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Article

Comparison of Chromatographic Conditions for Analysis of Selected Psychotropic Drugs in Human Serum

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Retention parameters of psychotropic drug standards were determined on different stationary phases: octadecyl silica, polar octadecyl silica, cyanopropyl silica and phenyl-hexyl silica using aqueous eluent systems containing acetonitrile, methanol or mixture of acetonitrile and methanol as organic modifiers; acetic buffer at pH 3.5 and diethylamine. The influence of stationary phases, kind of organic modifier and concentration of methanol and acetonitrile in mobile phases on retention, separation selectivity, peak symmetry and system efficiency was examined. These chromatographic parameters were significantly changed when analyses were performed on different stationary phases, when acetonitrile or methanol was used as organic modifier and when proportions of acetonitrile and methanol in eluent were different. The most efficient and selective systems were applied for quantification of the selected psychotropic drugs in fortified samples of human serum.

Introduction

Therapeutic drug monitoring is a useful tool for the clinical management of patients receiving a pharmacotherapy, especially in psychiatry. Hence, determination of drug concentration in biological fluids is important to rationally support physicians' decisions on drug dosage adjustments. However, on the basis of clinical experience, those antidepressants in overdose would cause several side effects such as myocardial depression and ventricular arrhythmia and sometimes cause patients death. It is well known that the positive effect of therapy and the incidence of side effects during psychotropic therapy are often dose related, and similar correlations have been found between plasma levels and therapeutic effects, at least for some psychotropic drugs (1). The additional problem in routine therapeutic drug monitoring at a psychiatric treatment is that only a small percentage of the patients are in monotherapy. A relevant percentage of the patients are comedicated with other drugs often another psychotropic, neuroleptics or tricyclic antidepressants. Rapid and reliable analytical assays are also required to detect and identify drugs of toxicological importance.

Various analytical methods were described for the qualitative and quantitative determination of different psychotropic drugs, for example, LC–DAD (2), LC–MS (3, 4), LC–MS-MS (5, 6) and UHPLC–MS-MS (7).

Currently, the most frequently for the analysis of these drugs were used columns with C18 (3, 4, 6–8) or C8 (5, 9–11) stationary phases. Some papers have been done in evaluating other phases for separation of psychotropic drugs, e.g. C18 Polar Plus (12), Phenyl (2), Cyclohexyl (13) and Phenyl-Hexyl (2). As eluents were most often used mixtures of acetonitrile (MeCN) or methanol (MeOH) with addition of acids (3, 6, 9) or buffers at acidic pH (7, 14, 15) when ionization of free silanol groups is suppressed, with buffers at basic pH when ionization of analytes is suppressed (5, 8), rarely with a silanol-blocking agent (16).

A very important and difficult problem is analysis of psychotropic drugs in biological samples. Numerous methods have been developed for the determination of these drugs by LC in different biological samples, e.g. in plasma (3, 14, 17, 18), urine (10) and breast milk (19).

Conventional C18 silica columns are the most widely used HPLC stationary phases for the analysis of drugs and pharmaceuticals, but often they exhibit peak tailing for basic compounds, especially by mobile phase containing only organic modifiers and water. In recent years, significant improvement has been made in the quality of bonded phases used in HPLC. However, a serious undesirable property of silica is its surface acidity due to the free silanol groups. Effects of free silanols on retention are difficult to control and are especially deleterious as the chromatographic behavior of basic analytes. For basic solutes, the kinetics of the ion-exchange interaction with free silanols may be slower than those with the alkyl ligands, giving asymmetric peaks. Interactions with the silanols can be reduced by use of mobile phases at low pH, when silanol ionization is suppressed, or at high pH, to suppress solute ionization, addition of ion-pair reagents to form a neutral associates or use of organic amines as silanol blockers. Additionally, interaction between basic compounds and silanol groups can be significantly reduced by changing of type of stationary phase.

The stationary phase interactions with analytes are controlled principally by the properties of chemically bonded ligands and by polar groups present in the support material, such as residual silanol groups on the surface of silica gel. In addition to hydrophobic interactions with non-polar bonded moieties, the retention of polar compounds may depend on dipole–dipole, donor–acceptor, hydrogen bonding and π-electron interactions. Moreover, the retention and separation selectivity of ionic substances may be affected by attractive or repulsive electrostatic interactions (20). The π–π interactions occur between compounds containing π-electrons and are significant when the stationary phase is electron-rich and the analyte is electron-poor, or when both have extensive π-bonding (21). The π–π interactions between aromatic moieties of solutes and π–π ligands on stationary phase are significant for retention on these columns and are partially blocking the interactions between basic analytes and free silanol groups.

