Application of Mobile Phases Containing Ionic Liquid for the Analysis of Selected Psychotropic Drugs by HPLC–DAD and HPLC–MS

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In reversed-phase liquid chromatography, in case of the absence of additives, cationic basic compounds give rise to asymmetrical and broad peaks as a result of interactions of analyte cations with residual free silanols on silica-based stationary phases. Ionic liquids, added to the mobile phase, have been suggested as alternatives to amines to block the activity of free silanols. The different parameters affecting the retention behavior, symmetry of peak, system efficiency, and separation selectivity of selected psychotropic drugs, especially the effect of concentration of ionic liquid, kind and concentration of organic modifiers of mobile phases, and kind of stationary phases were investigated. The most selective and efficient systems are used for separations of psychotropic drug standards' mixture and for determination of selected psychotropic drugs in human serum.

Keywords: HPLC-DAD, psychotropic drugs, ionic liquid, serum sample

Introduction

Therapeutic drug monitoring of antidepressants provides the possibility to reduce side effects and optimize the treatment of patients with depression both to optimize therapy and to avoid medical complications, intoxication, non-response or non-compliance [1]. For this reason, it is advantageous to use a reliable, highly sensitive, selective, accurate and precise methods for determination of psychotropic drugs in biological samples.

Various chromatographic systems were applied for determination of psychotropic drugs in different biological samples. Most analysis of psychotropic drugs were performed on C18 stationary phases with various composition of mobile phases. Addition of acids or buffers at acidic pH to mobile phases containing organic modifier and water was often applied for determination of the drugs [2–4].

Mobile phases at basic pH were rarely used for analysis of these compounds [5]. Some authors reported application of mobile phases containing organic modifier, water, and salts, e.g., ammonium acetate [6]. Sometimes addition of amines as silanol blocker was applied [7].

Currently, silica as support matrix for bonded stationary phases in reversed-phase liquid chromatography is predominantly applied due to its high efficiency, good reproducibility, and universality [8]. Despite the many advantages, stationary phases bonded to the silica matrix have always free silanol groups, owing to steric problems in the derivatization process of silica supports. These silanols are negatively charged within the pH range commonly used in reversed-phase liquid chromatography (2.5–7.5) and can additionally interact with cationic compounds by ion-exchange interaction. Ion-exchange process is slow compared to the hydrophobic interaction of compounds with the groups bonded on the surface of the stationary phase. For this reason, the chromatographic peaks of basic compounds are broad and tailing and low system efficiency and poor separation selectivity are usually obtained. The suppression of the disadvantageous “silanol effect” is very important to obtain a good systems efficiency, separation selectivity of analytes, and appropriate peak shapes for basic compounds. Undesirable interactions with the silanols can be reduced by use of mobile phases containing buffers at low pH, when silanol

ionisation is suppressed, or at high pH, to suppress solute ionisation, as well as by addition of anionic ion-pair reagents to form neutral associates. Good peak symmetry and system efficiency for analysis of basic compounds are also achieved by use of mobile phases containing organic amines as silanol blockers.

To suppress free silanol groups, an addition of ionic liquids (ILs) to mobile phases can be also successfully applied [9, 10]. The interaction mechanism in chromatographic systems with addition of ILs is more complex in comparison to the interactions in systems containing commonly used buffers or amines. Both cations and anions of ionic liquid play an important role in the retention of basic compounds. The cations of ionic liquids coated the surface of the stationary phase suppressing interactions with free silanol groups. Anions of ILs are also adsorbed on the surface of stationary phase and thus, with ionic liquid (IL) cations creating a bilayer, positively or negatively charged, improved the shape of peaks of analytes.

