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REVIEW

Sustainable advancement of high-performance thin-layer chromatography: integrating green, blue and white analytical chemistry with smart software solutions

Sreenath Venkatesan[1] · Padhma Shri Babu[1] · Sudharsan Krishnamoorthy[1] · Nivathra Venkat Narayana Raju[2]

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Abstract
High-performance thin-layer chromatography (HPTLC) is increasingly recognised as a versatile, low-solvent technique for routine quality control of food and herbal and pharmaceutical products. Recent developments in green analytical chemistry (GAC), blue analytical chemistry (BAC) and white analytical chemistry (WAC) have accelerated the transition of HPTLC from solvent-intensive workflows to sustainability-driven, decision-oriented platforms. The current review describes the basics of HPTLC but in the context of a single GAC–BAC–WAC framework, including the use of solvents that are eco-friendly, miniaturisation and greener methods of sample preparation, including quick, easy, cheap, effective, rugged and safe (QuEChERS), pressurised fluid extraction, dispersive liquid–liquid microextraction, ultrasound-assisted extraction and solid-phase microextraction. Sustainable validation practices, including plate miniaturisation, chamber pre-saturation, automated spotting, binary low-toxicity mobile phases and low-volume derivatisation, are critically discussed with respect to solvent consumption, analytical performance and regulatory robustness. Particular emphasis is placed on contemporary greenness and whiteness assessment tools (Green Analytical Procedure Index [GAPI]; Analytical Greenness Metric [AGREE]; Analytical Eco-Scale; National Environmental Methods Index [NEMI]; red, green, blue (RGB); Greenness Evaluation Metric for Analytical Methods [GEMAM]; and Blue Applicability Grade Index [BAGI]) and their application to HPTLC methods. This review examines software-assisted HPTLC workflows, persistent gaps (limited life-cycle assessments [LCA], trace sensitivity, digital standardisation), and prospects for smart software integration with GAC–BAC–WAC principles.

Keywords High-performance thin-layer chromatography (HPTLC) · Method validation · Eco-friendly analysis · Life cycle assessment · Green chemistry · Blue chemistry · White chemistry · Conventional metrics

1 Introduction

Analytical chemistry plays a leading role in quality control of food and pharmaceutical products, enabling the establishment and determination of their quality, safety, authenticity and therapeutic efficacy [1]. Modern analytical approaches must address increasingly complex natural matrices while simultaneously complying with stringent global regulatory requirements, which impose the need for methods with high levels of reliability, reproducibility and efficiency [2]. Traditional analytical approaches, however, frequently rely on toxic organic solvents, energy-intensive instrumentation and multi-step sample preparation procedures [3], leading to substantial generation of non-eco-friendly waste and an increased environmental burden. These drawbacks have driven a global transition towards more sustainable and environmentally responsible analytical practices.

✉ Nivathra Venkat Narayana Raju
nivearaj96@gmail.com

Sreenath Venkatesan
sreenathmithwinner@gmail.com

Padhma Shri Babu
padhmashri2003@gmail.com

Sudharsan Krishnamoorthy
sudharsankdharsan@gmail.com

[1] B. Pharmacy Final Year, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India

[2] Department of Pharmaceutical Chemistry and Analysis, School of Pharmaceutical Sciences, Vels Institute of Science Technology & Advanced Studies (VISTAS), Pallavaram, Chennai, Tamil Nadu, India

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Green analytical chemistry (GAC) aims to reduce the environmental impact of analytical methods by minimizing solvent toxicity, reducing reagent consumption, decreasing waste generation and lowering energy requirements without compromising analytical performance [4]. Over the past decade, GAC principles have increasingly been incorporated into chromatographic techniques, particularly in food and pharmaceutical analysis [5]. However, real-world sustainability in modern analytical research should not be limited solely to environmental considerations but should also include method efficiency, cost-effectiveness, operator safety, durability and data integrity. These evolving requirements have renewed interest in high-performance thin-layer chromatography (HPTLC), an advanced form of traditional TLC, which offers practical advantages that may support sustainable analytical practices when appropriately optimized.

HPTLC offers several inherent advantages, including relatively low solvent consumption, high sample throughput, shorter analysis time, simultaneous processing of multiple samples and permanent visual documentation of chromatographic results [6]. Consequently, chromatographic methods developed under GAC principles are increasingly explored for routine quality control (QC) of herbal pharmaceuticals and food products. Recent technological developments, including improved stationary phases, automated sample application systems, densitometric detection, environmentally considerate mobile phase selection and digital image-based analytical tools, have significantly enhanced the sensitivity, reproducibility and versatility of HPTLC [7]. Although several reviews have discussed green HPTLC strategies—primarily focusing on greener solvent substitution, waste minimization and eco-friendly method development—most existing reviews predominantly evaluate these approaches from a GAC perspective, while comparatively limited attention has been given to analytical performance optimization, practical feasibility, cost considerations and decision-support capabilities in routine analytical environments. In routine practice, HPTLC separations are predominantly performed on silica gel stationary phases using mixtures of organic solvents under normal-phase (NP) conditions, which remain the most widely applied format in pharmaceutical and herbal analysis [8].

This observation highlights a significant gap in literature and underscores the need for a more comprehensive and balanced sustainability framework for HPTLC. To address this gap, the present review evaluates GAC principles through the complementary frameworks of blue analytical chemistry (BAC) and white analytical chemistry (WAC) within the context of HPTLC. BAC emphasizes analytical efficiency, operational safety, economic feasibility and practical applicability in routine laboratory environments, thereby supporting the pragmatic implementation of sustainable analytical methodologies [9]. WAC provides a holistic sustainability assessment by integrating the green (environmental impact), blue (method efficiency), and red (analytical performance and quality) dimensions of analytical chemistry. This integrative framework broadens sustainability evaluation beyond traditional environmental considerations to include analytical performance, operational efficiency, operator safety, economic viability and data-driven decision-making [10]. The review further highlights the importance of method miniaturization, software-assisted analysis, data-oriented workflows and emerging artificial intelligence-based tools as promising strategies for reducing solvent consumption while improving analytical resolution, throughput and sensitivity. Overall, this green–blue–white analytical perspective positions HPTLC as a versatile and potentially sustainable platform for routine quality control in food, herbal and pharmaceutical analysis [11].

2 Fundamentals of HPTLC with the integration principle of green–blue–white

GAC is based on the 12 principles of green chemistry proposed by Anastas and Warner (1998) to promote environmental sustainability, as mentioned in Fig. 1 [12]. It aims to provide a structural framework to minimise chemical hazards, reduce waste and enhance the safety of the sample and analysis, thereby addressing the ecological disadvantages associated with the method on the basis of ecological aspects [13].

