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Dimethyl carbonate as a green alternative to acetonitrile in reversed-phase liquid chromatography. Part I: Separation of small molecules

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ABSTRACT

Nowadays, environmental problems are drawing the attention of governments and international organisations, which are therefore encouraging the transition to green industrial processes and approaches. In this context, chemists can help indicate a suitable direction. Beside the efforts focused on greening synthetic approaches, currently also analytical techniques and separations are under observation, especially those employing large volumes of organic solvents, such as reversed-phase liquid chromatography (RPLC). Acetonitrile has always been considered the best performing organic modifier for RPLC applications, due to its chemical features (complete miscibility in water, UV transparency, low viscosity etc); nevertheless, it suffers of severe shortcomings, and most importantly, it does not fully comply with Environmental, Health and Safety (EHS) requirements. For these reasons, alternative greener solvents are being investigated, especially easily available alcohols.

In this work, chromatographic performance of the most common solvents used in reversed-phase chromatography, i.e., acetonitrile, ethanol and isopropanol, have been compared to a scarcely used solvent, dimethyl carbonate (DMC). The analytes of interest were two small molecules, caffeine and paracetamol, whose kinetics and retention behaviour obtained with the four solvents have been compared, and all contributions to band broadening have been assessed. Results about kinetic performance are very promising, indicating that a small amount (7 % v/v) of DMC is able to produce the same efficiency as a 2.5-times larger ACN volume (18 % v/v), and larger efficiency than alcohols.

This paper reports, for the first time, fundamental studies concerning the mass transfer phenomena when DMC is used as an organic solvent in RPLC, and, together with the companion paper, represents the results of a research whose final aim was to discover whether DMC is suitable for chromatographic applications both in linear and preparative conditions.

1. Introduction

In recent years, the awareness of modern society on global threats such as climate change and environmental issues has constantly increased. Governments and authorities have promoted specific programmes to enable sustainable development, such as the European Green Deal which aims to reach climate neutrality by 2050 and to boost the economy through green technologies [1].

In this context, the twelve principles of Green Chemistry represent the basis to develop sustainable processes for the production of materials and goods [2–5]. In particular, the replacement of harmful solvents with greener ones (which is directly mentioned in the 5th principle and indirectly referred to in almost every other) has become one of the main driving factors in laboratories and industries to enable a sustainable growth. This special attention is attributable to the fact that large volumes of toxic, flammable, or hazardous solvents are handled daily in

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chemical processes. These characteristics are often related to specific requirements needed for the application. For instance, volatile solvents are often employed for extraction or purification purposes since they can be easily evaporated, but they can generate unwanted air emissions which increase the risk of workers exposure.

Early efforts to substitute toxic solvents have been especially focused on production/synthetic processes which could have the greatest environmental impact. However, in the last few years, there has been a growing interest in applying Green Chemistry principles to almost every sector of chemistry. One of these areas is analytical chemistry, by considering, especially, analytical laboratories where liquid chromatography (LC) is routinary employed. Indeed, it is estimated that a liquid analytical chromatography instrument, using a conventional LC column (15-25 cm long, 4.6 mm diameter, packed with 5 μm particles) at a mobile phase flow rate of 1 mL/min, produces about 1.5 L of waste per day, which means about 500 L of effluent per year. Therefore about 26-50 million litres of chemical waste are generated every year worldwide [6,7].

For these reasons, Green Analytical Chemistry (GAC) is increasingly considered a new and important sub-area of Green Chemistry, which has started emerging in the 2000s [8]. The goal of GAC is to adapt the concepts of Green Chemistry (which have been specifically formulated for synthetic processes) to the field of analytical chemistry to reduce its environmental impact without compromising outcome and performance [2]. Obviously, the ideal solution would be the development of innovative solventless analytical procedures, possibly based on the only use of aqueous solutions, or the recycling of the solvent in order to produce a minimal amount of waste and to reduce the environmental impact of the process. However, many analytical processes require the use of organic solvents, and, in those cases, GAC suggests replacing toxic, harmful chemicals with greener alternatives [2,4,6].

