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Review Article

Eco-friendly HPLC Strategies for Pharmaceutical Analysis

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ABSTRACT

The pharmaceutical business has a significant challenge in the worldwide need to adapt processes to green analytical chemistry (GAC) standards. One of the most common procedures employed at different points in the pharmaceutical manufacturing process is high-performance liquid chromatography (HPLC), which results in massive amounts of organic hazardous waste. Therefore, it is important to incorporate GAC concepts into pharmaceutical research and analysis. Although there has been a rise in recent years in the number of papers detailing green chromatography techniques, they have not yet been widely adopted by the pharmaceutical industry. Reasons for this include analysts' doubts about whether or not they have met the method's requirements, a lack of time to make the necessary adaptations to their standard HPLC instruments, and a lack of relevant experience. This chapter provides a high-level review of green strategies for liquid chromatography (LC) that can be simply applied to common instruments for creating environmentally friendly HPLC approaches in pharmaceutical analysis. The goal is to motivate the analytical community within the pharmaceutical sector business to not only develop new green methods but also to transition the well-established traditional HPLC methods into environmentally green sustainable alternatives.

Keywords: Green analytical chemistry, RP-liquid chromatography, Micellar liquid chromatography, Green solvents.

INTRODUCTION

Green chemistry (GC) is the practice of developing chemical methods and goods that minimize or do away with the production and consumption of hazardous materials and byproducts. In 1998, Paul Anastasi and John Warner outlined 15 tenets upon which the GC idea rests.[1] The ACS/American Chemical Society website provides a comprehensive introduction to these concepts. In 2001, "GAC was coined.[2] The GAC is shorthand for "green analytical chemistry," which is the study of how to conduct chemical analyses in a way that uses less energy and resources while yet yielding accurate results. The 15 green chemistry principles described above were adapted to define the fundamental factors that establish analytical chemistry's green character and its role as GAC. Eliminating (or at least drastically reducing) the need for reagents in analytical operations, minimizing energy use, managing analytical waste responsibly, and enhancing operator safety are the four basic tenets of the GAC.[3] There has been a steady increase in the number of papers published in various domains of GAC (principles, spectroscopy, electrochemistry, and separation techniques) because scientists, researchers, and analysts all over the world have realized the need to implement the principles of GAC in analytical methods. Even the pharmaceutical industry must adapt its procedures to satisfy GAC standards. Researchers at Canada's McMaster University found in 2019 that, worldwide, the pharmaceutical business was responsible for more greenhouse gas emissions than the automobile manufacturing industry.[4,5] HPLC/High-performance liquid chromatography is a typical method used in the pharmaceutical business for pharma analysis. Pharmaceutical life cycle analysis (PK/PD/bioequivalence studies), API/excipient quality control, manufacturing process control, final product quality control, and stability studies, among other aspects, benefit from the use of HPLC. The chromatographic parameters, including accuracy, precision, robustness, and analysis runtime, are prioritized throughout the development and validation of the chromatographic techniques. However, other considerations, such as the effect of the chromatographic process on the safety of the analyst and the environment, remain underappreciated. This article provides a concise summary of the green strategies (and their advantages and disadvantages) that analysts can use to create environmentally friendly chromatographic techniques applicable to existing HPLC equipment right away. This review will aid in the widespread adoption of the imperative to switch from the currently prevalent traditional HPLC techniques in the pharmaceutical business to "green" chromatographic procedures.[6,7]

These strategies aim to reduce the environmental impact of the analysis without compromising the accuracy and reliability of results.

Eco-Friendly Approaches for HPLC

Solvent selection

One of the critical factors influencing the environmental impact of HPLC is the choice of solvents. Green solvents, such as water or mixtures of water with eco-friendly organic solvents like ethanol or acetonitrile, should be considered whenever possible. These solvents are biodegradable and possess a reduced environmental impact compared to traditional organic solvents.[8-11]

Recycling and reusing solvents

Implementing solvent recycling systems can significantly reduce solvent waste generation and decrease the overall environmental footprint of HPLC analyses. Whenever feasible, collected solvents should be purified and reused in subsequent analyses.

Miniaturization

The use of micro or nano-HPLC systems can reduce solvent consumption and waste generation while maintaining or even improving analytical performance. Miniaturization not only saves solvents but also decreases analysis time and improves energy efficiency.