The aim of this work was systematic investigations of selected psychotropic drugs on C18, CN–silica and very rarely used for the analysis of psychotropic drugs Phenyl-Hexyl or Polar RP

columns by use of aqueous eluents containing methanol (MeOH), acetonitrile (MeCN) or mixture of both and diethylamine as silanol blocker to obtain sufficient selectivity of separation, system efficiency and peak symmetry. The use of the double protection (use of stationary phase with $\pi-\pi$ ligands and mobile phase containing addition of silanol blocker) against interaction between aromatic basic solutes and free silanol groups allows to obtain symmetrical peaks and good system efficiency. The influence of different types of chemically bonded stationary phases on chromatographic parameters was examined. The effect of application of MeOH, MeCN or mixture of them as organic modifier was also investigated.

Most selective chromatographic systems were used for qualitative and quantitative determination of selected psychotropic drugs in fortified samples of human plasma.

Experimental

Chemicals

Acetonitrile (MeCN), methanol (MeOH) of chromatographic quality and diethylamine (DEA) were from Merck (Darmstadt, Germany). Water was double distilled. The pH of acetate buffer used in experiments in 0.2 M/L concentration was measured in aqueous solution.

HPLC conditions

Analysis was performed using liquid chromatograph LC-10 ATVP Shimadzu equipped with a Shimadzu detector SPD−10 AVVP and a Rheodyne 20 μL injector. Detection was at wavelength 254 nm. All chromatographic measurements were carried out at 22°C with an eluent flow rate of 1.0 mL/min. The chromatographic separation was performed on XBridge C18 column from Waters (150 × 4.6 mm, 5 μm), XSELECT CSH Phenyl-Hexyl column from Waters (150 × 4.6 mm, 5 μm), ACE Excel 5 CN column from Altmann Analytik (150 × 4.6 mm, 5 μm) and Synergi Polar RP column from Phenomenex (150 × 4.6 mm, 5 μm).

All chromatographic parameters such as retention times, asymmetry factor (A_S) (calculated by 10% of peak height) and theoretical plate number (N/m) were calculated by software CLASS-VP 5.0 controlling the chromatograph.

Sample preparation

Solid-phase extraction (SPE) was carried out using Bakerbond SPE C18 endcapped columns and SPE chamber−Baker SPE-12G (J.T. Baker, Phillipsburg, NJ, USA). The SPE method was optimized, and the best procedures in terms of recovery and purification were selected for sample preparation.

Procedure 1

The procedure was used for preparation of human serum samples fortified by mirtazapine and olanzapine. Two milliliters of MeCN were added to 2 mL of human serum sample fortified by mirtazapine at the concentration of 0.25 μg/mL and olanzapine at the concentration of 0.125 μg/mL and incubated at 37°C for 60 min. Then, the samples were filtered and centrifuged (400 rmp). To the supernatant, 0.8 mL of ammonium buffer at pH 8.3 was added and the SPE was carried out. SPE columns were conditioned by elution of 3 mL of MeOH followed by 3 mL of mixture containing MeCN, water and ammonium buffer at pH 8.3 (5 : 5 : 2). Then, the supernatant containing investigated drugs was introduced to the SPE column at a speed of 2 mL/min. The column was prewashed with 3 mL of methanol−water solution (1 : 1). The extracted drugs were eluted with 3 mL of mixture containing 90% methanol in water and 2% acetic acid. In acidic solution, basic drugs forming cations were better dissolved in aqueous media and eluted from the SPE column. The samples were evaporated to dryness and dissolved in 0.5 mL of MeOH.

Procedure 2

The procedure was used for preparation of human serum samples fortified by quetiapine, risperidone and oxcarbazepine. Then, 0.4 mL of ammonium buffer at pH 8.3 was added to 2 mL of fortified human serum (drugs concentration 0.5 μg/mL) and incubated at 37°C for 60 min. Then, the SPE was carried out. The SPE column was conditioning by elution of 3 mL of MeOH followed by 3 mL of water and ammonium buffer at pH 8.3 (5 : 2). Then, the serum containing the investigated drugs was introduced to the column at a speed of 2 mL/min. The column was prewashed with 3 mL of MeOH−water solution (3 : 7). The extracted drugs were eluted with 3 mL of mixture containing 90% methanol in water and 2% acetic acid. The sample was evaporated to dryness and dissolved in 0.5 mL of MeOH.

Method validation

The proposed method was validated by linearity, limit of detection (LOD) and limit of quantification (LOQ). Method linearity was studied by analyzing solvent-based standard solutions in triplicate at six concentrations ranging from 0.5 to 10 μg/mL for mirtazapine, olanzapine and quetiapine and 1 to 10 μg/mL for risperidone and oxcarbazepine. All calibration curves were linear over the concentration ranges with correlation coefficients (r) >0.9995. LOD and LOQ were calculated according to the following formulas: $\mathrm{LOD} = 3.3\ (\mathrm{SD}/S)$ and $\mathrm{LOQ} = 10\ (\mathrm{SD}/S)$, respectively, where SD is the standard deviation of the response and S is the slope of the calibration curve.

