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Measuring Sustainability: Metrics And Methods In Green Analytical Chemistry

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ABSTRACT

Green chemistry has emerged as a vital framework in laboratories, prioritizing sustainable, energy-efficient practices. The field focuses on replacing conventional methods with environmentally friendly alternatives by utilizing greener materials and improved waste management. Anastas first defined green chemistry, aiming to reduce hazardous substances, while also pioneering green analytical chemistry (GAC), which emphasizes practices like miniaturization and reagent substitution. This review highlights sustainable practices in analytical chemistry, focusing on techniques such as HPLC and UV-Visible Spectroscopy. Various metrics, including the National Environmental Methods Index (NEMI) and Analytical GREEnness (AGREE), are discussed to evaluate the eco-friendliness of these methods. The need for continuous improvement of these metrics is essential for researchers aiming to enhance sustainability in analytical practices, particularly in industries like pharmaceuticals and chemicals, where reducing environmental impact is crucial.

Keywords: *Green chemistry; Sample preparation; Solvent used; Green metric tools*

1. INTRODUCTION

Green chemistry has become increasingly prominent in laboratories worldwide, emphasizing sustainable, energy-efficient processes. Chemists globally are working to replace conventional methods with environmentally friendly alternatives, such as redesigning experiments to use greener materials and better waste management practices. Anastas first defined green chemistry as the field dedicated to reducing or eliminating hazardous substance production in chemical processes. Although Anastas coined the term, Badami, Nameroff, and Keltz were instrumental in its early development. In 1995, Anastas also initiated green analytical chemistry (GAC), calling for the miniaturization, containment, and reagent substitution in analytical practices[1]. The effectiveness of green chemistry approaches is often evaluated against traditional methods using the Twelve Principles of Green Analytical Chemistry. Those are Utilize direct analysis methods whenever possible, Minimize sample size and quantity to the extent feasible, Conduct analyses on-site whenever practical, Integrate processes to streamline operations and conserve resources, Automate analyses where possible, Avoid derivatization procedures, Minimize waste production and prioritize effective waste management, Optimize methods to allow analysis of multiple analytes where feasible, Limit energy consumption throughout the process, Favor the use of renewable and sustainable reagents, Select safer, less toxic chemicals whenever possible and Prioritize operator safety at all times[2]. Analytical chemistry is increasingly adopting green chemistry principles in equipment design and procedural methods. A key focus is developing efficient, sustainable sample preparation methods, aiming to reduce waste by using minimal or no sample prep where possible. However, many analytes still require matrix extraction. In these cases, principles such as minimizing solvent use, energy consumption, waste production, and reducing hazardous chemical exposure are essential. Improving sample preparation methods can involve using smaller sample sizes, efficient extraction techniques, and avoiding toxic organic solvents. Chromatographic analysis—a major focus within green analytical chemistry (GAC)—requires minimizing harmful solvent use. Traditional chromatographic methods, often generating 1–1.5 L of organic waste daily, risk environmental and human health impacts due to solvent volatility and disposal challenges [3]. It is, therefore, critical for analytical scientists to reduce the environmental footprint of their methods.

This review discusses sustainable practices in analytical chemistry, including green solvents, sample preparation, and the application of GAC principles in validating analytical methods for HPLC, UHPLC, UV–Visible Spectroscopy, HPTLC, and TLC.

2. SAMPLE PREPARATIONS

Sample preparation often introduces the highest pollution in analytical methods due to the use of hazardous and volatile substances[4]. Adopting direct analytical techniques, which eliminate sample prep, aligns well with green chemistry principles. Techniques like GC or LC allow for the direct injection of samples, minimizing waste and reducing solvent use[5]. Advancements in column design have also mitigated the impact of water and salts on chromatographic columns, making direct aqueous injection more viable. Although these methods work best for cleaner matrices, they play a crucial role in reducing environmental impact.

Green Sample Preparation Techniques:

- Solid Phase Extraction (SPE) : SPE is widely used and environmentally friendly, as it employs minimal solvent and generates little waste. Small amounts of organic solvents are used to recover analytes from a sorbent, enhancing precision and reducing solvent waste. However, issues such as inconsistent sorbent packing and analyte recovery challenges must be optimized for effective results[6].
- QuEChERS Extraction : Known for being quick and low in solvent use, QuEChERS involves solvent extraction and clean-up, using salts like magnesium sulfate to manage moisture. This method is commonly used in blood sample analysis, extracting a range of compounds[7].
- Solid Phase Microextraction (SPME) : A solvent-free approach, SPME uses a coated fiber to concentrate analytes. It's cost-effective, fast, and can be paired with HPLC or GC. However, limitations include fiber fragility and potential sorbent degradation[8].
- Stir-Bar Sorptive Extraction (SBSE) : A solvent-free technique like SPME but with a higher sorptive phase volume, SBSE enhances sensitivity and is particularly suitable for aqueous matrices. Volatile analytes can be directly desorbed for GC analysis, while non-volatiles can be extracted with minimal solvent[9].
- Dispersive Liquid–Liquid Microextraction (DLLME) : DLLME creates a fine emulsion using minimal solvent, optimizing analyte transfer and allowing for high recovery rates. It is efficient and requires small sample volumes[10].
- Pressurized Fluid Extraction (PFE) :Also known as Accelerated Solvent Extraction (ASE), PFE uses high pressure and temperature to increase analyte solubility and extraction speed with eco-friendly solvents like ethanol[10].
- Microwave-Assisted Extraction (MAE) : MAE employs microwave energy to rapidly heat samples and extract analytes. This method is fast, reaching high temperatures quickly but is limited by solvent dielectric properties[11].
- Ultrasound-Assisted Extraction (UAE): UAE uses ultrasound to promote clean, efficient extraction with safe solvents, making it versatile and less resource-intensive [12].
- Supercritical Fluid Extraction (SFE): SFE is eco-friendly and automated, often using CO_2 as a non-toxic supercritical fluid. It preserves analyte activity, requires no additional clean-up, and is quick and selective[13].

These techniques offer green alternatives by reducing solvent use, waste, and energy, supporting the principles of Green Analytical Chemistry. Organic solvent consumption is high for DLLME and UAE, while it remains low for SPE, QuEChERS, SPME, PFE, and SFE. Energy consumption is similarly high for DLLME, PFE, UAE, and SFE, but low for SPE, QuEChERS, and SPME. Reusability is favorable in DLLME, PFE, and UAE, whereas it is lower for SPE, QuEChERS, SPME, and SFE. Laboratory waste production is high in SPE and QuEChERS, yet low in SPME, DLLME, PFE, UAE, and SFE. Lastly, automation potential is high in DLLME, UAE, and SFE but lower in SPE, QuEChERS, SPME, and PFE.