Many biological active compounds, e.g., components of plant extracts, and various drugs contain basic groups in their structure and in reversed-phase liquid chromatography (RPLC) are analyzed with application of mobile phases containing addition of ionic liquids as silanol blocker. The use of ILs additives was reported for analysis of some alkaloids, e.g., fangchinoline and tetrandrine in *Stephania tetrandra* [9], jatrorrhizine, palmatine, and berberine in Huangbo, the dried bark of Chinese Corktree [10]. ILs were also applied for analysis of some basic drugs such as β -blockers [11] and β -lactam antibiotics [12].

Besides the commonly used *n*-alkyl-type reversed-phase columns containing *n*-alkyl-type ligands onto a silica support, alternative hybrid RP-type phases providing additional interaction sites and properties due to embedded functional groups can be used [13, 14]. The introduction of hydrophobic π – π active aromatic moieties to the common *n*-alkyl chain RP-sites generates a concerted π – π reversed-phase retention mechanism, which is a consequence of the new functionality. These interactions are the type of electron donor–acceptor interaction between π electrons of the phenyl groups bonded on the stationary phase and the π electrons of the aromatic moieties of analyzed compounds. The π – π interactions may limit the ion exchange process on free silanol groups because of the preferred interactions of basic analytes with the phenyl. The π – π interactions typically involve the charge transfer of electrons from electron-rich (π -base) to

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electron-poor (π -acid) substances. The π - π interactions can also involve a simple overlap of π -orbitals in two interacting molecules. Ether linkage to alkyl chain (as in Polar RP column) increases aromaticity of the phenyl group and also provides π - π interactions between the phenyl ligands of stationary phase and aromatic moieties of solutes.

The aim of the paper is investigation of influence of IL applied as mobile phases additive on phenyl stationary phase (RP-Phe column) for twenty-two basic psychotropic drugs retention behavior. The effect of the concentration of IL or acetonitrile in mobile phases and effect of temperature was investigated and optimal eluent for analysis of psychotropic drugs in human serum was chosen.

Experimental

Chemicals and Drug Standards. Acetonitrile, methanol, tetrahydrofuran, dioxane, and 1-butyl-3-methylimidazolium tetrafluoroborate of chromatographic quality were from E. Merck (Darmstadt, Germany). Formic acid was of p.a. quality (Polish Reagents Gliwice, Poland).

Drug standards were obtained from the following sources: aripiprazole, Otsuka Pharmaceutical Europe Ltd. (Wexham, Great Britain); chlordiazepoxide, Tarachomińskie Zakłady Farmaceutyczne "Polfa" S. A. (Warsaw, Poland); citalopram, Ranbaxy Sp. z o. o. (Warsaw, Poland); desipramine hydrochloride, Temmler Pharma GmbH & Co. KG (Marburg, Germany); escitalopram and paroxetine, Gedeon Richter (Grodzisk Mazowiecki, Poland); carbamazepine, Sigma-Aldrich (Saint Louis, USA); clozapine, EGIS Pharmaceuticals PLC (Budapest, Hungary); quetiapine, Adamed Sp. z o.o. (Pieńków, Poland); levomepromazine, PROTERAPIA Sp. z o.o. (Warsaw, Poland); lamotrigine, Glenmark Pharmaceuticals (Prague, Czech Republic); moclobemide, Hoffmann LaRoche (Basel, Switzerland); mirtazapine, Orion Corporation (Espoo, Finland); olanzapine, Actavis Group PTC (Hafnarfjörður, Iceland); oxcarbazepine, Generics UK Ltd. (Potters Bar, Great Britain); perazine, HASCO-LEKSA. (Wrocław, Poland); perphenazine, MSD (Warsaw, Poland); risperidone, Janssen-Cilag International NV (Beerse, Belgium); sertraline, Biofarm (Poznań, Poland); venlafaxine, Polfa Pabianice S.A. (Pabianice, Poland); opipramol dihydrochloride; and zolpidem tartas, Polpharma (Stargard Gdański, Poland).