Accuracy of the method was tested by performing recovery studies. The average recovery was 87.2% for olanzapine, 103.11% for mirtazapine, 105.2% for quetiapine, 91.70% for risperidone and 98.77% for oxcarbazepine. Using the selected SPE procedures and HPLC systems, the investigated drugs were determined in fortified samples of human serum.

Results

The first part of our work was a search of retention behavior of investigated drugs on four columns with different chemical-bonded stationary phases in three eluent systems. Psychotropic drug standards were chromatographed on C18, Polar RP18, Phenyl-Hexyl and CN−silica columns by the use of aqueous mobile phases containing acetate buffer at pH 3.5, addition of 0.025 M DEA and MeOH, MeCN or mixture of MeOH and MeCN as organic modifiers. These chromatographic systems were compared in terms of retention of psychotropic drugs on stationary phases with different ligands that were selected according to their potential differences in retention mechanism

and hence possible changes in selectivity, peak shape and performance.

The weakest retention of the drugs has been obtained for eluent systems containing MeOH on the CN column, but on the Polar RP column investigated compounds were strongest retained. In eluents containing 30% MeCN, most drugs were weakest retained on the C18 column and strongest retained on the Polar RP stationary phase.

Different retention orders were observed depending upon whether MeCN or MeOH was used as the organic modifier (Figures 1 and 2). In eluent systems with MeOH compared with systems containing MeCN as organic modifier, greatest differences in investigated drugs retention on different columns were observed.

Great differences in peak shapes were obtained on different columns, e.g. for mirtazapine in eluent containing MeCN as organic modifier on the Polar RP column $A_S = 2.15$ but on Phenyl-Hexyl $A_S = 1.17$, while for desipramine in eluent with MeOH on the C18 column $A_S = 1.81$, but on Phenyl-Hexyl $A_S = 1.00$ (Tables I and II). The most symmetrical peaks were obtained on the Phenyl-Hexyl column using MeOH as a modifier in an aqueous mobile phase—for the 13 investigated drugs A_S values were in the optimal range, while the least symmetrical peaks were obtained on the Polar RP column using an MeCN—only for five drugs A_S values were optimal. In both eluent systems, more symmetrical peaks were on Phenyl-Hexyl column. On all tested columns, better peak shapes for most investigated compounds were obtained in eluent system containing methanol.

Depending on the type of stationary phase and organic modifier, great differences in system efficiency were obtained (Tables III and IV). Higher systems efficiency for most investigated psychotropic drugs were in eluent systems containing MeCN compared with systems with addition of MeOH, e.g. for desipramine on the C18 column in system with MeCN N/m = 38,300, but in system with MeOH N/m = 13,430, for oxcarbazepine N/m = 48,840 and 18,250 in eluents with MeCN and MeOH, respectively. Especially, highest efficiency in system with MeCN compared with system with MeOH was obtained on the C18 column—for 10 compounds N/m > 20,000, while in eluent containing MeOH for no compounds N/m > 20,000 and only for six compounds N/m > 10,000. On the Phenyl-Hexyl column N/m > 20,000 for five compounds in eluent containing MeOH and for nine in system with MeCN, on CN and Polar RP columns in system with MeOH N/m > 20,000 for four and nine drugs, respectively, but in system with MeCN for six and eleven

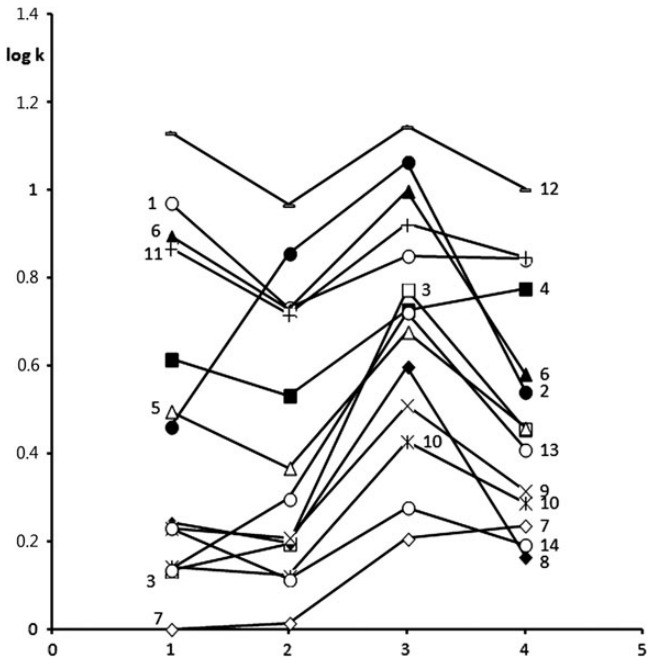

Figure 1. Graphical comparison of log *k* values obtained on (A) C18, (B) Phenyl-Hexyl, (C) Polar C18 and (D) CN columns in eluent system containing (A) 50% MeOH, (B) 55% MeOH, (C) 50% MeOH and (D) 35% MeOH; 20% acetate buffer at pH 3.5 and 0.025 M DEA.

