

## Article

# Greenness Assessment of HPLC Analytical Methods with Common Detectors for Assay of Paracetamol and Related Materials in Drug Products and Biological Fluids

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**Abstract:** Paracetamol is one of the most widely consumed analgesic and antipyretic medications worldwide. It is frequently analyzed in many quality control (QC) laboratories in pharmaceutical companies, either in raw materials or drug products. It was reported that paracetamol self-toxicity often occurs, leading to the frequent analysis of paracetamol in toxicological centers in biological fluids. Green analytical chemistry (GAC) is growing to be a global philosophy; therefore, the high frequency of paracetamol analysis poses potential concerns. Chromatographic analytical methods used for the daily analysis of paracetamol could be a potential risk to the environment or the health of the analysts if not thoroughly considered. The presented study aims to establish greenness assessments of nine HPLC methods used to assay paracetamol in raw materials and drug products and twenty-one HPLC methods. The reason for selecting HPLC methods of analysis to be the core of the study is the known reproducibility, reliability and availability in most QC laboratories. The most commonly used metric systems for greenness evaluation are the Analytical GREENness (AGREE), the eco-scale assessment (ESA) and the national environmental methods index (NEMI) which have been used in this comparative study. The greenest chromatographic method for the analysis of paracetamol in raw materials and drug products was introduced by Rao et al. (the obtained scores were ESA = 76 and AGREE = 0.62, while the greenest chromatographic method for the analysis of paracetamol in biological fluids was proposed by Modick et al.). The obtained scores were ESA = 85 and AGREE = 0.7. The NEMI tool proved to have limited performance compared to other metric systems, hence it could not be used alone. Accordingly, the collaboration of NEMI results with ESA and AGREE for greenness assessment is highly recommended to reach appropriate conclusions.

**Keywords:** AGREE; biological fluids; ESA; GAG; HPLC; NEMI; paracetamol



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## 1. Introduction

Paracetamol (acetaminophen) is one of the most commonly consumed medications globally [1]. Paracetamol is a well-known painkiller. In terms of pain, it lowers the temperature and relieves mild to moderate pain in adults and children. It is prescribed as an over-the-counter (OTC) medication in various cases of headache and fever [2]. Furthermore, physicians recommend it for its safety and efficacy as an analgesic and antipyretic for some critically ill patients, pregnant women, antihypertensive patients, children and others. Regardless of its extensive consumption for over a century, the exact mechanism of paracetamol in relieving pain and fever is not fully understood.

Nevertheless, acute poisoning has been recently reported in many countries due to the overconsumption and mistreatment of OTC medicines [3]. Plentiful medications with analgesic and antipyretic effects are possibly misused and have dangerous side effects [3]. As analgesics poisoning is an extensive medical emergency, paracetamol, which is regularly consumed, is responsible for around 30% of adult self-poisoning globally [4]. Recently, it has been reported from the electronic medical record system, the demographic statistics and poisoning history in the past five years that Saudi Arabia is on the list of paracetamol toxicity caused by overdose accidents [5]. According to earlier research, the average age of paracetamol overdose patients was 34 years, and about 53% were female. From these statistical data, 45% of patients had a history of alcohol misuse, and an average of 42% had a history of mental disorders [5]. A previously published study in Saudi Arabia between 2016 and 2021 presented that 492 adult patients and 1013 children were involved in paracetamol poisoning [5].

Moreover, paracetamol has been ranked on the top list of toxicity centers globally for misuse; therefore, it is being significantly analyzed in biological fluids [6]. Additionally, paracetamol is manufactured and analyzed by many pharmaceutical companies worldwide and frequently analyzed in their quality control (QC) laboratories, as a raw material or a final drug product [6]. In conclusion, paracetamol is found to be extensively analyzed in different phases, raw materials, pharmaceutical products and biological fluids by numerous analytical methods. According to a literature review in the analytical abstracts database published by the Royal Society of Chemistry (RSC), more than 200 studies have been published about the analysis of paracetamol by different methods, whether tackling its analysis solely or in mixtures [6].