Apparatus and HPLC Conditions. The high-performance liquid chromatography with diode array detection (HPLC-DAD)

analysis was performed using liquid chromatograph LC-10 ATVP Shimadzu equipped with column Synergi Polar RP 80A (RP-Phe column) 150 × 4.6 mm, 5 μ m. Chromatograph was equipped with Shimadzu detector SPD 10 AVVP and Rheodyne 20 μ L injector. The detection was carried out at 240 nm wavelength. All chromatographic measurements were controlled by CTO-10ASVP thermostat. Flow rate was 1.0 mL/min.

Preparation of Serum Samples. Samples were collected from psychiatric patients. After blood coagulation, the samples were centrifuged for 10 min at 1500 × *g*. The serum was separated and stored at -20 °C until analysis.

Solid-phase extraction (SPE) for serum sample preparation was performed using BAKERBOND™ spe Octadecyl (C18) J.T. Baker (Phillipsburg, USA) cartridges (100 mg/1 mL) and SPE chamber—Baker SPE-12G, J.T. Baker (Phillipsburg, USA).

Serum sample (1.5 mL) was diluted with 1.5 mL of ammonium buffer at pH 8.6. SPE column was activated with 1 mL of methanol and conditioned with 1 mL of mixture containing water and ammonium buffer at pH 8.6 in ratio 5:1. Then, the serum sample was introduced to the column at speed 1 mL/min. The column was prewashed with 1 mL of methanol solution (25% v/v) and dried applying vacuum for 3 min. The extracted drugs were eluted twice with 1 mL of mixture containing 98% methanol and 2% acetic acid. The eluted sample was evaporated to dryness and dissolved in 0.5 mL of methanol. Next, 20 μ L of the sample was injected into the HPLC column.

Results and Discussion

Psychotropic drug standards (Table 1) were chromatographed on RP-Phe column in eluent systems containing organic modifier, water, and ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate as free silanol blocker. Tested chromatographic systems were compared in terms of retention of investigated compounds, peak shape, system efficiency, and differences in separation selectivity. In the first step of investigations, commonly used mobile phase containing acetonitrile, water, and formic acid and mobile phase containing acetonitrile, water, and IL were applied. For comparison of chromatographic systems performance, important parameters, theoretical plate number and asymmetry factor, were calculated. Theoretical plate number (*N*) is an index that indicates chromatographic system efficiency and is calculated by Eq. (1).

$$N = 5.54 \times \left(\frac{t_R}{w_{0.5h}} \right) \quad (1)$$

Table 1. t_R and N/m values obtained for psychotropic drugs on Polar RP column with mobile phase containing 25% MeCN, water, and different concentrations of IL

