), methanol: water (60:40 v/v), methanol: water (65:35 v/v), methanol: water (70:30 v/v) and methanol: water with 1% v/v acetic acid (65:35 v/v) were done. All data from these trails were rejected based on higher retention time of Ethosuximide, reproducibility, appearance of extra peaks, interference with the blank and long stabilization time with high baseline noise. Moreover, using acetonitrile: water in the ratio of 40:60 and 50:50 v/v were also not considered due to high retention time and reproducibility. However, trial 8 demonstrated good chromatographic conditions for the selective and specific analysis of Ethosuximide in formulation and demonstrated excellent adherence to the guidelines. Therefore, trial 8 was chosen for the further experiments.

### Optimized analytical method

The RP-HPLC method was developed using the Shimpak Solar $C_{18}$ column (4.6 × 250 mm, 5 µm) with 60 % ACN and 40 % water; 1 mL/min flow rate and 210 nm analytical wavelength. The Ethosuximide peak was observed at 3.445 min having a tailing factor of 1.28 and theoretical plates of 9727 (Figure 2B).

### Method validation - System suitability

Six injections of standard Ethosuximide (30 µg/mL) exhibited consistent results (Table 1). The percentage RSD values for all the critical chromatographic parameters are within the acceptable range (% RSD < 2.0).

### Specificity

The chromatogram of the standard and sample showed identical retention times which is an indication of the absence of interference (Figure 2A-C). The peak purity index of Ethosuximide oral solution was 0.999 (Figure 2D). This further confirmed that the components of the oral solution did not interfere with Ethosuximide peak.

### Linearity

The standard calibration curve was plotted as mean peak area vs the different concentrations of Ethosuximide in the range of 10-70 µg/mL with correlation co-efficient ($r^2$) 0.9995 (Figure 3) showing the results were within the acceptable standards.

### Accuracy

The accuracy was confirmed by spiking the standard solution at three levels (80%, 100%, 120%) in the diluted sample solution. The results