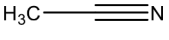
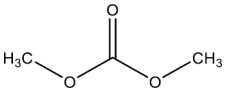
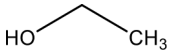
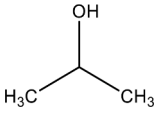
Among these processes it is worth mentioning separations in reversed-phase LC (RPLC) conditions, which represent the most popular mode of chromatography for quantitative and preparative applications, being estimated to be used in almost 80 % of the cases [9,10]. RPLC is based on the use of a hydrophobic stationary phase (typically made of silica particles functionalized with C_{18} or C_8 chains) and a more polar mobile phase, which is usually a mixture of water and an organic modifier. Acetonitrile (ACN) is by far the most employed organic solvent for RPLC due to its intrinsic properties such as low viscosity, excellent elution strength, UV-transparency (cut-off wavelength at 190 nm, see Table 1), and complete miscibility with water. Despite these characteristics, which are very convenient from a chromatographic point of view, acetonitrile also possesses other properties that make it unsuitable from a GAC perspective. Indeed, it shows dramatic shortcomings associated with the production of acrylonitrile, since ACN is produced as a by-product of its production [9,10]. Furthermore, ACN is associated to toxic effects on the human body due to the metabolic release of cyanide. For these reasons, the replacement of ACN with greener alternatives is becoming an urgent priority.

The greenness of an organic solvent can be defined on the basis of Environmental, Health and Safety (EHS) requirements and Life-Cycle Assessment (LCA). The first criterion comprehends environmental indicators (such as persistence and water/air hazards), health parameters (e.g., chronic toxicity) and safety specifications (including flammability, explosion risk and stability). LCA, on the other hand, evaluates the effects of a product on the environment over the entire period of its life, thus including production, uses, disposal and potential recycling. In order to help chemists in the choice of a green option, many pharmaceutical companies have drawn up solvent selection guides, which rank solvents according to their “greenness” [11,12]. Among these, ethanol (EtOH), isopropanol (IPA), acetone, but also ethyl lactate and propylene carbonate are popular green solvents which have already been tested as eluents in RPLC [2,13–15].

Very recently, the attention of scientists has also focused on other less popular options such as organic carbonates, which show a very low eco-toxicity and are completely biodegradable [16]. An example of these organic compounds is dimethyl carbonate (DMC). This is a nonpolar flammable transparent liquid, with a similar smell to that of methanol. It does not have irritating or mutagenic effects; therefore, it can be safely handled. It is extensively studied for applications in several product groups: synthesis of pharmaceuticals (such as taladafil [17]), coatings, and lithium-ion battery electrolytes [18]. In addition, it is commonly used as an environmentally friendly substitute of dimethyl sulphate and methyl halides for acid-catalysed carboxymethylation reactions [19]. According to EHS criteria, DMC is considered among the "recommended” solvents, therefore it belongs to the same class of water and alcohols [11]. However, the application of DMC in chromatography is still very limited. The first report is dated 2021, where this solvent has been used as an organic modifier in RPLC for the separation of different probe molecules of pharmaceutical interest by using inductively coupled mass spectrometry (ICP-MS) as detection method. It was found that, thanks to its higher hydrophobicity, a lower percentage of DMC was necessary to elute analytes in comparison to MeOH or ACN, allowing for a better stability of the plasma [20]. The other recent paper about DMC reports on its use as an organic solvent for normal phase LC (NPLC) and hydrophilic interaction chromatography (HILIC). The authors reported that DMC is a weaker solvent both for NPLC and HILIC applications, at least under the experimental conditions used in that case, but it allowed to obtain slightly better efficiencies [21].

In this work, fundamental studies concerning retention behaviour and kinetic performance of two different probe molecules, namely

Table 1
Chemo-physical characteristics of ACN, DMC, EtOH and IPA [42].

Properties	Acetonitrile (ACN)	Dimethyl carbonate (DMC)	Ethanol (EtOH)	Isopropanol (IPA)
Structure				
Pictograms				
Molecular weight (g/mol)	41.05	90.08	46.07	60.10
log K_{ow}	-0.34	0.35	-0.31	0.05
Boiling point (°C)	81.6	90.4	78.2	82.3
Melting point (°C)	-43.8	4.7	-114.1	-89.5
Density at 25°C (g/cm^3)	0.786	1.069	0.785	0.781
Viscosity at 25°C (mPa•s)	0.334	0.589	1.074	2.038
Cut-off (nm)	190	220	210	210

paracetamol and caffeine, were investigated under RPLC conditions with UV-Vis detection by using different organic modifiers in aqueous solutions, including the innovative DMC. Particularly, DMC performance was compared to traditional RPLC solvents, such as ACN, EtOH and IPA. A detailed investigation of all the contributions to band broadening has been performed in order to unravel mass transfer phenomena and diffusion properties of the analytes in DMC/H_2O as well as in all the other mobile phase compositions.