Name of compounds	0%		0.05%		0.1%		0.25%		0.5%		1.0%		1.5%		2.0%	
	t_R	N/m	t_R	N/m	t_R	N/m	t_R	N/m	t_R	N/m	t_R	N/m	t_R	N/m	t_R	N/m
Aripiprazole	65.128	16,100	72.268	37,600	79.837	50,100	84.102	64,300	78.924	70,000	79.743	68,400	79.743	63,800	73.746	58,700
Chlordiazepoxide	12.541	6200	18.447	11,700	14.119	66,300	13.728	62,600	7.878	51,000	7.506	46,100	7.506	40,800	8.042	36,900
Citalopram	14.872	15,300	19.033	33,100	19.442	37,300	21.565	30,400	20.678	33,100	21.394	32,200	21.394	30,700	19.568	29,800
Desimipramine	20.158	9800	24.807	17,000	25.371	18,900	28.344	46,900	26.787	41,100	26.390	37,400	26.390	33,600	24.899	31,400
Escitalopram	16.843	22,000	19.136	37,200	19.925	39,000	20.710	36,600	20.689	33,500	19.980	29,100	19.980	25,300	19.600	21,600
Carbamazepine	11.282	20,800	15.485	57,100	15.880	57,500	16.478	54,500	15.147	60,000	13.819	55,300	13.819	52,000	14.486	48,300
Clozapine	10.928	19,300	14.082	40,900	16.315	26,000	15.191	37,000	12.199	32,900	13.425	29,800	13.425	26,900	11.286	25,700
Quetiapine	15.152	14,700	17.291	28,600	18.123	19,900	17.935	43,900	17.302	49,000	13.573	38,100	13.573	30,500	16.295	25,800
Levomepromazine	41.284	23,100	46.722	63,800	42.133	71,000	44.982	56,800	42.261	62,000	42.141	51,800	42.141	44,200	39.170	36,600
Lamotrigine	4.121	10,800	4.759	25,100	4.817	26,500	4.769	17,500	4.661	16,600	4.609	17,400	4.609	17,300	4.601	18,900
Maclobenid	3.982	9500	4.503	19,600	3.921	15,500	3.893	22,700	3.913	15,800	3.985	16,200	3.985	17,600	3.900	18,100
Mirtazapine	5.758	7200	6.895	14,700	7.597	25,100	7.048	27,100	6.952	17,700	6.910	18,400	6.910	20,500	6.076	23,100
Olanzapine	3.301	4800	3.492	9400	3.543	6300	3.374	8700	3.522	10,300	3.468	12,800	3.468	12,100	3.389	11,300
Opipramol	19.321	15,900	21.218	32,500	21.594	35,000	21.266	50,200	16.006	44,500	19.503	40,300	19.503	38,200	18.959	37,000
Oxcarbamazepine	8.536	21,100	9.687	43,900	9.933	44,200	9.916	45,300	9.500	44,200	8.829	40,700	8.829	37,300	9.194	34,000
Paroxetine	27.158	18,500	31.251	26,800	33.305	30,500	35.739	48,600	33.600	51,600	32.985	43,300	32.985	37,100	31.107	31,200
Perazine	31.415	22,800	36.659	46,500	37.469	50,100	36.258	65,100	34.500	66,500	33.182	54,800	33.182	44,600	25.173	38,200
Perfenazine	47.129	17,800	53.601	35,800	54.689	40,200	37.602	65,300	36.376	72,000	38.041	63,500	38.041	54,700	41.692	49,500
Risperidone	12.186	16,300	15.287	35,700	13.717	31,700	13.476	43,100	12.427	40,200	11.601	39,800	11.601	39,100	12.831	38,200
Sertraline	39.158	10,100	46.871	25,400	49.045	24,200	55.726	57,800	50.578	59,100	47.987	55,200	47.987	51,400	49.008	48,500
Venlafaxine	6.722	11,200	7.203	30,900	6.562	31,900	6.849	24,400	6.685	16,100	6.829	14,900	6.829	11,900	7.024	10,200
Zolpidem	11.125	9800	13.554	21,900	10.665	18,900	9.770	41,100	9.624	35,900	9.322	32,200	9.322	29,100	9.044	27,200

where t_R is retention time and $w_{0.5h}$ is the peak width measured at 0.5 of peak high.

Asymmetry factor (A_s) is a coefficient that shows the degree of peak symmetry and is calculated by Eq. (2):

$$A_s = \frac{b}{a} \quad (2)$$

where b is distance from the point at peak midpoint to the trailing edge (measured at 10% of peak height) and a is

distance from the leading edge of peak to the midpoint (measured at 10% of peak height).

In system containing only acetonitrile, water, and formic acid, for most investigated drug, peaks were very wide and asymmetric; only for six out of twenty-two drugs, A_s values were in acceptable range ($0.8 < A_s < 1.5$). System efficiency in the mobile phase was also poor for most investigated compounds, only for seven drugs N/m values were higher than 25,000. The significant improvement of peak shape and systems efficiency was obtained in mobile phase system containing acetonitrile, water, and

Figure 1. Graphical comparison of A_s values of psychotropic drugs on the RP-Phe column with eluent systems containing 25% MeCN, water, and various concentrations of 1-butyl-3-methylimidazolium tetrafluoroborate