High-performance liquid chromatography (HPLC) is a reliable method of analysis. It is frequently used for all analysis purposes due to its accuracy, short time of analysis, high resolution for different components in different matrices, great sensitivity and high precision [6]. HPLC is frequently employed in the pharmaceutical field, starting from the analysis of the purity of raw material to the final product analysis in QC laboratories, in addition to the pharmacokinetics, pharmacodynamics and bioequivalence studies of pharmaceutical products in *in vivo* studies [6]. HPLC is coupled with several types of detectors but mostly with ultraviolet (UV) and mass spectrometry (MS) detectors. UV detectors are commonly used and practically delivered with all HPLC instruments in developed and developing countries. Mass spectrometry (MS) is a universal detection method for ionic or ionizable compounds with high speed, sensitivity and selectivity [6]. Consequently, the presented study will be more focused on HPLC methods coupled with MS and UV detectors.

A nonstop liquid chromatograph (LC) with a standard LC column and a mobile phase flow rate of 1 mL/min produces about 1.5 L of effluent daily, or nearly 500 L per year [7]. As a result, it is imperative to adhere to the principles of green analytical chemistry (GAC) and attempt to compute hazardous wastes. GAC is a global strategy that aims to find practical alternatives for removing waste produced by different analysis methods and replacing it with clean and green ones [7]. In GAC, a balance should be achieved between seeking accurate results and diminishing environmental distress when analytical procedures are practiced [7]. To reach this sense of balance, the twelve principles of GAC should be followed [8]:

Waste prevention should be preferred over cleaning up and treating waste after it has arisen. Waste at the molecular level should be reduced. Using safe chemical reactions and synthetic pathways is preferred. Safer chemicals should be suggested when designing a method of analysis. For each step of a chemical reaction and method of analysis, the safest and least amounts of solvents and auxiliaries should be used. Selecting the most energy-saving procedures is a critical choice. Chemicals derived from renewable or plant-based sources are far better. Derivatization should be avoided whenever possible.

On the other hand, using catalysts is recommended. Generating biodegradable chemicals that can be easily discarded is recommended to avoid toxicity, bioaccumulation and

persistence. Choosing and creating safer chemical procedures that reduce the risk of accidents for the working analyst is crucial.

Several metric systems for greenness assessment were developed based on the 12 GAC principles mentioned above. Three tools from these greenness assessment protocols will be used in our presented work: the analytical greenness metric (AGREE), the national environmental method index (NEMI) and the analytical eco-scale assessment (ESA) [6]. NEMI, which is the earliest in appearance in the literature, is a qualitative technique for estimating how eco-friendly analytical processes are. ESA computes numerical values and produces a final figure showing how environmentally friendly the system is. AGREE, a recent greenness assessment tool, is distinguished by using the 12 principles of GAC as its input criteria and has both qualitative and quantitative features.

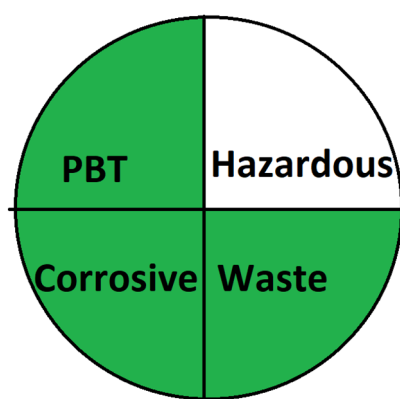
Finally, this study aims to select the most eco-friendly HPLC analytical method used for paracetamol analysis, whether in raw materials, drug products or biological fluids. Accordingly, and after a thorough literature review and filtration, the presented study aims to assess the greenness of 30 HPLC analytical methods using MS and UV detectors, whether solely or in the presence of common impurities, degradants or metabolites. Nine methods [9–17] were used to analyze paracetamol in raw materials and pharmaceuticals, and twenty-one methods [18–38] were applied for paracetamol analysis in biological fluids. Our proposed study helps introduce the greenest HPLC methods for paracetamol analysis to different analysts whether in QC laboratories in pharmaceutical companies or in toxicological centers. The study also highlights the advantages and disadvantages of the three greenness assessment tools.

## 2. Materials and Methods

Three greenness assessment methods are applied in the presented study that can be summarized as follows:

### 2.1. National Environmental Method Index (NEMI)

It is a metric system based on a circle that is separated into four sections; each section represents a particular criterion (waste prevention, hazardous, corrosive and persistence, and bio-accumulative and toxic compounds (PBT) (Figure 1) [39]. When a criterion's value is fulfilled, the quarter is colored green; otherwise, it is left blank. Based on the following requirements, the meant quarter of the NEMI circle is colored green [39].



