ISSN : 0974-7419 Volume 15 Issue 8

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 15(8) 2015 [304-310]

Retention behavior of selected basic, acidic and neutral analytes on three different chromatographic stationary phases in presence of chaotropic mobile phase additives

Hisham Hashem[1,2,*], Mohamed Al Bratty[2], Thomas Jira[3]

[1]Faculty of Pharmacy, Pharmaceutical Analytical Chemistry Department, Zagazig University, (EGYPT)

[2]Faculty of Pharmacy, Pharmaceutical Chemistry Department, Jazan University, (SAUDI ARABIA)

[3]Institute of Pharmacy, Pharmaceutical/Medicinal Chemistry, Ernst-Moritz-Arndt-University Greifswald, F.-L.-Jahn-Str. 17, D-17487 Greifswald, (GERMANY)

E-mail : hisham413@yahoo.com

ABSTRACT

In this study the effect of type and concentration of two chaotropic counter anions namely perchlorate and dihydrogen phosphate in mobile phase on retention behavior of three basic compounds (tricyclic neuroleptics), one acidic compound (acetyl salicylic acid) along with one neutral compound (triamcinolone) was studied. The effect of change of concentration of the two chaotropic counter anions and the role of organic modifier type (methanol and acetonitrile) were considered. All these parameters were studied for three different RP-columns (calixarene modified silica gel, monolithic and conventional RP-columns). The results indicate that all studied factors affected the chromatographic behavior of the studied compounds. The type and concentration of chaotropic counter anions have clear effect on the basic compounds retention while the retention of neutral and acidic compounds did not clearly change. The type of the used stationary phase was found to play an important role in retention behavior of the studied basic compounds.

KEYWORDS

Chaotropic additives;
Neuroleptics;
Neutral and acidic analytes;
Chromatographic behavior;
Three different stationary phases.

INTRODUCTION

Counter anions that increase the disorder of water are called chaotropic counter anions[1]. Although it was reported that the increase in the ionic strength of the mobile phase results in reduction of the retention time of the basic compounds due to the competetive interaction of the counter-cation with the residual silanol[2], C. Horvath *et al.*[3] found that increase in anion salt concentration led to increase in the retention factors through the increase in the surface tension of the mobile phase. R LoBrutto *et al.*[4] postulated that by increasing the counter anion concentration the retention factor increased for protonated basic compounds due to their electrostatic interaction with counter-anion (perchlorate and trifluoroacetate). This in turn led to increase hydrophobicity of the ion-associated neutral complex. Solvation shell of the basic analyte is disrupted due to formation of the ionic complex which in turn causes an increase in apparent analyte hydrophobicity. YV Kazakevich *et al.*[5] stated that a

multilayer-type adsorption of the organic modifier (acetonitrile) on the reversed-phase surface and its strong dispersive (or π-π) interactions with liophilic ions are responsible for significant retention of chaotropic counterions. L. Pan *et al.*[6] found that increase in chaotropic counter-anion concentration led to increase in symmetry of basic compounds and also increase in the efficiency of the C8-bonded silica column. In addition J. Dai and P. Carr[7] pointed out that the retention of basic compounds on conventional reversed phase can increase, decrease or effectively remain constant as the concentration of the additives increased. And this can be attributed to the presence of two opposing effects, one of them leads to elongate the retention times (ion pairing of the analytes with the chaotropic counter-anion) and the other results in shortening of the retention times (competetion of the buffer counter-cation with the analyte for the ionized silanols). In previous studies for other authors, the effect of chaotropic mobile phase additives on retention behaviour of basic drugs (beta-blockers) on conventional RP-columns was studied[4,8]. Hashem and Jira[9] have examined the retention behavior of beta-blockers on monolithic column and concluded that beside the type and concentration of the buffer counter-anion the properties of the basic analyte especially its hydrophobicity have an effective role determining to which extent the type and concentration of chaotropic anion will affect the retention of the protonated basic analytes and this was confirmed by the results of J. Flieger[10]. The role of chaotropic mobile phase additives was studied for separation and determination of ropinirole and its impurities[11] as well as a mixture containing levodopa, carpidopa, entacaptone and their impurities[12].

