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DOCTORAL THESIS

ECOLOGICAL (GREEN) ALTERNATIVES FOR LIQUID CHROMATOGRAPHY

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4.1. Retention study some oxime type compounds through liquid chromatography on a bimodal acting stationary phase, as an indirect alternative and green approach for hydrophobicity estimation

4.1.1. Introduction

The best known property that describes the behavior of a chemical species in a biological environment or in the environment is lipophilicity, defined by IUPAC as the affinity of a molecule or functional group for a lipophilic environment and which is usually expressed as the distribution between two phases of a liquid-liquid or liquid-solid system [62].

The lipophilicity of a drug, for example, is associated with its biological activity, its ability to bind to plasma proteins, metabolism, and last but not least, its toxicity, which occurs after accumulation in cell membranes.

On the other hand, lipophilicity influences the bioavailability and bioconcentration of chemicals that reach the environment, especially in water and soil, from where, through absorption, they reach the food chain. The distribution of chemicals between soil or sediment and water is an important factor in determining the risk factors associated with hazardous waste management and the correct use of chemicals to protect crops [63]

The most accepted and used way of expressing lipophilicity (or hydrophobic character) is made using the octanol-water partition coefficient ($K_{O/W}$) or log P.

One of the most well-known and used direct methods of experimental determination of log P is the "shake-flask" method which involves a long time of determination, the use of large volumes of solvents, is sensitive to the presence of impurities, but especially low accuracy at the extremes of the value's interval (for very high or very low values of log P), due to the process of emulsifying octanol in water. The slow agitation method can be used to prevent the formation of octanol emulsion in water, which, although it allows the determination of log P up to values of about 9, involves, compared to the initial method, a long analysis time and very laborious operations [64, 65].

These methods can be miniaturized and automated, but even in this case there are a number of issues that limit their use to a limited range of values of log P.

The need to process as many samples as possible in the shortest possible time, with the lowest possible cost and environmental impact, has led to the discovery of alternative methods that are easy to automate, require small amounts of samples, are insensitive to impurities, have good accuracy and provide reproducible results. One such widely accepted and recognized method is reverse phase liquid chromatography which is an indirect approach for hydrophobicity estimation [63].

Log P estimation by reverse phase liquid chromatography is accepted because the separation mechanism, which involves the partition of analytes between 2 immiscible phases, simulates the partition of analytes in the octanol-water system, namely: the stationary apolar phase can be assimilated with octanol, while the aqueous component of the mobile phase can be assimilated with water.

Log P is estimated from chromatographic data using the retention factor k. From a practical point of view, the k values are determined for different percentages of organic modifier in the mobile phase and then its value is extrapolated for the situation in which the mobile phase is composed entirely (100%) of the aqueous solvent, using the linear or polynomial regression technique [66].

The dependence of the retention factor on the percentage of organic solvent in the mobile phase can be described by equation:

$$logk = logk_w + S \times \varphi \qquad (13)$$

in which $logk_w$ it represents the extrapolated value of $logk$ to 100% aqueous solvent, and φ represents the percentage of organic solvent in the mobile phase. For a binomial regression, the dependence may be re-written as it follows:

$$logk = logk_w + a_1 \times \varphi \ + a_2 \times \varphi^2 \qquad (14)$$

where the significance of $log\ k_w$ and φ is the same as in the case of linear regression, a_1 and a_2 are the parameters of the regression and usually they don't have a specific physical significance [63].

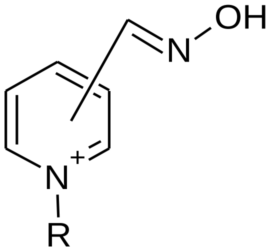
The aim of this study is to estimate hydrophobicity indices from chromatographic retention data and to correlate them with hydrophobicity descriptors calculated using dedicated software for a set of 30 compounds belonging to the mono-pyridine oxime class.

The stationary phase on which the study was conducted operates in a bimodal manner, depending on the percentage of organic solvent in the mobile phase: at values of up to about 40% organic solvent, for example, the separation is based on reverse phase mechanism (RP), and at values above 80% organic solvent, the separation mechanism is based on hydrophilic interaction (HILIC).

4.1.2. Reagents and equipments

The retention study was performed on a set of 30 compounds belonging to the mono-pyridine oxime class. Their chemical structures are shown in **Figure 23** (*numbering of figures were kept as in the thesis).

Pyridinium oxymes act as reactivators of acetylcholinesterase, representing cleavage agents between a former serine hydroxyl group situated on the active site of acetylcholinesterase (AChE), blocked by a phosphorus atom of a (thio)phosphonyl/ phosphoryl moiety belonging to a nerve agent or pesticide.



Substituent (R) Name	Substituent (R) Formula	Position of the oxime moiety	Acronym
Ethyl	$-C_2H_5$	2, 3, 4	2-PAE,3-PAE,4-PAE
Butyl	$-C_4H_9$	2, 3	2-PAB,3-PAB
Hexyl	$-C_6H_{13}$	2, 3, 4	2-PAH, 3-PAH, 4-PAH
Octyl	$-C_8H17$	2, 3, 4	2-PAO, 3-PAO, 4-PAO
Decyl	$-C_{10}H_{21}$	2, 3	2-PAD, 3-PAD
Dodecyl(Lauryl)	$-C_{12}H_{25}$	2, 3, 4	2-PAL, 3-PAL, 4-PAL
Benzyl	$-CH_2-C_6H_5$	2, 3, 4	2-PABn, 3-PABn, 4-PABn
Ethyl-phenyl	$-(CH_2)_2- C_6H_5$	2, 3, 4	2-PAPE, 3-PAPE, 4-PAPE
Propyl-phenyl	$-(CH_2)_3-C_6H_5$	3	3-PAPP
Butyl-phenyl	$-(CH_2)_4-C_6H_5$	3, 4	3-PAPB, 4-PAPB
4-Methylbenzyl	$-CH_2- C_6H_4-(CH_3)$	2, 3, 4	2-PAMB, 3-PAMB, 4-PAMB
4-t-buthylbenzyl	$-CH_2- C_6H_4-C(CH_3)_3$	3, 4	3-PATB, 4-PATB

Figure 23. Chemical structure of the compounds considered during the study.

The oximate anion acts as a nucleophilic agent, while the quaternary nitrogen atom contributes to the correct positioning of the reactivator molecule towards an anionic center representing a secondary binding site at the entrance of a narrow gorge of the enzyme.

Through different computational algorithms, with dedicated software, the values of some hydrophobicity descriptors (generically called log P) and some parameters of the biopharmaceutical classification system (BCS) - solubility in simulated biological fluids and gastrointestinal permeability- were calculated. The computed Log P values for the considered compounds and the reciprocal correlation between them are enlisted in **Table 1** (*numbering of tables were kept as in the thesis).

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4.1.3. Chromatographic experiments

An Acclaim Mixed-Mode HILIC-1 column was used and thermostated at 25° C. The stationary phase consists in spherical silicagel particles chemically functionalized with long alkyl chains (dodecyl) terminated by a polar environment created through the vicinal diol substitution at the lasting carbon atoms. Alkylic chains create the necessary conditions for hydrophobic interactions, while vicinal diol groups provide the hydrophilic environment for the hydrophilic interaction mechanism. Injection volume was 2 μL. Retention profiles of the analytes were obtained under isocratic conditions, with solvent B proportions from 5% to 90%.

Detection was made at 220, 254 and 300 nm (± 4 nm), with a reference wavelength of 480 nm (± 10 nm).

4.1.4. Results and discussions

Estimation of hydrophobicity indices from chromatographic retention data involved a number of steps. First step was the calculation of the retention factor $k=(t_R-t_0)/t_0$, where t_R is the absolute retention time, and t_0 represents the void time. The void time was determined through observation of the injection signal. Each injection was repeated three times. Mean absolute time retention and void time were used for calculation of the retention factor. The relative standard deviation (RSD) calculated for the retention factor were below 2%. For the Acclaim Mixed-Mode HILIC-1 column operated under the experimental conditions, the mean void time values was 1.23 min.

The values of the retention factor were used to plot the functional dependencies $k = f(\varphi)$ and $\log k = f(\varphi)$, where φ represents the percentage of organic modifier in the mobile phase. Examples of such dependencies are shown in **Figure 29** for 3-PAE-I and 3-PATB-Br.

The retention profiles are presented in **Figure 29.** Such profiles are U shaped. The left side of the functional dependency profile may be attributed to the reversed phase (RP) retention mechanism, when the retention decreases with the increase of the organic solvent in the mobile phase. The separation in RP is determined by the partition of the analyte between the polar mobile phase and the hydrophobic layer formed by the alkyl chains chemically bonded to the silicagel surface.

The right side of the plot can be attributed to the hydrophilic interaction mechanism (HILIC), when the retention increases with the increase of the organic component of the mobile phase. The separation, in this case, is determined by the partition of the analyte between the less polar mobile phase and an aqueous layer formed by means of the hydrogen bonds realized between the vicinal diol groups, positioned at the end of the alkyl chains of the stationary phase and water molecules. In this context, the mobile phase (with an important content of organic solvent, in this case acetonitrile) has a less polar character compared to the water film retained on the surface of the stationary phase.

Table 1. Log P values of studied pyridinium oximes computed through different algorithms and their reciprocal correlation.

