Solvents for Green Pharmaceutical Liquid Chromatography - Possibilities and Limitations

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Abstract

The pharmaceutical industry faces increasing pressure to adopt more sustainable practices, and liquid chromatography (LC), a cornerstone analytical technique in pharmaceutical analyses, is no exception. Traditional LC methods often rely on large volumes of hazardous organic solvents, posing significant environmental and health risks. This review explores the current landscape of eco-friendly solvent alternatives and strategies for their implementation in pharmaceutical analysis. We delve into the potential of green alternative solvents for reversed-phase liquid chromatography (RP LC), aqueous mobile phases, bio-based solvents, and supercritical fluid chromatography (SFC), examining their chromatographic performance, compatibility with existing instrumentation, and regulatory acceptance. The review indicates the advantages of alternative solvents and their applicability. It also critically assesses the limitations of these green approaches, including challenges in method development, separation efficiency, and detection. Furthermore, it discusses the economic implications and the crucial role of analytical method transfer in transitioning to greener pharmaceutical LC. Ultimately, this article aims to provide a comprehensive overview of the progress towards sustainable pharmaceutical analysis and highlight the future directions necessary for the widespread adoption of greener chromatographic practices.

Keywords: green chemistry; green chromatography; pharmaceutical separations; solvents;

1. Introduction

Chromatography is a fundamental analytical technique in the pharmaceutical industry, playing a key role in all stages of the drug life cycle. It is indispensable in the process of discovering new medicinal substances through the identification and isolation of potential drug candidates. In addition, chromatography is essential for developing pure reference materials and active substances, ensuring their appropriate quality and composition. In the analysis of existing drugs, chromatographic techniques allow for the monitoring of stability, the examination of impurity profiles, and the optimization of formulations to improve efficacy and reduce side effects. The most commonly used techniques include high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC), which are characterized by high resolution and sensitivity, enabling precise identification, separation, and quantification of drug components and detection of even trace amounts of impurities [1,2].

Pharmaceutical analysis is critical in ensuring the safety, efficacy, and quality of medicinal products. Traditionally, these analyses have relied on methods that frequently involve hazardous chemicals, toxic solvents derived from petrochemicals, and energy-intensive procedures [3]. It is well known that methanol (MeOH) and acetonitrile (ACN) are the most consumed organic solvents in the reversed-phase liquid chromatography (RP LC) mobile phase used in pharmaceutical analyses [4]. These solvents are hazardous and pose both acute and chronic toxic risks. Methanol, a toxic alcohol, can lead to retinal damage and severe acidosis [5,6]. Acetonitrile exposure – whether through inhalation of vapors or contact with skin and eyes – also presents significant health risks. In vivo, acetonitrile is metabolized into cyanide, resulting in cytotoxic anoxia [7]. Clearly, using these solvents in mobile phases raises serious health concerns for analysts and contributes to higher operational costs for analytical laboratories. Moreover, due to the large volumes of chemical waste generated, their substantial environmental footprint cannot be overlooked.

This reliance contributes significantly to environmental pollution by generating substantial waste and potentially releasing harmful substances. The pharmaceutical industry's overall contribution to global carbon emissions is also a growing concern, necessitating a shift towards more sustainable practices [8]. In response to these environmental challenges, the principles of green chemistry have been increasingly adopted within pharmaceutical analysis [9].

Green chemistry is defined as the design of chemical products and processes that reduce or eliminate the use or generation of hazardous substances. This philosophy extends across the entire lifecycle of a chemical product, from its initial design and manufacturing to its use and ultimate disposal. A particularly active area within green chemistry is the establishment of environmentally benign analytical methodologies, giving rise to the concept of Green Analytical Chemistry (GAC) [10–14].

The modern pharmaceutical industry faces the challenge of minimizing its negative environmental impact. Drug production generates significant amounts of waste, including used solvents, which can pollute the air, water, and soil, posing a threat to ecosystems and human health [15]. Therefore, searching for and implementing more sustainable solutions has become a priority. Green chemistry, as an approach aimed at designing chemical processes in a way that minimizes or eliminates the use and production of hazardous substances, is becoming increasingly important in the pharmaceutical industry. Among the various approaches within GAC, using green solvents is a crucial strategy for achieving sustainability in the pharmaceutical sector [16,17]. These solvents offer alternatives to conventional organic solvents, aiming to minimize the environmental and health hazards associated with their use. Green solvents can also contribute to improving the efficiency and reducing the costs of analytical processes [18,19]. Implementing these sustainable solutions is not only a matter of

environmental responsibility but also a response to growing regulatory requirements and social expectations regarding environmental friendliness and safety in the pharmaceutical industry [20].

The green solvent adoption aligns with increasing regulatory pressures focused on minimizing environmental footprints and a growing recognition within the pharmaceutical industry of its environmental responsibilities. Furthermore, the transition to bio-based solvents can lessen the dependence on finite fossil fuel resources and contribute to a lower overall carbon footprint. This review article aims to comprehensively understand the current landscape of green solvents in pharmaceutical analysis. It will delve into the definition of green pharmaceutical analysis, explore the diverse types of green solvents utilized, and critically assess their potential benefits and inherent limitations [21].

Green solvents for liquid chromatography methods are becoming increasingly popular. This is evidenced by research work and numerous review articles published recently [9,21–25]. Nevertheless, each new work of this type brings a critical perspective on previous achievements and new possibilities. In an excellent article by Davy Guillarme about trends in pharmaceutical analysis, one can read that "replacing ACN with a greener solvent in LC is highly challenging, if not impossible" [20]. In this article, we would like to prove that using green solvents in chromatography is indeed possible. We even believe that this solution will become very popular in the near future.

We would also state in the beginning that this review focuses only on greener organic solvents with high potential for use in HPLC. From the authors' point of view, more traditional solvents, such as ethanol, dimethyl carbonate, propylene carbonate, ethyl lactate, or even glycerol, are more likely to be used in the pharmaceutical industry. This work does not discuss solvents such as deep eutectic solvents, ionic liquids, etc.

2. Green Pharmaceutical Analysis

To define green pharmaceutical analysis, it should be stated that it is the application of green chemistry principles specifically to analytical methods within the pharmaceutical industry [26]. According to the 12 principles of green analytical chemistry, derived from the 12 green chemistry principles, the primary objective is to minimize or eliminate the environmental and health hazards associated with traditional analytical techniques [13]. This involves a concerted effort to reduce or entirely avoid the use and generation of hazardous substances, such as the toxic organic solvents and harmful reagents that have historically been prevalent in pharmaceutical laboratories. The core focus of green pharmaceutical analysis is to optimize analytical methodologies, making them more environmentally benign while ensuring the continued accuracy and reliability of the results. A central tenet of green pharmaceutical analysis is the importance of minimizing hazardous substances, waste generation, and energy consumption throughout the analytical process. Green Analytical Chemistry (GAC) specifically prioritizes minimizing the amount of waste produced, reducing the reliance on hazardous chemicals and solvents, and enhancing the overall efficiency of analytical procedures. This includes strategies such as employing fewer reagents, utilizing recyclable materials whenever possible, and substituting traditional solvents with less harmful alternatives [13,24].

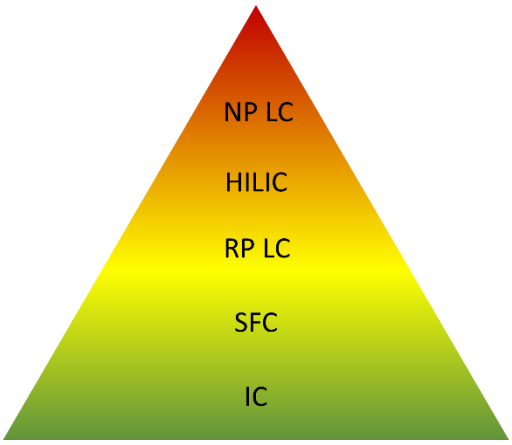

Figure 1. The greenness of chromatographic modes: NP. LC – normal-phase liquid chromatography, HILIC – hydrophilic interaction liquid chromatography, RP LC – reversed-phase liquid chromatography, SFC – supercritical fluid chromatography, and IC - ion chromatography.

