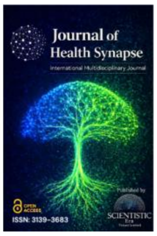
ISSN: 3139-3683

JOURNAL OF HEALTH SYNAPSE (JHS)
Peer Reviewed Refereed Open Access International Multidisciplinary Journal

Publisher: *Scientistic Era Private Limited*
Journal Hompage: *https://healthsynapse.org*



Green analytical techniques in drug development: Eco-friendly analytical methods, miniaturisation, and solvent reduction

Ishika [a*]

[a]*Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra, ishikaantil63@gmail.com*

ARTICLE INFO

KEYWORDS:
Green bioanalytical method , Separation Method , Supercritical , Environmental Impact.

ARTICLE HISTORY
Received Date: 15 April 2026
Revised Date: 28April 2026
Accepted Date: 29 May 2026
Published Date: 30 June 2026

CITATION
Ishika., 2026 Green analytical techniques in drug development: Eco-friendly analytical methods, miniaturisation, and solvent reduction. *Journal of Health Synapse (JHS), 1(2), 38-49. https://doi.org/*

ABSTRACT

The goal of green bioanalytical methods is to lessen or completely eradicate the hazardous waste that bioanalytical technologies generate. A methodical and practical approach to the development of bioanalytical methods has an significant addition to the green analysis. Choosing the right organic mobile phase components and sample extraction method .The separation method makes the bioanalytical approach environmentally friendly. Recent advancements in green analytical chemistry are highlighted in this study, including the application of green solvents like water, ionic liquids, supercritical carbon dioxide, bio-based substitutes, and energy-saving methods like processes that are photo-induced, ultrasonic, and microwave-assisted. Developments in green instrumentation, such as portable and compact instruments and the incorporation of Analytical procedures are now more efficient and have a smaller environmental impact because to automation and chemometric technologies.

I. Introduction

Modern analytical chemistry is facing new hurdles as the analytical world grows. Analysing complicated samples and ensuring the sustainability of those methods are current challenges facing the industry. Concerns about the environment have prompted the evaluation of novel analytical techniques that affect both the safety of nature and human welfare. However, the capacity to examine complex materials has improved thanks to sophisticated and compact analytical instruments coupled with the least amount of energy, consumables, and reagents. The most labour-intensive and time-consuming part of the analytic process is still sample treatment, which takes up to 60–80% of the overall time [1]. This stage also has an impact on the method's analytical performance (such as accuracy and precision) and green features [2].

Green analytical instruments, which are based on the ideas of green chemistry, were the answer to this issue. Additionally, as time went on, green analytical methods evolved into a profession that has reduced the negative effects of dangerous chemicals on the environment, and in addition to that Additionally, it has improved the speed, specificity, and efficiency of chemical analysis. Anastas coined the term "green chemistry" in 1991, and Badami, Nemeroff, and Kletz expanded on the idea.

In 1995, GAC (Green Analytical Chemistry) made its first move. Green analytical tools are those that require methods that reduce chemical usage, minimise the analysis time, as well as using combinative approaches and the direct method of analysis to get beyond the pre-analysis and sample preparation procedures. The use of green analytical tools for detection and analysis has been covered in this chapter.

Principles of Green Chemistry:

The green chemistry approach is an ideal way to reduce costs for society and industry. It increases the likelihood that waste will be accepted and disposed of so that no hazardous or poisonous materials are present generated from chemical-producing sources. Anastas and Warner presented "Twelve Principles of Green Chemistry" 3–4.

1. **Prevent Waste**: Recycling or waste cleansing is the procedure used following any Preventing such trash is preferable to treating it because research might lead to a variety of hazards. These can be accomplished through miniaturisation, direct solventless processes, and approaches to determination of extraction .
2. **Atomic Economy:** The goal of the synthetic approach is to make greater use of every material needed to create the finished product.
3. **Less Hazardous Chemical Synthesis:** Online analytical waste detoxification is a synthetic process that minimises toxicity to human health and the environment.
4. **Creating Safer Chemicals:** Create chemical products with the least amount of toxicity possible while still achieving their intended purpose.
5. **Safer Solvents and Auxiliaries:** Reduce the usage of supplements as much as possible and make them entirely safe. Solventless extraction methods, direct analysis, and the replacement of harmful solvents with less toxic ones.
6. **Energy Efficiency**: Design Energy requirements for chemical processes are reduced and synthetic methods at ambient temperature and pressure must be conducted if the possible application in microwave, ultrasound or pressure-assisted extraction. The goal of all these initiatives is to reduce energy use.
7. **Uses of Renewable Raw resources:** When necessary, use renewable raw resources.
8. **Reduce Derivatives:** Avoid needless derivatisation wherever feasible because it produces waste and calls for extra reagents.
9. **Catalysis:** Compared to stoichiometric reagents, catalytic reagents are superior.
10. **Design for Degradation:** Create chemical products that decompose into fewer byproducts that don't linger in the environment.
11. **Real-Time Prevention:** Development of Pollution Analytical Techniques can provide in-process monitoring, real-time analysis, and control prior to the development of hazardous materials to create methods that enable the acquisition of analytical results with (ideally) no delay.
12. **Chemistry That Is Inherently Safer to Prevent Accidents:** Chemical processes should employ substances and their types that have the ability to reduce the potential for the use of no solvent in chemical mishaps, such as leaks, explosions, and fires methods to avoid surveillance, miniaturisation, and exposure at work.

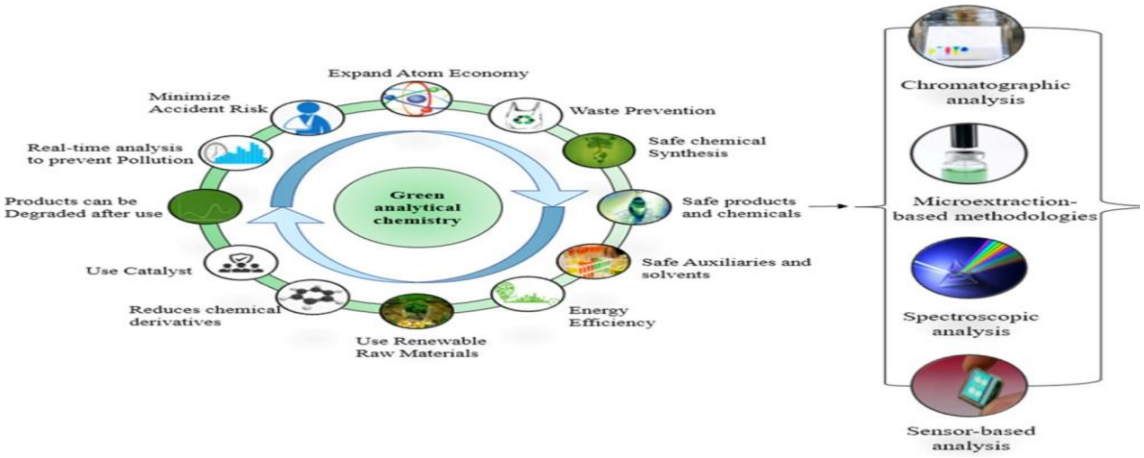


Figure 1: Concept and Principles of GAC (green analytical chemistry)

