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Greenness Assessment of Chromatographic Methods Used for Analysis of Empagliflozin: A Comparative Study

Eman Y. Santali [1], Ibrahim A. Naguib [1,*], Abdullah M. Alshehri [2], Yazeed A. Alzahrani [2], Abdullah E. Alharthi [2], Turki S. Alosaimi [2], Bandar D. Alsayali [2], Izzeddin Alsalahat [3,*], Albandary Almahri [4], Mohammed A. S. Abourehab [5,6] and Fatma F. Abdallah [7]

[1] Department of Pharmaceutical Chemistry, College of Pharmacy, Taif University, Taif 21944, Saudi Arabia
[2] College of Pharmacy, Taif University, Taif 21944, Saudi Arabia
[3] UK Dementia Research Institute Cardiff, School of Medicine, Cardiff University, Cardiff CF24 1TP, UK
[4] Department of Chemistry, College of Science and Humanities in Al-Kharj, Prince Sattam Bin Abdul Aziz University, Al-Kharj 11942, Saudi Arabia
[5] Department of Pharmaceutics, Faculty of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia
[6] Department of Pharmaceutics, College of Pharmacy, Minia University, Minia 61519, Egypt
[7] Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62541, Egypt
* Correspondence: i.abdelaal@tu.edu.sa (I.A.N.); alsalahati@cardiff.ac.uk (I.A.)

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Abstract: The analytical chemistry community is attempting to incorporate green chemistry concepts in the development of analytical techniques to redefine analytical methods and dramatically modify the philosophy of analytical technique development. Each greenness assessment method has its own benefits and drawbacks, as well as its own procedures. The results of each greenness assessment method produce numerous deductions regarding the selection of a greenest chromatographic method on which the determination of a greenness assessment tool depends. The current study examined the greenness behavior of 26 reported chromatographic methods in the literature for the evaluation of the medicine empagliflozin using three evaluation methods: the national environmental methods index (NEMI), the eco-scale assessment (ESA), and the green analytical procedure index (GAPI). This comparative study discussed the value of using more than one greenness evaluation methods while evaluating. The findings showed that the NEMI was a less informative and misleading tool. However, the ESA provided reliable numerical assessments out of 100. Despite the GAPI being a complex assessment compared to the others, it provided a fully descriptive three-colored pictogram and a precise assessment. The findings recommended applying more than one greenness assessment tool to evaluate the greenness of methods prior to planning laboratory-based analytical methods to ensure an environment friendly process.

Keywords: empagliflozin; green chemistry; NEMI; ESA; GAPI; HPLC; chromatography

1. Introduction

Diabetes is a critical medical condition that may cause many microvascular and macrovascular cardiovascular complications, including kidney diseases [1,2], and the presence of these factors is associated with increased rates of mortality [1,2]. Studies have shown that glucose lowering is good for decreasing the rates of incidence of cardiovascular events and death, in addition to renal complications, especially for patients with the second type of diabetes [1,2]. All these benefits can be achieved by using antidiabetic medications. In the present study, empagliflozin is the medicine of interest selected to treat the second type of diabetes. It acts by inhibiting sodium glucose cotransporter-2 (SGLT2), which leads to reducing the renal glucose threshold by preventing glucose reabsorption from the proximal convoluted tube (PCT), leading to blood glucose improvement [1,2].

Empagliflozin is a better choice to manage type 2 diabetes compared to canagliflozin and dapagliflozin due to its FDA-labeled use to decrease the mortality rate in patients with cardiovascular disease (CVD) and type 2 diabetes (DM2) [3]. Additionally, the selection of empagliflozin depends on randomized clinical trials that showed a 35% reduction in hospitalized heart failure patients who underwent an empagliflozin regimen [3].

The quantitative analysis of pharmaceutical active ingredients, whether in pure forms or drug products, is a routine activity in pharmaceutical companies and clinical research laboratories. Recently, researchers have preferred to follow green analytical chemistry (GAC) concepts, which aim to decrease or prevent the environmental impact of the chemical enterprise and achieve sustainability at the molecular level. Accordingly, it is not surprising nowadays that many industrial sectors pay attention to the greenness of chemical processes because of the great outcomes that decrease cost while increasing production and saving time and resources. The first green chemistry manual [4], written by Paul Anastas and John Warner in the 1990s, outlined a set of 12 proposed green chemistry principles based on the avoidance or stop of using toxic solvents in chemical methods and assessment, as well as the avoidance of residue generation from these methods.