- Solvents: Instead of using toxic solvents such as benzene or chloroform in the mobile phase, we can choose sustainable solvents, that is, ethanol and water, which should be used [14].

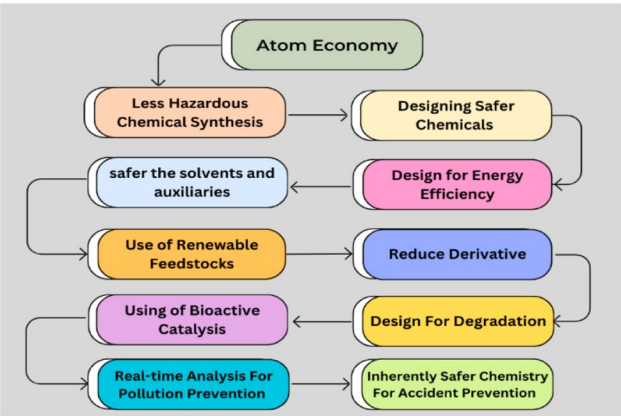


Fig. 1 Principles of green analytical chemistry

- Waste: Reduction of solvent usage and subsequent generation of waste, which is normally linked to HPTLC tests, can be done by reducing the size of the chromatographic plates and development lengths [15].
- Energy: The implementation of energy-saving protocols, such as HPTLC, which often work at ambient temperature and atmospheric pressure [16], leads to a decrease in total energy usage.

BAC aims to enhance the practical applicability of the methodology by emphasising aquatic sustainability and water conservation in analytical techniques. It complements green (ecological) and red (analytical performance) principles, thereby enhancing the analytical performance aspects by providing a holistic method evaluation. This discussion looks into the elements of usefulness and usability in real-world regulatory or quality control settings [17].

- Cost and time: The method is considered to be fast and cost-efficient, as it has the common features of HPTLC because it has very few equipment requirements and can be used to evaluate two or more samples simultaneously [18].
- Simplicity: It is better to prefer more streamlined procedures, which require fewer steps of the procedure and use more readily available reagents [19].
- Automation: The use of automation during densitometric measurement and application of samples can be more productive and, at the same time, minimise the error and interaction of operators [20].

WAC is based on overall sustainability and performance. WAC integrates three complementary analytical dimensions:

Red (analytical performance), green (environmental sustainability), and blue (practical applicability and efficiency). The main aim is to create a basic framework for evaluating and developing analytical methods that balance the ecological impact of green, the core performance of red and the economic factor of blue, thereby combining the advantages of the red, green, blue (RGB) components to achieve white balancing on the basis of RGB content, as shown using a colour wheel representation [21].

The red dimension (analytical performance) is evaluated through validation parameters such as accuracy, precision, robustness, linearity and sensitivity in accordance with the International Council for Harmonisation (ICH) Q2(R2) guidelines, yielding a percentage score (0–100%) [22]. The green dimension (environmental impact) is assessed using greenness metrics such as Analytical Greenness Metric (AGREE), Green Analytical Procedure Index (GAPI), National Environmental Methods Index (NEMI) and Analytical Eco-Scale, which evaluate solvent toxicity, waste generation, energy consumption and reagent safety (AGREE ≥ 80, Environmental Sustainability Assessment score (ESA) ≥ 75) [23]. The blue dimension (practical usability) is evaluated through operational parameters such as analysis time, cost per analysis, automation capability, energy consumption and operator safety using tools such as Blue Applicability Grade Index (BAGI) or related blue analytical indices [24]. The overall WAC score (whiteness index) is commonly expressed as the average contribution of the three dimensions:

$$W = (R + G + B)/3,$$

where R represents analytical performance, G represents environmental sustainability and B represents operational practicality. This integrated evaluation provides a balanced sustainability profile of analytical methods [25].

To create an optimised and advanced yet comprehensive approach, the green (ecological) and blue (practical) elements are combined rationally and practically with the red (analytical performance) elements. Performance of the technique is verified to retain high levels of resilience, sensitivity, accuracy and precision for quick validation metrics. Holistic evaluation is to evaluate the methodology's overall sustainability profile and use multi-tool testing metrics such as NEMI, Analytical Eco-Scale, AGREE, Complementary Green Analytical Procedure Index (Complex GAPI), BAGI, RGB tools and Greenness Evaluation Metric for Analytical Methods (GEMAM). In response to these challenges, modern analytical research has increasingly focused on sustainable analytical strategies, including the integration of green, blue and white analytical chemistry principles in HPTLC (Table 1).

For example, in herbal drug analysis, a conventional high-performance liquid chromatography (HPLC) method may require 100–150 mL of acetonitrile per run, whereas an optimized HPTLC method may use 5–10 mL of ethanol–ethyl acetate mixture, reducing solvent consumption by more than 90%. Similarly, miniaturised HPTLC plates (5 cm × 5 cm) allow simultaneous analysis of multiple samples, improving throughput while significantly reducing solvent waste and analysis time.

2.1 Sustainable solvents

Sustainable solvents are one of the most powerful strategies implemented to reduce environmental toxicity and improve laboratory safety by aligning with the principles of GAC and BAC [25, 26]. Green solvents are solvents that are less toxic in nature or non-toxic in activity, biodegradable, derived from natural and renewable resources, and found to be safe for human health and the environment [27]. The traditional solvents, such as benzene, toluene, methanol and hexane, are considered toxic as they exhibit carcinogenicity and

Table 1 Triadic integration of GAC–BAC–WAC in HPTLC workflows

HPTLC step	Green (GAC)	Blue (BAC)	White (WAC)	Metrics
Sample preparation	Eco-friendly solvents; minimal organic waste	Rapid extraction; energy-efficient; low cost	High reproducibility; minimal operator risk	E-factor < 10, extraction time < 30 min, solvent ≤ 5 mL
Plate development	Miniaturized plates (5 cm × 5 cm); chamber pre-saturation	Uniform migration; low sample volume	Standardised high-throughput	Rs > 2, RSD < 1.5%, solvent savings ~70%
Detection	Immersion derivatisation; low reagent use	Fast scanning; densitometry automation	Accurate, robust quantification	LOD < 0.5 ng spot^{-1}, precision RSD < 2%
Data analysis	Digital evaluation; GAPI/AGREE/Eco-Scale scoring	Automated peak integration, minimal human intervention	WAC RGB score; holistic sustainability	Whiteness index ≥ 0.85, predictive modelling via ML
Workflow integration	Minimal waste; solvent recycling	Time/cost optimised; energy-saving	Overall sustainability and analytical performance	Combined G/B/W index ≥ 0.8; validated per ICH Q2(R2)