According to the newest data, liquid chromatography consumes more than 150,000 tons of acetonitrile and methanol annually [27]. These figures are, of course, estimates, but they nevertheless illustrate the scale very well. Other sources indicate that about 26-50 million litres of chemical waste are generated annually [23,28–30]. Therefore, possible solutions to reduce solvent consumption in chromatographic analyses should be considered.

By far the simplest way to make chromatographic methods more environmentally friendly is to reduce the size of the chromatographic columns by shortening them and reducing their internal diameter [20]. Shortening the columns obviously reduces the resolution, which can be compensated for by reducing the particle size (and, unfortunately, increasing the pressure). Nevertheless, shortening the column and reducing the particle size makes it possible to shorten the method and thus reduce the amount of solvents used. Reducing the internal diameter of columns significantly reduces the consumption of solvents used in the mobile phase. Reducing the diameter from the classic 4.6 mm to 2.1 mm allows for a reduction in solvent consumption of up to 80% [31]. Additionally, using such a column with reduced flow increases the analytical sensitivity of the method. Regarding pharmaceutical analysis, the reduction of column diameter is restricted by Pharmacopeas. The United States Pharmacopoeia (USP) allows for greater flexibility with column diameter changes by requiring constant linear velocity, while the European Pharmacopeia (Ph. Eur.) has stricter limits and often restricts diameter changes to a ±25% range. In this case, changing the diameter from 4.6 to 2.1 mm is impossible. However, new methods can be developed based on columns with a reduced diameter.

The second solution is to replace classic solvents such as methanol and acetonitrile with their green counterparts, which are described in the next chapter. Of course, the best results will be achieved by combining a reduction in solvent consumption with a qualitative change in solvents. Most chromatographic analyses performed in the pharmaceutical industry are carried out in reversed-phase liquid chromatography based on methanol and acetonitrile. RP LC methods are greener than normal-phase liquid chromatography (NP LC) and hydrophilic interaction liquid chromatography (HILIC), but still are not as green as supercritical fluid chromatography (SFC) [32]. Unfortunately, the application of SFC at the current time is still limited [20]. For these reasons, it seems evident that there is a need to search for alternative solvents to RP LC. A comparison of the greenness of the different chromatographic modes is schematically shown in Fig. 1.

It is clear that eliminating toxic solvents from RP LC mobile phases and replacing them with greener alternatives plays a crucial role in advancing environmentally friendly HPLC methods. Furthermore, the solvents used during sample preparation for a given HPLC method should also be carefully considered.

3. Green solvents

The transition towards green pharmaceutical analysis necessitates the adoption of more environmentally friendly solvents. These green solvents can be broadly classified into several categories, each with distinct properties and applications within the pharmaceutical analytical context. The definition of a green solvent is not straightforward either. Currently, many assessment tools are available, and sometimes they give quite contradictory results [33,34]. Nevertheless, there are presently several solvents that can replace methanol and acetonitrile. These options include: dimethyl carbonate, propylene carbonate, ethanol, and ethyl lactate. There are, of course, others, but their application in the current situation seems very limited. These include Cyrene, acetone, 2-methyltetrahydrofuran, and many others, presented in the GSK Solvent Sustainability Guide [35,36], Pfizer guide [37], Sanofi's guide [38], and the American Chemical Society (ACS) and Green Chemistry Institute® Pharmaceutical Roundtable (GCIPR) guide [39]. Considering the possibilities and limitations, only a small proportion of green solvents can be used as eluents in liquid chromatography. These solvents must be compatible with the chromatographic system, both with the stationary phase and detection methods. These properties will be discussed in later sections of this article. It should be clearly stated here that this review only concerns green solvents with potential use as mobile phase components in pharmaceutical analyses (see Fig. 2). It should also be noted that the introduction of green solvents at the sample preparation stage, e.g., extraction, is also important for the development of sustainable methods [40–42].

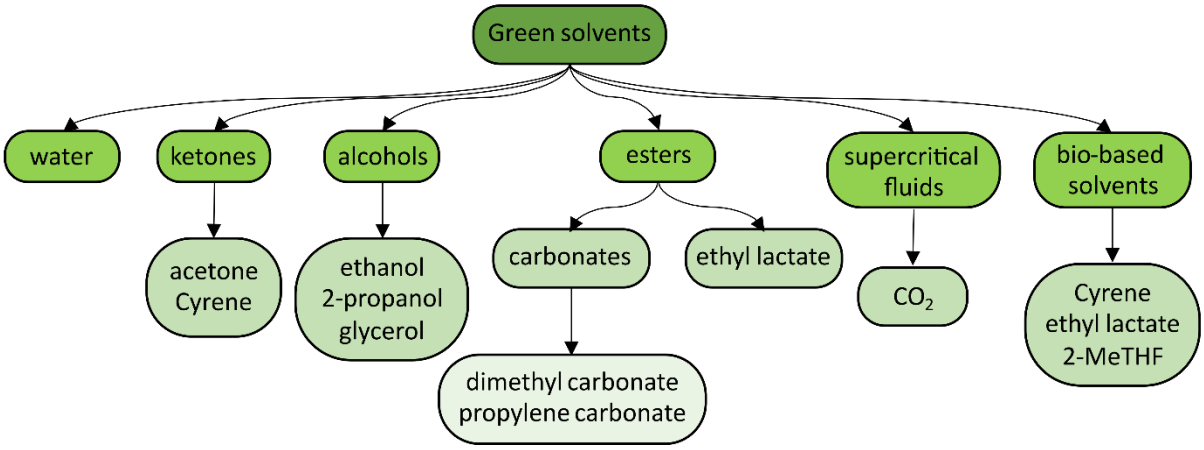

Figure 2. Potential green solvents for chromatographic analyses: 2-MeTHF - 2-methyltetrahydrofuran.

3.1. Water

The greenest solvent is water. Water is often considered the ultimate green solvent due to its abundance, non-toxicity, non-flammability, and environmental compatibility [43]. Aqueous mixtures, where water is the primary component, also fall under this category. Innovations in aqueous chromatography even allow using pure water as the mobile phase in liquid chromatography [23]. Interestingly, heating water to supercritical or subcritical conditions can enhance its solvency power for less polar compounds, potentially reducing the need for organic modifiers in chromatographic mobile phases [44]. However, a significant limitation of water is its poor ability to dissolve many natural

products and lipophilic pharmaceutical compounds. Nevertheless, water is extensively used in pharmaceutical analysis, particularly in RP LC.

An essential application of water is subcritical water chromatography (SBWC) [45,46]. SBWC was discussed in detail in the previous studies [47,48]. Even if SBWC is not as efficient at present, it is undoubtedly one of the greenest alternatives. In the authors' opinion, this chromatographic mode is unlikely to gain widespread popularity in routine analyses, especially pharmaceutical ones, due to its lower efficiency and lower resolution. The application of SBWC requires the adaptation of traditional equipment to HPLC. A special thermostat and, in most cases, other detectors are needed [44]. This can be one of the reasons that SBWC has not yet had a broader application in pharmaceutical analyses [9]. From an energy consumption perspective, SBWC is definitely more energy-intensive. Therefore, it is difficult to estimate whether eliminating organic solvents from the method is worth the higher energy consumption. In addition, this will depend on the specific analysis. So far, SBWC methods are characterized by lower efficiency and resolution than HPLC.

