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Performance evaluation of green and conventional solvents in reversed-phase liquid chromatography based on the separation of non-polar and polar substances

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This study investigated the potential for replacing conventional solvents such as acetonitrile (ACN) and methanol (MeOH) with greener alternatives – ethanol (EtOH) and dimethyl carbonate (DMC) – in chromatographic separations. The aim was to assess whether these environmentally friendly solvents could achieve comparable separation performance while reducing the environmental impact of the analyses. Chromatographic separations were carried out on two different mixtures: non-polar and polar, using three stationary phases with different surface properties (C18, diphenyl and perfluorinated phenyl). The Technique for Order of Preference by Similarity to Ideal Solution algorithm (TOPSIS) was used to select the optimal conditions for ultra-high performance liquid chromatography (UHPLC) separations, integrating multiple criteria, including chromatographic run time, tailing ratios, resolution and solvent-related environmental hazards. The results show that EtOH and DMC can effectively replace traditional solvents without compromising separation performance, confirming that sustainable analytical methods for mixtures of non-polar and polar compounds are achievable with green solvents.

Green foundation

1. The manuscript demonstrates the feasibility of using a green solvent such as dimethyl carbonate in liquid chromatography. This is the first comparison under equal analytical conditions of conventional solvents (methanol and acetronitrile) with green solvents (ethanol and dimethyl carbonate).
2. The results obtained show that alternative solvents allow obtaining equally good chromatographic separation parameters as with conventional solvents. The introduction of dimethyl carbonate and ethanol for serial analysis for example in pharmaceutics can significantly reduce the use of dangerous solvents such as methanol and acetonitrile.
3. We plan to continue research using gradient elution and more alternative solvents. The use of gradient elution should significantly shorten and optimize the methods making them more sustainable.

Introduction

Reversed-phase liquid chromatography (RP LC) is a widely used chromatographic technique that employs a nonpolar stationary phase and a more polar mobile phase. For a few decades, octadecyl-bonded silica gel (C18) has been the preferred stationary phase, while acetonitrile (ACN) and methanol (MeOH) have been the most common organic modifiers.[1–4] The popularity of C18 phases stems from their versatility and robustness. They can withstand a wide range of mobile phase conditions and effectively separate various analytes, including pharmaceuticals, environmental pollutants, food components, and biological molecules.[5–7]

Acetonitrile and methanol are commonly used organic modifiers of mobile phases due to their low viscosity, minimal UV absorption, and complete miscibility with water.[8,9] These properties have made them particularly popular in the pharmaceutical industry, which accounts for over 70% of global acetonitrile demand. However, these solvents pose significant con-

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cerns due to their toxicity and flammability.[10] Additionally, since ACN is mainly produced as a by-product of acrylonitrile synthesis, the availability and its price is fundamentally linked to trends in the acrylonitrile market, which remains sensitive to global economic and industrial conditions.[11,12]

To address these challenges, researchers have explored alternative organic solvents for use in analytical chemistry, especially sample preparation[13–15] and chromatography,[16] particularly RP LC, that align with Green Analytical Chemistry (GAC) principles. Green liquid chromatography emphasizes using environmentally friendly solvents, with candidates including ethanol (EtOH), isopropanol, acetone, dimethyl carbonate (DMC), propylene carbonate, glycerol, ethyl lactate, and Cyrene.[17–23] Among these, ethanol is the most widely used and extensively studied alternative with numerous publications demonstrating its effectiveness as a substitute for ACN and MeOH.[24–27]

Recently, dimethyl carbonate has also gained attention as a promising green solvent for RP LC applications, showing potential as an environmentally friendly alternative in chromatographic separations.[28–30] In a recent study by Felletti *et al.*,[28] the fundamental retention properties of DMC were studied and compared to other RP LC solvents (ACN, ethanol, and isopropanol). These four organic solvents were also compared under preparative conditions during the purification of a peptide of pharmaceutical interest.[29] In both cases DMC showed efficiency comparable to ACN, achieving similar retention with less solvent. Higher non-polarity of DMC allowed for its lower content in the mobile phase, which limits consumption, partially compensating for its poor solubility in water. Lajin *et al*.[31] also studied the potential of DMC in RP LC by comparing its elution behavior with ACN and MeOH. The study included a chlorine speciation application in urine using HPLC-ICP-MS. DMC facilitated the elution of hydrophobic compounds and improved recovery from the column without compromising detection. Recently, DMC has also been successfully used as a solvent to extract oleanolic acid from grape pomace.[32]

An increasing variety of stationary phases, offering selectivity other than the conventional C18 phase are emerging in the chromatographic market.[33] These materials differ in their surface chemistry, especially polarity. Among these, materials containing aromatic rings, such as phenyl, diphenyl, and biphenyl, are gaining popularity.[34,35] Additionally, stationary phases with perfluorinated groups[36] and those featuring embedded polar groups or polar-endcapped ones are also on the rise.[37,38]

When considering alternative green solvents, it is important to weight their environmental and sustainability benefits.[39,40] However, a key concern is whether replacing ACN or MeOH with a green solvent will not deteriorate chromatographic separation results (retention, selectivity, efficiency, resolution, peak symmetry, *etc.*). Thus, it is reasonable to investigate whether the chromatographic separation parameters obtained with green solvents can match (or exceed) those obtained with the commonly used ACN and MeOH.

There are greenness metric tools for the assessment of analytical procedures. Eco-scale,[41] GAPI,[42] AGREE[43] and others[44–46] are focused on the entire analytical processes, from sample collection to final analysis. If applied for HPLC separations their algorithms are not capable of discriminating differences in greenness of such systems.

For the assessment or optimization of the system with many responses the use of multicriteria decision analysis (MCDA) is beneficial.[47,48] This group of tools allows for ranking alternatives based on specified criteria. MCDA is widely used in decision-making processes across fields such as finance, management (including environmental management), and risk assessment. Its main advantages include providing a systematic and formalized approach to decision-making, while handling multiple, often conflicting, assessment criteria. MCDA tools are flexible, enabling users to define criteria based on specific goals and assign different weights to reflect their relative importance.[49] The results are straightforward to interpret, and the process can often be carried out using specialized software. Although applications of MCDA in chemical sciences are relatively rare,[50] it has been used in green chemistry for evaluating ionic liquids,[51] deep eutectic solvents,[52] more traditional solvents,[53] nanoparticles,[54] and analytical methods.[55] MCDA is particularly applied if the assessment criteria are contradictory – the better performance in some results in worse performance of others. Therefore, MCDA holds significant potential as a tool for simultaneous comparative greenness and efficiency assessment of liquid chromatography mobile phases.