AGREE Analytical Greenness Metric; *BAC* blue analytical chemistry; *GAC* green analytical chemistry; *GAPI* Green Analytical Procedure Index; *ICH* International Council for Harmonisation; *LOD* limit of detection; *ML* machine learning; *RGB* red, green, blue; *RSD* relative standard deviation; *WAC* white analytical chemistry

neurotoxicity, and contribute to volatile smog formation. It also directly impacts the rising pollution levels of the surrounding environment [28]. In practical HPTLC applications, the majority of separations are still performed on silica gel stationary phases using mixtures of organic solvents such as toluene, ethyl acetate, hexane or chloroform. Although greener solvent systems such as ethanol–water mixtures are increasingly explored, complete replacement of conventional normal-phase silica gel systems with reversed-phase (RP) HPTLC is not always feasible because analyte polarity, selectivity requirements and migration behaviour often favour normal-phase separations. Alternative solvents that are used in HPTLC, such as ethanol, ethyl acetate, water, cyclohexane and propylene carbonate bio-based solvents, are preferred as they are better aligned with sustainable principles. Although propylene carbonate is often classified as a green solvent owing to its low toxicity and low volatility, its relatively high viscosity and unfavourable evaporation characteristics make it unsuitable for conventional capillary-driven HPTLC development. High-viscosity solvents migrate slowly in planar chromatographic systems. Because solvent migration in planar chromatography occurs through capillary flow within the stationary phase layer, mobile phases with low viscosity are generally preferred to ensure uniform development and sharp chromatographic zones, resulting in prolonged development times and potential zone broadening during solvent evaporation. Consequently, its practical application in routine HPTLC separations remains limited (Table 2).

Examples of mobile phase in previous studies over green chemistry on HPTLC methods are in the ratio of ethanol–water (60:40, *V/V*), ethyl acetate–cyclohexane (1:1, *V/V* or 6:4, *V/V*) and ethanol–ethyl acetate (50:50, *V/V*) [29, 30]. Building on these sustainability principles, their practical implementation can be observed in the development and optimisation of modern HPTLC methodologies.

Table 2 Benefits of sustainable solvents usable in HPTLC

Sustainable solvent	Benefits
Ethanol	Renewable, biodegradable, low toxicity
Ethyl acetate	Less toxic, biodegradable
Water	Non-toxic, universally green
Cyclohexane	Less toxic than hexane
Bio-based solvents	Derived from plant materials

3 Eco-friendly and sustainable sample preparation methods for HPTLC

Sample preparation plays a major role in HPTLC analysis, significantly influencing analytical performance, environmental impact, analyst safety and operational efficiency. The recent improvement emphasises not only GAC principles, such as reduced solvent toxicity and waste, but also BAC, which focuses primarily on energy efficiency and resource conservation, and WAC [31], which predominantly balances the analytical performance, sustainability, economic feasibility and user safety. The GAC–BAC–WAC principles are interlinked with one another to achieve an optimised and enhanced overall HPTLC performance.

3.1 Use of green and sustainable solvents for the mobile phase

The selection of environmentally benign solvents is a fundamental consideration for developing sustainable methods in HPTLC. Water–ethanol-rich systems, particularly in reversed-phase HPTLC Reverse Phase-High Performance Liquid Chromatography (RP-HPTLC), have increasingly been explored as greener alternatives to conventional non-polar mobile phases used in normal-phase systems. These systems are compatible with a wide range of pharmaceutical active ingredients (APIs), botanical extracts and food matrices, and are frequently employed with end-capped reversed-phase plates to improve separation efficiency [32]. Most HPTLC separations are performed in normal-phase systems using silica gel plates with organic solvent mixtures such as toluene, hexane, ethyl acetate or chloroform. In contrast, reversed-phase HPTLC (RP-HPTLC) employs modified stationary phases such as RP-18 plates and requires polar mobile phases such as methanol–water, acetonitrile–water, or ethanol–water mixtures. Therefore, solvents such as toluene or hexane are generally restricted to normal-phase systems and are rarely used in RP-HPTLC separations.

Deep eutectic solvents (DES), especially choline chloride-based systems, have recently attracted interest in analytical chemistry owing to their low volatility, tunable polarity and relatively lower toxicity. However, their application in HPTLC remains limited because high viscosity and slow solvent migration may affect chromatographic reproducibility. In addition, removal of residual DES components prior to instrumental detection can be difficult, which further limits their routine application in HPTLC workflows. Therefore, viscosity control and optimisation of plate wetting are important when evaluating DES-based solvent systems for chromatographic development [33].

Greener alternatives for acetonitrile, tetrahydrofuran and toluene, such as bio-based solvents including ethyl lactate and 2-methyltetrahydrofuran, may provide reduced toxicity and favourable chromatographic behaviour in selected analytical applications. From a WAC perspective, the selection of such solvents should balance environmental safety with analytical robustness and method reliability. Additionally, eco-conscious derivatization strategies employing low-toxicity reagents and controlled spray application can reduce reagent consumption and waste generation. Modern digital densitometry further supports sustainable analysis by enabling accurate detection while minimizing excessive reagent use [34].

3.2 Quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction

QuEChERS is quick, easy, cheap, effective, rugged and safe. It was developed by Anastassiades et al. in 2002 and is widely regarded as a green–white hybrid extraction-based technique. It can be compared with conventional extraction methods, which can significantly reduce organic solvent consumption while integrating extraction and cleanup into two streamlined steps. During each extraction step, samples are vigorously shaken with acetonitrile in the presence of sodium chloride and anhydrous magnesium sulphate to induce the action of the salting-out process [35]. Buffering agents play a major role in protecting base-sensitive analytes. The dispersive solid-phase clean-up step efficiently removes interfering matrix components such as fatty acids and carbohydrates [36]. This method aligns with GAC, BAC and WAC by minimising solvent use; reducing processing time and energy; and maintaining analytical reliability and simplicity, respectively.

3.3 Pressurised fluid extraction (PFE)

PFE, also known as accelerated solvent extraction (ASE), is a modern sample-type preparation process that applies solvents at elevated temperature ranges (50–200 °C) and pressures (5–15 MPa) to achieve rapid analyte extraction [37]. It serves as a sustainable alternative to traditional extraction methods by reducing the solvent volume, extraction time and analyst exposure to various hazardous chemicals. The addition of BAC in this process is that it can improve energy efficiency through shorter extraction cycles, while the GAC benefits arise from reduced solvent waste [38]. Within the WAC type framework, PFE can balance speed, safety and analytical performance, making it particularly suitable for trace analysis before HPTLC analysis.