**Figure 1.** An example of an assessment score with the NEMI metric system [39].

1. The reagents and chemicals provided during the study should be neither persistent nor poisonous or bio-accumulative and meet the green chemistry specifications. These chemicals should not be listed on the TRI of the EPA list (accessed 6 November 2022) at [www.epa.gov/toxics-release-inventory-tri-program/tri-listed-chemicals](http://www.epa.gov/toxics-release-inventory-tri-program/tri-listed-chemicals).

2. The materials employed in the analysis should not be harmful. They should not be included in the Resource Conservation and Recovery lists (retrieved on 6 November 2022, from [www.toxicfreefuture.org](http://www.toxicfreefuture.org)).

3. The potential of hydrogen (pH) range should be maintained between 2 and 12 to avoid the corrosive effects of the chemicals and reagents in the study.

4. The waste amount should not exceed 50 g throughout the analysis.

Although NEMI is just a one-look metric system, it provides the analyst with preliminary feedback about the greenness of the method under investigation. However, the main drawback of NEMI is that the results are qualitative (either green or blank), and the hazard source is unclear in the graph.

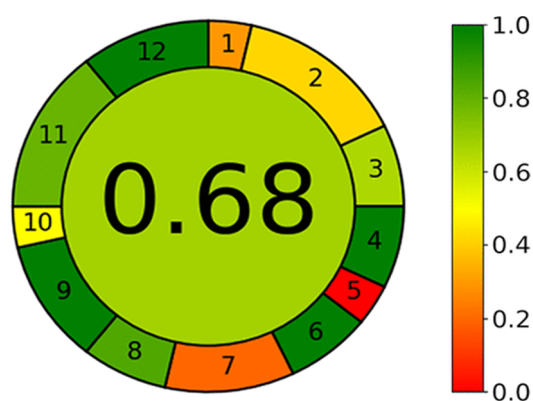
## 2.2. Analytical Eco-Scale Assessment (ESA) [40]

ESA employs numerical evaluation of the method under investigation based on penalty points [40], such that the process is considered ideal when its score is 100, which means that no penalty points were deducted. The analysis process should be carried out at room temperature and be safe for both the operator and the environment with 100% yield [40]. The higher score an organic preparation receives, the more economical and environmentally friendly it is [40]. Analytical process greenness is evaluated based on this perception. Penalty points are deducted from 100 if an analytical procedure's greenness assessment deviates from the ideal green measurements [40]. The final ESA score is determined by deducting the total penalty points for the entire procedure from 100 [40].

The greenness result is classified based on the resulting score; if it is more than 75, it signifies green evaluation; if the score is between 50–75, it denotes proper greenness assessment; if the score is lower than 50, this is an inadequate greenness assessment.

## 2.3. The Analytical Greenness Metric (AGREE) [41]

The analytical greenness metric (AGREE) is a novel system for greenness evaluation based on the twelve principles of green analytical chemistry. The 12 SIGNIFICANCE principles [42] are referenced in the input criteria, and the weights can be varied for each principle for more flexibility weights. The final assessment result is the sum of the assessments of each of the 12 input variables, which are interpreted into a score on a 0–1 scale [41]. As illustrated in Figure 2 [40,41], the result is a clock-like circle with the total score and color illustration in the center.



**Figure 2.** An example of AGREE analysis where the reference color scale is on the right, and the circle representing the outcome of the evaluation is on the left [41].

The red–yellow–green scale represents how well each principle is followed, and the width of the segment represents how important each principle is. Using user-friendly freely accessible software, the assessment can be completed quickly, where a report and a graph are generated automatically [41]. AGREE software is freely accessed by clicking the live link in the citation below (<http://www.mostwiedzy.pl/AGREE> retrieved on 6 November 2022).

The analytical greenness calculator software was utilized, with its latest version code accessible at [git.pg.edu.pl/p174235/AGREE](https://git.pg.edu.pl/p174235/AGREE) (accessed on 23 December 2022). The calculator produces a user-friendly graph that includes a comprehensive score.

Using the three greenness assessment protocols, NEMI, ESA and AGREE, the 30 chromatographic techniques under investigation to analyze paracetamol were assessed for their eco-friendliness. This evaluation was performed to determine the safest and most environmentally friendly analytical method for paracetamol in pharmaceutical dosage forms, biological fluids and raw materials.

