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DOCTORAL DISSERTATION

Ion - pair liquid chromatographic mechanism : retention behavior study of some cationic-type oximes

- SUMMARY -

Ph. D. student
Florea (married Rădulescu)
Medeea - Ioana

Doctoral advisor
Prof. Dr. Victor David

Dissertation committee:

Chairman: Prof. Dr. Camelia Bala, University of Bucharest

Doctoral advisor: Prof. Dr. Victor David

Members:

1. Prof. Dr. Andrei Medvedovici, University of Bucureşti
2. Conf. Dr. Costel Sârbu, Babeş-Bolyai University of Cluj-Napoca
3. C.P. I Dr. Dan Florin Anghel, „Ilie Murgulescu" Institute, Romanian Academy

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Table of contents

2.1. Introductive study regarding the ion-pairing retention mechanism applied to some guanidine based compounds

2.1.1. Introduction

Ion-pairing mechanism (IP) in reversed phase liquid chromatography (LC) is widely used in separation of very polar or ionic compounds. This retention mechanism is based on fact that the ionic analyte will interact with an counter-ion, an ion-pair agent (IPA), forming an ion-pair which will partion between the two phases. The ion-pair is characterized by an enhanced hydrophobicity.

The retention process of very polar or ionic compounds in IP-LC depends on many parameters such as the amount and the hydrophobicity of the ion-pairing agent, the concentration of the organic modifier, the nature of the organic modifier, ionic strength, the stationary phase nature, or the mobile phase pH.

The RP-IP retention process is described by two main models, partition and electrostatic models. Although they offer a different image of the interaction between analyte and stationary phase, they may be used as complementary models in explaining different influences of experimental parameters on the retention data obtained from the ion pairing process.

Within this thesis we investigated the behavior of four very hydrophilic compounds with guanidine moiety or tertiary amine moieties. The process of choosing these model compounds was based on their importance in pharmaceutical research, and their ability to form ion pairs due to their high polar character. The compounds structures are shown in **Fig.2.1.1**.

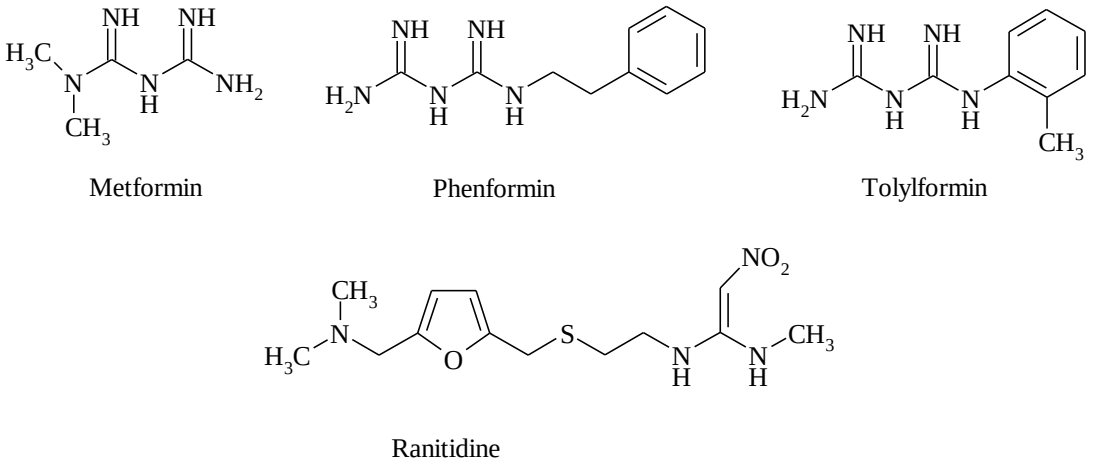


Fig.2.1.1. The compounds structures analized by RP-IPC : metformin, phenformin, tolylformin and ranitidine.

According to the electrostatic model of the ion-pairing mechanism, the retention factor (k') of an charged analyte $A^{\pm z_A}$ with the charge denoted by z_A, in the presence of ion-pairing agent (with charge denoted by z_{IPA}) in mobile phase in a concentration denoted by C_{IPA}, is related to its retention factor in absence of IPA (k^0) by the following dependence:

$$\ln k' = \ln k^0 + \left(\frac{-z_A z_{IPA}}{z_{IPA}^2 + 1}\right)\left[\ln\left(\frac{n_0 K_{IPA} C_{IPA}}{\kappa}\right) + \ln\left(\frac{F^2}{RT\varepsilon_0\varepsilon_r}\right) + 1\right] \quad (1)$$

Where: ln is the natural base logarithm; n_0 – the monolayer of the stationary phase surface for the ion-pair agent (as mol/m^3); κ - inverse Debye length (m^{-1}); F – Faraday constant (C/mol); T – absolute temperature (in K); R – gas constant (J/mol K); ε_0 – permittivity of vacuum (F/m); ε_r – dielectric constant of the mobile phase.

2.1.3. Results and discussions

The influence of the organic modifier concentration on the retention process

The methanol content of the mobile phase influences the adsorption equilibrium of IPA at hydrophobic sites from stationary phase. Linear dependences between the ten-base logarithm (log) of the retention factor and the methanol content of the mobile phases (%MeOH) were obtained for all studied compounds and the four alkylsulphonates used as IPA. In Fig.**2.1.4.** and **2.1.5** are illustrated the dependences obtained for metformin and fenformin, two of the studied compounds.

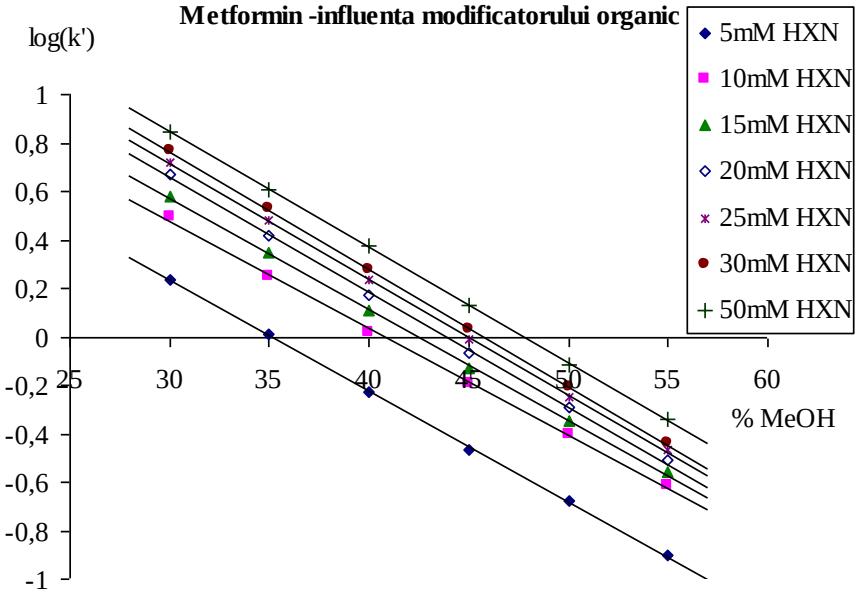


Fig.2.1.4. Dependences log (k') vs %MeOH obtained for metformin analyzed by RP-IPC on Zorbax Eclipse XDB C18 stationary phase, Mobile Phase: Aq (0,1% H_3PO_4 + x mM $C_6H_{13}SO_3Na$) + MeOH

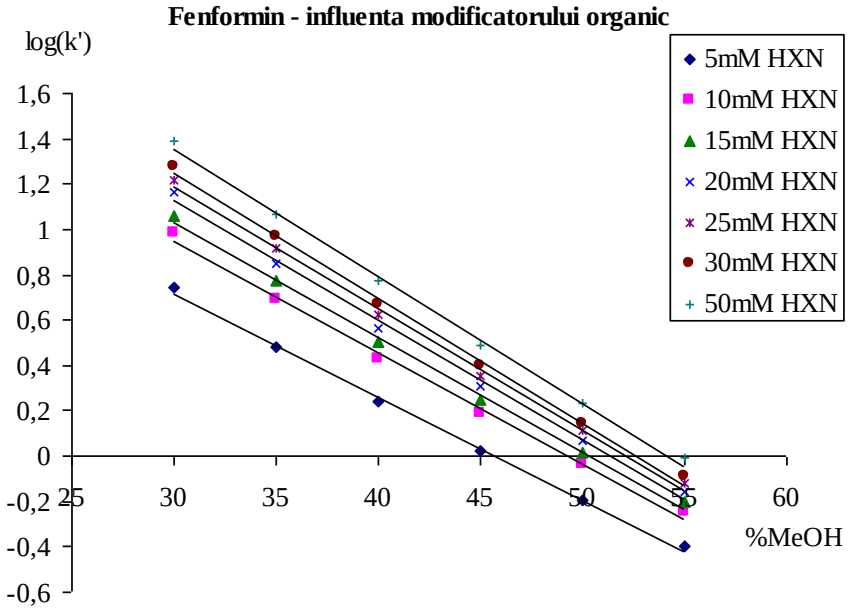


Fig.2.1.5. Dependences log (k') vs %MeOH obtained for fenformin analyzed by RP-IPC on Zorbax Eclipse XDB C18 stationary phase, Mobile Phase: Aq (0,1% H_3PO_4 + x mM $C_6H_{13}SO_3Na$) + MeOH

The intercept of this regression represents the extrapolated values of $\lg k'$ for 100% aqueous component as mobile phase and it is proportional to the partition constant of the ion pair between mobile phase and stationary phase, for the used concentration of IPA in mobile phase and for the used pH of the aqueous component. These values for $C_6H_{13}SO_3^-$, for example, used as anion pairing agent are given in Table 2.1.1.

