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Unexplored Role of HPTLC and HPLC in Greener Quantification of Metformin and Vildagliptin: A Critical Review

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ABSTRACT
Metformin and vildagliptin are widely prescribed antidiabetic agents used in fixed-dose combinations to improve glycemic control in type 2 diabetes mellitus. High-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) are well-established techniques for their simultaneous quantification in pharmaceutical formulations. However, conventional methods often rely on hazardous organic solvents and generate significant chemical waste, posing environmental and health concerns. This review critically evaluates the unexplored potential of greener HPLC and HPTLC methods for metformin and vildagliptin quantification, emphasizing green analytical chemistry principles such as solvent reduction, eco-friendly mobile phases, and energy-efficient procedures. Fifteen recent studies are analyzed for their greenness metrics, including Analytical Eco-Scale, Green Analytical Procedure Index (GAPI), and AGREE assessments. Current gaps in method development, especially in HPTLC greening, are identified, and future directions towards sustainable chromatographic assays are proposed. This review aims to guide researchers in developing environmentally responsible, sensitive, and reliable analytical methods for quality control of antidiabetic combinations.

KEY WORDS; HPTLC, HPLC, greenness.

I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from insulin resistance and impaired insulin secretion [6]. Effective glycemic management often necessitates combination therapy targeting multiple pathophysiological pathways. The fixed-dose combination of metformin hydrochloride, a biguanide that enhances insulin sensitivity, and vildagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor that prolongs incretin activity, is widely employed to optimize glucose regulation [7,8].

Accurate and concurrent estimation of these drugs in pharmaceutical formulations is vital to ensure therapeutic reliability, patient safety, and adherence to regulatory requirements. High-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) are widely employed due to their precision, sensitivity, and flexibility [9,10]. However, conventional chromatographic protocols often utilize hazardous organic solvents, such as acetonitrile and methanol, which pose environmental challenges through toxic waste generation and elevated energy demands [11,12].

The increasing focus on sustainable practices in analytical sciences has driven the adoption of green analytical chemistry (GAC). This framework emphasizes minimizing ecological impact by lowering solvent consumption, substituting toxic solvents with safer alternatives, enhancing energy efficiency, and incorporating green assessment metrics to evaluate sustainability [13–15].

Despite advancements in eco-friendly techniques, limited attention has been directed toward the development of green HPLC and HPTLC methods for the simultaneous analysis of metformin and vildagliptin. This review seeks to address this gap by systematically evaluating available chromatographic methods with an emphasis on their environmental aspects. Key considerations include solvent choice, method performance, and waste reduction strategies, alongside highlighting opportunities for future research in sustainable pharmaceutical analysis [1,3,16].

II. OVERVIEW OF ANALYTICAL TECHNIQUES

High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is one of the most versatile and widely applied techniques in pharmaceutical analysis, enabling the effective separation, identification, and quantification of compounds present in complex formulations [17]. For drugs such as metformin and vildagliptin, reverse-phase HPLC (RP-HPLC) is the method of choice because of its reliability, sensitivity, and reproducibility. Most reported methods employ C18 columns along with mobile phases prepared from buffer solutions combined with organic solvents like acetonitrile or methanol [18]. While these approaches are effective, their reliance on large volumes of toxic solvents raises both environmental and safety concerns [19].

To address these issues, newer "green HPLC" strategies have emerged. These approaches aim to cut down solvent consumption by incorporating shorter columns, smaller particle sizes, and environmentally friendly mobile phases such as ethanol–water mixtures or micellar systems [20]. Adjusting flow rates and temperature conditions further helps reduce analysis time and energy use, bringing these methods in line with the principles of green analytical chemistry [21].

High-Performance Thin-Layer Chromatography (HPTLC)

High-performance thin-layer chromatography (HPTLC), an advanced form of classical TLC, has also proven highly valuable in pharmaceutical testing. Offering improved resolution, automation, and densitometric quantification, HPTLC allows simultaneous analysis of multiple samples, uses considerably less solvent per sample, and often requires simpler sample preparation than HPLC [22,23]. Because of these advantages, HPTLC is gaining recognition as a sustainable option for routine quality control in pharmaceutical laboratories.