Column selection

Choosing the appropriate HPLC column may result in improved separation efficiency and reduced analysis time, resulting in energy and solvent savings. Furthermore, columns with longer lifetimes can decrease the frequency of replacements and waste generation.

Green Detectors

Selecting environmentally friendly detectors can contribute to environmentally sustainable HPLC techniques. For instance, UV/vis detectors are commonly used and do not generate harmful waste, unlike some other detectors, such as mass spectrometers.

Green sample preparation

Optimizing sample preparation methods can decrease the amount of sample and reagents required for analysis. This can result in reduced waste generation and lower energy consumption.

Energy efficiency

HPLC instruments can consume significant amounts of energy. Using energy-efficient instruments, turning off equipment when not in use, and optimizing method parameters can help minimize overall energy consumption.

Green chemistry principles

Embrace the green chemistry principles in method development, such as minimizing waste, using safer reagents, and designing processes with higher atom economy.

Analytical quality assurance

Guaranteeing the precision and dependability of outcomes can prevent unnecessary re-analyses and, consequently, reduce resource consumption and waste generation.

Lifecycle assessment

Perform a lifecycle evaluation of the HPLC technique to identify areas of enhancement regarding ecological consequences. This assessment can help in making informed decisions to enhance the method's green credentials.

Figure 1 illustrates the various types of sustainable analytical methods, emphasizing environmentally friendly approaches that minimize solvent use, reduce waste, and enhance energy efficiency. These methods, which include green chromatography, miniaturized techniques, and solvent-free sample preparation, are increasingly important in modern analytical chemistry for promoting environmental sustainability while maintaining analytical performance.

Instruments for Assessing the Eco-Friendliness of Chromatographic Techniques

There are several tools and metrics available to evaluate the environmental sustainability of chromatographic techniques regarding their environmental impact. These tools help researchers and analysts assess the environmental sustainability of their analytical techniques and guide them in making greener choices. Here are some common tools for assessing the environmental sustainability of chromatographic techniques:[12-15]

GAPI / Green analytical procedure index

GAPI is a scoring system that assesses the greenness of an analytical method. It considers various factors such as solvent consumption, waste generation, energy consumption, and the use of hazardous materials. The GAPI score provides a numerical value that indicates the environmental impact pertaining to the analytical procedure, allowing for easy comparison with other methods.

Analytical eco-scale

The analytical eco-scale is a tool used to evaluate the ecological ramifications of analytical methodologies, including chromatographic techniques. It considers factors like the type and amount of solvents used, waste generated, and the use of energy. The eco-scale provides a rating ranging from E (most environmentally harmful) to A (most environmentally friendly) for the method being evaluated.

Environmental factor (EF)

The environmental factor is a parameter proposed by the United States Environmental Protection Agency (EPA) for assessing The

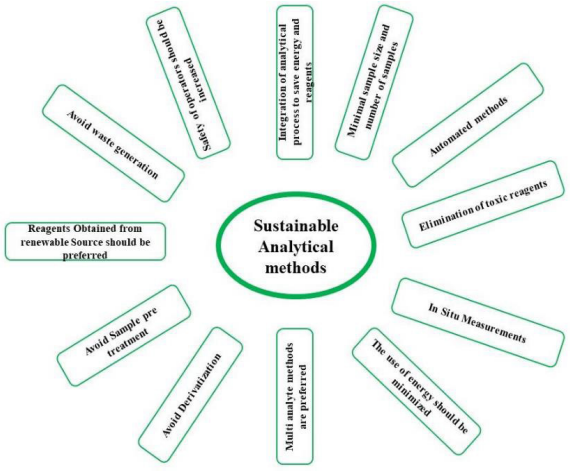

Figure 1: Types of sustainable analytical methods

Table 1: Mobile phase in using green solvents RP- HPLC[18-22]

