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Green Analytical Chemistry to Eco-Friendly HPLC Techniques in Pharmaceutical Analysis: A Review

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GREEN Chemistry (GC) is designing chemical processes and products to decrease the use of harmful materials, based on 12 theories established in 1998. The pharmaceutical industry faces a significant problem due to the worldwide requirement to comply with Green Analytical Chemistry (GAC) requirements. High-pressure liquid chromatography (HPLC) is a widely used process in the medicine industry, but it produces massive quantities of organic hazardous waste. Analytical techniques must be optimized for precision, sensitivity, repeatability, effortlessness, cost effectiveness, versatility, and speed. Though, other factors such as analysts' security and ecological effects are rarely considered. Although there are more publications about green chromatography techniques, there has not been much adoption of eco-friendly HPLC techniques in the pharmaceutical business. The major causes of this are the need for adapting the standard HPLC apparatus, a lack of time, knowledge, or analysts' confusion over the fulfilment of the method requirements. An overview of green approaches for liquid chromatography (LC) that may be quickly applied to traditional instruments was provided to create eco-friendly HPLC procedures for pharmaceutical analysis.

Keywords: Green Analytical Chemistry, HPLC, Pharmaceutical analysis.

Introduction

Green Chemistry (GC) is designing chemical processes and products to decrease the use of harmful materials, based on 12 theories established in 1998 by Anastas and Warner [1]. On the website of the American Chemical Society (ACS), there is a thorough summary of these concepts [2]. In 2001, "green analytical chemistry" (GAC) became a recognized term [3].

Analytical techniques must be optimized for precision, sensitivity, repeatability, effortlessness, cost effectiveness, versatility, and speed. Though, other factors such as analysts' security and ecological effects are rarely considered. This paradoxical scenario occurred in the 1990s, where the analytic chemicals used were even more hazardous than the species being identified. Using hazardous chemicals and reagents in environmental analysis have become unsustainable due to public concern on environmental matters. To prevent contaminating water and discharging with urban garbage, laboratories have moved to manage wastes and collect leftovers. However, there is a significant issue that makes managing harmful leftovers challenging: the sheer volume of them [4].

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nanomaterials will affect in vivo and in vitro biomedical applications [3].

The GAC aims to develop new analytical methodologies to lower dangerous chemicals as well as chemical waste while enabling faster and more energy-effective chemical analysis. The four primary subjects that make up the essential characteristics defining analysis chemistry's green aspect were created by modifying the 12 principles of ecological chemistry; Reagent consumption should be reduced, energy consumption should be minimized, waste should be effectively managed, and safety should be increased for operators [2]. GAC principles were recognized by scientists, researchers, and analysts, leading to an increase in papers published in various GAC fields (fundamentals, spectroscopy, electrochemistry, and separation methods) [5]. The pharmaceutical industry is facing a global need to change activities to meet GAC measures, as a study exposed by McMaster University in 2019 showed that it emits more greenhouse gases than the automotive production sector [6]. The pharmaceutical industry places various emphases on pharma analysis, and HPLC is one of the most often utilized methods. It is utilized across the whole lifespan of a drug, including Pharmacokinetic, pharmacodynamic, and bioequivalent studies, as well as manufacturing process, finished product, stability, and quality control of active pharmaceutical ingredients. Developing and authentication of chromatographic techniques focus on accuracy, precision, robustness, analysis runtime, but other aspects such as the impact on the analyst's security and the environment are not considered.

Green Analytical Chemistry (GAC) is an effort to find workable solutions to off-line waste management in order to replace dirty practices with hygienic ones [6].

Green strategies are available for analysts to develop environmental-friendly chromatographic techniques that can apply to traditional HPLC tools. This review article helped to promote the need for transforming existing HPLC methods into "green" methods in the pharmaceutical industry.