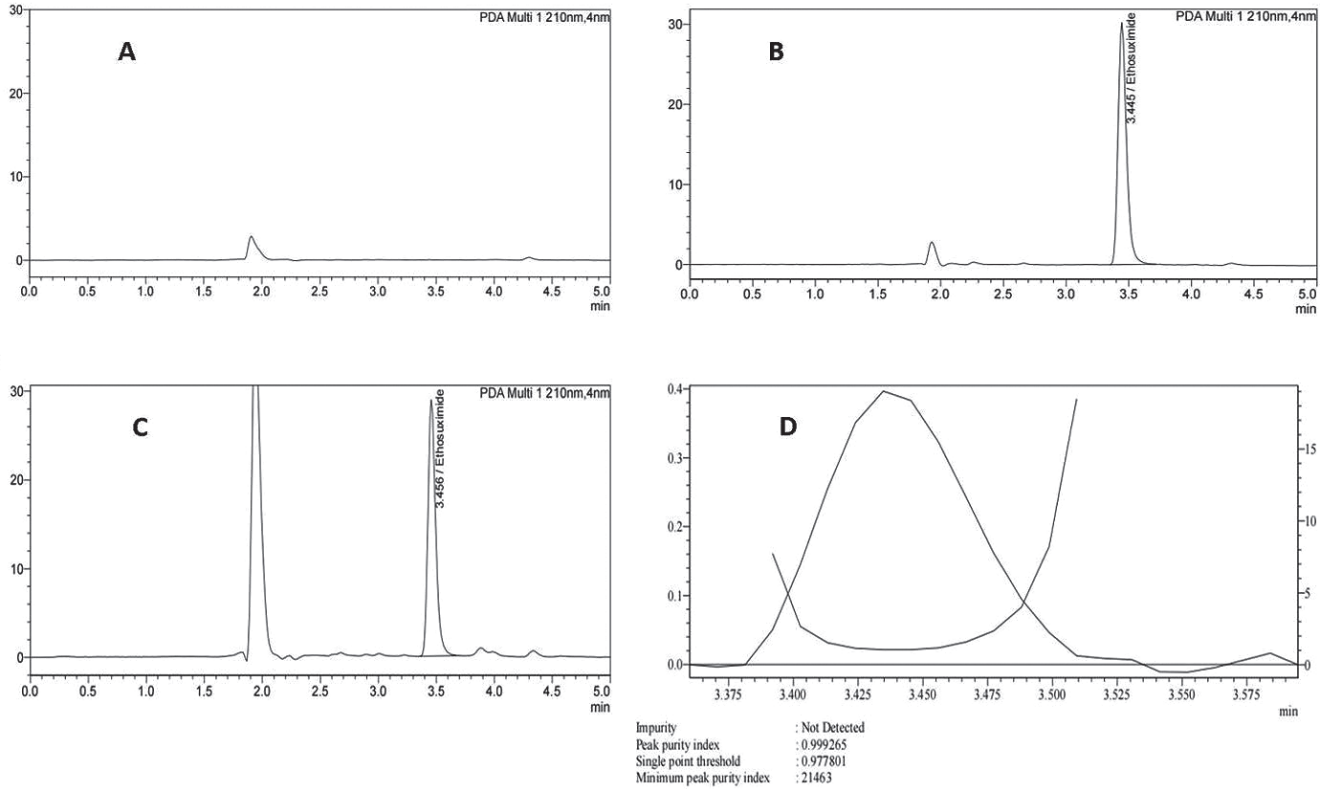



**Figure 2:** A) Blank chromatogram; B) Ethosuximide standard (30 µg/mL; RT 3.445 min; NTP 9896; TF 1.2); C) Ethosuximide sample (30 µg/mL; RT 3.456 min; NTP 9954; TF 1.2); and D) Peak purity profile of Ethosuximide.

**Table 1.** System suitability of Ethosuximide

| Injection (n=6) | Retention time | Peak area | Tailing factor | NTP |
|---|---|---|---|---|
| Mean | 3.44 | 132917 | 1.322166 | 9817.5 |
| **SD** | 0.0055 | 1630.53 | 0.024879 | 95.6843 |
| **% RSD** | 0.1592 | 1.2267 | 1.8817 | 0.9746 |

of % recovery (Table 2) for the three levels are within the acceptable range (98%-102%).

### Instrument precision and method precision

Repeatability was confirmed by consistent results for all the investigated chromatographical parameters with six injections. Intermediate precision was established by evaluation of intra- and inter-day experiments. Similarly, the method precision was measured by performing an assay on both the intra- and inter-day experiments.

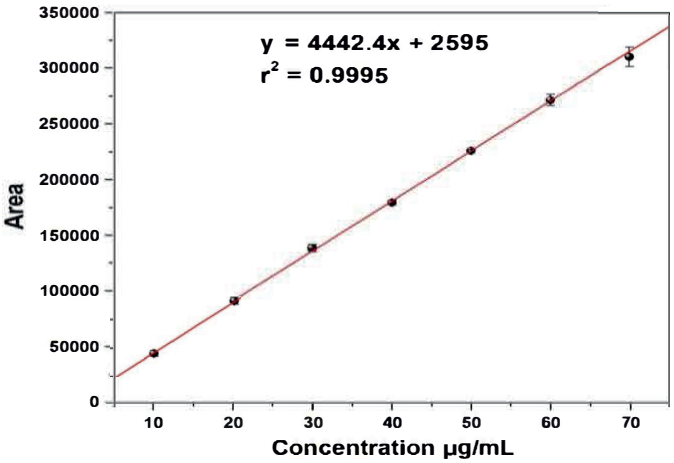



**Figure 3:** Standard calibration curve of Ethosuximide.

All the critical chromatographic parameters were within the acceptance criteria. Thus, all of the precision data revealed that the proposed analytical method is reproducible (Table 3).

### Robustness

The robustness was done by introducing minor variations in the experimental settings such as a change in the flow rate (± 0.1 mL/min), mobile phase composition (± 5 mL), and wavelength (± 2 nm). The % RSD for all three factors were inside the acceptance limit of less than 2 (Table 4).