Solvent	*Advantages*	*Challenges*	*Applications*
Ethanol (EtOH)	- Biodegradable and less toxic than ACN and MeOH - Lower vapour pressure (safer for inhalation) - Cost-effective alternative	- Higher viscosity increases column backpressure (can be mitigated by increasing temperature or reducing flow rate)	Pharmaceutical analysis, widely used as a green alternative
Propylene carbonate (PC)	- Derived from CO_2 (environmentally friendly) - High polarity, suitable for replacing ACN, DMF, and DMSO - UV cutoff at 210 nm (acceptable for UV detection)	- Poor miscibility with water (solved by mixing with ethanol or methanol)	Used in liquid chromatography as an organic modifier
Glycerol	- Non-volatile (safe for analysts) - Non-flammable and highly stable - Biodegradable and renewable - Higher UV cutoff (207 nm) and complete miscibility with water	- High viscosity requires sonication before use to ease mixing and reduce pump strain	Used in green LC techniques, pharmaceutical and biochemical analysis
Water	- Environmentally benign (no waste disposal concerns) - Can be used in high-temperature chromatography (SHWC/SCWC) - Low-cost alternative	- Requires thermally stable stationary phases for subcritical water chromatography	Used in subcritical and superheated water chromatography
Surfactants (Micellar Liquid Chromatography - MLC)	- Biodegradable and non-toxic - Can modify the stationary phase polarity for tailored separations -Reduces environmental impact of solvents	- Requires careful removal from the system after analysis to avoid contamination - Limited compatibility with some columns	Used in micellar liquid chromatography (MLC) for eco-friendly separations
Isopropanol (2-Propanol)	- Lower toxicity and environmental impact than ACN or MeOH - Good solubility for various analytes - Provides sharp peaks and low UV absorbance	- Some UV absorbance can interfere with UV detection - Requires compatibility testing with specific columns	Used in HPLC mobile phases, often mixed with water or ACN for enhanced separation
Acetone	- Easily available and biodegradable - High elution strength (reduces analysis time) - Lower toxicity than ACN and MeOH	- High UV cutoff (330 nm) limits UV detection - High viscosity increases column backpressure	Used in green chromatography where UV detection is not required
Ethyl acetate	- Biodegradable and less toxic than ACN and MeOH - Readily available and cost-effective - Useful for non-polar analytes	- High UV cutoff (260 nm) limits UV detection - Can cause high backpressure	Used in normal-phase chromatography and green alternatives to toxic solvents
Methanol (MeOH)	- Less toxic than acetonitrile - Biodegradable - Good solubility for a variety of analytes	- Toxic in high doses - Can be absorbed through the skin - Volatile (risk of inhalation exposure)	Used in pharmaceutical and environmental analysis
Butanol	- Low toxicity and biodegradable - Good miscibility with water - Cost-effective	- Higher viscosity may increase system pressure - Limited use in UV detection due to absorbance issues	Used in green chromatography for non-polar analytes
Tetrahydrofuran (THF)	- High elution strength - Useful for polymer and hydrophobic compounds - Miscible with water	- Peroxide formation risk (needs stabilizers) - Higher toxicity than some other green solvents	Used in pharmaceutical and polymer analysis

ecological consequences of analytical methodologies. It quantifies the amount of organic solvent used in the analysis. Lower EF values indicate greener methods with reduced solvent usage.

Green chromatography metrics

These metrics involve evaluating specific aspects of a chromatographic method that contribute to its greenness. Some common green chromatography metrics include solvent efficiency (amount of solvent used per analysis), sample throughput (number of samples analyzed per unit time), and column efficiency (resolution per unit analysis time).

Solvent sustainability metrics

These metrics focus on assessing the sustainability regarding the solvents employed in chromatographic methods. Parameters like the solvent greenness index (SGI) and the eco-scale for solvents provide a measure of the ecological consequences of solvents, encouraging the use of greener alternatives.

Life cycle assessment

Life cycle assessment (LCA) is a comprehensive tool used to evaluate the environmental impact of an entire chromatographic process throughout its lifecycle, including raw material production,

instrument manufacturing, analysis, and waste disposal. LCA provides a holistic view of the environmental footprint of the method.

Green star rating

The green star rating system, developed by the Analytical Chemistry Division of the ACS, is used to assess the sustainability of green analytical methodologies. It considers factors like waste generation, solvent usage, and energy consumption and assigns a star rating from one (least green) to five (most green).

These tools can be utilized individually or in combination to comprehensively evaluate the sustainability of chromatographic techniques. By employing these assessment techniques, researchers and analysts can make informed decisions to develop and optimize eco-friendly and sustainable analytical methods.