Evaluation tools of the greenness chromatographic methods

The pharmaceutical industry's development plan includes the incorporation of green analytical procedure (GAP) principles into the creation of green chromatographic methods and the conversion of traditional HPLC methods into environmentally friendly alternatives. To assess how green the methods are, an analyst should evaluate the suggested method's environmental friendliness and contrast it with the ones currently in use. This evaluation provides numerical information on how well each of the method's components adheres to the GAC principles, which can be used to develop the approach further in terms of important method parameters.

Over time, chromatographic methods have become better in applying the GAC principles. The National Environment Methods Indexing and the analytical eco-scale are two instruments used to assess the greenness of the methods and their adherence to the GAC principles. The most popular instruments for assessing the greenness of the Chromatographic approach are briefly described in this section.

The National Environment Methods Index (NEMI) is also one of the earliest instruments for evaluating how green an approach is [7]. With this procedure, the technique is assessed in four separate categories: corrosive, hazard, PBT, and waste. A method's greenness profile is displayed as a straightforward pictogram with 4 components. If the phrase satisfies the requirements, the color of each segment will be green; otherwise, it will be black. However, as one of the GAC principles, energy usage is not considered by the NEMI index. Furthermore, the creation of the symbols takes longer when non-standard chemicals are utilized.

An analytical eco-scale indicator, a quantified tool, had been introduced in 2012 [8]. This index assigns 100 points to a perfectly green method, while a lower number indicates a greater deviation from the principles of GAC and inferior greenness. By deducting the penalty points from 100, which are computed on various criteria, calculations can be made for the analytical eco-scale points. For instance, the quantity and type of reagents utilized determine the penalty points for the reagents. The type of instrumentation utilized affects the penalty points allotted for energy use. Titration, for instance, is the method that uses smallest energy, but gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), the analytical eco-scale points are calculated by calculating the total penalized points for the various steps in the process. A score above 75 points indicates an excellent green method, a score between 75 and 50 indicates an acceptable green method, and a score below 50 indicates inadequate green analysis. (LC-MS) are the procedures that use the most energy and carry larger penalty points. The analytical eco-scale index is a quantified tool that is used to compare greenness of methodologies and calculate penalty points to assess nonconformity with GAC principles. Scientists widely accept it.

The green analytical procedure index (GAPI) is a tool that makes it possible to assess how environmentally friendly the entire analytical process is, from the collection of the sample to the result. It is presented in terms of five pentagrams, which represent the different segments of the analytical procedure and can be colored green, yellow, or red

depending on their environmental impact. The first pentagram refers to the sample, which is subdivided into four parts, the type of method, the sample preparation, the reagents, and the instrumentation. The GAPI index is incredibly thorough and may be used as both a qualitative and a quantitative instrument for the method of evaluating greenness. The GAPI tool not only offers the user/reader an immediately apparent perspective but also in-depth details on analyzed operations [7].

The Analytical Method Greenness Score (AMGS) metric is a free, online calculator to supercritical fluid chromatography (SFC) and liquid chromatography (LC). Hicks *et al.,* [9] developed this programme to evaluate the solvents' effects on the environment, human health, and safety (EHS), as well as the energy requirements of the instruments. When users submit details about the approach, the score is automatically determined, and the 3 basic categories (solvent energy, instrument energy, and solvent safety) may be painted red, yellow, or green depending upon its contribution score. However, when there are more than three components utilized in the mobile phase, it is not applicable.

In 2020, the analytical greenness (AGREE) metrics method and software have been posted [10]. The approach evaluates 12 GAC principles independently, transforming them into a 0-1 score within the metric system. The individual scores for each GAC principle were combined to create the AGREE score, which is displayed as a symbol. The outer portion of the pictogram shows 12 segments in a color scale (red-yellow-green) representing the method's success in relation to the 12 variables. Because it takes into account each of the 12 principles, the AGREE technique is a thorough tool for the greenness methodology assessment. It is also a straightforward instrument, facilitating simple findings explanation. The AGREE measures for the sample preparation were used in a lesson written by the same group of authors.