#	Compound	ALOGPs	AC logP	miLogP	KOWWIN	XLOGP2	XLOGP3	ALOGP	Hy	MLOGP	SlogP	SlogD7.4	LogD7
1	2-PAE	-2.33	1.12	-2.47	-0.80	1.02	1.34	1.41	-0.12	0.81	-2.37	-2.90	-4.56
2	3-PAE	-2.76	1.01	-2.76	-0.80	0.93	1.00	0.98	-0.12	0.81	-2.54	-3.60	-4.06
3	4-PAE	-2.81	1.02	-3.73	-0.80	0.93	1.00	0.98	-0.12	0.81	-2.40	-3.29	-4.19
4	2-PAB	-1.69	2.05	-1.41	0.18	1.95	2.22	2.39	-0.20	1.43	-1.50	-2.19	-3.69
5	3-PAB	-2.22	1.94	-1.70	0.18	1.86	1.89	1.96	-0.20	1.43	-1.62	-2.82	-3.20
6	2-PAH	-0.71	2.97	-0.40	1.16	3.09	3.30	3.30	-0.27	2.00	-0.56	-1.37	-2.90
7	3-PAH	-1.46	2.87	-0.69	1.16	3.00	2.97	2.87	-0.27	2.00	-0.66	-2.01	-2.40
8	4-PAH	-1.35	2.87	-1.65	1.16	3.00	2.97	2.87	-0.27	2.00	-0.54	-1.73	-2.53
9	2-PAO	0.54	3.90	0.61	2.14	4.23	4.39	4.21	-0.31	3.45	0.45	-0.47	-2.11
10	3-PAO	-0.71	3.80	0.32	2.14	4.14	4.05	3.78	-0.31	3.45	0.35	-1.18	-1.61
11	4-PAO	-0.67	3.80	-0.64	2.14	4.14	4.05	3.78	-0.31	3.45	0.45	-0.88	-1.74
12	2-PAD	1.51	4.83	1.62	3.13	5.36	5.47	5.12	-0.35	3.95	1.47	0.46	-1.32
13	3-PAD	0.41	4.73	1.33	3.13	5.27	5.14	4.69	-0.35	3.95	1.37	-0.34	-0.82
14	2-PAL	2.43	5.76	2.63	4.11	6.50	6.55	6.04	-0.39	4.43	2.47	1.40	-0.52
15	3-PAL	1.40	5.66	2.35	4.11	6.41	6.22	5.61	-0.39	4.43	2.37	0.50	-0.03
16	4-PAL	1.27	5.66	1.38	4.11	6.41	6.22	5.61	-0.39	4.43	2.44	0.87	-0.15
17	2-PABn	-1.10	2.34	-1.25	0.41	1.96	2.63	2.64	-0.29	2.02	-1.08	-1.69	-3.13
18	3-PABn	-1.79	2.23	-1.54	0.41	1.87	2.30	2.21	-0.29	2.02	-1.22	-2.35	-2.63
19	4-PABn	-2.00	2.23	-2.51	0.41	1.88	2.75	0.32	-0.29	1.95	-1.07	-2.07	-2.75
20	2-PAPE	-0.74	2.40	-1.04	0.91	2.47	2.93	2.96	-0.31	2.29	-0.84	-1.54	-2.87
21	3-PAPE	-1.81	2.29	-1.33	0.91	2.39	2.59	2.53	-0.31	2.29	-0.97	-2.19	-2.38
22	4-PAPE	-1.82	2.29	-2.30	0.91	2.38	2.59	2.53	-0.31	2.29	-0.82	-1.89	-2.50
23	3-PAPP	-1.59	2.76	-0.81	1.40	2.74	2.95	2.99	-0.33	2.54	-0.60	-1.88	-1.98
24	3-PAPB	-1.31	3.22	-0.54	1.89	3.31	3.31	3.45	-0.35	2.79	-0.16	-1.50	-1.58
25	4-PAPB	-1.34	3.22	-1.51	1.89	3.31	3.31	3.45	-0.35	2.79	-0.02	-1.23	-1.71
26	2-PAMB	-0.78	2.65	-0.81	0.96	2.40	3.00	3.13	-0.31	2.29	-0.71	-1.34	-2.66
27	3-PAMB	-1.48	2.55	-1.09	0.96	2.31	2.66	2.70	-0.31	2.29	-0.86	-2.00	-2.16
28	4-PAMB	-1.71	2.55	-2.06	0.96	2.31	2.66	2.70	-0.31	2.29	-0.71	-1.71	-2.29
29	3-PATB	-0.35	3.75	0.17	2.32	3.69	3.97	3.61	-0.37	3.04	0.41	-0.92	-1.00
30	4-PATB	-0.22	3.75	-0.80	2.32	3.69	3.97	3.61	-0.37	3.04	0.55	-0.62	-1.13
Correlation coefficient		ALOGPs	AC logP	miLogP	KOWWIN	XLOGP2	XLOGP3	ALOGP	Hy	MLOGP	SlogP	SLogD7.4	LogD7
ALOGPs			0.9477	0.9490	0.9227	0.9430	0.9655	0.9394	-0.7188	0.9118	0.9417	0.9760	0.8140
AC logP				0.9421	0.9920	0.9935	0.9945	0.9523	-0.8029	0.9739	0.9968	0.9718	0.9353
miLogP					0.9226	0.9405	0.9459	0.9434	-0.7169	0.9083	0.9233	0.9158	0.8363
KOWWIN						0.9848	0.9832	0.9505	-0.8479	0.9804	0.9955	0.9610	0.9634
XLOGP2							0.9892	0.9549	-0.7543	0.9639	0.9889	0.9594	0.9115
XLOGP3								0.9438	-0.7983	0.9715	0.9934	0.9832	0.9138
ALOGP									-0.7602	0.9302	0.9467	0.9439	0.8663
Hy										-0.8638	-0.8302	-0.8091	-0.9095
MLOGP											0.9805	0.9507	0.9487
SlogP												0.9760	0.9474
SlogD7.4													0.8797

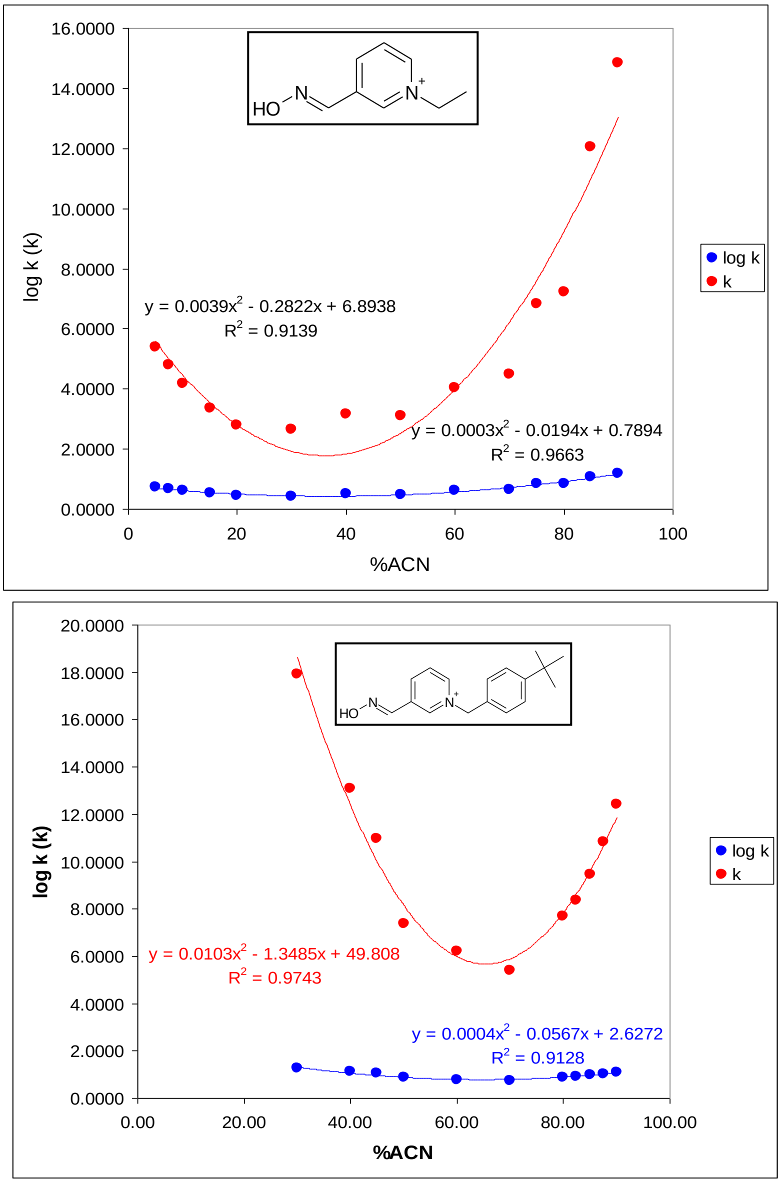


Figure 29. Retention profiles of 3-PAE-I şi 3-PATB-Br presented as functional dependencies between the content of the organic modifier in the mobile phase and the retention factor (k) or logarithm of the retention factor (log k).

A special interest was given to the bottom of the U shaped retention profiles which corresponds to the minimum retention (k_{min} or log k_{min}) and to the composition of the mobile phase enabling the minimal retention (φ corresponding to k_{min} or log k_{min}). This composition has been arbitrarily named ISOELUT. The prefix LOG will be added to the estimated hydrophobicity indices from the functional dependencies log k = f (φ).

4.1.4.1. Chromatographic derived hydrophobicity indices

The minimum retention factor (**k_{min}**) and the corresponding logarithm (**log k_{min}**) can be determined from the experimental data set for each analyte.