### Ruggedness

This test required two different analysts to carry out the experiments which involved six replicate injections of Ethosuximide into the HPLC. The ruggedness results (Table 5) showed that the % RSD for all the parameters, performed by both analysts, are within the acceptable range of less than 2.

### Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ were determined to be 1.74 µg/mL and 5.81 µg/mL, respectively, based on the signal to noise ratio.

### Statistical comparison of the proposed and reported method

A statistical comparison of the assay results from the proposed and reported methods was carried out using the Student t-test (One tail)

**Table 2.** Accuracy results

| Spike level | Sample taken (µg/mL) | Standard conc. added (µg/mL) | Concentration found (µg/mL) | % Recovery | Average recovery ± SD | %RSD |
|---|---|---|---|---|---|---|
| | | 24 | 24.21 | 100.85 | | |
| 80% | 30 | 24 | 24.22 | 100.92 | 101.18 ± 0.51 | 0.5061 |
| | | 24 | 24.43 | 101.77 | | |
| | | 30 | 30.45 | 101.47 | | |
| 100% | 30 | 30 | 30.24 | 100.80 | 101.12 ± 0.33 | 0.3322 |
| | | 30 | 30.33 | 101.09 | | |
| | | 36 | 36.46 | 101.02 | | |
| 120% | 30 | 36 | 36.01 | 100.05 | 100.77 ± 0.60 | 0.6034 |
| | | 36 | 36.42 | 101.17 | | |

**Table 3.** Precision results

| Repeatability (n=6) | | | | |
|---|---|---|---|---|
| Mean RT ± SD (% RSD) | 3.44 ± 0.005 (0.1592) | | | |
| Mean Area ± SD (% RSD) | 132917 ± 1630.53 (1.2267) | | | |
| Mean TF ± SD (% RSD) | 1.3221 ± 0.02 (1.8817) | | | |
| **Instrumental Precision (30 µg/mL) (n=6)** | | | | |
| Chromatographic parameters | Intra-day | | Inter-day | |
| | Time point 1 | Time point 2 | Day 1 | Day 2 |
| Mean RT ± SD (% RSD) | 3.4383 ± 0.0052 (0.1502) | 3.4505 ± 0.0060 (0.1746) | 3.4383 ± 0.0052 (0.1502) | 3.44 ± 0.0055 (0.1592) |
| Mean Area ± SD (% RSD) | 133655.8 ± 2621.562 (1.9614) | 132044.3 ± 1267.643 (0.9600) | 133655.8 ± 2621.562 (1.9614) | 132916.8 ± 1630.676 (1.2268) |
| Mean TF ± SD (% RSD) | 1.3225 ± 0.0252 (1.9055) | 1.2661 ± 0.0214 (1.6927) | 1.3225 ± 0.0252 (1.9055) | 1.3223 ± 0.0248 (1.8792) |
| Method Precision using oral solution (30 µg/mL) (n=6) | | | | |
| Mean % Assay ± SD | 98.94 % ± 0.38 | 98.97% ± 0.31 | 98.94 % ± 0.38 | 99.20 % ± 0.41 |

**Table 4.** Robustness results

| Parameter | Mean RT ± SD (% RSD) | Mean area ± SD (% RSD) | Mean TF±SD (% RSD) |
|---|---|---|---|
| Change in flow rate (1 ± 0.1 mL/min) | | | |
| 0.9 mL/min | 3.936 ± 0.000 (0.000) | 158650 ± 2382.963 (1.5020) | 1.4246 ± 0.0200 (1.4092) |
| 1.1 mL/min | 3.2463 ± 0.0051 (0.1590) | 133116.7 ± 898.5748 (0.6750) | 1.3623 ± 0.0161 (1.1832) |
| Change in mobile phase (60:40 ± 5%) | | | |
| 55:45 (ACN: Water) | 3.6081 ± 0.0030 (0.0848) | 148923.3 ± 743.0838 (0.4989) | 1.2885 ± 0.0116 (0.9034) |
| 65:35 (ACN: Water) | 3.3035 ± 0.00251 (0.0759) | 131478.8 ± 978.2602 (0.7440) | 1.3026 ± 0.0053 (0.4081) |
| Change in wavelength (210 ± 2 nm) | | | |
| 208 nm | 3.4366 ± 0.0040 (0.1187) | 242524.3 ± 4451.173 (1.8353) | 1.3276 ± 0.0234 (1.7661) |
| 212 nm | 3.4401 ± 0.0087 (0.2536) | 92482 ± 1279.657 (1.3836) | 1.3231 ± 0.0235 (1.7810) |