To the best of our knowledge, this is the first work reporting on a fundamental study of retention mechanisms in RPLC by using DMC as an organic modifier and it represents the basis to further understand the possibility to also use this solvent for chromatographic applications also of industrial relevance. These further aspects will be the object of the second part of this work.

2. Theory

2.1. Efficiency

The evaluation of kinetic performance of a chromatographic column is commonly done through the well-known van Deemter equation which correlates the efficiency, in terms of plate height (H), to the mobile phase linear velocity. The reduced version of this equation involves the use of adimensional coordinates which allow the evaluation of kinetic performance independently from particle diameter (d_p) and type of analyte:

$$h = a(\nu) + \frac{b}{\nu} + c_s\nu \tag{1}$$

being h ($=H/d_p$) the reduced plate height and ν the reduced interstitial velocity, expressed as:

$$\nu = \frac{F_v d_p}{\pi r^2 \varepsilon_e D_m} \tag{2}$$

where F_v is the flow rate, r the column radius, D_m the molecular coefficient of the analyte in the mobile phase and ε_e the interstitial porosity of the packed bed. The latter is defined as the ratio between the interstitial volume, V_e (which can be calculated through Inverse Size Exclusion Chromatography [22]), and the geometrical volume of the column, V_{col}.

The three terms appearing in Eq. (1) refer to the main sources of band broadening in a chromatographic column and they can be independently evaluated. The b-term is the longitudinal diffusion which is defined as:

$$b = 2(1+k_1)\frac{D_{eff}}{D_m} \tag{3}$$

where D_{eff} is the effective diffusion coefficient in the porous zone (see later on) and k_1 is the zone retention factor, which is referred only to the interstitial volume. It is correlated to the retention factor k with the following relationship:

$$k_1 = \frac{(1+k)\varepsilon_t}{\varepsilon_e} - 1 \tag{4}$$

being ε_t the total porosity, which is the ratio between the void volume, V_0, and V_{col}.

The interpretation of D_{eff} in light of a proper model of diffusion in porous media [23–28] allows to evaluate the different contributions to diffusion in the adsorbent, including the intraparticle diffusivity, D_{part}, which accounts for diffusion in the intraparticle volume. In this work, the more advanced Effective Medium

Theory (EMT) [23–25,27] has been employed. According to the Maxwell's model, D_{eff} can be written as:

$$D_{eff} = \frac{1}{\varepsilon_e(1+k_1)}\left[\frac{1+2(1-\varepsilon_e)\beta}{1-(1-\varepsilon_e)\beta}\right]D_m \tag{5}$$

where β is the polarizability constant, defined as:

$$\beta = \frac{\alpha_{part}-1}{\alpha_{part}+2} \tag{6}$$

and α_{part} is the relative permeability:

$$\alpha_{part} = \frac{D_{part}K_{part}}{D_m} = \frac{D_{part}k_1\varepsilon_e}{D_m(1-\varepsilon_e)} \tag{7}$$

with D_{part} the diffusion coefficient through the porous particles and K_{part} the whole particle-based equilibrium distribution constant.

The c_s-term reported in Eq. (1) accounts for the solid-liquid mass transfer resistance, which represents the main source of band broadening at high flow rates. Its expression is the following:

$$c_s = \frac{1}{30}\,\frac{k_1}{(1+k_1)^2}\,\frac{D_m}{D_{part}} \tag{8}$$

$a(\nu)$ can be calculated by subtraction of b- and c_s-terms from accurately measured h-values:

$$a(\nu) = h - \frac{b}{\nu} - c_s\nu \tag{9}$$

3. Materials and methods

3.2. Columns and materials

Caffeine and paracetamol were purchased from Merck Sigma-Aldrich (Darmstadt, Germany). All solvents and reagents were purchased from Carlo Erba Reagents (Milan, Italy), except for DMC which was from Thermo Fisher Scientific (Waltham, Massachusetts, USA), with a purity $\geq$ 99 %. A Kromasil C18 column (250×4.6 mm i.d.) packed with 5 µm fully porous particles was purchased from Merck Sigma-Aldrich (Darmstadt, Germany). A 33 × 4.6 mm Micra column (Eprogen, Inc., Downers Grove, IL, USA) packed with 1.5 µm non-porous silica particles was purchased from DBA Italia s.r.l. (Milan, Italy) and employed for the estimation of bulk molecular diffusion coefficients.