3.4 Dispersive liquid–liquid microextraction (DLLME)

DLLME is a miniaturised sample preparation technique based on the use of micro- to low-millilitre volumes of solvents. In this approach, a small volume of extraction solvent (microlitre scale) is dispersed into an aqueous sample using a disperser solvent (typically 100–1000 µL) [39], forming fine droplets and a large interfacial surface area for rapid analyte partitioning. Following the centrifugation or settling, the now mixed and enriched extract phase (in tens of microlitres) is collected for analysis. DLLME strongly supports the combination of GAC, BAC and WAC as it optimises the overall process by enabling extremely low solvent requirement, inducing rapid equilibrium and

minimal energy input [40] and providing high enrichment factors with simple instrumentation, respectively.

3.5 Stir bar sorptive extraction (SBSE)

SBSE is a solvent-minimised technique in which analytes are extracted directly from liquid samples using a magnetic stir bar coated with a sorptive polymer, commonly polydimethylsiloxane (PDMS) [41]. After extraction, analytes are recovered with the help of thermal desorption or with a minimal amount of solvent. This technique is easily recyclable, offers high extraction efficiency for semi-volatile and non-polar components and significantly reduces the overall solvent consumption [42]. The SBSE methodology is found to strongly align with GAC and WAC principles. From the WAC perspective, it provides a robust, convenient and practical alternative to the conventional liquid–liquid extraction method.

3.6 Microwave-assisted extraction (MAE)

MAE is a type of extraction that is used to accelerate sample preparation with the help of microwave energy to heat polar solvents such as water or ethanol. It also enhances the diffusion of the analyte into the solvent matrix. Conventional MAE systems typically operate at 2.45 GHz, which enables rapid heating and decreased extraction times [43]. MAE mostly supports the BAC principle by lowering overall energy consumption compared with prolonged conventional heating, while GAC benefits can be determined from the use of aqueous or alcohol-based solvents. Yet, it can have a few limitations, including unequal heating and dependency on solvent dielectric characteristics [44]. When WAC is taken into consideration, MAE offers an efficient way of balancing speed, efficiency and environmental results.

3.7 Ultrasound-assisted extraction (UAE)

UAE is an eco-friendly, sustainable methodology that aims to enhance the separation of analytes from solid or semi-solid type of matrices using environmentally friendly solvents with the help of ultrasonic waves (20–100 kHz). Acoustic cavitation leads to the formation of bubbles and their collapse due to ultrasonic waves [45]. UAE efficiently extracts the secondary metabolites of plants such as phenolics, flavonoids and alkaloids using eco-friendly solvents, while offering reduced extraction time and solvent use. This is due to the disruption of cell walls, which leads to enhanced mass transfer and solvent penetration [46]. This method also combines GAC, BAC and WAC through solvent reduction and low energy requirements by providing a simple and scalable approach, respectively, which enhances overall workflow involved in this method of extraction.

3.8 Solid-phase microextraction (SPME)

SPME is a solvent-free sample preparation method that aims to isolate volatile and semi-volatile analytes with the help of a coated fibre, which is exposed to the sample matrix [47]. This methodology is highly preferred for HPTLC workflows owing to its decreased environmental footprint and increased sensitivity. SPME strongly combines the advantages of GAC, BAC and WAC by eliminating solvent use, through passive extraction without heating, by offering reproducibility, ease of use and compatibility with diverse matrices, respectively, which in turn enhances the overall experience involved in the extraction process [48]. In addition to sustainable sample preparation strategies, optimisation of chromatographic conditions and validation practices further contribute to environmentally responsible HPTLC workflows.

4 Sustainable aspects in validation of HPTLC

Some of the main aspects involved in sustainable validation of HPTLC include reduction of HPTLC plates, use of pre-saturation chambers, automated spotting, efficient mobile phases and low-volume derivatization [49]. This can be achieved by reducing the HPTLC plate size by using smaller TLC plates (e.g. 5 cm × 5 cm or 10 cm × 5 cm) instead of the traditional procedure (20 cm × 10 cm plates), which requires less solvent volume for development. It also reduces solvent use by up to 70%, used particularly when developing new methods, and is effective for routine analysis. These validation parameters are evaluated in accordance with internationally recognised regulatory guidelines such as ICH Q2(R2), US Pharmacopeia (USP) < 203 > and European Pharmacopoeia recommendations for chromatographic method validation. This is a vital green chemistry methodology that promotes economy, sustainability and efficiency [50], as presented in Table 3.

Table 3 Comparison of the standard and miniaturised HPTLC

Feature	Standard HPTLC	Miniaturised HPTLC
Plate size	20 cm × 10 cm	5 cm × 5 cm or 10 cm × 5 cm
Solvent volume	20–30 mL	5–10 mL
Sample volume	5–10 µL	0.5–2 µL
Time per run	30–40 min	10–20 min
Environmental impact	Higher	Significantly lower

4.1 Sample and reagent consumption in GAC–BAC–WAC frameworks

HPTLC demonstrates the principles of GAC through ultralow sample volumes (typically 1–2 µL band^{-1}) and reduced mobile phase consumption (approximately 5–10 mL plate^{-1}). Compared with conventional HPLC methods that may consume > 100 mL solvent per run, HPTLC can reduce organic solvent usage and associated waste generation by up to 90–95%, thereby lowering the environmental burden and eco-toxicity risks when greener solvent systems such as ethanol-based mixtures are used [51].

Within the BAC perspective, validated HPTLC methods comply with regulatory requirements such as ICH Q2(R2), demonstrating acceptable analytical performance, including linearity ($R^2 \geq 0.999$), precision (relative standard deviation [RSD] < 2%) and accuracy within ± 1.5% without compromising analytical reliability [52].

From a WAC perspective, HPTLC provides a balanced platform for routine quality control by integrating environmental sustainability with regulatory compliance and analytical performance. This approach combines low environmental impact (e.g. reduced solvent consumption and waste generation) with method robustness and operational efficiency, as summarised in Table 4.

4.2 Chamber pre-saturation for enhanced sustainability and reproducibility

Pre-saturation of the development chamber allows equilibration of the mobile phase vapour within the chamber environment, which stabilizes chromatographic conditions and reduces solvent evaporation during plate development. This practice contributes to GAC principles by improving solvent utilization efficiency and minimizing unnecessary solvent loss.

From the BAC perspective, chamber pre-saturation enhances chromatographic reproducibility by promoting uniform solvent front migration and consistent R_F values, thereby improving separation efficiency and resolution (Rs > 2.0) while maintaining inter-plate reproducibility (RSD < 1.5%) [53]. In the WAC framework, pre-saturation supports reliable and reproducible separations while optimizing resource utilization, enabling efficient workflows and consistent analytical outcomes in routine laboratory applications, as reflected in Table 4.