Green solvents as a mobile phase

A substantial number of organic solvents are needed as eluents at mobile phase of the Reversed Phase-High Pressure Liquid Chromatography (RP-HPLC) process.

Methanolic acid (MeOH) and acetronitrile cluster (ACN) are the most used chemical solvents in the RP-HPLC mobile phase. Methanolic acid is a poisonous alcohol that can result in optic nerve damage and disorders [11], while acetronitrile cluster toxicity manifests through contact with eyes and skin or inhaled [12]. ACN is a byproduct of acrylonitrile, which leads to reduced manufacturing and ACN shortage [13]. The use of acetonitrile in the mobile phase raises health issues and increases expenses for analytical labs. It also has a negative ecological impact due to the massive quantity of toxic waste produced annually by HPLC devices.

The development of green HPLC techniques depends on replacing hazardous solvents in RP-HPLC mobile phases with more environmental alternatives. Therefore, when conducting an environmental evaluation, HPLC waste ought to be considered.

Organic solvents are utilized as environmentally friendly replacements for traditional organic solvent in the mobile phase, including ethanol (EtOH), acetone, ethyl acetate, glycerol, 2-propanol, and propylene carbonate (PC), nevertheless not all of them have the same rewards. Due to their high viscosity and high UV cutoffs, acetone and ethyl acetate have the disadvantage of being incompatible with UV detectors, which increases column back pressure [14].

Green organic solvents with high chromatographic performance that are inexpensive include EtOH, PCs, and glycerol.

In RP-HPLC mobile phases, ethanol can be utilised as an environmentally friendly solvent instead of ACN [15, 16]. It belongs to the same group as methanol and has several advantages over these two solvents [17]. Because EtOH has a lower pressure of vapour, it has less harmful side effects when inhaled, and is biodegradable, with less adverse environmental influence than ACN and MeOH. Since EtOH has a higher eluotropic strength, less of it is required to obtain a similar retention time. The UV cutoff is acceptable, but higher column temperatures or reduced flow rates can be used to overcome the column backpressure. GAP principles can be used to reduce reagent consumption. Ethanol is cheaper than acetonitrile and methanol, resulting in minimal technique expenses and waste removal fees, leading to a reduction in expenses in pharmaceutical analysis. EtOH is the most favored green alternate for ACN and MeOH and is used in RP-HPLC in drug analysis. Yabré *et al.* [14] reviewed pharmaceutical applications.

Propylene carbonate is a high-polarity green aprotic reagent that can take the role of harmful aprotic polar reagents [18, 19]. PC was employed for the 1st time as an organic modulator in 2011 [20]. Utilization of it in the chromatographic mobile phase has been limited [21- 23]. The investigation showed that a mix of PC with ethanol or MeOH could solve the limited miscibility of PC with water, with acceptable viscosity and UV cutoff of 210 nm. PC can be used to separate 39 medicinal substances mixture using two-dimensional liquid chromatography (2D LC). Despite having a substantially shorter duration, the study indicated that PC's effective peak distribution and peak capacity as an organic modifier were equivalent to those of ACN and MeOH (52 minutes for ACN and MeOH against

32 minutes for PC) [24]. PC Propylene carbonate is an effective substitute for acetonitrile, providing greater separation power in 2D LC, opening the possibility for wider applications.

Glycerol is an organic solvent used in green chromatographic methods to separate antiviral medicines and as a mobile phase changer for the quantification of vitamin C and L-glutathione in pills [25]. Glycerol is a nonvolatilizable safe diluent with low flammability and high stability, making it suitable for eco-friendly LC methods. It is also accessible from sources that are renewable and decomposable [26, 27]. Glycerol's higher viscosity can be beneficial, as it can be premixed with water and sonicated to reduce pump load and facilitate the mixing process. Higher column temperature can reduce column back pressure and increase chromatographic performance. A glycerol-based mobile phase with a higher viscosity will have less longitudinal diffusion and eddy diffusion, resulting in better chromatographic performance. Elution power of glycerol is in the medium among water and ACN and MeOH.