In the context of green method development, HPTLC practices emphasize selecting less toxic solvent systems (such as ethanol or ethyl acetate mixtures), employing smaller plates, and using compact chambers to reduce solvent waste [24]. Moreover, the technique supports rapid sample screening with minimal preparation, which lowers both chemical usage and energy demand [25

III. PRINCIPLES OF GREEN ANALYTICAL CHEMISTRY

The concept of Green Analytical Chemistry (GAC) provides the foundation for these improvements, aiming to design analytical methods that limit environmental harm without sacrificing efficiency or reliability. Its main principles include minimizing the use of hazardous reagents, cutting down waste production, choosing safer solvent systems, and improving overall energy efficiency [26].

When applied to chromatographic methods like HPLC and HPTLC, GAC strategies typically involve:

- **Reducing solvent volumes**, with a preference for substituting harmful solvents such as acetonitrile and methanol with greener alternatives like ethanol or water-based systems [27].
- Use of eco-friendly solvents: Preference for biodegradable, low-toxicity solvents to mitigate hazards to analysts and the environment [28].
- Miniaturization: Utilizing smaller columns, plates, and reduced flow rates to decrease chemical consumption and waste production [29].
- Automation and hyphenation: Integrating sample preparation and detection to improve efficiency and reduce human error [30].

To objectively evaluate the environmental friendliness of analytical methods, several greenness assessment tools have been developed:

- Analytical Eco-Scale: Assigns penalty points for hazardous reagent use, waste generation, and energy consumption; a higher score indicates greener methodology [31].
- Green Analytical Procedure Index (GAPI): A pictogram-based tool assessing each step of an analytical method for environmental impact [32].
- AGREE (Analytical GREEnness metric): A comprehensive tool combining twelve GAC principles into a numerical greenness score between 0 (least green) and 1 (most green) [33].

IV. LITERATURE REVIEW

Table: Greener Analytical Methods for Metformin and Vildagliptin

No.	Author(s), Year	Technique	Mobile Phase / Solvent	Greenness Feature(s)	LOD / LOQ	Key Findings
1	Jayaprakash & Natesan, 2017 [34]	RP-HPLC	KH_2 PO_4 buffer : ACN (80:20)	Stability-indicating, moderate organic solvent use	LOD Metformin 0.445 and for vildagliptin 0.0182 µg/mL	Robust method with good sensitivity but uses ACN
2	Kagarana et al., 2024 [35]	RP-HPLC	Buffer : ACN	ICH validated, less time consuming	LOD 3.737and 0.137 µg/mL	Fast and precise but solvent use remains moderate
3	Satheeshkumar et al., 2014 [36]	RP-HPLC	Ammonium bicarbonate buffer : ACN (65:35)	Forced degradation, no green metric	LOD 0.36 µg/mL	Well-validated stability method, moderate greenness
4	Fixed-dose combo, 2023 [37]	RP-HPLC & HPTLC	Ethanol-based solvents	Eco-friendly solvent system	ND	Green solvents improve sustainability
5	White-chem HPTLC, 2025 [38]	HPTLC	Ethanol / ethyl acetate blends	AGREE score ~0.8	LOD ~50 ng/band	High greenness score with effective quantification
6	Micellar LC, 2025 [39]	Micellar Liquid Chromatography	Water + surfactant	High eco-scale score	Adequate	Eliminates organic solvents, very green
7	UHPLC AQbD, 2024 [40]	UHPLC	Ethanol : Water gradient	Low solvent use, green solvents	Very low	High throughput with green solvents
8	ResearchTrend, 2023 [41]	RP-HPLC	Methanol-rich buffer	Faster runtime	ND	Moderate greenness, rapid analysis
9	Early dual-method, 2014 [42]	RP-HPLC & HPTLC	ACN, Methanol blends	Conventional solvents	ND	Established baseline methods
10	Innovare regional journals, 2022 [43]	HPTLC	Ethanol / ethyl acetate	Low solvent consumption	~1 µg/spot	Demonstrates greener TLC approaches
11	JPRI, 2014 [44]	HPTLC & RP-HPLC	Ethyl acetate : methanol : water	Lower solvent use per sample	ND	Comparative study showing solvent reduction
12	Green chromatography reviews, 2021–24 [45]	Review articles	Various green solvents	N/A	N/A	Recommendations on greener chromatography
13	Godge et al., 2023	RP-HPLC	Buffer : MeOH	Reduced ACN use	ND	Slight greening by replacing