Subcritical water exhibits distinct properties compared to water at ambient temperatures. As the temperature increases, its dielectric constant, viscosity, and surface tension decrease, reducing polarity. Consequently, in chromatographic systems, subcritical water behaves more like organic solvents. This allows for the elution of non-polar compounds at higher temperatures, while polar compounds require lower temperatures for effective elution [9]. However, due to the thermal limitations of conventional reversed-phase columns, using pure water under subcritical conditions necessitates thermally stable stationary phases capable of withstanding temperatures above 200 °C. Suitable stationary phases for SBWC include those based on polymers (e.g., polystyrene and divinylbenzene), zirconia, or carbon [49–53]. Traditional stationary phases used in RP LC obtained on the silica support degrade under such conditions. The reverse conversion is much simpler; the phases used in SBWC can be successfully applied in RP LC. Analyte stability at high temperatures is also an important issue that has to be considered. [54–56]. It is necessary to reduce the analysis time not to affect the analytes' stability. It allows the separation of even compounds that degrade at high temperatures if the residence time in the elevated-temperature column is short [52]. Conducting analyses at high temperatures obviously leads to reduced retention and selectivity, which is why the selection of the stationary phase, which is mainly responsible for the separation selectivity, plays an important role here. An example of a pharmaceutical application of SBWC is the separation and analysis of dextromethorphan hydrobromide, chlorpheniramine maleate, doxylamine succinate, phenylephrine hydrochloride, acetaminophen, and guaifenesin present in cold medications [45].

Theoretically, the most environmentally friendly solution would be to conduct analyses in pure water as a mobile phase within the so-called normal temperature range, generally below 60°C, which is feasible on most column thermostats and most stationary phases for RP LC. These are modes per aqueous liquid chromatography (PALC), the water-only reversed-phase liquid chromatography (WRP-LC). Term PALC is dedicated to systems containing silica-based stationary phases, whereas WRP-LC uses polar-embedded or polar-endcapped stationary phases [57,58]. It should be noted that in PALC, water is usually the dominant, but not the only, component of the mobile phase.

The separation of organic components in pure water as a mobile phase is possible, as proved in our study [59]. It is worth noting that the data obtained were fully satisfactory and did not differ in quality from those obtained in a traditional chromatographic system. However, one should be aware of the limitations of this situation. Firstly, there is the problem of the solubility of analytes in water as the mobile phase. Secondly, a single-component mobile phase does not allow for optimization and does not affect retention and resolution. Optimization can only be achieved in such a situation by selecting the stationary phase and temperature or changing the pH of the mobile phase. The third limitation is

the selection of stationary phases, which must be compatible with the mobile phase, which consists only of water. Of course, all three conditions can be met, but the number of potential methods that meet all three limitations will be significantly limited.

In situations where the mobile phase composition is highly restricted – such as when using pure water or water-rich mixtures – selectivity is primarily determined by the choice of the stationary phase. In addition, the stationary phase must remain compatible in aqueous conditions. Thus, various polar-embedded and polar-endcapped stationary phases have become available in recent years. These phases differ in their solvation characteristics, leading to variations in selectivity [49]. In the literature, examples of the separation of various substances in water as a single-component mobile phase: nucleic bases, nucleosides, purine alkaloids, amino acids, etc., using polar-embedded stationary phases can be found [59–61]. Such stationary phases enhance the capability to separate compounds with diverse polarities, thereby making WRP-LC a more appealing and environmentally friendly option for method development.

To remain objective despite the desire to promote chromatography in water as a single-component mobile phase, it must be stated that the likelihood of introducing such chromatographic methods in pharmacy is relatively low, primarily due to the small number of commercially available stationary phases that operate in aqueous conditions. For this reason, a move towards alternative green solvents is a much more likely direction.

3.2. Alcohols

The most obvious group of green solvents is alcohols. Ethanol [62–64] and isopropanol are commonly employed as greener alternatives to solvents like methanol and acetonitrile in HPLC mobile phases and extraction procedures. Ethanol, often produced through the fermentation of renewable resources like bio-waste, is biodegradable and exhibits low toxicity. Isopropanol is also considered a safer alternative to methanol in various applications. These alcohols offer a good polarity balance and are miscible with water, making them suitable for a wide range of analytes in RP LC [65]. Glycerol, derived from renewable sources, is another nontoxic and biodegradable alcohol ideal for green chemistry, though its high viscosity can present challenges in HPLC [66,67]. Biobutanol, produced by sugar fermentation, is another example.

Among alcohols, ethanol is the most promising alternative to methanol and acetonitrile [23,68–70]. This was influenced by its physical and chemical properties and its availability in HPLC-grade purity. In terms of human health, ethanol has a lower vapor pressure, which reduces the risk of toxic effects from inhalation. Its toxicity is primarily associated with long-term consumption rather than its use as a laboratory reagent. From an environmental perspective, ethanol is biodegradable and has a lower environmental impact compared to acetonitrile and methanol.

The high viscosity is the only disadvantage of ethanol. It is discussed later in this paper. Even its UV cut-off of 210 nm does not pose a serious analytical problem. For the above reasons, most pharmaceutical separation methods using green solvents utilize ethanol. It was used in various applications, for separation antipsychotics (quetiapine fumarate, aripiprazole, asenapine maleate, and chlorpromazine hydrochloride) [71], separation of artesunate and amodiaquine [63], impuritiesthe analysis of atorvastatin in tablets, and in the presence of its degradation products [72], the determination of escitalopram and etizolam in tablets [73], the determination of impurities of artesunate and amodiaquine as APIs [63], the determiantion of famotidine, paracetamol, thiocolchicoside as APIs [74], determination of two different moxifloxacin combinations (moxifloxacin/dexamethasone and moxifloxacin/prednisolone) [75], the separation of nirmatrelvir and

ritonavir [76] and determination of eight water-soluble B vitamis [77]. Ethanol was also applied for chiral separations, e.g., for the simultaneous determination of timolol and latanoprost in eye drops [78]. Other recent applications deals with the determination of 3,4-methylenedioxymethamphetamine (MDMA) [79], analisis of ketoconazole and beclomethasone [80], determination of pyridoxine HCl and doxylamine succinate in pure and pharmaceutical dosage forms [81], analysis of tafluprost in its pure form and ophthalmic formulation [82], quantification method of lamivudine, zidovudine and nevirapine with identification of related substances in tablets [83], analysis of rosuvastatin [84], and for quantification of secnidazole in tablets [85]. A considerable number of methods using ethanol have been published in recent years. This indicates the very high potential of ethanol in creating green analytical methods for pharmaceutical analysis.

Glycerol possesses several characteristics that make it well-suited for developing environmentally friendly liquid chromatography methods. Firstly, it is a nonvolatile and safe solvent, minimizing the risk of inhalation exposure for analysts. Regarding laboratory safety, glycerol is advantageous due to its low flammability and high chemical stability under standard storage conditions. Environmentally, it is biodegradable and derived from renewable, cost-effective sources [66,86].

Despite its undoubted problems resulting from high viscosity, more and more methods that use glycerol as a mobile phase modifier are emerging. These applications are also being developed for pharmaceutical products. Some examples are: glutathione and ascorbic acid determination in pharmaceutical tablets [87], the separation of four antiviral medicines under reversed-phase chromatographic conditions [66], and the simultaneous determination of methionine and paracetamol in pharmaceutical tablets [88].

Isopropanol (isopropyl alcohol) is increasingly recognized as a viable green solvent for HPLC, offering a favorable balance between performance and environmental sustainability [89]. Compared to many traditional organic solvents, isopropanol exhibits lower toxicity and reduced environmental impact, making it an attractive option for green analytical chemistry [90]. It is commonly blended with water, acetonitrile, or other solvents to tailor mobile phase compositions for various analytical separations, depending on the nature of the analytes and the stationary phase used. Several advantages support its application in HPLC, including good solubility for a wide range of analytes, moderate volatility that facilitates efficient evaporation and shorter run times, and the ability to produce sharp, symmetrical peaks. Furthermore, isopropanol has low UV absorbance at commonly used detection wavelengths, enhancing its suitability for UV-based detection methods. From a green chemistry perspective, its biodegradability and relatively low toxicity make it an environmentally friendly solvent. However, certain limitations must be considered. Isopropanol does absorb UV light at some wavelengths, which may interfere with detector baselines; this can be mitigated through method optimization and baseline correction. Additionally, not all chromatographic columns or analytes are compatible with isopropanol, highlighting the importance of preliminary compatibility testing. The most crucial disadvantage is the high viscosity that causes high backpressure. Lower diffusion and consequently broader signals at higher viscosity mobile phases are also observed. Ultimately, with careful method development and optimization, isopropanol can be an effective and sustainable choice for HPLC mobile phases, supporting analytical performance and environmental responsibility [91].