For the above reasons, this work aimed to compare chromatographic separations using conventional solvents (MeOH and ACN) and two green solvents (EtOH and DMC). The study was performed for two mixtures of compounds: non-polar and polar. Separations were carried out on three stationary phases with different surface properties. Chromatographic materials with higher polarity than C18 combined with non-conventional solvents like dimethyl carbonate represent an unexplored area of liquid chromatography that can provide valuable insights. The resulting aim was to select the greenest and efficient separation system from all combinations of stationary and mobile phases.

Experimental

Materials

Acetonitrile, methanol, and ethanol were HPLC grade purchased from J. T. Baker (Avantor, Radnor, PA, USA). Dimethyl carbonate was purchased from Sigma-Aldrich (Saint Luis, MO, USA). Water was prepared with a Milli-Q Water Purification System (Millipore Corporation, Bedford, MA, USA). Standard of analytical substances for non-polar mixture (benzene, naphthalene, anthracene, phenanthrene, pyrene, triphenylene, chrysene) and polar mixture (aniline, benzyl alcohol, 4-hydroxybenzaldehyde, 4-aminacetophenone, *p*-cresol, *p*-chlorophenol) were purchased from Sigma-Aldrich (Saint Luis, MO, USA).

Three chromatographic columns were used in the study: Kinetex XB-C18 1.7 μm, 2.1 × 100 mm (Phenomenex, Torrance, CA, USA), Fortis SpeedCore DiPhenyl 2.6 μm, 2.1 × 100 mm (Fortis Technologies, Neston, Great Britain), and Kinetex F5 1.7 μm, 2.1 × 100 mm (Phenomenex, Torrance, CA, USA).

Apparatus

The chromatographic assessments were conducted utilizing the Shimadzu Nexera UHPLC system (Kioto, Japan). This system features a binary solvent delivery pump (LC-30AD), an autosampler equipped with a 20 μL volume loop (SIL-20AC), a column temperature controller (CTO20AC), and a diode-array UV-Vis-detector (SPD-M20A). Data acquisition and instrument control were managed using LabSolutions LC/GC 5.65 software developed by Shimadzu (Kioto, Japan).

Methods

Two different mixtures of compounds (non-polar and polar) were prepared and subjected to detailed chromatographic analysis. The chromatographic separation of the substances in both cases was carried out under identical conditions to obtain comparable results and ensure maximum precision in the measurements. Measurements were carried out at a flow rate of 0.3 mL min^{-1} for diphenyl stationary phase and 0.25 mL min^{-1} for C18 and F5 stationary phases. The column temperature was maintained at 30 °C. A sample volume of 1 μL was injected into the column during each analysis. Spectrophotometric detection of each analyzed mixture was performed at 254 nm. Chromatographic separations were performed using a two-component mobile phase except for one, which was carried out using a ternary mobile phase. Each time, the mobile phase consisted of an organic solvent (or a mixture of two) and water. In the case of non-polar mixture separation solvent were mixed online at high pressure mixer. For separation of polar mixture, acetonitrile, dimethyl carbonate and ethanol were premixed with water (10/90) and next mixed online with to obtain certain concentration. Methanol was mixed online with water. Chromatographic separation conditions were chosen to allow best possible separation of all analytes (ideally to the baseline).

Tailing factor (T_f) were calculated according to equation:

$$T_\mathrm{f} = \frac{a+b}{2a}$$

where: a: distance from the leading edge of the peak to the peak midpoint, measured at 5% of peak height; b: distance from the peak midpoint to the trailing edge of the peak, measured at 5% of peak height. The number of theoretical plates was calculated as:

$$N = 16\left(\frac{t_\mathrm{r}}{w_\mathrm{b}}\right)^2$$

where: t_r: retention time of the peak (in minutes); w_b: peak width at the base (in minutes).

TOPSIS analysis

For the evaluation of the different mobile phases, the TOPSIS algorithm (Technique for Order of Preference by Similarity to Ideal Solution) has been selected due to its ease of interpretation and relatively straightforward mechanism. Originally developed by Hwang and Yoon,[56] this method aims to identify the optimal option among a set of alternatives based on a ranking system. The best alternative is determined by its shortest distance from the positive ideal solution and its greatest distance from the negative ideal solution. This mathematical approach allows for the integration of various (often conflicting) criteria into a single score, resulting in a ranked list of alternatives. The ranking is based on similarity to the ideal solution, with values ranging from 0 to 1 for each alternative. A score of 0 represents the least ideal option (with the worst values across all criteria), while a score of 1 indicates the ideal solution, with the best values for all criteria.

Typically, the input data for TOPSIS analysis consists of a matrix of n alternatives, each evaluated based on m criteria. The procedure follows these steps:

Construction of the normalized matrix:

$$r_{ij} = x_{ij} \div \sqrt{\sum x_{ij}{}^2}, \quad i = 1, 2, \ldots, \quad m \wedge j = 1, 2, \ldots, n$$

where x_{ij} and r_{ij} are original and normalized scores in decision matrix, respectively.

Construction of the weighted normalised decision matrix:

$$v_{ij} = r_{ij} \times w_j, \quad i = 1, 2, \ldots, \quad m \wedge j = 1, 2, \ldots, n$$

where w_j is the weight of the criterion j and the sum of all weights must equal to $1 - \sum_{j=1}^{n} w_j = 1$

Determination of positive ideal (A^*) and negative ideal (A^-) solutions:

$$\begin{aligned} A^* &= \left\{ \left(\max_i v_{ij} \vee j \in C_\mathrm{b} \right), \left(\min_i v_{ij} \vee j \in C_\mathrm{c} \right) \right\} \\ &= \left\{ v_i^* \vee j = 1, 2, \ldots, m \right\} \end{aligned}$$

$$\begin{aligned} A^- &= \left\{ \left(\min_i v_{ij} \vee j \in C_\mathrm{b} \right), \left(\max_i v_{ij} \vee j \in C_\mathrm{c} \right) \right\} \\ &= \left\{ v_j^* \vee j = 1, 2, \ldots, m \right\} \end{aligned}$$

Calculation of the separation scores for each of the alternatives:

$$S_i^* = \sqrt{\sum_{j=1}^{m} \left(v_{ij} - v_j^* \right)^2} \quad j = 1, 2, \ldots, m$$

$$S_i^- = \sqrt{\sum_{j=1}^{m} \left(v_{ij} - v_j^- \right)^2} \quad j = 1, 2, \ldots, m$$

Calculation of the relative closeness to the ideal solution

$$C_i^* = \frac{S_i^-}{S_i^* + S_i^-}, \quad i = 1, 2, \ldots, \quad m \wedge 0 < C_i^* < 1$$

Arrangement of alternatives in order of the closest to ideal to furthest from ideal that is the creation of a ranking. The alternative with C_i^* closest to 1 is the best preference among the possible options.