Greener organic solvents are vital in analytical chemistry, where using no solvent is the most eco-friendly option. However, solvents are generally needed for sample preparation and analysis to make samples liquid and facilitate separation. To reduce the environmental impact of traditional solvents—known for volatility, flammability, and toxicity—advances in green chemistry have introduced new solvent alternatives. Solvent Selection Guides (SSGs) help identify environmentally friendlier solvent, such as amphiphilic solvents (like alcohols, carboxylic acids, surfactants), ionic liquids (ILs), and deep eutectic solvents (DESs), all of which are applied in sample preparation and liquid chromatography.

Solvents used in Green chemistry

Alternative Solvents for Sample Preparation

Water remains the most eco-friendly, economical, and safe solvent. Methods like maceration and water-based extraction can be enhanced with additives like surfactants or hydrotropes to improve solubility of nonpolar compounds. Techniques such as subcritical water extraction (SWE) allow for effective nonpolar extractions. Natural Deep Eutectic Solvents (NADESs)

are low-melting combinations of natural solids, ideal for green extraction due to their hydrogen-bonding properties, recyclability, and environmental benefits[14].

Bio-based solvents, derived from agricultural products, include ethanol, ethyl acetate, and 2-methyl tetrahydrofuran. Liquefied gases (e.g., dimethyl ether, n-propane, n-butane) provide eco-friendly extraction with low-temperature volatility, preserving sensitive compounds. Supercritical fluids, especially CO_2, enable selective extraction without residual solvents, due to their adaptable density and near-zero surface tension [15].

Sustainable Solvents as Mobile Phases

Chromatography requires large amounts of high-purity organic solvents as mobile phases. Switching from normal-phase to reversed-phase chromatography, which uses less toxic solvents, was an initial green chemistry advancement. Environmentally favorable options in reversed-phase chromatography include ethanol, acetone, and ethyl acetate, which are less toxic than acetonitrile and methanol[16]. Despite higher viscosity, ethanol is slightly less harmful, while acetone provides a viable, efficient alternative. With modifications like water-triethylamine or polyethylene glycol, chromatographic separations can achieve high precision with minimal ethanol usage.

For nonpolar and non-volatile samples (like lipids) analyzed in normal-phase systems, greener solvents like cyclopentyl methyl ether, hexamethyldisiloxane, isopentyl acetate, and 2-methyltetrahydrofuran are effective alternatives The polar solvent mixture of EtOH/Water (H_2O) is often used in high-performance liquid chromatography (HPLC). Polar liquid water serves in micellar and submicellar liquid chromatography, while Milli-Q water, also polar, is utilized in subcritical water chromatography. The polar combination of $CHCl_3$ and ethylene glycol is applied in modified micellar chromatography. Nonpolar supercritical CO_2 is employed in supercritical fluid chromatography, whereas nonpolar liquid CO_2 is used in flash chromatography[17,18].

Green metric tools for analytical methods:

Green chemistry metrics help identify characteristics of chemical processes that align with green principles. Various tools, such as NEMI, Analytical Eco-Scale, GAPI, and AGREE, evaluate the "greenness" of analytical methods. This article summarizes these metrics, covering their history, methodologies, principles, and case studies on techniques like chromatography, spectroscopy, and titration. It emphasizes the need for continuous improvement and updating of these metrics to better assess the sustainability of analytical practices, serving as a useful resource for researchers focused on green chemistry principles.

Industries such as chemicals, pharmaceuticals, and engineering rely on various testing methods, prompting researchers to develop eco-friendly analytical techniques based on green chemistry principles to minimize pollution. To assess the "greenness" and eco-friendliness of these methods, several metrics have been established, including the National Environmental Methods Index (NEMI), Analytical Eco-Scale (AES), Green Analytical Procedure Index (GAPI), and Analytical GREEnness (AGREE).

***NEMI*:**

The National Environmental Methods Index (NEMI), established in 2002, is a searchable database that provides scientists and managers with access to various environmental techniques, protocols, and analytical methods for monitoring. Since its inception, NEMI has undergone several updates to incorporate new technologies and methodologies. It features a four-part pictogram that includes categories for Persistent Bioaccumulative Toxic (PBT) substances, hazardous materials, corrosive substances, and waste management. The color of the pictogram indicates the assessment outcome, which follows specified criteria. Although NEMI is user-friendly, it lacks software support and relies on a manual evaluation process without quantification measures[19]. The NEMI rules and criteria for assessing analytical methods consist of four key components:

a. Persistent Bioaccumulative Toxic (PBT): The method must not involve any persistent, bioaccumulative, or toxic components.
b. Hazardous Materials: The use of chemicals listed under the Toxic Release Inventory (TRI) and the Resource Conservation and Recovery Act (RCRA), specifically those classified as D, F, P, or U, is prohibited.
c. Corrosiveness: The pH of the method should be maintained between 2 and 12 to avoid corrosive substances.
d. Waste Generation: The method should produce less than 50 grams of waste.

Modified NEMI:

The modified National Environmental Methods Index (NEMI), developed by Raynie and Driver in 2009, features a pentagonal pictogram divided into five sections representing health, safety, waste quality, environmental impact, and energy usage[20]. Health and safety metrics are based on quantifiable values from National Fire Protection Association (NFPA) standards, while the other three sections lack measurable criteria. The modified NEMI employs a pictogram with five sections, each evaluating different criteria related to analytical methods. The health section uses the National Fire Protection

Association (NFPA) health hazard value, assigning green for scores of 0 to 1, yellow for 2 to 3, and red for 4 and above. Safety is assessed using the NFPA flammability or instability values, with the same color-coding system. The quality of waste is determined by the total waste generated during the process, categorized as green for samples ≤50g, yellow for ≤250g, and red for samples exceeding 250g. Environmental risk is measured by the amount of polluted waste, where less than 50g is marked green, 50 to 250g yellow, and more than 250g red. Lastly, energy consumption for one sample is evaluated, with green for ≤0.1 kWh, yellow for ≤1.5 kWh, and red for more than 1.5 kWh.Like the original NEMI, this modified version is user-friendly, but it does not provide software for quantifying waste quality, environmental impact, or energy consumption.