It was recommended in a previous study[13]that the effect of chaotropic mobile phase on the retention of basic analytes other than beta-blockers should be studied on different stationary phases. Hashem and Jira[14] were surprised with the results in which increase in buffer concentration led to increase in retention factor of tricyclic neuroleptics on calixarene stationary phase then decreased with further increase in buffer concentration. Therefore it was important to compare among three types of columns including calixarene and monolithic stationary phases concerning with the effect of the chaotropic mobile phase additives on retention behavior of selected basic, acidic and neutral analytes and this is the main goal of this paper.

Calixarenes are macrocyclic molecules including phenol units linked by alkylidene groups and belong to the class of [1n] cyclophanes. They have the ability to make reversible complexes with metals and organic molecules[15,16].

Monolithic columns were introduced as an alternative to particle-based columns and are made of a single porous piece (rod). Using organic polymer as polymethacrylate and polystyrene or inorganic polymer as silica and through a polymerization process these rods are prepared. These columns possess a biporous structure consisting of larger macropores (2 μm) permitting high flow rates with low back pressure and smaller mesopores (13 nm) ensuring a high surface area for high efficiency[17].

EXPERIMENTAL

Chemicals

HPLC grade methanol (MeOH) was purchased from Mallinckrodt Baker B.V.(Deventer, Netherland). Acetonitrile (ACN) was HPLC grade and purchased from LGC Promochem (Wesel, Germany). Water was deionized and doubly distilled. Sodium hydroxide, perchloric acid, phosphoric acid, sodium perchlorate and sodium dihydrogenphosphate were purchased from Merck KgaA (Darmstadt, Germany).

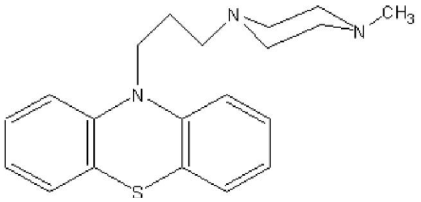
Analytes

Triamcinolone was obtained from Schering AG (Berlin, Germany). Acetyl salicylic acid is obtained from Sigma. Perazine, Chlorpromazine and Promazine were friendly supplied by Tropon (Cologne, Germany).

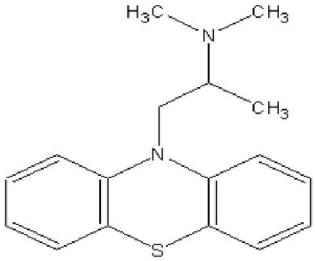
Equipments

HPLC

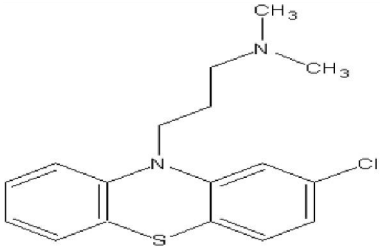
Full Paper



A : Perazine (pKa = 8.34 and 6.30)



B : Chlorpromazine (pKa = 9.71)



C : Promazine (pKa = 9.73)

Chemical structure of the examined neuroleptics

Agilent HPLC series 1200 (Agilent technologies, Germany) consisting of solvent pump (model G1311A), autosampler (model G1329A), column compartment (model G1316A) and UV detector (model G1314A).

pH-meter

Knick Elektronische Meßgeräte GmbH & Co. (Berlin, Germany).

Analytical columns

Chromolith® Performance RP-18e, 100 x 4.6 mm I.D. (friendly supplied by Merck KGaA (Darmstadt, Germany). CALTREX®AIE column (250 x 4 mm I.D.) was friendly supplied by Syntrex GbR (Greifswald, Germany). The calixarene stationary phase contains silica-bonded calix[4]arene (basic silica: endcapped Kromasil Si 100 A° pore diameter, 5 µm particles; manufacturer: EKA Chemicals (Bohus, Sweden). C18 Kromasil 125 mm and 5 µm particles packed and supplied by Syntrex GbR (Greifswald, Germany).