4.3 Automated sample spotting: precision at the nanoscale

Automated applicators, such as the CAMAG (Muttenz, Switzerland) ATS4, enable highly precise sample application with nanolitre to microlitre accuracy and reduced variability (< 5% RSD). This aligns with GAC principles by minimizing sample wastage and reducing the need for repeated spotting and additional solvent consumption.

From a BAC standpoint, automated application improves band geometry and analytical performance, resulting in improved band symmetry (asymmetry < 1.2), enhanced detection sensitivity (limit of detection [LOD] < 1 ng band^{-1}) and reduced operator-induced variability compared with manual spotting techniques [54]. Within the WAC perspective, automation facilitates standardized and reproducible analytical workflows suitable for routine quality control laboratories, improving analytical reliability while maintaining sustainable laboratory practices, as presented in Table 4.

4.4 Mobile phase optimisation: binary systems for improved efficiency

Selection of simplified mobile phase systems, such as binary solvent mixtures (e.g. ethanol–ethyl acetate), supports GAC principles by reducing solvent complexity and minimizing the volume of organic solvents required for chromatographic development (typically 5–10 mL run^{-1}). From the BAC viewpoint, optimized binary solvent systems can provide adequate chromatographic performance, including improved selectivity ($\alpha > 1.5$) and satisfactory plate efficiency ($N > 10{,}000$ m^{-1}), while maintaining analytical robustness [55]. Under the WAC framework, solvent-efficient mobile phase systems allow laboratories to maintain regulatory-compliant analytical performance while reducing environmental impact and operational costs, as summarized in Table 4.

Table 4 Comparative HPTLC paradigms under GAC–BAC–WAC metrics

Approach	Solvent use (mL run^{-1})	Analytical performance (R^2/RSD)	Overall sustainability (E-factor)
Traditional (manual)	20–30	Moderate (0.995/< 5%)	Low (> 50)
Green (optimised)	5–10	High (≥ 0.999/< 2%)	High (< 10)
Micro-HPTLC	2–5	High (≥ 0.999/< 2%)	Very high (< 5)

4.5 Derivatization techniques and sustainability considerations

Derivatization is frequently employed in HPTLC to enhance visualization and detection sensitivity. Immersion derivatization provides rapid and uniform impregnation of the chromatographic plate; however, immersion chambers generally require relatively large volumes of derivatization reagent to ensure complete plate coverage.

Spray-based derivatization systems, such as the CAMAG nebulizer derivatizer, allow controlled application of derivatization reagents and typically require smaller reagent volumes. This approach reduces reagent consumption and operator exposure, making it more consistent with GAC principles while maintaining adequate analytical performance and reproducibility. Therefore, the selection of a derivatization technique should balance analytical sensitivity, reagent consumption and operational safety in accordance with sustainable analytical practices [56]. The automated CAMAG derivatizer typically consumes only 2–4 mL plate^{-1}. For the manual applicator, 50- and 100-mL reagent bottles are included with the standard CAMAG TLC or HPTLC Sprayer (manual, electro-pneumatic). Although the bottle may carry up to 100 mL, the user determines how much is used. It is intended for a fine aerosol mist (0.3–10 µm) for minimal consumption. Other approaches often require 3–6 mL for post-chromatography derivatization using other techniques.

Although immersion derivatization provides uniform reagent distribution, it requires relatively large reagent volumes to fully immerse the chromatographic plate. Spray-based derivatization systems such as the CAMAG nebulizer derivatizer are generally more consistent with green analytical principles because they apply significantly smaller reagent volumes while maintaining adequate detection sensitivity (Table 4).

4.6 Comparative sustainability of HPTLC and conventional chromatographic techniques

Compared with conventional liquid chromatographic techniques such as HPLC, HPTLC offers several sustainability advantages. HPLC methods frequently consume 50–150 mL of organic solvents per analytical run, whereas HPTLC typically requires 5–10 mL of mobile phase per plate, enabling solvent savings of up to 90–95%. In addition, HPTLC allows simultaneous analysis of multiple samples on a single plate, improving analytical throughput and reducing total energy consumption per analysis. These characteristics strongly align HPTLC with the principles of GAC while maintaining acceptable analytical performance for routine quality control applications.

4.7 Miniaturisation: synergising resolution and resource thrift

Microplates at 5 cm × 5 cm shrink migration distances, enforcing the GAC solvent severity (2–5 mL run^{-1} and diffusion-limited waste). BAC can be enhanced further by amplified mass transfer (HETP < 20 µm) and sensitivity gains. WAC implements miniaturisation for high-throughput quality control, and fuses ultragreen metrics standards with cost-normalised, better performance [57].

4.8 Validation in integrated green–blue–white regimes

WAC validation unifies the BAC rigour at accuracy ±1%, RSD < 1.5% and robustness per ICH Q2(R2) with GAC audits (e.g. AGREE prep scores > 0.8 and waste < 5 g analyte^{-1}). The empirical HPTLC data affirm $R^2 > 0.999$ and LOD < 1 µg mL^{-1} under green limitations, which is evidence of no sustainability penalty [58].

4.9 Atom economy in streamlined HPTLC workflows

Analytical atom economy mirrors synthetic ideals, which can be explained with the following example: 77% for ibuprofen is streamlining steps, curbing cumulative E-factors through omitted extractions or derivatisation [59]. By GAC–WAC alignment, error propagation of BAC is reduced to a variance < 2%, thereby yielding efficiency with the low-waste protocols.

4.10 Detection modalities aligned with GAC–BAC–WAC

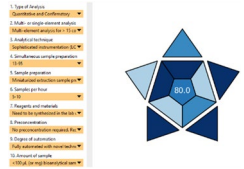
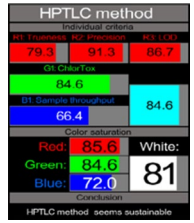
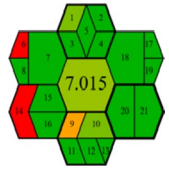
Ultraviolet–visible (UV–Vis) detection can be reagent-less, with low-energy consumption (GAC) quantitation (BAC: linearity > 0.999), while the densitometry bolsters WAC to be robust. Eco-reagents such as vanillin-ethanol (log $P < 2$) through immersion ensure GAC compliance, BAC sensitivity (LOD < 0.1 ng) and WAC practicality [60].

Beyond analytical performance, evaluating the environmental impact of chromatographic methods has become increasingly important in modern analytical chemistry (Table 5).