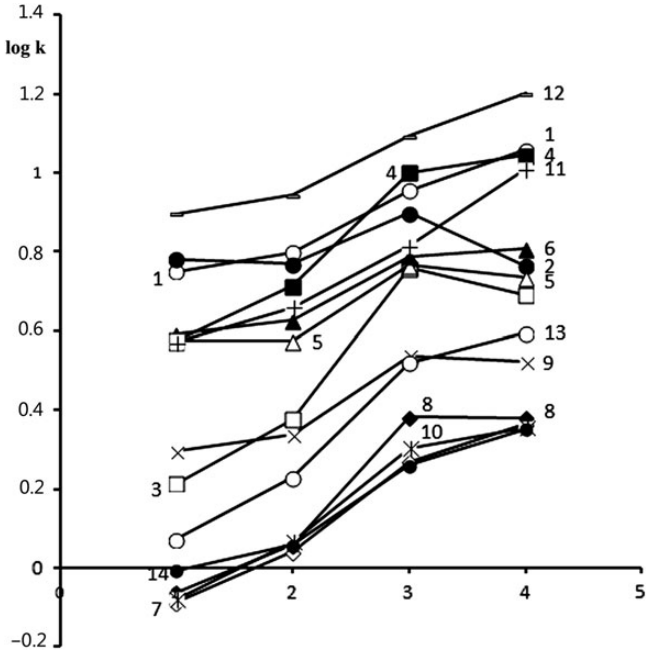

Figure 2. Graphical comparison of log *k* values obtained on (A) C18, (B) Phenyl-Hexyl, (C) Polar C18 and (D) CN columns in eluent system containing (A) 30% MeCN, (B) 30% MeCN, (C) 30% MeCN and (D) 30% MeCN; 20% acetate buffer at pH 3.5 and 0.025 M DEA.

Table I

A_S Values for the Psychotropic Drugs on Different Columns with Mobile Phases Containing MeOH, 20% Acetate Buffer at pH 3.5 and 0.025 M DEA

No.	Name of compounds	C18 column 50% MeOH	Phenyl-Hexyl column 55% MeOH	CN column 35% MeOH	Polar C18 column 50% MeOH
1	Desipramine	1.81	1.00	1.19	1.09
2	Chlordiazepoxide	1.32	0.93	0.67	1.03
3	Donepezil	0.96	0.96	1.19	1.36
4	Haloperidol	1.68	1.24	1.48	1.71
5	Carbamazepine	1.03	0.81	0.35	0.91
6	Quetiapine	1.35	1.02	1.00	1.11
7	Lamotrigine	0.87	0.70	0.99	0.78
8	Mirtazapine	1.36	1.08	1.22	1.82
9	Oxcarbazepine	0.89	0.82	0.68	0.84
10	Olanzapine	1.13	1.04	1.14	1.31
11	Opipramol	1.60	1.18	1.15	1.26
12	Perazine	1.85	1.28	1.30	1.76
13	Risperidone	1.02	1.05	1.48	1.53
14	Venlafaxine	1.05	0.92	1.46	1.17

drugs, respectively. In both eluent systems, highest N/m values for most investigated compounds were obtained on the Polar RP column.

For almost all investigated compounds on all tested columns in systems containing MeOH as organic modifier, better peak shapes were obtained compared with systems with MeCN, but the higher systems efficiency was obtained in eluent systems with MeCN. Due to the fact, mixtures of MeOH and MeCN in aqueous eluents were applied. In this eluent system for near all compounds, intermediate A_S values were obtained, e.g. for perazine on the Phenyl-Hexyl column in eluent containing MeOH $A_S = 1.28$, in eluent with MeCN $A_S = 1.73$ and in eluent containing mixture of organic modifiers $A_S = 1.44$ (Table V). Most symmetrical peaks in eluent system with mixture of MeOH and MeCN were on the Phenyl-Hexyl column—for 11 of 14 investigated drugs A_S values were in optimal range.

In system containing mixture of MeOH and MeCN, intermediate N/m values were obtained on all tested columns, e.g. for desipramine on the Polar RP column in eluent containing MeOH N/m = 25,060, in system with MeCN N/m = 38,500 and in system with mixed modifier N/m = 30,600 (Table VI). High efficiency in this eluent system was obtained on the Polar RP column—for 13 of 14 psychotropic drugs N/m > 20,000.