### 3. Results and Discussion

Using the three greenness evaluation tools, NEMI, ESA and AGREE, the environmental impact of each of the 30 chromatographic techniques for measuring paracetamol in pharmaceutical formulations, raw materials and biological fluids has been evaluated. Tables 1 and 2 show the findings of evaluating the environmental friendliness of all the methodologies under investigation. The pictogram of the NEMI results has four circle quadrants and is colored blank–green, where green represents the safety and environmental friendliness of the method. The ESA results are expressed as a number out of 100, where higher values indicate that the analytical process is more environmentally friendly. The colors red, yellow and green are depicted in a symbol for the “AGREE method”. The overall greenness of the approach is represented by each of the 12 sections in the outer and inner circles, each with different color intensities and a score between 0 and 1.

**Table 1.** Chromatographic methods for paracetamol analysis in raw material and drug products.

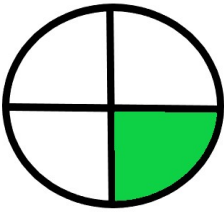

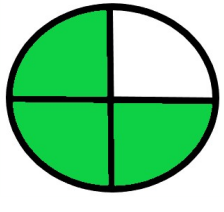

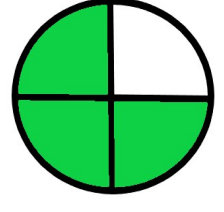

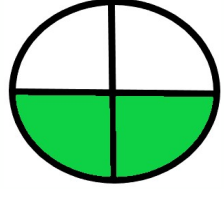

Study Number	Applied Instrument and Chromatographic Method	ESA	NEMI Pictogram	AGREE Pictogram
1.1. [9]	HPLC/DAD The mobile phase: 10 mM ammonium acetate/acetic acid (pH 6) as solvent A and acetonitrile as solvent B, using a flow rate of 1.0 mL/min.	33		
1.2. [10]	HPLC/UV The mobile phase: a mixture of water-methanol (3:1) with a flow rate of 1.0 mL/min.	18		
1.3. [11]	HPTLC The mobile phase: methanol:ethyl acetate:glacial acetic acid (8:0.8:0.6:0.2, v/v/v/v) at 1.0 mL/min.	77		
1.4. [12]	HPLC/DAD The mobile phase: potassium dihydrogen phosphate buffer (pH 3.0) and acetonitrile at 1.0 mL/min. LOD range (0.05–0.08 ug/mL) LOQ range (0.145–0.197 mg/mL)	72		

Table 1. Cont.

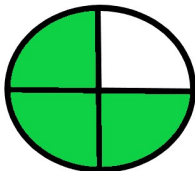

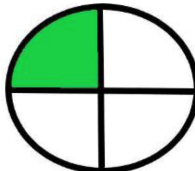

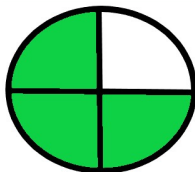

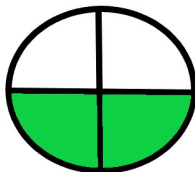
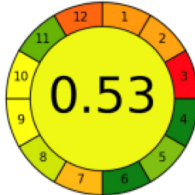
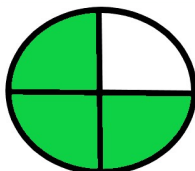

Study Number	Applied Instrument and Chromatographic Method	ESA	NEMI Pictogram	AGREE Pictogram
1.5. [13]	HPLC/UV The mobile phase: 99% formic acid, 0.2% <i>v/v</i> and 1% methanol at 1.0 mL/min.	79		
1.6. [14]	HPLC/UV The mobile phase: solvent A: 0.01 M phosphate buffer at pH 3.0 and solvent B: methanol at a flow rate of 1.0 mL/min.	27		
1.7. [15]	HPLC/UV The mobile phase: a 15:85 mixture of methanol, 50 mM potassium phosphate, monobasic (pH = 3.25) aqueous solution with a flow rate of 1.0 mL/min. LOD 0.034 mg/mL	81		
1.8. [16]	HPLC/UV The mobile phase: a mixture of phosphate buffer (pH = 4.88) and methanol at a flow rate of 1.0 mL/min.	44		
1.9. [17]	HPLC/UV The mobile phase: an isocratic mixture of 80/20 ( <i>v/v</i> ) acetonitrile/0.05 M potassium phosphate buffer (pH 5.5) with flow velocity of 1.0 mL/min.	67		

Table 2. Chromatographic methods for paracetamol analysis in biological fluids.