Tabel 2.1.1. Partition constants values obtained by RP-IP mechanism
IPA : Sodium 1-hexanesulfonate

Analyte	5mM	10mM	15mM	20mM	25mM	30mM	50mM	**log P EPI Suite**
Metformin								
log k'w	1.6003	1.7947	1.9449	2.0739	2.1441	2.2181	2.2742	
log P	1.7953	1.9897	2.1399	2.2689	2.3391	2.4131	2.4692	-2.6399
R^2	0.9996	0.9989	0.9994	0.9992	0.9997	0.9998	0.9999	
Phenformin								
log k'w	2.0745	2.4128	2.5478	2.7019	2.7909	2.89	3.0293	
log P	2.2695	2.6078	2.7428	2.8969	2.9859	3.085	3.2243	-0.6522
R^2	0.9982	0.9965	0.9975	0.996	0.997	0.9969	0.9972	
Tolylformin								
log k'w	2.3913	2.8024	2.9445	3.1199	3.2216	3.337	3.4929	
log P	2.5863	2.9974	3.1395	3.3149	3.4166	3.532	3.6879	1.0514

R^2	0.9969	0.9951	0.9961	0.9948	0.9960	0.9960	0.9967	
Ranitidine								
log k'w	2.2041	2.5871	2.7631	2.934	3.0204	3.1335	3.2563	0.2938
log P	2.3991	2.7821	2.9581	3.129	3.2154	3.3285	3.4513	
R^2	0.9932	0.9905	0.9929	0.9912	0.9931	0.9930	0.9937	

The influence of the IPA concentration on the retention process

Keeping constant all the other parameters, the dependence of the retention factor on this parameter must be a linear logarithmic dependence with a slope 0.5 for ionic charge for both analyte and IPA of 1 ($\frac{z_A z_B}{z_A^2+1} = -\frac{1}{2}$). This equation can be written in the form:

$$\ln k' = \chi + \frac{1}{2}\ln C_{API} \qquad (2.1.8)$$

The regression parameters obtained for different composition of the mobile phase, within 30%-55% MeOH, are listed in Table 2.1.2.

Tabelul 2.1.2. Regression parameters for the dependences of $\ln k'$ on $\ln C_{IPA}$ (IPA: C6) for several compositions of the mobile phase.

% MeOH	Intercept (χ)	Slope	R^2
Metformin			
30	-0.344	0.611	0.9716
35	-0.862	0.602	0.9801
40	-1.415	0.601	0.9852
45	-1.890	0.576	0.9663
50	-2.301	0.537	0.9557
55	-2.784	0.530	0.9333
Phenformin			
30	0.705	0.650	0.9917
35	0.192	0.590	0.9938
40	-0.263	0.526	0.9977
45	-0.672	0.464	0.9961
50	-1.079	0.414	0.9948
55	-1.498	0.382	0.9918
Tolylformin			
30	1.082	0.719	0.9915
35	0.499	0.652	0.9951

40	0.003	0.576	0.9987
45	-0.438	0.502	0.9990
50	-0.857	0.439	0.9991
55	-1.275	0.394	0.9980
Ranitidine			
30	0.444	0.720	0.9861
35	-0.281	0.677	0.9895
40	-0.853	0.597	0.9966
45	-1.326	0.524	0.9947
50	-1.759	0.464	0.9933
55	-2.220	0.432	0.9868

Such dependences can be used in estimating the extrapolated value of the retention factor of the analyte in presence of IPA having a concentration of 1 (moles/L). At this concentration value of IPA the value of $\ln C_{IPA}$ becomes 0, and the value of $\ln k'$ becomes χ.

In **Fig.2.1.12.** there can observed the influence of IPA concentration on the retention process and the chromatographic peak shapes. The enhanced amount of the IPA leads to an enhancement of the retention times; the peaks maintain their symmetry, but are more stretched.

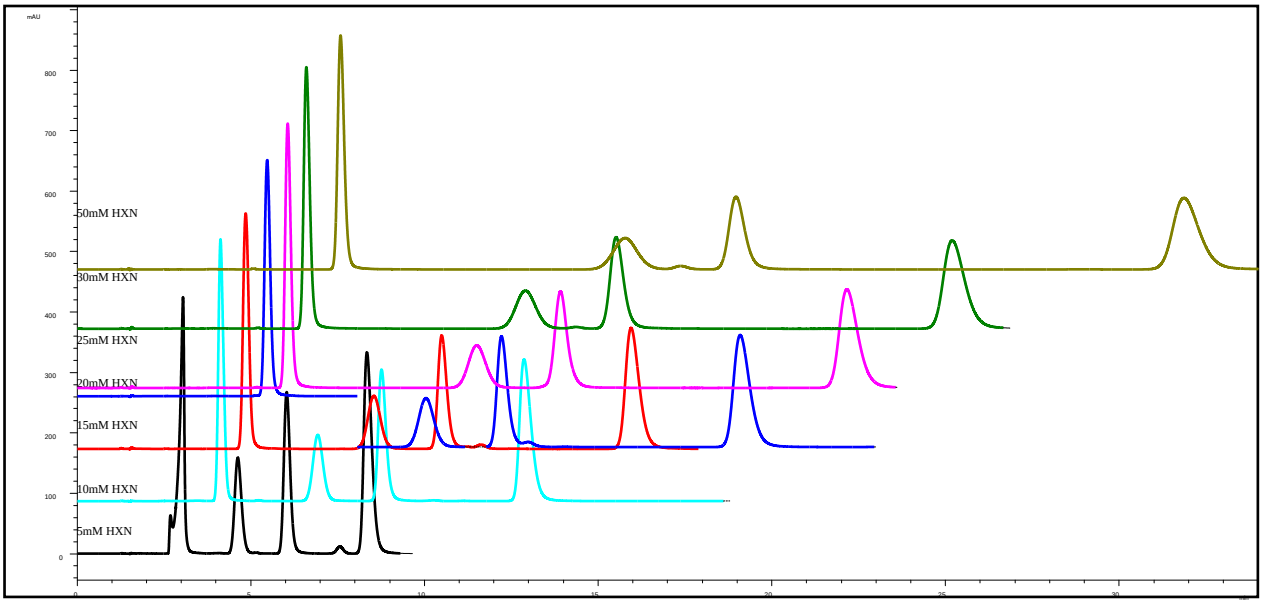


Fig.2.1.12. Overlaid chromatograms obtained for the analysed compounds by RP-IPC on Zorbax Eclipse XDB C18 stationary phase, Mobile Phase: 65%Aq (0,1% H_3PO_4 + x mM $C_6H_{13}SO_3Na$) + 35%MeOH

The influence of the IPA hydrophobicity on the retention process

The hydrophobicity of IPA increases with the number of C atoms and thus the value of the retention times increases with the number of C atoms. Four alkylsulphonates were studied in order to find out the influence of the number of C atoms on the retention of studied compounds (C5 – C8). A linear dependence was observed between log k' the number of C atoms, with correlations higher than 0.98, excepting ranitidine, whose correlation was however poorer (see **Fig.2.1.13**)

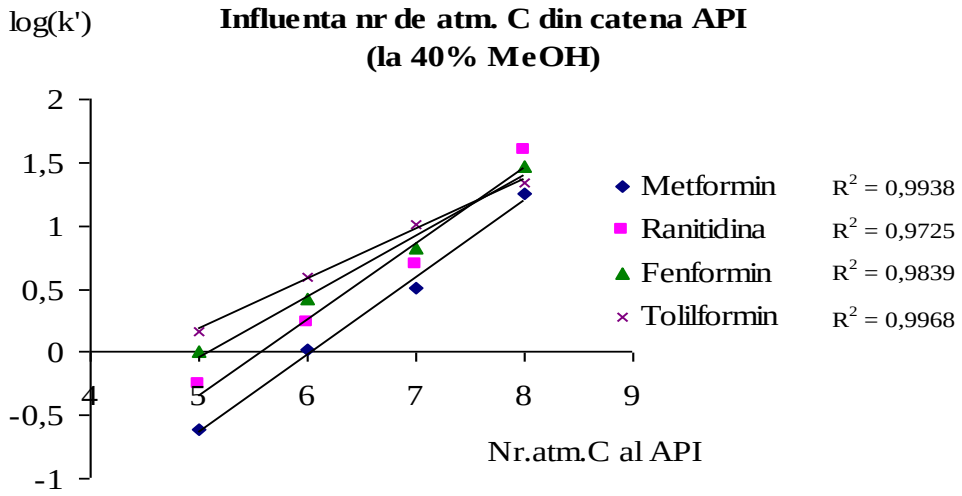


Fig.2.1.13. Dependence of the retention of studied compounds on the number of C atoms in ion pairing molecule (Experimental conditions: 10 mM IPA; pH = 2; 40% MeOH).