5 Applications based on case studies of GAC, BAC and WAC in contemporary HPTLC

In recent years, research in food, herbal, pharmaceutical and bioanalytical fields has focused on advancing HPTLC towards a sustainability-driven and decision-oriented

Table 5 Sustainability assessment tools for evaluating green, blue and white analytical chemistry compliance in HPTLC methods

Tool	Assessment scope	Scoring system and thresholds	Validated applications	Visualisation format of each tool
NEMI	Regulatory compliance against EPA criteria (toxicity, corrosivity, waste generation)	Binary quadrant evaluation: all four green = fully compliant	Ethanol/phosphate buffer HPTLC (complete compliance); pharmaceutical screening; low-waste HPTLC	
Eco-Scale	Penalty point accumulation for non-green parameters (solvents, energy, waste)	0–100 scale: > 75 (excellent green), 50–75 (acceptable), < 50 (inadequate); score = 100 − total penalty points	Lenvatinib RP/NP-HPTLC (80–85); MIR/TAM HPTLC (0.85); modafinil TLC–densitometry (82); general pharmaceutical HPTLC	Eco-Scale score = 100 − total penalty points (PP) is numerical data
AGREE	Comprehensive 12-principle compliance evaluation	0–1 scale: ≥ 0.70 (green compliant), 0.50–0.69 (partial), < 0.50 (non-compliant)	Tenoxicam HPTLC (0.82); cilnidipine analysis (0.88); trifluridine/tipiracil QbD-HPTLC (0.81)	 Twelve-segment circular pictogram
Complex GAPI	15 green analytical chemistry criteria-based (sample preparation through operator safety)	0–1 scale: ≥ 0.80 (excellent green), 0.60–0.79 (good), < 0.60 (requires optimisation)	MIR/TAM HPTLC (0.85); paracetamol/aspirin/diphenhydramine HPTLC; trifluridine/tipiracil HPTLC	 Five-segment pentagon (colour-coded)
BAGI	Integrated environmental impact, analytical performance, and operational efficiency	0–40 points: > 30 (outstanding), 24–29 (acceptable), < 24 (suboptimal), based on blue analytical metrics	Thiocolchicoside/aceclofenac HPTLC (32/80%); High Performance Liquid Chromatography-Mass Spectroscop HPLC–MS multi-analyte; HPTLC optimization	
RGB	Whiteness assessment using the red–green–blue additive colour model for overall sustainability	0–1 scale based on 12 principles, colour-coded for greenness/whiteness. It is based on white analytical chemistry	Favipiravir bioanalysis LC methods; multi-analyte pharmaceutical assays	
GEMAM	Comprehensive LCA of workflow: sample prep/reagents (1–8), performance (9–13), instrumentation (14–16), waste (17–19), safety (20–21); adjustable weight	0–10 weighted average: ≥ 8.0 (excellent green), 6.0–8.0 (good, e.g. 7.015), < 6.0 (inadequate)	HPTLC-firefly chemometrics (7.015); RP-HPLC/nano formulations; MEPS-microextraction	

BAGI Blue Applicability Grade Index; *EPA* Environmental Protection Agency; *GEMAM* Greenness Evaluation Metric for Analytical Method; *MEPS* Micro-Extraction by Packed Sorbents; *MIR/TAM* mirabegron/tamsulosin; *NEMI* National Environmental Methods Index

analytical platform by incorporating the principles of GAC–BAC–WAC. These developments reflect a paradigm shift from solvent-intensive, single-analyte separations to resource-efficient, information-rich and regulatory-relevant analytical workflows.

5.1 Case studies based on phytochemicals

These studies aimed to develop a rapid and sustainable HPTLC method for quantitative determination on the basis of phytochemicals of herbals.

5.1.1 Case study 1: piperine in Drakshadi Ghrita

A 2025 HPTLC study measured piperine in Drakshadi Ghrita. Separation was determined and calculated on silica gel 60 F_{254} plates utilizing toluene–ethyl acetate mobile phase (7:3, *V/V*). The analyte exhibited an R_F value of 0.52 ± 0.02. The R_F value was further quantified with the help of densitometric detection at 340 nm. This method demonstrated linearity in the range of 0.5–10 µg spot^{-1}, with precision (RSD < 2%), thereby making it suitable for routine herbal quality assurance. Integration of automated densitometry and decreased use of solvent favoured the principles of green and sustainable analytical practices, thereby yielding an Analytical Eco-Scale score of 85 [61].

5.1.2 Case study 2: polyphenols in *Bergenia* species

A 2022 research study executed HPTLC fingerprinting of bergenin, catechin and gallic acid from *Bergenia ciliata* and *Bergenia ligulata* extracts. Chromatographic separation was recorded and reported on silica gel 60 F_{254} plates with the help of ethyl acetate–methanol–water mobile phase (8:1:1, *V/V*). The compounds exhibited R_F values ranging from 0.45 to 0.72, and were detected deploying densitometric scanning at 280 nm. This methodology recorded limits of detection below 100 ng spot^{-1}, thereby enabling sensitive determination and identification of phytochemicals and detection of adulteration in herbal raw materials [62].

5.1.3 Case study 3: ginsenosides in *Panax ginseng* formulations

A 2023 HPTLC study analysed ginsenosides present in *Panax ginseng* formulations for species authentication. Separation was analysed and recorded with the help of silica gel 60 F_{254} plates with chloroform–methanol–water as mobile phase (65:35:10, *V/V*). The R_F values of major ginsenosides were observed and recorded between 0.30 and 0.65. The detection of the phytoconstituent was determined with the help of densitometric scanning at 203 nm following on-plate derivatization. This method established reproducible chromatographic profiles upon consecutive analysis and yielded an Analytical Eco-Scale score of 82. This testing allowed the differentiation of API from American ginseng adulterants [63]. Solvent-free or water-based methods, such as green extraction HPTLC techniques of plant volatiles and herbal amino acid reduction techniques used in the evaluation of nanomaterial purity also highlight the growing green nature of planar chromatography [64].

5.2 Case studies based on pharmaceutical sector

These studies aimed to develop a rapid and sustainable HPTLC method for quantitative determination based on the pharmaceutical drug sector.

5.2.1 Case study 1: carvedilol quantification

A 2025 green HPTLC study measured and reported carvedilol with the help of mobile phase toluene–isopropanol–ammonia (7.5:2.5:0.1, *V/V*) using silica gel 60 F_{254} plates. The R_F value observed and recorded was found to be 0.46 ± 0.02. Densitometric detection was evaluated at 242 nm. This methodology is based on GAC principles through limited solvent consumption, while concepts of BAC were favoured with the incorporation of aqueous traces. Automated densitometric evaluation under WAC optimisation yielded an AGREE > 0.8, which is considered an excellent NEMI rating. It also recorded linearity (20–120 ng band^{-1}), and waste generation < 10 mL run^{-1}, thereby establishing enhanced sustainability when compared with traditional analytical methods [65].

