

## Article

# Ethyl Lactate as a Greener Alternative to Acetonitrile in RPLC: A Realistic Appraisal

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## Abstract

Appropriate substitution of acetonitrile (ACN) in mobile phases for reversed-phase liquid chromatography (RPLC) by low toxicity, ecologically friendly alternative solvents emerges as a greener approach in separation sciences. Ethyl lactate is considered as a green solvent in organic synthesis, industrial extraction processes and many other applicative fields. Its ability to substitute ACN in mobile phases for RPLC applications was barely investigated. The feasibility of such a replacement was tested for the separation of the mixture of 16 polycyclic aromatic hydrocarbons listed by the Environmental Protection Agency. The analytical approach was found to be achievable, with some compromises in terms of elution order, peak efficiency and UV detectability. Thermodynamic aspects of the chromatographic process were also comparatively assessed. Correlations between the elution order and some molecular descriptors were also discussed.

## Introduction

The sense of a “green” chromatographic solvent expresses the goal to minimize the environmental impact resulting from its use in the separation process. The degree of greenness of an organic solvent is provided by its environmental, health and safety (EHS) properties and the life cycle assessment (LCA) (1, 2).

Chromatographic techniques have the potential to be greener during all steps of the analysis, from sample collection and preparation to separation and final determination. The approaches to get greener separations differ according to the type of chromatography. In liquid chromatography, the main direction focuses on the reduction of solvent consumption and replacement of toxic and environmentally hazardous solvents with more benign alternatives (3). The most popular green solvents are water, supercritical carbon dioxide (CO<sub>2</sub>) and ionic liquids. In spite of the increasing interest, less attention was given to bio-based solvents and today some of them are already commercialized, i.e., propylene carbonate, ethyl lactate (EL) and D-limonene (4).

The reduction in solvent consumption has been already discussed (5), and some environmental friendly solvents have been also used: water (6), ethanol or iso-propanol (7), supercritical CO<sub>2</sub> (8) and propylene carbonate (9–11).

EL is one of the most promising candidates among the known alternative green organic solvents (12, 13). It is *in vivo* readily metabolized to ethanol and lactic acid due to the esterase enzymatic activity. The ecotoxicity of EL is also very low, as it rapidly and completely undergoes biodegradability. In case of vapor release, EL is a non-ozone-depleting fluid. Its production from carbohydrate feedstock as starting materials makes it economically attractive. EL has been successfully used in industrial extractive processes (14), as dispersion solvent in magnetic tape coating (15), as co-solvent in organic synthesis (16, 17) or surfactantless microemulsions (18) and constituent in skin protective formulations. A single report about the use of EL as an organic modifier in reversed-phase liquid chromatography (RPLC) was identified in the existing literature (19). The application refers to the separation of paracetamol, aspirin and caffeine in pharmaceutical products. The content of EL in the mobile phase was limited to 10%, the separation required acidic addition in the eluent (3% acetic acid) and the column was thermostated at 60°C.

The aim of the present work was to have a deeper insight about the possibility of ACN replacement by EL in RPLC applications. The set of the analytes was increased to 16 and consists in a mixture of polycyclic aromatic hydrocarbons (PAHs). The elution was carried out at

**Table II.** Functional Correlation Between Molecular Descriptors and Experimental Retention Data Obtained Under ACN and EL Elution Conditions

Organic modifier	Descriptor Function	$M_w$ $M_w = f(\log k)$	$V_w$ $V_w = f(\log k)$	$\log P$ $\log P = f(\log k)$	$S$ $\log S = f(\log k)$	$\chi$ $\chi = f(\log k)$	$F$ $F = f(\log k)$	$L/B$ $L/B = f(\log k)$
ACN	Mean $r_{xy}$	0.9941	0.9918	0.9937	-0.9701	0.9964	0.9974	0.3540
	St dev	0.0010	0.0017	0.0009	0.0007	0.0006	0.0010	0.0111
EL	Mean $r_{xy}$	0.9800	0.9852	0.9865	-0.9584	0.9832	0.9874	0.4367
	St dev	0.0040	0.0043	0.0024	0.0048	0.0021	0.0023	0.0156

The functional correlation is expressed as the mean value of the correlation coefficients ( $r_{xy}$ ) considering retention data under the different tested mobile phase compositions.