After that, in order to reduce the environmental effect of new methods and analytical tools, Paul Anastas stressed the need of incorporating these 12 principles [4]. As a result, one of the most active areas of green chemistry study and research is the creation of analytical methods. To better correlate with green analytical chemistry, Galuszka, Migaszewski, and Namienski updated the 12 green chemistry principles in 2013 [5]. As a result, the 12 concepts of green analytical chemistry presented in this study are described as SIGNIFICANCE [6].

In accordance with green chemistry's 12 principles, three types of greenness assessment methods are applied in this study, which are the national environmental method index (NEMI), the analytical eco-scale assessment (ESA), and the green analytical procedure index (GAPI). The NEMI is the earliest method and is simple and easy with less accuracy compared to the others. The ESA tool is based on numerical final values that determine the greenest applied method. Moreover, it is simple and more accurate than the NEMI. The GAPI is a relatively new comprehensive tool that provides a more precise estimation of greenness [7–10].

Empagliflozin is analyzed using different chromatographic and nonchromatographic methods. A full literature review for the analysis of empagliflozin shows that there are only 26 published chromatographic studies for its quantitative analysis up to the date of this study. The chromatographic method is a regular routine in quality control laboratories and therapeutic drug monitoring, where it offers high precision and reproducibility. Therefore, the presented study aims to compare the 26 published chromatographic techniques for analyzing empagliflozin, considering greenness evaluation to reduce hazardous substances and waste with the three proposed tools: the NEMI, the ESA, and the GAPI [11–31]. Recently published chromatographic methods are included in the comparative greenness study [32–35].

2. Experimental Methods

The Royal Society of Chemistry (RSC) [36] analytical abstract service was used to collect analytical articles and reviews on the estimation of empagliflozin. After that, the greenness evaluation was performed for the resulting chromatographic methods belonging to empagliflozin analysis using the selected greenness assessment tools, namely the national environmental method index (NEMI), the analytical eco-scale assessment (ESA) method, and the green analytical procedure index (GAPI). Below is a brief description of the three greenness assessment tools.

2.1. National Environmental Method Index (NEMI)

This tool is industrialized by the methods and data comparability board (MDCB). It has the biggest ecological analytical database. The NEMI has free database access through www.nemi.gov (accessed on 14 February 2021) for environmental methods. This tool was fully described by Keith et al. in 2007 [7], who stated that the NEMI is expressed by a circle called the greenness profile, which is divided into 4 equal parts, as shown in Figure 1. The first part of the circle expresses PBT, which is the abbreviation of three items: persistent, bio-accumulative, and toxic. The second part expresses the hazardous aspect. The third and the fourth parts express the corrosiveness and the waste, respectively. Each part may take a green color, which reflects the greenness of the method, or may take a blank color, which reflects the lack of greenness. The greenness profile takes into consideration many important factors, such as compounds with specific properties, pH, and waste amount. Following that, the analyst may simply visually compare the greenness of different analytical techniques to evaluate the greenness and eco-friendliness degree of each one [7].

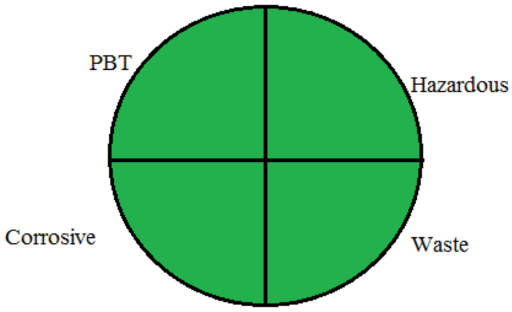


Figure 1. Pictogram of an ideal green analytical method assessed by NEMI tool.

2.2. Analytical Eco-Scale Assessment (ESA)

This measure relies on overall points, which might represent the degree of greenness of the analytical procedure used. Beginning with 100 points (symbolizing the most environmentally friendly degree with no penalties), by highlighting the negative effects of additives and excipients, such as dangerous solvents used in a procedure, as well as the effects on the environment and the energy used, a penalty point lowers the overall score. The technique is deemed green if the final score exceeds 75 points; nevertheless, if the final score is between 50 and 75 points, the approach is deemed acceptable. If the score is lower than 50, it indicates that the green analytical approach is unsatisfactory. Hazard penalty points are determined as follows: nonhazardous is denoted by 0 penalty points and a lack of pictogram, while only one penalty point is given regarding as less serious hazardous chemical, and more serious danger is indicated when the points rise over one [5,8,37].

2.3. Green Analytical Procedure Index (GAPI)

This is a brand new instrument that J. Potka-Wasylka presented in 2018 that may assess how environmentally friendly an entire analytical procedure is, from the collection of samples to the final outcome. In accordance with the GAPI, each analytical operation begins with sample collection; then, it is protected against harmful chemical and physical modifications in the second step. After that, it is determined and quantified using analytical techniques in the third and final step. The GAPI tool contains a pictogram that uses a color pattern (green, yellow, and red) to categorize the ecological value of each step of an analytical method. The green color implies a safe procedure, while the red color indicates a procedure that is not environmentally friendly. The GAPI sign uses five pentagrams for evaluation, with fifteen specific categories [9,10,37], as shown in Figure 2.