5.2.2 Case study 2: ertugliflozin in tablets

In a 2025 research study protocol, ertugliflozin was examined with the help of RP-HPTLC with mobile phase ethanol–water (7:3, *V/V*). The R_F value recorded was 0.54 ± 0.02, and densitometric detection was evaluated at 225 nm. This methodology established various nanogram-level detection limits and robustness (RSD < 2%). GAPI pictograms helped with confirming the environmentally friendly characteristics of the method. The combination of GAC solvent minimisation, BAC efficiency considerations and WAC-based digital densitometry ensured and allowed for reliable quantification of the drug and its related impurities in pharmaceutical formulations [66].

5.2.3 Case study 3: trifluridine and tipiracil

A 2025 quality-by-design (QbD)-assisted eco-friendly HPTLC method quantified trifluridine and tipiracil using the mobile phase ethyl acetate–methanol–water (8:1:1,

V/V). The analytes produced R_F values of 0.38 ± 0.02 (trifluridine) and 0.61 ± 0.02 (tipiracil) and were detected by densitometric scanning at 270 nm. The method demonstrated AGREE score of 0.81, favourable Complex GAPI evaluation, linearity over 100–1000 ng spot^{-1} and analysis time < 15 min, supporting its application in pharmaceutical quality assurance and stability testing [67].

5.3 Case studies based on food products

These studies aimed to develop a rapid and sustainable HPTLC method for quantitative determination based on food products.

5.3.1 Case study 1: aflatoxins in nuts

A 2025 green HPTLC method quantified aflatoxins B1, B2, G1 and G2 in peanuts and pistachios using the mobile phase ethyl acetate–methanol–water (7:2:1, *V/V*). The aflatoxins showed R_F values between 0.32 and 0.68 and were detected using densitometric scanning at 366 nm after derivatization. The method achieved AGREE scores > 0.85, linearity (0.1–5 µg kg^{-1}), LOD < 0.5 µg kg^{-1} and solvent waste < 5 mL run^{-1}, enabling rapid screening for food safety analysis [68].

5.3.2 Case study 2: Sudan dyes in spices

In a 2024 RP-HPTLC study, Sudan dyes I–IV were analysed in chilli and curry powders using an ethanol–water mobile phase (8:2, *V/V*). The dyes produced R_F values between 0.40 and 0.75, and detection was achieved using fluorescence densitometry at 500 nm. The method showed GAPI low-impact pictograms, precision (RSD < 1.5%), linearity (5–50 ng band^{-1}) and analysis time < 10 min, supporting efficient monitoring of adulteration in spice quality control [69].

5.3.3 Case study 3: capsaicinoids in peppers

A 2025 QbD-optimised HPTLC study evaluated capsaicin and dihydrocapsaicin present in extracts of *Capsicum* using toluene–ethanol–water (6:3:1, *V/V*) as the mobile phase. The compounds capsaicin and dihydrocapsaicin produced R_F values of 0.48 ± 0.02 and 0.62 ± 0.02, respectively. The values were detected by densitometry at 280 nm. This method established and recorded Eco-Scale score of 83/100, LOD of 20 ng spot^{-1} and time of analysis < 15 min, thereby enhancing reliable food authentication and pungency profiling [70].

Taken together, these developments demonstrate that modern HPTLC approaches extend beyond traditional green chemistry claims by integrating environmental sustainability (GAC), resource and energy efficiency (BAC), and analytical performance with regulatory applicability (WAC) [71]. In this context, HPTLC represents a versatile analytical platform capable of aligning sustainability metrics, operational efficiency and analytical performance within the combined GAC–BAC–WAC framework [72].

Recent advancements in digital technologies and smart analytical software further support sustainable method development and improve data management in HPTLC analysis.

6 Difficulties and limitations faced in sustainable HPTLC research

Despite the multiple merits and benefits of applying GAC–BAC–WAC principles in HPTLC, having various advantages and enhance overall analytical research, several practical challenges continue to remain.

6.1 GAC limitations

Some of the GAC-based adaptations, such as the use of eco-friendly solvents and solvent-reduction methodologies, often increase difficulties and impose constraints on the polarity range and strength of elution, thereby limiting their overall applicability when applied to highly complex or hydrophobic analytes. Micro-HPTLC or miniaturised HPTLC systems have the advantage of compromising separation efficiency and reproducibility in multicomponent mixtures and providing appropriate peak resolutions, all while reducing solvent consumption, especially when analysing any complex herbal, polyherbal or biological matrices. Sustainable sample preparation remains a critical bottleneck; many natural matrices still demand extensive extraction, cleanup or derivatisation, which contradicts the principles of minimal environmental impact. Green HPTLC decreases the need for hazardous solvents but, in return, faces various disadvantages, for example, lower resolution with mobile phases, such as ethanol–water mobile phases, when compared with acetonitrile, thereby impacting separation efficiency for complex herbal- or polyherbal-based samples. At times, the software for densitometric analysis lacks automation for real-time greenness scoring (e.g. AGREE metrics), thereby leading to manual biases and reduced scalability in routine labs. Real-world applicability is obstructed by the biological sample's matrix effects, as generally green methods tend to show higher loss on drying (LODs) when compared with traditional ones, consequently limiting their use in trace analysis.

6.2 BAC limitations

From the perspective of BAC, various methodologies and strategies, such as the adoption of artificial intelligence (AI)-driven workflows, chemometrics and smart software solutions, are largely in experimental stages. There are not many globally followed standardised protocols, and inter-laboratory reproducibility is minimal owing to various factors such as differences in data formats, operator expertise and instrument calibration. Data scarcity further restricts the development of robust predictive models and digital twins.

6.3 WAC limitations

In terms of WAC, HPTLC is still largely operator-dependent. Therefore, harmonisation and automation are currently facing difficulties owing to variations in sample application, development and scanning. Moreover, sustainability evaluation frameworks (e.g. GAPI, AGREE, RGB models) often provide inconsistent or subjective outcomes, rarely covering full life-cycle impacts, energy consumption or waste management. Regulatory acceptance of green HPTLC methodologies remains limited, impeding their broader adoption in standard analytical laboratories.

6.4 Challenges in software assessment

Various recent software programs, such as winCATS or visionCATS (CAMAG), assess and calculate plate images but often tend not to integrate GAC tools for lifecycle assessment, accordingly leading to underestimation of energy usable for scanning. Validation as per ICH guidelines reveals robustness issues under various humidity conditions, common in non-climate-controlled settings. The need for AI-driven standardisation ideas emerged owing to various subjective baseline corrections in open-source tools, which directly impact the inter-laboratory reproducibility.

Considering these technological advancements, future developments in sustainable chromatographic techniques are expected to integrate GAC principles with intelligent analytical platforms.

