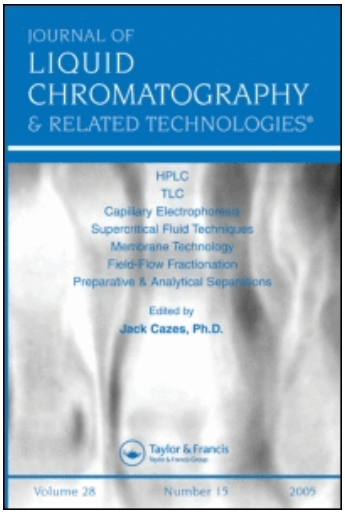

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Effect of 1-Alkyl-3-methylimidazolium Tetrafluoroborate on the Adsorption Behavior of Amine and Carboxylic Compounds in Reversed-Phase Liquid Chromatography

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Abstract: Ionic liquids have recently been introduced as mobile phase additives to separate ionized solutes and to improve separation abilities in reversed-phase liquid chromatography. In this study, four ionic liquids that are composed of different lengths of alkyl chain (ethyl, butyl, hexyl, and octyl) in 1-alkyl-3-methylimidazolium cation and tetrafluoroborate anion were used as the mobile phase additives and five ionizable solutes (benzylamine, benzoic acid, 4-aminobenzoic acid, L-phenylalanine, and L-trypophan) that include carboxyl and/or amine were used as analytes. By changing the hydrophobic property of the ionic liquid with the length of alkyl chain of imidazolium cation, one can control the interaction between 1-alkyl-3-methylimidazolium cation and the hydrophobic stationary phase. To explain the role of ionic liquids on the retention behaviors of ionized analytes, two adsorption mechanisms, the ion pairing forming mechanism and the dynamic ion-exchange mechanism, were

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presented. When 1-ethyl-3-methylimidazolium tetrafluoroborate was used as the additive, the retention behavior dominantly complied with the ion pairing forming mechanism and when other ionic liquids with long alkyl chain were used, the retention behavior can be explained by the dynamic ion-exchange mechanism.

Keywords: Ionic liquids, Mobile phase additive, Adsorption behavior, Reversed-phase liquid chromatography, Preparative chromatography

INTRODUCTION

In reversed-phase liquid chromatography (RPLC), many salts and surfactants are used as mobile phase additives to enhance the selectivity and separation capacity. The ionized analytes cannot have enough interaction with the hydrophobic adsorbent such as C_{18} stationary phase. To separate easily ionized analytes, it is necessary to prevent analytes from being ionized. When the pH (or pOH) of the mobile phase is controlled lower than pK_a (or pK_b) values of analytes, or salts that have special functions are dissolved in mobile phase, the analytes probably exist as nonionic forms; therefore, it can interact with hydrophobic stationary phases and increase the separation capacity.[4] However, these methods are rarely allowed in preparative chromatography, because it is difficult to maintain the pH of mobile phase; the analytes can be destroyed under acidic or basic condition, and the used salts must be separated from the products.

Ionic liquids that exist as liquid phases at room temperature are entirely composed of ionic species, especially large organic cations, and inorganic or organic anions. Especially, the physicochemical properties of ionic liquids can be designed by varying the lengths and branching the alkyl chains of the anionic core and cationic precursor. With some main physicochemical properties such as very low vapor pressure, non-flammability, high ionic mobility, and excellent chemical stability, ionic liquids have recently gained recognition as environmentally benign solvents, as well as good solvents, for a wide range of organic and inorganic materials.[1–6]