Scope

- Basic advancements enabling environmentally friendly analytical chemistry techniques and technologies
- The creation of biosensors and environmentally friendly sensors
- Reusing the gadgets to cut down on waste
- Alternative solvents, substituting dangerous substances, and solventless extraction methods
- Miniaturisation allows for a significant reduction in the quantity of trash produced and reagents used.
- Minimising or avoiding analytical procedures' adverse consequences
- Cutting time and effort
- Eco-friendly methods for preparing samples
- Development of analytical instruments and sampling procedures on-site .

- Methods that can be fully or partially automated to accurately, safely, quickly, and efficiently analyse the parameters of biological and environmental samples.
- Cells of flow
- Green structural and physicochemical analysis
- Teaching Green Chemistry
- Developing evaluation techniques and validation criteria, as well as adhering to sustainable development goals, are new approaches to ensuring quality in analytical chemistry [8].

Classification:

Four categories have been established for green analytical approaches based on analytical strategies :

1. Chromatographic instruments
2. Methods based on microextraction
3. Spectroscopic instruments
4. Tools based on sensors

Sustainable development and green analysis complement each other. In essence, the idea has to do with industrial-scale procedures and goods. At first, it was limited to organic synthesis in the chemical and pharmaceutical industries. In 2000, green chemistry evolved into green analytical chemistry. This has evolved into a new strategy where researchers concentrate on creating environmentally friendly laboratory procedures. Later, it advanced into improving the tools and procedures required to improve the quality of chemical analyses while reducing their adverse environmental consequences. The use of green analytical techniques has positioned labs to support sustainable activities. Maintaining an environmentally benign method while increasing the efficiency of the outcomes was the chemists' main problem [3–4].

This has happened as a result of the scientific community being more conscious of the idea of green analytical chemistry. This includes creating innovative goods, procedures, and methods that get past different social, economic, and environmental barriers. This concept's primary goals were to guarantee that the developed method or product is safe for the environment, uses cleaner production techniques, preserves a sustainable ecology, and reduces pollution. The majority of solvents used in analytical procedures are volatile organic compounds, which have drawbacks such as being extremely flammable, poisonous, and classified as hazardous air pollutants.

Because of this, they pose a serious risk to the environment, human health, and safety. They also include ecological toxicity and waste management problems. Consequently, while summarising the idea of green analytical procedures, they are defined as methods that reduce chemical toxicity and hazards as well as samples, solvents, and reagents with improved energy efficiency and shorter analysis times [5].

The following are some of the several approaches that industries are using for greener analysis. These also apply to broad green chemistry principles.

- Solvent recycling, clean effluent recycling, downsizing, and distillation of the entire analytical process are methods to reduce the amount of solvent used.
- To employ direct methods of analysis and minimise the use of reagents and derivatising agents.
- Use of analytical methods that guarantee sample preparation is not necessary, such as non- destructive (NIR and Raman Spectroscopy).
- High-throughput analysis is used to quickly analyse several components.
- Use of analytical or combinative methods that reduce sample size and yield quick findings.
- Reduction or improvement of pre-treatment and sample preparation processes.

Green sample preparation

1. Solid Phase Extraction (SPE) : SPE is a widely used technique for sample preparation. In SPE, solutes are adsorbed onto a short column of a compatible solid sorbent after an aqueous sample is run over it**.** The analysed materials are enriched when they are extracted from the absorbent in trace amounts of highly eluting organic solvents [25]. Solid phase extraction uses small amounts of solvent and generates little waste. As a result, it is thought to be an environmentally advantageous approach.

Simple, inexpensive equipment can be used to mechanise SPE, increasing laboratory throughput while improving accuracy and precision. Notwithstanding the benefits of this approach, several potential disadvantages should be considered to avoid inefficient analyte extraction. The uneven bed of the packing material is one of the issues that could lead to a decrease in efficiency. Using commercial cartridges is a safe way to address this problem. The limited selectivity of some conventional sorbents may also lead to inadequate retention of highly polar compounds. Analyte recovery is significantly impacted by the conflict between analytes and sample matrix over retention, which is another issue. Therefore, to ensure successful analyte extraction, strict process optimisation is required [26].

2. **QuEChERS Extraction Methodology**

The well-known extraction method QuEChERS is renowned for being quick, easy, affordable, effective, reliable, and secure. The complete QuEChERS approach was developed in 2002 by Anastassiades et al. [27]. Since it uses fewer organic solvents than other extraction techniques, this approach is considered a green extraction method. QuEChERS methods consist of two crucial stages: Solvent extraction and sample cleaning. The sample, buffer (to protect base-sensitive analytical agents inside the sample), anhydrous magnesium sulphate, and sodium chloride (to salt out) are all vigorously agitated during the solvent extraction procedure. To eliminate Using "primary secondary amine" (PSA), a weak anion exchanger, and magnesium sulphate to soak any leftover water Rapid dispersive SPE is improving the extract's purity by using absorbent as the dispersive solid-phase extraction agent [28].

QuEChERS was employed in a number of applications to extract analytes of interest from blood tests, including the removal of various contaminants from human blood sample [29], and eliminating cocaine, amphetamine, and opiates from blood using liquid chromatography and tandem mass spectroscopy [30], as well as the recently finished extraction of THC [31].

3. **Solid Phase Microextraction (SPME)**

Solvent-free sample preparation can be achieved by combining the extraction and enrichment processes with SPME. Arthur and Pawliszyn developed the first iteration of this method in 1990. This technique uses silica fibre coated with the proper adsorbent phase. Both concentration on the fibre layer and direct extraction of the analyte from the solution are employed [32]. The efficacy of the analyte preliminary concentration in the SPME technique may differ based on a number of factors, in addition to the type of fibre, sample stirring, length of extraction, etc. [33]. By combining SPME with GC/mass spectrometry (MS), high-performance liquid chromatography (HPLC), GC, and LC–MS, It is possible to isolate chemicals from food samples. Low cost, simplicity of use, and elimination of solvent disposal costs, short sample preparation times, reliability, susceptibility, and Perceptiveness is one of SPME's primary advantages. However, in order to avoid negative consequences, certain flaws must be found.