Pure water as a mobile phase

Pure water is an eco-friendly option for LC mobile phase. It is the greenest solvent of all [28].

The pure water usage as an LC elution solvent is made possible by two methods. The 1st one is to elevate heats alongside stable stationary phases that can be used to custom pure water as the LC elution solvent **[29]**. This type of chromatography is named superheated water chromatography (SHWC) or subcritical water chromatography (SCWC) [30]. When compared to water at room temperature, subcritical water has various characteristics, such as a lower dielectric constant, surface tension, and viscosity [31]**.** This calls for the use of stationary phases that are heat stable and capable of withstanding temperatures exceeding 200 °C. The requirement for adapting traditional HPLC apparatus, such as unique column heaters and detectors other than UV and Vis, is relevant to the use of SHWC. This method hasn't been used much in drug analysis yet [31].

The 2nd one is predicated on using clean water in settings of room temperature (lower than 60°C) [32]. If silica-based stationary phases are used, refer to the process as per aqueous liquid chromatography (PALC) [33]. PALC is recognized from hydrophilic interaction liquid chromatography (HILIC) because of the extreme participation of siloxane groups. Water-only reversed-phase liquid chromatography (WRP-LC), a variation of PALC, utilizes polar-embedded or polar-end capped stationary phases rather than silica-based columns.

The stationary phase (LC-column) type determines the selectivity of a mobile phase when the composition is only one of two options (high-water content or pure water). Various polar-embedded and polar-end capped stationary phases have entered the market over the past few years. These phases have different solvation properties, resulting in differences in selectivity. Purified water was employed as the mobile phase in a mixed-mode polar embedded column for the separation of polar compounds (nucleosides, nucleic bases, and purine alkaloids). The long alkyl chains that make up this stationary phase's reverse phase and the ion-exchange functional groups connected to the chain's terminal end are what give this column its ion-exchange and reversed phase properties [34]. This type of stationary phase could expand the options for component separation with various polarities, making WRP-LC a more appealing strategy for the development of green chromatographic methods.

Surfactants as a mobile phase

Surfactants are two-sided compounds that decrease the tension on their surface. When present in low concentrations, they form micelles. There are several uses for surfactants in the field of analytical chemistry, such as micellar liquid chromatography (MLC). MLC is non-hazardous, ecological, and has components with low environmental biological concentration, making it an efficient approach for greening HPLC techniques. At a specific monomer quantity, micelles develop, known as the critical micellar concentration (CMC) [35].

MLC is a reversed-phase chromatography using nonpolar stationary phases and an aqueous solution of surfactants [35]. The mobile phase forms micelles and surfactant monomers, which modify the surface of the stationary phase. Based on how the analytes partition between the modified stationary phase, pseudo phase, and bulk solvent, the analytes are separated [36, 37].

The anionic sodium dodecyl sulphate (SDS), the cationic cetyl trimethyl ammonium bromide (CTAB), and the nonionic polyoxyethylene-23-lauryl ether (Brij-35) are the surfactants most frequently employed as eluents in MLC. The mobile phase's acceptable viscosity is made possible by these surfactants' low CMC. Stationary phase temperature, surfactant concentration and type, and co-eluents are the three most crucial chromatographic factors. To increase separation effectiveness and shorten the retention period, the working column temperature should be higher than the Kraft point [38- 40].

Surfactant concentration has a reverse influence on retention time, but Brij-35 quantity and retention period are positively correlated because phenolic chemicals and Brij-35's hydroxyl groups form a hydrogen bond [41].

In the last few years, In the mobile phases, more polar surfactants, including Brij-35, have taken the

role of organic alcohols. This better MLC is known as micellar liquid chromatography (MLC) in mixed mode. Brij-35 shortens the amount of time polar compounds are kept in solution by reducing the polarity of the stationary phase. Brij-35 lowers the negative charge that SDS, an anionic surfactant, leaves on the stationary phase's surface, keeping the column at a neutral charge [41].