In analytical chemistry, applications of ionic liquids as mobile phase additives in liquid chromatography have been an attractive topic in many recent studies. In 2003, He et al. used 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) ionic liquid as the mobile phase additive to separate ephedrines,[7] and to investigate the adsorption mechanism of 1-alkyl-3-methyl-imidazolium tetrafluoroborate ([Rmim]BF_4) ionic liquids as mobile phase additves in HPLC on the separation of some amines.[4] Recently, some imidazolium tetrafuoroborate ionic liquids have been studied for the possibility of supression of deleterous effects of free silanols in liquid chromatography.[8,9] In these studies, the silanol suppressing potency of imidazolium tetrafluoroborates ionic liquids were demonstrated to markedly exceed that of the standard mobile phase additives,

like triethylamine, dimethyloctylamine, and ammonia. It is, however, possible to design the hydrophobic properties of ionic liquids. This proves that ionic liquids can contribute to the hydrophobic interaction between the analytes, especially ionizable solutes, and reversed-phase stationary phase by controlling the hydrophobicity.

In fact, the adsorption mechanism of ionized solutes with the presence of polar or charged additives in mobile phase is quite complex and depends on many experimental factors, especially on the nature of additives.[10,11] Some additives with high hydrophobic property participate mainly in the mechanism of dynamic ion exchange, while some hydrophilic additives affect the solute retention by the mechanism of ion pairing forming.[12] In this work, 4 kinds of ionic liquids which have different lengths of alkyl chain in imidazolium cation are used. These differences vary the hydrophobicity of ionic liquids. Therefore, the retention of solutes can be explained by the dynamic ion exchange or the ion paring forming mechanism. With the increasing interests on the application of ionic liquids in chromatography separation, understanding the effect mechanism of ionic liquids on the single functional group is useful for further studies. For this purpose, the experiments in this work were carried out with using some amine and carboxylic compounds, which are simple in structure as analytes, as well as 4 kinds of 1-alkyl-3-methylimidazolium tetrafluoroborate ([emim][BF_4], [bmim][BF_4], [hmim][BF_4], and [omim][BF_4]) ionic liquids with various lengths of alkyl chain as mobile phase additives. The interaction of ionic liquids between ionized analytes and C_{18} stationary phase was discussed as an aspect of the adsorption mechanism.

EXPERIMENTAL

Reagents

Ionic liquids (1-ethyl-3-methylimidazolium tetrafluoroborate [emim][BF_4], 1-butyl-3-methylimidazolium tetrafluoroborate [bmim][BF_4], 1-hexyl-3-methylimidazolium tetrafluoroborate [hmim][BF_4], and 1-octyl-3-methylimidazolium tetrafluoroborate [omim][BF_4]) were supplied from C-tri (Korea). All of five compounds, L-phenylalanine, L-tryptophan, benzoic acid, benzylamine, and 4-aminobenzoic acid, were purchased from Sigma-Aldrich (USA). Mobile phases were prepared with methanol (HPLC grade, J. T. Baker Co., USA) and deionized water (Milli-Q Purification System, Millipore, USA). Potassium phosphate monobasic (KH_2PO_4) (Duksan Pure Chemical Co., Korea) was used as buffer agent in mobile phase at the concentration of 25 mM. Hydrochloric acid (Matsunoen Chemical Co., Japan) and ammonia solution 28.0–30.0% (Samchun Pure Chemical Co., Korea) were added dropwise as needed to adjust pH 4.0.

Apparatus

The HPLC system was composed of a pump (LC-6AD Shimadzu, Japan), a PDA detector (SPD-M10Avp Shimadzu, Japan), an autoinjector (SIL-10ADvp, Shimadzu, Japan), and a column oven (CTS-30 Younglin, Korea). The pH meter and pH electrode were from Thermal Orion (USA). A prepacked C_{18} column (1 cm $\times$ 10 cm, Kromasil, 100 Å, 25 μm, Spherical) was used.