These include the brittleness of the fibre, which could cause it to break when handled, the possibility of coating degradation from frequent competitive absorption while using coatings of the sorbent kind, the significant influence of temperature and mass transmit variables on matrix effects, equalisation, and other aspects. [34].

4. Stir-Bar Sorptive Extraction (SBSE)

SBSE is a solvent-free, environmentally friendly extraction technique that was introduced in 1999 as a replacement for SPME. The initial goal of SBSE was to separate and purify volatile analytes from the water-based matrix. Its applications were then broadened to cover non-volatile analytes in combination with HPLC and the analysis of the headspace above samples that are gaseous, liquid, or solid.

Like SPME, SBSE employs sorptive extraction. Analytical materials are absorbed into a matrix of polymers, like polydimethylsiloxane. In this case, however, it is wrapped around a magnetic stir rod rather than a fibre. matrix components that interfere, such as fatty acids and carbohydrates, the stage of sample washing is crucial. When opposed to SPME, SBSE uses a significantly larger quantity of the sorptive phase main difference between the two. The result is improved sensitivity, especially when using large sample volumes or materials with low partition coefficients.

In this case, there is no solvent at all because the coating's capacity to absorb volatile analytes enables them to be immediately thermally desorbed into a GC. Tiny amounts of a diluent are used to desorb non-volatile analytical material, maintaining the method's eco-friendliness [35].

5. Dispersive Liquid–Liquid Microextraction (DLLME)

An aqueous sample, an extracting solvent that is immiscible with water, and a dispersive fluid that is soluble in each phase make up the three-phase solution used in the procedure. An incredibly fine emulsion is produced by combining the two different solvents and rapidly introducing the resulting solutions into the samples. Analytes may therefore be transported in the scattered extraction solution very rapidly. The emulsion is centrifuged once it has been formed.

Using a micro syringe, the phase of increasing extraction density is extracted and added to a desired analytical apparatus. A small sample volume, little solvent consumption, a high enrichment factor, outstanding repeatability, and good recovery are only a few advantages of DLLME.

Massive regions of closeness between the solvent mixture and the watery portion are created by the emulsion produced after it was added to the liquid amalgam.

Consequently, the extraction is efficient and the equilibration occurs rapidly [36].

6. Pressurised fluid extraction (PFE)

This process, known as accelerated solvent extraction (ASE), entails carrying out the extraction at temperatures and pressures higher than the solvent's boiling point at atmospheric pressure. At the higher temperature, the solvent's viscosity and surface tension drop while the analytes' solubility and diffusion rate increase. The extractant can more easily enter the matrix pores under increased pressure. Because PFE consumes less solvent it makes it possible to employ environmentally friendly solvents such as ethanol, methanol, and accomplishes efficient extraction in a shorter time frame with less energy [36].

7. Microwave-Assisted Extraction (MAE)

In MAE, microwave radiation is used to expedite the extraction process. The MAE principle depends on polar molecules absorbing microwaves, which heats the solution. It allows analytes to be extracted into a solvent or a water-based solution by heating the solvent that interacts with the substance. While conventional ovens should only operate at 2.45 GHz, the full microwave use are its main advantages. The limited heating of the examined solvent due to the dielectric constant is its only disadvantage [37].

8. Ultrasound-Assisted Extraction (UAE)

Given that traditional or other more recent methods have a number of disadvantages, including expensive capital expenditure, energy use, and the usage of hazardous organic chemicals for Over the past ten years, there has been an increase in the usage of ultrasound for extraction. A method called ultrasound-assisted extraction (UAE) makes use of ultrasound in the preapplication procedures, and it might be preferred because it is an environmentally responsible and clean extraction method [38]. Consequently, the use of UAE is increasingly recognised as a secure and useful substitute for conventional food production techniques. Thus, compared to other extraction methods, ultrasound is an easy-to-use, multidirectional, adaptable, and low-cost technology. Using ultrasonography increases the variety of solvents that are generally thought to be safe.

9. Supercritical Fluid Extraction (SFE)

Supercritical fluid extraction (SFE) is a straightforward, completely automated, and eco-friendly analytical method. A procedure that uses water or other non-toxic solvents is referred to be a "environmentally benign sample preparation method" (such as "typical SFE"). Reduced temperatures, pressures, and extraction times, along with the capacity to maintain the SFE can virtually eliminate the chance of activity loss by maintaining the integrity of food's functional components [39]. Clean extract is one of SFE's advantages since it uses less solvent and requires less time to extract. One benefit of SFE is clean extract because it uses less solvent and takes less time to extract. The most common use of nontoxic and non-polluting extraction fluids is made possible by this approach's sample pretreatment stage [40]. Nitrous oxide, carbon dioxide, ethane, propane, n-pentane, ammonia, and sulphur hexafluoride are a few of the most widely used supercritical fluids. Because CO2 doesn't have any abrasive or explosive qualities, it is the most frequently used petrol. The main advantages of the SFE are quantitative, simple, fast, selective, and environmentally friendly [41].

Greener organic solvents in analytical chemistry

The most durable and environmentally friendly solvent that might be utilised in analytical Without a doubt, chemistry has no solvent. However, in order to liquidise samples and make it Solvent is required for both sample preparation and analytical methods, however it is simple to separate relevant components.

To modify conventional organic solvents, which are frequency range of 300 MHz to 100 GHz can be utilised. The MAE's rapid heating, high temperatures, and ease of known for their excellent volatility, combustibility, and toxicity, recent advancements in adopting the ideas of green New solvents are created and used as a result of integrating chemistry into analytical processes [42]. Green organic solvent selection is aided by the use of solvent selection guidelines (SSGs) [43].

An alternative to conventional organic solvents, highlighting the three most Amphiphilic solvents, which include alcohols and carboxylic acids, are important forms of green solvents, and surfactants), deep eutectic solvents (DESs) [45], ionic liquids (ILs) and analogues [44]. Analytical samples have been prepared using each of these solvents (as extraction solvents) and liquid chromatography (LC) (mostly as pseudo stationary phases or as mobile phase enhancers).

A. Alternative solvents for sample preparation

The most sustainable, economically feasible, environmentally sound, and safe solvent is water. The simplest and most used technique for extraction using water as the solvent is maceration. It is possible to slightly change the properties of water by using certain chemicals. Water must be combined with another material, either a hydrotrope or a surfactant for hydrotropic and micellar extractions. By generating aggregates and micelles, In aqueous settings, these amphiphilic compounds can enhance the dissolution of substances that dislike water [46, 47].