Green Solvents as a Mobile Phase

A wide variety of organic solvents are needed for use in the mobile phase of the chromatographic technique known as reversed-phase high-performance liquid chromatography (RP-HPLC). It is common knowledge that the most often used organic solvents in the mobile phase of RP-HPLC are acetonitrile (ACN) and methanol (MeOH). Both the short-term and long-term exposure to these solvents are dangerous. Retinal damage and severe acidosis can be brought on by methanol exposure.[16] Inhalation of fumes alternatively interacts with skin and eyeballs, causing the toxicity of acetonitrile. In living organisms, acetonitrile is converted to cyanide, which then causes cytotoxic hypoxia. The ACN's market pricing is also a problem. As a byproduct of acrylonitrile, ACN was in short supply due to a drop in production following a decline in acrylonitrile demand in 2008. As a result, following the ACN crisis, the cost of acetonitrile skyrocketed. It is clear that using these mobile phase solvents increases costs for analytical laboratories and poses risks concerning the well-being of the analysts. Furthermore, the vast quantity of chemical waste means that the detrimental effects of these solvents ecological effect cannot be ignored. HPLC instruments produce about 34 million liters of chemical waste annually. In light of these findings, it is clear that the elimination of these hazardous solvents from the RP-HPLC mobile phases and the substitution of greener alternatives play a crucial role in the creation of environmentally friendly HPLC techniques. It is equally essential to think about the solvents that are utilized in the various stages of sample preparation for a single HPLC technique. In order to get symmetrical chromatographic peaks, it is common knowledge that the solvents used in sample preparation should have identical polarity as the mobile phase. This means that the solvent used in sample preparation should have a composition that is consistent with the mobile phase. In light of the foregoing, it's clear that an ecological evaluation of the HPLC technique must consider the waste produced throughout the entire procedure. In the mobile phase, several organic solvents are utilized as environmentally friendly substitutes for traditional organic eluents. These include 2-propanol, glycerol, ethanol (EtOH), acetone, ethyl acetate, and propylene carbonate (PC). Even while there are many green organic alternatives to standard HPLC techniques, not all of them are created equal in terms of their benefits and the ease with which they can be transferred. As their UV cutoffs are so high, acetone and ethyl acetate at wavelengths of 330 and 260 nm, respectively, are largely incompatible with UV detectors. Also problematic is the increased column back pressure caused by their high viscosity. Green organic solvents, like propylene carbonate, glycerol, and ethanol, are explored in this chapter because of their low cost and high chromatographic performance.[17] The selection and characteristics of these green solvents used in RP-HPLC mobile phases are summarized in Table 1.[18-22]

Ethanol

The benefits of using ethanol as a sustainable solvent in RP-HPLC mobile phases have been discussed in detail by a number of review articles. Snyder places ethanol with methanol in his table of organic solvents, however, this solvent can also stand in for acetonitrile. There are a few reasons why ethanol is preferable to these other solvents. Because of its lower vapor pressure, ethanol poses less of a threat to human health when inhaled. Long-term use of ethanol, rather than its usage as a reagent, is what causes its harmful effects. When in comparison to acetonitrile and methanol, ethanol has a smaller detrimental influence on the environment because it is biodegradable.[14] Ethanol has greater eluotropic strength than acetonitrile or methanol; hence, less of it is required to accomplish the same retention period in an analysis. UV cutoff of about 210 nm is satisfactory.[23] The biggest negative is that the ethanol-water mobile phase has a higher viscosity, which increases column backpressure. Our tests have shown that increasing the column temperature to 35 or 40°C or decreasing the flow rate significantly alleviates this backpressure. The decreased flow rate won't be a factor problem but rather an advantage of the green technique, given the principles of GAP for decreasing reagent usage. Ethanol has a cheaper technique cost than acetonitrile or methanol since its market price is lower. Since ethanol is non-toxic, it also saves money on waste management. This helps with the overarching goal of decreasing pharmaceutical research costs. Because of these factors, ethanol has surpassed acetonitrile and methanol as the most popular environmentally friendly alternative. Green RP-HPLC mobile phases based on ethanol have been used extensively in the pharmaceutical industry.[24]