By altering the level of either the organic solvent or the surfactant, the gradient mode of elution in MLC is feasible. This lengthens the amount of time required for the column's re-equilibration as well as for analysis. However, as more micelles are formed as a result of the higher monomer concentration, the monomer concentration stays constant and remains close to the CMC value [42]. This mode can be overridden by adding an organic solvent or a more polar surfactant, however it is not appropriate for nonpolar hydrophobic substances.

It is crucial for analysts to offer details regarding the surfactant mobile phase flushing, column conditioning, and column maintenance. Studies of the literature can be used to find comprehensive directions for maintaining the chromatographic system in MLC [43]. To get rid of any remaining organic solvent, the reversed-phase column must first be flushed with 100% water for at least 30 column volumes. The system should then be switched over to the mobile phase based on surfactants. The pumps should run continuously throughout the night and the flow rate should be adjusted to the minimum (for example, 100 µL/min) to avert column congestion or system blockage. The HPLC system and the column of chromatography must be cleaned gradually up to 100% water once the analysis is finished to eliminate the surfactant. The column should then be restored in accordance with the manufacturer's recommendations for conditioning the column [44].

Conclusions

Labs are increasingly adopting green approaches for environmentally friendly HPLC procedures in pharmaceutical examination. Ethanol is the most widely used green organic diluent, offering better method performances than conventional organic solvents. Propylene carbonate, glycerol, and pure water are also eco-friendly solvents for mobile phase LC. Modern materials have increased the pertinence of WPR-LC and PALC as environmentally friendly HPCL methods. MLC techniques based on surfactant mobile phases satisfy GAC criteria but are challenging to develop. Environmentally friendly HPLC techniques outperform traditional methods in terms of energy use, operator safety, and ecological effect. They also offer higher technique performance, attracting the pharma business to adopt the Green Analytical Chemistry (GAC) approach. The use of green practices in drug analysis benefits analysts, the pharma sector, and the micro community.

Conflict of interest

There are no declared conflicts of interest. The corresponding author is ready to provide any detailed data upon request.

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الكيمياء التحليلية الخضراء إلى تقنيات HPLC الصديقة للبيئة في التحليل الدوائي: مراجعة

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تقوم الكيمياء الخضراء (GC) بتصميم العمليات والمنتجات الكيميائية لتقليل استخدام المواد الضارة، بناءً على 12 نظرية تم وضعها في عام 1998. تواجه صناعة الأدوية مشكلة كبيرة بسبب المتطلبات العالمية للامتثال لمتطلبات الكيمياء التحليلية الخضراء (GAC). تعد عملية الفصل اللوني للسوائل عالية الضغط (HPLC) عملية مستخدمة على نطاق واسع في صناعة الأدوية، ولكنها تنتج كميات هائلة من النفايات العضوية الخطرة. يجب تحسين التقنيات التحليلية من حيث الدقة والحساسية والتكرار والسهولة والفعالية من حيث التكلفة والتنوع والسرعة. ومع ذلك، نادرًا ما يتم أخذ عوامل أخرى مثل أمن المحللين والتأثيرات البيئية في الاعتبار. على الرغم من وجود المزيد من المنشورات حول تقنيات الفصل اللوني الأخضر، لم يكن هناك الكثير من اعتماد تقنيات HPLC الصديقة للبيئة في مجال الأعمال الصيدلانية. الأسباب الرئيسية لذلك هي الحاجة إلى تكييف جهاز HPLC القياسي، أو نقص الوقت، أو المعرفة، أو ارتباك المحللين بشأن استيفاء متطلبات الطريقة. تم تقديم نظرة عامة على الأساليب الخضراء للتحليل اللوني السائل (LC) التي يمكن تطبيقها بسرعة على الأدوات التقليدية لإنشاء إجراءات HPLC صديقة للبيئة للتحليل الصيدلاني.

الكلمات الدالة: الكيمياء التحليلية الخضراء، HPLC، التحليل الدوائي.