3.2. Equipment

All measurements were carried out on an Agilent 1290 Infinity LC System, equipped with a binary solvent pump (max pressure: 1200 bar), a column thermostat, an autosampler and a photodiode array detector. Detection wavelength was 254 nm. Temperature was set at 25°C.

3.3. Retention studies

The dependence of the retention factor, k, on the fraction of organic modifier, Φ, was evaluated at different mobile phase compositions for two small molecules (paracetamol, caffeine). ACN, DMC, IPA and EtOH were investigated as organic modifiers. It is worth noting that Φ_{DMC} ranged from 0.01 to 0.1 due to its miscibility limit in water. The flow rate was 1 mL/min. Injection volume was 0.5 µL. Retention factors, k, were corrected for the extra-column residence time. The hold-up time, t_0, was calculated with inverse size exclusion chromatography, ISEC (for further details the reader is referred to Ref. [29]).

3.4. Peak parking experiments

Peak parking measurements were employed for the purpose of estimating diffusion coefficients, D_{eff} and D_m, of caffeine and paracetamol with all the organic solvents tested in this study (ACN, DMC, IPA, EtOH)

[30,31]. D_{eff} was calculated by considering that the spatial peak variance $\Delta\sigma_x^2$ is directly proportional to the parking time, t_{park}, through the following relationship:

$$\Delta\sigma_x^2 = 2D_{eff}t_{park} \quad (10)$$

Experimentally, spatial peak variance was calculated as $\Delta\sigma_x^2 = L^2/N$, where L is the column length and N the number of theoretical plates given by the software (determined with the method of moments). All the data obtained were corrected for the extra-column peak variance. Parking times employed were 0, 2, 5, 7, 10, 15, 20, 25, and 30 min and the flow rate was 0.2 ml/min.

Molecular diffusion coefficients, D_m, of probe molecules in all the solvents mentioned above were estimated by performing peak parking experiments in a column packed with non-porous particles (Micra column) at a flow rate of 0.2 ml/min.

In this case:

$$D_m = \frac{D_{eff}}{\gamma_e} \quad (11)$$

where γ_e is the external obstruction factor, a geometrical parameter which describes the constriction and the tortuosity of inter-particle channels [32]. In order to determine the value of γ_e an experiment of peak parking was carried out using a molecule whose D_m is known from literature. Thiourea dissolved in pure water was chosen for this purpose ($D_m = 1.33 \times 10^{-5}$ cm^2/s) [33]. The value of γ_e was found to be 0.65.

3.5. van Deemter curves measurements

van Deemter curves were measured for each organic modifier by choosing an appropriate mobile phase composition to keep the retention factors of paracetamol and caffeine constant at around 0.65 and 1.20, respectively. The mobile phase compositions were the following: (i) ACN/H_2O 18:82 % (v/v); (ii) DMC/H_2O 7:93 % (v/v); (iii) EtOH/H_2O 18:82 % (v/v) and (iv) IPA/H_2O 10:90 % (v/v).

All measurements were performed through stepwise increments of flow rate, starting from 0.02 mL/min, up to 1 ml/min with ACN and DMC or up to 0.7 ml/min with EtOH and IPA, due to higher back pressures. Retention time and column efficiency (given as number of theoretical plates) of eluted peaks were automatically processed by the software (calculated through the method of moments) and corrected for the extra-column contribution.

4. Results and discussion

Firstly, physico-chemical properties of DMC were evaluated in order to understand possible advantages and limitations related to its applicability in LC. By looking at Table 1, it can be evinced that DMC is characterised by a cut-off wavelength of 220 nm, which is the highest among the four solvents that were considered in this study. This could represent a limitation when dealing with complex samples that would require wavelength below this value but for most applications in LC (involving molecules with aromatic rings or with absorption maxima at higher wavelengths) it does not represent an issue. Also, it must be kept in mind that the maximum amount of DMC in an aqueous mobile phase is around 10 %, therefore the baseline noise caused by DMC absorption at wavelengths below 220 nm should be very limited.