Analytical Ecoscore:

The Analytical Eco-Scale, developed by Koen Van Aken et al. in 2006, is a semi-quantitative method for assessing the eco-friendliness of organic synthesis reactions. It assigns penalty points based on factors such as chemicals used, energy consumption of instruments, occupational hazards, and waste generation[21]. The GREEnness score is derived by subtracting total penalty points from 100; scores above 75 are excellent, above 50 are acceptable, and below 50 are deemed non-eco-friendly. Despite its utility, the Analytical Eco-Scale lacks dedicated software for calculations, relying on manual assessments that may overlook certain factors, thus making it susceptible to human error[22].

The penalty points for the Analytical Eco-Scale are calculated based on various criteria:

- Chemicals or Reagents: Points are assessed by checking the hazard symbols and signal words in the Material Safety Data Sheet (MSDS), as well as the volume of the chemical used.
    - If the signal word is “DANGER,” each pictogram counts as 2 points; if it’s “WARNING,” each symbol counts as 1 point.
    - The volume used also affects the score: less than 10 mL incurs 1 point, 10 to 100 mL incurs 2 points, and over 100 mL incurs 3 points.
    - The final penalty points for each chemical are calculated by multiplying the points from the hazard symbols by those from the volume used.
- Instruments Energy Consumption: Penalty points are assigned based on energy usage: less than 0.1 KWh results in 0 points, less than 1.5 KWh results in 1 point, and more than 1.5 KWh results in 2 points.
- Occupational Waste: Points depend on vapor emissions: no vapor release earns 0 points, while releasing vapors earns 3 points.
- Waste Management: The total waste generated is scored based on volume: less than 1 mL incurs 1 point, 1 to 10 mL incurs 2 points, and more than 10 mL incurs 5 points. For waste management methods, recycling earns 0 points, degradation earns 1 point, passivation earns 2 points, and no treatment earns 3 points.

GAPI:

The Green Analytical Procedure Index (GAPI), created by Płotka-Wasylka in 2018, is a qualitative assessment tool that evaluates various aspects of analytical methods, including sample sourcing, method type, sample preparation, reagents and chemicals, and instrumentation[23]. Represented as a pictogram with red, yellow, and green sections, GAPI's color coding reflects the eco-friendliness of the procedures used. However, it lacks a standardized software for evaluation, making it somewhat challenging to interpret, as users may select pictogram colors subjectively[24].

The GAPI rules and criteria for assessing analytical methods are organized into several sub-parts, each with specific conditions for color selection based on environmental impact.

- Sample Sourcing:
    - Collection: The method is categorized as In-Line (green), Online (yellow), or Offline (red).
    - Preservation: No preservation (green), use of either chemical or physical methods (yellow), or both methods (red).
    - Transport: No transport requirements (green) or required (yellow).
    - Storage: Normal conditions (green) or special conditions (yellow).
- Method Type:
    - Direct/Indirect: No preparation (green), simple preparation (yellow), or required extraction (red).
- Sample Preparation:
    - Scale of Extraction: Nano (green), Micro (yellow), or Macro (red).
    - Solvents/Reagents: No solvents (green), green solvents (yellow), or non-green solvents (red).

  - Additional Treatments: None (green), simple treatments (yellow), or advanced treatments (red).
- Reagents and Chemicals Used:
  - Amount: Less than 10 mL (green), between 10 and 100 mL (yellow), or more than 100 mL (red).
  - NFPA Health Hazard Value: Ranges from 0 to 1 (green), 2 to 3 (yellow), or 4 and above (red).
  - NFPA Flammability or Instability Value: Same as above.
- Instrumentation:
  - Energy Usage: Less than 0.1 KWh per sample (green), less than 1.5 KWh (yellow), or more than 1.5 KWh (red).
  - Occupational Hazard: None (green) or vapors released (red).
  - Waste: Less than 1 mL (green), 1 to 10 mL (yellow), or more than 10 mL (red).
  - Waste Treatment: Recycling (green), degradation (yellow), or no treatment (red).
- Symbol:
  - If present, indicates the method is suitable for both qualitative and quantitative purposes; if absent, it is for qualitative purposes only.

Complex GAPI:

Complex GAPI, also referred to as the complementary green analytical procedure index, is an extended version of the GAPI that incorporates a hexagonal glyph at the bottom of the main pictogram. This technique includes dedicated software to assess the eco-friendliness of methods, covering both GAPI and Complex GAPI evaluations. Although the software does not provide a specific numerical value, it offers a pictorial representation of GREEnness. The hexagonal glyph consists of six sections: the first three have single parts, while the fourth and sixth include two subparts, and the fifth has three subparts. Assessment methods utilizing Complex GAPI have been reported in various studies as green solutions[25,26].

The Complex GAPI rules for assessing analytical methods include various criteria represented in a pictogram with distinct color coding. For yield, a green indicator signifies greater than 89%, yellow indicates a range of 70% to 89%, and red denotes less than 70%. For temperature and time, a green condition is for reactions at room temperature and less than one hour, while yellow reflects heat for less than one hour or cooling to 0 °C; red indicates heating for over one hour or cooling below 0 °C. The number of rules met is also assessed, with green indicating 5 to 6 rules, yellow for 3 to 4, and red for 1 to 2. In terms of reagents and solvents, the health hazard is classified based on the NFPA health hazard value: green for slightly toxic (0 or 1), yellow for moderately toxic (2 or 3), and red for serious injury risks (4). The safety hazard is assessed similarly, with green indicating no special hazards and red indicating a high flammability risk. Instrumentation is evaluated based on the technical setup, with green for common setups, yellow for additional or semi-advanced instruments, and red for specialized equipment. Energy consumption follows the same color scheme, where green denotes ≤0.1 KWh per sample and red is for ≥1.5 KWh. Occupational hazards are also included, where green reflects hermitization of the analytical process, while yellow and red indicate vapor release into the atmosphere. For the workup and purification of the endpoint, a simple process earns a green rating, standard purification yellow, and advanced purification red. Purity levels above 98% are green, between 97% and 98% yellow, and below 97% red. Waste generation is similarly categorized, with green for less than 1 mL, yellow for 1–10 mL, and red for more than 10 mL. An additional field, the E factor, is calculated as the total mass of waste generated from the process divided by the total mass of the product.

AGREE:

The Analytical GREEnness (AGREE) tool, developed by Gdańsk University of Technology in Poland, assesses the eco-friendliness of processes based on the 12 principles of green chemistry. It features a circular pictogram divided into 12 sections, each representing a different principle, with values ranging from 0 to 1 that change color from green to red according to the method's conditions. The tool provides both qualitative and quantitative results, but it does not account for general instruments like balances and pH meters, which may complicate its use and understanding[27, 28].