The data for TOPSIS analysis were different mobile phase compositions as alternatives. The assessment criteria were the retention time of the last eluting compound + 1 minute, the tailing factors for every analytical peak, resolutions between peaks and organic solvent consumption with the risk based approach included. Namely the volumes of organic solvents were multiplied by the scores obtained from CHEMS-1[57] and ChlorTox[58] analyses. TOPSIS offers assigning weights to differentiate the importance of criteria. In this study all four groups of criteria – analysis time, tailing factors, resolutions and organic solvent consumption – were given equal (0.25) weights. Tailing factors were equal for all analytes resulting in assigning weight equal to $0.25/n$, resolutions were also equal resulting in weights $0.25/(n-1)$, where n is equal to number of analytes. Such application of weights results in treating all criteria as equally significant, without putting too much stress on green analytical chemistry nor chromatographic performance aspects. It should be clearly noticed that some of considered criteria are contradictory to each other.

Results and discussion

Comparison of solvents

Each solvent possesses unique properties that affect its behavior in chromatographic processes, as detailed in Table 1. Methanol is the most volatile solvent. It has low viscosity which can facilitate rapid separations. However, its low elution strength can extend analysis time, and its highest toxicity (among the compared solvents) restricts its use in some applications. Acetonitrile is less toxic than methanol and more effective due to its lower viscosity and higher elution strength, which can accelerate chromatographic separations. The highest viscosity of ethanol causes the highest back-pressure in chromatographic system. These three solvents mix with water without restriction.

Dimethyl carbonate, with its high elution strength, higher density, and stability, is a promising choice for specific separation conditions. However, its limited solubility in water and higher UV absorption limit may restrict detection range and versatility. Ethanol, on the other hand, exhibits properties that represent a particular compromise. It has a moderate density and elution strength, allowing the separation process to be carried out relatively quickly and efficiently, and its lower toxicity compared to methanol makes it a safer choice for many analytical applications. Its primary disadvantage is its relatively high viscosity compared to other solvents, which can lead to increased pressures, requiring reduced flow rates and possibly extending analysis time.

It is also worth mentioning that the solubility of many compounds, especially pharmaceuticals, varies significantly depending on the solvent used, which is influenced by factors such as polarity or hydrogen bonding capacity. Of the solvents compared, methanol shows the most desirable properties. Ethanol and acetonitrile are effective for many compounds, but some may exhibit limited solubility. On the other hand, dimethyl carbonate will be incompatible with many pharmaceuticals due to its significant non-polarity. However, in liquid chromatography, injection volumes are usually very small, often in the microliter range. This means that even if a compound has limited solubility in a greener solvent such as DMC, it can still be dissolved in a more efficient solvent such as methanol for sample preparation. The small injection volume minimizes the impact on the overall solvent system, allowing the use of greener solvents in the mobile phase without compromising separation quality. This approach balances the need for efficient solubilization of analytes with the environmental benefits of using more sustainable solvents during chromatographic analysis.

Each solvent thus offers certain advantages and disadvantages, making them unique in chromatographic separation processes. Therefore, the choice of an optimal solvent depends on the analysis's specific requirements, such as separation speed, toxicity, stability, type of analytes and elution properties (Fig. 1).

In order to compare the environmental harmfulness of the solvents used, the chloroform-oriented toxicity assessment scale (ChlorTox Scale)[58] and the CHEMS-1 tool[57] were applied. The CHEMS-1 model uses toxicological and exposure data found in safety data sheets (SDS) to calculate hazard values associated with the reagents. This tool can be particularly useful in the early stages of procedure development when sol-

Table 1 Properties of compared organic solvents

Property	Methanol	Acetonitrile	Ethanol	Dimethyl carbonate
Boiling point [°C]	64.7	81.0	78.5	90.0
Melting point [°C]	−98.0	−46	−114.1	0.5
Flash point [°C]	9.7	6.0	13.0	17.0
Density at 25 °C [g cm^{-3}]	0.796	0.782	0.789	1.070
Viscosity at 20 °C [mPa s]	0.60	0.37	1.20	0.63
Solubility in H_2O [g dm^{-3}]	Miscible	Miscible	Miscible	114.7
Elution strength	Low	High	Moderate	High
Dielectric constant	32.7	37.5	24.5	3.1
Dipole moment [D]	2.87	3.44	1.69	0.93
UV cut-off [nm]	200	190	205	220

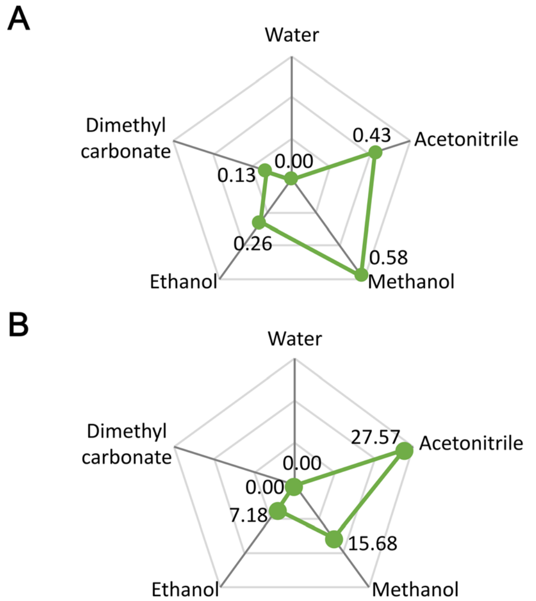

Fig. 1 Comparison of the environmental friendliness of the solvents used according to (A) ChlorTox Scale; (B) CHEMS-1 tool, calculated per one gram of solvent.[58]

vents are selected. ChlorTox Scale, on the other hand, is a tool that provides a straightforward way to assess risk by measuring the harmfulness of reagents used in an analytical method based on hazard categories also found in SDS. This is done by comparing the substance in question with chloroform as a reference. Like the CHEMS-1 tool, the ChlorTox Scale thus focuses solely on the harmfulness of the reagents and the amount of wastewater generated by them. These tools were chosen since the greenness of chromatographic separation is determined by the type and amount of solvent used.[59]

Comparison of separations

Two mixtures, one non-polar composed of aromatic hydrocarbons, and one polar, consisting of benzene derivatives with different functional groups, were separated using three chromatographic columns (Diphenyl, C18, and perfluorinated phenyl). The separations were performed in hydro-organic mobile phases with varying concentrations of organic solvents (ACN, MeOH, EtOH, and DMC), achieving baseline separation of all components in most cases.