Chromatography

The experiments were performed with isocratic elution mode. The binary mobile phase consisted of different proportions of MeOH or ACN in the aqueous solution. The two components of mobile phase (aq. and organic) were mixed 1st time inside the apparatus. pH values were measured in aq. component of the mobile phase. Phosphoric acid and perchloric acid were used for pH adjustment in case of NaH_2PO_4 and $NaClO_4$ respectively. The eluents were degassed with helium gas before running. In all cases, the column temperature was set at 40 °C. The hold-up times (t_0) were determined via MeOH peak under each mobile phase composition. Detection was achieved at 254 nm.

RESULTS AND DISCUSSION

In this study several experiments were carried out, in all of them the concentrations of counter anions ($H_2PO_4^-$ and ClO_4^-) were gradually increased and the changes in retention factor of examined analytes were observed. In all cases mobile phase of pH 3.5 is applied. This pH value is lower than the pKa values of the used neuroleptics ensuring complete or partial protonation of the analytes which is very important for ion-pair complex formation between buffer counter-anion and basic analytes cation.

It can be seen that in case of using methanol as organic modifier (Figure 1 A-C and TABLE 1) raising of NaH_2PO_4 concentration in the mobile phase leads to increase in K´-values of neuroleptics (basic analytes) on Kromasil and monolith stationary phases. Using caltrex as stationary phase, K'-values of neuroleptics increase when NaH_2PO_4 concentration in the mobile phase increases in the range of 1.562-12.5 mM and then decrease with further increase in NaH_2PO_4 concentration in the mobile phase.

For the three columns the increase in NaH_2PO_4 concentration in the mobile phase did not lead to significant change in K'-values of the neutral analyte (Triamcinolone) and acidic analyte (Acetyl salicylic acid).

Bliesner and Sentell[18] suggested that different or-

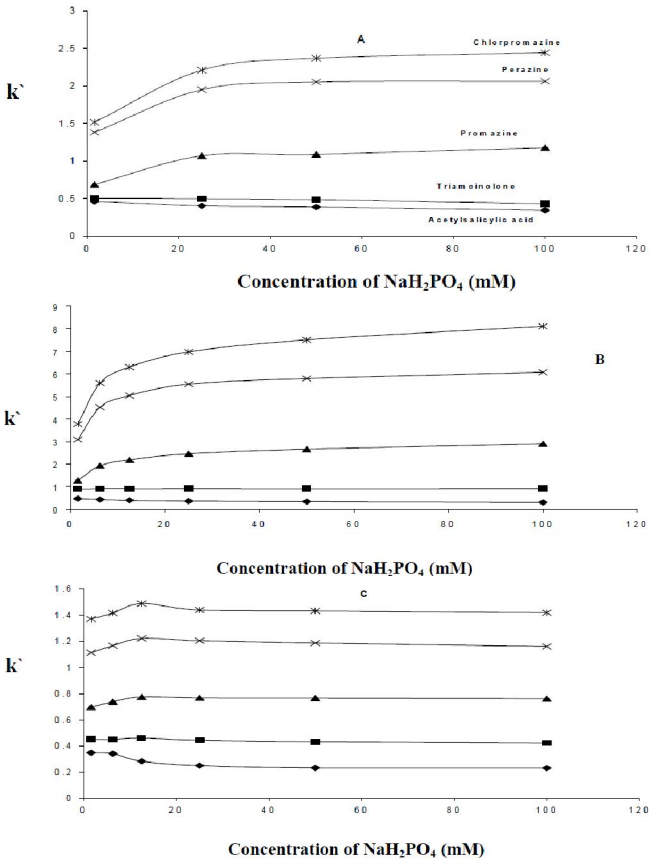


Figure 1 : Behavior of k'-values of analytes upon increase in NaH_2PO_4 concentration using 65% MeOH as organic modifier; A : On Kromasil C-18 RP; B : Monolithic stationary phase; C : Caltrex AIE stationary phase