AGREEprep:

AGREEprep is a software tool designed to evaluate the eco-friendliness of sample preparation procedures[29], developed by Wojciech Wojnowski et al. in 2022. It is a modified version of the AGREE tool, focusing specifically on sample preparation. AGREEprep comprises 10 sections, with values ranging from 0 to 1, where colors change from green to red based on the entered conditions. Although it provides both qualitative and quantitative assessments, users may find it challenging to input values and adapt the tool to specific analytical methods. The AGREEprep and AGREE tools can be integrated to provide a comprehensive evaluation of the overall process's GREEnness[30].

HPLC- EAT:

HPLC-EAT (Environmental Assessment Tool) is a software tool developed in 2011 by Yasser Gaber et al., specifically for evaluating the eco-friendliness of HPLC methods[31]. This quantitative tool focuses on assessing the environmental health and safety of solvents used in liquid chromatography, but it does not provide a visual representation of GREEnness. The eco-friendliness score is calculated using a specific equation (Score = $S_1m_1+H_1m_1+E_1m_1+S_2m_2+H_2m_2+E_2m_2+$ … …$+S_nm_n + H_nm_n + E_nm_n$, where, S as safety, H as health, and E as the environment; m expresses the mass of the solvents, and n is the number of solvents) that incorporates safety, health, and environmental factors, alongside the mass of the solvents used[32].

AMVI:

Analytical Method Volume Intensity (AMVI) is a quantitative green metric introduced[33] in 2011 by R. Hartman et al. This tool is designed for evaluating HPLC techniques but lacks a visual representation of GREEnness. The %AMVI value is determined by the ratio of total solvent consumption to the number of chromatographic peaks of interest.

To calculate solvent consumption, the following equations are used:

1. For HPLC solvent consumption:
    a. HPLC solvent consumption=Flow rate×Analysis time×Number of injections
2. For solvent consumption in sample preparation:
    a. Solvent consumption=(Volume of standard preparation×Number of standards)+(Volume of sample preparatio n×Number of samples)+(Volume of system suitability preparation×Number of systems).
3. Total solvent consumption combines both HPLC solvent consumption and Solvent consumption in sample preparations, multiplying with replicates.
4. Finally, AMVI is calculated as:
    a. AMVI=Total Solvent consumption / Number of chromatographic peaks

While AMVI shares similarities with HPLC-EAT, it does not incorporate the 12 principles of green chemistry and is exclusive to liquid chromatography techniques[34].

***AMGS*:**

The Analytical Method GREEnness Score (AMGS) is a quantitative tool developed[35] by Michael B. Hicks et al. in 2019, designed to assess the GREEnness of analytical methods. The tool evaluates the instrument energy score, solvent energy score, and solvent environmental health and safety (EHS) score, with a lower GREEnness score indicating a greener method. The AMGS was developed by referencing similar tools like AMVI and HPLC-EAT. Users input method conditions, and the spreadsheet computes the relevant scores, providing a user-friendly experience[36].

GREEnness Index with Spider Diagram:

The GREEnness index, illustrated through a spider diagram, assesses the environmental impact of solvents and chemicals based on their safety data sheets (SDS). It evaluates five key attributes: health impact, general properties, odor, fire safety, and stability, with scores ranging from -5 to +5 for each attribute. These scores generate a green index table, forming the basis of the spider diagram. If SDS lacks complete information for certain solvents, those sections are scored as zero. While the primary spider diagram can contain up to 30 hypothetical parameters, the secondary diagram focuses on the five main attributes. This tool is primarily for comparing the GREEnness of reagents but is not suitable for a comprehensive evaluation of method GREEnness and does not adhere to the 12 principles of green chemistry. Its complexity may hinder its application in analytical methods[37,38].

Green Solvent Selection Tool (GSST):

Developed by members of the ACS Pharma Roundtable, the GSST addresses the importance of solvent selection in process mass intensity. This interactive tool allows users to choose solvents based on critical characteristics, with similar solvents grouped on a principal component analysis (PCA) map. While it provides valuable data on physical, environmental, safety, and health properties, it does not align with the 12 green principles, making its application in analytical methodologies challenging [39].

Innovation Green Aspiration Level (iGAL):

The Green Chemistry Innovation Scorecard Calculator emphasizes waste reduction alongside process mass intensity (PMI). Developed collaboratively by the IQ Consortium, ACS GCI Pharmaceutical Roundtable, and academic experts, this web tool helps demonstrate how green chemistry innovations can minimize waste during pharmaceutical production. While it does not adhere to the 12 green principles, it is beneficial for evaluating drug synthesis and manufacturing processes[40]

Process Mass Intensity - Environmental Life Cycle Assessment Tool (PMI-LCA):

The PMI-LCA tool, available as an Excel sheet, provides a high-level estimator of process mass intensity and environmental life cycle assessment for small molecule active pharmaceutical ingredients (APIs). Although it offers a streamlined approach for benchmarking greener manufacturing processes, it is not exhaustive. Key categories include mass net, energy, global warming potential, and water depletion, with additional considerations for acidification and eutrophication[41] .

Biopharma Process Mass Intensity Tool:

The Biopharma PMI metric quantifies the water, raw materials, and consumables needed to produce 1 kg of biological drug substances (APIs). This standardized metric facilitates industry benchmarking, transparency in process development, and objective comparisons across processes[42].

3. ANALYTICAL WORKS

Recent studies highlight the trend toward eco-friendly HPLC methods through green solvents and analytical Quality by Design (AQbD) frameworks. For example, Elsheikh et al. (2023) optimized a reliable, cost-effective HPLC method using ethanol and water as a mobile phase to detect zonisamide alongside its degradation products[43]. The method, utilizing a Kromasil C18 column with a photodiode array, achieved robust separation with an eco-scale of 91.

Kokilambigai et al. (2022) applied AQbD to analyze atorvastatin, optimizing factors like ethanol concentration to improve peak sharpness and symmetry, resulting in a green method with an eco-scale score of 90[44]. Similarly, Perumal et al. developed a rapid and specific HPLC technique to detect escitalopram and etizolam using ethanol-phosphate buffer, achieving notable eco-friendliness[45] (AGREE score of 0.78).

Vieira-Sellai et al. (2022) achieved eco-friendly quantification of zidovudine, lamivudine, and nevirapine with a reduced solvent volume and shorter analysis time. This technique, using ethanol as a mobile phase and yielding accurate recoveries, scored 75 on the ECO Scale. Each of these studies underscores the shift toward sustainable chromatography practices through optimized methodologies that minimize environmental impact while maintaining analytical reliability[46].