Separation of non-polar mixture

The most optimal conditions for separating the components of the non-polar mixture for each of the solvents used, were obtained using the following composition of mobile phases: 62% MeOH/38% water, 41% ACN/59% water, 50% EtOH/50% water, and 19.5% DMC/19.5% EtOH/61% water on Diphenyl stationary phase; 77% MeOH/23% water, 67% ACN/33% water, 58% EtOH/42% water, and 26.5% DMC/26.5% EtOH/47% water on C18 stationary phase, and 57% MeOH/43% water, 39% ACN/61% water, 46% EtOH/54% water, and 19% DMC/19% EtOH/62% water on F5 stationary phase. Data are illustrated in Fig. 2.

In the separation processes conducted for the non-polar mixture using MeOH, ACN, EtOH, and DMC as organic solvents, successful separation was achieved with three of the four solvents when mixed in appropriate volume ratios with water.

Separation was impossible, however, with a mobile phase of dimethyl carbonate and water alone, as DMC proved to be an inefficient solvent for separating this mixture, mainly due to its low solubility in water. This characteristic made it impossible to obtain an adequate solvent concentration sufficient to separate non-polar compounds effectively. The maximum achievable DMC concentration in water was about 12%, which proved insufficient since separating this mixture requires higher organic solvent content in the mobile phase for optimal results.

A ternary mobile phase consisting of dimethyl carbonate, ethanol and water was created to overcome this limitation. Ethanol was introduced to increase DMC's solubility in water, allowing a higher proportion of organic solvent in the mobile phase, which enable effective separation of the test mixture. Optimal conditions were achieved with a 1 : 1 mixture of DMC and EtOH in water. The resulting chromatograms, presented in Fig. 3, demonstrate that aromatic hydrocarbons could be successfully separated across nearly all combination of mobile phases and stationary phases. The only incomplete separation occurred with the perfluorinated stationary phase when MeOH/water was used as the mobile phase.

When comparing classical solvents, chromatograms obtained with acetonitrile proved to be better than those with methanol as an organic modifier when used with C18 and F5 phases. With the diphenyl phase, analysis time was more satisfactory when MeOH was used, but peak symmetry was considerably poorer. As expected, conventional solvents among all solvents performed most effectively in combination with the C18 stationary phase. What is more, the C18 stationary phase

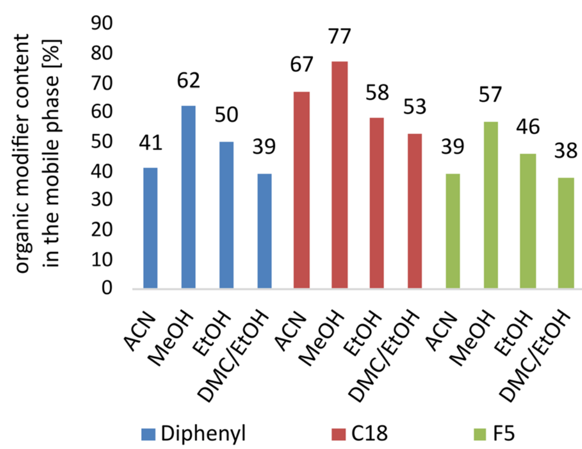

Fig. 2 Concentrations of organic solvent allowing the best separation of non-polar mixture.

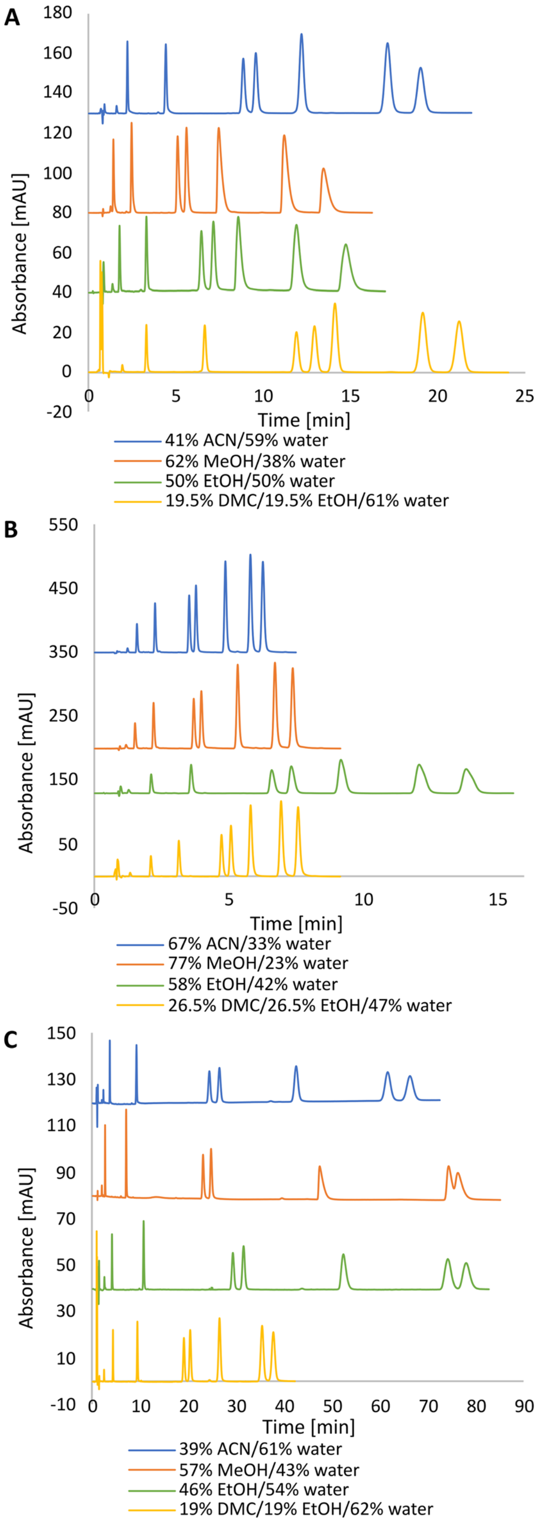

Fig. 3 Chromatograms of non-polar mixture separation obtained on Fortis SpeedCore Diphenyl (A), C18 (B), and Kinetex F5 (C) columns.

provided better separation of aromatic hydrocarbons compared to the diphenyl phase.

Complete separation was achievable on all stationary phases, changing the organic modifier to ethanol; however, it has to be emphasized that peak shapes showed greater tailing compared to ACN, with detailed data of peak asymmetry presented in Fig. 4. The least favorable results for ethanol were observed with the C18 stationary phase, where peak asymmetry of critical pair peaks required a reduction in ethanol content in the mobile phase (in order to effectively separate them), leading to a considerable increase in separation time.