Chromatographic Conditions

Mobile phase was prepared by adding 25 mM KH_2PO_4 buffer agent into 20% methanol aqueous solution that was adjusted to pH 4.0 by using hydrochloric acid. Each analyte was dissolved in mobile phase solution with 5 concentrations: 1 mM; 5 mM; 10 mM; 20 mM; and 30 mM. Four ionic liquids ([emim][BF_4], [bmim][BF_4], [hmim][BF_4], and [omim][BF_4]) were added directly into mobile phase solution with the fixed concentrations of 17.5 mM. All of the experiments were carried out at the controlled temperature of 30°C. The mobile phase flow rate is 3.0 mL/min, and injection volume is 100 μL.

RESULTS AND DISCUSSION

Figure 1 shows the molecular structure of 1-alkyl-3-methylimidazolium tetrafluoroborate. With the quaternary amine in imidazolium, it has a strong positive charge. In this class of ionic liquids, four kinds of ionic liquids having different length of the alkyl chain such as ethyl ([emim][BF_4]), butyl ([bmim][BF_4]), hexyl ([hmim][BF_4]), and octyl ([omim][BF_4]), are commercially used. By changing the length of alkyl chain of the imidazolium cation, the hydrophobic property of the ionic liquid can be designed. When these ionic liquids are used as mobile phase additives, long alkyl chain ionic liquids have a stronger interaction with reversed-phase stationary phase surface than short

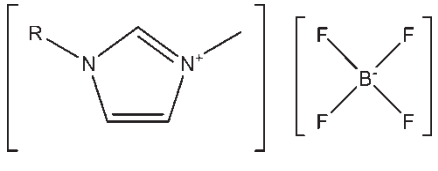

Figure 1. Chemical structure of 1-alkyl-3-methyl imidazolium tetrafluoroborate. R = ethyl:[emim][BF_4], R = butyl:[bmim][BF_4], R = hexyl:[hmim][BF_4], and R = octyl: [omim][BF_4].

alkyl chain ionic liquids. This implies that the hydrophobic property of the reversed-phase stationary phase surface can be controlled by the concentration of ionic liquid in mobile phase and the length of alkyl chain in the imidazolium cation. To survey the interaction of ionic liquid between analytes and stationary phase surface, five kinds of amine, carboxylic acid, and zwitterions were used.

Figure 2 showed the molecular structure of five analytes including (a) benzoic acid, (b) benzylamine, (c) 4-aminobenzoic acid, (d) L-phenylalanine, and (e) L-tryptophan. Among them, benzylamine and benzoic acid have only one functional group, amine and carboxylic acid, respectively. Therefore, these two analytes were used to investigate the retention behavior of cationic and anionic compounds between the hydrophobic stationary phase and hydrophilic mobile phase added ionic liquids. Three zwitterions including 4-aminobenzoic acid, L-phenylalanine, and L-trpytophan, were used to investigate the interaction between different functional groups in the same molecule. The 4-aminobenzoic acid and L-phenylalanine have one amine and one carboxylic acid. Two functional groups of 4-aminobenzoic acid located opposite the site of the benzene ring, L-phenylalanine, however, have amine and carboxyl acid functional groups in the same carbon site. Unlike L-phenylalanine, L-tryptophan has an additional indol ring as a side chain in the benzene ring.

When the pH of the mobile phase was not controlled, three analytes, except L-phenylalanine and L-tryptophan, have irregular peak shapes. It was

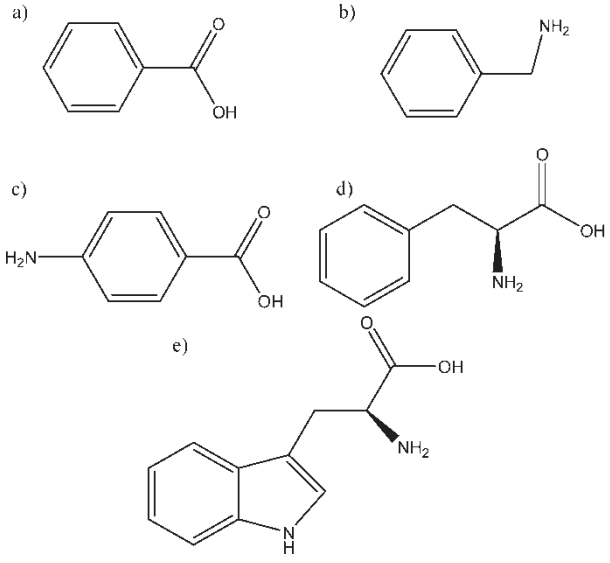

Figure 2. Molecular structures of analytes. a) benzoic acid, b) benzylamine, c) 4-aminobenzoic acid, d) L-phenylalanine, and e) L-tryptophan.