Hydrotropic extraction just incorporates a continuous liquid phase, whereas micellar extraction imitates a two-liquid-phase technique. Subcritical water extraction (SWE) is another method of extracting nonpolar compounds with water. Combinations of naturally occurring solids having melting points significantly lower than any of their individual components are known as Natural Deep Eutectic Solvents (NADESs). The creation of hydrogen bonds between molecules that link hydrogen bond donors and acceptors is the main reason for this uniqueness. Because NADESs are readily recyclable and environmentally friendly solvents [48,49].

The agro-sector from which they originated claims that the "bio-based solvents" gather an vast quantity of biomolecules that can be divided into three categories: cereal/sugar, oleo-proteaginous, and wood. Ethanol, ethyl acetate, lactic acid esters, derivatives of succinic acid, furfural, and its derivatives, including 2-methyl-tetrahydrofuran and cyclopentyl methyl ether (CPME), are examples of bio-based solvents [50,51]. Liquefied gases are those that have the potential to liquefy in their vapour pressure within a pressured vessel at a pressure between 1 and 100 bar. Their volatility makes it easy to distinguish them from the extracts and offer the advantages of dissolving organic components at temperatures that are relatively low, preventing deterioration of sensitive components. A growing body of research documents the use of liquefied gases, such as Supercritical fluids (SFs) are substances at temperatures and pressures above their critical values.

The main advantage of an SFs is that it can be customised for density by varying pressure and/or temperature, which enables the selective extraction of particular analytes. Furthermore, because SFs have almost no surface tension, they can penetrate microporous surfaces and leave very little solvent residue in the finished extract. Carbon dioxide (CO2) is the most widely utilised SF due to its somewhat substantial temperature and pressure of 31 °C and 74 bar, respectively. This fluid is also inert, tasteless, odourless, inexpensive, and not environmentally friendly [53].

B. Using more sustainable solvents as mobile phase

As the mobile phase in chromatography, high-purity organic solvents are required in very large amounts. The switch from normal phase to reversed phase chromatography, which employed toxic, nonpolar solvents as the first step toward eco-friendly chromatography [54]. Acetonitrile (ACN), ethanol (EtOH), methanol (MeOH), acetone, and ethyl acetate, or their mixes with water, or their combinations, are frequently employed in reversed phase liquid chromatography.

EtOH, acetone, and ethyl acetate are the most favoured of these solvents in terms of environmental friendliness, and some efforts are undertaken to substitute them with the more dangerous acetonitrile and methanol [55]. The rather high viscosity of ethanol is a drawback as compared to acetonitrile [56]. One organic mobile phase component that is somewhat hazardous is ethanol. Acetone is another greener substitute for acetonitrile, even though the separation efficiency is statistically same [57]. All four basic and acidic compounds were successfully partitioned using polyethylene glycol as the stationary phase and water with 0.04% triethylamine as the mobile phase [58]. In extreme temperature liquid chromatography, a water-based mobile phase required just 0.85 mL of EtOH per chromatographic run [59, 60]. Because normal phase systems are used to evaluate some nonpolar and non-volatile compounds (such lipids), it is imperative to focus on employing more environmentally friendly solvents in this field. Cyclo pentyl methyl ether, hexa methyl disiloxane, isopentyl acetate, and 2-methyltetrahydrofuran have all been used effectively as mobile phase components [61, 62].

C. Solvent Replacement:

This method substitutes greener solvents for hazardous and combustible ones. The following methods are used in solvent replacement:

1. Replacement of Acetonitrile with Ethanol: dimethyl ether, n-propane, and n-butane, as environmentally acceptable extraction solvents [52]. Acetonitrile has numerous drawbacks from the perspective of green analytical chemistry, including flammability, high cost, and volatility. Because ethanol is readily available and non-toxic, it is used in place of acetonitrile.

Ethanol's high viscosity is one of its drawbacks, however this can be addressed by raising the mobile phase's temperature or employing UPLC equipment 2.

2. Superheated Water Chromatography: Instead of combining water at high temperatures (80–250 °C) with organic solvents like Eluents include water-acetonitrile and water methanol. Water can take the role of organic modifiers because its polarity diminishes with warmth. Superheated water has the following benefits: it is readily available, affordable, non-flammable, environmentally acceptable, and has a low UV cutoff that permits detection at a lower wavelength. It also requires less disposal. However, there are certain restrictions, such as the requirement for high temperatures, which causes thermally liable materials to degrade. More temperature-resistant packing materials, such as polymeric phases [32–33], are needed because lipophilic chemicals are insoluble in water.

3. Supercritical Fluid Chromatography

Similar to supercritical water chromatography, carbon dioxide is employed as a supercritical fluid that provides an alternative to organic solvent. The advantage of using pressurised carbon dioxide in the supercritical state as an eluent is that it has solvent qualities comparable to hydrocarbons obtained from petrochemicals, making it a more environmentally friendly option than often used normal-phase solvents (such as hexane or heptanes). Additionally, the low viscosity of carbon dioxide leads to higher flow rates and quicker separations.

4. Enhanced Fluidity

These methods include the addition of large amounts of soluble gases to polar liquids, such alcohol. Low viscosity, higher solute diffusion coefficients, accurate solvent strength control, and high efficiency are the benefits of this technique.

Additionally, liquid chromatography has been utilised in a variety of separation methods, including size exclusion chromatography, chiral separations, and normal- and reversed-phase LC. Ionic liquid: An ionic liquid is a liquid salt.

When salt melts without breaking down or evaporating, ionic liquids are created.

Quaternary nitrogen cations are the main component of various ionic liquids. As a result, it is seen as a green substitute for solvents. Ionic solvents are good solvents for organic, inorganic, and polymeric materials; they are easy to purchase and prepare; they have no discernible vapour pressure; they are thermally resilient; they produce nearly no waste products; and they are non-flammable.

D. Solvent Reduction

There are numerous ways to utilise less solvent, which also results in less trash being produced. These methods include the following:

1. **Use of Short Column with Small Particle Size:** Reducing the analysis time by cutting the run time was thought to be the most straightforward method of lowering the quantity of solvent consumption. The same separation profile and efficiency can be achieved by reducing the column length as the particle size decreases, long column with larger particle sizes, yet it takes less time to analyse, which results in using less solvent. The primary disadvantage of this was that it causes column back pressure to rise [29].