The influence of proportion of MeOH and MeCN in aqueous mobile phase on the Phenyl-Hexyl column was also examined. Figure 3 presents plots of log k vs. fraction of MeCN in mixtures of MeOH and MeCN used as organic modifiers. The linear dependencies were obtained. The retention of investigated drugs decreased by the change of MeCN concentration from 0 to 100% of MeCN as organic modifier. Moreover, the change

Table II

A_S Values for the Psychotropic Drugs on Different Columns with Mobile Phases Containing 30% MeCN, 20% Acetate Buffer at pH 3.5 and 0.025 M DEA

No.	Name of compounds	C18 column	Phenyl-Hexyl column	CN column	Polar C18 column
1	Desipramine	1.35	1.50	1.37	2.07
2	Chlordiazepoxide	0.85	0.99	0.47	1.03
3	Donepezil	0.90	1.27	1.37	2.11
4	Haloperidol	2.55	2.10	1.96	2.44
5	Carbamazepine	0.76	0.95	0.69	0.96
6	Quetiapine	1.26	1.41	1.05	1.80
7	Lamotrigine	0.85	0.96	1.01	1.04
8	Mirtazapine	1.21	1.17	1.43	2.15
9	Oxcarbazepine	0.74	0.94	0.75	0.95
10	Olanzapine	1.05	1.24	1.33	1.60
11	Opipramol	2.28	1.97	1.34	1.94
12	Perazine	2.35	1.73	1.68	2.59
13	Risperidone	1.24	1.34	1.78	2.21
14	Venlafaxine	1.50	1.83	1.93	2.45

Table III

N/m Values for the Psychotropic Drugs on Different Columns with Mobile Phases Containing MeOH, 20% Acetate Buffer at pH 3.5 and 0.025 M DEA

No.	Name of compounds	C18 column 50% MeOH	Phenyl-Hexyl column 55% MeOH	CN column 35% MeOH	Polar C18 column 50% MeOH
1	Desipramine	13,430	20,880	25,830	25,060
2	Chlordiazepoxide	18,800	26,680	9,180	34,300
3	Donepezil	6,470	12,800	14,080	22,220
4	Haloperidol	9,360	18,140	20,580	19,420
5	Carbamazepine	9,050	13,690	8,870	24,140
6	Quetiapine	16,700	25,446	13,360	31,230
7	Lamotrigine	5,180	8,620	13,150	13,240
8	Mirtazapine	12,900	22,980	15,380	22,090
9	Oxcarbazepine	5,800	11,850	7,480	18,250
10	Olanzapine	7,260	14,440	15,540	19,760
11	Opipramol	12,290	19,590	20,220	26,770
12	Perazine	14,460	31,260	23,170	28,960
13	Risperidone	7,380	18,220	16,010	23,760
14	Venlafaxine	3,580	9,320	10,220	7,800

Table IV

N/m Values for the Psychotropic Drugs on Different Columns with Mobile Phases Containing 30% MeCN, 20% Acetate Buffer at pH 3.5 and 0.025 M DEA

No.	Name of compounds	C18 column	Phenyl-Hexyl column	CN column	Polar C18 column
1	Desipramine	38,300	39,520	37,850	38,500
2	Chlordiazepoxide	44,480	50,510	13,440	69,260
3	Donepezil	18,750	26,520	21,900	28,190
4	Haloperidol	16,540	24,100	28,840	25,960
5	Carbamazepine	30,220	34,400	9,940	58,910
6	Quetiapine	42,640	39,350	22,060	42,280
7	Lamotrigine	20,660	24,080	18,830	37,840
8	Mirtazapine	28,540	33,290	21,890	23,330
9	Oxcarbazepine	21,320	30,050	6,290	48,840
10	Olanzapine	21,440	25,380	25,140	27,740
11	Opipramol	10,500	24,100	32,500	38,240
12	Perazine	21,140	34,190	35,920	34,080
13	Risperidone	22,470	33,620	25,570	20,210
14	Venlafaxine	9,360	10,300	12,570	7,300

Table V

A_S Values for the Psychotropic Drugs on Phenyl-Hexyl Column with Mobile Phases Containing 40% Mixtures of MeOH and MeCN, 20% Acetate Buffer at pH 3.5 and 0.025 M DEA

No.	Name of compounds	40% MeOH	30% MeOH 10% MeCN	20% MeOH 20% MeCN	10% MeOH 30% MeCN	40% MeCN
1	Desipramine	1.56	1.52	1.51	1.47	1.28
2	Chlordiazepoxide	1.00	0.99	0.98	1.01	1.01
3	Donepezil	1.11	1.15	1.22	1.22	1.14
4	Haloperidol	3.12	2.73	2.51	2.04	1.48
5	Carbamazepine	0.91	0.94	0.95	1.00	1.01
6	Quetiapine	1.52	1.40	1.31	1.23	1.15
7	Lamotrigine	0.60	0.9	0.99	0.93	0.88
8	Mirtazapine	1.35	1.33	1.26	1.23	1.02
9	Oxcarbazepine	0.88	0.92	0.96	1.03	0.98
10	Olanzapine	1.19	1.20	1.30	1.24	0.96
11	Opipramol	3.34	3.27	2.66	2.19	1.22
12	Perazine	2.24	2.18	2.24	2.08	1.40
13	Risperidone	1.62	1.49	1.44	1.33	1.02
14	Venlafaxine	2.34	2.69	2.08	1.49	0.87