7 Discussion for a proper integration approach

Structured workflow to integrate HPTLC into smart software under GAC–BAC–WAC principles, prioritising sustainability and automation:

- Adopt GAC, metrics first. Use tools such as Analytical Eco-Scale, AGREE and NEMI to score methods pre-integration, selecting ethanol-based RP-HPTLC over chloroform Normal Phase High Performance Thin Layer Chromatography (NP-HPTLC) for higher level greenness (e.g. scores > 80/100).
- Leverage a BAC for miniaturisation to employ benchtop HPTLC plates (e.g. 10 cm × 10 cm) with lower volumes of sorbents, interfacing through USB scanners to software such as ImageJ plugins for real-time data capture and waste reduction.
- Implement WAC for holistic whiteness to integrate RGB tools based on 'whiteness' assessment from current metrics into software dashboards, combining greenness solvents, blueness (automation) and economic viability. Use Python scripts for API links to cloud validators.
- Smart software pipelines are developed with hybrid solutions with ML-type models (e.g. through R or MATLAB) for peak deconvolution, auto-greenness calculation and predictive modelling of method failures. It is validated against ICH Q2(R2) for linearity ($R^2 > 0.99$).
- Real-world deployment condition pilot in quality control laboratories with API endpoints for laboratory information management systems (LIMS), ensuring < 1 mL solvent per run and digital twins for virtual optimisation. This approach aims to transform HPTLC from a static and basic tool into a smart, sustainable platform, consequently addressing various review gaps while promoting practical adoption of the methodology.

Collectively, these developments highlight the growing importance of sustainable analytical strategies in modern chromatographic science.

8 Future prospects and research gaps in green HPTLC: method validation of GAC, WAC and BAC

HPTLC, which is based on eco-solvent and low-waste philosophy of GAC, is challenged by critical bottlenecks such as manual optimisation, limited scalability, inconsistency in the validation of measures such as process mass intensity (PMI), life-cycle assessments (LCA), poor detection of trace analytes in complex samples (e.g. natural products) and the fragility of hyphenation with sustainable bioassays or non-toxic imaging to suit high-throughput requirements [72, 73]. The less-is-more paradigm of WAC reveals additional gaps in reagent-free protocols and energy-efficient workflows, whereas the automation focus of BAC demonstrates poorly developed real-time digital interfaces and operator-safety measures. Future directions are dependent on AI–machine learning (ML) fusion; random forest or neural network models trained on solvent-chromatographic data to predict

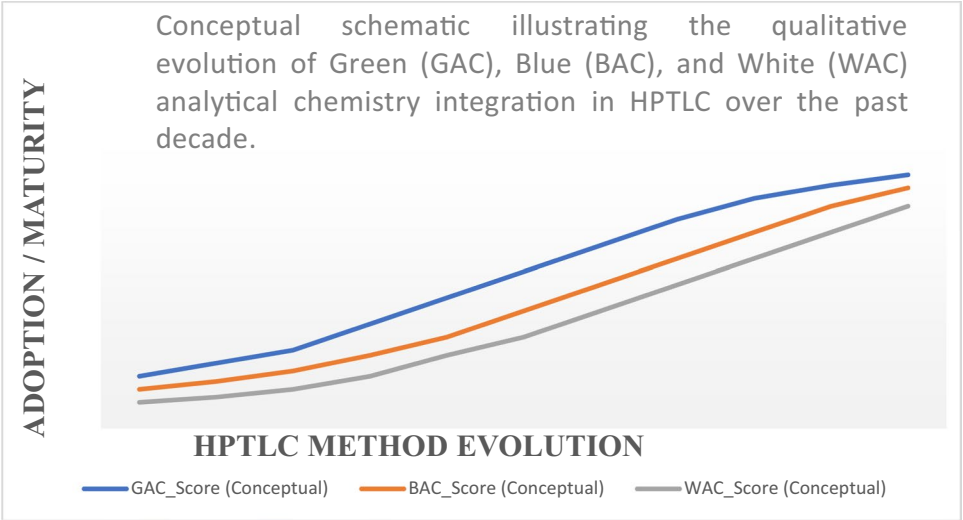


Fig. 2 Theory-based conceptual trend illustrating the progressive integration and relative maturity of GAC–BAC–WAC principles in HPTLC-based analytical methods. The curves represent a normalised, literature-derived adoption index and do not correspond to real-time or experimentally measured data

GAC-compliant mobile phase optimisation 70% faster [74]; convolutional neural network (CNN)-based plate imaging with a 95%+ accuracy with no stains [75]; and digital twins with simulated triadic GAC–WAC–BAC workflows including LCA-inculcated predictions [76, 77]. Recommendations prioritise emerging approaches under investigation, such as federated learning for global HPTLC datasets, blockchain-certified consumables with ML anomaly detection [78, 79] and solar-powered edge devices for site global framing, aiming for future targets of 90% hazardous waste reduction with the help of inter-laboratory pilots that holistically advance sustainable, efficient and safe analytical chemistry [80].

The conceptual model assumes progressive adoption of sustainability principles on the basis of the feasibility of implementation, methodological complexity and relevance to routine analytical practice. The best order to be followed is GAC > BAC > WAC [80, 81] (Fig. 2).

9 Conclusions

Each and every perspective that has been presented in this review aims to summarise the knowledge of the sustainable advancement of HPTLC through the synergistic integration of green–blue–white analytical chemistry. By prioritising eco-friendly solvents such as ethanol–water systems; miniaturised plates that reduce solvent use by 70–95%; and automated workflows supported by tools such as AGREE (≥ 0.80), Eco-Scale (> 75) and BAGI (> 30), HPTLC achieves exceptional resource efficiency without compromising ICH-validated performance ($R^2 \geq 0.999$, RSD < 2%). Eco-friendly sample preparation techniques such as QuEChERS, UAE and DLLME are applied to further decrease waste production (E-factor < 10) and enable robust applications in food authentication, herbal fingerprinting and pharmaceutical quality control. Despite challenges, such as resolution trade-offs and software limitations, various solutions such as AI-driven innovations, digital twins and federated learning promise up to 90% hazardous waste reduction and are being thoroughly experimented. The growing evidence supporting the applicability of GAC–BAC–WAC-guided HPTLC approaches is attributed to the integration of green analytical chemistry principles, decreased solvent consumption, enhanced environmental safety, cost-efficiency and definitive analytical performance without compromising the methodology's accuracy, precision, sensitivity and reproducibility. In this context, a holistic paradigm in analytical science safeguards the environment while protecting it, facilitating laboratories worldwide with the intention of achieving accurate, ethically responsible analyses, consequently strongly establishing the fact that sustainability is the highest standard of excellence.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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