The determination of **ISOELUT** or **LOG ISOELUT** may be achieved by applying a binomial fitting to all experimental values. By correlating k or log k with φ through a binomial relationship $c + b \times \varphi + a \times \varphi^2$, it can be determined the minimum as $-\frac{b}{2a}$.

The same algorithm can be applied for the two sides of the U shaped profile. If a linear or power fitting model is applied , the isoelution composition can be determined through equalization of the linear equations, and is named **ISOELUT1** or **LOG ISOELUT 1**, or through the equalization of the power equations, and is named **ISOELUT2** or **LOG ISOELUT2.**

In order to make a comparison to hydrophobicity scales already in use, the values of the retention factor (k or log k) were extrapolated to a hypothetical mobile phase containing 100% water, in which case k or log k become k_w and log k_w , respectively). Extrapolation to $\varphi=0$ was made through linear or binomial fitting models, in the RP side of the U shape, and in this case the hydrophobicity indices are noted as **k_w^{lin}, logk_w^{lin} , k_w^{bin}** and **log k_w^{bin}**. For the HILIC side a hydrophilicity scale was proposed through extrapolation to $\varphi=$ 100%. The corresponding indices are denoted as **HYL** or **LOG HYL (HYL** comes from hydrophilic character).

In **Table 7** are enlisted the correlation coefficients established between the indices derived from the chromatographic retention data and computed log P values.

Table 7. Correlation between the hydrophobicity indices derived from the chromatographic retention data and the computed log P values.

	r_{xy}									
	ALOGPs	AClogP	miLogP	KOWWIN	XLOGP2	XLOGP3	Hy	MLOG P	ALOGP	Log D7
k_{min}	**0,958**	**0,977**	**0,934**	**0,963**	**0,977**	**0,983**	-0,758	**0,935**	**0,943**	***0,884***
log k_{min}	**0,938**	**0,977**	**0,927**	**0,973**	**0,964**	**0,978**	***-0,894***	**0,954**	**0,944**	**0,918**
ISOELUT	***0,842***	**0,915**	**0,835**	**0,942**	***0,895***	***0,899***	***-0,891***	**0,914**	**0,904**	**0,931**
LOG ISOELUT	***0,809***	***0,881***	***0,810***	**0,904**	***0,865***	***0,873***	***-0,874***	***0,880***	***0,885***	***0,864***
ISOELUT1	0,479	0,512	0,460	0,508	0,484	0,529	-0,518	0,577	0,393	0,537
LOG ISOELUT1	0,678	0,740	0,661	0,752	0,700	0,750	**-0,817**	**0,806**	0,640	0,788
ISOELUT2	0,552	0,598	0,526	0,602	0,558	0,621	-0,730	0,668	0,493	0,642
LOG ISOELUT2	0,193	0,209	0,117	0,214	0,201	0,226	-0,267	0,240	0,135	0,232
k_w^{lin}	0,788	0,784	0,767	0,753	0,793	0,787	-0,546	0,803	0,770	0,652
log k_w^{lin}	***0,847***	***0,862***	***0,836***	***0,843***	***0,862***	***0,863***	-0,672	***0,878***	***0,840***	0,766
k_w^{bin}	0,754	***0,812***	0,756	0,787	***0,817***	***0,800***	-0,570	***0,814***	0,778	0,714
log k_w^{bin}	0,139	0,278	0,202	0,304	0,246	0,239	-0,377	0,257	0,259	0,374
HYL	0,488	0,472	0,479	0,413	0,458	0,502	-0,327	0,520	0,363	0,363
LOG HYL	0,430	0,405	0,420	0,346	0,400	0,440	-0,245	0,464	0,298	0,291

In **Table 7,** the bold values correspond to correlation coefficients above 0,9 and the bold and italic values correspond to the correlation coefficients between 0,8 and 0,9.

In the same table one can observe that k_{min}, log k_{min}, ISOELUT and LOG ISOELUT are better correlated to computed log P values compared to the other determined indices.

4.1.4.2. Thermodynamic considerations

The shift from RP to HILIC separation mechanisms was evaluated, from a thermodynamic point of view, for compound 3-PAB. The compositions of the mobile phases used in the study were: 10, 40, 55, 80 and 90% ACN in water while the column was thermostated at 20, 25, 30, 35, 40, and 45 ^{o}C. The van't Hoff plots (ln k = f(1/T)) were obtained for the target analyte for each of the elution conditions being mentioned, allowing computation of ΔH^0, ΔS^0 and ΔG^0. Due to the fact that almost identical retention was observed on both sides of the U shaped retention profile, through equalizing the van't Hoff equation on RP and HILIC side, one can deduce that the water layer volume retained on the surface of the stationary phase is somehow similar to the volume of the bulk volume of the hydrocarbonate chains.

4.1.5. Conclusions

In the retention study of a series of alkyl pyridinium mono-oximes on a stationary phase working in bimodal conditions (RP and HILIC) a series of hydrophobicity indices was investigated.

It has been demonstrated that the minimum retention, expressed by the retention factor and its logarithm, respectively (k_{min}, log k_{min}) and the composition of the mobile phase corresponding to the minimum retention (ISOELUT and LOG ISOELUT) can function as descriptors of hydrophobicity. The values of these indices correlate statistically well with the calculated log P values. Moreover, the correlation of these newly proposed indices is often superior to those estimated by procedures already in use, namely by extrapolating the retention to an exclusively aqueous mobile phase composition (φ = 0%), using linear or binomial regressions.

Estimation of hydrophobicity indices from chromatographic retention data on a bimodal stationary phase can be useful in elaboration of hydrophobicity scales for compounds with certain structural particularities and for which a single separation mechanism approach may be difficult or inconclusive.

4.2. Propylene carbonate as green alternative for organic phase modifier in liquid chromatography

4.2.1. Introduction

The impact of chromatographic techniques on the environment can be reduced only if, during the entire analysis process, starting with sampling, processing (by extraction, derivatization and any other process is necessary for analysis), and continuing with the analysis itself, the principles of green chemistry are thoroughly considered.

Chromatographic approaches have the potential to be greener in all steps of the analysis. In liquid chromatography the focus should be oriented toward the reduction of solvent consumption and replacement of toxic and environmentally hazardous solvents with more benign alternatives [22].

Evaluation of the greenness degree of an organic solvent is based on two main criteria: life cycle assessment (LCA) and environmental, health and safety concerns (EHS) [69, 70].

Water, critical carbon dioxide (CO2), and ionic liquids are less harmful and environmental friendly solvents.

In the last decade, a number of solvents are beginning to be used in practice and consequently to be more and more present in the literature due to their green character and their use in sustainable processes. The results obtained by using these solvents proved to be superior, compared to those obtained in the case of solvents obtained from conventional sources, especially from oil.

Solvents from renewable sources have become commercially available, and the best known are propylene carbonate (PC), ethyl lactate (EL) or d-limonene [71].

In spite of the commercial availability as chromatographic grade solvents, they have been under-investigated as potential alternative solvents in HPLC.

Propylene carbonate (R,S-4-methyl-1,3-dioxolan-2-one) is a carbonate ester derived from propylene glycol, synthesized by means of more or less green processes [72-75], often used as a polar aprotic solvent in analytical chemistry and organic synthesis [76-78].

The uses of propylene carbonate (PC) and methanol (MeOH) for replacement of acetonitrile (ACN) in reversed phase liquid chromatography (RPLC) was already reported [79-82].

However, the referenced works mainly focused on validation aspects of RPLC applications in environmental and pharmaceutical fields when using PC/MeOH organic modifiers instead of ACN, rather than treating fundamental aspects of such an action.

Consequently, the present study refers to a systematic evaluation of the possibility to replace ACN by PC in RPLC.

A comparative overlook on ACN and PC properties and MeOH and ethanol (EtOH) respectively, one can easily deduce that a PC/EtOH mixture represents a greener alternative compared with a PC/MeOH, and obviously much greener compared to ACN.

Substitution of ACN by PC was evaluated according to the following aspects:

- necessary conditions to obtain full miscibility between the components of the mobile phase;
- pressure drop in the chromatographic system;
- equivalence between ACN and PC contents to achive similar retention;
- tolerability against inorganic buffers;

- optimal flow rates - Van Deemter plots;
- thermodynamic data comparison;
- the influence of PC on UV detection;
- the feasibility of ACN replacement by PC was evaluated in applications designed for Ion Pair Liquid Chromatography (IPLC) and Hydrophilic Interaction Liquid Chromatography (HILIC) separation mechanisms.

4.2.4. Results and discussions

A comparative overlook on ACN and PC properties leads to conclusions that both solvents are aprotic and polar, with increased molecular dipole moments, and exhibiting similar Hansen and Hildebrand solubility parameters.

Apart from these similarities, there are 2 aspects that may raise some concerns. The first one refers to the partial miscibility of PC with water and subsequently the aqueous PC mobile phases cannot be obtained over the whole mixing proportion range. The second concern may arise when look at the superior densities and viscosities values for PC, compared with ACN. Consequently it is to be expected that the pressure drop on the chromatographic column is higher as well as the resistance to the mass transfer in the mobile phase, probably leading to a decreased chromatographic efficiency.