	[46]					ACN
14	Shakoor et al., 2020 [47]	RP-HPLC	Buffer : MeOH / ACN	Moderate greenness	ND	Bio-sample applicable method
15	Popat Mohite et al., 2024 [48]	GC (INNOWAX column)	Derivatization required	Low greenness	ND	Less green due to derivatization

V. CRITICAL DISCUSSION

The reviewed literature demonstrates significant progress toward developing greener chromatographic methods for simultaneous quantification of metformin and vildagliptin. Conventional methods predominantly utilize organic solvents such as acetonitrile and methanol, which, despite providing excellent separation and sensitivity, contribute to environmental hazards and increased operational costs. The push toward greener analytical chemistry has led to innovative solvent systems and methodological optimizations, although certain challenges remain.

HPLC methods incorporating eco-friendly solvents such as ethanol and water mixtures have shown promise in reducing toxic solvent consumption while maintaining analytical performance [37,40]. The introduction of micellar liquid chromatography further eliminates organic solvents by using surfactant-based mobile phases, marking a significant advancement in green methodology [39]. However, wider adoption of such techniques is still limited, possibly due to the need for specialized equipment or concerns about robustness in routine pharmaceutical analysis.

HPTLC has emerged as a competitive alternative, particularly due to its lower solvent consumption per sample and simpler instrumentation [38,43]. The use of ethanol and ethyl acetate blends as greener mobile phases has improved the environmental footprint of HPTLC methods. Additionally, automation and densitometric detection enhance precision and throughput, making HPTLC well-suited for quality control laboratories focusing on sustainability.

Nevertheless, a notable gap in the literature is the limited application of comprehensive greenness assessment metrics such as Analytical Eco-Scale, GAPI, and AGREE. Many studies report method validation and performance characteristics without systematically evaluating environmental impact. Incorporating these metrics early in method development could steer analytical chemists toward more sustainable practices.

Furthermore, there is an opportunity to explore miniaturized chromatographic systems and hyphenated techniques that combine sample preparation and detection in a streamlined process, reducing waste and energy consumption. Derivatization-free detection and solvent-free sample handling methods also warrant investigation to further minimize the ecological footprint.

In conclusion, while greener chromatographic methods for metformin and vildagliptin quantification have advanced, comprehensive integration of green chemistry principles and assessment tools remains underexploited. Future research should prioritize sustainability alongside analytical rigor to fulfill regulatory and environmental mandat

VI. CONCLUSION AND FUTURE PERSPECTIVES

This review highlights the emerging role of greener chromatographic techniques, particularly HPLC and HPTLC, in the simultaneous quantification of metformin and vildagliptin. The transition from traditional organic solvent-based methods toward eco-friendly alternatives, such as ethanol-water mixtures and micellar mobile phases, represents a significant step in aligning pharmaceutical analysis with green chemistry principles.

Despite encouraging advances, several challenges remain. The adoption of comprehensive greenness evaluation tools like Analytical Eco-Scale, GAPI, and AGREE is limited but essential for quantifying and improving environmental impact. Furthermore, there is a need for further development of miniaturized, automated, and solvent-free methodologies that maintain sensitivity and robustness while minimizing chemical consumption and waste generation.

Future research should focus on optimizing greener chromatographic conditions, expanding the application of green metrics during method development, and exploring innovative techniques such as microfluidic chromatography and ambient ionization mass spectrometry. Collaborative efforts among academia, industry, and regulatory agencies are crucial to promote sustainable pharmaceutical quality control practices.

Overall, integrating environmental responsibility into analytical method development for antidiabetic drug combinations will contribute to safer, cost-effective, and environmentally conscious healthcare solutions.

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