**Table 5.** Ruggedness results

| Parameter | Analyst 1 (n=6) | | | | Analyst 2 (n=6) | | | |
|---|---|---|---|---|---|---|---|---|
| | Retention time | Area | Tailing factor | NTP | Retention time | Area | Tailing factor | NTP |
| Mean | 3.4523 | 131544.5 | 1.2196 | 9959.5 | 3.4366 | 135095.7 | 1.351 | 9842.5 |
| SD | 0.0056 | 1385.921 | 0.0180 | 145.3035 | 0.0040 | 2521.639 | 0.0225 | 77.8684 |
| % RSD | 0.1645 | 1.0535 | 1.4809 | 1.4589 | 0.1187 | 1.8665 | 1.6663 | 0.7911 |

**Table 6.** Statistical comparison of assay data for the proposed and reported method[6]

| Parameter | Proposed method | Reported method[6] |
|---|---|---|
| Assay | 99.22 | 100.19 |
| | 99.10 | 100.35 |
| | 98.50 | 99.20 |
| Mean±SD | 98.94±0.386 | 99.91±0.623 |
| % RSD | 0.390 | 0.623 |
| Student t-test (one-tailed) | t = 2.301; df = 4; P = 0.0414[a] | |
| Variance ratio F test | F = 2.608; df = 2; P = 0.5544[b] | |

Values for three determinations (n=3). [a]Proposed method exhibited significant (P<0.05) difference compared to the reported method[6]. [b]No statistical significance between the proposed and reported method[6].

and the variance ratio F test (Table 6). The statistical comparison reveals that there was a significant difference, using the Student t-test (One tail), between the proposed method and the reported method. However, a comparison using the variance ratio F test, exhibited an insignificant difference.

## Green evaluation of the optimized analytical method

### *Analytical Eco-Scale*

Analytical Eco-Scale data for the developed and reported methods are presented in Tables 7 and 8. The penalty points for reagents/solvents were calculated by multiplying the number of hazard pictograms listed in the material safety data sheet against the score assigned to the signal word (warning =1, danger =2). Here, acetonitrile has two pictograms and the signal word 'danger', hence the proposed method was assigned four penalty points in the reagent utilization section. The energy consumption for a single analysis is considered to be the total power consumption. The developed method consumed less than 1.5 kWh per sample, so 1 penalty point was assigned. For waste production, the penalty points are calculated by multiplying run time multiplied against the flow rate. The proposed method received 3 penalty points, whereas reported methods 1 and 2 received 5 penalty points each. The proposed method involved an oxidative degradation of acetonitrile into non-toxic/safer by-products, hence it received 2 penalty points. However, no waste treatment was specified in the reported methods, therefore each got 3 penalty points. The AEC scores collectively demonstrated that the proposed method had a higher eco-score and better greenness than the reported methods.

### *AGREE model*

The optimized approach has an AGREE value of 0.58, which is greater than the other two reported methods (Table 8). In the developed method, segments 2, 4, 6, and 9 were dark green, indicating a small sample size, few steps in sample preparation (such as sonication and filtering), and no derivatization process that consumed less than 1.5 kWh of energy, respectively. Red for the 3$^{rd}$ segment represented an analytical device was offline. The 8$^{th}$ segment has a light green shade because a single analyte was used per run. Segments 11 and 12 were orange, which was due to the use of hazardous reagents like acetonitrile. Yellow appeared for the 1$^{st}$, 5$^{th}$, 7$^{th}$, 10$^{th}$ segments representing the sampling method (off-line), manual handling of the instrument, waste generation (5 mL), and use of some bio-based reagent.