Table 1. Ionization constant of analytes and isoelectric point of zwitterions (25°C, 1 atm)

Chemical name	$pK_{a(COOH)}$	$pK_{a(NH2)}$	pI
Benzoic acid	4.21	—	—
Benzylamine	—	9.37	—
4-Aminobenzoic acid	4.65	9.35	7.00
L-Phenylalanine	2.20	9.09	5.50
L-Tryptophan	2.38	9.34	5.89

difficult to find the effect of ionic liquids on the retention behavior of ionized analytes. Therefore, potassium phosphate monobasic (KH_2PO_4) salt was dissolved in the mobile phase, with controlled pH 4.0, as 25 mM. Table 1 showed the ionization constants and isoelectric points of analytes. Under the pH 4.0 condition, carboxylic functional groups of benzoic acid and 4-aminobenzoic acid have a tendency to exist as anionic forms, those of L-phenylalanine and L-tryptophan are, however, hard to exist as charged forms. On the contrary, all amines of analytes tend to have positive charges. Therefore, in this mobile phase condition, benzoic acid has a strong interaction with the stationary phase, but other analytes have weak interaction with the stationary phase. When [emim][BF_4] that has the shortest alkyl chain among the four ionic liquids was dissolved in the mobile phase, this ionic liquid acted as the ion suppressing agent. As shown in Table 2, the retention times of all analytes were slightly increased. When [emim][BF_4] ionic liquid was added to the mobile phase, the ion pairing forming mechanism dominantly effected the retention behaviors of analytes.

Figure 3 showed the changes of retention time of benzoic acid and benzylamine with four ionic liquids as mobile phase additives. When long alkyl chain imidazolium ionic liquids were used as the mobile phase additive, the

Table 2. Increment of retention time of analytes with [emim][BF_4] ionic liquid as mobile phase additive

Additive	Control	[emim][BF_4]	Increament (%)
Retention time (min)			
L-phenylalanine	3.99	4.05	1.45
L-tryptophan	5.92	6.07	2.50
Benzoic acid	36.81	37.36	1.51
Benzylamine	3.38	3.65	7.90
4-Aminobenzoic acid	5.28	5.34	1.12

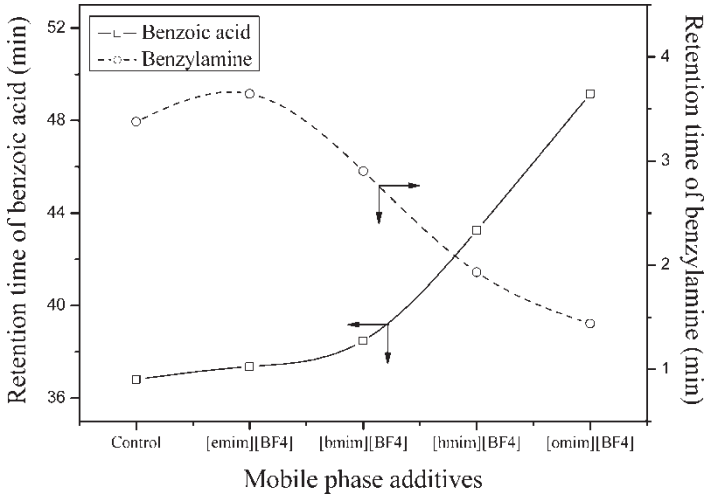

Figure 3. Changes of retention time of benzoic acid and benzylamine with four ionic liquids as the mobile phase additives. Open square is benzoic acid and open circle is benzyl amine.