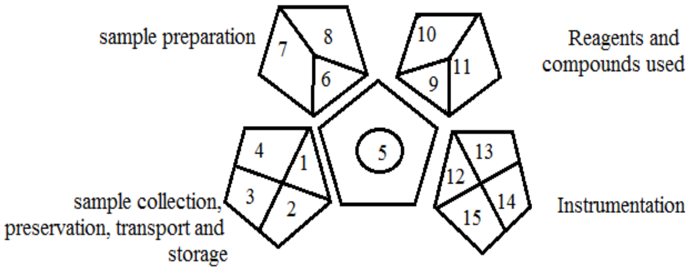


Figure 2. Green Analytical Procedure Index pictogram with description.

2.4. Application of Three Greenness Evaluation Tools to Empagliflozin Assessment Methods

The greenness of the 26 empagliflozin analyses reporting chromatographic methods was assessed by applying the three greenness investigation methods individually. Table 1 includes an overview of the results for comparison, as well as complete reports for the NEMI, ESA, and GAPI tools. Table 1 also offers thorough explanations of each chromatographic method, as well as the citations that are necessary. The NEMI is expressed by a circle divided into four quarters colored with green or left blank according to a procedure's greenness. The ESA has no figure, but it is explained by numerical values, with a maximum score of 100. The GAPI, like the NEMI, is expressed by a pictogram divided into 15 sections; each section is shaded with one of three colors (green, yellow, and red) according to the greenness criteria of each particular technique. A complete environmentally friendly section takes the green color, a moderate environmentally friendly section takes the yellow color, while a non-ecofriendly section takes the red color.

Table 1. NEMI, ESA, and GAPI for evaluation of analytical procedures from published studies of empagliflozin.

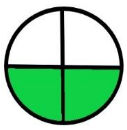
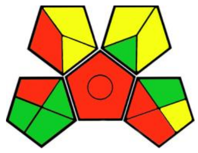
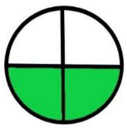
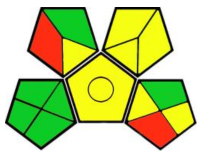
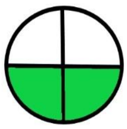
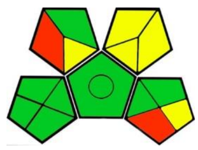
Study	Applied Instrument and Chromatographic Conditions	NEMI	Eco-Scale	GAPI
1. Mixed-mode solid phase extraction combined with LC-MS/MS for determination of empagliflozin and linagliptin in human plasma [11].	Analytical method: (HPLC method) Mobile phase: gradient elution (2 M ammonium acetate buffer and acetonitrile) with flow rate: 0.4 mL/min		Ammonium acetate buffer 0 Acetonitrile 4 Methanol 6 Occupational hazard 0 Waste 3 Energy 2 Total penalty: 15 Eco-scale: 85 (Green method)	
2. The application of quality by design in the development of a liquid chromatography method to determine empagliflozin in the presence of its organic impurities [12].	Analytical method: (HPLC method) Mobile phase: acetonitrile: water mixture (72: 28) with flow rate: 0.84 mL/min^{-1}		Acetonitrile 4 Methanol 6 Phosphoric acid 2 Triethylamine 8Occupational hazard 0 Waste 3 Energy 1 Total penalty: 24 Eco-scale: 76 (Green method)	
3. Simultaneous estimation of empagliflozin and metformin with high-performance thin-layer chromatography using quality-by-design approach [13].	Analytical method: (HPTLC method) Mobile phase: ammonium acetate (2%): methanol: acetonitrile: ethyl acetate (3:1:4.5:1.5)		Acetonitrile 4 Methanol 6 Ethyl acetate 4 Ammonium acetate 1 Occupational hazard 0 Waste 3 Energy 0 Total penalty: 18 Eco-scale: 82 (Green method)	