4.2.4.1. Miscibility study

PC is not fully miscible with water over the whole mixing range, so the choice of a third solvent, miscible with both PC and water become evident. Such a solvent can be found among the low aliphatic alchools. Keeping in mind that the aim of the study was to evaluate a possible green solvent, EtOH has been chosen as the third component of the system. In order to obtain an aqueous PC mobile phase over the whole mixing range two alternatives become available:

- for rich aqueous mobile phases a premix of PC/EtOH was combined with water;
- for poor aqueous mobile phases Pc was added to premixed EtOH/water.

The observation made after this approach was that a full miscibility, between the three components is attained when the mixing ratio between PC or water and ethanol was at least 7/3 in volumetric proportions.

4.2.4.2. Column pressure drop

When substituting ACN/water with PC/EtOH/water the pressure drop on the chromatographic column increases in a range from 1,1 to 9,2 folds.

A higher increase is observed when PC is added to a premix of EtOH/water (7/3 v/v) compared to mobile phases obtained by combining premixed PC/EtOH (7/3 v/v) with water.

It is to mention that the upper limit of 400 bar accepted in “classic” HPLC systems was not overcome.

4.2.4.3. Equivalence between ACN and PC contents to produce similar retention

The shifting from the ACN/water elution mode to the PC/EtOH/water elution mode is, from a practical point of view, conditioned by a fast and easy transfer without compromising the performance criteria or alter the elution order.

In the transfer evaluation study three sets of compounds have been considered:

- A set of compounds having acidic character: fenofibric, lovastatic and simvastatic acids;
- A set of compounds having neutral character: toluene, fluorene and fluoranthene;
- A set of compounds having basic character: carbamazepine, diltiazem, nicergoline.

A Purospher STAR RP-18e (75 mm L x 4 mm i.d. x 3 μm d.p.), thermostated at 25 °C was used. The flow rate was 0.5 mL/min, under isocratic conditions.

When ACN was the organic modifier of the mobile phase, the aqueous component was 0,1 % formic acid for acidic compounds, pure water for neutral compounds and aqueous 50mM borate buffer for basic compounds.

In the alternative elution mode PC was added to a premix of water/EtOH (7/3 v/v), with the same additives as in the ACN elution mode.

The UV detection was used by monitoring the 238 nm wavelength, for acidic compounds, 254 nm for neutral ones and 220 nm for the basic compounds.

The first step of the transfer method was the replacement of ACN with PC and the water with water/EtOH (7/3 v/v) followed by the recording of the corresponding chromatograms. In **Figure 39.** are illustrated the chromatograms for all categories of compounds, in both elution modes, where one can observe that a relative similar elution behavior is exhibited when the proportions of PC and EtOH equalize the content of ACN.

It was considered that the equivalence between PC and ACN content, in order to produce similar retention is achieved when the sum of the differences between retention times obtained under PC and ACN elution conditions was minimal (relation 21).

$$\Delta t_R^{total} = \sum_{i=1}^{n} \left(t_R^{PC} - t_R^{ACN} \right) = \min \qquad (21)$$

Furthermore, the variation of retention times for fenofibric acid, toluene and diltiazem with the content of the organic modifier t_R=f (% org. mod.) were studied. The resulting functional dependencies were not linear and the linearization was made through double logarithmation. As a consequence the following relationships were obtained:

$$log\left(t_{R_i}^{ACN} \right) = B_i log\left(\%ACN \right) + A_i \qquad (22)$$

$$log\left(t_{R_i}^{Org} \right) = b_i log\left(\%Org \right) + a_i \qquad (23)$$

Taking into the consideration that

$$\%Org = 0,7 \times \%PC + 30 \qquad (24)$$

and having as study objective the equalization of the retention times ($t_R^{ACN} = t_R^{Org}$), the PC content is given by the following equation:

$$\%PC = \left[\left(1/0,7\right) \times 10^{\frac{A_i - a_i}{b_i}} \times \%ACN^{\frac{B_i}{b_i}} \right] - \left(30/0,7\right) = \left[1,43 \times 10^{\frac{A_i - a_i}{b_i}} \%ACN \right] - 42,78 \qquad (25)$$

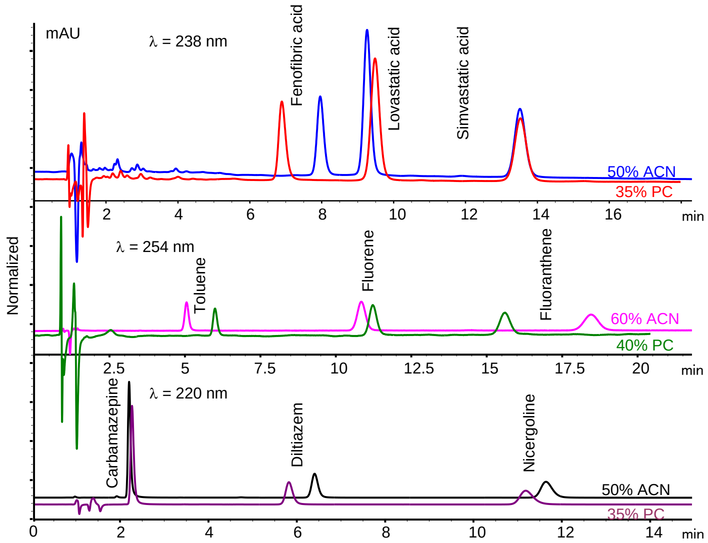


Figure 39. Chromatograms for all categories of compounds, in both elutions modes.

The equivalent of % PC was calculated based on equation (25), for a mobile phase containing 30-80% ACN for the selected compounds and a general relationship (26) was obtained.

$$\%PC = 1{,}37(\pm 0{,}06)\times \%ACN - 36{,}7(\pm 4{,}4) \qquad (26)$$

In equation (26) the numbers under parenthesis are standard deviations of the slope and intercept, respectively.

The data presented in **Figure 39.** are confirmed by this algorithm.

4.2.4.4. Tolerability against inorganic buffers

Separation of the basic analytes, in both elution alternatives (ACN and PC-EtOH, respectively) were achieved by varying the concentration of the borate buffer in the aqueous component of the mobile phase, at 10, 25, 75 and 100 mM concentration levels. For each concentration level variations of retention times, efficiencies and peak symmetries were monitored.

No solubility problems were observed on increasing buffer concentration in mobile phases composed by PC/EtOH. Salting-out effects are significantly higher for compounds with increased retention in mobile phases based on PC-EtOH compared to ACN. However, above 50 mM borate buffer, retention stabilizes as depicted in **Figure 41.**

Higher chromatographic efficiencies, as depicted in **Figure 43.** were obtained in ACN/water mobile phases. This verifies the previously mentioned concern, relating to a decreased diffusion of analytes within PC containing mobile phases.

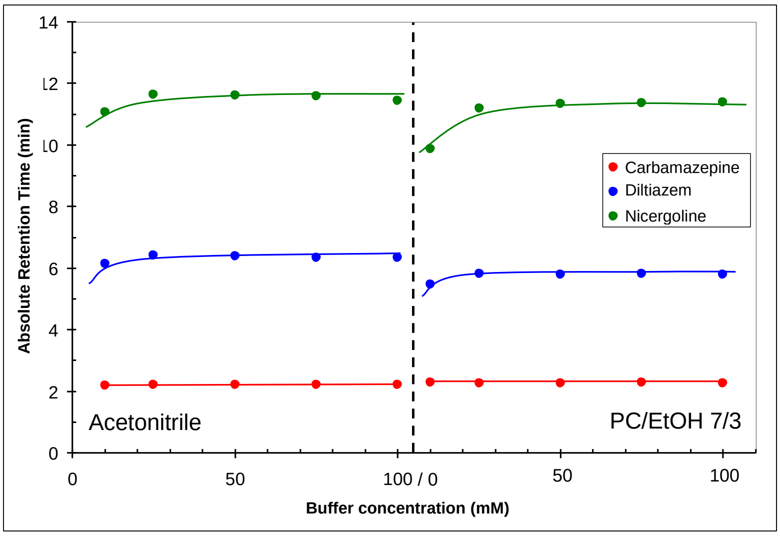


Figure 41. Variation of the retention time with the increased buffer concentration

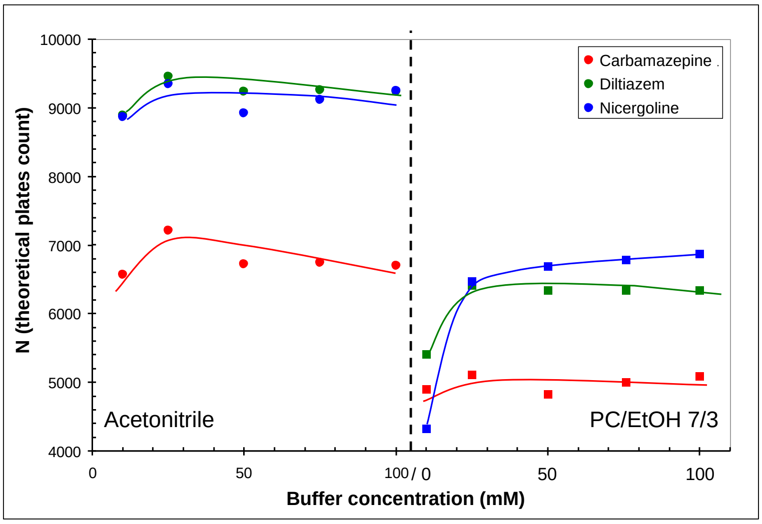


Figure 43. Variation of chromatographic efficiency (theoretical plate count) with the buffer concentration

4.2.4.5. Van Deemter plots

In order to have a better insight on the relationship between chromatographic efficiency, expressed as HETP and the mobile phase velocity (and consequently flow rate), the Van Deemter plots were constructed for fluoranthene and simvastatic acid, in both elution modes (ACN and PC/EtOH, respectively).