retention time of benzylamine decreased whereas that of benzoic acid increased. A long alkyl chain imidazolium cation is easy to adsorb on the stationary phase, therefore, ionized benzoic acid that has a negative charge is attracted to the adsorbed imidazolium cation. To the contrary, ionized benzylamine that has a positive charge is repulsive to the adsorbed imidazolium cation. Therefore, it can be explained that the retention behaviors of benzylamine and benzoic acid were dominant to the dynamic ion-exchange mechanism when strong hydrophobic ionic liquids, such as [bmim][BF_4], [hmim][BF_4], and [omim][BF_4] were used as the mobile phase additives. Figure 4 showed the changes of retention time of three zwitterions with four ionic liquids as the mobile phase additives. The retention behavior of L-phenylalanine and L-tryptophan were similar to the behavior of benzylamine (Fig. 3); therefore, it can be explained with the dynamic ion-exchange mechanism. The ionization constants of carboxylic functional group of two amino acids, L-phenylalanine and L-tryptophan, are 2.20 and 2.38, respectively (Table 1). Because of these small ionization constants, it is hard for ionization to occur in weak acidic conditions (pH 4.0), but the amine functional groups of the two amino acids are easily ionized to cationic form because the pH is lower than the ionization constants of the amines ($pK_a > 9$) in the two amino acids (Table 1). Therefore, the retention behaviors of two amino acids are similar to the benzylamine. However, the retention time of 4-aminobenzyoic acid increases when the long alkyl chain ionic liquids are used as the mobile phase additives, even though the amine functional group exists as a positive charged form. The

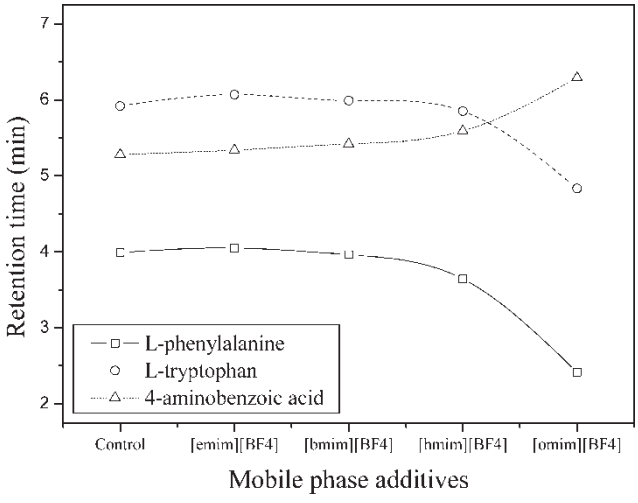

Figure 4. Changes of retention time of zwitterions with four ionic liquids as the mobile phase additives. Open square is L-phenylalanine, open circle is L-tryptophan, and open triangle is 4-aminobenzoic acid.

ionization constant of the carboxylic functional group of 4-aminobenzoic acid is 4.65 (Table 1). In weak acidic condition (pH 4.0), two functional groups, amine and carboxylic, of 4-aminobenzoic acid are easily changed to the ionized forms and these are located on opposite sides of the benzene ring. The 4-aminobenzoic acid molecule is attracted to the imidazolium ion adsorbed on the stationary phase, even though it has a positive charged amine. It is impossible for the positive charged amine to have some interaction when the negative charged carboxyl is headed to the imidazolium cation, because of the location of amine and carboxyl in 4-aminobenzoic acid.