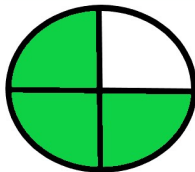

Study Number	Applied Instrument and Chromatographic Method	ESA	NEMI Pictogram	AGREE Pictogram
2.1. [18]	HPLC/UV The mobile phase: 40 mM ammonium acetate (pH 4.8): methanol [87:13 <i>v/v</i> ] at a flow rate 1.0 mL/min. The sample type was human liver.	74		

Table 2. Cont.

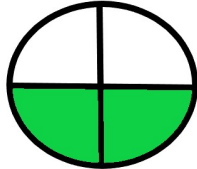

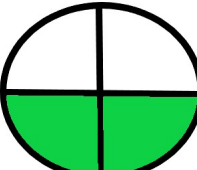
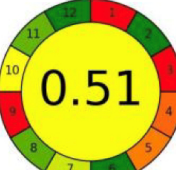
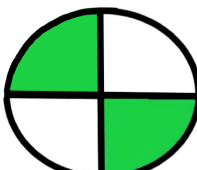
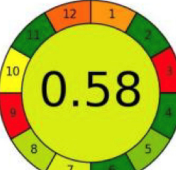
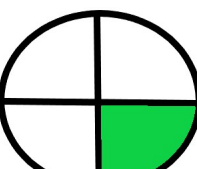

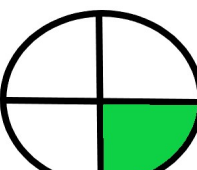

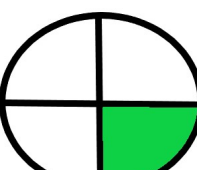

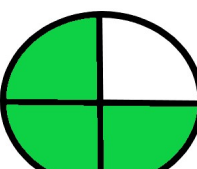

Study Number	Applied Instrument and Chromatographic Method	ESA	NEMI Pictogram	AGREE Pictogram
2.2. [19]	HPLC/UV The mobile phase: a mixture of methanol and acetic acid at a flow rate 1.0 mL/min. The sample type was human plasma LOD 0.17 mcg L <sup>-1</sup> LOG 0.4 mcg L <sup>-1</sup>	31		
2.3. [20]	HPLC/MS The mobile phase: 0.1% (v/v) formic acid and acetonitrile at a flow rate of 0.2 mL/min. The sample type was mouse urine. LOD 0.66 mol/L	84		
2.4. [21]	HPLC/UV The mobile phase: 20 mM ammonium formate buffer pH 3.5 (A) and methanol (B) (pH 3.5) at a flow rate of 0.8 mL/min. The sample type was blood spots.	67		
2.5. [22]	HPLC/UV The mobile phase: aqueous buffer solution and methanol at a flow rate of 1.0 mL/min. The sample type was human liver.	58		
2.6. [23]	HPLC/UV The mobile phase: 35% water and 20% methanol at a flow rate 1.0 mL/min. The sample types were human plasma, urine and saliva.	79		
2.7. [24]	HPLC/MS The mobile phase: ammonium acetate, buffers, formate buffers and methanol at a flow rate of 0.25 mL/min. The sample types were human plasma and urine.	66		
2.8. [25]	HPLC/UV The mobile phase consisted of water and methanol at a flow rate of 1.0 mL/min. The sample type was human urine LOQ 0.96 mcg/L <sup>-1</sup> .	37		

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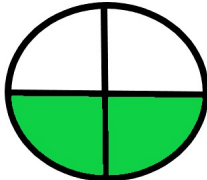
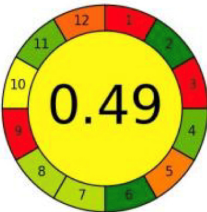
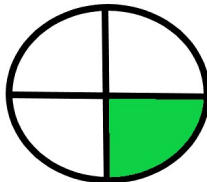
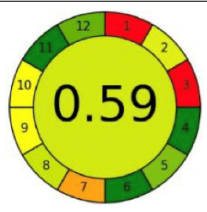
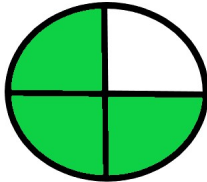