Table 2. A_s and N/m values obtained for psychotropic drugs on Polar RP column with mobile phase containing different concentration of MeCN, water, and 0.25% IL

Name of compounds	10		15		20		25		30		35		40		45	
	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m
Aripiprazole	—	—	—	—	—	—	1.11	64,300	1.12	58,500	1.17	56,300	1.19	47,900	1.23	43,700
Chlordiazepoxide	—	—	0.98	59,500	0.68	40,400	0.60	12,600	0.66	22,100	1.59	17,800	1.66	19,100	—	—
Citalopram	—	—	—	—	0.89	40,400	0.90	30,400	0.93	29,100	1.20	42,700	1.05	31,200	—	—
Desimipramine	—	—	—	—	1.24	52,400	1.24	46,900	1.10	42,300	1.24	45,800	1.35	38,800	1.37	34,500
Escitalopram	—	—	—	—	0.93	43,400	0.92	36,600	0.91	27,400	1.07	35,800	1.06	29,200	—	—
Carbamazepine	—	—	—	—	0.95	57,900	0.97	54,500	1.00	50,100	1.03	47,100	1.09	47,500	1.14	40,800
Clozapine	—	—	0.92	45,000	0.97	48,900	0.91	37,000	0.93	35,400	1.08	67,000	1.19	18,700	—	—
Quetiapine	—	—	—	—	1.06	51,100	1.09	43,900	1.06	33,500	1.22	46,000	1.24	36,700	—	—
Levomepromazine	—	—	—	—	—	—	1.29	56,800	1.22	50,900	1.30	51,400	1.50	51,400	1.40	39,700
Lomatrigine	0.75	22,900	0.75	18,800	0.78	15,500	0.89	17,500	0.85	17,500	1.19	29,900	—	—	—	—
Maclobenid	1.14	16,900	0.79	15,500	1.18	31,000	1.04	22,700	1.12	24,600	1.30	25,600	—	—	—	—
Mirtazapine	0.97	36,500	1.28	40,000	1.11	15,800	0.98	27,100	1.03	28,900	1.61	27,200	—	—	—	—
Olanzapine	0.62	4100	1.07	23,400	1.14	19,000	1.33	8700	1.35	20,400	1.34	14,200	—	—	—	—
Opipramol	—	—	—	—	1.14	57,200	1.06	50,200	1.03	42,500	1.22	43,100	1.27	36,200	—	—
Oxcarbamazepine	—	—	0.97	51,700	0.95	48,900	0.96	45,300	1.01	43,300	1.04	41,000	—	—	—	—
Paroxetine	—	—	—	—	—	—	1.14	48,600	1.13	43,500	1.18	52,000	—	—	—	—
Perazine	—	—	—	—	—	—	1.14	65,100	1.10	56,100	1.25	48,200	1.39	43,400	1.37	39,200
Perfenazine	—	—	—	—	—	—	1.15	65,300	1.16	58,500	1.28	53,100	1.10	30,900	1.29	38,300
Risperidon	—	—	1.13	53,600	1.13	49,300	1.11	43,100	1.13	40,400	1.20	39,500	1.07	33,700	—	—
Sertraline	—	—	—	—	—	—	1.23	57,800	0.98	57,700	1.45	55,200	1.38	31,600	1.40	22,600
Venlafaxine	0.95	34,400	0.81	36,700	0.85	24,800	0.93	24,400	0.95	21,900	1.14	31,600	—	—	—	—
Zolpidem	—	—	1.15	47,800	1.08	40,400	1.14	41,100	1.15	38,800	1.31	37,100	1.32	33,100	—	—

—, Very strong or weakly retention.