Table 3 showed the retention time distribution of analytes with different concentrations of injected sample. The injection concentrations were varied from 1 mM to 30 mM. For four analytes (benzoic acid, 4-aminobenzoic acid, L-phenylalanine, and L-tryptophan) the retention time was not changed, while the injected concentration was increased up to 30 mM, 30 times of the smallest injected concentration. The percentage of standard deviation from the average was not over 1.5%. For all experiments, the concentration of ionic liquid was fixed to 17.5 mM. When no ionic liquids were used as mobile phase additives, the retention time of analytes was not changed within 1 to 30 mM of injected concentration range of analytes. In these experiments, therefore, the effect of ionic liquids as mobile phase additives was maintained by the low concentration of ionic liquid, even though the concentrations of analytes increased up to 30 mM.

Table 3. Retention time distribution of analytes with different injected concentrations of analytes

	Retention time (min)																			
	Benzoic acid					4-Aminobenzoic acid					L-Phenylalanine					L-Tryptophan				
[a]Conc. (mM)	[b]Ctrl	[b]EB	[b]BB	[b]HB	[b]OB	[b]Ctrl	[b]EB	[b]BB	[b]HB	[b]OB	[b]Ctrl	[b]EB	[b]BB	[b]HB	[b]OB	[b]Ctrl	[b]EB	[b]BB	[b]HB	[b]OB
1	36.81	37.32	38.50	43.33	49.07	5.28	5.34	5.42	5.59	6.29	4.00	4.05	3.97	3.65	2.42	5.92	6.07	5.98	5.86	4.84
5	36.71	37.68	—	42.26	48.76	5.28	5.36	5.38	5.56	6.27	3.98	4.05	3.98	3.63	2.42	5.91	6.12	6.04	5.75	4.84
10	37.24	38.30	38.53	42.86	48.58	5.31	5.40	5.31	5.56	6.24	3.99	4.07	3.95	3.65	2.42	5.94	6.17	5.94	5.85	4.83
20	37.64	38.43	38.54	42.94	48.38	5.21	5.36	5.29	5.56	6.21	4.03	4.11	3.95	3.65	2.41	6.04	6.28	5.96	5.89	4.84
30	37.39	38.51	38.68	43.07	48.13	5.31	5.36	5.29	5.55	6.18	4.01	4.13	3.97	3.66	2.42	6.05	6.30	6.00	5.91	4.82
Average	37.16	38.05	38.56	42.89	48.58	5.28	5.36	5.34	5.56	6.24	4.00	4.08	3.96	3.65	2.42	5.97	6.19	5.98	5.85	4.83
[c]SD	0.35	0.47	0.07	0.35	0.32	0.04	0.02	0.05	0.01	0.04	0.02	0.03	0.01	0.01	0.00	0.06	0.09	0.03	0.06	0.01
[c]SD (%)	0.94	1.23	0.18	0.83	0.66	0.69	0.37	0.99	0.24	0.64	0.43	0.80	0.30	0.27	0.17	1.01	1.44	0.58	0.94	0.17

[a]The concentrations of injected analytes.
[b]Ctrl = no ionic liquid is used as additive, EB = [emim][BF_4], BB = [bmim][BF_4], HB = [hmim][BF_4], and OB = [omim][BF_4].
[c]SD = Standard deviation and SD (%) = SD/Average × 100.

CONCLUSION

In reversed-phase liquid chromatography, mobile phase additives must be used to separate ionized or highly hydrophilic solutes. The ionic liquids have recently been used as mobile phase additives in the reversed-phase liquid chromatography, but the role of ionic liquids on adsorption mechnism is not clearly identified. In this study, the two well known mechanisms about ionized solutes and ionized additives are referred in order to explain the role of 1-alkyl-3-methylimidazolium tetrafluoroborate ionic liquid. One is the ion paring forming mechanism. When short alkyl chain imidazolium, such as [emim][BF_4], is used as a mobile phase additive, the retention behaviors of analytes can be explained with the ion paring forming mechanism. The other is the dynamic ion-exchange mechanism. This adsorption mechanism explains the retention behaviors of analytes when long alkyl chain ionic liquids ([bmim][BF_4], [hmim][BF_4], and [omim][BF_4]) are used. The effect of ionic liquids as mobile phase additives is comparatively maintained with low concentration ($<$20 mM).