The first pharmaceutical analyses using isopropanol were performed a long time ago, such as determining doxorubicin hydrochloride [92]. Methods using isopropanol are also currently being developed, such as simultaneous determination of dorzolamide, brinzolamide, and timolol [91]. Isopropanol may also be applied as an organic modifier in subcritical chromatography, for example, in simultaneous enantioseparation and simulation studies of atenolol, metoprolol, and propranolol [93]. However, it should be noted that its use is significantly less common than that of ethanol. This is

probably due to the higher viscosity of isopropanol and the generation of significantly higher pressures in the chromatographic system.

3.3. Ketones

Acetone, which is less toxic and more biodegradable, has received significantly higher rankings than acetonitrile in solvent selection guides created by pharmaceutical companies that promote more sustainable research and practices [94]. According to Pfizer's solvent selection guide, acetone is considered the second most environmentally friendly solvent, with only water ranked higher [37]. Due to its comparable physical and chemical properties to acetonitrile – such as complete miscibility with water, its role as a hydrogen bond acceptor, similar solvatochromic behavior, and nearly identical viscosity – acetone is a potential substitute for acetonitrile in numerous RP LC applications [95].

A cyclic ether-ketone is Cyrene (dihydrolevoglucosenone). Cyrene is biodegradable, nontoxic, and nonmutagenic, making it an environmentally friendly alternative to classical solvents. It is essential that it is a biobased solvent. Cyrene was applied as a solvent in liquid chromatography as a co-solvent to ethanol by El Deeb [96] to separate metronidazole and moxifloxacin. While this application was exciting from the point of view of liquid chromatography, the practical application of Cyrene is significantly limited. High viscosity (14.5 cP), low water solubility (52.6 g/L), and a high UV cut-off (250-270 nm or 350 nm), which significantly limits (or almost makes impossible) its use with UV detection.

3.4. Carbonates

Propylene carbonate is a cyclic carbonate solvent derived from carbon dioxide. As a green, highly polar aprotic solvent, it is a safer alternative to traditional toxic aprotic polar solvents like acetonitrile, dimethylformamide, and dimethyl sulfoxide [90,97]. Propylene carbonate was first introduced as an organic modifier in LC mobile phases in 2012. Since then, only a few studies have explored its use in a pharmaceutical context [98–102]. It has to be emphasized that propylene carbonate is offered in the market in HPLC-grade, which is undoubtedly an advantage.

Research has shown that propylene carbonate has limited miscibility with water, but it can be effectively addressed by combining it with methanol or ethanol. In line with the GAC principles, ethanol is recommended as the third component in a propylene carbonate/water mobile phase. The resulting propylene carbonate/ethanol/water mixture offers acceptable viscosity and is compatible with UV detection due to propylene carbonate's suitable UV cut-off of 210 nm [9,102].

One very interesting solvent that can be used in chromatography is dimethyl carbonate. In recent research, dimethyl carbonate has been investigated as a promising green mobile phase modifier for RP-LC, with four key objectives guiding its evaluation. First, it was demonstrated that its limited water solubility range (2–10%) is adequate for effectively separating polar aromatic compounds. Second, the solvent strength of dimethyl carbonate was successfully extended beyond 12% by incorporating ethanol as a co-solvent, thereby broadening its applicability in various chromatographic conditions. Third, the use of dimethyl carbonate contributed to a reduction in both analysis time and organic solvent consumption during the separation of highly hydrophobic analytes. Lastly, the studies confirmed that DMC is compatible with fluorescence detection, further supporting its utility as a sustainable alternative in GAC [103,104]. It was also proven that dimethyl carbonate in a mixture with ethanol may be applied in HILIC and normal-phase chromatography [105]. It should be mentioned here that its use in a normal-phase system improves the sustainability aspect of the normal-phase system. However, in the authors' opinion, it is definitely not in a green chromatographic mode.

Dimethyl carbonate was popularized into RP LC by Felleti et al. for the analysis of small molecules and the purification of therapeutic peptides [29,106]. Dimethyl carbonate was also used in an analytical procedure to determine the caffeine and theobromine content in tea, which was the only organic solvent used in the SPE extraction and chromatographic analysis stages in the RP LC system [107]. Dimethyl carbonate may also be applied in sub/supercritical fluid chromatography of psychoactive drugs [104].

3.5. Esters

Among esters, the greatest hopes are pinned on ethyl lactate. It can be sourced from biomass via fermentation [108]. Its chromatographic application was demonstrated as early as 2015 [109], but its popularity has not increased significantly over the next 10 years. Ethyl lactate exhibits slightly higher elution strength than acetonitrile. However, high cut-off and significant increase of backpressure make the application of ethyl lactate to replace acetonitrile rather impossible at the current time. Ethyl lactate is biodegradable and exhibits low toxicity. However, its application in HPLC is hampered mainly by a high UV cut-off and susceptibility to hydrolysis.

3.6. Bio-based solvents

Bio-based solvents, distinguished by their derivation from renewable biological resources rather than finite fossil fuels, represent a cornerstone of sustainable chemistry, offering environmentally benign alternatives to conventional petroleum-based counterparts. Within this burgeoning field, several compounds stand out for their versatility and potential [110–113]. Ethanol, widely recognized for its production via biomass fermentation, serves as a highly effective polar protic solvent across diverse sectors, including pharmaceuticals, food, and fine chemicals, benefiting from its biodegradability, low cost, and minimal toxicity. Glycerol, an abundant by-product of biodiesel synthesis, offers a nontoxic, nonvolatile, and highly polar medium. It is an attractive solvent and reaction platform for numerous organic transformations, leveraging its robust hydrogen-bonding capabilities. A recent innovation, Cyrene, is gaining prominence as a safer, biodegradable alternative to problematic dipolar aprotic solvents. Derived from cellulose waste, Cyrene exhibits comparable solvation properties, proving effective in advanced material synthesis, peptide chemistry, and various organic reactions [114]. Similarly, ethyl lactate, an ester formed from bio-derived lactic acid and ethanol, is celebrated for its low toxicity, biodegradability, and broad solvency. It finds application in cleaning formulations, pharmaceuticals, and as a reaction medium. The potential applications of these solvents were discussed above.

2-Methyltetrahydrofuran is derived from biomass sources like levulinic acid or furfural. 2-MeTHF is a promising green ether. It exhibits properties somewhat analogous to THF but with better stability and reduced peroxide formation tendency (though stabilizers are often added in HPLC grades). It can be an alternative to dichloromethane. Its UV cut-off is around 210-220 nm [115]. While its availability in HPLC grade is increasing, it remains significantly more expensive than traditional solvents [116]. 2-Methyltetrahydrofuran was used as an acetonitrile additive to separate +epinephrine, tetramisole, acebutolol, labetalol, +verapamil [117]. It constituted a 5% addition to the mobile phase, although this reduced the consumption of acetonitrile, which should be emphasized.

3.7. Alternatives for normal-phase system

NP LC often relies on significant amounts of volatile, flammable, and toxic organic solvents, which present environmental, safety, and disposal challenges. This is where greener alternatives become particularly attractive. Supercritical fluid chromatography (SFC) is a prime alternative to NP LC, especially when considering green chemistry principles [118–120]. SFC uses a supercritical fluid as the

primary mobile phase, most commonly supercritical carbon dioxide ($scCO_2$), mixed with a polar organic co-solvent (like methanol or ethanol). Especially $scCO_2$ with ethanol is a green solution [121]. Another alternative organic modifier may be dimethyl carbonate [105].

SFC significantly reduces the use of organic solvents, often by 75-90% compared to LC methods. The primary mobile phase, $scCO_2$, is nontoxic, non-flammable, inexpensive, and can be easily recycled or vented into the atmosphere without significant environmental impact. The supercritical mobile phase's low viscosity and high diffusivity lead to much quicker analyses and higher sample throughput. This is a considerable advantage in drug discovery and development, where speed is critical [122].