2. **Ultra-High Performance Liquid Chromatography (U-HPLC)**

HPLC, which was developed to address the high back pressures caused by small particle size, gradually replaced the traditional HPLC devices. Reducing the column's length shortens its run time, uses less solvent, and produces less waste. Additionally, a shorter run time of thirty is achieved by utilising a short column with a tiny internal diameter.

3. **Reversed Hydrophilic Interaction Liquid Chromatography**

The use of organic solvents is reduced in this instance because the stationary phases are inverted to non-polar and the mobile phase used has a high percentage of water.

4. Microflow and Capillary HPLC: Using microscale and capillary columns reduced flow rates, which saved solvent 2.
5. Elevated Temperature: There are several benefits associated with this method by lowering the quantity of organic modifier required and giving water a non-polar nature at high temperatures. As a result, the overall quantity of garbage generated decreases. Reduced viscosity results in higher flow rates, quicker separations, and less backpressure. enhances peak symmetry overall and increases efficiency 31.

Green analytical chemistry tools:

1. **NEMI, or the National Environmental Method Index** The Methods and Data Comparability Board (MDCB) has industrialised this instrument. The largest ecological analytical database is found there. For environmental techniques, the NEMI offers free database access via www.nemi.gov (accessed February 14, 2021).

Keith et al. (2007) provided a detailed description of this instrument, stating that the NEMI is expressed by the greenness profile is a circle with four equal segments. PBT, an acronym for three things, is expressed in the first portion of the circle: poisonous, bioaccumulative, and persistent. The dangerous aspect is expressed in the second section.

The corrosiveness and waste are expressed in the third and fourth sections, respectively. Each component may be given a green hue to represent the method's greenness or a blank hue to represent its absence of greenness. The greenness profile accounts for a number of significant variables, including waste quantity, pH, and substances with particular characteristics. After that, the analyst can easily assess the degree of eco-friendliness and greenness of various analytical methods by visually comparing them [11–12].

2. **Analytical eco-scale/eco-scale assessment (AES/ESA)** AES was introduced in 2012 as a semi-quantitative instrument due to its integration of descriptive and Researchers can reasonably estimate the method's greenness profile using numerical data and the outcomes produced.

The score calculation for evaluating greenness using AES begins with a 100-point scale and is deducted from 100 with specific penalty points. The optimal level of greenness is represented by 100 points, while the penalty points are produced in accordance with the specifications, including the amount of trash, energy consumption, and solvent/reagent. The approach is considered to have good greenness if the total score is greater than 75. The method's greenness is deemed satisfactory if the overall score falls between 50 and 75. The method's greenness is insufficient if the overall score is less than 50. The AES tool's primary benefits are its user-friendliness, quantitative data on environmental implications, and assessment of different environmental impacts. It may have trouble differentiating specifics of the analytical processes, even when it seems to provide conclusive results. Furthermore, the overall AES score cannot be considered a reliable indicator of the root cause of adverse effects; Consequently, it becomes challenging to identify the aspects of the process that require modification. But compared to previous tools, AES is superior. The AES tool is regarded as the most widely used, along with GAPI and AGREE.

3. **Green analytical Procedure Index (GAPI)** GAPI is an integrated technique that assesses the sustainability of a process from determination to sampling. The results of the GAPI assessment, which was first proposed in 2018, are shown as a pictogram

with four extra pentagons encircling a central pentagon. The general kind of procedure, sample evaluation's findings indicate that the pictogram uses the colours red, yellow, and green. GAPI is used to evaluate fifteen distinct criteria. This tool's primary benefit is its simplicity, which incorporates a wide range of analytical procedure parameters. GAPI's shortcomings include producing qualitative data and offering a complicated representation, despite overcoming the major shortcomings of the NEMI tool [13].

4. **Analytical Greenness metric (AGREE**) Twelve green analytical chemistry principles serve as the foundation for the evaluation criteria of the AGREE instrument, which was initially presented by Pena-Pereira et al. in 2020 [14]. With twelve windows to examine each concept, the AGREE tool is a free web program. The outcome is a pictogram with a circle in the center that represents the final score between 0 and 1, surrounded by 12 portions that correspond to each parameter. Red, yellow, and green tones in the pictogram can also be used to interpret the results. On the final scoring scale, a score of 0 represents unsatisfactory and a score of 1 represents satisfactory. The following is a list of the AGREE tool's most significant benefits: - Because it covers every aspect of green analytical chemistry, it is more thorough. Because users can make some changes, it is more adaptable. The comprehensive pictogram makes it simple to analyse the method's advantages and disadvantages. It provides both quantitative and qualitative findings. The software provides quick results and is simple to use. The fact that each section's weight is not explained is the tool's biggest drawback. One of the most popular tools for evaluating greenness nowadays is the AGREE tool [15–16].
5. **The Blue Applicability Grade Index (BAGI)** In 2023, Natalia Manousi et al. presented BAGI as a new metric for evaluating the applicability of analytical techniques inside the White Analytical Chemistry WAC framework. BAGI focuses on ten essential characteristics that are essential for method application, in contrast to typical green metrics that emphasise environmental effect. These consist of the kind of analysis, the capacity to identify several analytes at once, sample preparation, the quantity of samples that may be handled at once, the necessary analytical methods and equipment, throughput in samples per hour, selection of materials and reagents, required for preconcentration, level of automation, and sample size. the primary characteristics and BAGI index score points. Both a pictogram and a numerical score are produced using the BAGI metric tool. An asteroid pictogram with a number in the middle represents the overall assessment. The handling, sample preparation, solvents/reagents, and instruments are the five factors that affect them. The pictogram's colour gradient shows how effectively the approach satisfies the requirements: High compliance is represented by dark blue, medium compliance by blue, low compliance by light blue, and no compliance by white. The analytical method's overall score, which ranges from 25 to 100, is represented by the number inside the BAGI symbol. The lowest level of applicability is represented by a score of 25, whilst exceptional performance is indicated by a score of 100.

6. Recent applications of green technology

By using green solvents in HPLC

Elsheikh et al. have created and refined simple, precise, and trustworthy stability-indicating chromatographic techniques, like the The Central Composite Design is used in conjunction with the HPLC method to provide a cost-effective and ecologically responsible solution. Here, he tried to use safe solvents to find zonisamide (ZNS) even while its breakdown product was present. Using a safe and environmentally friendly mobile phase (MP) with EtOH, Kromasil MS: ZNS was chromatographically separated from its degradation product using H2O (30:70%v/v), at (1 mL/min), and a photodiode array detector set to 280 nm for detection. ZNS 50 g/mL HPLC chromatogram (Rt-2.806 retention time) and its degraded form 10 g/mL (Rt-1.553). Results in the range of 2–10 μg/band were reproducible. The recently created HPLC methods have moderate green hues and ECO Scale scores of 91. The suggested procedures were more environmentally friendly, as shown by the comparison of the findings with the approved USP approach (81 eco scale score) [72].