Iqbal et al. 2023 developed a UHPLC-MS/MS method for the rapid quantification of umifenovir in plasma, emphasizing its environmental impact. Using an ACQUITY UPLC BEH C18 column (2.1 × 100 mm, 1.7 µm), the mobile phase comprised 15 mM ammonium acetate and acetonitrile in an 80:20 (% v/v) ratio, pumped at 0.3 mL/min. Umifenovir and the internal standard (IS) were separated in under 2.5 minutes, with the auto-sampler and column oven set at 10 °C and 40 °C, respectively. Positive electrospray ionization was employed for ionization. The method exhibited strong linearity across a concentration range of 1.32–625 ng/mL, with accuracy between 90.5% and 105.8%. Its environmental score, assessed using a greenness metric, was 0.77, indicating a favorable procedure[47].

In another study, Iqbal et al. 2021 established a UHPLC-MS/MS assay for delafloxacin (DFX). Separation was performed on a UHPLC BEH C18 column (50 × 2.1 mm, 1.7 µm) using gradient elution with a mobile phase of 0.1% formic acid in acetonitrile and water, at a flow rate of 0.3 mL/min and an injection volume of 5 µL. The column oven temperature was set to 35 °C. A triple quadrupole mass detector with positive ionization was utilized for quantification, yielding retention times of 1.72 and 1.79 minutes for DFX and IS, respectively. The method demonstrated linearity from 2.92 to 6666 ng/mL, with a correlation coefficient (R^2) of at least 0.995. Accuracy ranged from 94.4% to 106.1%, and the greenness assessment using AGREE software yielded a score of 0.78, indicating excellent environmental performance[48].

Prajapati et al. 2018 utilized the AQbD methodology to create an eco-friendly HPTLC method for quantifying thiocolchicoside (THC) in medicinal formulations. Through Box-Behnken Design, they optimized essential parameters. Compared to HPLC, HPTLC requires less mobile phase (MP) for analyzing multiple samples simultaneously, which reduces time and costs. The chromatographic separation was performed using a toluene-acetone-water (1.5:7.5:1.0, %v/v/v) MP on 10 cm × 10 cm aluminum plates coated with 250 µm silica gel 60 F254 at 25 °C and 35% relative humidity. The chamber saturation time was set to 15 minutes with a displacement distance of 90 mm. Degradation studies revealed that THC is more prone to oxidative degradation and acidic-alkaline hydrolysis, while it is less affected by photolysis, dry heat, and water hydrolysis. The spots for THC, acidic product DP1, and DP2 had retardation factors (Rf) of 0.53, 0.65, and 0.80, respectively. Recovery rates ranged from 99.39% to 101.65%, with linearity between 100 and 500 ng/spot (R^2 = 0.9979). The limit of detection (LOD) was 4.03 µg/band, and the limit of quantification (LOQ) was 12.21 µg/band[49].

Naguib et al. 2020 developed a rapid, cost-effective HPTLC method to analyze a novel combination of mebendazole (MBZ) and quinfamide (QF). Distinct bands were scanned at 254 nm using silica gel HPTLC F254 plates with a simple MP of methanol and toluene (2:6, %v/v). The Rf values for MBZ and QF were 0.45 and 0.75, respectively. Linearity ranged from 0.2 to 2.5 µg/band for QF and 0.1 to 2 µg/band for MBZ, with accuracies of 99.62% and 100.10%, respectively. LOD and LOQ for QF were 0.055 and 0.168 µg/band, while for MBZ, they were 0.031 and 0.094 µg/band. Additionally, an isocratic RP-HPLC method was employed using a Phenomenex C18 column with a green MP of double distilled water and methanol

(30:70 %v/v) at a flow rate of 0.8 mL/min over 4 minutes, detecting at 254 nm. This method significantly reduced waste to 3.2 mL/run using a short column (100 mm) with small particle size (3.5 µm), indicating a greener process. The retention times for MBZ and QF were 2.69 and 3.43 minutes, respectively, with linearity of 1 to 60 µg/band for QF and 2 to 80 µg/band for MBZ. Accuracies were 100.04% for QF and 99.87% for MBZ, with LOD and LOQ values for QF at 0.323 and 0.979 µg/band, and for MBZ at 0.643 and 1.948 µg/band, respectively[50].

Elsheikh et al. 2022 established stability-indicating TLC-Densitometric methods for modafinil and its acid-induced degradation product. Utilizing 10 × 20 cm silica gel G60 F254 plates (0.25 mm thick), they employed a dichloromethane-methanol (90:10, %v/v) developing solution. The chromatograms were scanned at 254 nm, revealing an Rf of 0.48 for modafinil and 0.77 for its degradation product. The method demonstrated linearity in the range of 1–10 µg/band, with an accuracy of 100.11%. The limits of detection (LOD) and quantification (LOQ) were 0.110 and 0.335 µg/band, respectively. The TLC-densitometry procedures scored 80 on the Eco Scale, indicating a relatively eco-friendly approach[51].

Additionally, Elsheikh et al. 2023 developed a reliable stability-indicating TLC-densitometric technique for zonisamide (ZNS) in the presence of its degradation product. The method employed aluminum plates coated with silica gel 60F254 and utilized a developing solution of chloroform, methanol, and acetic acid (80:15:5 %v/v/v), with detection at 240 nm under UV light. The Rf values were 0.76 for ZNS and 0.11 for its degradation product. This TLC-densitometry method received an Eco Scale score of 84, reflecting its eco-friendly characteristics[52].

Using UV spectrophotometric techniques, Chanduluru et al. 2022 introduced a simple, cost-effective, and environmentally friendly stability-indicating method for assessing Chlorthalidone (CTD) and Cilnidipine (CIL). They replaced methanol with propylene carbonate as a green solvent, which is less harmful to water, air, and health. The absorbance ranges, based on area under the curve analysis, were 218–227 nm for CTD and 224–232 nm for CIL. The linearity was established at 7–13 µg/mL for CTD and 8.75–16.25 µg/mL for CIL, with a simultaneous equation score of 0.91 according to the AGREE program[53].

Fawzy et al. 2022 developed accurate and sensitive spectrophotometric methods for the simultaneous estimation of saxagliptin hydrochloride and dapagliflozin propanediol monohydrate. The first method, the induced dual-wavelength approach (IDW), utilized substitute equality factors to isolate the effects of each drug during measurement. The second, the ratio difference method (RDM), calculated amplitude differences on the ratio spectrum using divisor concentrations of 25 µg/mL for dapagliflozin and 20 µg/mL for saxagliptin[54]. A third method employed plateau subtraction multiplied by the divisor concentration to compute saxagliptin at λmax 221 nm. This method achieved an ECO Scale score of 88.