ganic modifiers solvate the alkyl ligands of the stationary phase to different extents. S.Kunsági-Máté *et al.*[19] postulated that the solvent affects the stability of the complex between calixarene and drug; since the solvation energies are proportional to the solvent permittivity, a solvent of high permittivity leads to a decreased stability of the inclusion complexes. LoBrutto *et al.*[4] Pointed out that due to its ability to form hydrogen bonds MeOH may actually participate in the analyte solvation as if compared with ACN, therefore, analyte solvatation with methanol would increase the analyte hydrophobicity and aid in analyte retention process (using conventional C18-RP column). In this case[4] the presence of methanol molecules and increase of perchlorate anion concentration produced a synergistic effect on the analyte retention. This maybe true for the conventional RP-columns but for calixarene it will be different because it differs from the conventional RP-column in that it can form reversible complexes with the analytes[15,16].

Using NaH_2PO_4/ACN (Figure 2 A-C and TABLE 1) the increase in the concentration of the salt in the mobile phase from 0 to 25 mM leads to increase in K´-values of Perazine while further increase in the salt concentration in the mobile phase results in decrease in K´-values of Perazine. At too low concentration of salt in the mobile phase Perazine elutes before Promazine and Chlorpromazine in case of Caltrex and Kromasil stationary phases while at greater concentration of salt in the mobile phase Perazine elutes after Promazine and before Chlorpromazine. In case of Monolith stationary phase the elution order is the same at all concentrations of the salt in the mobile phase (Promazine, Perazine and then Chlorpromazine). In case of Monolith stationary phase the increase in the concentration of NaH_2PO_4 in the mobile phase leads to unclear increase in K´-values of Promazine and Chlorpromazine. In case of the other two stationary phases the increase in the concentration of NaH_2PO_4 in the mobile phase leads to decrease of K´-values of Promazine and Chlorpromazine. The increase in the salt concentration in the mobile phase does not significantly change K´-values of Triamcinolone and Acetyl Salicylic acid. The elution order of Triamcinolone and Acetyl Salicylic acid on the three columns in case of NaH_2PO_4/ACN is different from that in case of NaH_2PO_4/MeOH and $NaClO_4$/MeOH (Figure 1, 2 and 3). So it is clear that use of ACN instead of MeOH could be the main reason for the different elution order of Triamcinolone and Acetyl Salicylic acid and that of Perazine by low concentration of salt in the mobile phase. The different behavior of Perazine than other two neuroleptics can be attributed to its lower pKa value[20]. Roses et al.[21] postulated that the pKa of basic compounds is shifted to lower value and pH of the aqueous phase increases under addition of organic modifier to the mobile phase. These two factors result in presence of perazine, which has lower pKa than the others two neuroleptics, in partial or even unionized form and hence increase its hydrophobicity.

It was found that the differences observed in the analytes retention by increasing the salts concentra-

Full Paper

TABLE 1: Effect of mobile phase compositon and stationary phase type on retention of analytes