Auspicious results were obtained for a mixture of dimethyl carbonate with ethanol, particularly on the diphenyl and C18 stationary phases, where performance was comparable to that with ACN. Notably, on the perfluorinated stationary phase the mixture of DMC/EtOH/water enabled the fastest separation with high efficiency, outperforming ACN as an organic modifier. Preliminary analyses of chromatograms suggests that this organic mixture can effectively replace classical solvents in separating non-polar substances.

Notably, when operating with alternative green solvents, it is essential to consider stationary phases other than those typically paired with classical solvents. For example, the perfluorinated phase (Kinetex F5), performs less effectively with ACN and MeOH compared to C18. However, pairing DMC with EtOH provides prominent separation results while significantly reducing the environmental impact of this analysis. Although the DMC/EtOH mixture achieved the shortest separation time – twice as fast as the other solvents tested – the separation of this particular mixture on the F5 phase still required 40 minutes, making it relatively time-consuming. In contrast, the classical C18 phase allowed a significant reduction in separation times. Nonetheless, using DMC with EtOH on the C18 phase also provided results comparable to those obtained with conventional solvents.

When analyzing separation efficiency, it is important to note that although the column dimensions were the same, the particle sizes differed, so comparisons are made only between solvents. Unfortunately, ethanol consistently demonstrated lower efficiency than the other solvents, likely due to its highest viscosity among the modifiers tested (see Fig. 4).In the case of the diphenyl phase, ACN allowed to achieve the highest efficiency, with the DMC/EtOH mixture showing slightly lower but comparable performance. Similar trends were observed for the C18 phase, although methanol also demonstrated high efficiency in this case. Surprisingly, on the F5 stationary phase, methanol provided the highest efficiencies; however, the peak shape was not satisfactory and the highest tailing factor was also registered with methanol (Fig. 4 C2).

On the diphenyl stationary phase, the most symmetrical peaks were obtained using a mixture of DMC/EtOH/water, supporting the potential of greener solvents in liquid chromatography. For the C18 phase, peak symmetry obtained with DMC/EtOH/water is only slightly less favourable than with acetonitrile, while for the F5 phase, the peak symmetry values were nearly identical. Table 2 lists the volumes of organic solvents used for each separation and the overall chemical risk associated with each method, quantified by ChlorTox Scale and CHEMS-1 tool. Overall, the lowest chemical risk values were obtained for the C18 stationary phase, which is related to

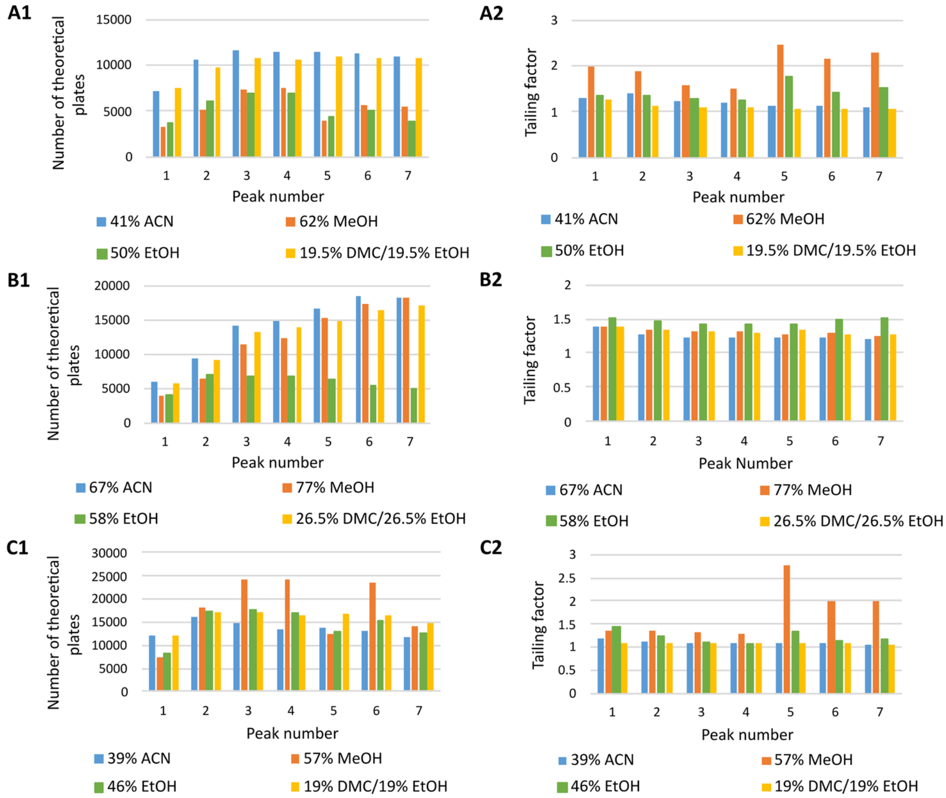

Fig. 4 Number of theoretical plates (1) and tailing factor (2) for components of a hydrophobic mixture separated on Fortis SpeedCore Diphenyl (A), C18 (B), and Kinetex F5 (C) columns.

Table 2 Volumes of organic solvents used for non-polar mixture separation and their greenness evaluation with ChlorTox Scale and CHEMS-1 tool

	Diphenyl			C18			F5		
	Volume [mL]	ChlorTox (WHN)	CHEMS-1 score	Volume [mL]	ChlorTox (WHN)	CHEMS-1 score	Volume [mL]	ChlorTox (WHN)	CHEMS-1 score
ACN	2.46	0.96	66.1	1.22	0.47	32.6	6.55	2.55	175.6
MeOH	2.69	1.53	42.2	1.61	0.92	25.3	11.00	6.27	172.2
EtOH	2.36	0.61	17	2.15	0.56	15.5	9.07	2.36	65.3
DMC/EtOH	2.60	0.51	9.4	1.14	0.22	4.1	3.68	0.72	13.2

the lowest amounts of mobile phases used (and short analysis times). According to the ChlorTox Scale, methods using methanol pose the highest risk, while CHEMS-1 rated methods using acetonitrile the most harmful. The application of green solvents generally reduced the risk, especially for methods with DMC, which received the lowest ChlorTox values

Table 3 The results of TOPSIS rankings with CHEMS-1 and ChlorTox risk assessment data for non-polar compounds separation