Experiments were performed on a Purospher STAR RP-18e (75 mm L x 4 mm i.d. x 3 µm d.p. column, thermostated at 25°C.

The flow rates were varied from 0.1 to 1.0 mL/min for PC/EtOH containing mobile phases and from 0.3 to 1.2 mL/min for ACN containing mobile phases, increments of 0.1 mL/min being applied in both cases.

As indicated in **Figure 44**, optimum flow rate intervals under PC elution mode (the red colour) are lower than the corresponding values produced in ACN elution conditions (the blue colour). The right side of the Van Deemter curve mainly controlled upon the mass transfer processes in mobile and stationary phases is less influenced by the flow rate increase when using ACN, compared to PC/EtOH.

This observation only supports the theoretical statement made earlier in this chapter that due to the high density and viscosity of PC (compared to ACN) it is expected that the diffusion of the analytes will be more difficult, which entails an increased resistance to the mass transfer of the analytes in the mobile phase.

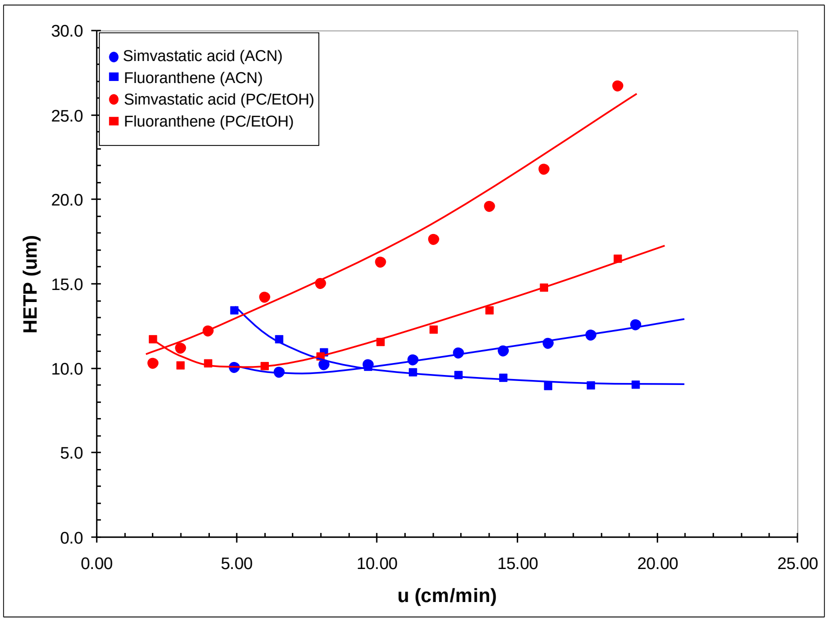


Figure 44. Van Deemter plots for simvastatic acid and fluoranthene in both elution modes

4.2.4.6. Thermodynamic aspects

Replacement of ACN with PC was investigated from a thermodynamic point of view. The same chromatographic column, as in the paragraph 4.2.4.3., thermostated in a temperature interval ranging from 15 to 45°C with 5°C increments was used. Van't Hoff plots lnk=f(1/T) were obtained for fenofibric acid, toluene, simvastatic acid, fluorene and fluoranthene.

Mobile phases were composed by ACN/aqueous 0,1% formic acid (6/4 v/v) and respectively PC combined with 0,1% formic acid in a premix of water/EtOH (7/3 v/v) in a volumetric ratio of 4/6 v/v.

The resulting van't Hoff retention temperature plots were linear and characterized by correlation coefficients higher than 0,99, except for simvastatic acid, especially under PC elution conditions. The linearity of the above mentioned plots indicate that the separation mechanism doesn't change with the variation of the temperature.

4.2.4.7. UV detection

The shift from ACN to PC-EtOH based mobile phases raises natural concerns about the way the sensitivity under UV detection conditions may be affected. Consequently, cut-off wavelengths (wavelength corresponding to 1 unit of absorbance for a pathlength of 1 cm) were determined at different mixing ratios between PC/EtOH/water. Results are contained in **Table 16**. For making comparison easier, the same Table contains cut-off wavelengths for ACN/ aqueous 0.1% formic acid mobile phases.

One can conclude that no significant sensitivity loss should be produced when ACN is substituted by PC-EtOH in mobile phases.

Table 16. Cut-off wavelengths at different mixing ratios between PC/EtOH/water and ACN/ formic acid 0,1%

Solvent A	Solvent B	Mobile phase composition (A/B v/v)	Cut-off wavelenght (nm)*
PC/EtOH (7/3 v/v)	Water	9/1	214
		4/1	212
		7/3	211
		3/2	210
		1/1	209
		2/3	207
		3/7	205
		1/4	203
		1/9	200
PC	Water/EtOH (7/3 v/v)	9/1	216
		4/1	214
		7/3	213
		3/2	212
		1/1	211
		2/3	209
		3/7	208
		1/4	206
		1/9	202
ACN	Aq. 0,1% formic acid	9/1	190
		4/1	191
		7/3	191
		3/2	191
		1/1	191
		2/3	192
		3/7	192
		1/4	213
		1/9	213

**mesurements were performed on a diode array spectrometre Specord S600 (Analytic Jena, Germany).*

4.2.4.8. Applications based on different other separation mechanisms

One of the major aims of our study was to test the replacement of ACN by PC for other separation mechanisms, for instance RPIP and HILIC.

HILIC is an antipodal separation mechanism to RP, and it represents a solution for separation of polar compounds. The process is achieved through partition of hydrophilic analytes between a mobile phase rich in organic modifier and a layer of water retained at the surface of a polar stationary phase.

The application referred in the text illustrates the separation of nicotinamide, pyridoxine and thiamine on Luna 3u HILIC column, thermostated at 25 °C, exploited under HILIC conditions.

The results of the two separation alternatives are illustrated in **Figure 49**. When ACN is the organic modifier of the mobile phase the peak symmetry and the efficiency are better compared to the situation when PC is the organic modifier of the mobile phase. With PC as the major component of the mobile phase, selectivity and resolution are better.

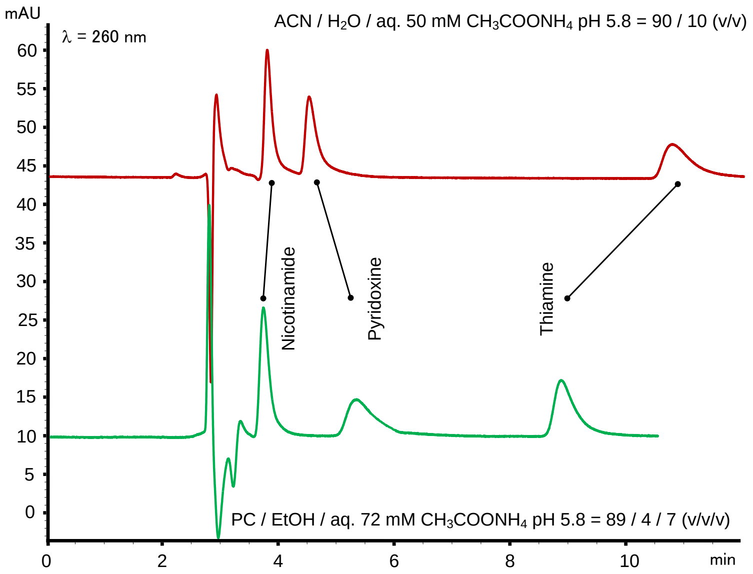


Figure 49. The corresponding chromatograms for the both elution modes (ACN and PC/EtOH respectively) in the HILIC mechanism, for the considered application.

4.2.4.9. Conclusions

ACN substitution with PC alone or premixed with ethanol can be made directly and simply.

For the both elution modes tolerance against inorganic buffers is similar.

The optimal flow rates under PC elution conditions are lower compared to ACN based mobile phases, due to a reduced mass transfer of analytes in mobile phase.

Replacement of acetonitrile by propylene carbonate/ethanol mixtures in liquid chromatography is affordable and should be considered as a step toward greening analytical chemistry, without any compromise in terms of performance criteria.

4.3. Evaluation of ethyl lactate as a green alternative for organic modifier in liquid chromatography: Application on PAHs separation

4.3.1. Introduction

Organic esters are a very important class of chemicals having applications in a variety of areas in the chemical industry, such as perfumes, flavors, pharmaceuticals, plasticizers, solvents and intermediates. One of these esters is ethyl lactate (Ethyl -2-hydroxypropanoate). Ethyl lactate (EL) represents a viable alternative not only economically but also from the environmental perspective.

These affirmations can be sustained by some arguments like:

- It is naturally present in small amounts in certain fruits, chicken or wines, and is even approved by the FDA for use as an additive in the food industry;
- It is not teratogenic and is easily metabolized under the enzymatic action of esterases, to ethanol and lactic acid, the latter compound being a product of human metabolism;
- It is completely and quickly biodegradable in the environment and it is not ozone depleting;
- EL is obtained by the esterification reaction between lactic acid and ethyl alcohol. These reagents can be obtained from completely renewable sources such as carbohydrates generated as part of biomass. Biomass processing is done industrially in what are called bio-refinery, which is based on a concept similar to that of oil processing. [83, 84].