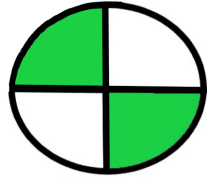
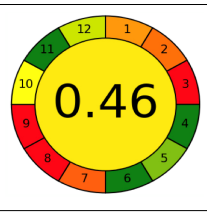
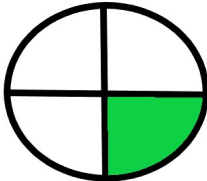

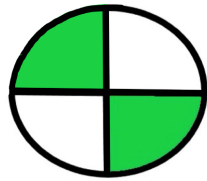

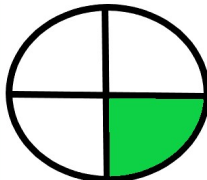
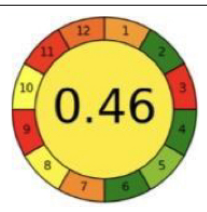
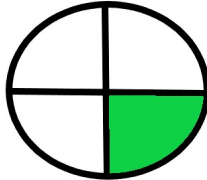

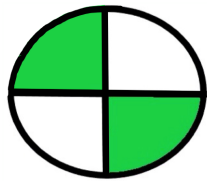

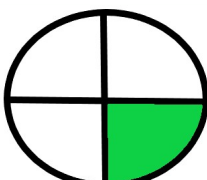

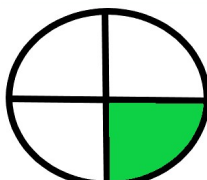

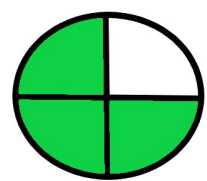

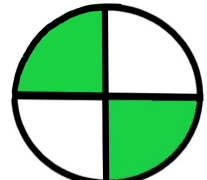

Study Number	Applied Instrument and Chromatographic Method	ESA	NEMI Pictogram	AGREE Pictogram
2.9. [26]	HPLC/MS The mobile phase: methanol-water containing 0.0875% formic acid at a flow rate of 1.5 mL/min. The sample types were human plasma and urine.	72		
2.10. [27]	HPLC/UV The mobile phase: 0.3% methanol at a flow rate of 0.25 mL/min. The sample type was cell culture representing an in vitro model of blood–brain barrier.	79		
2.11. [28]	HPLC/UV The mobile phase: A gradient consisting of 0.1% formic acid and water at a flow rate of 0.25 mL/min. The sample type was human urine sample.	75		
2.12. [29]	HPLC/UV The mobile phase: 0.1 M potassium dihydrogen orthophosphate, acetic acid and propane-2 at a flow rate of 1.5 mL/min. The sample types were human plasma and urine.	65		
2.13. [30]	HPLC/MS The mobile phase: methanol and phosphate buffer (0.05 M) at a flow rate of 1.0 mL/min. The sample type was human serum. LOQ 7.41 ng/mL.	67		
2.14. [31]	HPLC/MS The mobile phase: 75% water and 25% methanol at a flow rate of 1.0 mL/min. The sample type was human urine. LOQ 0.75 mcg/L.	85		
2.15. [32]	HPLC/UV The mobile phase: aqueous buffer solution of KH <sub>2</sub> PO <sub>4</sub> (0.05 M) containing 1% CH <sub>3</sub> COOH (pH 6.5) and methanol at a flow rate of 1.5 mL/min. The sample types were rabbit plasma and urine.	63		



Table 2. Cont.

Study Number	Applied Instrument and Chromatographic Method	ESA	NEMI Pictogram	AGREE Pictogram
2.16. [33]	HPLC/MS The mobile phases: ammonium acetate (10 mM; adjusted to pH 10 with ammonia) and methanol at a flow rate 0.25 mL/min. The sample type was human dried blood spots.	85		
2.17. [34]	HPLC/MS The mobile phase: methanol degassed with ultra-sonication at flow rate of 1.0 mL/min. The sample type was saliva.	92		
2.18. [35]	HPLC/MS The mobile phase: 10 mM ammonium formate containing 0.3% ammonia and methanol at a flow rate of 0.25 mL/min. The sample type was dog dried blood spots.	91		
2.19. [36]	HPLC/MS The mobile phase: a gradient consisting of 0.1% formic acid in water and 0.1% in methanol at a flow rate of 0.25 mL/min. The sample type was human serum.	86		
2.20. [37]	HPLC/MS The mobile phase: 25% methanol and 75% citrate-phosphate buffer (pH 3.0) at a flow rate of 1.0 mL/min. The sample type was serum.	19		
2.21. [38]	HPLC/MS The mobile phase: 0.1% trifluoroacetic acid in water (A), and methanol (B), at a flow rate of 1.0 mL/min. The sample type was urine–bile.	83		