Table VI

N/m Values for the Psychotropic Drugs on Phenyl-Hexyl Column with Mobile Phases Containing 40% Mixtures of MeOH and MeCN, 20% Acetate Buffer at pH 3.5 and 0.025 M DEA

No.	Name of compounds	40% MeOH	30% MeOH, 10% MeCN	20% MeOH, 20% MeCN	10% MeOH, 30% MeCN	40% MeCN
1	Desipramine	39,020	37,500	35,690	34,510	34,330
2	Chlordiazepoxide	49,440	49,090	45,590	41,330	36,720
3	Donepezil	16,430	17,230	19,140	22,450	26,120
4	Haloperidol	15,400	17,200	18,590	18,860	23,160
5	Carbamazepine	24,080	24,430	26,940	28,400	31,570
6	Quetiapine	44,230	43,160	30,180	39,340	35,650
7	Lamotrigine	9,530	12,270	17,040	19,710	19,690
8	Mirtazapine	28,250	28,527	29,760	28,960	22,040
9	Oxcarbazepine	15,800	17,510	21,470	24,160	28,610
10	Olanzapine	17,834	19,100	22,470	22,410	17,730
11	Opipramol	14,650	14,880	23,940	12,280	22,600
12	Perazine	20,130	27,640	32,270	26,450	30,290
13	Risperidone	24,270	23,300	25,040	26,980	23,450
14	Venlafaxine	6,970	7,920	9,560	11,150	7,170

of type of organic modifier proportion in mobile phase changes the selectivity of separation. The theoretical plate number increased with the increase of MeCN proportion of organic modifier and in system containing only MeCN for 11 compounds N/m > 20,000.

On the basis of the results of chromatographic systems' optimization, systems for the analysis of selected psychotropic drugs in human serum were selected. Quetiapine, risperidone and carbamazepine were quantified on the Phenyl-Hexyl column in eluent system containing 30% MeCN, acetate buffer at pH 3.5 and 0.025 M DEA; mirtazapine and olanzapine on the Polar C18 column with eluent containing 20% MeOH, 20% MeCN, acetate buffer at pH 3.5 and 0.025 M DEA. In Figure 4, chromatograms obtained for fortified human serum samples are presented. It is seen that a good separation of the drugs load in human serum was obtained.

Before HPLC analysis, fortified human serum samples were prepared by the procedure described in the "Experimental" section. The quantitative analysis was performed by a calibration curve method. Table VII presents parameters of the calibration curves for investigated drugs. The identities of analyte peaks in human plasma samples were confirmed by comparison of their UV spectra with the spectra of standards (Figure 5).

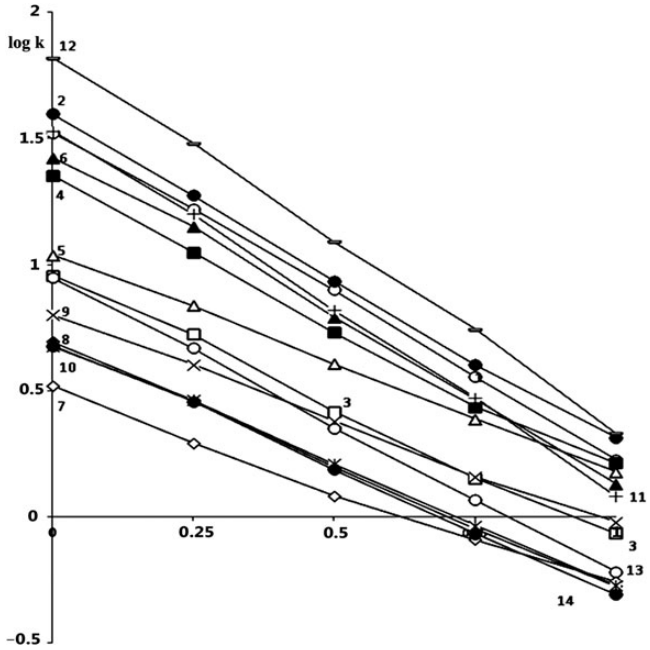

Figure 3. Dependence of psychotropic drugs log *k* values vs. fraction of MeCN in MeOH mixture as organic modifier. Mobile phase containing 40% organic modifier, 20% acetate buffer at pH 3.5 and 0.025 M DEA.

Discussion

Taking into account results of chromatographic experiments, it can be observed that the strongest retention obtained in both eluent systems (with MeOH or MeCN) on Polar RP stationary phase relays on strongest interactions between investigated drugs and surface ligands, which are an ether-linked phenyl base with polar endcapping.