SFC has become the go-to technique for chiral separations in the pharmaceutical industry for analytical and large-scale preparative purposes. Its efficiency and ability to dissolve chiral modifiers make it superior to many LC methods for separating enantiomers, which is crucial for drug safety and efficacy [123–126].

In cases where SFC does not replace the normal-phase system, it remains to look again for alternative solvents. While it is challenging to completely replace traditional NP LC solvents, research is ongoing to find "greener" alternatives or reduce their consumption. Ethanol and isopropanol are common modifiers in NP LC mobile phases. Still, they can sometimes serve as a higher proportion of the mobile phase, or even the primary solvent, in specific NP LC applications (e.g., using specific diol or bare silica columns) to reduce the reliance on more hazardous solvents. They are undoubtedly less toxic and more biodegradable than many common NP LC solvents. Ethyl acetate and methyl acetate are generally less toxic and flammable than hexane or chloroform [127]. They can be explored as alternative bulk solvents for specific NP-LC separations, though their applicability depends heavily on the particular compounds and stationary phases. Some of the newest alternatives for NP LC are: D-limonene, isopentyl acetate, cyclopentyl methyl ether, and 2-MeTHF [128,129].

Currently, the only option is optimizing traditional NP-LC methods to use the lowest possible percentage of hazardous solvents, and maximizing less toxic ones (like alcohols or even small amounts of water with specialized stationary phases) can reduce environmental impact.

3.8 Tools and metrics

The evolution GAC has led to the development of sophisticated metrics to quantitatively and visually assess the environmental impact of analytical procedures, with solvent selection and consumption being a critical, dedicated component across most tools. AGREE (Analytical GREEnness Metric Approach) [130], structured around the 12 Principles of GAC, evaluates solvent greenness through a specific principle, transforming criteria into a unified 0–1 scale for its final, easily interpretable pictogram score. The Modified GAPI (MoGAPI) and its more complex variant, ComplexMoGAPI, utilize a multi-segment pictogram (often a pentagram or star) that includes a distinct subsection for reagents and solvents, color-coded (green, yellow, red) based on toxicity, quantity, and source, to quickly highlight environmental weak points, while also providing a numerical total score derived from numerous predefined questions. Similarly, Analytical Green Star Area (AGSA), an extension of the AGREE concept, integrates solvent and reagent criteria into its assessment, providing a comprehensive, built-in scoring system and a star-shaped visual output resistant to user bias. Other tools like the ChlorTox Scale are focused explicitly on quantifying the environmental hazard of solvents containing chlorine, offering a targeted toxicity assessment, while broader frameworks like CHEM21 and the Carbon Footprint Reduction Index (CaFRI) incorporate solvent-related metrics, such as use of renewable/bio-based solvents and overall mass/volume efficiency, to gauge the method's sustainability and climate impact, collectively advancing the systematic adoption of eco-friendly

analytical practices. Other tools are HPLC-EAT [30], NEMI [131], Eco-scale [132], and RGB model [133]. These tools have already been the subject of numerous reviews and comparisons [34,134–137].

As a summary, a pictograms of GEARS score [138] are listed in Figure 3. The "Green environmental assessment and rating for solvents" tool compares toxicity, biodegradability, renewability, volatility, thermal stability, flammability, environmental impact, efficiency, recyclability, and affordability of solvents.

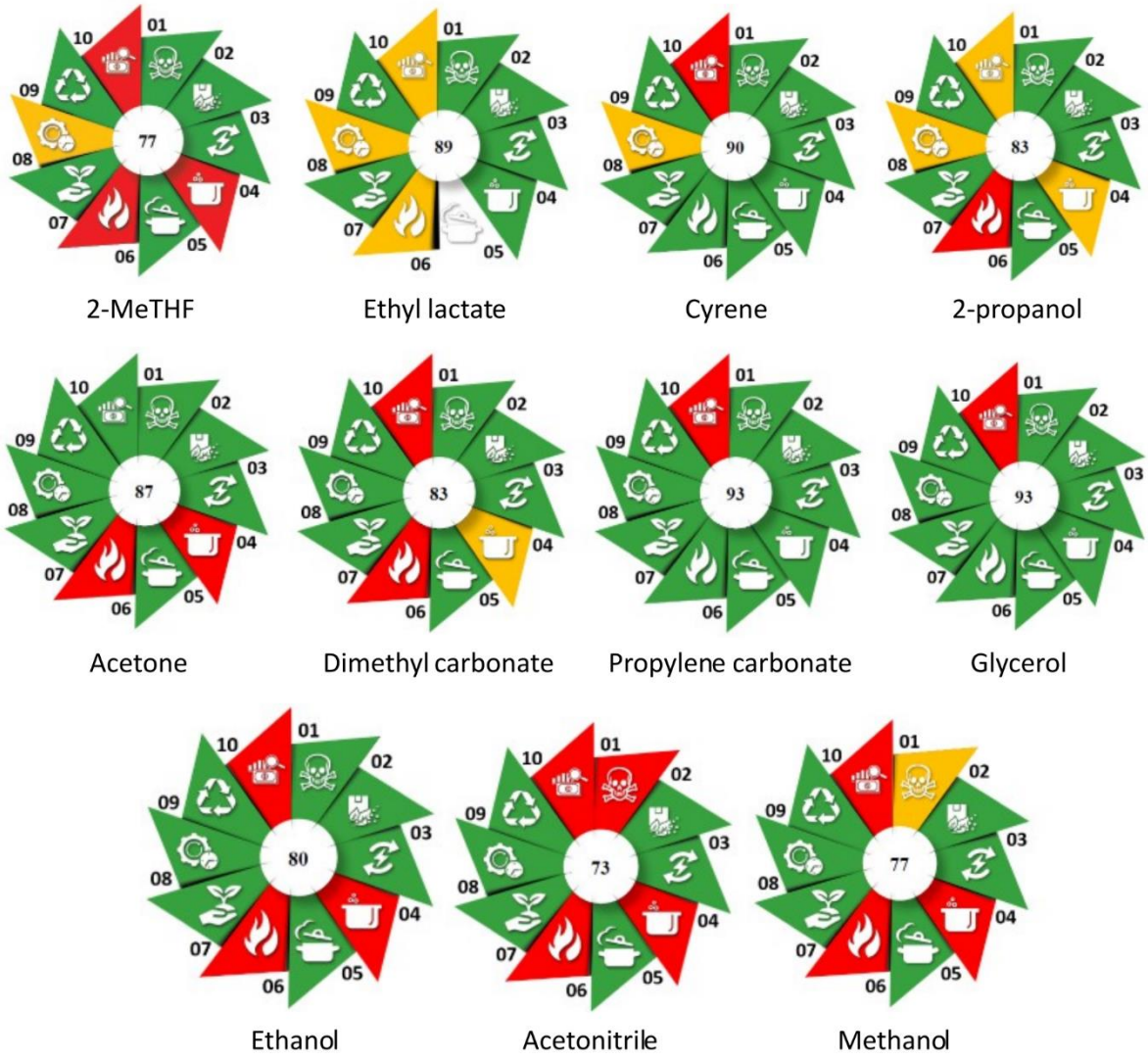

Figure 3 Pictograms of GEARS score for discussed solvents.

4. Possibilities

Detailed properties of green solvents were presented in detail in other works [21,22]. In this work, the authors focused on demonstrating the possibilities of their application and the most important limitations.