Table 1. *Cont.*

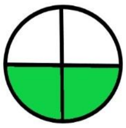
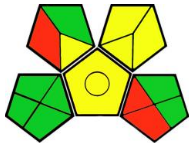
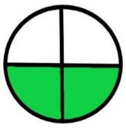
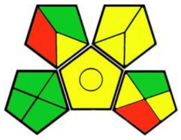
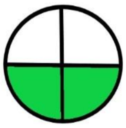
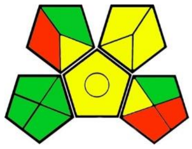
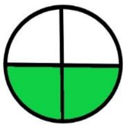
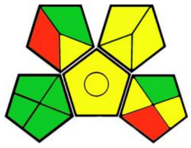
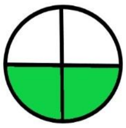
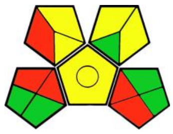
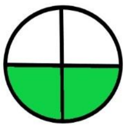
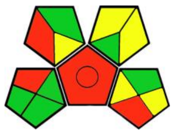
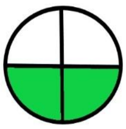
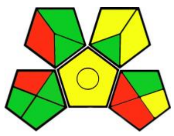
Study	Applied Instrument and Chromatographic Conditions	NEMI	Eco-Scale	GAPI
4. LC-MS/MS determination of empagliflozin and metformin [14].	Analytical method: (HPLC method) Mobile phase: gradient elution (0.1% aqueous formic acid and acetonitrile) with flow rate: 0.2 mL/min^{-1}		Acetonitrile 4 Formic acid 6 Methanol 6 (applying but not a mobile phase) Occupational hazard 0 Waste 1 Energy 2 Total penalty: 19 Eco-scale: 81 (Green method)	
5. Validated liquid chromatographic method for the determination of canagliflozin, dapagliflozin, or empagliflozin and metformin in the presence of 1-cyanoguanidine [15].	Analytic method: (HPLC method) Mobile phase: NaH_2PO_4 buffer (10 mM, pH 2.8): acetonitrile (18.5:81.5, *v*/*v*) with flow rate: 2 mL/min^{-1}		Acetonitrile 4 NaH_2PO_4 (not classified as a hazardous chemical) Orthophosphoric acid 2 Occupational hazard 0 Waste 3 Energy 1 Total penalty: 10 Eco-scale: 90 (Green method)	
6. Densitometric simultaneous estimation of combination of empagliflozin, linagliptin, and metformin hydrochloride used in the treatment of type 2 diabetes mellitus [16].	Analytical method: (HPTLC method) Mobile phase: n-butanol: water: glacial acetic acid (6:3:1, *v*/*v*)		n-butanol 6 Glacial acetic acid 4 Occupational hazard 0 Waste 3 Energy 2 Total penalty: 15 Eco-scale: 85 (Green method)	
7. Liquid chromatographic and spectrofluorimetric assays of empagliflozin applied to degradation kinetic study and content uniformity testing [17].	Analytical method: (HPLC method) Mobile phase: gradient elution (acetonitrile and NaH_2PO_4 buffer) with flow rate: 1 mL/min		Acetonitrile 4 NaH_2PO_4 0 Orthophosphoric acid 2 Methanol 6 Formic acid 6 Occupational hazard 0 Waste 3 Energy 0 Total penalty: 22 Eco-scale: 78 (Green method)	
8. Simple, fast, and robust LC-MS/MS method for the simultaneous quantification of canagliflozin, dapagliflozin, and empagliflozin in human plasma and urine [18].	Analytic method: (HPLC method) Mobile phase: gradient elution (20 mM ammonium acetate and acetonitrile) with flow rate: 0.8 mL/min		Acetonitrile 4 Methanol 6 Ammonium acetate 1 Occupational hazard 0 Waste 1 Energy 2 Total penalty: 14 Eco-scale: 86 (Green method)	
9. Ultrasound-assisted dispersive liquid–liquid microextraction for determination of three gliflozins in human plasma through HPLC/DAD [19].	Analytic method: (HPLC method) Mobile phase: acetonitrile: aqueous 0.1% trifluoroacetic acid (40:60, *v*/*v*) with flow rate: 1 mL/min		Acetonitrile 4 Trifluoroacetic acid 4 Methanol 6 Occupational hazard 0 Waste 3 Energy 1 Total penalty: 18 Eco-scale: 82 (Green method)	
10. A new HPLC-MS/MS method for the simultaneous quantification of SGLT2 inhibitors and metformin in plasma and its application to a pharmacokinetic study in healthy volunteers [20].	Analytic method: (HPLC method) Mobile phase: gradient elution (water and acetonitrile, both containing 1 mM ammonium formate and 0.1% formic acid) with flow rate: 0.2 mL min^{-1}		Acetonitrile 4 Formic acid 6 Ammonium formate 1 Methanol 6 Occupational hazard 0 Waste 3 Energy 2 Total penalty: 22 Eco-scale: 78 (Green method)	