Rank	CHEMS-1 ranking	Similarity to ideal solution	Rank	ChlorTox ranking	Similarity to ideal solution
1	C18 26.5% DMC + 26.5% EtOH	0.854	1	C18 67% ACN	0.876
2	C18 77% MeOH	0.837	2	C18 26.5% DMC + 26.5% EtOH	0.872
3	C18 58% EtOH	0.825	3	C18 77% MeOH	0.849
	Diphenyl 50% EtOH	0.822	4	C18 58% EtOH	0.848
5	C18 67% ACN	0.818	5	Diphenyl 50% EtOH	0.844
6	Diphenyl 19.5% DMC + 19.5% EtOH	0.816	6	Diphenyl 19.5% DMC + 19.5% EtOH	0.836
7	Diphenyl 62% MeOH	0.754	7	Diphenyl 41% ACN	0.827
8	F5 19% DMC + 19% EtOH	0.731	8	Diphenyl 62% MeOH	0.763
9	Diphenyl 41% ACN	0.687	9	F5 19% DMC + 19% EtOH	0.758
10	F5 46% EtOH	0.427	10	F5 39% ACN	0.489
11	F5 57% MeOH	0.175	11	F5 46% EtOH	0.472
12	F5 39% ACN	0.163	12	F5 57% MeOH	0.152

and CHEMS-1 scores not only in methods that used the lowest amounts of the organic mixture but also in the case of the diphenyl column where the highest volume of this organic modifier was used.

TOPSIS analysis results for non-polar compounds separation

The results of TOPSIS ranking presented in Table 3 demonstrate the performance of each chromatographic system when greenness and chromatographic separation quality of mixture of non-polar compounds are simultaneously considered. The rankings for two scenarios differ in the details because of different assumptions in CHEMS-1 and ChlorTox models but the general patterns are similar. It is clear that the first discriminator in selection of green and efficient separation is the type of stationary phase. Here the selection order is C18, diphenyl and F5 stationary phases. A closer look on the values of similarities to ideal solution is also needed. For both rankings it is seen that the overall performance is comparable for all C18 and diphenyl conditions and when DMC/EtOH mobile phase is applied with F5 stationary phase. For the remaining three mobile phases with F5 stationary phase the overall performance is indicatively lower. The second discriminator in the ranking is the type of mobile phase. The general pattern is that DMC/EtOH mobile phase is preferential, following are both alcohols and ACN. Not all alternatives follow this scheme what shows that the assessment of mobile phase together with stationary phase is needed. None of the chromatographic conditions reached the value of similarity to ideal solution equal to 1 what means that other alternatives have better performance within some of the assessment criteria.

Separation of polar mixture

In the separation processes conducted for the polar mixture using MeOH, ACN, EtOH, and DMC as organic solvents, successful separation was achieved with all of the four solvents when mixed in appropriate volume ratios with water: 15% MeOH/85% water, 2% ACN/98% water, 8% EtOH/92% water, and 1% DMC/99% water on diphenyl stationary phase; 15% MeOH/85% water, 2% ACN/98% water, 6% EtOH/94% water, and 1% DMC/99% water on C18 stationary phase and 16% MeOH/84% water, 8% ACN/92% water, 7% EtOH/93% water, and 3% DMC/97% water on F5 stationary phase. In the case of

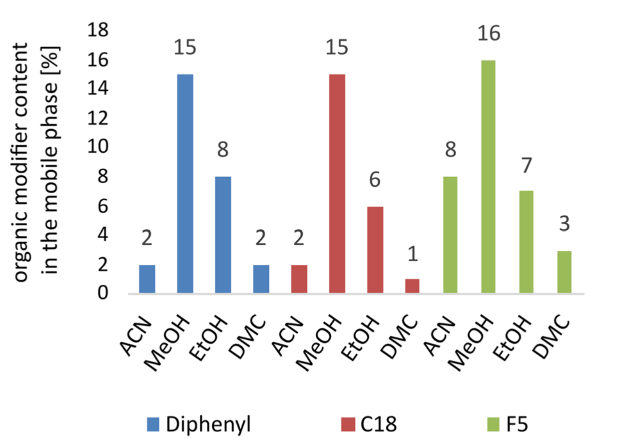

Fig. 5 Concentrations of organic solvent allowing the best separation of polar mixture.

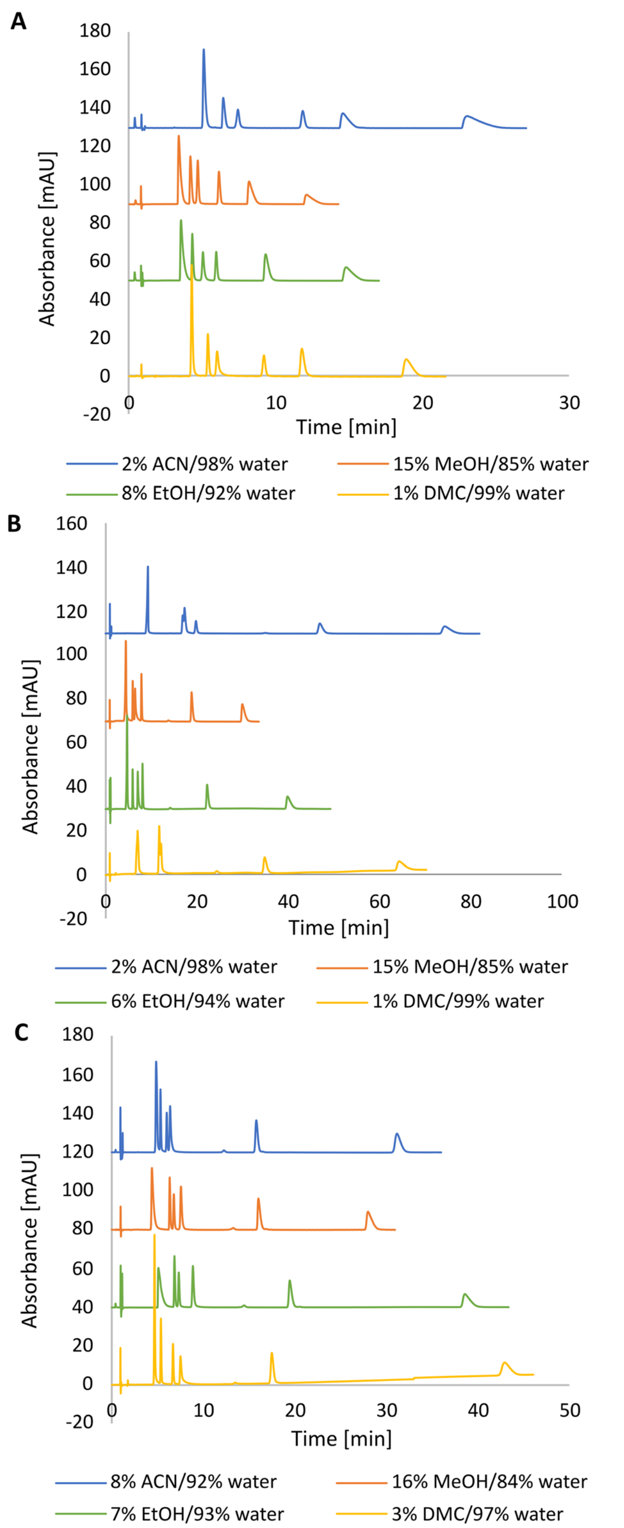

Fig. 6 Chromatograms of polar mixture separation obtained on Fortis SpeedCore Diphenyl (A), C18 (B), and Kinetex F5 (C) columns.