The differences in retention of investigated drugs in systems with methanol and acetonitrile on stationary phases may be the result of the fact that acetonitrile impedes the selective π−π interactions between the analyte molecules and the π-ligand in the stationary phase. When a π-ligand columns are used, and an acetonitrile added to mobile phase, π−π interactions between

Table VII
Parameters of Calibration Curves for Quantitative Analysis of Selected Psychotropic Drugs: Calibration Curves' Equations, Concentration Range, Regression Coefficient (*r*), LOD and LOQ

Name of compounds	Concentrations range (μg/mL)	Equation of calibration curve	*r*	LOD	LOQ
Mirtazapine	0.5–10	$y = 73{,}573x - 6{,}983$	0.9998	0.29	0.89
Olanzapine	0.5–10	$y = 247{,}717x - 20{,}479$	0.9999	0.13	0.39
Quetiapine	0.5–10	$y = 214{,}831x - 7{,}140$	0.9997	0.34	1.04
Risperidone	1–10	$y = 94{,}948x - 2{,}452$	0.9996	0.37	1.12
Oxcarbazepine	1–10	$y = 151{,}559x - 29{,}245$	0.9995	0.43	1.30

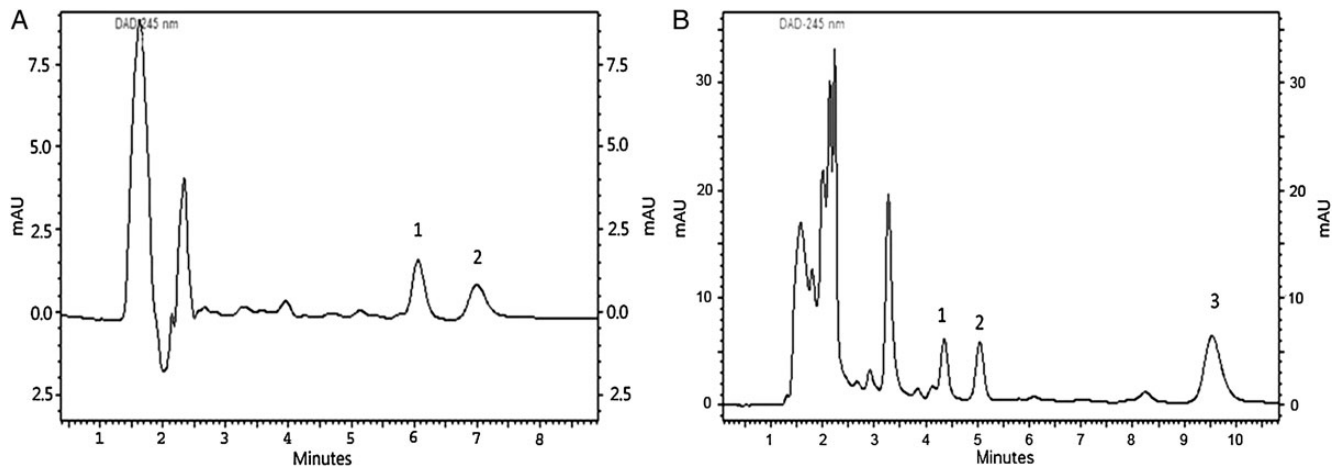

Figure 4. Chromatograms obtained for human serum samples fortified: (A) olanzapine—1 and mirtazapine—2 obtained on the Polar RP column in eluent system containing 20% MeOH, 20% MeCN, 20% acetate buffer at pH 3.5 and 0.025 M DEA. (B) Risperidone—1, oxcarbazepine—2 and quetiapine—3 obtained on the Phenyl-Hexyl column in eluent system containing 30% MeCN, 20% acetate buffer at pH 3.5 and 0.025 M DEA.

the compounds and the stationary phase ligands are weakened by the acetonitrile molecules, thus the retention order will be more significantly determined by the hydrophobic interactions. On different columns, especially when MeOH was added to eluent, great differences in investigated drugs separation selectivity were obtained, e.g. carbamazepine and chlordiazepoxide were practically not separated on the C18 column, but separated on the CN column and very well separated on the Phenyl-Hexyl and Polar RP columns, while carbamazepine and donepezil were poorly separated on the CN column, good separated on Polar RP stationary phase and very well separated on the C18 or Phenyl-Hexyl columns.

Mixtures being separated and determined in human serum are often used in multidrug therapy. The elaborated method gives the possibility for determination of psychotropic drugs with the following recoveries: 87.2% for olanzapine, 103.1% for mirtazapine, 105.2% for quetiapine, 91.7% for risperidone and 98.8% for oxcarbazepine.