Figure 2. The dependencies of psychotropic drugs $\log k$ values versus concentration of acetonitrile. System: RP-Phe column eluted with a mixture of MeCN, water, and 0.5% 1-butyl-3-methylimidazolium tetrafluoroborate

Figure 3. The dependencies of psychotropic drugs $\log k$ values versus temperature on the RP-Phe column eluted with a mixture of MeCN, water, and 0.5% 1-butyl-3-methylimidazolium tetrafluoroborate

addition of even small amount of IL (0.05%). In mobile phases containing IL, significantly higher theoretical plate number (N) was also obtained. In eluent system containing only 0.05% IL for fifteen out of twenty-two investigated psychotropic drugs, N/m was higher than 25,000.

Further increase in IL concentration resulted in further changes in retention, peaks' symmetry, and system efficiency. The retention was slightly decreased with the increase of IL concentration for most investigated compounds. The separation selectivity for all drugs was similar in all range of IL concentration. However, great differences were obtained in peaks' symmetry with the change in IL concentration for investigated drugs (Figure 1). In systems with 0.25% or higher concentration of IL, peaks for almost all investigated drugs were symmetrical. For example, in system containing 0.25% IL, symmetrical peaks were obtained for 21 drugs, except chlordiazepoxide, and for 16 drugs symmetry of peaks was excellent; in system with 0.5% IL, only for lomatrigine, peak was asymmetrical, and for 14 compounds, symmetry was excellent. Slight improvement of peaks shape was obtained when mobile phases containing higher concentration of IL were applied. In system containing 1% of IL, excellent peak symmetry was obtained for 15 compounds, in system with 1.5% for 17 and in system

containing 2% of IL for 15 investigated drugs. In mobile phase systems containing 1–2% IL for all investigated drugs, peaks of A_s values were in acceptable range.

Significant increase of systems' efficiency was observed in eluent containing only 0.05% IL in comparison to system without the additive (Table 1). An increase of the IL concentration caused increase of systems efficiency for all investigated psychotropic drugs. For most investigated drugs, the highest N/m values were obtained for eluent systems containing 0.1–0.5% IL. On the basis of the obtained results, in further investigations, mobile phases containing 0.25% IL were applied.

In the next step of experiments, an effect of acetonitrile concentrations in aqueous mobile phases containing water and 0.25% IL on chromatographic parameters was examined (Figure 1, 2 and Table 2). Retention of psychotropic drugs decreased with increase of organic modifier concentrations in the investigated range of 10–45% MeCN. Separation selectivity also decreased with the increase of MeCN concentration for most investigated drugs. In systems with 10 or 15% MeCN, most drugs are strongly retained on RP-Phe column.

With the change of MeCN concentration, changes in peak symmetry and systems efficiency were also observed. However,

Table 3. A_s and N/m values obtained for psychotropic drugs on Polar RP column with mobile phase containing 25% MeCN, water, and 0.25% IL at different temperature