Propylene carbonate

The chemical compound known as propylene carbonate is generated from carbon dioxide and is a member of the family of cyclic carbonated solvents. This solvent, which is an aprotic solvent but has a high level of polarity and is environmentally friendly, can be utilized as a substitute for harmful aprotic polar solvents, including acetonitrile, dimethylformamide, and dimethyl sulfoxide. As 2011 was the year that PC was utilized for the very first use as an organic modifier in the liquid chromatography mobile phase.[25] Since that time, there have only been a select few articles published on the subject of utilising this environmentally friendly solvent in the LC mobile phase.[26] According to the findings of the investigations, the problem of PC's inadequate miscibility with water can be remedied through the utilization of a mixture of PC with either methanol or ethanol. Ethanol ought to be regarded as a tertiary solvent in the PC/water mobile phase when the GAC idea is taken into consideration. The viscosity of the mixture of PC, EtOH, and water is satisfactory. The PC possesses a UV cutoff at 210 nm, which is an acceptable value.[27,28]

Glycerol

Green chromatographic techniques have lately incorporated the organic solvent glycerol. In 2021, four antiviral drugs were separated using glycerol and reversed-phase chromatography. For ascorbic acid and glutathione determination in tablets, this group of authors employed this solvent as a modifier of a mobile phase in the same article.[29] This solvent is well-suited for the research and improvement of green LC techniques because of several properties. To begin, glycerol poses less of a threat to the analyst's health than other volatile solvents because it does not evaporate easily. Glycerol's low flammability and good stability under typical laboratory storage conditions is a benefit. It's beneficial for the environment because it decomposes quickly and comes from low-cost, renewable resources.[30] Glycerol is preferable to ethanol and PC from an analytical perspective due to its higher UV cutoff and higher miscibility with water. In particular, glycerol's UV cutoff of 207 nm is slightly lower than that of ethanol, and unlike PC, it is entirely miscible with water. Although glycerol's increased viscosity has certain potential drawbacks, it also has some advantages. Because of its greater viscosity, glycerol is best sonicated after being pre-mixed with the water phase. This will ease the mixing process and lessen the strain on the LC system's pumps. Glycerol's elution strength in RP-HPLC falls between that of water and that of the stronger eluents acetonitrile and methanol. Because of this quality, glycerol can be used by analysts to fine-tune the mobile phase's elution strength and improve selectivity.[31]

Water

No other change can be made to the chromatographic analysis that would have a smaller impact on the environment than using water as the LC mobile phase. The analytical community has been aware of this concept for over 30 years, but it has just gained traction in the last decade. Water may serve as an LC mobile phase in two different ways. The first method involves using high temperatures in combination with stable stationary phases. Temperatures exceeding 100℃ or water heated below the critical point (374℃ and 218 atmospheres) must be considered. This chromatography technique has been referred to in the literature as subcritical water chromatography (SCWC) or superheated water chromatography (SHWC).[32,33] The properties of subcritical water are distinct from those of water at room temperature. Specifically, the water's dielectric constant, surface tension, and viscosity all drop as the temperature rises. Since its polarity has decreased, water now acts more like organic solvents in the chromatographic system. Thus, non-polar molecules are eluted at higher water temperatures, while polar compounds are eluted at lower temperatures.[34] Pure water in subcritical conditions requires the employment of thermally robust stationary phases that can resist temperatures beyond 200°C, which is beyond the temperature stability of classical RP columns. Employing a mixed-mode polar embedded column with water as the mobile phase, four amino acids (hydroxyproline, proline, glycine, and alanine) were analyzed. Long alkyl chains with ion-exchange functional groups connected at their termini make up the stationary phase employed in this application, giving the column its reverse phase and ion-exchange characteristics.[35]

Surfactants as a Mobile Phase

Amphiphilic molecules have bifunctional ends—hydrophilic (polar) and hydrophobic (non-polar)—that allow them to reduce surface tension, making them surfactants. At low levels in a dispersive environment, they are present as monomers. Upon rising concentration, they form micelles when the critical micelle concentration (CMC) has been attained. While micelles would ideally be present at some discrete monomer concentration, in reality, they occur over a small range of concentrations. Surfactants are useful in analytical chemistry, especially as environmentally friendly eluents in mobile phases.[36] Micellar liquid chromatography (MLC) utilizes surfactants in the mobile phase and is thus an environmentally friendly alternative to RP-HPLC because of its non-toxic, biodegradable nature and low environmental footprint.[37,38] Similar to conventional reversed-phase chromatography, MLC utilizes non-polar stationary phases (e.g., C18, C8). Surfactants, added in excess of their CMC, exist as micelles and monomers in the aqueous mobile phase. These also adsorb on the stationary phase, and their hydrophobic tails anchor on the surface while their hydrophilic heads point toward the mobile phase in an open micelle-like arrangement.[39,40] This configuration alters the polarity and charge of the stationary phase based on the surfactant type (anionic, cationic, or nonionic). Separation is achieved as analytes partition between the stationary phase, micellar pseudophase, and bulk solvent, allowing varied retention mechanisms—hydrophobic, ionic, and steric—based on analyte characteristics. Following analysis, extensive rinsing with water is necessary to eliminate surfactant residues, followed by column reconditioning according to manufacturer recommendations.[41,42]