The influence of the pH value on the retention process

pH value of the aqueous component plays a major role in ion-pair formation and thus in enhancing the analyte retention under this mechanism. This parameter was studied on a broad pH range (1.2 – 8). Sigmoidal shapes were obtained for several compositions of the mobile phase (35; 40 and 45% MeOH, respectively), in accordance with the partition model; the process can be described by the equation:

$$k_A^{API} = k_A^0 + k_{A^+API^-} \frac{K_b 10^{-pH}}{K_w + K_b 10^{-pH}} \qquad (2.1.9)$$

An example of such shapes is illustrated in **Fig. 2.1.15** (for 35% MeOH in mobile phase), where the experimental values situated in the pH domain within 3 – 7 can be fitted by means of Boltzmann function. This behavior is well explained by partition model. If we extend the *k* versus

pH dependence on 2 – 8 a double sigmoidal shape can be observed for metformin, phenformin and tolylformin, which might be explained by the possibility of protonation at two =NH sites of the molecule, and leading to a double pair, $AH_2^{2+}(IPA^-)_2$. This double pair between analyte and ion pairing agent is more hydrophobic than AH^+IPA^- and can be formed for very acidic pH of the aqueous component (pH between 1 and 2).

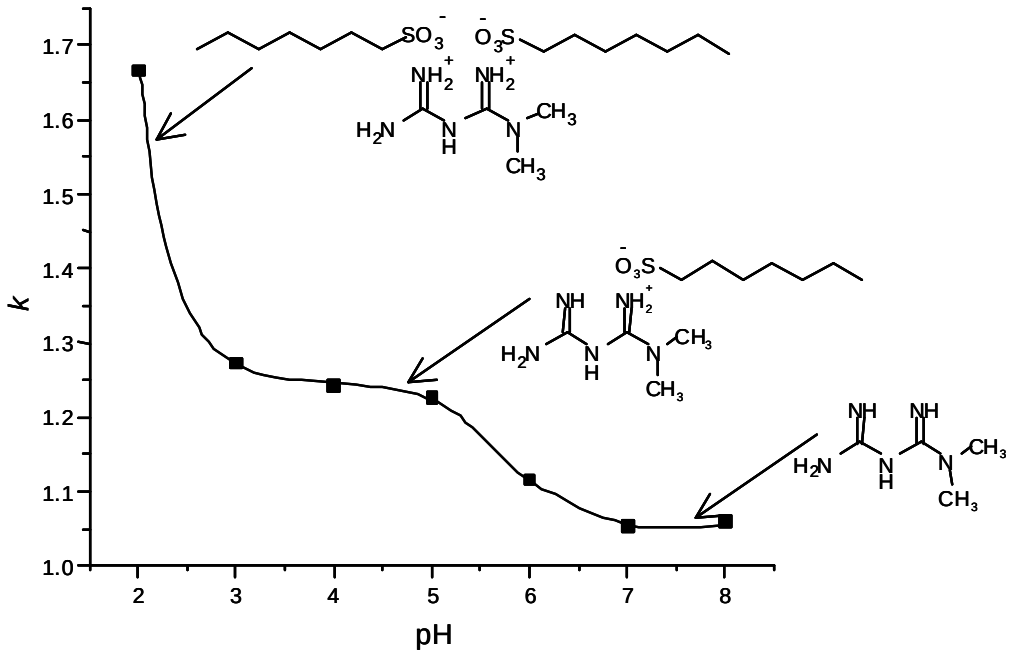


Fig.2.1.15. Dependence of the capacity factor for studied compounds on the pH of the aqueous mobile in IP mechanism (m.p.: 35% MeOH + 65% aqueous component consisting of 10 mM $C_7H_{15}SO_3Na$ + 0.1% H_3PO_4 adjusted with 10% NaOH solution).

The increase of the retention of phenformin, tolylformin and ranitidine for pH > 7 can be explained by the deprotonation of the basic sites from their molecules, and thus the ion pairing does no longer take place. For pH > 7, these molecules participate to the retention process as free molecules according to the simple reversed-phase mechanism owing to the hydrophobic interaction between hydrocarbon moieties of their molecules and C18 chains from stationary phase. This effect was also observed for metformin, but its retention enhancement was less significant compared to the other model compounds because the two methyl groups are less involved in such interaction.

2.1.4. Conclusions

The experimental parameters studied for some model compounds containing basic functional groups, under ion-pairing mechanism in reversed-phase liquid chromatography, can be

explained by both theoretical models, i.e. electrostatic and partition model. Thus, the effects of the nature of the ion pairing agent and its concentration in mobile phase are fairly explained by the electrostatic model, and the dependences predicted by this model did not reveal any deviation from theory. Good correlations were obtained for the dependences between experimental parameters and the chromatographic outcome as predicted by this theoretical model.

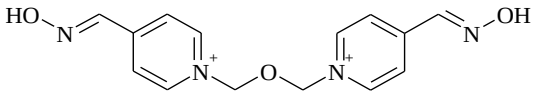
The effect of the methanol and pH was found to be well explained by the partition model, and the functional dependences between experimental parameters and the chromatographic outcome were very well correlated. Moreover, these correlations can be used in estimating of some extrapolated values for the chromatographic outcome, which can be used in characterizing the ion pair between analyte and ion pairing agent by means of the hydrophobicity descriptor. The effect of pH on the retention is however more complex in the case of complex molecules with two or more basic sites and other functional groups with electronic effects on their molecule.

2.2. Retention study of some cationic-type oximes under RP-IP mechanism

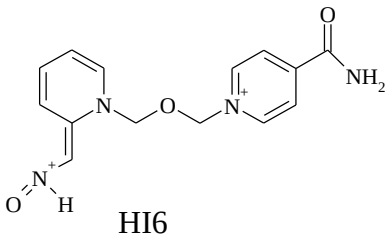
2.2.2. Retention study of some cationic-type oximes on Zorbax Eclipse XDB C18 stationary phase

2.2.2.1. Introduction

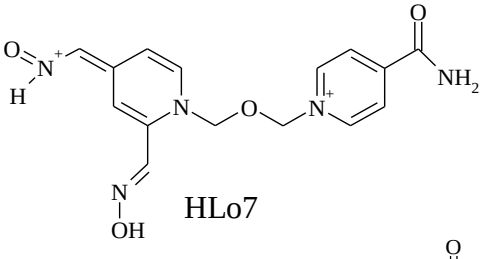
In this study we investigated the retention behavior of eight cationic-type aldoximes (with structures given in **Fig. 2.2.3**) used as acetylcholinesterase (AChE) reactivators by ion-pairing mechanism with common alkylsulphonate anions in order to compare the results with behavior predicted by partition and electrostatic models.



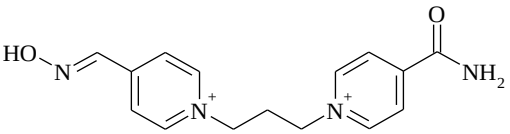
Obidoxime



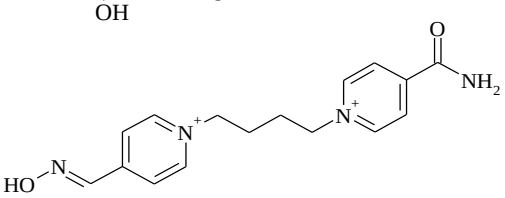
HI6



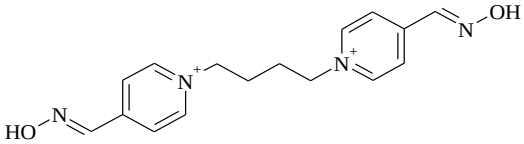
HLo7



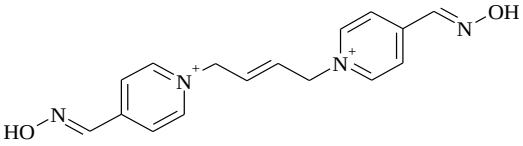
K27



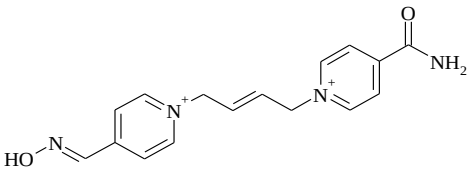
K48



K74



K75



K203

Fig.2.2.3. Structures of the cation-type oximes analysed under RP-IPC on C18 Zorbax Eclipse XDB stationary phase.

2.2.2.3. Results and discussions

The influence of the organic modifier concentration on the retention process

In reverse phase liquid chromatography, the effect of the organic modifier content on the retention process can be described by the following equation:

$$\log(k') = \log(k')_w + \sum_{i=1}^{2} \alpha_i C_m^i \qquad (2.2.2)$$

Where: α_i are regressions parameters, C_m – the organic modifier concentration (volumetric percentage).

- For pH = 2 were obtained linear dependences and the regressions parameters were listed in Table 2.2.1.a. The intercept of this regression represents the extrapolated values of

$\lg k'_o$ for 100% aqueous component as mobile phase and it is proportional to the partition constant of the ion pair between mobile phase and stationary phase, for the used concentration of IPA in mobile phase and for the used pH of the aqueous component.