Additionally, Fawzy et al. 2022 created sustainable and reliable spectrophotometric and chemometric methods for the simultaneous quantification of Fluticasone propionate and Azelastine. While methanol served as a diluent, the method remained eco-friendly in terms of instrumentation, chemicals used, and waste disposal[55]. The absorbance for both medications was measured at 258 nm (λiso), with an ECO Scale score of 88 for this approach

This review underscores the importance of green analytical techniques, including GAC examples with HPLC, HPTLC, UHPLC, and TLC, as a foundation for sustainable advancements in analytical practices. It serves as a valuable reference for researchers and industry professionals aiming to adopt eco-friendly and cost-effective methods across various sectors, such as pharmaceuticals and environmental monitoring. Liquid chromatography, commonly used in pharmaceutical quality control, often relies on significant organic solvent use, contributing to waste and toxicity[56]. Shifting towards greener techniques is essential for minimizing environmental impact. Integrating green chemistry principles and utilizing assessment tools can help optimize methods by focusing on solvents, energy consumption, and waste generation[57,58]. Incorporating green solvents like sodium dodecyl sulfate and propylene carbonate, as well as adopting miniaturized techniques like UHPLC, can further enhance sustainability.

4. CONCLUSION

The review also identifies areas for innovation, encouraging research into new green solvents, alternative separation methods, and advanced instrumentation. By promoting customized green analytical protocols, it aims to foster ongoing development in the field. Additionally, the review advocates for prioritizing sustainability within the scientific community and beyond, encouraging policymakers and industry stakeholders to adopt greener practices in chemical analysis. Overall, it seeks to catalyze positive change and inspire collaboration for a more sustainable approach to analytical chemistry.

REFERENCES

[1] Anastas PT (1999) Green chemistry and the role of analytical methodology development. Crit Rev Anal Chem 29:167–175. https://doi.org/10.1080/10408349891199356

[2] Ibanez E, Cifuentes A (2020) Moving forward to greener extraction techniques. TrAC Trends Anal Chem 122:115698. https://doi.org/10.1016/j.trac.2019.115698

[3] Reyes-Garces N, Gionfriddo E, Gomez-Rios GA, Alam MN, Boyaci E, Bojko B, Singh V, Grandy J, Pawliszyn J (2018) Advances in solid phase microextraction and perspective on future directions. Anal Chem 90:302–360.

https://doi.org/10.1021/acs.analchem.7b04502

[4] Grob K (1984) Further development of direct aqueous injection with electron-capture detection in gas chromatography. J Chromatogr A 299:1–11. https://doi.org/10.1016/S0021-9673(01)95206-4

[5] Tobiszewski M, Mechlinska A, Zygmunt B, Namiesnik J (2009) Green analytical chemistry in sample preparation for determination of trace organic pollutants. TrAC Trends Anal Chem 28(8):943–951. https://doi.org/10.1016/j.trac.2009.06.001

[6] Broekaert JAC (2015) Daniel C. Harris: Quantitative chemical analysis, 9th ed. Anal Bioanal Chem 407:8943–8944. https://doi.org/10.1007/s00216-015-9059-6

[7] Dybowski MP, Dawidowicz AL (2018) Application of the QuEChERS procedure for analysis of Δ9-tetrahydrocannabinol and its metabolites in authentic whole blood samples by GC–MS/MS. Forensic Toxicol 36:415–423. https://doi.org/10.1007/s11419-018-0419-8

[8] Andrascikova M, Matisova E, Hrouzkova S (2015) Liquid phase microextraction techniques as a sample preparation step for analysis of pesticide residues in food. Sep Purif Rev J 44(1):1–18. https://doi.org/10.1080/15422119.2013.872125

[9] Raut P, Bhosle D, Janghel A, Deo S, Verma C, Kumar SS, Agrawal M, Amit N, Sharma M, Giri T (2015) Emerging pressurized liquid extraction (PLE) techniques as an innovative green technologies for the effective extraction of the active phytopharmaceuticals. Res J Pharm Technol 8(6):800–810. https://doi.org/10.5958/0974-360X.2015.00129.4

[10] Armenta S, la Guardia M, de, (2016) Green chromatography for the analysis of foods of animal origin. TrAC Trends Anal Chem 80:517–530. https://doi.org/10.1016/j.trac.2015.08.011

[11] Tiwari BK (2015) Ultrasound: a clean, green extraction technology. TrAC Trends Anal Chem 71:100–109. https://doi.org/10.1016/j.trac.2015.04.013

[12] Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M (2017) Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. Ultrason Sonochem 34:540–560. https://doi.org/10.1016/j.ultsonch.2016.06.035

[13] Meskar M, Sartaj M, Sedano JAI (2018) Optimization of operational parameters of supercritical fluid extraction for PHCs removal from a contaminated sand using response surface methodology. J Environ Chem Eng 6(2):3083–3094. https://doi.org/10.1016/j.jece.2018.04.048

[14] Mazaud A, Lebeuf R, Laguerre M, Nardello-Rataj V (2020) Hydrotropic extraction of carnosic acid from rosemary with short-chain alkyl polyethylene glycol ethers. ACS Sustain Chem Eng 8:15268–15277. https://doi.org/10.1021/acssuschemeng.0c05078

[15] Kanda H, Hoshino R, Murakami K, Wahyudiono ZQ, Goto M (2020) Lipid extraction from microalgae covered with biomineralized cell walls using liquefied dimethyl ether. Fuel 262:116590. https://doi.org/10.1016/j.fuel.2019.116590

[16] Funari CS, Carneiro RL, Khandagale MM, Cavalheiro AJ, Hilder EF (2015) Acetone as a greener alternative to acetonitrile in liquid chromatographic fingerprinting. J Sep Sci 38(9):1458–1465. https://doi.org/10.1002/jssc.201401324

[17] Prache N, Abreu S, Sassiat P, Thiebaut D, Chaminade P (2016) Alternative solvents for improving the greenness of normal phase liquid chromatography of lipid classes. J Chromatogr A 1464:55–63. https://doi.org/10.1016/j.chroma.2016.08.034

[18] Dogan A, Eylem CC, Akduman NEB (2020) Application of green methodology to pharmaceutical analysis using eco-friendly ethanol-water mobile phases. Microchem J 157:104895. https://doi.org/10.1016/j.microc.2020.104895