mobile phases containing less than 10% of one of the solvents, the solvent was pre-diluted with water (in the appropriate volume ratio) to ensure mixing precision and achieve optimal and reproducible separation. Results are listed in Fig. 5. But, what is important to mention is that separating a polar mixture presents significantly different challenges than separating a non-polar one.

Without pH stabilization provided by mobile phase additives, such as phosphate buffers or ammonium salts, polar substances often exhibit peak tailing, complicating baseline separation. Despite these challenges, the separation of polar benzene derivatives was mostly successful (see Fig. 6). In two cases, one for the acetonitrile, second for the dimethyl carbonate, complete separation could not be achieved, since reducing the organic solvent percentage in the mobile phase significantly increased analysis time and still did not provide successful separation.

To obtain comprehensive data on each component of the mixture (and perform TOPSIS analysis), Peak Deconvolution Analysis (i-PDeA II) was applied in the case where DMC did not separate all compounds (on the C18 stationary phase). Using photodiode array (PDA) detectors, this unique software feature uses 3D PDA data to resolve peaks that are not separated on-column. Peak deconvolution is particularly useful for analyzing co-eluting compounds by reconstructing individual component peaks, enabling detailed analysis even in complex separations.[60,61]

As expected, among the stationary phases tested, the diphenyl phase provided the most optimal results across all four solvents, with higher selectivity and the shortest separation times. In contrast, the C18 phase showed the longest retention times and the greatest separation challenges, particularly with acetonitrile and DMC, which could not fully separate the critical pair of compounds. However, this outcome is understandable, as C18 phases are not optimized for polar substances, especially without buffer additives in the mobile phase. The F5 column performed moderately well, offering satisfactory separation of all compounds, but requiring a longer analysis time.

When comparing solvents, dimethyl carbonate showed generally superior separation efficiency when paired with the diphenyl and F5 phases (see Fig. 7, part 1). DMC also provided similar peak symmetry among the solvents tested, especially on the C18 and F5 phases and poorer peak shapes on the diphenyl phase (see Fig. 7, part 2). Unfortunately, in combination with the C18 phase, this green solvent performed the worst as it failed to fulfill the primary purpose – it was impossible to separate not only the critical pair of compounds but also the two other components of the mixture. Also it can be concluded from the lowest separation efficiency of the first two compounds.

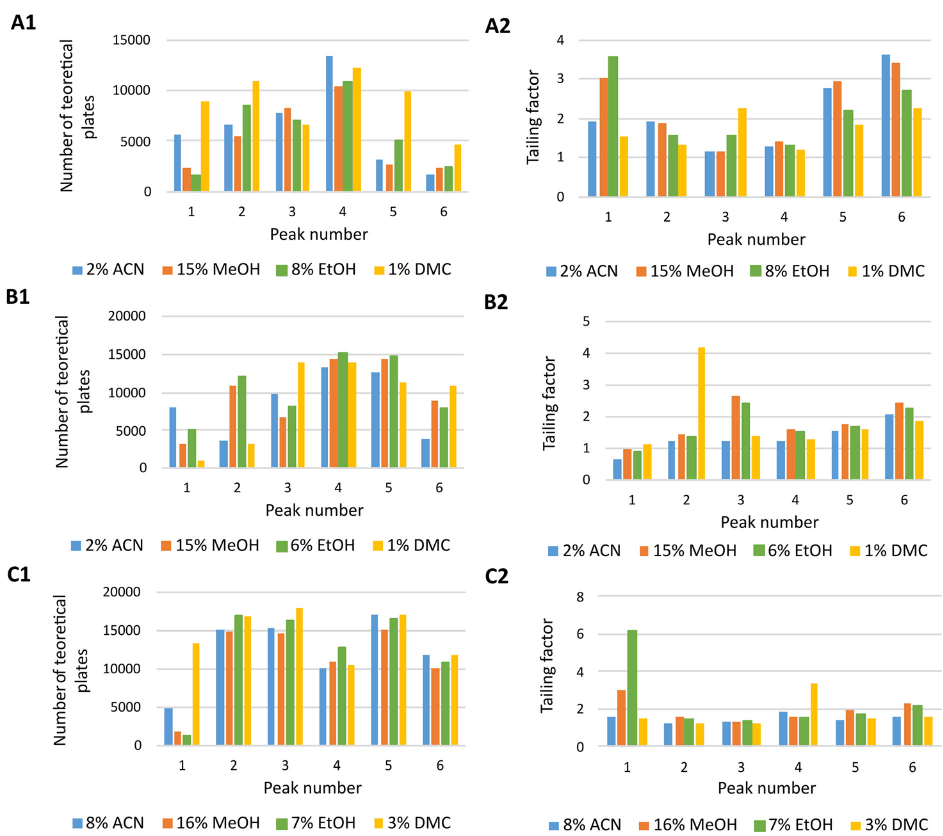

Fig. 7 Number of theoretical plates (1) and tailing factor (2) for components of a polar mixture separated on Fortis SpeedCore Diphenyl (A), C18 (B), and Kinetex F5 (C) columns.

When it comes to alcohols, both performed similarly in terms of peak symmetry (typically, however, with a slight advantage for ethanol) and separation efficiency, though ethanol showed slightly better separation efficiency than methanol, especially on the C18 and F5 phases. The least favorable results for ethanol were observed for the F5 stationary phase due to the significantly high asymmetry of the first peak and rather long analysis time. However, alcohols achieved the shortest retention times for polar mixture components on the diphenyl and C18 phases, with methanol delivering the fastest analyses across all stationary phases, although its percentage in the mobile phase was about twice that of ethanol. Contrarily, ACN resulted in the longest analysis times when combined with the diphenyl and C18 phases, (moreover, it could not satisfactorily separate the critical pair of compounds on C18 stationary phase). On the other hand, comparing the separation efficiency of acetonitrile, it presented similar to the other organic solvents when combined with the F5 phase except for the first peak, where dimethyl carbonate presented a considerably better result. Comparable conclusions can be also drawn for the symmetry of the peaks, where all solvents obtained similar asymmetry factors – except for the first peak.