Tabel.2.2.1.a. Regressions parameters obtained for the dependences log(k') vs. %MeOH

API	Intercept	Slope	R^2	API	Intercept	Slope	R^2
Obidoxime				**HI6**			
PNT	2,3256	-0,0559	0,9792	PNT	2,3213	-0,0575	0,9669
HXN	3,4253	-0,0681	0,9788	HXN	3,0257	-0,0618	0,9879
HPT	3,3648	-0,0576	0,9843	HPT	3,2370	-0,0561	0,9937
OCT	4,2943	-0,0653	0,9993	OCT	4,0115	-0,0614	0,9992
HLo7				**K27**			
PNT	2,5867	-0,0618	0,9557	PNT	2,3331	-0,0569	0,9732
HXN	3,5411	-0,0710	0,9526	HXN	3,6358	-0,0728	0,9548
HPT	3,3737	-0,0578	0,9824	HPT	3,5888	-0,0616	0,9909
OCT	4,1739	-0,0637	0,9993	OCT	-0,0624	4,1165	0,9993
K48				**K74**			
PNT	2,7684	-0,0658	0,9376	PNT	2,7407	-0,064	0,9476
HXN	3,1790	-0,0645	0,9884	HXN	3,2747	-0,0651	0,9823
HPT	3,1704	-0,0546	0,9760	HPT	3,5419	-0,0609	0,9780
OCT	4,0602	-0,0620	0,9993	OCT	4,1797	-0,0635	0,9997
K75				**K203**			
PNT	2,8053	-0,0656	0,9444	PNT	2,9373	-0,0693	0,9200
HXN	3,5753	-0,0711	0,9372	HXN	3,3307	-0,0674	0,9622
HPT	3,6113	-0,0621	0,9927	HPT	3,6699	-0,0640	0,9528
OCT	4,1274	-0,0629	0,9996	OCT	3,9957	-0,0611	0,9998

For several values of pH were verified the correlations between $\log k'$ and C_m; for pH = 2, 3, 4, and 5, respectively, linear regressions were obtained; for pH = 7 the dependences followed a binomial pattern. The regressions parameters were listed in Table 2.2.2.

Tabel.2.2.2. Regression parameters obtained for the studied oximes for pH= 2, 3, 4 and 5, respectively (a); for pH= 7 (b); sodium octanesulphonate (10mM) was used as IPA

a.

pH	log k_w	α_1	R^2	pH	log k_w	α_1	R^2
Obidoxime				**HI6**			
2	4,1259	-0,0641	0,9996	2	3,8788	-0,0608	0,9995
3	4,0428	-0,065	0,9999	3	3,8638	-0,0632	0,9989

4	4,1081	-0,0652	0,9977	4	3,8708	-0,0616	0.998
5	3,3309	-0,0533	0,9935	5	3,2657	-0,0542	0,9971
HLo7				**K27**			
2	4,0510	-0,0633	0,9997	2	3,9946	-0,0621	0,9998
3	4,0464	-0,0658	0,9994	3	4,0119	-0,0649	0,9994
4	4,0086	-0,0641	0,9973	4	4,0132	-0,0639	0,9979
5	3,2905	-0,0537	0,9977	5	3,3561	-0,0543	0,9981
K48				**K74**			
2	3,9508	-0,0619	0,9998	2	4,0997	-0,0639	0,9999
3	3,9648	-0,0647	0,9992	3	4,1265	-0,0668	0,999
4	3,9573	-0,0635	0,9981	4	4,1325	-0,066	0,9981
5	3,3279	-0,0544	0,9982	5	3,5249	-0,0572	0,9985
K75				**K203**			
2	4,0586	-0,0634	0,9998	2	3,9173	-0,0615	0,9998
3	4,0739	-0,0662	0,9992	3	3,9212	-0,0641	0,9992
4	4,0869	-0,0654	0,9979	4	3,9345	-0,0633	0,9974
5	3,4367	-0,0558	0,9982	5	3,1898	-0,052	0,9965

b.

pH 7

Analyte	**log k_w**	**α_1**	**α_2**	**R^2**	**$(C_m)_{min}$**	**$\log(k')_{min}$**
OBI	7,3202	-0,2234	0,0016	0,9988	69,8	-0,4779
HI6	5,9708	-0,1911	0,0015	0,9993	63,7	-0,1157
HLo7	6,0495	-0,1916	0,0014	0,9997	68,4	-0,5060
K27	7,6263	-0,2342	0,0019	0,9994	61,6	0,4092
K48	7,5766	-0,2325	0,0018	0,9993	64,6	0,0688
K74	7,1518	-0,2109	0,0016	0,9995	65,9	0,2020
K75	7,0474	-0,2088	0,0015	0,9995	69,6	-0,2188
K203	7,9774	-0,2473	0,0020	0,9997	61,8	0,3327

The influence of the IPA concentration on the retention process

According to the electrostatic model that explains the compounds retention under ion-pairing mechanism, the functional dependence for a doubly charged analyte ions and a singly charged pairing ions follow the equation:

$$\ln k' = \chi + \ln c_{API} \quad (2.2.9)$$

, where χ is a constant depending on the hydrophobicity and the charge of the analyte and pairing ion, organic modifier and ionic strength.

These dependences were observed for tree IPAs, i.e.sodium haxane-, heptane-, and octane-sulphonate, for an acid pH of the aqueous component of the mobile phase. The plots revealed a good linear correlation (R^2 >0,99), with the slope values within 0.6 – 0.8. This values obtained from the experimental data denotes that in this case are some deviations from the electrostatic model, which predicts a unit value.

Such dependences can be used in estimating the extrapolated value of the retention factor of the analyte in presence of IPA having a concentration of 1 (moles/L). At this concentration value of IPA the value of $\ln C_{IPA}$ becomes 0, and the value of $\ln k'$ becomes χ.

The influence of the pH value on the retention process

The pH value of the aqueous component of the mobile phase is an important parameter that infuences the retention behavior of the ionic/ionizable compounds in RP- IPC mechanism. Sigmoidal shapes were obtained for several compositions of the mobile phase (45; 50, 55 and 60% MeOH, respectively).

An example of such shapes is illustrated in **Fig.2.2.35** (K48; for 50% MeOH in mobile phase), where the experimental values situated in the pH domain within 3 – 7 can be fitted by means of Boltzmann function. If we extend the *k* versus pH dependence on 2 – 8 a double sigmoidal shape can be observed which might be explained by the possibility of the analyte ions to constitute a double pair with the IPA, $A^{2+}(API^-)_2$. This double pair between analyte and ion pairing agent is more hydrophobic than $A^{2+}API^-$ and can be formed for very acidic pH of the aqueous component.

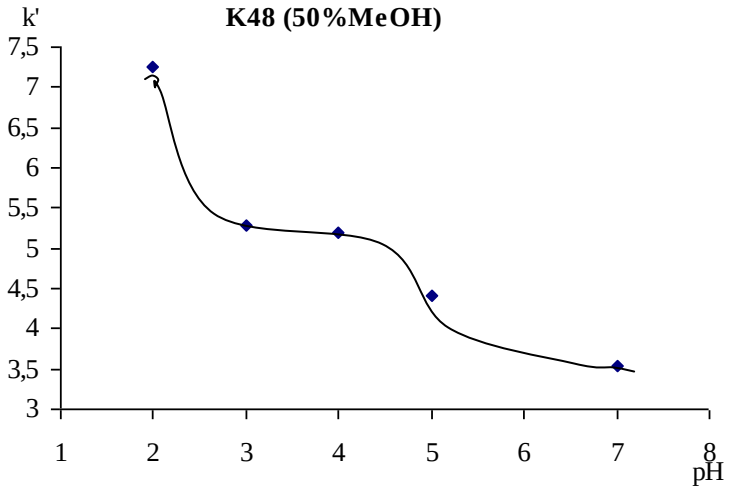


Fig.2.2.35. Functional dependences (k') vs. pH obtained for K48 solution under RP-IPmechanism on Zorbax Eclipse XDB C18 stationary phase, M.P. 50% Aq (10 mM OCT) + 50% MeOH

This retention behavior may rely on different structures that can be assigned to this compounds due to the tautomeric equilibrium, as exemplified in **Fig.2.2.37**. The ionic form (Structure I) is stabilized in a very polar environment and is able to form a double pair with IPA ions. Increasing the pH value of the aqueous component of the mobile phase, Structure II is more stable, so that a single pair is formed. A neutral pH value shifts the analyte structure toward nitroso form (Structure III); in this case, the analyte is unable to interact with IPA, these molecules participate to the retention process as free molecules according to the simple reversed-phase mechanism owing to the hydrophobic interaction between hydrocarbon moieties of their molecules and C18 chains from stationary phase.