Green spectroscopy methods

Kokilambigai et al. proposed an environmentally friendly spectrophotometric simultaneous equation method for measuring paracetamol (PCM), aceclofenac (ACF), and medications containing thiocolchicoside (THC) in combination. Since the diluent phosphate buffer with pH 7.8 has no effect on the UV spectral characteristics of the medications, it was used as an environmentally friendly solvent in place of methanol. Maximum absorptions (λmax) for PCM, ACF, and THC were found at wavelengths of 243, 274, and 259 nm, respectively. The recovery quantity ranged from 99.56 to 100.99% w/w, indicating that the developed process was accurate. For paracetamol, aceclofenac, and thiocolchicoside, the linear range was determined to be 5–15 μg/mL, 1–5 μg/mL, and 1–5 μg/mL, respectively. The limits of detection for PCM, ACF, and THC were 0.24 μg/mL, 0.33 μg/mL, and 0.32 μg/mL, respectively. The LOQ values fell within the following ranges: 0.78 μg/mL, 1.01 μg/mL, and 0.95 μg/mL. The greenness profile score was determined using the ECO Scale tool, which yielded a score of 100 [120].

Milestones in Green Analytical Chemistry

Three strategies have been used to lessen the negative environmental effects of analytical techniques:

i) a decrease in the quantity of solvents needed for sample pretreatment;
ii) a decrease in the quantity and toxicity of chemicals and solvents used in the measurement phase, particularly through automation and miniaturisation; and,
iii) the creation of substitute direct analytical techniques that don't call for reagents or solvents [7].

The White Analytical Chemistry principles: In order to create the 12 WAC principles, we suggest combining the 12 recognised GAC principles into four broad "green" rules that address the most significant and independent facets of GAC (G1-G4). The 12 WAC principles are made up of these four concepts plus the four "red" principles (R1–R4) and four "blue" principles (B1–B4) that deal with analytical efficiency and practical/economic factors, respectively. In order to preserve the concept of sustainability, it is vital to stress at the outset that, in accordance with the WAC philosophy, all hues and concepts are equally important. However, only some principles—such as LOD and cost-efficiency—that are essential in a particular scenario and related conditions may establish a method's true use and functioning. As a result, every principle should be taken into account while evaluating the approach, but the most crucial ones for a given application should be evaluated more carefully in order to meet the requirements. A quantitative indicator known as "whiteness," which is a simplified measure of how well the approach suits the intended application, expresses the method's overall compliance with the suggested principles [10].

References

1. Gandhi MY, Prasad SB, Kumar V, Soni H, Rawat H, Mishra SK, Grewal J, Singh S, Charde V, Gupta A, Jha SK. Quantification of phytochemicals and metal ions as well as the determination of volatile compounds, antioxidant, antimicrobial and antacid activities of the Mimosa pudica L. leaf: Exploration of neglected and under-utilized part. Chemistry & biodiversity. 2023 Oct;20(10):e202301049.
2. Shamim, Ali S, Ali T, Sharma H, Kishor BN, Jha SK. Recent advances in monodisperse gold nanoparticle delivery, synthesis, and emerging applications in cancer therapy. Plasmonics. 2025 Sep;20(9):7121-41.
3. Gandhi Y, Kumar V, Singh G, Prasad SB, Mishra SK, Soni H, Rawat H, Singh S, Charde V, Gupta A, Dhanjal DS. Chemoprofiling and medicinal potential of underutilized leaves of Cyperus scariosus. Scientific Reports. 2024 Mar 27;14(1):7263.
4. Jha SK, Islam M, Kumar R, Rana L, Saifi MA, Ali S, Alam N. Evaluation of Vernonia amygdalina del. containing phyto constituents a medicinal plant compound as new potential inhibitors of Monkey pox virus using molecular docking analysis. World J Adv Res Rev. 2023;17(1):1112-22.
5. Singh A, Jha SK, Dwivedi PC, Srivastava U, Pal A. Revolutionizing healthcare: Integrating electronics, AI, traditional, and conventional methods. World J Adv Res Rev. 2024;23:2426-34.
6. Lohiya GV. Conjunctivitis in unusual populations: A review of rare cases and challenges in diagnosis and management. World Journal of Advanced Research and Reviews. 2023 Sep 29.
7. Lohiya GV. Conjunctivitis in unusual populations: A review of rare cases and challenges in diagnosis and management. World Journal of Advanced Research and Reviews. 2023 Sep 29.
8. Doshi R, Lohiya G. Synthesis, in-silico design and spectral characterization, elucidation of Cannabis sativa L. cannabaceae containing phytoconstitutents demonstrating novel therapeutic efficacy against epilepsy.
9. Jha SK, Mishra AK, Kumar V, Dane G, Kumari S, Charde V, Saddam M, Jagtap C, Chuhan S, Chaturvedi SK, Narasimhaji CV. Ecological and Behavioral Impacts of COVID-19 on Human Existence and potential preventive measures through traditional and alternative medicine–A Narrative review. Pharmacological Research-Natural Products. 2024 Jun 1;3:100042.
10. Sonkar V, Jha SK, Tiwari S, Akarsh A, Priya S, Sharan P, Sharan A. Characterization and explanation of the soil fertility state of Sakaldiha block in the Chandauli District of Uttar Pradesh's industrial area. World J Adv Res Rev. 2023;18:063-72.
11. Singh NK, Sengar AS, Jha SK. A review of Ayurvedic measures for preventing COVID-19 and promoting health during pregnancy. Journal of Indian System of Medicine. 2024 Apr 1;12(2):61-5.
12. Chauhan S, Jha SK, Tamta A, Sharma V, Kumar C, Lohiya G, Gadgul A, Satpute K, Chalmale N, Chauhan P, Chaturvedi SK. Descriptive analytical study based on profiling, morphological, pomological and pharmacological traits to identify the genotypes of the promising mango Mangifera indica L. World J Adv Res Rev. 2023;19:1544-53.
13. Khan R, Maheshwari D, Chauhan S, Lohiya GV, Kumar C, Antil I, Sharma S, Garg Y, Chauhan P, Jha SK. Exploring the potential therapeutic value of

Solanum lycopersicum L. phytoconstituents for Parkinson’s disease through molecular docking analysis. World J Adv Res Rev. 2023;20:488-501.

approaches in oncology: bridging ayurvedic medicine and modern cancer therapeutics. Frontiers in Natural Products. 2025 Aug 22;4:1635197.