DMC shows a higher boiling point and density if compared to the other three solvents, an intermediate viscosity between ACN and EtOH, but no harmful effects. These points should be carefully evaluated when considering the overall greenness, environmental impact, and feasibility of a chromatographic method. Furthermore, the energy and material required for the production of the organic solvent has to be taken into account. In this contest, lowering the amount of organic solvent used into the chromatographic run, such in the case of DCM (max 10 % v/v is used), will lower the environmental impact coming from its production. On the basis of these points, DMC can be considered a comparable, even if not better in some feature, alternative solvents from a greenness point of view respect to the other ones investigated in our study.

4.1. Effect of mobile phase composition on retention

The dependence of the retention factor on the amount of organic modifier (Φ) was evaluated for caffeine and paracetamol (Fig. 1) by using the four different organic modifiers (ACN, DMC, IPA, EtOH).

In reversed phase chromatography, retention decreases by increasing the percentage of organic modifier in mobile phase due to the increase of solubility and affinity of analytes with the organic-rich phase, or in other words, to the reduction of the partition coefficient [34,35]. By comparing the figures, it can be evinced that DMC shows the highest elution strength in all cases, indeed, for the same Φ, DMC shows smaller k if compared to other solvents. This represents a clear advantage, especially from the industrial and large-scale viewpoint, in terms of environmental impact, solvent consumption and solvent disposal costs, since a limited amount of this solvent would be sufficient for the elution.

This behaviour can be explained by taking into account the polarity, in terms of log K_{ow} (Table 1), of the organic modifiers used. From these data it is clear that EtOH and ACN are the most polar solvents, having very low (and similar) values of log K_{ow} (around -0.3), followed by IPA (0.05) and DMC (0.35). Therefore, DMC polarity is the closest to the C_{18}

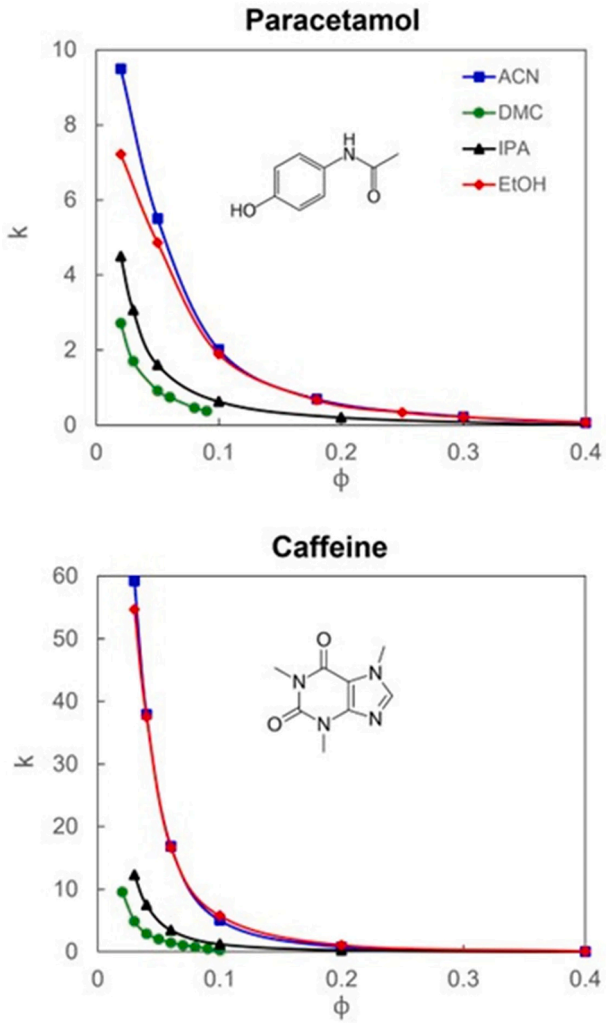


Fig. 1. Dependence of the retention factor (k) on the amount of organic modifier (Φ) for paracetamol (top) and caffeine (bottom). ACN (blue squares), DMC (green points), IPA (black triangles) and EtOH (red diamonds).

stationary phase, with respect to the other solvents. This leads to a decrease of the partition coefficient, hence of retention, since analytes show high affinity towards DMC.