Name of compounds	10 °C		15 °C		20 °C		25 °C		30 °C		35 °C		40 °C		45 °C	
	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m	A_s	N/m
Aripiprazole	1.20	55,300	1.16	58,400	1.01	61,000	1.12	64,800	1.21	61,200	1.35	59,700	1.39	56,200	1.51	54,200
Chlordiazepoxide	1.73	10,400	1.54	10,700	1.35	12,400	0.90	12,600	0.78	12,700	0.76	12,100	0.74	11,300	0.73	10,300
Citalopram	1.21	39,600	1.17	36,800	1.10	32,300	0.90	30,100	0.83	28,100	0.80	25,900	0.79	22,800	0.74	22,300
Desimipramine	1.19	45,700	1.10	46,200	1.13	46,300	1.24	46,900	1.46	45,200	1.53	43,200	1.57	40,700	1.62	40,100
Escitalopram	0.93	34,000	1.03	34,800	1.01	35,200	0.90	36,300	0.81	36,700	0.79	35,700	0.75	34,200	0.72	33,300
Carbamazepine	1.03	41,900	1.06	45,800	1.02	48,000	0.97	53,900	0.90	52,100	0.85	50,800	0.81	45,600	0.79	41,800
Clozapine	1.33	44,100	1.27	45,100	1.23	42,400	0.91	37,000	0.84	35,300	0.81	34,000	0.77	30,900	0.75	30,200
Quetiapine	1.04	37,800	1.05	39,400	1.05	41,300	1.11	43,700	1.24	42,800	1.31	41,300	1.37	37,600	1.47	36,500
Levomopromazine	1.31	49,200	1.24	51,800	1.10	54,600	1.31	56,100	1.51	53,900	1.68	52,100	1.73	46,500	1.75	46,100
Lomatrigine	1.05	22,400	1.10	19,600	1.11	18,400	0.88	17,600	0.81	16,400	0.78	15,900	0.73	14,700	0.71	13,800
Maclobenid	1.24	24,800	1.17	23,300	1.06	23,100	1.02	22,500	0.95	21,100	0.90	20,800	0.84	18,900	0.80	18,300
Mirtazapine	1.14	15,900	1.19	18,800	1.21	22,800	0.97	27,400	0.92	26,900	0.87	26,400	0.82	24,800	0.79	19,200
Olanzapine	1.34	13,700	1.33	12,700	1.30	11,700	1.34	8700	1.48	8100	1.56	7900	1.63	7700	1.60	7800
Opipramol	1.01	42,000	1.04	41,500	1.05	46,400	1.07	50,800	1.19	49,700	1.28	48,200	1.35	45,200	1.39	41,900
Oxcarbamazepine	0.95	38,400	0.98	40,900	1.02	26,400	0.94	45,300	0.87	46,100	0.83	45,600	0.80	40,100	0.78	37,900
Paroxetine	1.18	40,500	1.14	34,700	1.09	46,400	1.14	48,800	1.27	48,300	1.35	47,700	1.47	43,500	1.53	40,300
Perazine	1.11	36,900	1.12	44,800	1.13	52,800	1.16	65,700	1.25	60,800	1.30	54,800	1.41	41,200	1.49	35,800
Perfenazine	1.23	53,500	1.20	55,100	1.15	59,300	1.18	65,100	1.27	63,200	1.42	61,200	1.49	58,300	1.51	51,100
Risperidon	1.09	38,200	1.10	39,000	1.08	40,200	1.11	43,800	1.20	41,100	1.29	40,300	1.37	37,200	1.42	36,700
Sertraline	1.36	51,300	1.25	53,200	1.08	55,300	1.25	56,800	1.39	54,600	1.50	52,800	1.64	49,900	1.67	48,600
Venlafaxine	1.03	25,500	1.01	26,500	0.91	26,300	0.93	24,400	0.84	21,900	0.78	20,500	0.77	19,700	0.75	18,900
Zolpidem	1.36	35,100	1.28	37,800	1.17	40,200	1.15	41,100	1.05	40,700	0.92	38,100	0.89	36,400	0.85	34,600

Figure 4. Chromatogram of psychotropic drug standards mixture obtained on the RP-Phe column eluted with a mixture of MeCN, water, and 0.5% 1-butyl-3-methylimidazolium tetrafluoroborate

Figure 5. Chromatograms obtained on the RP-Phe column eluted with a mixture of MeCN, water, and 0.5% 1-butyl-3-methylimidazolium tetrafluoroborate. A, Perazine standard; B, blank human serum; C, human serum fortified by 0.4 $\mu\text{g/mL}$ of perazine; D, human serum from patient medicated by perazine

these changes have not been such significant as these obtained when concentration of IL was changed. The most symmetrical peaks were obtained in mobile phase containing 25% or 30% of MeCN; for 21 drugs, A_s values were optimal ($0.8 < A_s < 1.5$). Slight differences were also observed in system efficiency with the change of MeCN concentrations. For most cases, N/m values decreased with the increase of MeCN concentration. For most of investigated compounds, the highest N/m values were obtained with mobile phases containing 20% MeCN, but in the system, some psychotropic drugs were still strongly retained on RP-Phe column stationary phase ($t_R > 100$ min). The sufficient system efficiency for most investigated drugs was obtained when concentrations of MeCN were in range 25–35%. In mobile phases containing 25% or 30% of acetonitrile and in system containing 35% MeCN for 17 and for 20 compounds, respectively, N/m values were $>25,000$.