Mobile phase	Salt conc. (mM)	k´								
		Caltrex AIE			Monolith			Kromasil C-18		
		TRI	ASA	PER	TRI	ASA	PER	TRI	ASA	PER
(A) NaH_2PO_4 and ACN	0.781	*	*	*	*	*	*	0.490	0.870	0.863
	1.562	0.777	1.051	1.485	0.480	0.576	1.680	0.465	0.865	0.957
	6.25	0.768	1.010	1.562	0.444	0.571	2.285	0.448	0.882	1.424
	12.5	0.721	0.951	1.886	0.448	0.585	2.606	*	*	*
	25	0.744	0.927	1.933	0.443	0.495	2.820	0.495	0.846	1.558
	50	0.779	0.927	1.904	0.443	0.464	2.803	0.468	0.823	1.439
	100	0.746	0.819	1.669	0.430	0.459	2.409	0.461	0.786	1.223
(B) NaH_2PO_4 and MeOH	1.562	0.450	0.347	1.134	0.893	0.468	3.095	0.503	0.463	1.381
	3.125	*	*	*	*	*	*	0.498	0.448	1.533
	6.25	0.449	0.340	1.167	0.903	0.428	4.526	*	*	*
	12.5	0.459	0.282	1.222	0.905	0.395	5.048	0.498	0.434	1.809
	25	0.442	0.249	1.205	0.915	0.357	5.546	0.497	0.403	1.950
	50	0.431	0.231	1.187	0.911	0.335	5.795	0.484	0.387	2.053
	100	0.421	0.230	1.161	0.916	0.299	6.074	0.433	0.344	2.063
(C) $NaClO_4$ and ACN	1.562	0.429	0.584	0.429	0.153	0.301	0.347	0.425	0.800	0.606
	6.25	0.397	0.603	0.630	0.169	0.299	0.523	0.396	0.783	0.758
	12.5	0.424	0.630	0.766	0.218	0.341	0.696	0.381	0.775	0.896
	25	0.422	0.648	0.908	0.176	0.301	0.751	0.358	0.755	1.035
	50	0.403	0.658	0.921	0.193	0.326	0.911	0.317	0.748	1.185
	100	0.401	0.651	1.008	0.181	0.324	1.033	0.347	0.757	1.243
(D) $NaClO_4$ and MeOH	1.562	0.597	0.419	1.353	0.522	0.325	1.725	0.387	0.373	0.756
	6.25	0.600	0.469	1.576	0.531	0.329	2.423	0.369	0.360	0.977
	12.5	0.604	0.473	1.680	0.531	0.339	2.728	0.366	0.360	1.078
	25	0.600	0.450	1.744	0.538	0.351	3.063	0.364	0.367	1.178
	50	0.585	0.438	1.806	0.539	0.346	3.339	0.357	0.358	1.261
	100	0.584	0.421	1.915	0.529	0.336	3.531	0.351	0.335	1.333

Conditions for: CaltrexAIE : A: NaH_2PO_4/ 40% ACN; B: NaH_2PO_4/ 70% MeOH; C:$NaClO_4$/ 55% ACN; D: $NaClO_4$/ 65% MeOH; Monolith: A: NaH_2PO_4/ 40% ACN; B: NaH_2PO_4/ 35% MeOH; C:$NaClO_4$/ 40% ACN; D: $NaClO_4$/ 50% MeOH; Kromasil C-18: A: NaH_2PO_4/ 45% ACN; B: NaH_2PO_4/ 65% MeOH; C:$NaClO_4$/ 50% ACN; D: $NaClO_4$/ 70% MeOH

tion (having different types of anions) may be attributed to the extent of anions hydration which differs from ion to ion.

The anion that was least capable of being solvated leads to greatest disruption of the analyte solvation and hence the increase of the analyte hydrophobicity. Dihydrogen phosphate anion in aqueous environment is highly solvated due to its hydrogen bonding capabilities. Perchlorate anion has four electron withdrawing oxygen atoms which lead to delocalization of the charge density. Hence being lower solvated than dihydrogen phosphate anion[8].

In case of $NaClO_4$/MeOH (Figure 3 A-C), increase in the salt concentration of the mobile phase leads to increase in K´-values of the three neuroleptics. At too low concentration of ClO_4^- (1.562 mM) Chlorpromazine and Perazine co-eluted and by increase in the salt concentration in the mobile phase they give two separate peaks. For the three columns the increase in $NaClO_4$ concentration in the mobile phase does not lead to significant change in K'-values of the neutral analyte (Triamcinolone) and acidic analyte (Acetyl salicylic acid). It is noticed also that the elution order in case of NaH_2PO_4/MeOH and