4.2. Efficiency

The effect of organic modifiers on efficiency was evaluated for the two small molecules (caffeine and paracetamol) by calculating their van Deemter curves (Fig. 2). Firstly, from these plots it can be evinced that, for paracetamol ($k = 0.65$), relatively good kinetic performance (with h values as low as 2.6) was achieved with all solvents. It is worth noting that, at high ν, IPA leads to the best efficiency, followed by ACN and DMC, while EtOH leads to the worst performance. Conversely, van Deemter curves of caffeine ($k = 1.20$) are not overlapping. In this case, a different trend is observed, indeed IPA leads to the worst kinetic performance. As an example, at $\nu = 20$, h values of 8.5, 7.0, 5.3 and 5.6 were measured for IPA, EtOH, ACN and DMC, respectively. Moreover, the c-branch of van Deemter curves obtained with alcohols for the two molecules are parallel, with almost the same performance obtained with EtOH. Surprisingly, IPA leads to very high efficiency for paracetamol but, when the analyte is caffeine, a huge loss of efficiency is observed.

It is noteworthy that van Deemter curves of ACN and DMC are perfectly superimposable, independently from the probe molecule under study, indicating that DMC is able to produce the same efficiency as ACN. In this case, the c-branch is steeper for paracetamol.

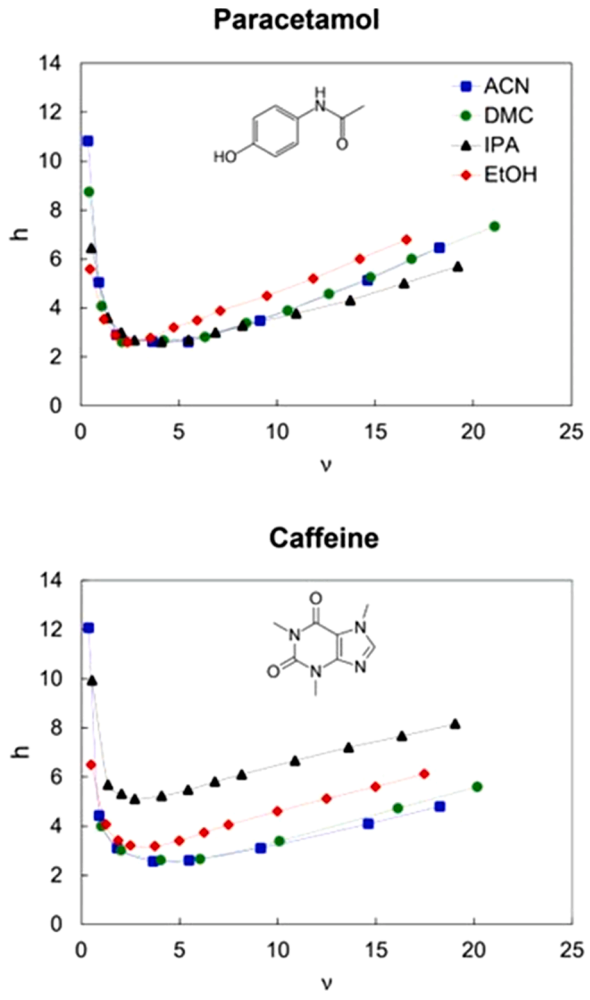


Fig. 2. van Deemter curves of paracetamol (top) and caffeine (bottom) using four different organic modifiers: ACN (blue squares), DMC (green points), IPA (black triangles) and EtOH (red diamonds).

In the following, the individual terms of the van Deemter equation (Eq. (1)) will be independently evaluated by combining stop-flow experiments (peak parking) and the Maxwell's model [23]. Once effective and molecular diffusion coefficients, D_{eff} and D_m, have been calculated through the peak parking method, longitudinal diffusion terms of the two probe molecules were then evaluated through Eq. (3). The application of the Maxwell's model permits to calculate the diffusion in the porous zone (D_{part}), allowing to access the c_s term (Eq. (8)). All these data are reported in Table 2. As it can be easily noticed, diffusion coefficients depend only on the nature of the organic modifier used, being practically the same for the two sample molecules. As expected, b-terms are slightly larger for caffeine than for paracetamol, due to the higher retention. Overall, the b-term represents approximately 40-45 % of $h_{\min}$, a value in line with what is reported in literature [36]. The contribution of b on $h_{\min}$ is more pronounced for less viscous solvents, like ACN and DMC, with respect to EtOH and IPA (see Table 1). Alcohols, on the contrary tend to disfavour diffusion process due to their large viscosity, hence D_{eff} follows the trend: ACN > DMC > EtOH > IPA. The c_s-term represents only 2-3 % of h(min). From Eq. (8) one can notice that it depends on retention and on the ratio between D_m and D_{part}. The ratio D_m/D_{part}, and as a consequence c_s, follow the trend: IPA > EtOH > DMC > ACN.