Table 1. *Cont.*

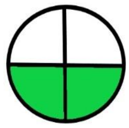
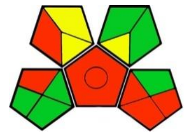
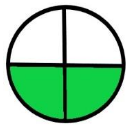
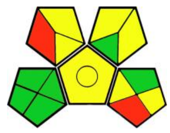
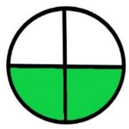
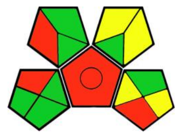
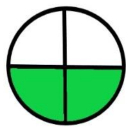
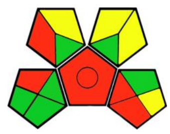
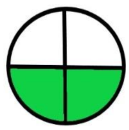
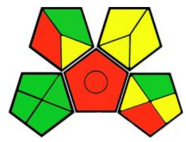
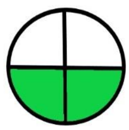
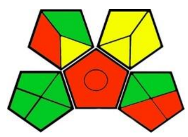
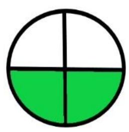
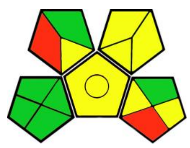
Study	Applied Instrument and Chromatographic Conditions	NEMI	Eco-Scale	GAPI
11-Separation of achiral antidiabetic drugs using sub- or supercritical fluid chromatography with a polysaccharide stationary phase: thermodynamic considerations and molecular docking study [21].	Analytic method: (supercritical fluid chromatography) Mobile phase: CO_2 and 0.1% diethylamine in methanol and isopropanol 50:50 (*v*/*v*) in 60:40 ratios with flow rate: 4.0 mL/min		CO_2 2 Isopropanol 4Diethylamine 6 Methanol 6 Occupational hazard 0 Waste 5 Energy 2 Total penalty: 25 Eco-scale: 75 (Inadequate green method)	
12. New LC-UV method for pharmaceutical analysis of novel antidiabetic combinations [22].	Analytical method: (HPLC method) Mobile phase: 0.1 % formic acid: methanol: acetonitrile (40:20:40) by volume with flow rate: 2.0 mL/min^{-1}		Acetonitrile 4 Formic acid 6 Orthophosphoric acid 2 Methanol 6 Occupational hazard 0 Waste 3 Energy 1 Total penalty: 22 Eco-scale: 78 (Green method)	
13. Silica-coated magnetic iron oxide functionalized with hydrophobic polymeric ionic liquid: a promising nanoscale sorbent for simultaneous extraction of antidiabetic drugs from human plasma prior to their quantitation by HPLC [23].	Analytical method: (HPLC method) Mobile phase: NaH_2PO_4 and sodium dodecyl sulfate (0.01 M): acetonitrile (55:45, *v*/*v*) with flow rate: 1 mL/min^{-1}		Acetonitrile 4 NaH_2PO_4 0 Sodium dodecyl sulfate 6 Occupational hazard 0 Waste 3 Energy 1 Total penalty: 14 Eco-scale: 86 (Green method)	
14. Challenges in simultaneous extraction and chromatographic separation of metformin and three SGLT-2 inhibitors in human plasma using LC-MS/MS [24].	Analytical method: (HPLC method) Mobile phase: acetonitrile and 10 mM ammonium formate buffer (75:25, *v*/*v*) with flow rate: 0.9 mL/min		Acetonitrile 4 Ammonium formate 1 Occupational hazard 0 Waste 3 Energy 2 Total penalty: 10 Eco-scale: 90 (Green method)	
15. Stability-indicating pH- and pKa-dependent HPLC-DAD method for the simultaneous determination of weakly ionizable empagliflozin, dapagliflozin, and canagliflozin in pharmaceutical formulations [25].	Analytical method: (HPLC method) Mobile phase: gradient elution (acetonitrile and 0.1% formic acid buffer) with flow rate: 1 mL/min^{-1}		Acetonitrile 4 Formic acid 6 Occupational hazard 0 Waste 3 Energy 1 Total penalty: 14 Eco-scale: 86 (Green method)	
16. Stability-indicating ultra-performance liquid chromatography method development and validation for simultaneous estimation of metformin, linagliptin, and empagliflozin in bulk and pharmaceutical dosage forms [26].	Analytical method: (HPLC method) Mobile phase: gradient elution (mixture solution of 40% phosphate buffer and 60% acetonitrile) with flow rate: 0.6 mL/min		Acetonitrile 4 Phosphate buffer 0 Occupational hazard 0 Waste 5 Energy 0 Total penalty: 11 Eco-scale: 90 (Green method)	
17. Determination of empagliflozin in the presence of its organic impurities and identification of two degradation products using UHPLC-QTOF/MS [27].	Analytical method: (UHPLC method) Mobile phase: acetonitrile: water mixture (72:28), with flow rate: 0.84 mL/min^{-1}		Acetonitrile 4 Methanol 6 Phosphoric acid 2 Triethylamine 8 Occupational hazard 0 Waste 3 Energy 0 Total penalty: 23 Eco-scale: 77 (Green method)	