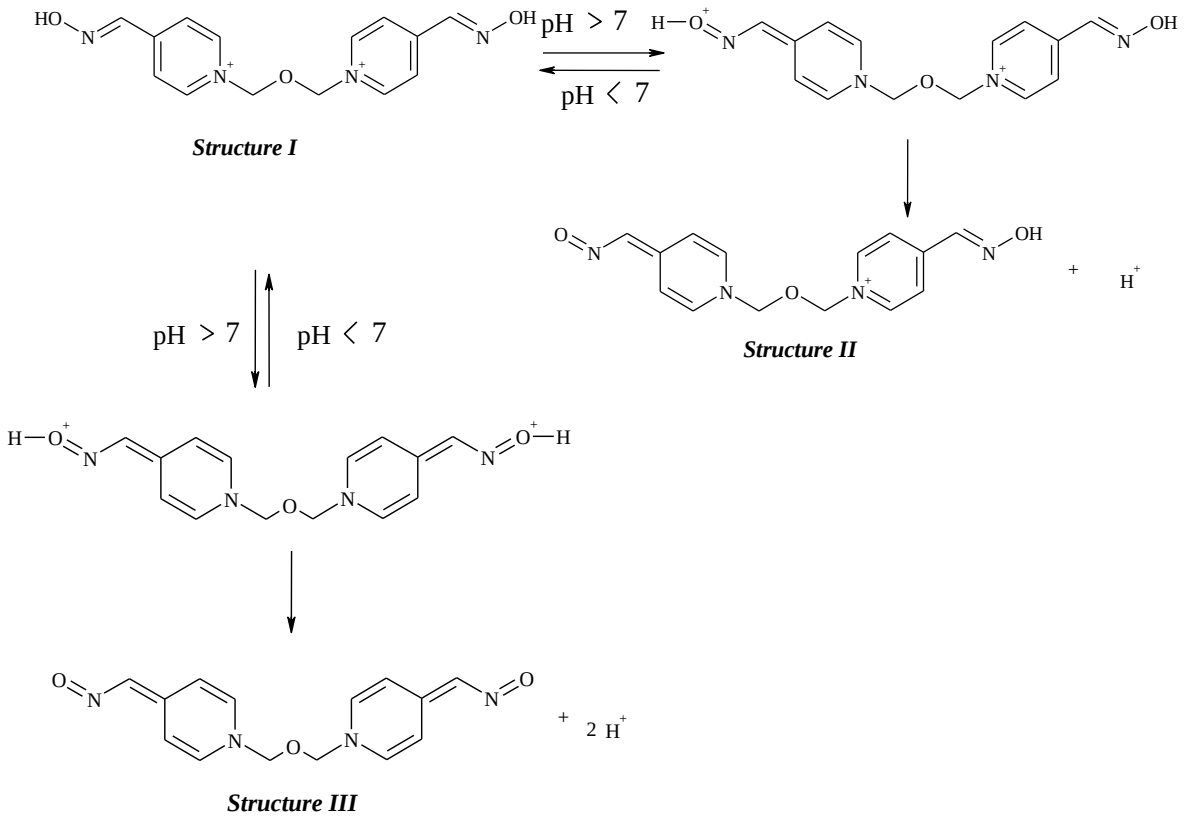


Fig.2.2.37. Tautomeric equilibrium exemplified for obidoxime.

2.2.2. Retention study of some cationic-type oximes on Zorbax ODS C18 stationary phase

The aim of this study was to investigate the retention behavior of some cationic-type oximes, used as AChE reactivators, under ion-pair reversed phase elution mechanism. The studied compounds are pralidoxime (PAM), obidoxime (OBI) and pyridostigmine (PDST) –does not belong to the oximes class, but it has been considered during the experiments due to its former use as an AChE reactivator.

2.2.2.3. Results and discussions

The influence of the organic modifier concentration on the retention process

The functional dependences between the ten base logarithms of the retention factors and the concentration of the organic modifier in the mobile phase were studied, using sodium hexane, heptane- and octane-sulphonate as IPA (15 mmoles/L) – see Fig. **2.2.49**.

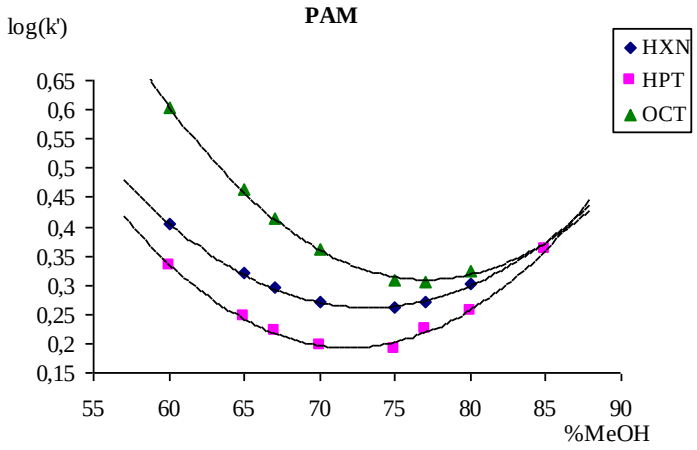


Fig.2.2.49. Functional dependences log (k') vs %MeOH obtained for PAM analyzed under RP-IP mechanism on C18 Zorbax ODS stationary phase, M.P. Aq (0,1% H_3PO_4 + 15mM IPA) + MeOH.

The functional dependence obtained is given by a second order polynomial regression according to the following equation:

$$\log(k') = \log(k')_w + \alpha_1 C_m + \alpha_2 C_m^2 \qquad (2.2.15)$$

where k_w represents the extrapolated value of the capacity factor corresponding to a hypothetical mobile phase containing only the aqueous component and α_1, α_2 are the regression parameters of the functional dependence.

Mathematically, this dependence has a minimum, which can be obtained from the relationship $\frac{\partial \log(k')}{\partial C_m} = 0$. This minimum of the plot is characterized by the value of $(C_m)_{min}$ and $\log(k')_{min}$ according to these relationships:

$$(C_m)_{\min} = -\frac{\alpha_1}{2\alpha_2} \qquad (2.2.5)$$

$$\log(k')_{\min} = \alpha_0 - \frac{\alpha_1^2}{4\alpha_2} \qquad (2.2.6)$$

The graphical representations between the ten base logarithm of the retention factor (log k) for each ionic liquid and the volume percentage of methanol in mobile phase (C_m) showed a characteristic "U" shape with a minimum value within studied interval of mobile phase composition (from 0 to 100%).

The values of the polynomial regression parameters and calculated minimum value

$(C_m)_{min}$, $log(k')_{min}$ are given in Table 2.2.5, where good correlations can be observed for the data fitting.

Tabelul.2.2.5. Regression parameters, according to binomial equation (2.2.15), obtained for the target analytes on retention studies made in acidic conditions (0.1% H_3PO_4, pH ~ 2) and methanol as organic modifier.

Analit	Conc.API	log k_w	$log(k')_{min}$	α_1	A_2	R^2	$(C_m)_{min}$
IPA: Sodium hexane sulphonate							
PAM	15mM	4.655	0.261	-0.1199	$8.18x10^{-4}$	0.9967	73.3
	10mM	3.854	0.294	-0.1024	$7.36 x10^{-4}$	0.9946	69.5
	5mM	3.349	0.447	-0.0875	$6.59 x10^{-4}$	0.9968	66.4
OBI	15mM	5.447	0.347	-0.1684	$1.39 x10^{-3}$	0.9961	60.6
	10mM	3.848	0.483	-0.1206	$1.08 x10^{-3}$	0.9996	55.8
	5mM	2.609	0.690	-0.0789	$8.10 x10^{-4}$	0.9986	48.7
PDST	15mM	2.787	0.577	-0.0727	$5.98 x10^{-4}$	0.9973	60.8
	10mM	2.454	0.580	-0.065	$5.63 x10^{-4}$	0.9995	57.7
	5mM	2.118	0.716	-0.0519	$4.80 x10^{-4}$	0.9965	54.0
IPA: Sodium heptane sulphonate							
PAM	15mM	5.348	0.193	-0.1432	$9.94 x10^{-4}$	0.9978	72.0
	10mM	4.786	0.315	-0.1256	$8.81 x10^{-4}$	0.9979	71.2
	5mM	3.937	0.502	-0.1022	$7.60 x10^{-4}$	0.999	67.3
OBI	15mM	6.366	0.526	-0.1841	$1.45 x10^{-4}$	0.9976	63.5
	10mM	6.044	0.587	-0.1785	$1.46 x10^{-4}$	0.99197	61.1
	5mM	4.403	0.828	-0.1315	$1.21 x10^{-4}$	0.9998	54.4
PDST	15mM	3.187	0.548	-0.0845	$6.76 x10^{-4}$	0.9967	62.5
	10mM	3.184	0.617	-0.0837	$6.82 x10^{-4}$	0.9973	61.4
	5mM	3.047	0.764	-0.0799	$6.99 x10^{-4}$	0.9916	57.1
IPA: Sodium octane sulphonate							
PAM	15mM	6.327	0.296	-0.1561	$1.01 x10^{-3}$	0.9968	77.3
	10mM	5.738	0.337	-0.1464	$9.92 x10^{-4}$	0.9979	73.8
	5mM	5.182	0.497	-0.1331	$9.45 x10^{-4}$	0.9964	70.4
OBI	15mM	12.725	0.657	-0.3439	$2.45x10^{-3}$	0.9952	70.2
	10mM	9.440	0.746	-0.2598	$1.94 x10^{-3}$	0.9986	66.9
	5mM	8.287	0.960	-0.2391	$1.95 x10^{-3}$	0.9979	61.3
PDST	15mM	5.089	0.680	-0.1316	$9.82 x10^{-4}$	0.999	67.0
	10mM	4.218	0.679	-0.1093	$8.43 x10^{-4}$	0.9995	64.8
	5mM	3.595	0.796	-0.0921	$7.58 x10^{-4}$	0.9955	60.8

The tentative explanation for such unusual retention behavior refers to the influence of the mobile phase composition on the interaction between the ionic analyte and the pairing ions (Fig.**2.2.52**).