Finally, $a(\nu)$ was calculated by subtracting b and c_s terms from h (Eq. (9)). The results are plotted in Fig. 3 for ACN and DMC (top) and alcohols (bottom). By this mean of representation, it can be easily evinced that, once again, ACN and DMC show the same eddy dispersion contributions for the two probes, with smaller values obtained for caffeine, result which is consistent with literature, where the higher the retention the smaller the eddy dispersion [37,38]. Nevertheless, when using alcohols, especially IPA, the opposite behaviour is observed. Indeed, the eddy dispersion contribution measured for caffeine with IPA is consistently higher than that of paracetamol. IPA turns out to be the solvent with the highest eddy dispersion for caffeine over the whole range of ν investigated, followed by EtOH. As an example, at ν= 19 the use of IPA leads to a 60 % loss of efficiency if compared to ACN or DMC.

In order to explain this behaviour, further aspects have been evaluated. Firstly, a more detailed description of molecular diffusion occurring on the surface of the porous particle has been investigated. To this end, the contributions of pore and surface diffusions to intraparticle diffusion have been separately calculated by assuming the parallel diffusion model [30,39]:

$$D_{part} = \varepsilon_p \gamma_p F(\lambda_m) D_m + (1 - \varepsilon_p) K_A D_s \tag{12}$$

with ε_p being the particle porous zone porosity, γ_p the internal obstruction factor, $F(\lambda_m)$ the hindrance diffusion factor, K_A the Henry's constant of adsorption and D_s the surface diffusion coefficient. Calculated D_s coefficients are shown in Table 2, where it can be noticed that: (i) D_s follows the trend already observed for other diffusion coefficients (D_{part} and D_{eff}), i.e. ACN > DMC > EtOH > IPA, (ii) caffeine shows D_s values which are more than 30 % smaller than paracetamol, (iii) D_s coefficients measured with ACN are more than 6-fold higher than with IPA for both molecules. These results indicate that solvents like ACN or DMC, thanks to their small viscosity, are able to speed up the molecule diffusion along the hydrophobic surface of the particle, while alcohols (EtOH and IPA) limit the surface mobility of the analytes. It is interesting to notice that the ratio D_{part}/D_s is systematically larger for caffeine with respect to paracetamol (Table 2). This may be linked to a stronger interaction between caffeine and the layer of organic modifier adsorbed on the surface of the porous particles, the so-called excess adsorption [40]. This effect is more pronounced with alcohols, probably due to the possibility to form hydrogen bonds. Caffeine, as a consequence, will spend more time on the stationary phase, with respect to paracetamol. This, in combination with a smaller contribution of surface diffusion that does not favour the reduction of concentration gradients originating from velocity variations occurring inside the packed bed, will cause an

Table 2
Effective (D_{eff}), molecular (D_m), particle (D_p) and surface (D_s) diffusion coefficients, longitudinal diffusion (b) and solid-liquid mass transfer resistance term (c_s) for caffeine and paracetamol measured for the four organic modifiers.

Compound	Paracetamol				Caffeine			
Organic modifier	ACN	DMC	EtOH	IPA	ACN	DMC	EtOH	IPA
D_{eff} (cm^2/s)	5.2×10^{-6}	4.1×10^{-6}	2.8×10^{-6}	2.4×10^{-6}	5.1×10^{-6}	4.1×10^{-6}	2.8×10^{-6}	2.3×10^{-6}
D_m (cm^2/s)	7.4×10^{-6}	6.5×10^{-6}	5.4×10^{-6}	4.9×10^{-6}	7.5×10^{-6}	6.6×10^{-6}	5.5×10^{-6}	4.9×10^{-6}
D_{part} (cm^2/s)	4.2×10^{-6}	2.9×10^{-6}	1.7×10^{-6}	1.3×10^{-6}	4.1×10^{-6}	3.1×10^{-6}	1.9×10^{-6}	1.5×10^{-6}
D_s (cm^2/s)	4.1×10^{-6}	2.8×10^{-6}	1.0×10^{-6}	0.6×10^{-6}	2.8×10^{-6}	1.7×10^{-6}	0.6×10^{-6}	0.4×10^{-6}
D_m /D_{part}	1.8	2.2	3.2	3.9	1.8	2.1	3.0	3.4
D_{part} /D_s	1.0	1.1	1.6	2.1	1.5	1.8	3.0	3.3
b	4.0	3.4	2.9	2.6	4.5	4.3	4.0	3.5
c_s	0.014	0.017	0.025	0.030	0.013	0.015	0.018	0.022