### 3.1. Paracetamol Assay in Drug Products and Raw Material

The results in Table 1 represent the chromatographic methods for paracetamol analysis in drug products and raw materials, where complete data for the NEMI, ESA and AGREE tools are compared. The five NEMI methods (1.2 [10], 1.3 [11], 1.5 [13], 1.7 [15] and 1.9 [17]) had three green quadrants (PBT, corrosive and waste), whereas methods 1.4 [12] and 1.8 [16] had two green quadrants (corrosive, and waste). Method 1.1 [9] had one green quadrant (waste), and method 1.6 [14] had a green quadrant (PBT). The greenest method could not be discriminated through NEMI. However, AGREE and ESA tools could determine the greenest method among the analytical methods, where among the nine analytical

chromatographic methods, the greenest method according to ESA score was found to be method 1.7 [15] with the highest score of 81 then method 1.5 [13] with an ESA score of 79. In contrast, method 1.2 [10] had the lowest ESA score, 18. For the results based on AGREE method, which is considered the most critical assessment tool, method 1.4 [12] showed the highest AGREE score (0.62), and methods 1.5 [13] and 1.7 [15] had the second-greenest AGREE score (0.60). In contrast, method 1.9 [17] had the least AGREE score (0.43).

According to the AGREE method, derivatization, energy usage, analysis throughput and device location are the main factors in most procedures that make them the least environmentally friendly. The outcomes are in line with earlier studies [39].

The following provides a detailed illustration of how the three greenness assessment tools are applied for the greenest method number 1.4 [12]:

- NEMI tool:

In this method, a circle symbol with four quarters was designed as a pictogram; each quarter signified a component of the method that could potentially negatively influence the environment. The hazardous quarter was empty since acetonitrile is a well-known toxic liquid. During analysis, the mobile phase had no corrosiveness that threatened the environment because the pH was 3, and, accordingly, the pH quarter was green. The waste quarter was also green because less than 50 g of waste was produced.

- ESA tool:

According to Rao et al. [12], pictograms and signal words should be considered when assessing the risks posed by chemicals employed in analytical procedures for ease and simplicity. One or more of the nine pictograms can be used to characterize each chemical. Penalty points are allocated to each pictogram, e.g., flame over the circle, corrosion, gas cylinder, skull and crossbones, exclamation mark, environment and health hazard. The globally harmonized system (GHS) uses the terms “danger” (a more severe hazard, category 1 or 2) and “warning” as its two signal words (less hazard, other types). The following system is used to determine the penalty points for risks: none (no pictogram) = 0 penalty points, less severe hazard = 1 penalty point, and more severe hazard = 2 penalty points. Acetic acid had four pictograms in method 1.4 [12] and was more dangerous, hence its penalty points were 4. HPLC-RP used 1.5 kWh of energy for each sample, resulting in one penalty point. Waste was estimated by multiplying the flow rate by the time used. According to one penalty point for waste, 15 mL of waste per sample in method 1.4 [12] was generated. By deducting all penalty points from 100, the ESA score was determined to be 72.

- AGREE tool:

The 12 SIGNIFICANCE principles are referenced in the input criteria, which can be given various weights to accommodate some flexibility. Each of the 12 input variables is converted into a scale with a 0–1 range, and the assessment of method 1.4 [12] generated a score of 0.62.

Based on the NEMI results, only the greenest approach could not thus be distinguished. Hence, a collaboration of AGREE and ESA tools with NEMI was required to choose the analytical method that is the most environmentally friendly. Among the nine analytical methods, method 1.4 [12], with an ESA score of 76 and an AGREE score of 0.62, was found to be the greenest, whereas method 1.9 [17], with an ESA score of 67 and an AGREE score of 0.43, was the least green.