The influence of temperature on retention, peaks' shape, and systems efficiency was also investigated. The experiments were performed by using mobile phase containing 25% MeCN, water, and 0.25% IL at temperatures in the range of 10–45 °C. In applied system, linear decrease of retention with the increase of temperature has been obtained for all investigated psychotropic drugs according to van't Hoff's equation (Figure 3). These dependencies are typical for unionized compounds, which may indicate a predominant influence of ion-pairs formation on the retention in the chromatographic system. Separation selectivity was similar for most investigated drugs in all range of temperature.

Symmetry of peaks changed significantly with the change of temperature (Table 3). At temperatures 10 and 15 °C (only peak for chlordiazepoxide was asymmetrical) for 13 and 16 compounds, respectively, excellent shape of peaks was obtained. When experiments were performed at 20 or 25 °C for all investigated drugs, symmetrical peaks were observed, and for 18 and 17 compounds, respectively, symmetry of peaks was excellent. Further increase of temperature caused deterioration in the shape of the peaks especially when it was higher than 45 °C when only for 6 drugs symmetrical peaks were obtained.

High systems efficiencies were obtained for most psychotropic drugs in whole range of examined temperatures. For most compounds, N/m values slightly increased with increase of temperature and reached its maximum at 25 °C and then decreased again. For example, for aripiprazole, at 10 °C, N/m was 55,300; at 25 °C, 64,800; and at 45 °C, 54,200.

Usefulness of chromatographic system containing IL for analysis of basic psychotropic drugs was confirmed by application of it for analysis of mixture of psychotropic drugs' standards and for analysis of perazine in human serum from patient. For this purpose, mobile phase containing 25% MeCN, water, and 0.25% IL was applied. Experiments were performed at 22 °C. Figure 4 presents chromatogram obtained for separation of mixture of psychotropic drug standards. All components of the mixture were separated, and symmetrical peaks were obtained.

The same chromatographic system was applied for analysis of perazine. Figure 5A shows chromatogram obtained for

standard of perazine. In Figures 5B and 5C, chromatograms obtained for blank human serum and for human serum fortified by 0.4 µg/mL of perazine were presented. Before HPLC analysis, blank human serum and fortified human serum samples were prepared by procedure described in Experimental section. There was no significant interference for the drug and components of matrix. Chromatogram obtained for serum sample from patient medicated with perazine was presented in Figure 5D.

Conclusions

The mobile phases containing addition of IL 1-butyl-3-methylimidazolium tetrafluoroborate as silanol blocker were successfully applied for analysis of basic psychotropic drugs by HPLC on RP-Phe column.

In mobile phases without addition of IL, the obtained peaks were asymmetrical and system efficiencies were low. The application of mobile phases containing even low concentration of IL (0.05%) significantly improves both shape of peaks and system efficiency. The most symmetrical peaks, highest N/m values, and sufficient separation selectivity were obtained for drugs on Polar RP column with mobile phase containing 25% MeCN, water, and 0.25% IL. This is due to the masking of residual free silanol groups on the surface of the silica-based stationary phases and creation of ion-pair between cations of analytes and anions of IL.

Additionally, application of stationary phases with π - π interactions, e.g., containing phenyl groups such as RP-Phe column, allows double protection against deleterious interaction between basic analytes and free silanol groups.

Systematic optimization of retention behavior of the investigated drugs enables a choice of the best chromatographic systems for separation of mixture of psychotropic drugs and analysis of one of them (perazine) in human serum samples.

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