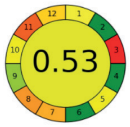
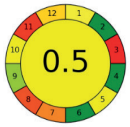
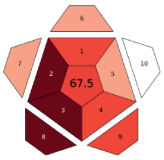
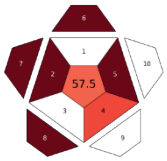
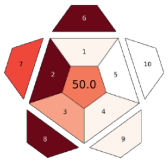
### *Modified GAPI*

Modified GAPI provided the quantification of greenness of an analytical method using a scoring system for each segment as well as for the overall system (Table 8). In the proposed method, greener segments were due to the lack of sample preservation, transportation, storage and additional treatment. Further, the method used less than 10 mL of solvent per run and a hermetic sealing process. The yellow segments were related to simple procedures (filtration, sonication),

**Table 7.** Analytical Eco-Scale score comparison: proposed vs reported methods

| Solvent/Properties | Penalty points (PP) | | |
|---|---|---|---|
| | Proposed method | Reported method 1[5] | Reported method 2[6] |
| Solvents/Reagents utilization | | | |
| Methanol | - | 6 | - |
| Acetonitrile | 4 | - | 4 |
| Glacial acetic acid | - | - | 4 |
| Orthophosphoric acid | - | 4 | - |
| Energy utilization/waste management | | | |
| Energy consumption | 1 | 1 | 1 |
| Waste produced | 3 | 5 | 5 |
| Waste management | 2 | 3 | 3 |
| Total penalty points | 10 | 19 | 17 |
| Analytical Eco-Scale score (100 PP) | 90 | 81 | 83 |

**Table 8.** Overall comparison of the proposed and reported methods

| Parameter | Proposed method | Reported method 1[6] | Reported method 2[7] |
|---|---|---|---|
| Mobile Phase | ACN:Water (60:40) | $NaH_2PO_4$[a]:$CH_3OH$ (90:10) | ACN:Water:GAA[b] (25:75:1) |
| Flow rate | 1 mL/min | 1.2 mL/min | 1 mL/min |
| Retention time | 3.45 min | 4.00 min | 7.20 min |
| Run time | 5.00 min | 15.00 min | 30. 00 min |
| AES | 90 | 81 | 83 |
| AGREE |   |   |   |
| MoGAPI |   |   |   |
| BAGI |   |   |   |
| RAPI |   |   |   |

[a]Concentration 0.05M; [b]Glacial acetic acid.

selected solvent (acetonitrile) and a health-safety NFPA hazard score of 2 or 3. In addition, energy of less than 1.5 kWh was used and less than 10 mL of waste was generated. The remaining red segments were due to offline sample collection, use of non-green solvent (ACN). The developed method displayed a total score of 79 which is considered as green compared to the two reported methods having scores of 70 and 66, respectively.

### *BAGI model*

The proposed method had a comparatively higher Blue Applicability Grade Index (BAGI) score of 77.5 than reported methods 1 and 2 which had scores of 67.5 and 70.0, respectively (Table 8). The BAGI pictogram has ten parts or sections. Sections 1, 5, 6, 7, 8, and 10 are dark blue, revealing that the proposed method was very much compliant for: quantitative analysis; on-site sample preparation; rate of analysis of more than ten samples per hour; use of common commercially available reagents such as ACN; no pre-concentration required and small sample size. The 3rd section had a moderate blue shade since the use of the HPLC-UV instrument implies reasonable compliance. Light blue for the 9th section implies semi-automatic instrumentation. White for sections 2 and 4 was due to single compound analysis and no simultaneous sample preparation (Table 8).

### *RAPI Tool*

The Red Analytical Performance Index (RAPI) focuses primarily on the evaluation of the analytical performance by considering the