Isopropanol

In high-performance liquid chromatography, isopropanol (or isopropyl alcohol) can function as a mobile phase in HPLC. In relation to other organic solvents, it has a lower toxicity and less environmental impact, making it a viable option for use in high-performance liquid chromatography. It is common practice to mix isopropanol with other solvents, such as water or acetonitrile, to create mobile phase compositions that are amenable to various analytical separations when employing HPLC. The analytes being tested, together with the stationary phase of the chromatographic column, will dictate the mobile phase's chemical makeup.[43-45]

Isopropanol offers several advantages as a mobile phase in HPLC.[46,47]

- *Solubility*

Isopropanol has good solubility for a diverse array of analytes, rendering it appropriate for the separation of various compounds.

- *Volatility*

Isopropanol is relatively volatile, allowing for efficient evaporation during the chromatographic process, which contributes to shorter analysis times.

- *Peak shape*

Isopropanol can provide excellent peak shapes, leading to sharp and well-defined chromatographic peaks.

- *Low UV absorbance*

Isopropanol exhibits low UV absorbance at typical HPLC wavelengths, which is advantageous for UV detection applications.

- *Green solvent*

Isopropanol is considered a green solvent due to its biodegradability and reduced toxicity compared to some other organic solvents used in HPLC.

Despite its benefits, there are some considerations when using isopropanol as a mobile phase inside HPLC: Because isopropanol absorbs UV light at some wavelengths, it can affect the accuracy of UV detectors' baselines. Baseline adjustments and method optimization can assist in reducing the severity of the problem. It's possible that some chromatographic columns and types of analytes won't work with isopropanol.[47,48] Separations can only go smoothly if compatibility testing is performed first. Separation efficiencies and selectivities for target analytes can be maximized, as with any mobile phase, through technique development and optimization. For the HPLC mobile phase, isopropanol can be a practical and environmentally friendly choice. It is essential to take into account the chromatographic circumstances, analyte qualities, and column compatibility when choosing a solvent if you want to get accurate and trustworthy chromatographic findings.[49-52]

CONCLUSION

This chapter demonstrates how ordinary LC equipment can be used to effectively implement the green techniques necessary for the advancement of environmentally sustainable HPLC procedures in pharmaceutical analysis. The most popular eco-friendly organic solvent, ethanol, improves method performances (lower runtime, lower limits of detection, lower limits of quantitation, etc.) compared to traditional organic solvents (acetonitrile and methanol). The findings (in terms of the technique accuracy and precision) achieved using ethanol-based techniques were shown to be statistically indistinguishable from those obtained with the methodologies grounded in the standard LC mobile phase in many of the published studies. While propylene carbonate is not yet widely used as an eco-friendly green substitute, it has been proven in recent articles that analysts may want to regard this solvent as a viable option to acetonitrile. The elution potency of glycerol, the most recently utilized eco-friendly substitute for traditional organic solvents, falls somewhere between that of water and that of methanol/acetonitrile. Micellar liquid chromatography (MLC) techniques utilizing a surfactant mobile phase have been shown in numerous publications to be capable of separating chemicals of varying degrees of polarity, making them a perfect fit for the GAC criterion. The method development procedure is the primary difficulty of this green chromatography. When developing an MLC technique, using the DoE strategy helps optimize the key chromatographic parameters for greater efficiency. The GAC concept is being implemented in the pharmaceutical industry's research and development and quality control laboratories because eco-friendly procedures not only have environmental and financial benefits but also have higher method performances. Analysts, the pharmaceutical business, and the local society as a whole stand to gain from the adoption of eco-friendly practices in the analytical phase of drug development and testing (minimized adverse environmental effects).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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