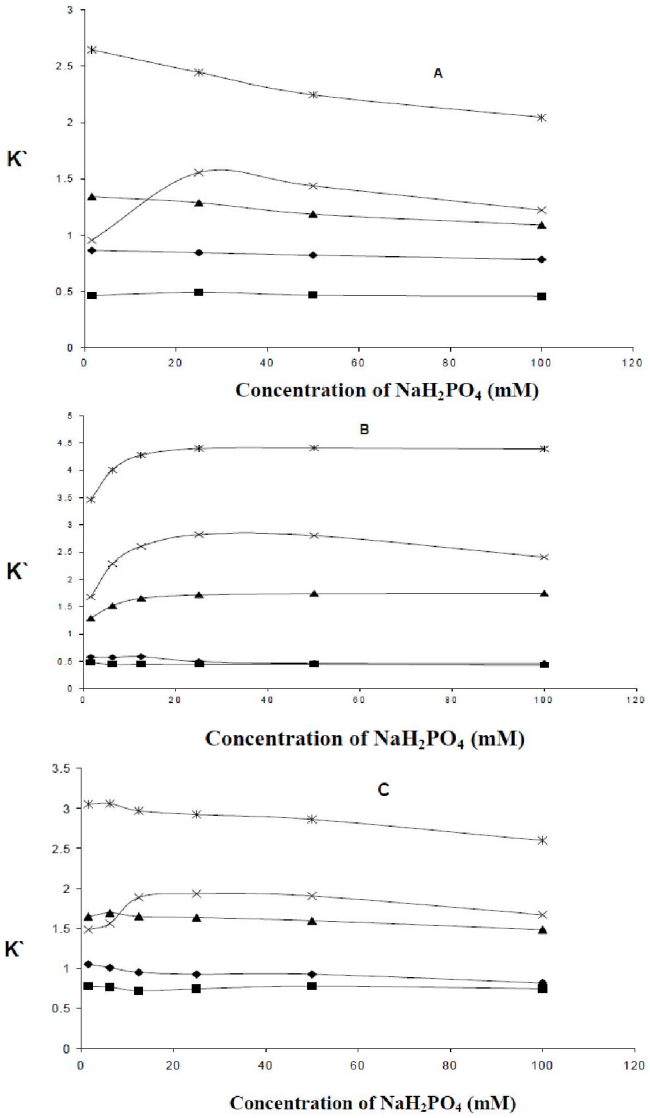


Figure 2: Behavior of k'-values of analytes upon increase in NaH_2PO_4 concentration using 45% ACN as organic modifier; A : On Kromasil C-18 RP; B : Monolithic stationary phase; C : Caltrex AIE stationary phase