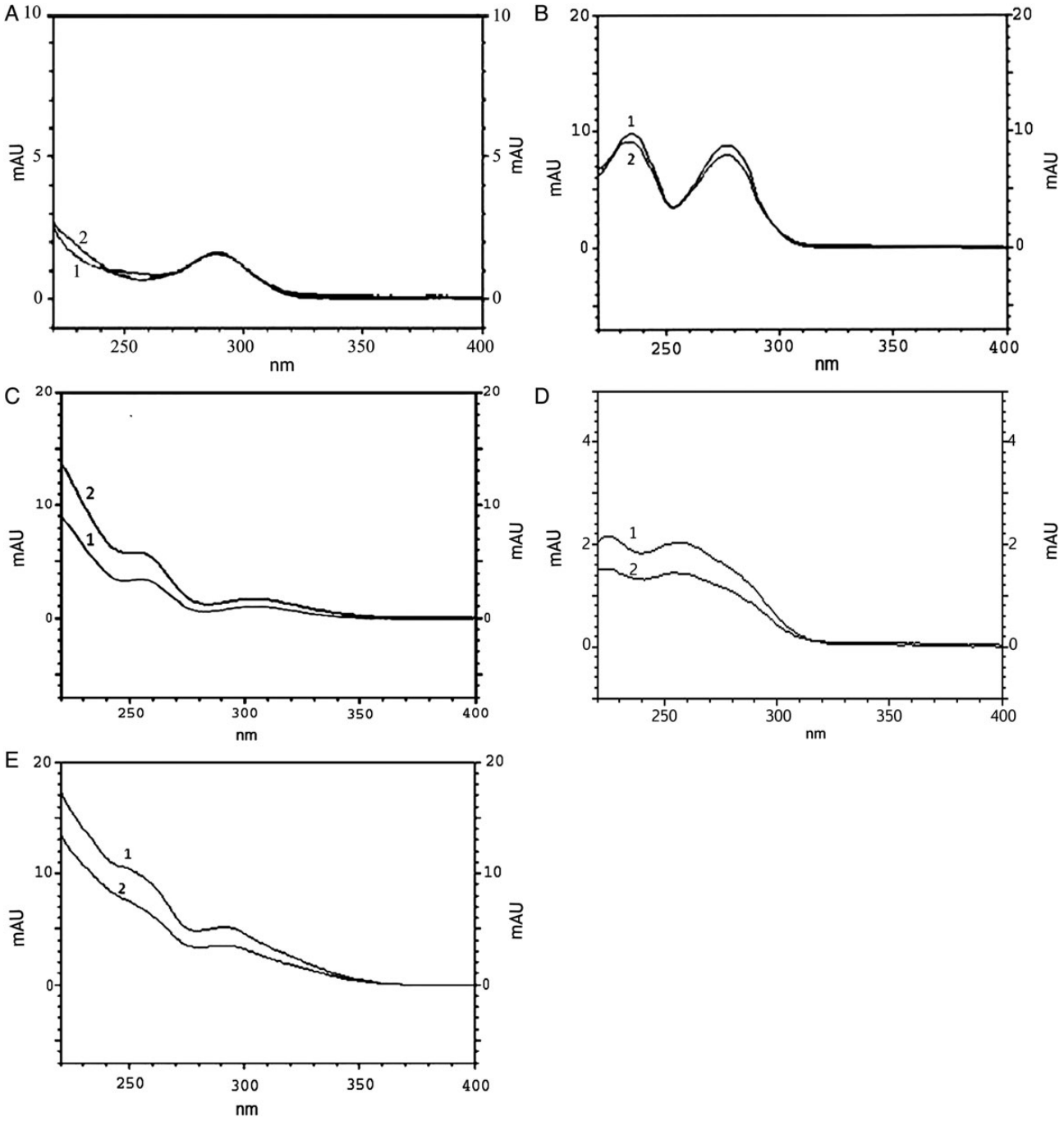

Figure 5. Spectra of psychotropic drug standards (1) and psychotropic drugs from human serum samples (2). (A) Mirtazapine, (B) risperidone, (C) oxcarbazepine, (D) quetiapine and (E) olanzapine. For chromatographic systems, see Figure 4.

Conclusion

Great differences in investigated psychotropic drugs retention, peak shapes and systems efficiency were obtained on chemically bonded stationary phases with different ligands, especially when MeOH was used as organic modifier in buffered mobile phases containing addition of DEA.

The most symmetrical peaks on the Phenyl-Hexyl column in eluent system containing MeOH as organic modifier were obtained, but efficiency was on the Polar RP column in mobile phase containing MeCN.

Good peaks' symmetry and simultaneously high systems efficiency for most investigated drugs were obtained in eluent systems containing a mixture of MeOH and MeCN, especially on the Phenyl-Hexyl and Polar RP columns.

Systematic optimization of retention behavior of the investigated drugs enables a choice the best chromatographic systems to their qualitative and quantitative analysis in human serum samples.

Application of the SPE method for sample preparation and optimal HPLC systems to analysis allowed us to obtain good recoveries in the range of 87.2–105.1% for the five selected psychotropic drugs quantified in human serum samples.

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