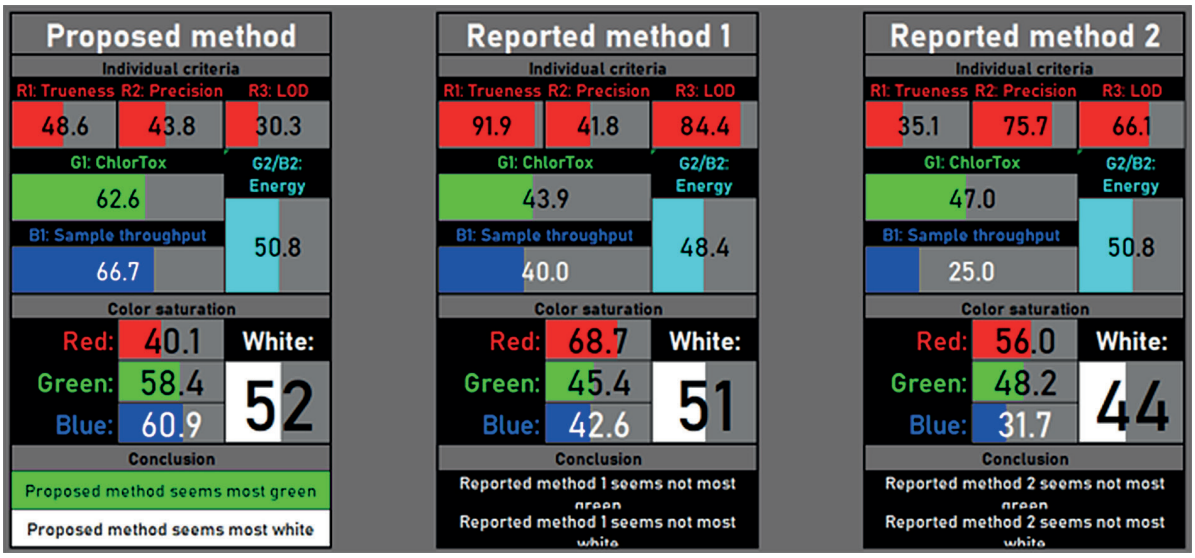



**Figure 4:** Evaluation data for the proposed and reported methods from the RGB fast model.

ten parameters as per the ICH $Q_2R_1$ guidelines. The 2nd, 3rd, and 8th sections were dark red for the proposed method since the % RSD for intermediate precision and reproducibility was less than 10 % and standard linearity was greater than the 0.99 correlation coefficient (Table 8). Sections 1, 4, and 9 were red. Section 1 represented the repeatability which was less than 10% RSD; section 4 showed the trueness (<1.0), and section 9 showed the robustness which consisted of three factors (change in the mobile phase ratio, flow rate, and wavelength). Sections 5, 6 and 7 were a lighter shade of red. Section 10 was white which demonstrated that there was no selectivity. Overall, the proposed method was analytically more efficient (RAPI score 67.5) than the two reported methods (RAPI scores of 57.5 and 50.0, respectively) (Table 8).

### *RGB fast model*

The proposed method had a score of 40.1 for analytical efficiency (Red). The ChlorTox scale, which is related to greenness, was 58.4. The last section, which was blue, exhibited economic efficiency with a score of 60.9 due to the sample throughput. The whiteness score for the proposed method was evaluated by considering all three aspects and was determined to be 52. The proposed analytical method performed significantly better in adhering to white principles than the two reported methods (white scores of 51 and 44, respectively) (Figure 4).

## CONCLUSION

An improved method for estimating Ethosuximide in bulk and oral solution was developed and validated to meet all the pre-determined acceptance conditions of the ICH $Q_2R_1$ guidelines. The developed method is rapid, simple, precise and environmentally friendly, with good analytical performance in terms of green and white metric principles. Therefore, this method can be used for regular quality control analysis of Ethosuximide in pharmaceutical formulations.

## SUPPLEMENTARY INFORMATION

This manuscript does not have Supplementary Information. All the data is presented in the article.

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## AUTHOR CONTRIBUTIONS

Concept: V.P.V.; Design: V.P.V., R.R., D.P.; Supervision: V.P.V.; Resources: V.P.V.; Materials: D.P., S.R.P.; Data collection and/or processing: R.R, S.R.P.; Analysis and/or interpretation: V.P.V., R.R.; Literature search: R.R., V.P.V.; Writing: R.R.; Critical reviews: V.P.V., S.R.P., D.P.

## DECLARATION OF COMPETING/FINANCIAL INTERESTS

The authors declared no conflict of interests.

## DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

During the preparation of the manuscript, AI-assisted tool Grammarly was used for grammar checks or revisions.

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