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REFERENCES

1. Earle, M.J.; Seddon, R.K. Green solvents for the future. Pure Appl. Chem. **2000**, *72*, 1391–1398, Ionic liquids.
2. Gorman, J. New liquids take aim at old-fashioned chemistry. Sci. News **2001**, 160:156.
3. Pandey, S. Analytical applications of room-temperature ionic liquids: A review of recent efforts. Anal. Chim. Acta. **2006**, *556*, 38–45.
4. Xiaohua, X.; Liang, Z.; Xia, L.; Shengxiang, J. Ionic liquids as additives in high performance liquid chromatography. Analysis of amines and the interaction mechanism of ionic liquids. Anal. Chim. Acta. **2004**, *519*, 207–211.
5. Berthod, A.; Carda-Broch, S. Use of ionic liquids in analytical chemistry. MCFA annals. **2002**, 3.
6. Zhao, H.; Xia, S.; Ma, P. Use of ionic liquids as 'green' solvents for extractions. J. Chem. Technol. Biotechnol. **2005**, *80*, 1089–1096.
7. He, L.; Zhang, W.; Zhao, L.; Liu, X.; Jiang, S. Effect of 1-alkyl-3-methylimidazolium-based ionic liquids as the eluent on the separation of ephedrines by liquid chromatography. J. Chromatogr. A. **2003**, *1007*, 39–45.
8. Kaliszan, R.; Marszałł, M.P.; Markuszewski, M.J.; Ţaczek, T.; Pernak, J. Suppression of deleterious effects of free silanols in liquid chromatography by imidazolium tetrafluoroborate ionic liquids. J. Chromatogr. A. **2004**, *1030*, 263–271.

9. Ruiz-Angel, M.J.; Carda-Broch, S.; Berthod, A. Ionic liquids versus triethylamine as mobile phase additives in the analysis of beta-blockers. J. Chromatogr. A. **2006**, *1119*, 202–208.
10. Knox, J.H.; Hartwick, R.A. Mechanism of ion-pair liquid chromatography of amines, neutrals, zwitterions and acids using anionic hetaerons. J. Chromatogr. A. **1981**, *204*, 3–21.
11. Horváth, Cs.; Melander, W.; Molnár, I.; Molnár. Enhancement of retention by ion-pair formation in liquid chromatography with nonpolar stationary phases. Anal. Chem. **1977**, *49*, 2295–2305.
12. Dai, J.; Carr, W.P. Role of ion pairing in anionic additive effects on the separation of cationic drugs in reversed-phase liquid chromatography. J. Chromatogr. A. **2005**, *1072*, 169–184.
13. Li, S.; Fritz, S.J. Organic modifiers for the separation of organic acids and bases by liquid chromatography. J. Chromatogr. A. **2002**, *964*, 91–98.
14. Marszałł, M.P.; Ąaczek, T.; Kaliszan, R. Reduction of silanophilic interactions in liquid chromatography with the use of ionic liquids. Anal. Chim. Acta. **2005**, *547*, 172–178.
15. Montgomery, E.M.; Wirth, J.M., Jr. Spectroscopic study of the molecular basis of wetting of C_{18} surface by long-chain n-alcohol. Anal. Chem. **1994**, *66*, 680–684.
16. Cecchi, T.; Pucciarelli, F.; Passamonti, P. Ion interaction chromatography of neutral molecules. Use of a potential approximation to obtain a simplified retention equation. J. Liq. Chromatogr. & Rel. Technol. **2001**, *24*, 291–302.
17. Cecchi, T.; Cecchi, P. The dipole approach in ion interaction chromatography of zwitterions. Use of the linearized potential expression for low surface potential. J. Liq. Chromatogr. & Rel. Technol. **2002**, *25*, 415–420.

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