### 3.2. Paracetamol Assay in Biological Fluids

As for the NEMI, four methods (2.1 [18], 2.8 [25], 2.11 [28] and 2.20 [37]) had three green quadrants (PBT, corrosive and waste), three methods (2.2 [19], 2.3 [20] and 2.9 [26]) had two green quadrants (corrosive and waste), whereas five methods (2.4 [21], 2.12 [29], 2.14 [31], 2.17 [34] and 2.21 [38]) had green PBT and waste quadrants. The nine methods 2.5 [22], 2.6 [23], 2.7 [24], 2.10 [27], 2.13 [30], 2.15 [32], 2.16 [33], 2.18 [35] and 2.19 [36] had

one green quadrant (waste). Accordingly, the greenest method could not be determined via the NEMI method. However, AGREE and ESA tools could determine the greenest among the analytical methods. Among the twenty-one analytical techniques, method 2.17 [34], with an ESA score of 92, was found to be the greenest method, and then method 2.18 [35], with an ESA score of 91. In contrast, method 2.20 [37] had the lowest ESA score (19). On the other hand, according to AGREE, which is the most significant assessment tool, method 2.14 [31] had the highest AGREE score (0.7), and then method 2.6 [23] with an AGREE score of 0.67, whereas method 2.7 [24] had the lowest AGREE score (0.36). Derivatization, analysis throughput, energy usage and device location are the main factors in most procedures that render analytical methods less environmentally friendly. The outcomes are in line with earlier studies [39].

The following provides a detailed illustration of how the three greenness assessment tools were applied in the evaluation of the greenest method number 2.14 [23]:

- NEMI tool:

The pictogram for this approach was a circle with four parts. Each quarter represented a part of the procedure that might harm the environment. Ammonium acetate is a toxic substance, hence the hazardous quarter was left unfilled. The mobile phase did not pose a corrosive threat to the environment during analysis because the pH was 6.5 to 6.8, hence the pH quarter was green. Moreover, the produced wastes were less than 50 g, and the waste quarter was presented as green.

- ESA tool:

Pictograms and signal words should be considered when evaluating the dangers caused by the compounds used in analytical methods for ease and simplicity according to Modick et al. [31]. Each of the nine pictograms can be used to describe a particular chemical: flame, flame over the circle, corrosion, gas cylinder, skull and crossbones, exclamation mark, environment and health hazard. For each of these pictograms, a certain number of penalty points is given. The two signal phrases used by GHS are “danger” (a more severe hazard, category 1 or 2) and “warning” (less hazard, other categories). The following system calculates the penalty points for hazards as none (no pictogram) = 0 penalty points, less severe hazard = 1 and more severe hazard = 2. As acetic acid is a hazard in method 2.14 [31], its penalty points were 4. Energy penalty points were earned because HPLC-RP required 1.5 kWh of energy for each sample. Waste was calculated by dividing the flow rate by the duration of use. Method 2.14 [31] generated 15 mL of waste per sample, corresponding to one waste penalty point. The ESA score was 85 when all the penalty points were subtracted from 100.

- AGREE tool:

The 12 SIGNIFICANCE principles are referenced in the input criteria, and different weights can be assigned to them to provide some flexibility. After transforming each of the 12 input variables into a scale with a 0–1 range, process 2.14 [31] produced an evaluation result of 0.7.

#### 4. Conclusions

In conclusion, this study aimed to identify the greenest analytical method for assaying paracetamol using various assessment methodologies. Thirty chromatographic analytical methods were compared using the NEMI, ESA and AGREE tools.

The NEMI tool, although it is straightforward, was found to be the least useful in providing details about the environmental impact of the analytical procedures. All 30 chromatographic methods exhibited almost comparable NEMI diagrams, making it difficult to differentiate between them based on their NEMI greenness results.

On the other hand, the ESA rating offered a semi-quantitative evaluation of the environmental impact of the analytical methods, taking into account the volume of reagents used and the waste generated, but there was a usual gap considering the effect on the

analyst and the source of hazard. On the other hand, the most valuable tool for evaluating the greenness of the analytical methods was found to be the AGREE tool. The synergistic performance of the three metric systems provided comprehensive and in-depth information and offered qualitative and quantitative estimations.

According to the results from the ESA and AGREE tools, method number 1.4 [12] by Rao et al. and method 2.14 [31] by Modick et al. were the most eco-friendly. Based on the former findings, employing multiple assessment tools for evaluating the greenness profiles to achieve accurate greenness profiling for analytical methods is highly recommended. By implementing eco-friendly methods, researchers and analysts can promote sustainability in the field of chemical analysis, leading to the desired economic and social benefits.

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