15. Tamta A, Jha SK. *Screening of Germplasms and Population Dynamics of Major Insect Pests of Spring Green Gram Vigna radiata (Linn.)* (Doctoral dissertation, Doctoral Dissertation, Doctoral Dissertation, GB Pant University of Agriculture and Technology, Pantnagar-263145 (Uttarakhand)).
16. Mehta V, Zargar AA, Attri P, Jha SK. Bagging the role of herbal drugs in the management of cervical cancer. Ayush Journal of Integrative Oncology. 2025 Jan 1;2(1):35-43.
17. Trivedi LM, Saxena S, Sharma S, Beniwal M, Jha SK, Rao AK, Khan MR. Characterization, profiling, and molecular docking analysis of phytochemicals derived from Daucus carota for evaluating their potential role in cardiovascular disease (CVD) assessment. World J Adv Res Rev. 2023;20:159-75.
18. Patel S, Singh S, Gupta AK, Dalimbe AY, Muthoju SM, Pawar AR, Jha SK, Gupta AJ. Ayurveda and common Indian spices: A natural alternative for cancer therapy. Ayush Journal of Integrative Oncology. 2025 Apr 1;2(2):91-102.
19. Jha SK, Gupta A, Huddar VG. Botanical breakthroughs: The growing impact of plant-derived compounds in cancer treatment. Ayush Journal of Integrative Oncology. 2025 Apr 1;2(2):62-7.
20. Kumar R, Verma H, Ali M, Midha T, Kumar D, Jha SK. Review of mitigating cancer risk through Ayurvedic practices: A holistic approach to combating sedentarism. Ayush Journal of Integrative Oncology. 2025 Apr 1;2(2):86-90.
21. Sharma A, Jha SK, Huddar VG. Integrative role of Ayurvedic phytochemicals in cancer treatment: Targeting signaling pathways, boosting chemosensitivity, and utilizing traditional therapeutics. Ayush Journal of Integrative Oncology. 2025 Apr 1;2(2):77-85.
22. Jha SK, Charde V, Kumar V, Narasimhaji CV. Vitiligo treatment with natural bioactive: A narrative review. The Natural Products Journal. 2026 Mar;16(3):e22103155337960.
23. Chawla S, Gupta R, Jha SK, Kashid S, Jha KT. Stereoisomerism in Chemistry and Drug Development: Optical, Geometrical, and Conformational Isomers. Medicinal Chemistry (Shariqah (United Arab Emirates)). 2025.
24. Shrivastav S, Tyagi R, Singh M, Jha S. The effectiveness of curcumin on dysmenorrhea. International Journal of Medical Sciences and Pharma Research. 2022 Dec 15;8(4):8-12.
14. Jha SK, Singh N, Shanker OR, Antil I, Baghel JS, Huddar V, Tripathi R. A review on integrative
25. Gupta AJ, Huddar VG, Jha SK, Soni U. Traditional wisdom in modern oncology: Ayurvedic pain relief and supportive care. Ayush Journal of Integrative Oncology. 2025 Jul 1;2(3):178-87.
26. Chauhan S, Jha SK, Huddar VG, Ahlawat A, Sharma S, Tripathi A, Khurana B, Antil I, Soni U. Targeting cancer at the molecular level: A biochemical and medicinal chemistry approach. Ayush Journal of Integrative Oncology. 2025 Jul 1;2(3):169-77.
27. Antil I, Bangarwa S, Jha SK, Gupta AJ, Soni U, Huddar VG, Tripathi A, Sharma S. Exploring the chemopreventive and anticancer effects of green tea. Ayush Journal of Integrative Oncology. 2025 Jul 1;2(3):158-68.
28. Attri P, Zargar AA, Mehta V, Mushtaq SU, Jha SK, Soni U. Genetic basis of cancer: Harnessing natural remedies for therapeutic potential. Ayush Journal of Integrative Oncology. 2025 Jul 1;2(3):188-94.
29. Zargar AA, Mehta V, Gupta R, Bhandari K, Sharma MC, Balaji P, Jha SK. Incretin hormones: Mechanisms, therapeutic implications, and future directions in glucose regulation and diabetes management. Curr Proteom. 2025 Apr 1;22:100014.
30. Dhiman S, Thakur N, Dwivedi PC, Pande P, Jha SK, Lohiya GV. Phytochemicals: investigating bioactive compounds for antifungal uses and quality issues in functional foods and therapeutics.
31. Sandrepogu J, Khandelwal P, Tiwari SK, Jha SK, Chauhan S, Sharma H, Goel P. A holistic paradigm for cancer care: Merging Ayurveda with contemporary oncology. Ayush Journal of Integrative Oncology. 2025 Oct 1;2(4):254-61.
32. Sharma S, Yadav C, Chawla S, Jha SK. Beyond chemotherapy: Exploring the potential of phytomedicine in oncology. Ayush Journal of Integrative Oncology. 2025 Jul 1;2(3):133-45.
33. Ahlawat A, Chauhan S, Antil I, Jha SK, Huddar VG. Examining the impact of the microbiome on cancer immunotherapy outcomes: A review. Ayush Journal of Integrative Oncology. 2025 Jul 1;2(3):118-24.
34. Shamim PT, Khatri N, Yadav AK, Mishra B, Khan MR, Behera SK, Jha SK. Role of human chorionic gonadotropin in frozen-thawed embryo transfer cycles for secretory transformation: a narrative. Eur Chem Bull. 2023;12(8):5477-93.
35. Sharma H, Mahto RR, Jha SK, Chauhan S, Khandelwal P, Tiwari SK. Rasayana-Based Adjuvant Strategies in Cancer Care: An Ayurvedic–Oncologic Perspective. Iranian Journal of Blood and Cancer. 2025 Feb 30;17(4):35-68.
36. Raj H, Jha SK, Negi M, Khandelwal P, Chauhan S, Sharma H, Goel P, Tiwari SK. Integrative oncology: A comprehensive comparative analysis of herbal and

allopathic medicine in cancer treatment. Current Pharmaceutical Research (CPR). 2025;1:427-5.

37. Kumar C, Chauhan P, Chauhan S, Jha SK, Lohiya G. Phyto-Pharmacognostic Experimental Study of Technol. 2023;8(6):254-60.