Table 1. *Cont.*

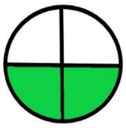
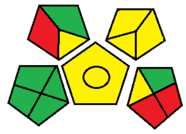
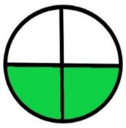
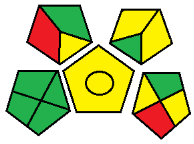
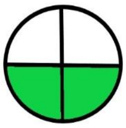
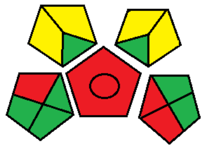
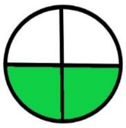
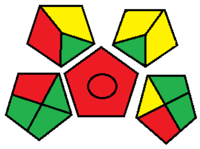
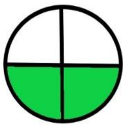
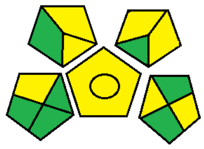
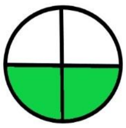
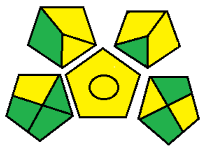
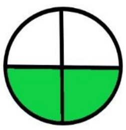
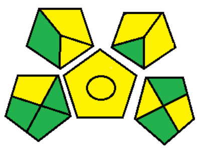
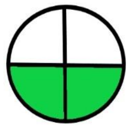
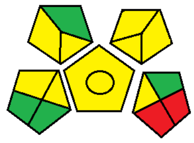
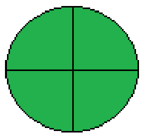
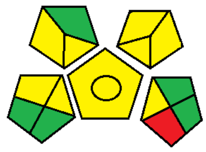
Study	Applied Instrument and Chromatographic Conditions	NEMI	Eco-Scale	GAPI
18. Application of experimental design in HPLC method optimization and robustness for the simultaneous determination of canagliflozin, empagliflozin, linagliptin, and metformin in tablet [31].	Analytical method: (HPLC method) Mobile phase: Na_2HPO_4 buffer (0.05 M, adjusted to pH 6 using o-phosphoric acid): acetonitrile: methanol (50:25:25, $v/v/v$) with flow rate: 1.5 mL/min		Na_2HPO_4 buffer 0 o-phosphoric acid (pH 6) 0 Acetonitrile 4 Methanol 6 Waste 5 Energy 1 Total penalty: 16 Eco-scale: 84 (Green method)	
19. Development of an HPLC-UV method to assay empagliflozin tablets and identification of the major photoproduct by quadrupole time-of-flight mass spectrometry [30].	Analytical method: (HPLC method) Mobile phase: methanol, acetonitrile, and purified water (60:5:35 v/v) with flow rate: 1 mL/min^{-1}		Purified water 0 Acetonitrile 4 Methanol 6 Waste 3 Energy 1 Total penalty: 14 Eco-scale: 86 (Green method)	
20. Validation of a novel UPLC-MS/MS method for estimation of metformin and empagliflozin simultaneously in human plasma using freezing lipid precipitation approach and its application to pharmacokinetic study [29].	Analytical method: (UPLC method) Mobile phase: formic acid (0.01%): acetonitrile (70:30 v/v) with flow rate: 0.3 mL/min		Formic acid (0.01%) 0 Acetonitrile 4 Waste 1 Energy 2 Total penalty: 7 Eco-scale: 93 (Green method)	
21. Innovative TLC densitometric method with fluorescent detection for simultaneous determination of ternary antidiabetic mixture in pharmaceutical formulations and human plasma [28].	Analytical method: (HPTLC method) Mobile phase: toluene: methanol: ethyl acetate (4:3:2 $v/v/v$) Separation of the cited drugs was performed on aluminum plates precoated with silica gel 60 F_{254}		Toluene 3 Methanol 6 Ethyl acetate 2 Waste 3 Energy 0 Total penalty: 14 Eco-scale: 86 (Green method)	
22. LC-PDA method for the simultaneous quantification of metformin, empagliflozin, and linagliptin in pharmaceutical dosage form [32].	Analytical method: (HPLC method) Mobile phase: acetonitrile: triethylamine (TEA) (70:30) with flow rate adjusted to 1 mL/ min		Acetonitrile 4 TEA 3 Energy 1 Waste 3 Total penalty: 11 Eco-scale score: 89 (Green method)	
23. Stability-indicating RP-HPLC for determination of gliflozins in their mixture with metformin [33].	Analytical method: (HPLC method) Mobile phase: acetonitrile: 0.1% orthophosphoric acid (65:35) with flow rate adjusted to 1 mL/min		Acetonitrile 4 Energy 1 Waste 3 Total penalty: 8 Eco-scale score: 92 (Green method)	
24. Analytical quality by design based on design space in reversed-phase high-performance liquid chromatography analysis for simultaneous estimation of metformin, linagliptin, and empagliflozin [34].	Analytical method: (HPLC method) Mobile phase: 0.043 M NaH_2PO_4 buffer premixed with 0.05% v/v TEA (buffer pH 3.79 adjusted using orthophosphoric acid): methanol (34.4:65.6, v/v) with flow rate: 1 mL/min		Orthophosphoric acid 1 Methanol 6 Energy 1 Waste 3 Total penalty: 11 Eco-scale score: 89 (Green method)	