[19] L.P. Kowtharapu, N.K. Katari, C.A. Sandoval, S.K. Muchakayala, V.K. Rekulapally (2022) Unique green chromatography method for the determination of serotonin receptor antagonist (Ondansetron hydrochloride) related substances in a liquid formulation, robustness by quality by design-based design of experiments approach. J. Separ. Sci., 45; 1711-1726. https://doi.org/10.1002/jssc.202100979

[20] D. Raynie, J.L. Driver (2009), Green Assessment of Chemical Methods, In13th Green Chem Conf., USA. https://doi.org/10.1021/cr068359e

[21] K. Van Aken, L. Strekowski, L. Patiny(2006), EcoScale, a semi-quantitative tool to select an organic preparation based on economical and ecological parameters, Beilstein J. Org. Chem., 2. https://doi.org/10.1186/1860-5397-2-3

[22] S.S. Panda, S. Dutta, R.K.V.V. Bera, S. Jammula(2021), Analytical eco-scale and quality by design-oriented

liquid chromatography method for simultaneous quantification of metoprolol succinate, telmisartan, and cilnidipine in their fixed-dose combination, Separ. Sci., 4: 128-136. https://doi.org/10.1002/sscp.202000041

[23] J. Płotka-Wasylka (2018), A new tool for the evaluation of the analytical procedure: green Analytical Procedure Index, Talanta, 181: 204-209. https://doi.org/10.1016/j.talanta.2018.01.013

[24] Y.A. Sharaf, A.E. Ibrahim, S. El Deeb, R.A. Sayed (2023), Green chemometric determination of cefotaxime Sodium in the presence of its degradation impurities using different multivariate data processing tools; GAPI and AGREE GREEnness evaluation, Molecules, 28: 2187. https://doi.org/10.3390/molecules28052187

[25] J. Płotka-Wasylka, W. Wojnowski (2021), Complementary green analytical procedure index (ComplexGAPI) and software, Green Chem., 23: 8657-8665. https://doi.org/10.1039/D1GC02008F

[26] M.A.E. Hamd, M.A. Albalawi, H. Gomaa, B.S. Mohammad, R.F. Abdul-Kareem, R.H. Obaydo, W.T. Alsaggaf, S.F. Saleh, M.A. Alossaimi, M.A. Abdel-Lateef (2023), Ziziphus spina-christi leaf-derived carbon dots as a fluorescence nanosensor to evaluate rifaximin antibacterial via inner filter effect: GREEnness and whiteness studies, Chemosensors, 11: 275. https://doi.org/10.3390/chemosensors11050275

[27] F. Pena-Pereira, W. Wojnowski, M. Tobiszewski, (2020) AGREE—analytical GREEnness metric approach and software, Anal. Chem., 92: 10076-10082. https://doi.org/10.1021/acs.analchem.0c01887

[28] Mostafa, H. Shaaban, A.M. Alqarni, M. Alghamdi, S. Alsultan, J. Saleh Al-Saeed, S. Alsaba, A. AlMoslem, Y. Alshehry, R. Ahmad, (2022) Vortex-assisted dispersive liquid–liquid microextraction using thymol based natural deep eutectic solvent for trace analysis of sulfonamides in water samples: assessment of the GREEnness profile using AGREE metric, GAPI and analytical eco-scale, Microchem. J., 183: Article 107976. https://doi.org/10.1016/j.microc.2022.107976

[29] W. Wojnowski, M. Tobiszewski, F. Pena-Pereira, E. Psillakis (2022), AGREEprep – analytical GREEnness metric for sample preparation, TrAC, Trends Anal. Chem., 149: Article 116553. https://doi.org/10.1016/j.trac.2022.116553

[30] A.K. El-Deen, H. Elmansi, K. Shimizu (2022), Utilization of hydrophilic and hydrophobic deep eutectic solvents for dispersive liquid-liquid microextraction of bicalutamide from water and spiked human plasma, Sustainable Chemistry and Pharmacy, 29:Article 100825. https://doi.org/10.1016/j.scp.2022.100825

[31] Y. Gaber, U. Törnvall, M.A. Kumar, M. Ali Amin, R. Hatti-Kaul (2011), HPLC-EAT (Environmental Assessment Tool): a tool for profiling safety, health and environmental impacts of liquid chromatography methods, Green Chem., 13: 2021. https://doi.org/10.1039/C0GC00667J

[32] M. Gamil, N.M. El Zahar, N. Magdy, A.M. El-Kosasy (2022), Green, bioanalytically validated chromatographic method for the determination and quantification of photoinitiators in saliva in contact with baby bibs, teethers and pacifiers, Microchem. J., 181: Article 107841. https://doi.org/10.1016/j.microc.2022.107841

[33] R. Hartman, R. Helmy, M. Al-Sayah, C.J. Welch (2011), Analytical Method Volume Intensity (AMVI): a green chemistry metric for HPLC methodology in the pharmaceutical industry, Green Chem., 13:934. https://doi.org/10.1039/C0GC00524J

[34] M.Y. Fares, M.A. Hegazy, G.M. El-Sayed, M.M. Abdelrahman, N.S. Abdelwahab (2022), Quality by design approach for green HPLC method development for simultaneous analysis of two thalassemia drugs in biological fluid with pharmacokinetic study, RSC Adv., 12: 13896-13916. https://doi.org/10.1039/D2RA01320A

[35] M.B. Hicks, W. Farrell, C. Aurigemma, L. Lehmann, L. Weisel, K. Nadeau, H. Lee, C. Moraff, M. Wong, Y. Huang, P. Ferguson (2019), Making the move towards modernized greener separations: introduction of the analytical method GREEnness score (AMGS) calculator, Green Chem., 21:1816-1826. https://doi.org/10.1039/C8GC03875H

[36] K.P. Kannaiah, A. Sugumaran, H.K. Chanduluru (2023), Simultaneous estimation of crotamiton and hydrocortisone by RP-UPLC using green analytical principles embedded analytical quality by design (AQbD) method, Microchem. J., 184:Article 108166. https://doi.org/10.1016/j.microc.2023.108166

[37] Y. Shen, C. Lo, D.R. Nagaraj, R. Farinato, A. Essenfeld, P. Somasundaran (2016), Development of GREEnness Index as an evaluation tool to assess reagents: evaluation based on SDS (Safety Data Sheet) information, Miner. Eng., 94: 1-9. https://doi.org/10.1016/j.mineng.2016.04.001

[38] Y. Shen, P. Somasundaran, (2019) GREEnness index evaluation of fracking chemicals using SDS (safety data sheet) information, J. Environ. Chem. Eng., 7:Article 102989. https://doi.org/10.1016/j.jece.2019.102989