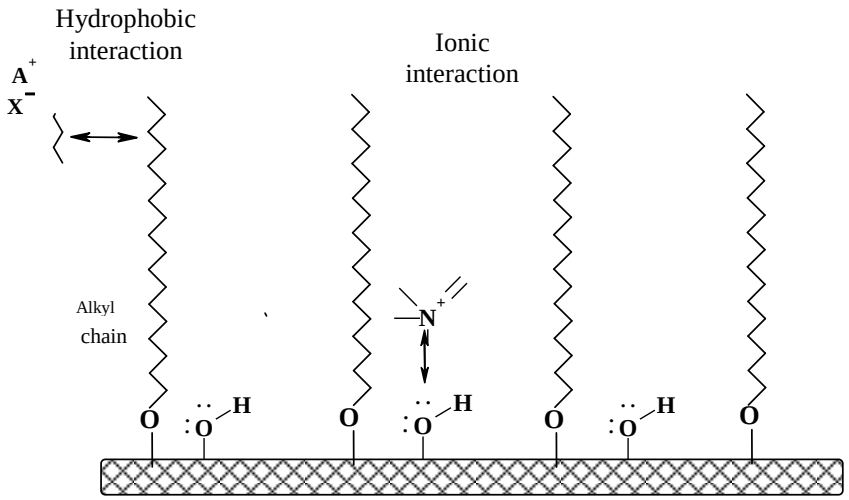
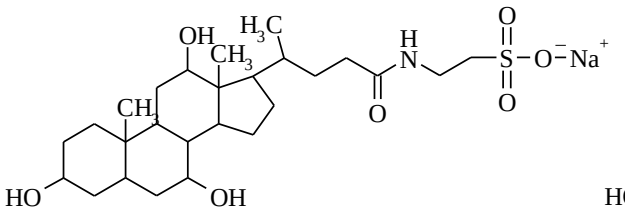


Fig.2.2.52. Potential interactions which may occur between cationic analytes and stationary phase C18 Zorbax ODS, studied through RP-IPC mechanism.

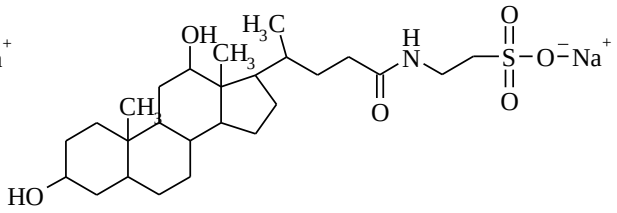
2.3. Bile acids sodium salts used as Ion Pairing Agents in Liquid Chromatography

2.3.1. Introduction

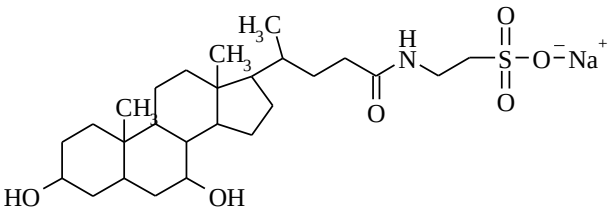
In this thesis we studied the retention behavior of some cationic-type aldoximes used as AChE reactivators, under ion-pair reversed phase elution mechanism using some bile acid sodium salts (with structures given in **Fig.2.3.1**) as ion pairing agents.



Sodium taurocholate



Sodium taurodeoxycholate



Sodium taurochenodeoxycholate

Fig.2.3.1. Structures of bile acids as sodium salts used as ion pairing agents in this study.

2.3.3. Results and discussions

The influence of the organic modifier concentration on the retention process

The experimental retention plots between log k' and the content of organic modifier in mobile phase obtained for the investigated compounds, using sodium taurodeoxycholate as ion pairing agents for acidic pH in the aqueous component of the mobile phase are given in **Fig. 2.3.2.**

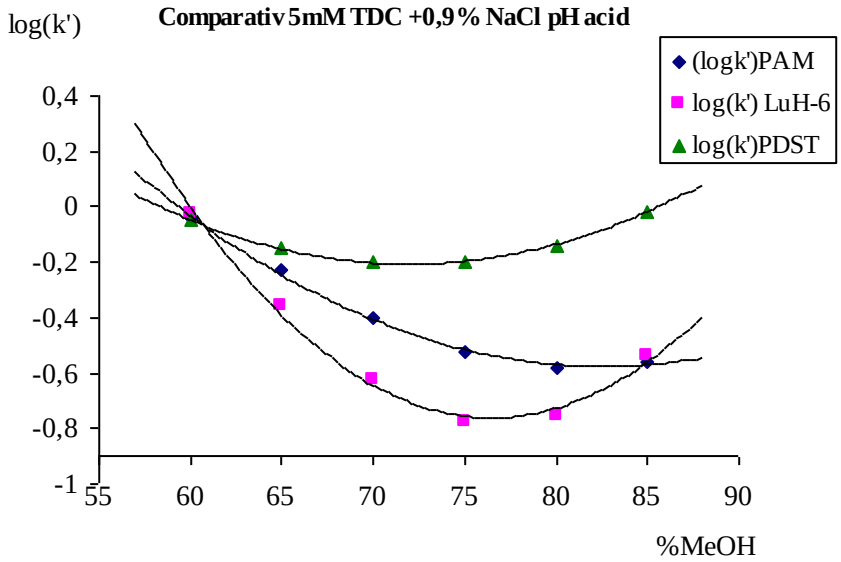


Fig.2.3.2. Functional dependences log (k') vs %MeOH obtained for the solutes analyzed under RP-IP mechanism on C18 Zorbax ODS stationary phase, M.P. Aq (0,1% H_3PO_4 + 5mM TDC+ 0.9% NaCl) + MeOH

These functional dependences followed a binomial pattern (U – shaped), with a minimum positioned within the 70-85% methanol percentage content. This shape is rather uncommon for such dependences.

These dependences can be useful in estimating some molecular descriptors. Thus, the logarithms of the retention factor extrapolated at 0% or 100% organic solvent can be used in estimating different kinds of hydrophobicity descriptors assigned to the ion-pairs of these compounds with mentioned agents. These descriptors can be useful in modeling the membrane crossing process in order to explain their use in the therapy related to intoxication with organophosphorus AChE inhibitors.

The regression parameters for studied compounds for the three bile acids used as ion-pairing agents are given in Table 2.3.1.

Table 2.3.1. Regression parameters and some extrapolated retention values obtained from these regressions.

Ion pairing agent: sodium taurodeoxycholate							
Analyte	$\log k_w$	α_1	α_2	r^2	$(\log k)_{Co=100}$	$(\log k)_{min}$	C_o^{min}
PAM	6.868	-0.180	1.10·10-3	0.9972	-0.201	-0.577	84.6
LuH-6	15.76	-0.432	2.82·10-3	0.9966	+0.758	-0.785	76.6
PDST	5.352	-0.154	1.08·10-3	0.9979	+0.599	-0.138	71.3
Ion pairing agent: sodium taurocholate							
PAM	6.298	-0.176	1.12·10-3	0.9967	-0.089	-0.616	78.6
LuH-6	14.73	-0.411	2.72·10-3	0.9970	+0.779	-0.795	75.6
PDST	4.921	-0.148	1.06·10-3	0.9937	+0.679	-0.245	69.8
Ion pairing agent: sodium taurochenodeoxycholate							
PAM	7.090	-0.182	1.16·10-3	0.9978	+0.519	-0.048	78.4
LuH-6	16.06	-0.440	2.94·10-3	0.9983	+1.419	-0.402	74.8
PDST	5.301	-0.147	1.02·10-3	0.9994	+0.769	+0.005	72.1

The influence of the pH value on the retention process

The value of pH of aqueous component from mobile phase may influence the tautomeric equilibriums of two of the studied compounds, according to the representation given in **Fig. 2.3.4**. Changing the pH of aqueous component from acid to neutral led to a change of the retention dependence shape. The retention curve obtained at pH = 7 may be explained by the lack of ion pairing formation between analyte and bile acid, which means that analyte may change its structure.

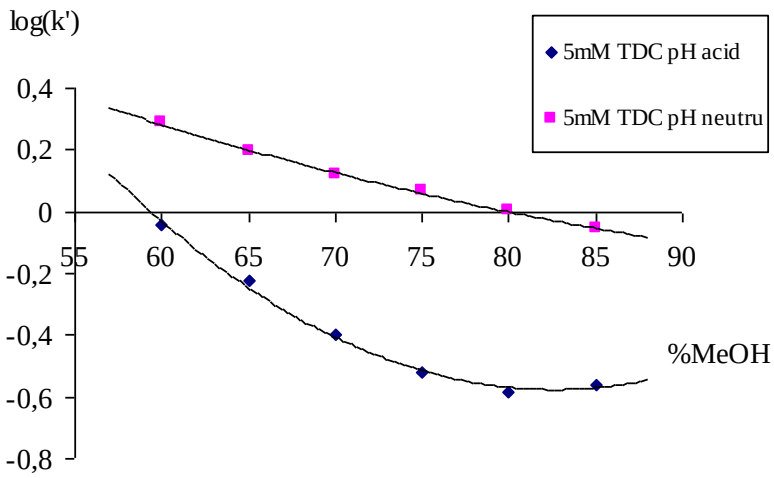


Fig.2.3.4. Modification of functional dependence (log k' on %MeOH) for PAM, when pH of aqueous component was brought to 7.

2.3.4. Conclusions

This study revealed the possibility of forming ion pairs between bile acids and different cationic compounds used as acetylcholinesterase reactivators. An unusual retention behavior was observed for studied cationic compounds eluted under the ion pair formation mechanism with mentioned ion pairing agents. "U-shaped" functional dependencies between log k' and the methanol concentration in the mobile phase were obtained for all studied compounds, under acidic conditions.

2.4. Study of some ionic liquids under reversed-phase ion-pairing mechanism

2.4.3. Results and discussions

The influence of the organic modifier concentration on the retention process

The experimental retention plots between log k' and the content of organic modifier in mobile phase were acquired for the nine compositions of mobile phase on a wide interval (50 – 80% MeOH, v/v).