Ethyl lactate has already found use in a number of processes, such as extraction [85], as a dispersion solvent in magnetic stripe production processes [86], as a solvent in organic syntheses [87, 88] or in obtaining solvent-free emulsions [89].

Given the different properties of EL from those of ACN it was necessary to evaluate comparatively the pressure drop in the column and the cut-off wavelength values for various compositions EL / water.

The equivalence between ACN and EL content in the mobile phase to produce similar retention and/or selectivity, a comparison between thermodynamic effects arising on substitution of ACN with EL and the correlation between resulting retention data and some molecular descriptors of PAHs were also considered.

4.3.2. Reagents and equipments

Chromatographic experiments involved a mixture of 16 polycyclic aromatic hydrocarbons (PAHs): naphthalene—A; acenaphthylene—B; fluorene—C; acenaphthene—D; phenanthrene—E; anthracene—F; fluoranthene—G;pyrene—H; chrysene—I; benzo(a)anthracene—J; benzo(b)fluoranthene—K; benzo(k)fluoranthene—L; benzo(a)pyrene—M; dibenzo (a,h)anthracene—N; benzo(g,h,i)perylene—O; indeno(1,2,3-c,d)pyrene—P).

Cutoff wavelength determination of different mixtures EL/water was achieved by using a Varian 100 Bio Cary UV–Vis spectrometer.

4.3.3. Chromatographic experiments

A Zorbax Eclipse XDB C18 column, thermostated at 25°C was used. Elution was achieved under isocratic conditions at a flow rate of 0,3 mL/min, as it resulted from Van Deemter plots (**Figure 50**) obtained for analyte E under elution with ACN/water = 70/30 and EL/water = 50/50 (v/v).

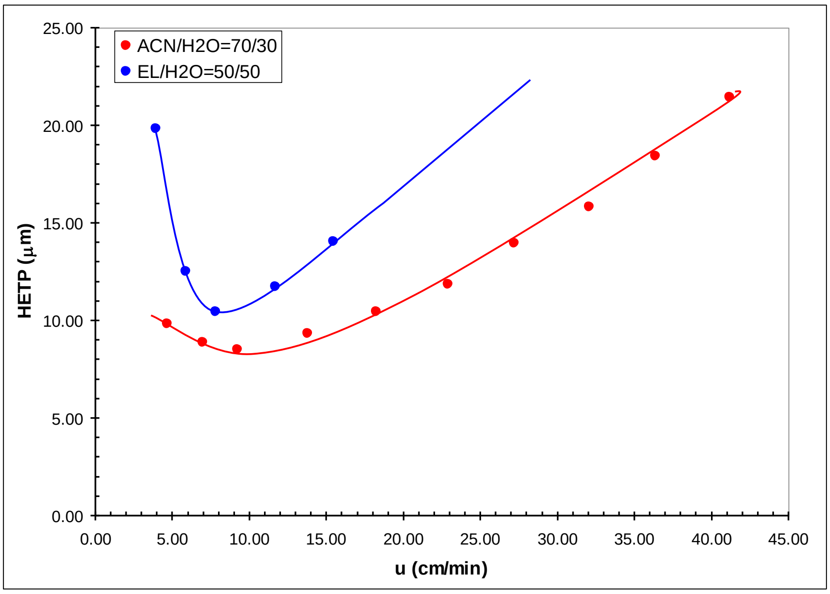


Figure 50. van Deemter plots for phenanthrene in both elution modes

4.3.4. Results and discussions

The separation of a PAHs mixture was not the subject of this work. This issue was from long date discussed in the literature.

The aim of the present study was to obtain an extended image of the possibility to replace ACN by EL, as a possible green organic modifier alternative.

4.3.3.5. Column pressure drop

As a consequence of the higher density and especially higher viscosity of EL compared with ACN an elevated pressure regime is expected. Indeed, as it may be observed from **Figure 51**, the increase in the column pressure drop by a factor of 2–10 was registered when shifting from ACN to EL over the interval 40–100% organic modifier in the mobile phase.

One can mention that all experiments realized during the present study using EL as the organic modifier in the mobile phase, the HPLC system was exploited under a maximal pressure drop regime set up at 400 bar.

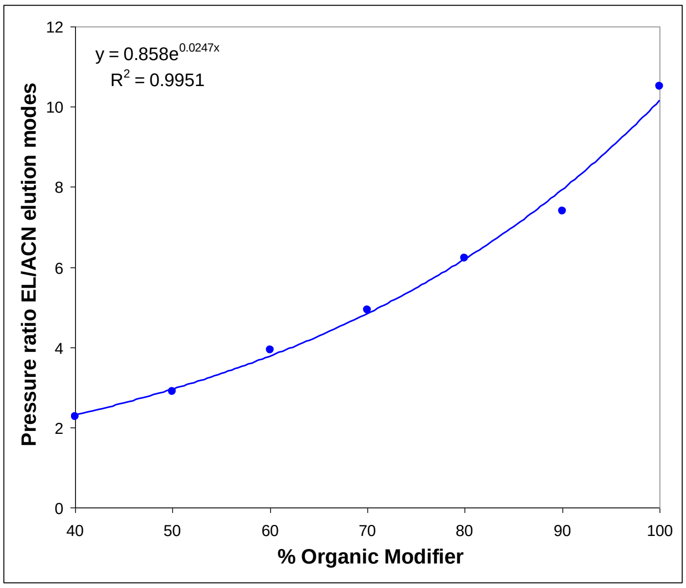


Figure 51. Column's backpressure increase on shifting from ACN to EL as organic modifier in the mobile phase

4.3.3.6. Cut-off wavelenghts

Shifting from ACN to EL based elution mode may influence UV detection due to EL different cut-off wavelengths compared to ACN. The EL cut-off wavelength is expected to be higher than that of ACN, due to the contained chromophore groups, on one hand, and due to possible solvent impurities in the solvent (EL used in this study is not a chromatographic grade solvent).

The cut-off wavelengths recorded for different mixtures EL/water are illustrated in **Figure 52**, where, as expected, an increase in EL percentage in the mobile phase content is followed by an increase in light absorbsion.

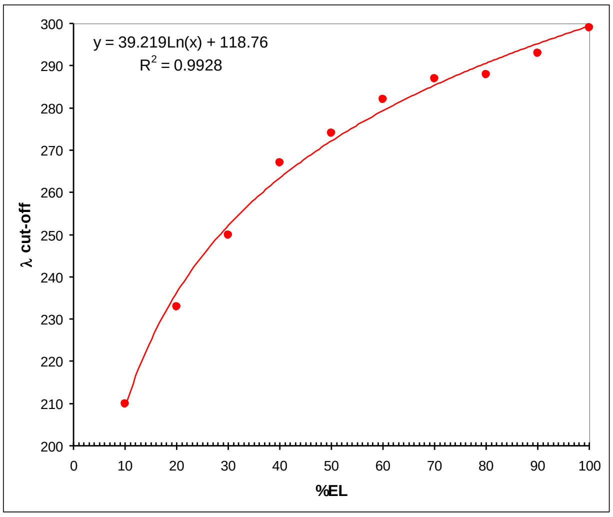


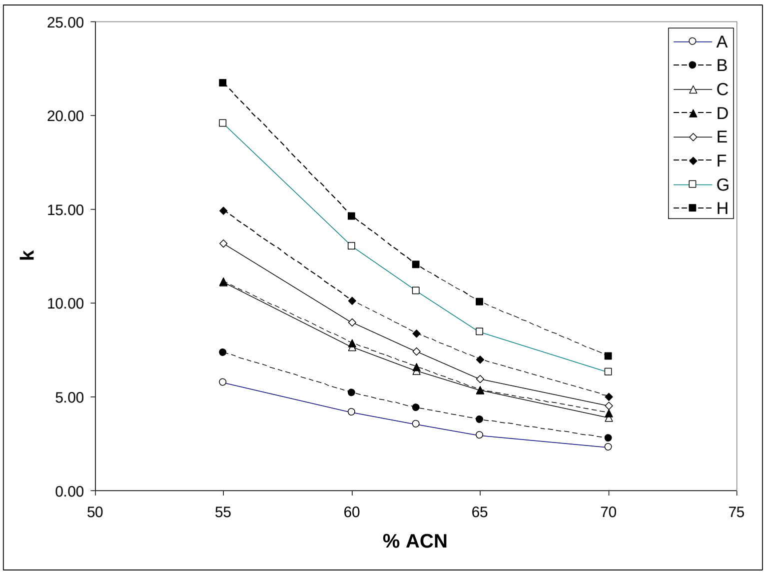
Figure 52. Cut-off wavelengths measured for different volumetric ratios between EL and water

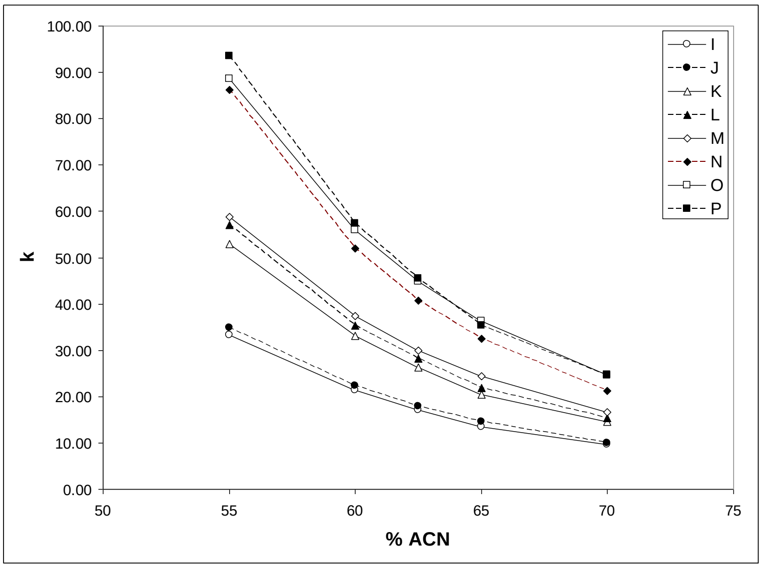
4.3.3.7. Equivalence between ACN and EL contents to produce similar retention