The volumes of organic solvents used for separating the polar mixture and the overall chemical risk quantified for the particular methods using the ChlorTox Scale and CHEMS-1 tool are presented in Table 4. In general, the lowest harmfulness values were obtained for the diphenyl stationary phase, which is related to the lowest amounts of mobile phases used (short analysis times). As with non-polar mixtures, according to the ChlorTox Scale, methods using methanol present the highest risk. However, this time the CHEMS-1 tool also rated methanol most unfavorably. According to the ChlorTox Scale, the use of ethanol generally provided similar results to ACN, due to the higher consumption volumes of ethanol. In contrast, the CHEMS-1 tool discriminated far better between these two solvents – with an advantage for ethanol. The use of dimethyl carbonate significantly reduced chemical risk since the lowest ChlorTox values and CHEMS-1 scores of zero were obtained.

These results suggest that DMC can be an effective solvent for the analysis of polar substances, with an added advantage over acetonitrile and methanol in terms of lower environmental impact. The minimal chemical risk associated with DMC further underscores that analyses of polar substances can be both more efficient and sustainable by using this green solvent. The results obtained for the most commonly used octadecyl stationary phase indicate, as seen with the non-polar mixture, that when replacing conventional solvents with greener alternatives, it is beneficial to consider and test columns with different packings, as alternative solvents may achieve better performance on different stationary phases.

TOPSIS analysis results for polar compounds separation

Table 5 presents the ranking results of separation quality of the mixture of polar compounds with different stationary and mobile phases. Similarly to rankings for the separation of non-polar compounds mixture the first discriminator is the type of stationary phase. However, the order is as such since the performance of diphenyl it the highest, while there is no clear differentiation between the two other stationary phases. More clear is the differentiation of mobile phase types as DMC and

Table 4 Volumes of organic solvents used for polar mixture separation and their greenness evaluation with ChlorTox Scale and CHEMS-1 tool

	Diphenyl			C18			F5		
	Volume [mL]	ChlorTox (WHN)	CHEMS-1 score	Volume [mL]	ChlorTox (WHN)	CHEMS-1 score	Volume [mL]	ChlorTox (WHN)	CHEMS-1 score
ACN	0.14	0.06	3.86	0.38	0.15	10.09	0.64	0.25	17.23
MeOH	0.59	0.34	9.25	1.16	0.66	18.23	1.16	0.66	18.19
EtOH	0.38	0.10	2.73	0.61	0.16	4.41	0.69	0.18	4.98
DMC	0.06	0.02	0	0.16	0.02	0	0.33	0.04	0

Table 5 The results of TOPSIS rankings with CHEMS-1 and ChlorTox risk assessment data for polar compounds separation

Rank	CHEMS-1 ranking	Similarity to ideal solution	Rank	ChlorTox ranking	Similarity to ideal solution
1	Diphenyl 2% DMC	0.810	1	Diphenyl 2% DMC	0.823
2	Diphenyl 8% EtOH	0.762	2	Diphenyl 2% ACN	0.790
3	Diphenyl 2% ACN	0.732	3	Diphenyl 8% EtOH	0.778
4	F5 3% DMC	0.699	4	F5 3% DMC	0.714
5	C18 6% EtOH	0.644	5	C18 6% EtOH	0.666
6	Diphenyl 15% MeOH	0.625	6	F5 7% EtOH	0.643
7	F5 7% EtOH	0.621	7	F5 8% ACN	0.633
8	C18 1% DMC	0.549	8	Diphenyl 15% MeOH	0.615
9	F5 8% ACN	0.404	9	C18 1% DMC	0.579
10	F5 16% MeOH	0.402	10	C18 2% ACN	0.515
11	C18 15% MeOH	0.395	11	F5 16% MeOH	0.372
12	C18 2% ACN	0.365	12	C18 15% MeOH	0.365

EtOH (with indication on DMC) have better overall performance over MeOH and ACN. The ranking results again show that the optimization of mobile phase and stationary phase system should be performed simultaneously, not one after another. As with the results for the non-polar mixture, none of the chromatographic conditions reached the value of similarity to ideal solution equal to 1.

Conclusions

This study investigates alternative approaches to liquid chromatography, exploring more sustainable solvent and stationary phase combinations, which supports global initiatives such as the European Green Deal, which aims to achieve climate neutrality by 2050 and stimulate economic growth through green technologies. The potential of greener solvents – ethanol and dimethyl carbonate – to replace conventional solvents, such as acetonitrile and methanol, was systematically evaluated across three chromatographic stationary phases: C18, diphenyl, and perfluorinated phenyl. By comparing solvent performance for both non-polar and polar analyte mixtures, it was observed that greener alternatives, particularly DMC and its mixture with ethanol, often achieved comparable or superior separation efficiency, peak symmetry, and environmental impact when combined with the appropriate stationary phase.

The multicriteria decision analysis using the TOPSIS algorithm reveals that the selection of the optimal chromatographic configuration for both non-polar and polar compound mixtures strongly depends on the combined choice of stationary and mobile phases. The TOPSIS analysis allowed to consider together many conflicting assessment criteria to select the efficient and green chromatographic system for separation of the components of both mixtures.

Overall, DMC and EtOH have shown promise as environmentally friendly alternatives capable of achieving effective and sustainable separations of compounds with differing polarity. These studies provide a better understanding of the chromatographic properties of dimethyl carbonate and confirm the potential for greener analytical methods in liquid chromatography without compromising separation quality.

While water remains the safest solvent in liquid chromatography, both, the ChlorTox Scale and CHEMS-1 confirms that ethanol and dimethyl carbonate are considerably safer for both human health and the environment compared to MeOH and ACN. However, solvent safety alone does not ensure suitability as an eluent in liquid chromatography; it must also deliver satisfactory separation parameters such as retention, selectivity, efficiency, and peak symmetry, to be considered feasible for analytical applications.

Author contributions

Conceptualization, S. B., O. K., M. T.; methodology, O. K., G. H., M. T.; validation, S. B., O. K., investigation, O. K., G. H., M. T.; resources, S. B.; data curation, S. B.; writing – original draft preparation, S. B.,O. K., M. T.; writing – review and editing, S. B., O. K., M. T.; visualization, O. K.; supervision, S. B.; funding acquisition, S. B. All authors have read and agreed to the published version of the manuscript.

Data availability

The data presented in this study are available on request from the corresponding author.

Conflicts of interest

There are no conflicts to declare.

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