*n*-octanol and water ( $\log P$ ). Well-known geometrical descriptors for PAHs are the van der Waals volume ( $V_w$ ) and the van der Waals surface area ( $A_w$ ). Other descriptors refer to the molecular shape (length to breadth ratio— $L/B$ ) or to the molecular topology (the connectivity index— $\chi$  and the correlation factor— $F$ ). More often, by plotting the experimental retention factors versus these descriptors and finding the descriptors that produce the highest correlation, one can determine the most important factors controlling retention (23, 24).

Our aim was to compare correlations obtained between some molecular descriptors (Supplementary Material, Part 1) and the retention behavior of PAHs under ACN and EL elution conditions. If such correlations do not substantially differ, one can consider that the shift from ACN to EL in the mobile phase composition does not alter interactions of the studied compounds with the stationary phase. Correlation data are given in Table II.

One can observe that linear relationships were obtained between the considered molecular descriptors and the logarithms of the retention factors, except for  $L/B$ . Better correlations are always obtained when ACN is used as an organic modifier. However, correlations obtained under EL elution conditions are not significantly lower, meaning that interactions solutes–stationary phase are basically the same fact that confirms also the thermodynamic aspects before mentioned.

If assuming that the chromatographic separation of the target compounds is driven by hydrophobic interactions and also considering the experimental findings with respect to enthalpy/entropy compensation (illustrating that the replacement of the organic modifier has little influence on the analyte/stationary phase interactions), the reversal of the elution order from some compounds under EL/water elution conditions merits at least a tentative explanation. The reversal of the elution order arises among D/C, M/L, and O/P/N compounds (illustrating a change in selectivity under EL/water elution conditions) characterizes analytes with very similar or identical  $\log P$  (for instance, compounds M and L have  $\log P$ -values of 5.52, whereas compounds N, O and P have  $\log P$ -values of 6.22). However, their water solubilities are different (Supplementary Material, Part S1). Information about their solubilities in ACN and EL is missing in the existing literature, in order to assess some correlations with the elution order. It may be possible that the specific different solubilities in the mobile phases (ACN/water and EL/water, respectively) are responsible with the reversal in the elution order within some groups of compounds characterized by very similar or identical  $\log P$ .

## Conclusion

Replacement of ACN by EL in mobile phases for RPLC appears to be feasible. This may be considered an useful issue in the actual general tendency of greening chromatographic processes. EL is fully miscible with water and produces pressure drop regimes accepted by the usual available instrumentation existing on the market. For the tested

solutes, similar retention was obtained in mobile phases containing ~15% of less EL compared with ACN-based ones. However, for a similar selectivity of the separation process, ~20–25% of less EL compared with the ACN elution conditions is needed. Although optimized selectivity with EL in the mobile phase does not differ substantially from that obtained under ACN elution conditions, the required duration of the separation process is larger. As observed from the Van Deemter plot, the increased resistance to the mass transfer in the mobile phase, when using EL, imposes working conditions close to the optimal speed of the eluent through the column, further increase in the flow rate generating an important reduction of efficiency. Two major drawbacks make ACN replacement by EL to be considered with precautions. The first one refers to detection reasons: the cutoff wavelength of EL is significantly much higher than that of ACN. Consequently, when using UV detection, especially for rich organic modifier mobile phase compositions and for separation of solutes lacking of chromophores, detectability under EL elution conditions may be fully compromised. When monitoring chromatograms at lower analytical wavelengths, under EL gradient elution conditions, the shift of the baseline and the noise will seriously affect the analytical results. The second drawback refers to the lack of availability (at least for the moment) on the market of chromatographic grade EL. Another sensible issue, which was not addressed in this work, deals with the chemical stability of EL under using acidic or alkaline conditions in the mobile phases. To conclude, ACN replacement by EL in mobile phases used in liquid chromatography is possible, but should be considered with precaution.

## Supplementary material

Supplementary materials are available at *Journal of Chromatographic Science* (<http://chromsci.oxfordjournals.org>).

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