Glycerol has emerged as an environmentally friendly mobile phase modifier with strong potential to replace traditional solvents used in HPLC. Due to its higher polarity than other organic modifiers, glycerol enables precise adjustment of mobile phase strength, enhancing the retention and separation

of polar and non-polar compounds. Its advantages include a low UV cut-off, low flammability, minimal volatility, and biodegradability, making it a sustainable option for chromatographic applications. Glycerol also bridges the gap in eluotropic strength between strong eluents like acetonitrile, methanol, or ethanol, and weaker ones like water or buffers. Incorporating glycerol into standard mobile phases allows for improved control over elution strength. However, its main drawback is its high viscosity, which can increase system pressure when used in larger amounts. This issue can be mitigated by diluting glycerol with water or other solvents. Research investigating the impact of glycerol's viscosity on the Van Deemter equation parameters revealed that a more viscous glycerol-based mobile phase reduced both eddy diffusion (A term) and longitudinal diffusion (B term). These effects may contribute to improved chromatographic performance, including enhanced peak symmetry, higher column efficiency, and better resolution. Thus, the higher viscosity of glycerol-based mobile phases can be considered a potential advantage in this context [66].

From an economic perspective, ethanol is more cost-effective than acetonitrile and methanol due to its lower market price. Unfortunately, the price of ethanol is also affected by excise duty. Additionally, its lower toxicity results in reduced waste disposal costs. These factors contribute to an overall decrease in expenses associated with pharmaceutical analysis [9]. These advantages have made ethanol the leading green alternative to acetonitrile and methanol. Numerous studies have demonstrated the use of ethanol-based mobile phases in reversed-phase HPLC for pharmaceutical analysis. Even better results are obtained when ethanol is used in a mixture with dimethyl carbonate, but this combination has not yet been used in pharmaceutical analyses [139]. Combining these two green solvents virtually eliminates the adverse effects of the high viscosity of ethanol.

Dimethyl carbonate and propylene carbonate are promising green solvents with high chromatographic potential. They have relatively low viscosity and a UV cut-off around 210-220 nm. The only drawback is the limited miscibility with water, which may be overcome by mixing with ethanol [139]. Similar effects can be achieved by blending with methanol, but in terms of sustainability, the use of ethanol seems more justified.

5. Problems and limitations

The use of green solvents carries specific problems and limitations due to the operation of the chromatographic system, detection methods, etc. A summary of these limitations and characteristic properties is shown in Table 1.

5.1. Viscosity

In the case of glycerol, its main drawback is its high viscosity, which can increase system pressure when used in larger amounts. This issue can be mitigated by diluting glycerol with water or other solvents. A similar problem may be observed with ethanol. In this case, the solution may be to increase the temperature of the separation process up to 40-50°C, as viscosity decreases with increasing temperature. Increasing the column temperature by 10°C led to a 20% decrease in column back pressure [9]. At the same time, the temperature increase will reduce the analyte's adsorption, which may, in a sense, "increase" the eluting power of the mobile phase. In this case, reducing organic solvent content in the eluent will be possible. In the case of viscous mobile phases, increasing the temperature may also improve separation efficiency, but it should be expected that a decrease in retention will result in a decrease in selectivity. The final change in resolution will depend on many factors: the extent of the temperature increase, the type of stationary phase, the mobile phase, and the analytes being separated. Another way to reduce high back pressure is to use monolithic columns. Such columns

exhibit significantly lower flow resistance and can operate with more viscous mobile phases at relatively low back pressures. The final solution may also be to operate a traditional column at reduced flow rates, but this may reduce separation efficiency and will certainly increase analysis times.

5.2. Purity

Working with alternative solvents is extremely difficult if separation is impossible under isocratic conditions. Switching to gradient elution, which allows for greater resolution, poses serious detection problems, especially when using a spectrophotometric detector and mass spectrometer (MS). Green solvents are definitely less pure than special solvents dedicated to gradient analysis. Although some are pure enough for HPLC, their purity is still within the standard for isocratic elution (ethanol, propylene carbonate). Many solvents, such as dimethyl carbonate or ethyl lactate, are unavailable in HPLC-grade purity at all, which generally does not pose a problem under isocratic conditions. However, under gradient elution conditions, the increase in the baseline makes detection practically impossible. One solution could be, for example, the use of selective detectors, such as a fluorometric detector, but its use is limited to analytes that exhibit fluorescence. Another disadvantage of green solvents may be the extent of the UV cut-off. It should be noted that cut-off values vary depending on the source, which is most likely due to differences between the theoretical value for a given solvent and its observed value resulting from the presence of impurities.

For example, the main drawback of acetone compared to acetonitrile is that acetone's UV cut-off extends to 330 nm. It restricts its usage with the spectrophotometric detection to a narrow range of wavelengths [140]. Spectrophotometry in the UV range is the most popular detection method in LC. Hopefully, the fast popularization of MS detectors in chromatography and the new generation of aerosol-based detectors will give new opportunities to apply acetone instead of acetonitrile [141].

Cyrene has an even higher UV cut-off equal to 350 nm. Ethyl lactate absorbs UV light up to 300 nm. In addition, the practical cut-off value may increase due to the presence of contaminants. It should be noted that most of these alternative solvents are only 99% pure (from a chromatographic point of view). In the case of Cyrene and ethyl lactate, changing the detector to a mass spectrometer is impractical given these solvents' current purity.

Problems may be visible even in the case of green solvents with a much lower cut-off. For example, ethanol can be applied to analyze artesunate and its impurities because the low UV absorption maximum of the analytes is equal to 210 nm, the same as the ethanol cut-off [63]. This is therefore a significant limitation of green solvents, fortunately limited only to specific analytes. In most cases, the absorption maxima are shifted towards longer wavelengths than the ethanol cut-off.

Without a doubt, the challenge for the chemical industry is the production of green solvents with increased purity, enabling work in gradient elution and using MS as a detector. The availability of such solvents will undoubtedly accelerate the development of green chromatographic methods.

5.3. Miscibility

Probably the most significant limitation of green solvents is their limited miscibility with water. Methanol and acetonitrile mix with water in any proportion, facilitating their use as mobile phases. Carbonates, such as dimethyl carbonate and propylene carbonate, Cyrene, and many other green solvents, exhibit limited miscibility with water, which in many cases is very low, e.g., 114,7 g/L for dimethyl carbonate. This means that the elution strength of the mobile phase will be pretty low even at the maximum concentration of the organic solvent. In such cases, it can be applied to analytes with significant polarity, such as the aforementioned analysis of caffeine and theobromine [107]. Such a

mobile phase will not be able to elute an analyte with significant hydrophobicity, unless we reduce the hydrophobicity of the stationary phase, e.g., by replacing C18 with C8.

However, this problem can be solved by mixing alternative solvents. An exciting solution was the use of a three-component mobile phase containing water and a mixture of two green solvents, dimethyl carbonate and ethanol [103]. Firstly, mixing these solvents resulted in a mobile phase with relatively low viscosity (compared to the ethanol-water mixture) and allowed analyses to be carried out at normal pressures. Secondly, the separation results obtained were better than those for the ethanol-water mobile phase. Regarding retention, peak symmetry, and efficiency, the separation results obtained for the dimethyl carbonate-ethanol-water mixture did not differ in quality from the data obtained for the acetonitrile-water system [139]. It is reasonable to assume that if such results were obtained for various mixtures of substances with varying hydrophobicity, there is a real chance of applying them to pharmaceutical analyses.