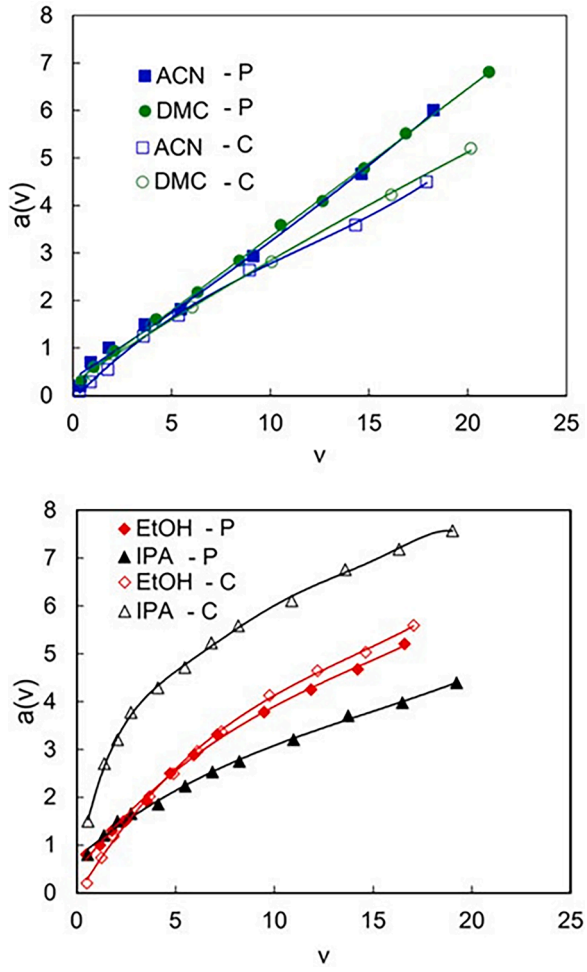


Fig. 3. Eddy dispersion curves ($a(\nu)$) of paracetamol (full points) and caffeine (empty points) measured with ACN (blue squares), DMC (green points), IPA (black triangles) and EtOH (red diamonds).

enhancement of eddy dispersion. This effect has been recently observed also in chiral chromatography [41].

5. Conclusions

In this work, for the first time, the evaluation and the comparison of kinetic properties and mass transfer characteristics of DMC with more traditional solvents commonly employed as organic modifiers in RPLC have been performed with two probe molecules. Results are very promising, indicating that DMC is able to produce comparable efficiency to ACN, obtaining the same retention with much smaller volume of solvent. On the other hand, efficiency measured with EtOH and IPA, which are considered common green alternatives to ACN, is deeply influenced by the analyte chemistry.

All these data indicate that DMC can be considered as an optimal candidate for the replacement of ACN in RPLC, since it leads to similar kinetic performance without detrimental effects on the column back-pressure, due to a very similar viscosity. The main issue is related to the scarce solubility of DMC with water, but this aspect cannot represent a critical drawback by considering the high elution strength of DMC.

Results of this work aim to lay the foundations for further studies involving the use of DMC for more specific fields, such as the separation of biomolecules of industrial interest and their purification through preparative LC. These aspects are discussed in the companion paper.

CRediT authorship contribution statement

Simona Felletti: Methodology, Investigation, Formal analysis, Visualization, Writing – original draft. **Matteo Spedicato:** Investigation, Writing – original draft. **Desiree Bozza:** Investigation, Validation. **Chiara De Luca:** Methodology, Writing – review & editing. **Francesco Presini:** Validation, Data curation. **Pier Paolo Giovannini:** Validation, Data curation. **Marco Carraro:** Methodology, Data curation, Resources. **Marco Macis:** Methodology, Data curation, Resources. **Alberto Cavazzini:** Supervision, Funding acquisition, Writing – review & editing. **Martina Catani:** Conceptualization, Visualization, Writing – original draft, Writing – review & editing. **Antonio Ricci:** Conceptualization, Resources, Supervision, Project administration, Writing – review & editing. **Walter Cabri:** Project administration, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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