38. Raj H, Sharma S, Tripathi A, Huddar VG, Jha SK. Herbal medicine in cancer therapy: A comprehensive review of phytoconstituents, mechanisms, and clinical applications. Ayush Journal of Integrative Oncology. 2025 Jul 1;2(3):146-57.

39. Antil I, Jha SK, Negi M, Sharma H, Tiwari SK. Baicalein from Scutellaria baicalensis as natural therapeutic agent for glioblastoma. Current Pharmaceutical Research (CPR). 2025;1:411-26.

40. Mandal P, Kumar J, Bora D, Hashmi SA, Tiwari M, Gupta R, Behera AK, Amoli M, Singh J, Jha SK. Literature review on forensic implications of illicit drug metabolism: A medicinal chemistry perspective. Journal of Forensic and Legal Medicine. 2026 Apr 24:103158.

41. Bansal KS, Mahto RR, Soni H, Tiwari SK, Goel P, Sharma H, Vishwakarma N, Jha SK, Chauhan S, Khandelwal P. Ayurveda in Renal Cell Carcinoma: A Narrative Review of Supportive Care During Targeted Therapy and Immunotherapy.

42. Bansal KS, Mahto RR, Soni H, Tiwari SK, Goel P, Sharma H, Vishwakarma N, Jha SK, Chauhan S, Khandelwal P. Ayurveda in Renal Cell Carcinoma: A Narrative Review of Supportive Care During Targeted Therapy and Immunotherapy.

43. Mahto RR, Bansal KS, Soni H, Tiwari SK, Sandrepogu J, Bhatted SK, Jha SK, Sharma H, Khandelwal P. Efficacy of Vidangadi Loha and Phalatrikadi Ghanavati with and without the prior administration of Virechana Karma in Madhumeha (Obese Diabetic): A Randomised Controlled Trial.

44. Shaaban H, Gorecki T (2015) Current trends in green liquid chromatog raphy for the analysis of pharmaceutically active compounds in the environmental water compartments. Talanta 132:739–752. https:// doi. org/ 10. 1016/j. talan ta. 2014. 09. 050

45. Funari CS, Carneiro RL, Khandagale MM, Cavalheiro AJ, Hilder EF (2015) Acetone as a greener alternative to acetonitrile in liquid chromato graphic fingerprinting. J Sep Sci 38(9):1458–1465. https:// doi. org/ 10. 1002/ jssc. 20140 1324

46. Satinsky D, Brabcova I, Marouskova A, Chocholous P, Solich P (2013) Green chromatography separation of analytes of greatly differing properties using a polyethylene glycol stationary phase and a low-toxic water-based mobile phase. Anal Bioanal Chem 405:6105–6115. https:// doi. org/ 10. 1007/ s00216- 013- 7003-1

47. Ordonez EY, Rodil R, Quintana JB, Cela R (2015) Determination of artificial sweeteners in beverages

Epimedium Sagittatum and Gloriosa Superba L. for the Treatment of Hypogonadism. Int J Innov Sci Res

with green mobile phases and high temperature liquid chromatography–tandem mass spectrometry. Food Chem 169:162–168. https:// doi. org/ 10. 1016/j. foodc hem. 2014. 07. 132

48. Prache N, Abreu S, Sassiat P, Thiebaut D, Chaminade P (2016) Alternative solvents for improving the greenness of normal phase liquid chroma tography of lipid classes. J Chromatogr A 1464:55–63. https:// doi. org/ 10. 1016/j. chroma. 2016. 07. 083

49. Koranya MA, Mahgouba H, Haggaga RS, Ragaba MA, and Elmallahc OA: Green chemistry: Analytical and chromatography. Journal of Liquid Chromatography & Related Technologies 2017; 1-14.

50. Yang Y: Subcritical water chromatography: a green approach to high-temperature liquid chromatography. J Sep Sci 2007; 30: 1131-40.

51. Smith RM: Superheated water chromatography—a green technology for the future. J Chromatogr A 2008; 1184: 441-55.

52. Kaljurand M and Koel M: Recent advancements on greening analytical separation. Crit Rev Anal Chem 2011; 41: 2-20.

53. Chen S and Kord A: Theoretical and experimental comparison of mobile phase consumption between ultra high-performance liquid chromatography and high performance liquid chromatography. J Chromatogr A 2009; 1216: 6204-09.

54. Jones BA: Temperature Programmed Liquid Chromato graphy. J Liq Chrom Rel Technol 2004; 27: 1331-52.

55. Divya Patel, Khushbu S. Patel, Dr. Khushbu Patel and Dr. C. N. Patel. Green Analytical Chemistry: An Overview. World Journal of Pharmacy and Pharmaceutical Sciences. 2024;13(8):419-429.

56. Barot Suhani, Dhruvi Modi, Anjali Patel, Mayuri Pathak, Dr. Khushbu Patel and Dr. C. N. Patel. A Comprehensive Review on Green Analytical Chemistry. World Journal of Pharmaceutical Research. 2024;13(10): 232-246.

57. S. Irem Kaya, Goksu Ozcelikay-Akyildiz, Sibel A. Ozkan. Green metrics and green analytical applications: A comprehensive outlook from developing countries to advanced applications. Green Analytical Chemistry 11 (2024) 100159.

58. F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, AGREE - Analytical GREEnness Metric Approach and Software, Anal. Chem. 92 (2020) 10076–10082, https://doi. org/10.1021/acs.analchem.0c01887.

59. M.S. Imam, M.M. Abdelrahman, How environmentally friendly is the analyticalprocess? A paradigm overview of ten greenness assessment

metric approaches for analytical methods, Trends Environ. Analyt. Chem. 38 (2023) e00202, https://doi.org/10.1016/j.teac.2023.e00202.

60. M. Locatelli, A. Kabir, M. Perrucci, S. Ulusoy, H.I. Ulusoy, I. Ali, Green profile tools:current status and future perspectives, Adv. Sample Preparation 6 (2023) 100068, https://doi.org/10.1016/j.sampre.2023.100068.
61. Elsheikh SG, Hassan AME, Fayez YM, El-Mosallamy SS (2023) Green ana lytical chemistry and experimental design: a combined approach for the analysis of zonisamide. BMC Chem 17:38. https:// doi. org/ 10. 1186/ s13065- 023- 00942-1
62. Kokilambigai KS, Lakshmi KS (2021) Utilization of green analytical chemistry principles for the simultaneous estimation of paracetamol, aceclofenac and thiocolchicoside by UV spectrophotometry. Green Chem Lett Rev 14:99–107. https:// doi. org/ 10. 1080/ 17518 253. 2020. 18623 11.