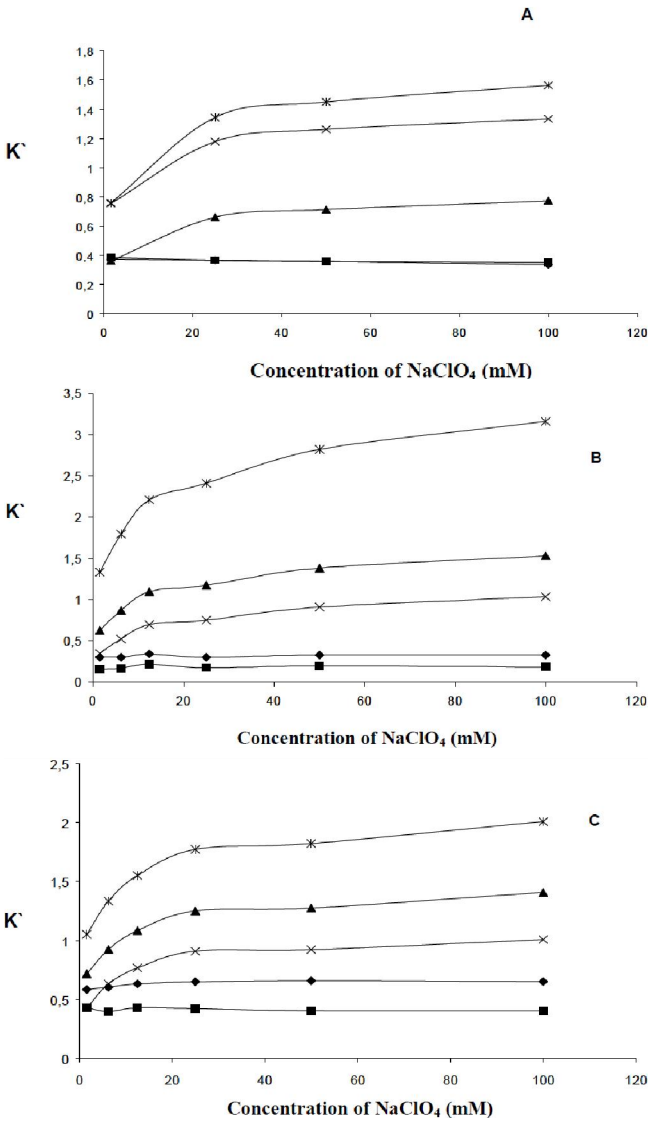


Figure 3: Behavior of k'-values of analytes upon increase in $NaClO_4$ concentration using 70% MeOH as organic modifier; A : On Kromasil C-18 RP; B : Monolithic stationary phase; C : Caltrex AIE stationary phase

$NaClO_4$/MeOH is the same on the three columns. In case of $NaClO_4$/ACN (Figure 4 A-C) elution order of Triamcinolone and Acetyl Salicylic acid is still different from the first two cases (in which MeOH is used). On all of three columns Perazine elutes before Promazine and Chlorpromazine opposite to the first two cases. Opposite to in case of NaH_2PO_4/ACN the increase in the concentration of perchlorate leads to increase in k'-values of neuroleptics on the columns. There is no clear change in k'-values of Triamcinolone and Acetyl Salicylic acid upon the increase in perchlorate concentration.

It can be noted that in case of $NaClO_4$ (Figure 3 and 4) the increase in k' by increasing in salt concentration is higher than that in case of NaH_2PO_4 (Figure 1 and 2). This can be explained according to Jones *et al.*[8], as they found that perchlorate is stronger chaotropic agent than dihydrogen phosphate.

CONCLUSIONS

The counter anion has been found to have high effect on separation of basic compounds. The type and concentration of these anions are essential to determine in which extent the retention factor of the basic compounds will change. The results indicated that the type