Table 1. *Cont.*

Study	Applied Instrument and Chromatographic Conditions	NEMI	Eco-Scale	GAPI
25. Green chromatographic methods for simultaneous microdetermination of empagliflozin and linagliptin with metformin and its pharmacopeial impurities in pure form and triple-combination tablets: a comparative study (HPLC) [35].	Analytical method: (HPLC method) Mobile phase: methanol and 0.01 M sodium dihydrogen orthophosphate buffer at pH 2.55 (adjusted with orthophosphoric acid) eluted in a gradient mode		Methanol 6 Orthophosphoric acid 1 Energy 1 Waste 5 Total penalty: 12 Eco-scale score: 88 (Green method)	
26. Green chromatographic methods for simultaneous microdetermination of empagliflozin and linagliptin with metformin and its pharmacopeial impurities in pure form and triple-combination tablets: a comparative study (HPTLC) [35].	Analytical method: (HPTLC method) Mobile phase: ethanol: ethylacetate: water (3.5:3:1, *v*/*v*/*v*, respectively) as mobile phase using Merk TLC plates precoated with 60 F_{254} silica gel on aluminum sheet as the stationary phase		Ethanol 6 Ethylacetate 1 Energy 0 Waste 5 Total penalty: 12 Eco-scale score: 88 (Green method)	

3. Results and Discussion

Three assessment tools were applied in this environmental safeness comparison, including the NEMI, ESA and GAPI tools. The NEMI tool showed nondistinctive results, as all the methods had two green parts in their pictograms, except the TLC method [35], as the mobile phase consisted of ethanol and ethylacetate. The TRI (Toxic Release Inventory) list and the RCRA's (Resource Conservation and Recovery Act's) list do not include either ethanol or ethylacetate. According to the ESA tool, it presented more detailed results. By applying the ESA tool, methods 20 [29] and 23 [33] came in first place in the arrangement, with 93 and 92 ESA scores, respectively, followed by method 5 [15] and method 14 [24], with 90 ESA scores. The ESA score of each method was calculated by subtracting the total penalty points of each constituent of the mobile phase, the energy consumption of the instrument used, and the amount of waste from 100. An ideal green method must have a score of 75 or more. The method with the highest ESA score became the greenest method. To apply a comprehensive comparison, the GAPI tool was also used, ensuring that method 22 [32], method 23 [33], and method 24 [34] were in the top, with pictograms free of red color and nine green parts.

All the distinctive data of the chromatographic methods under the study are included in Table 1. From Table 1, it is clearly seen that the high-performance liquid chromatographic method of number 23 presented by [33] was the greenest based on the GAPI, with nine green subcategories and zero red subcategories in the pictogram, indicating an eco-friendly and green method. Additionally, an ESA score of 92 was obtained for method number 23, indicating a safe procedure. The mobile phase applied in this method was almost green, as shown in Table 1, with less energy consumption.

Finally, based on the above summary of the most relevant data in Table 1, the NEMI was an ineffective tool for assessing greenness on its own, and it must be supplemented with other techniques for an accurate and exact conclusion. Furthermore, the GAPI was one of the most important instruments for evaluating greenness since it gave extensive and in-depth information when comparing various analytical methodologies. The ESA rating offered a semiquantitative environmental evaluation of the analytical methods, including the amount of consumed reagents and the amount of waste formed. In addition, the ESA had the advantage of being easier to use than the GAPI. Further details about the green analytical procedure index (GAPI) for empagliflozin analysis are presented in Table S1. In order to produce fair results, the results of the GAPI tool and the ESA were combined, and the final results concluded that method 23 [33] was in the lead.