Table 1: Comparison of key properties for selected HPLC solvents

Solvent	Typical UV cut-off (nm)	Relative Price (HPLC Grade, MeOH=1)	Viscosity (cP at 20-25°C)	Key limitation(s) for RP-LC	Green Credentials
Methanol (MeOH)	~205	1	~0.6	Toxicity, petrochemical origin, UV > ACN	Moderate
Acetonitrile (ACN)	~190	1.5 - 3+	~0.4	Cost, price, volatility, toxicity, petrochemical origin	Low
Water (H_2O)	<190	<0.1 (running cost)	~1.0	Weak eluent (RP LC), requires a purification system	Excellent
Ethanol (EtOH)	~210	1 - 1.5	~1.2	UV > MeOH/ACN	Good (bio-based potential, biodegradable)
Glycerol	~210	0.7	>900	Extremely high viscosity	Excellent (bio-based, biodegradable)
2-MeTHF	~210-220	3 - 6+	~0.5	Cost, peroxide potential (stabilized)	Good (bio-based)
Dimethyl Carbonate (DMC)	~210-220	1.5 - 2.5	~0.6	Limited water miscibility	Good (low toxicity, biodegradable)
Propylene Carbonate (PC)	~210-220	1.5 - 3	~2.5	High viscosity, limited water miscibility	Good (low toxicity)
Ethyl Lactate (EL)	~250-260	2 - 4	~2.6	High UV cut-off, hydrolysis risk, high viscosity	Good (bio-based, biodegradable)
Cyrene™	~250-270	10 - 20+	~4.0	Very high UV cut-off, high viscosity, very high cost, low purity	Excellent (bio-based, biodegradable)

6. Case studies

The glycerol-water (7:93) mobile phase was successfully applied for the separation of a mixture of four antiviral drugs (ribavirin, ganciclovir, acyclovir, and penciclovir) using a C18 column in isocratic conditions. The mixture was separated in 13 min with acceptable resolution and peak shapes meeting

the requirements of the ICH guidelines [66]. For a 250 mm × 4.6 mm, 5 µm particles column at 38°C, for a flow rate of 1 mL/min, the backpressure was 106 bar. This example clearly shows that glycerol can be a good alternative for analytes that do not require a high elution strength of the mobile phase (see Fig. 4).

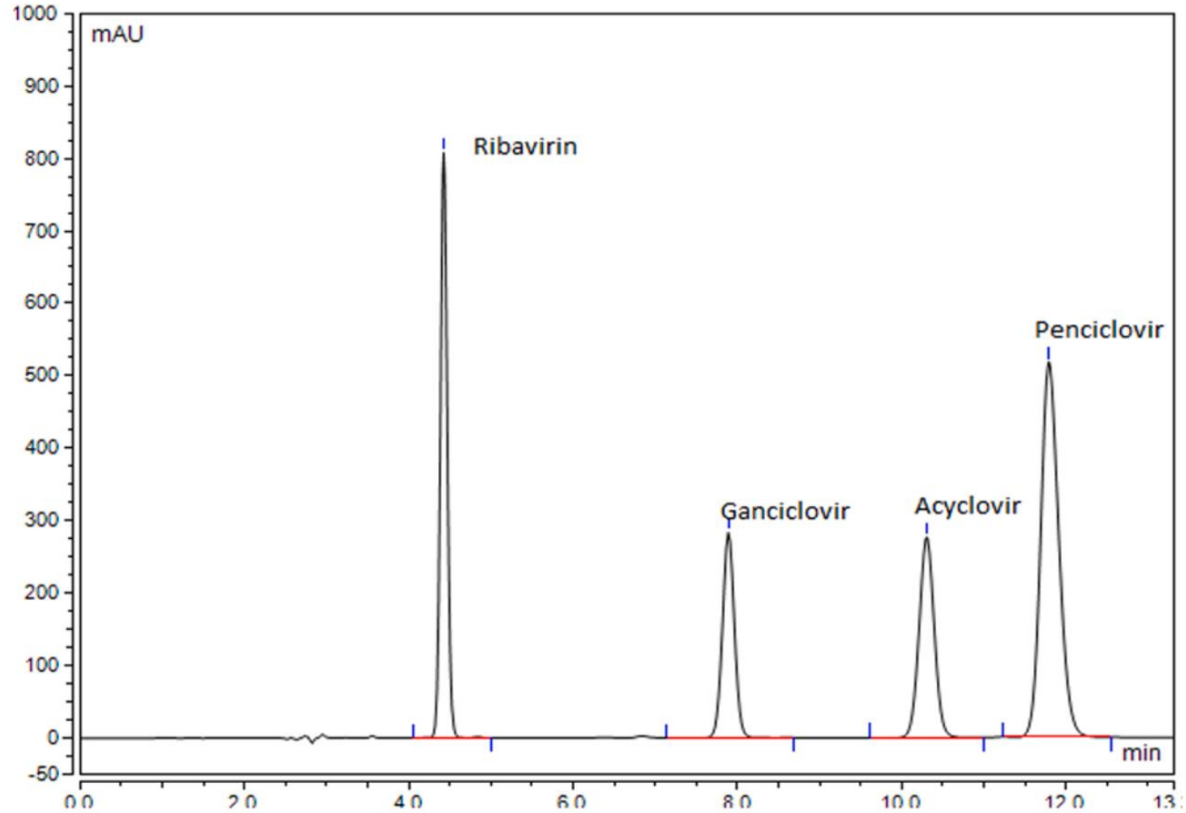

Figure 4 Chromatographic separation of RBV, GCV, ACV and PCV using 7% glycerol as a mobile phase modifier. Reprinted with permission from [66].

As an example of ethanol application in pharmacy, one can show the separation of two antimalarial APIs: amodiaquine and artesunate, described by Yabre et al. [62]. The method development was challenging due to the basicity of amodiaquine, leading to peak asymmetry. UV detection was also difficult due to the baseline drift observed with ethanol in gradient mode. Ethanol was also applied to the quantitative analysis of impurities of both artesunate and amodiaquine drugs using gradient elution [63]. Finally, the method was applied to analyze amodiaquine and artesunate impurities in raw materials and formulations. The generated column backpressure under 200 bars for a 150 mm × 3 mm, 5 µm particle column and reduced flow rate of 0.4 mL/min showed that this analysis can be performed using conventional HPLC equipment and could be implemented in resource-limited laboratories.

When developing methods using alternative solvents, comparing them with conventional methods is very important. A green method cannot have significantly worse validation parameters than a traditional method, because green chemistry aspects cannot compromise quality. An interesting example is a comparison of two HPLC methods for analyzing four antipsychotic drugs – quetiapine fumarate, aripiprazole, asenapine maleate, and chlorpromazine hydrochloride [71]. The researchers developed a green and a conventional RP HPLC method to determine these compounds in bulk materials and pharmaceutical tablet formulations. The green method utilized a mobile phase composed of ethanol and 20 mM sodium dihydrogen phosphate (35:65, v/v, pH 5.0), with isocratic elution completed within 11 minutes. In contrast, the conventional method employed acetonitrile

instead of ethanol and used a gradient elution to achieve separation in 15 minutes. Both methods operated at a 1 mL/min flow rate and were validated according to the ICH Q2R1 guidelines. What is very important is that the results showed that the green method provided superior limits of detection (LOD) and quantification (LOQ) for the target analytes. Statistical analysis indicated no significant differences in the accuracy and precision between the two methods. The environmental impact of the methods was assessed using the Green Analytical Procedure Index (GAPI). The green method yielded nine green and six yellow pentagrams, whereas the conventional method yielded six green, six yellow, and three red pentagrams. Overall, the study highlights that using ethanol as a mobile phase solvent not only enhances the environmental friendliness of the method but also improves performance metrics such as shorter analysis time and better sensitivity. A similar conclusion can be drawn from comparing the determination of two different moxifloxacin combinations using classical and ethanol-based methods [75].

Some studies have reported successful substitution of acetonitrile with acetone in RP HPLC coupled with MS for peptide analysis after a tryptic digest of bovine serum albumin (BSA) [142] and some peptide standards [143]. More recently, Hutchinson et al. explored the feasibility of replacing acetonitrile with acetone using HILIC and a corona charged aerosol detector for eleven sugar separation (sucralose, ribose, fructose, sorbitol, glucose, galactose, sucrose, maltitol, maltose, lactose, and maltotriose) [141]. Their findings indicated that while acetonitrile provided superior column efficiency and lower detection limits, acetone could still separate the same number of components.

7. Regulatory Landscape and Guidelines

The regulatory landscape surrounding the use of solvents in pharmaceutical analysis is becoming increasingly focused on sustainability and environmental impact. Various regulatory bodies and international organizations, such as the FDA, EMA, and ICH, have established guidelines and regulations that indirectly or directly influence the adoption of green solvents. While specific regulations mandating the use of green solvents are not yet widespread, the increasing emphasis on minimizing hazardous substances, reducing waste, and promoting environmentally responsible practices within these frameworks encourages the pharmaceutical industry to explore and adopt greener alternatives.