Full Paper

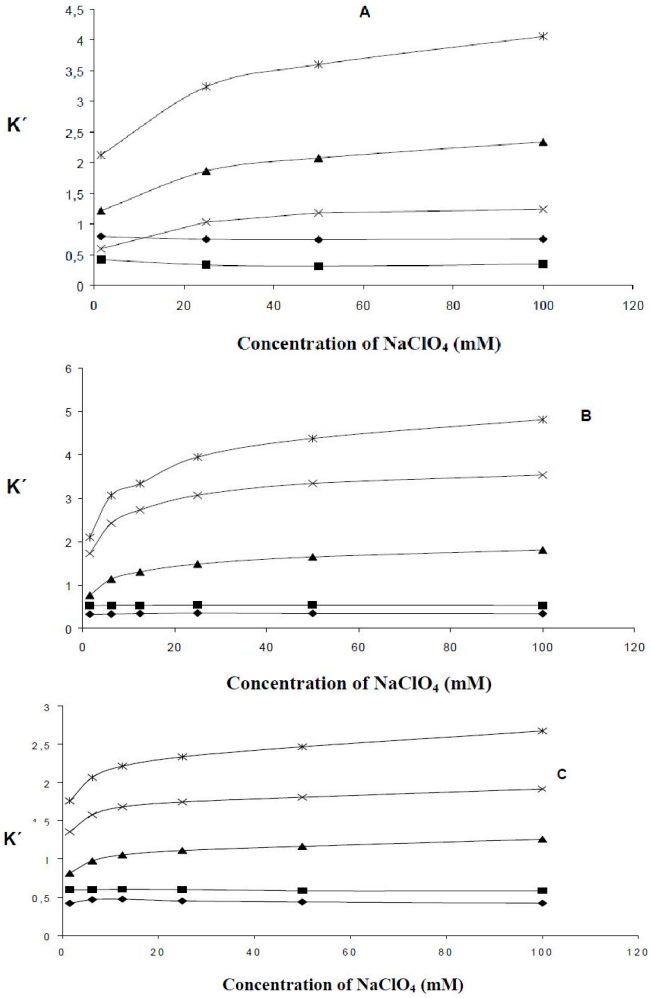


Figure 4 : Behavior of k'-values of analytes upon increase in $NaClO_4$ concentration using 50% ACN as organic modifier; A : On Kromasil C-18 RP; B : Monolithic stationary phase; C : Caltrex AIE stationary phase

of the used stationary phase plays an important role with the other factors. The effect of chaotropic additives on the retention of basic compounds is of practical importance because of their potential for improvement towards HPLC method development. The retention of the acidic and neutral analytes does not change upon increase in salt concentration in mobile phase.

ACKNOWLEDGEMENTS

The authors thank the above mentioned companies for the friendly supply of analytes and columns.

REFERENCES

[1] Y.Hatefi, W.G.Hanstein; Proc.Natil. Acad.Sci.USA., **62**, 1129 **(1969).**
[2] L.R.Snyder, J.J.Kirkland, L.J.Gljach; Optimizing the reversed-phase separation, In: Practical HPLC Method Development' Wiley; New York, **(1997).**
[3] C.Horvath, W.Melander, I.Molnar; J. Chromatogr., **125**, 129 **(1976).**
[4] R.LoBrutto, A.Jones, Y.V.Kazakevich; J. Chromatogr.A., **913**, 191 **(2001).**
[5] Y.V.Kazakevich, R.LoBrutto, R.Vivilecchia; J. Chromatogr.A., **1064**, 9 **(2005).**
[6] L.Pan, R.LoBrutto, Y.V.Kazakevich, R.Thompson; J. Chromatogr.A., **1049**, 63 **(2004).**
[7] J.Dai, P.W.Carr; J.Chromatogr.A., **1072**, 169 **(2005).**
[8] A.Jones, R.LoBrutto, Y.V.Kazakevich; J. Chromatogr.A., **964**, 179 **(2002).**
[9] H.Hashem, Th.Jira; J. Sep.Sci., **29**, 986 **(2006).**
[10] J.Flieger; J. Chromatogr.A., **1113**, 37 **(2006).**
[11] A.Vemić, A.Malenović, M.Medenica; Talanta, **123**, 122 **(2014).**
[12] A.Vemić, B. J.Stojanović, I.Stamenković, A.Malenović; J. Pharm.Biomed.Anal., **77**, 9 **(2013).**
[13] H.Hashem, Th.Jira; J. Chromatogr.A., **1133**, 69 **(2006).**
[14] H.Hashem, Th.Jira; Pharmazie, **60**, 186 **(2005).**
[15] C.D.Gutsche, R.Muthukrishnan; J. Org.Chem., **43**, 4905 **(1978).**
[16] V.Böhmer, M.A.McKervey; Chem.Unserer Zeit, **25**, 195 **(1991).**
[17] K.Cabrera; J. Sep.Sci., **27**, 843 **(2004).**
[18] D.M.Bliesner, K.B.Sentell; Anal.Chem., **65**, 1819 **(1993).**
[19] S.Kunsági-Máté, I.Bitter, A.Grün, G.Nagy, L.Kollár; Anal.Chim.Acta., **461**, 273 **(2002).**
[20] J.Flieger, R.S´wieboda; J. Chromatogr.A., **1192**, 218 **(2008).**
[21] M.Roses, I.Canals, H.Allemann, K.Siigur, E.Bosch; Anal.Chem., **68**, 4094 **(1996).**