Therefore, it is advised to use more than one greenness assessment tool for accurate and reliable results of greenness assessment. Moreover, laboratories should plan for greenness analytical methods prior to practical trials to ensure safe and eco-friendly procedures with less waste and hazards to the environment.

The following explains in detail the application of the three greenness assessment tools for method number 23 [33]:

– NEMI tool:

In this method, a circle symbol of four quarters was drawn (pictogram); each quarter represented one method aspect that could have a possible hazardous environmental impact. Acetonitrile is bio-accumulative and toxic (PBT) to the environment, as it is listed on the TRI (Toxic Release Inventory) list, as well as that of the RCRA (Resource Conservation and Recovery Act) [38]. Consequently, the hazardous quarter and the PBT quarter were blank.

The pH during analysis was 3.5, so the mobile phase had no corrosiveness threatening the environment during analysis, and the pH quarter was green. If the pH of the mobile phase is below 2 or more than 12, the method is corrosive, and the pH quarter is blank. The amount of waste generated was below 50 g, so the waste quarter was green as well.

– ESA tool:

Referring to Gałuszka et al. [8], for convenience and simplicity, pictograms and signal words should be included in the evaluation of hazards posed by reagents used in an analytical procedure. Each reagent can be characterized by one or more of nine pictograms (flame, flame over circle, corrosion, gas cylinder, skull and crossbones, exclamation mark, environment, and health hazard). For each pictogram, penalty points are assigned. Two signal words are used in GHS: "danger" (more severe hazard, category 1 or 2) and "warning" (less hazard, other categories). The following system can be used to calculate the penalty points of hazards: none (no pictogram) = 0 penalty points, less severe hazard = 1 penalty point, and more severe hazard = 2 penalty points. In method 23 [33], acetonitrile was had two pictograms and was a more severe hazard, so its penalty points were 4, i.e., amount pp x hazard pp. For energy consumption penalty points, HPLC-RP consumed 1.5 kwh energy per sample, referring to one penalty point [8]. Regarding waste, it was calculated by multiplying the flow rate and time consumed. A total of 5 mL waste was produced per sample in method 23 [33], referring to three penalty points for waste [8]. The ESA score was calculated by subtracting all the penalty points from 100, as shown in Table 1.

– GAPI tool:

In accordance with the GAPI, each analytical operation began with sample collection. The GAPI tool contains a pictogram that uses a color pattern (green, yellow, and red) referring to the ecological value of each step of an analytical process. The details of the construction of the GAPI pictogram are explained in Table S1.

4. Conclusions

Several assessment techniques were presented for selecting the greenest analytical method applied to determine empagliflozin. A comparative study was conducted on 26 chromatographic analytical methods. Although the NEMI tool was simple, it was the least effective at delivering information about the greenness of the analytical techniques, with all 26 chromatographic methods displaying identical NEMI diagrams, with two quarters green and two quarters blank. On the other hand, the ESA rating offered a semiquantitative environmental evaluation of the analytical methods, including the amount of consumed reagents and the amount of waste formed. Furthermore, the GAPI was one of the most important tools for evaluating greenness, since it gave extensive and in-depth information when comparing various analytical methodologies. According to the results, suggestions for the ESA and GAPI tools were created, which were informative and complete tools that gave trustworthy and consolidated results concerning the greenness of analytical procedures. The findings indicated that method number 23 was the greenest in accordance with the ESA and GAPI tools. It is highly advised when comparing the greenness profiles of different chromatographic methods to use more than one assessment tool to ensure reliable greenness profiling for analytical trials, as indicated when the NEMI, ESA, and GAPI tools were coupled for comparison.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations9100275/s1, Table S1: Green analytical procedure index GAPI in details for Empagliflozin analysis.

Author Contributions: Conceptualization, A.M.A. and I.A.N.; methodology, Y.A.A.; software, A.E.A.; formal analysis, F.F.A.; investigation, T.S.A.; resources, B.D.A.; data curation, I.A.N. and F.F.A.; writing—original draft preparation, E.Y.S.; writing—review and editing, I.A. and A.A.; visualization, M.A.S.A.; supervision, E.Y.S., I.A.N., F.F.A., and I.A.; project administration, I.A.N.; funding acquisition, A.A. and M.A.S.A. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

HPLC	high-performance liquid chromatography
RSC	the Royal Society of Chemistry
NEMI	national environmental methods index
MDCB	methods and data comparability
ESA	eco-scale assessment
GAPI	green analytical procedure index
PBT	persistent, bio-accumulative, and toxic
PCT	proximal convoluted tube
CVD	cardiovascular disease
SGLT2	sodium glucose cotransporter-2
DM2	diabetes type 2
GAC	green analytical chemistry

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