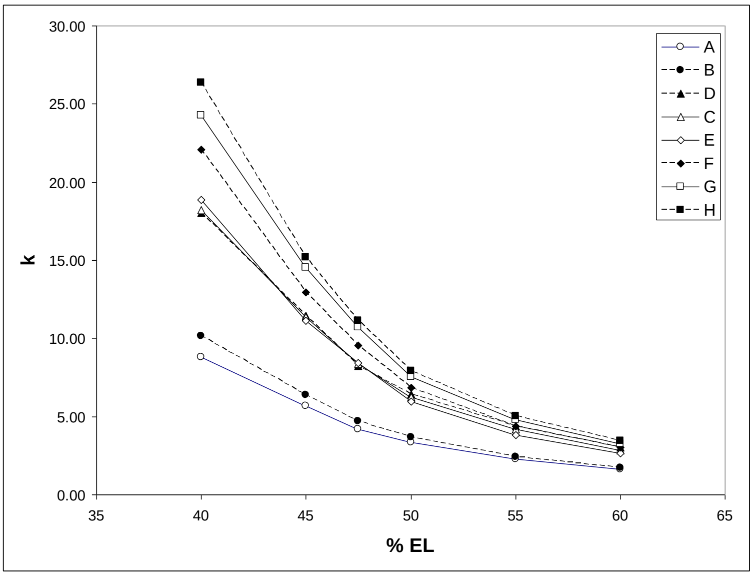
Determination of the equivalent amount of EL in the mobile phase producing similar retention to that obtained with ACN was performed by studying the retention behaviour of all target compounds.

The compositions of the mobile phase containing ACN/water in volumetric ratios of 55/45, 60/40, 62,5/37,5, 65/35 and 70/30 v/v and respectively EL/water in volumetric ratios of 40/60, 45/55, 47,5/52,5, 50/50, 55/45 and 60/40 v/v were used for determination of the retention factors (k).

The functional dependencies of the retention factor versus % of organic modifier are presented in **Figure 53 A-D.**







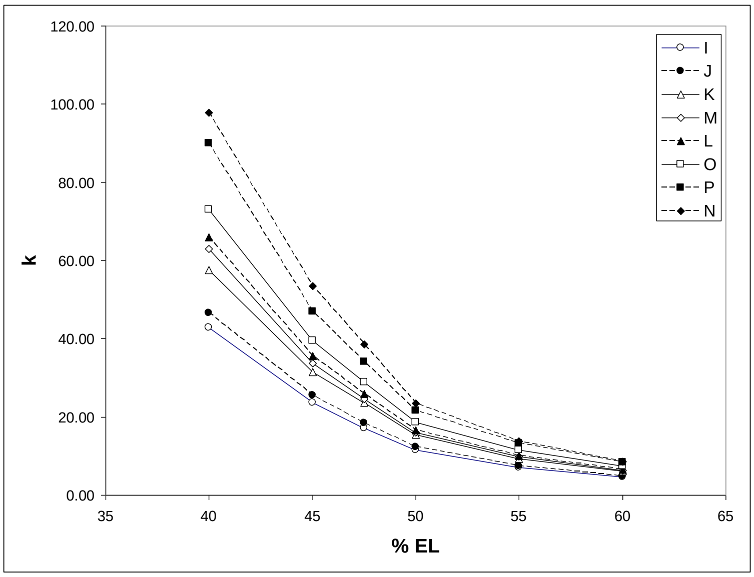


Figure 53. Variation of the retention factor as a function of the percentage of the organic modifier in the mobile phase

The linearisation of the above presented functional dependencies was made through double logaritmation and the folowing equations were obtained:

$$logk^{ACN} = B^{ACN} \times log(\%ACN) + A^{ACN} \qquad (29)$$

$$logk^{EL} = B^{EL} \times log(\%EL) + A^{EL} \qquad (30)$$

By equalizing the equations (29) and (30) a general equation is obtained:

$$(\%EL) = (\%ACN)^{B^{ACN}/B^{EL}} \times 10^{\left(A^{ACN} - A^{EL}\right)/B^{EL}} \qquad (31)$$

The equivalence between the content of EL and ACN in the mobile phase producing similar retention, according to (31) is presented in **Table 21.**

Considering the mean values derived from the retention equivalence for all compounds over the investigated intervals of organic modifier in the mobile phase a general relationship is obtained:

$$\%EL = 0{,}7 \times \%ACN + 3{,}6 \qquad (32)$$

Table 21. Equivalent EL and ACN content in the mobile phase producing similar retention for the target compounds

Compound	% EL				
% ACN	**70**	**65**	**62.5**	**60**	**55**
A	55.1	51.5	49.7	47.9	44.3
B	53.5	50.1	48.3	46.6	43.1
C	56.0	52.2	50.3	48.4	44.7
D	56.0	52.2	50.3	48.4	44.6
E	53.8	50.2	48.5	46.7	43.1
F	53.8	50.4	48.6	46.8	43.3
G	52.7	49.1	47.3	45.6	42.0
H	51.6	48.3	46.6	45.0	41.6
I	52.5	49.0	47.2	45.5	42.0
J	52.6	49.2	47.4	45.7	42.2
K	51.4	47.9	46.1	44.3	40.8
L	51.7	48.2	46.4	44.7	41.2
M	50.5	47.2	45.5	43.9	40.5
N	51.6	48.2	46.4	44.7	41.2
O	48.4	45.2	43.6	42.0	38.8
P	49.9	46.6	44.9	43.2	39.8
Mean	***52.6***	***49.1***	***47.3***	***45.6***	***42.1***
SD	***2.10***	***1.96***	***1.90***	***1.83***	***1.70***

Resulting chromatograms for all the target compounds**,** in both elution modes are presented in **Figure 55.**

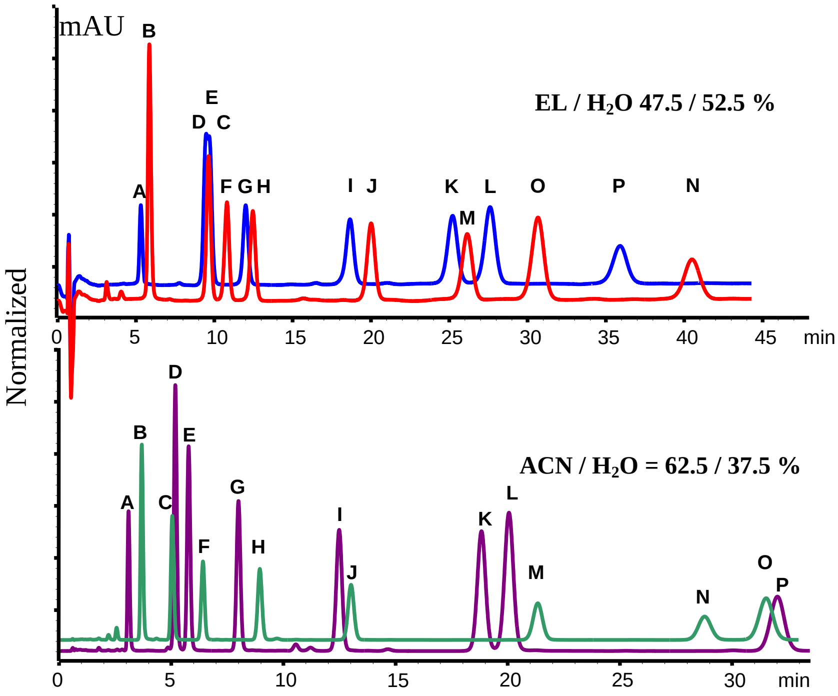


Figure 55. Tipical chromatograms for all the target compounds in both elution modes

4.3.3.8. Thermodynamic aspects

Replacement of ACN with EL was investigated from a thermodynamic point of view. The elution conditions were ACN/water 70/30 and EL/water 45/55, corresponding to similar selectivity conditions. The temperature interval 15-50°C was considered. Variation of temperature was made in steps of 5°C. Van't Hoff plots lnk=f(1/T) were found linear for all studied compounds and characterized by high correlation coefficients (r>0,98), illustrating that the separation mechanism does not change with temperature.

4.3.3.9. Correlation of the retention data with different molecular descriptors

In liquid chromatography, the correlation of the retention factor (k) with different physicochemical, topological or geometrical properties of the solutes, expressed by means of quantities known as descriptors, represents an opportunity to have an image of those solute properties which controls retention.

The best known and used physicochemical descriptors are: molecular weight (Mw), the solubility in water (S) and octanol-water partition coefficient, which is a measure of hydrophobicity (log P). In the case of PAHs, the best known geometric descriptors are the van der Waals volume (Vw) and the van der Waals surface area (Aw). Other descriptors refer to the molecular shape (length to breadth ratio—L/B) or to the molecular topology (the connectivity index—χ).

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.

The values of the mentioned descriptors are enlisted in **Table 23** and the correlations between these descriptors and the retention factors can be found in **Table 24.**

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.