The graphical representations between the ten base logarithm of the retention factor (log k') for each ionic liquid and the volume percentage of methanol in mobile phase (C_{MeOH}) showed a characteristic "U" shape with a minimum value within studied interval of mobile phase composition. This unusual shape can be described by a second order polynomial regression, according to the following equation:

$$\log(k') = \alpha_0 + \alpha_1 C_{MeOH} + \alpha_2 C_{MeOH}^2 \quad (2.4.1)$$

where α_0 is the intercept and it represents the extrapolated value of the retention factor corresponding to a hypothetical mobile phase containing 100% aqueous component (for C_{MeOH} = 0 $\rightarrow \alpha_0 = \log k_w$), while α_1 and α_2 are the regression parameters of the given functional dependence.

Extrapolation of dependences (2.4.1) to a mobile phase consisting in MeOH only (log k' for C_{MeOH} = 100 is denoted by $\log(k')_o$) corresponds to an adsorption of simple ionic compound from methanol to C18 hydrophobic sites from stationary phase. This adsorption proved to be significant as can be seen from the values of $\log(k')_o$. The extrapolated value $\log(k')_w$ describes the adsorption of the pair between the ionic pair of ionic liquid and $R\text{-}SO_3^-$.

2.4.4. Conclusions

An unusual chromatographic retention behavior has been observed: the functional dependences between the logarithm of the retention factor and the methanol content in the mobile phase followed a binomial pattern (U – shaped), with a minimum positioned within the interval 60 – 75% methanol. This may be explained by ion-dipole interactions occurring between pyridinium and imidazolium cations and residual silanols on the surface of stationary phase and also by hydrophobic interactions between the hydrophobic moiety of the cations and the bonded chains of the stationary phase.

2.5. Analytical applications of the ion-pairing mechanism

2.5.1. Separation and simultaneous quantization of the active ingredients in an injectable drug, Algifen

2.5.1.1. Introduction

The aim of the present work was to develop and validate a rapid, sensitive and selective RP-LC method for the simultaneous determination of three active pharmaceutical ingredients (metamizole sodium, fenpiverine bromide and pitofenone hydrochloride) and the main degradation compound of metamizole (metamizole impurity C) in an analgesic injectable solution (Algifen®) available on the Romanian pharmaceutical market.

2.5.1.3. Results and discussion

Separation of metamizole sodium, fenpiverine bromide, pitofenone hydrochloride and metamizole impurity C together with quantization of fenpiverine at its low concentration level proved to be very challenging analytical tasks. All four analytes have high polarity meaning low retention in RP-LC. Concerning their structures, metamizole (MZ) contains both an acidic sulphonic moiety and a basic amino one, while metamizole impurity C (IC), fenpiverine (FP) and pitofenone (PT) have basic amino or quaternary ammonium salt moieties (**Fig. 2.5.2**.).

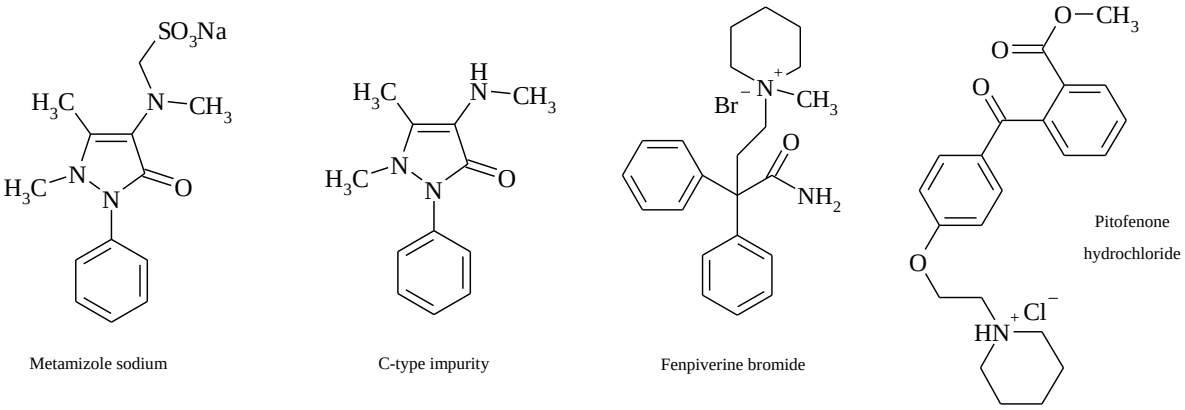


Fig 2.5.2. Molecular structures of the investigated analytes

An ion-pair mechanism using an alkyl sulphonate anion (10 mM) in the mobile phase at pH 3 was chosen to increase retention and provide good separation of metamizole impurity C, fenpiverine and pitofenone. Although metamizole has an amino group in its molecule, it does not form ion-pair with hexyl sulphonate anion because of its own sulphonic group which generates electrostatic repulsion. An ionic liquid (1-butyl-1-methyl-pirrolidinium tetrafluoroborate), also at 10 mM, was added in the aqueous component of the mobile phase to improve symmetry and increase retention for MZ. The ionic liquid interfered in the ion-pair formation process of IC, FP and PT with hexyl sulphonate anion leading to retention decrease for these analytes but without significant effects on their separation or efficiency. (see Fig. **2.5.3**)

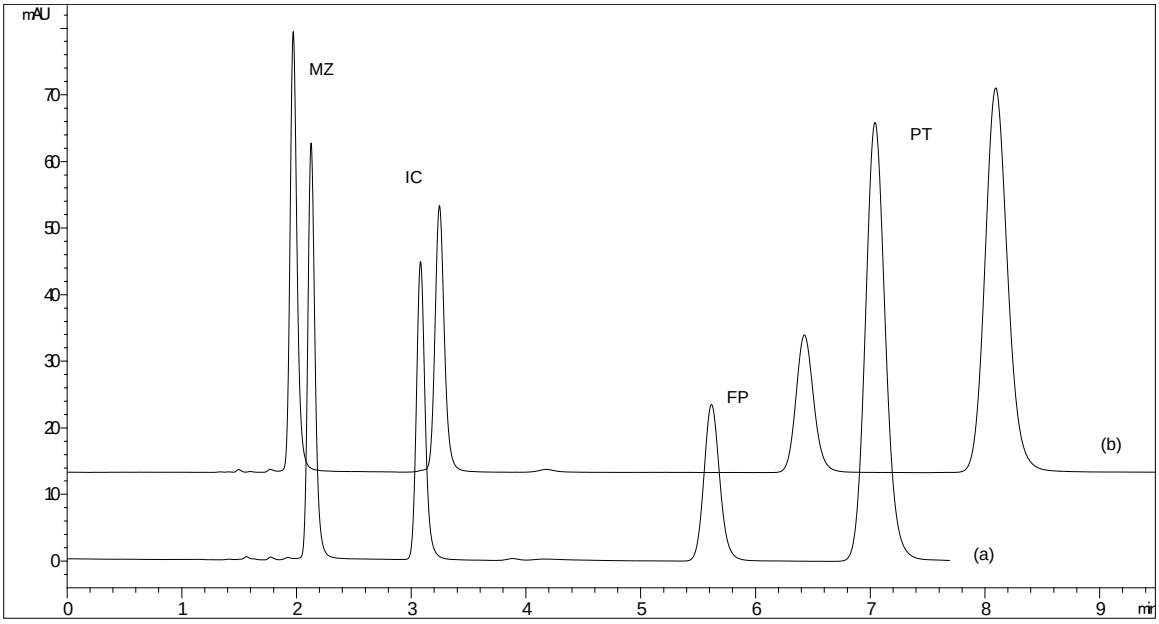


Fig 2.5.3. Comparison between retention of target compounds when the aqueous mobile phase contains: (a) both sodium hexane sulphonate and ionic liquid at 10 mM and (b) only 10 mM sodium hexane sulphonate.

Optimum concentration of the ion-pairing agent and the ionic liquid in the mobile phase was established following a retention study of analytes with variation of concentration of these additives (**Fig. 2.5.4**). Increasing concentration of sodium hexane sulphonate leads to a polynomial increase in retention of IC, FP and PT due to ion-pair formation and polynomial decrease in retention of MZ due to electrostatic repulsion between ion-pairing agent and MZ dissociated sulphonic group.

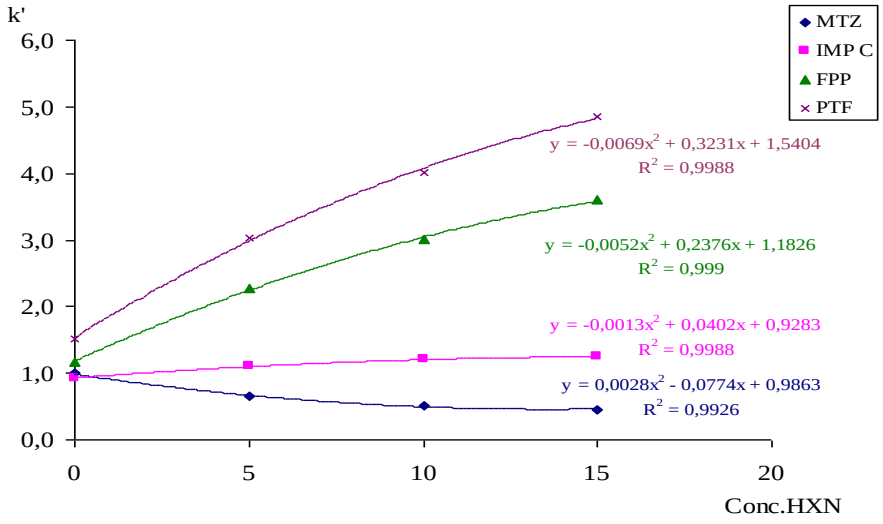


Fig 2.5.4. Retention factor dependence for target compounds function of ion-pairing agent concentration in the mobile phase. Aqueous mobile phase (pH 3) contains also 0.2%TEA and 10 mM 1-butyl-1-methyl-pirrolidinium tetrafluoroborate.