The approval process for novel green methods can be lengthy and complex, potentially hindering their widespread adoption. Furthermore, in some regions, there might be a lack of established guidelines and acceptance criteria specifically for methods employing certain types of green solvents, creating uncertainty for pharmaceutical companies seeking to implement these approaches. Ensuring that green solvents meet regulatory standards can be a long process [144].

There is a growing number of initiatives promoting the adoption of green chemistry principles, including using green solvents, within the pharmaceutical industry [145,146]. Pharmaceutical companies are increasingly recognizing the importance of sustainability, both from an ethical standpoint and due to growing public and investor expectations. Many companies are setting ambitious goals for reducing their environmental footprint, including transitioning to greener solvents and implementing more sustainable manufacturing and analytical processes. Research institutions and industry collaborations also foster the development and implementation green solvent technologies. According to SNS Insider, the green solvents market is poised to reach USD 3.9 billion by 2032 [147].

The regulatory environment is clearly evolving to place greater emphasis on environmental sustainability within the pharmaceutical industry. This shift is creating both opportunities and challenges for companies. The need to comply with increasingly stringent regulations regarding solvent use and waste disposal drives the exploration and adoption of greener alternatives. Simultaneously,

the regulatory approval process for new analytical methods, including those utilizing novel green solvents, can be complex and time-consuming. The growing awareness of corporate social responsibility and the increasing public demand for sustainable products are also significant factors influencing the pharmaceutical industry's move towards greener practices. Companies recognize that adopting environmentally friendly approaches can enhance their reputation and appeal to an increasingly eco-conscious market.

8. Trade-off between greenness and practice

The pursuit of Green Liquid Chromatography methods, while laudable for its aims to reduce environmental harm and occupational hazards by minimizing or replacing toxic solvents, intrinsically involves a trade-off with practicality and analytical efficiency. Achieving high "greenness," often measured by metrics like the Analytical Eco-Scale [132] or AGREE [130], frequently requires substituting traditional, high-performing solvents (e.g., acetonitrile) with greener, safer alternatives (e.g., water, ethanol). This substitution, however, introduces significant practical challenges: the higher viscosity of green solvents increases column back-pressure, limiting flow rates that were discussed above. It decreases sample throughput. The altered physicochemical properties of the chromatographic system (mobile phase) often lead to compromised selectivity and resolution, necessitating extensive and complex re-method development to re-establish the required separation quality; and the adoption of novel green technologies (like miniaturized columns or new stationary phases) can increase instrumentation cost and operational complexity.

Therefore, a method that scores highly on greenness alone may be entirely impractical for routine, high-throughput industrial and quality control laboratories, especially in pharmaceutical industry, where efficiency, robustness, and cost-effectiveness—the core tenets of practicality quantified by metrics such as the Blue Applicability Grade Index (BAGI) [148] and the Click Analytical Chemistry Index (CACI) [149] —remain paramount. The true virtue lies in developing methods that harmonize these conflicting demands, achieving a balanced state of White Analytical Chemistry (WAC) [150], ensuring that environmental responsibility does not compromise the necessary rigor and efficiency of analytical results.

9. Perspectives

Adopting green solvents in pharmaceutical analysis presents numerous potential benefits, contributing to a more sustainable and environmentally responsible industry. These possibilities span across reduced environmental impact, enhanced cost-effectiveness, and improved safety for laboratory personnel and the environment.

The analysis presented in this review underscores the significant potential of green solvents to revolutionize pharmaceutical analysis, offering a pathway towards a more sustainable and environmentally responsible industry. The possibilities associated with the adoption of these solvents are compelling, encompassing reduced environmental impact through lower toxicity, enhanced biodegradability, and decreased VOC emissions; improved cost-effectiveness via reduced solvent consumption, lower disposal costs, and increased energy efficiency; and enhanced safety for laboratory personnel due to reduced flammability and lower exposure risks. However, the implementation of green solvents is not without its limitations. Challenges related to the solubility of certain pharmaceutical compounds [151] and compatibility with existing analytical techniques like HPLC and UHPLC.

The drive towards sustainable laboratory practices is undeniable. Regulatory pressures (e.g., REACH in Europe [152]), corporate sustainability initiatives, and growing environmental awareness are accelerating the search for and adoption of green HPLC solvents. Investment continues to develop efficient, cost-effective methods for producing high-purity bio-based solvents such as ethanol and 2-MeTHF, and novel candidates are being explored. We can anticipate improved availability and potentially decreasing costs for the most promising green alternatives as production scales up and purification technologies advance. Developing analytical methods specifically designed around green solvents is also crucial for facilitating their wider adoption.

Implementing green solvents can also lead to significant cost savings in pharmaceutical analysis. While the initial purchase price of some specialized green solvents might be higher, the potential for lower overall solvent consumption and reduced expenses associated with hazardous waste disposal can result in long-term economic benefits. Many green solvents, such as ethanol and water, are readily available and relatively inexpensive compared to traditional organic solvents like acetonitrile.

Analyzing the prices of green solvents compared to traditionally used acetonitrile and methanol is crucial for assessing economic viability. Currently, methanol remains the most affordable choice, while acetonitrile, despite its popularity, is characterized by unstable and often high prices, which were particularly evident during availability crises such as that of 2008-2009 [58,89,153,154]. These crises, caused by a decline in acrylonitrile production (of which ACN is a by-product) and disruptions in supply chains, resulted in drastic price increases, forcing laboratories to seek alternatives. Green solvents such as dimethyl carbonate, propylene carbonate, ethanol, and ethyl lactate, although historically more expensive than methanol, are becoming increasingly competitive in price relative to acetonitrile. Future price trends for these alternatives will be strongly correlated with the production scale. The increased demand for sustainable solutions and advances in green solvent synthesis and purification technologies, including the development of bio-based processes, should lead to further reductions in unit costs. In the long term, with increasing supply and process efficiency, green solvents are expected to become a more stable and economically attractive option, reducing dependence on the volatile ACN market.

10. Conclusions

Replacing conventional HPLC solvents such as methanol and acetonitrile with greener alternatives is a desirable but complex goal. While water remains the ideal green solvent, its use is limited by chromatographic principles. Among organic modifiers, ethanol stands out as a readily available, often cost-effective, and chromatographically competent substitute for many applications, provided its ~210 nm UV cut-off is acceptable. Their HPLC purity is also an advantage. Dimethyl carbonate and propylene carbonate also show promise with favourable UV cut-offs (~210-220 nm) and good green credentials. However, factors like low water miscibility (DMC) and relatively high viscosity (PC) must be considered. Other solvents such as 2-MeTHF offer bio-based routes but come at a higher cost, while ethyl acetate, ethyl lactate, and Cyrene are currently limited primarily by their poor UV transparency for standard HPLC detection methods. In the authors' opinion, glycerol deserves special attention. It is a solvent that is less associated with liquid chromatography, yet it makes it possible to obtain surprisingly good chromatographic separations in pharmaceutical applications. At the same time, it is a highly green and bio-based solvent.

No particular green solvent has emerged as a universal replacement for acetonitrile or methanol across all HPLC applications. The selection process must remain application-specific, carefully weighing the required analytical performance (especially detector compatibility), method validation efforts, solvent properties, cost, availability, and environmental benefits. The path towards greener HPLC will likely involve a combination of strategies, including substituting conventional solvents where feasible (with candidates such as ethanol or DMC), optimizing methods to reduce overall solvent consumption, and continued innovation in green solvent technology and compatible analytical instrumentation. What is also important is that changing the conventional solvents for green ones can be implemented using existing laboratory equipment.

Nevertheless, the future of green solvents in the pharmaceutical industry appears promising, with continuous advancements in developing novel bio-based solvents and purifying existing solvents. We believe the availability of alternative solvents such as dimethyl carbonate and ethyl lactate in HPLC purity will significantly accelerate the development of chromatographic methods using green solvents.

Acknowledgement

The authors are grateful to the National Science Centre (Cracow, Poland) for financial support under the Opus project (2023/51/B/NZ7/00537).

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