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.

Table 23. The molecular descriptors values for the studied compounds.

Compound	Acronim	M_w	V_w	Log P	S mg/L	χ	L/B
Naphthalene	A	128	73.96	3.18	31.70000	3.405	1.24
Acenaphthylene	B	152	83.44	3.48	16.10000	4.149	1.08
Fluorene	C	166	93.67	4.01	1.90000	4.612	1.57
Acenaphthene	D	154	87.44	3.82	3.93000	4.445	1.06
Phenanthrene	E	178	99.56	4.2	1.10000	4.815	1.46
Anthracene	F	178	99.56	4.2	0.06200	4.809	1.57
Fluoranthene	G	202	109.04	4.5	0.26500	5.565	1.22
Pyrene	H	202	109.04	4.5	0.01320	5.559	1.27
Chrysene	I	228	125.16	5.22	0.00190	6.226	1.72
Benzo(a)anthracene	J	228	125.16	5.22	0.01460	6.220	1.58
Benzo(b)fluoranthen	K	252	134.64	5.52	0.00150	6.976	1.40
Benzo(k)fluoranthen	L	252	134.64	5.52	0.00081	6.970	1.48
Benzo(a)pyrene	M	252	134.64	5.52	0.00162	6.970	1.50
Dibenzo(a,h)anthracene	N	278	150.76	6.24	0.00056	7.631	1.79
Benzo(g,h,i)perylene	O	276	-	6.22	0.00026	7.720	1.12
Indeno(1,2,3-c,d)pyrene	P	276	-	6.22	0.00019	7.720	1.40

Table 24. Functional correlation between molecular descriptors and experimental retention data obtained under ACN and EL elution conditions.

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

4.3.3.10. Conclusions

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 the pressure drop is compatible with the maximum operating limit accepted by most chromatographic systems in laboratories (400 bar).

The cut-off the wavelength of EL is significantly much higher than that of ACN and it can be a major drawback when using UV detection, especially for rich organic modifier mobile phase compositions and for separation of solutes lacking of chromophores.

5. Thesis conclusions

The aim of this thesis was to evaluate two main directions through which liquid chromatography can contribute to the trend of becoming "green", more and more present in recent years.

The first direction was to estimate hydrophobicity indices from chromatographic retention data. This method of estimating hydrophobicity is an alternative to classical practices, which involve a high consumption of solvents, a long experimental time and are quite laborious. Chromatographic methods have the advantage of being faster, require small amounts of sample, determinations are made in a shorter time.

The proposed hydrophobicity indices correlate acceptably at the statistic level, with those calculated using dedicated software. Some of the newly proposed indices correlate even better with the computed indices, compared to those estimated by extrapolation, in the reverse phase mechanism.

The usefulness of such hydrophobicity indices, estimated from chromatographic retention data, on a bimodally acting stationary phase can be applied in the development of hydrophobicity scales for compounds with certain structural features and for which a single separation mechanism approach can be difficult or inconclusive.

The second direction was to evaluate greener solvents as possible substitutes for acetonitrile (ACN).

The first solvent evaluated was propylene carbonate (PC).

This thesis provided a more detailed assessment of some aspects of such a replacement. These issues related to the conditions required for PC to be miscible with water in any proportion, the additives required and their proportion for complete miscibility, the hydrodynamic resistance developed by the columns, the equivalence between ACN content and PC in the mobile phase, necessary to obtain a similar retention and optimal flow. The tolerability against inorganic buffers, necessary for the separation of basic compounds, was also investigated.

The second solvent evaluated as a possible green alternative to ACN was ethyl lactate (EL).

In the case of the thesis, the additional contributions were, as in the case of PC, the detailed assessment of a number of issues related to the replacement of ACN with EL.

Given the higher viscosities and densities of EL compared to ACN, in the case of both solvents it should be noted that in the studied concentration range the pressure limit of 400 bar, specific to most HPLC systems, was not exceeded.

Replacing ACN with PC or EL may be feasible. In addition to the advantage of replacing ACN with greener solvents, there are also some details, such as the EL cut-off wavelength, which can be a major drawback depending on the specific applications in which it is used.

References:

1. P. Anastas, N. Eghbali, Green Chemistry: Principles and Practice, Chem. Soc. Rev., 2010, 39, 301
2. J. Curyło, W. Wardencki, J. Namieśnik, Green Aspects of Sample Preparation – a Need for Solvent Reduction, Polish J. of Environ. Stud., 2007, 16, 5;
3. M. Kaljurand, M. Koel, Recent Advancements on Greening Analytical Separation, Crit. Rev. Anal. Chem., 2011, 41, 2;
4. U. D. Neue, HPLC columns: theory, technology, and practice, Wiley-VCH, New York, 1997;
5. S. Fekete, K. Ganzler, J. Fekete, Efficiency of the new sub-2μm core–shell (Kinetex) column in practice, applied for small and large molecule separation, J. Pharm. Biomed. Anal., 2011, 54, 482;
6. D. T.-T. Nguyen, D. Guillarme, S. Rudaz, J. Veuthey, Chromatographic behaviour and comparison of column packed with sub-2 μm stationary phases in liquid chromatography, J. Chromatogr., A, 2006, 1128, 105;
7. R. E. Majors, Fast and ultrafast HPLC on sub-2-μm porous particles—where do we go from here?, LCGC North Am., 2005, 23, 8;
8. A. D. Jerkovich, J. S. Mellors, J. W. Jorgenson, The use of micrometer-sized particles in ultrahigh pressure liquid chromatography, LCGC North Am., 2003, 21, 600;
9. J. R. Mazzeo, U. D. Neue, M. Kele, R. S. Plumb, Advancing LC performance with smaller particles and higher pressure, Anal. Chem., 2005, 77, 460A;
10. T. H.Walter, R. W. Andrews, Recent innovations in UHPLC columns and instrumentation, Trends Anal. Chem., 2014, 63, 14;
11. I. Ali, V. D. Gaitonde, A. Grahn, Halo Columns: New Generation Technology for High Speed Liquid Chromatography, J. Chromatogr. Sci., 2010, 48, 386;
12. V. Gonzalez-Ruiz, A. I. Olives, M. A. Martin, Core-shell particles lead the way to renewing high-performance liquid chromatography, Trends Anal. Chem., 64, 2015, 17;
13. G. Guiochon, F. Gritti, Shell particles, trials, tribulations and triumphs, J. Chromatogr., A,

1218, 2011, 1915;
14. F. Gritti, G. Guiochon, Facts and legends about columns packed with sub-3 μm core–shell particles, LCGC North Am., 2012, 30, 586;
15. S. Heinisch, J. L. Rocca, Sense and nonsense of high-temperature liquid chromatography, J. Chromatogr. A, 2009, 1216, 642;
16. L. T. Taylor, Supercritical fluid chromatography for the 21st century, J. Supercrit. Fluid., 2009, 47, 566;
17. C.West, E. Lesellier, A unified classification of stationary phases for packed column supercritical fluid chromatography, J. Chromatogr. A, 2008, 1191, 21;
18. H. Kataoka, Recent Advances in Solid-Phase Microextraction and Related Techniques for Pharmaceutical and Biomedical Analysis, Curr. Pharm. Anal., 2005, 1, 65;
19. F. Pena-Pereira, I. Lavilla, C. Bendicho, Liquid-phase microextraction techniques within the framework of green chemistry, Trends Anal. Chem., 2010, 29, 617;
20. S. K. Poole , C. F. Poole, Separation methods for estimating octanol–water partition coefficients, J. chromatogr., B, 2003, 797, 3;
21. G. Mercey, T. Verdelet, J. Renou, M. Kliachyna, R. Baati, F. Nachon, L. Jean, P.-Y. Renard, Reactivators of Acetylcholinesterase Inhibited by Organophosphorus Nerve Agents, Acc. Chem. Res., 2012, 45, 756.

Published papers:

1. F. Tache, Ş. Udrescu, F. Albu, F. Micăle, A. Medvedovici Greening pharmaceutical applications of liquid chromatography through using propylene carbonate–ethanol mixtures instead of acetonitrile as organic modifier in the mobile phases, J. Pharm. Biomed. Anal., 2013, 75, 230-238, F.I. (2013) = 2,829, F.I. (2020) = 3,209

2. V. Voicu, C. Sârbu, F. Tache, F. Micăle, Ş. F. Rădulescu, K. Sakurada, H. Ohta, A. Medvedovici, Lipophilicity indices derived from the liquid chromatographic behavior observed under bimodal retention conditions (reversed phase/hydrophilic interaction): Application to a representative set of pyridinium oximes, Talanta, 2014, 122, 172-179, F.I. (2014) = 3,545, F.I. (2020) = 5,339

3. F. Micăle, F. Albu, E. E. Iorgulescu, A. Medvedovici, F. Tache, Ethyl lactate as a greener alternative to acetonitrile in RPLC: a realistic appraisal, , J. Chromatogr. Sci., 2015, 53, 1701–1707, F.I. (2015) = 1,320, F.I. (2020) = 1,280

Participation at conferences and symposia:

1. F. Micăle, Log P descriptors from bimodal chromatographic behavior, Anniversary Symposium " Faculty of Chemistry – 150 years of tradition", 2014, Bucharest, oral presentation.

2. A. Medvedovici, F. Micăle, F. Tache, Green chromatographic assay of PAH in dietary supplements and food staff, International workshop- "Food Chemistry & Engineering", 2015, Constanţa, oral presentation.