Influence of IL concentration in the mobile phase on retention and peak symmetry for MZ was also studied. Mobile phases containing 0, 5, 10 and 15 mM IL were prepared without ion-pairing agent addition. MZ peak symmetry increased significantly with the increase in IL concentration in the mobile phase. Retention factor remained practically the same with IL concentration increase, leading to the conclusion that MZ does not from ion-pair with this additive. The IL generates MZ retention increase only when combined with ion-pairing agent in the mobile phase by reducing the salting-out effect of the latter against MZ.

Liquid-liquid extraction was required to provide good method sensitivity for fenpiverine due to the very low concentration of this analyte in the pharmaceutical formulation (20 µg/mL), with respect to the other active drugs (metamizole 500 mg/mL and pitofenone 2 mg/mL) and also due to intrinsic low UV absorbtivity of the former.

1-octanol was the solvent of choice for the selective liquid-liquid extraction of fenpiverine with respect to metamizole due to high hydrophobicity of this solvent compared to metamizole. 1-octanol is also more hydrophobic than all other three analytes which made it a suitable choice for direct injection of the extracted sample in the chromatographic column without the need to perform solvent evaporation and dry residue re-dissolution in a mobile phase compatible solvent. The extraction leads to a reasonable 48% recovery for FP and almost no recovery (0.4%) for MZ. The chromatogram obtained after 1-octanol extraction contains detector overloaded peaks of MZ, IC and PT which do not have analytical significance. Picric acid peak is also present in the chromatogram closed to IC peak but no interference with FP is observed (**Fig. 2.5.6**).

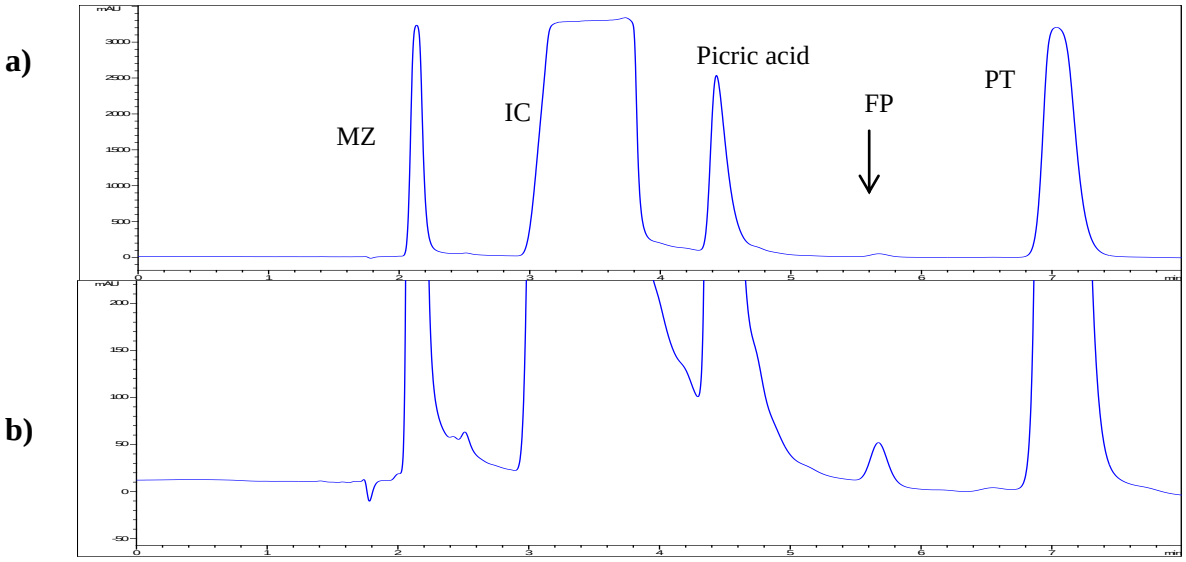


Fig.2.5.6. . Overlaid and zoomed chromatograms obtained after 20 µL injection of extracted standard solution containing FP, MZ and PT.

2.5.1.4. Conclusions

A rapid, sensitive, selective, and accurate ion-pair RP-LC method was developed for simultaneous determination of metamizole sodium, fenpiverine bromide, pitofenone hydrochloride and metamizole impurity C from an analgesic injectable solution. Liquid-liquid extraction in 1-octanol was used to increase method sensitivity and selectivity for fenpiverine bromide present at a low concentration in the injectable drug. Fenpiverine was extracted as ion-pair with picric acid in 1-octanol and the organic layer was directly injected (20 μL) in the chromatographic column without any other sample preparation step. The chromatographic method was successfully validated and applied to assay all four compounds in Algifen® injectable solution.

2.6. Dissertation conclusions

The main aim of this dissertation was to study the retention behavior of some polar or cationic-type compounds under reversed-phase ion pairing mechanism. This analytical technique is widely used in separation of ionic or ionizable compounds by enhancing their retention with the aid of ion-pairing agent added in mobile phase. The target compounds were a very polar one (belong to guanidine class) or a cationic-type (oximes or ionic liquid class).

Two of the main models describing the retention process in ion-pairing liquid chromatography (partition and electrostatic model) were used as complementary models in explaining different influences of experimental parameters on the retention data obtained from the ion pairing process. The dissertation studies tried to find out a correlation between theoretical models and experimental values investigating the main experimental parameters of the ion pairing mechanism: the nature and concentration of ion pairing agent, pH and mobile phase composition, the ionic strength or the nature of the stationary phase.

Generally, the effects of the nature of the ion pairing agent and its concentration in mobile phase were fairly explained by the electrostatic model, and the dependences predicted by this model did not reveal any deviation from theory. Good correlations were obtained for the dependences between experimental parameters and the chromatographic outcome as predicted by this theoretical model. The effect of the organic modifier content and pH was found to be fairly explained by the partition model, and the functional dependences between experimental parameters and the chromatographic outcome were very well correlated.

Concerning the cationic type-oximes analyzed on a C18 Zorbax ODS stationary phase an unusual chromatographic retention behavior has been observed: the functional dependences between the logarithm of the retention factor and the methanol content in the mobile phase followed a binomial pattern (U – shaped), with a minimum positioned within (0 - 100)% organic modifier. The tautomerism of the investigated structures and the stationary phase morphological modification caused by the adsorption of the ion-pairing agent may explain the experimental findings. Similar retention behavior was observed for the ionic liquids studied.

List of publications

1. V. Voicu, A. Medvedovici, M. Rădulescu, E. E. Iorgulescu, V. David, *Unusual retention behavior of some cationic-type aldoximes used as AChE reactivators under ion-pairing liquid chromatographic mechanism*. Analytical Letters, 43 (7-8), 1267-1276 (2010).

2. M. Rădulescu, V. Voicu, A. Medvedovici, V. David, *Retention study of some cation-type compounds using bile acid sodium salts as ion pairing agents in liquid chromatography*. Biomedical Chromatography, 25 (8), 873-878 (2011).

3. A. Medvedovici, I. D. Sora, M. Rădulescu, V. David, *Discontinuous double mechanism for the retention of some cation-type oximes on hydrophilic stationary phase in liquid chromatography*. Analytical Methods, 3 (2), 241-244 (2011).

4. M. Rădulescu, E. E. Iorgulescu, C. Mihailciuc, V. David, *Comparative study of the retention of pyridinium and imidazolium based ionic liquids on octadecylsilica stationary phase under ion pairing mechanism with alkylsulphonate anions*. Revue Roumaine de Chimie, 57 (1), 61-67 (2012).

5. M. Rădulescu, V. David, *Partition versus electrostatic model applied to the ion-pairing retention process of some guanidine based compounds*. Journal of Liquid Chromatography and Related Technologies, 35(14), 2042-2053 (2012).

6. T. Galaon, M. Rădulescu, A. Medvedovici, V. David, *Use of an immiscible diluent in ionic liquid / ionic pair LC for the assay of an injectable analgesic.* Central European Journal of Chemistry, 10(4), 1360-1368 (2012).

7. M.-C. Radulescu, A. Chira, M. Rădulescu, B. Bucur, M. P. Bucur, G. L. Radu, *Determination of Silver(I) by Differential Pulse Voltammetry Using a Glassy Carbon Electrode Modified with Synthesized N-(2-Aminoethyl)-4,4'-Bipyridine,* Sensors, *10*, 11340-11351 (2010)

List of presentations

1. A. Medvedovici, V. A. Voicu, M. Rădulescu, V. David, *Hydrophobic/hydrophilic character of some cation-type aldoximes studied by liquid chromatography under ion-pairing and HILIC partition mechanisms*, 11th International Congress of Clinical Pharmacology, Therapeutics and Toxicology, Oradea, Romania (2010)

2. T. Galaon, M. Rădulescu, A. Medvedovici, V. David, *Liquid-liquid extraction of Fenpiverine in 1-octanol followed by direct injection in LC and simultaneous quantitation of Fenpiverine, Metamizole, Metamizole impurity C and Pitofenone in an injectable drug by ion-pair RP-LC*, Recent Developments in Pharmaceutical Analysis, 14th International Meeting, Pavia, Italia (2011)