[39] M. González-Miquel, I. Díaz, (2021) Green solvent screening using modeling and simulation, Current Opinion in Green and Sustainable Chemistry, 29: Article 100469. https://doi.org/10.1016/j.cogsc.2021.100469

[40] L.J. Diorazio, P. Richardson, H.F. Sneddon, A. Moores, C. Briddell, I. Martinez(2021), Making sustainability

assessment accessible: tools developed by the ACS green chemistry institute pharmaceutical roundtable, ACS Sustain. Chem. Eng., 9:16862-16864. https://doi.org/10.1021/acssuschemeng.1c06235

[41] C.H. Benison, P.R. Payne (2022), Manufacturing mass intensity: 15 Years of Process Mass Intensity and development of the metric into plant cleaning and beyond, Current Research in Green and Sustainable Chemistry, 5: Article 100229. https://doi.org/10.1016/j.crgsc.2022.100229

[42] S.R. Madabhushi, N.D.S. Pinto, H. Lin (2022), Comparison of process mass intensity (PMI) of continuous and batch manufacturing processes for biologics, N. Biotech., 72: 122-127, https://doi.org/10.1016/j.nbt.2022.07.004

[43] Elsheikh SG, Hassan AME, Fayez YM, El-Mosallamy SS (2023) Green analytical chemistry and experimental design: a combined approach for the analysis of zonisamide. BMC Chem 17:38. https://doi.org/10.1186/s13065-023-00920-0

[44] Kokilambigai KS, Lakshmi KS (2022) Analytical quality by design assisted RP-HPLC method for quantifying atorvastatin with green analytical chemistry perspective. J Chromatogr Open 2:100052. https://doi.org/10.1016/j.jcoa.2022.100052

[45] Perumal DD, Krishnan M, Lakshmi KS (2022) Eco-friendly based stability-indicating RP-HPLC technique for the determination of escitalopram and etizolam by employing QbD approach. Green Chem Lett Rev 15(3):671–682. https://doi.org/10.1080/17518253.2022.2109780

[46] Vieira-Sellai L, Quintana M, Diop O, Mercier O, Tarrit S, Raimi N, Ba A, Maunit B, Galmier MJ (2022) Green HPLC quantification method of lamivudine, zidovudine and nevirapine with identification of related substances in tablets. Green Chem Lett Rev 15(3):695–704. https://doi.org/10.1080/17518253.2022.2109780

[47] Iqbal M, Imam F, Ali EA, Kalam MA, Alhudaithi SS, Anwer MdK (2023) A validated UPLC-MS/MS method for rapid quantification of umifenovir in plasma samples and its greenness assessment. Separations 10(7):379. https://doi.org/10.3390/separations10070379

[48] Iqbal M, Ezzeldin E, Anwer MdK, Imam F (2021) Eco-friendly UPLC-MS/MS quantitation of delafloxacin in plasma and its application in a pharmacokinetic study in rats. Separations 8(9):146. https://doi.org/10.3390/separations8090146

[49] Prajapati PB, Bodiwala KB, Shah SA (2018) Analytical quality-by-design approach for the stability study of thiocolchicoside by eco-friendly chromatographic method. JPC J Planar Chromatogr Mod TLC 31(6):477–487. https://doi.org/10.1556/1006.2018.31.6.8

[50] Naguib IA, Hassan ES, Emam AA, Abdelaleem EA (2020) Development and validation of HPTLC and green HPLC methods for determination of a new combination of quinfamide and mebendazole. J Chromatogr Sci 58(1):16–21. https://doi.org/10.1093/chromsci/bmz070

[51] Elsheikh SG, Hassan AME, Fayez YM, El-Mosallamy SS (2022) Greenness assessment of two validated stability-indicating chromatographic methods for estimating modafinil using the analytical eco-scale. J AOAC Int 105(2):379–386. https://doi.org/10.1093/jaoacint/qsab148

[52] Elsheikh SG, Hassan AME, Fayez YM, El-Mosallamy SS (2023) Green analytical chemistry and experimental design: a combined approach for the analysis of zonisamide. BMC Chem 17:38. https://doi.org/10.1186/s13065-023-00920-0

[53] Chanduluru HK, Sugumaran A, Kannaiah KP (2022) Multiple spectrophotometric determinations of Chlorthalidone and Cilnidipine using propylene carbonate—as a step towards greenness. Anal Biochem 657:114890. https://doi.org/10.1016/j.ab.2023.114890

[54] Fawzy MG, Hassan WE, Mostafa AA, Sayed RA (2022) Different approaches for the assessment of greenness of spectrophotometric methodologies utilized for resolving the spectral overlap of newly approved binary hypoglycemic pharmaceutical mixture. Spectrochim Acta A Mol Biomol Spectrosc 272:120998. https://doi.org/10.1016/j.saa.2022.120998

[55] Fawzy MG, Saleh H, Reda A, Bahgat EA (2022) A green spectrophotometric method for the simultaneous determination of nasal binary mixture used in respiratory diseases: applying isosbestic point and chemometric approaches as a resolving tool, greenness evaluation. Spectrochim Acta A Mol Biomol Spectrosc 283:121585. https://doi.org/10.1016/j.saa.2022.121585.

[56] Avasarala, H., Jayanthi, V. R., & Dinakaran, S. K. (2018). Fast and sensitive quantification of asenapine maleate by high-performance thin layer chromatography. Lat. Am. J. Pharm, 37(2), 330-3. https://www.researchgate.net/publication/323186272_Fast_and_sensitive_quantification_of_asenapine_maleate_by_high-performance_thin_layer_chromatography.

[57] Dinakaran, S.K., Nitesh, Y., Kothapalli, U.L.S.T., Alekhya, R., Avasarala, H.(2024) Eco-friendly assessment

of turmeric-assisted approach in developing and validating a method for the quantification of atropine sulphate in bulk and tablet dosage form using UV–Visible spectrophotometry, Green Analytical Chemistry, 9, 100114. https://doi.org/10.1016/j.gac.2023.100114

[58] Kumar D. Sathis, Bhavitha K., Harani A., Meenakshi V, Rajendran Parvathy, Lee It Ee, Awadh Mohammed Al, Yuheng Lu, Prasad N.E Chandra,(2025) Green analytical comparison and central composite design optimization for simultaneous estimation of pain management drugs using RP-liquid chromatography, Microchemical Journal, 208, 112309. https://doi.org/10.1016/j.microc.2024.112309.