Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 1 pp. 39–47, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

EFFECT OF CHROMATOGRAPHIC CONDITIONS ON RETENTION BEHAVIOR AND SYSTEM EFFICIENCY FOR HPTLC OF SELECTED PSYCHOTROPIC DRUGS ON CHEMICALLY BONDED STATIONARY PHASES

ANNA PETRUCZYNIK, KAROL WRÓBLEWSKI and MONIKA WAKSMUNDZKA-HAJNOS*

Department of Inorganic Chemistry, Medical University of Lublin, Staszica 4a, 20-093 Lublin, Poland

Abstract: Selected psychotropic drug standards have been chromatographed on RP18, CN and diol layers with a variety of aqueous and nonaqueous mobile phases. The effect of buffers at acidic or basic pH, acetic acid, ammonia and diethylamine (DEA) in aqueous mobile phases on retention, efficiency and peak symmetry was examined. Improved peak symmetry and separation selectivity for investigated compounds were observed when ammonia or DEA were used as mobile phase additives. The effect of diethylamine concentration in aqueous eluents on retention, peak symmetry and theoretical plate number obtained on CN plates was also investigated. Because of the strong retention of these basic drugs on stationary phases bonded on silica matrix, nonaqueous eluents containing medium polar diluents, strongly polar modifiers and silanol blockers (ammonia or diethylamine) were applied. Aqueous and nonaqueous eluent systems with the best selectivity and efficiency were used for separate psychotropic drug standards' mixture on CN layer by 2D TLC.

Keywords: HPTLC, psychotropic drugs, system efficiency, 2D-TLC

Psychotropic drugs are prescribed to treat a variety of mental health problems when these problems cause significant impairment to healthy functioning. Psychotropic drugs typically act by changing the secretion of important chemicals in the brain called neurotransmitters. This group of drugs is one of the most toxic groups among different groups of substances used in medicine. In case of poisoning by them, there are serious health and life hazards. Diagnostics of poisoning by psychotropic drugs, determination of their content in pharmaceutical preparations and monitoring of their concentration in biological fluids have special significance.

Different chromatographic techniques play an important role in the analysis of these drugs e.g., gas chromatography (GC) (1, 2), high performance liquid chromatography (HPLC) (3–5), thin layer chromatography (TLC) (6, 7). TLC is particularly useful for rapid analysis of large number of samples.

Most often, psychotropic drugs have been analyzed by TLC on silica gel with mobile phase of high eluent strength containing e.g., mixture of toluene and methanol (6, 8), butanol, acetic acid and water (9), methanol, toluene and acetic acid (10), methanol and chloroform (7), acetone, methanol and triethylamine (11), hexane, dioxane and propylamine (12), ethyl acetate, methanol and ammonia (13), methanol, toluene, ammonia (14), ethanol, hexane, ammonia (15).

Alkyl bonded plates were also used for analysis of these drugs e.g., on C18 layers with eluent system containing tetrahydrofuran and phosphate buffer at pH 9.0 (12), on C8 layers eluted with tetrahydrofuran and phosphate buffer at pH 3.5 (16).

Rarely, these compounds were chromatographed on other chemically bonded stationary phases e.g., on aminopropyl (NH_2) and cyanopropyl (CN) layers with eluents containing acetone, diethylamine, dioxane, ethanol, isopropanol, or tetrahydrofuran in *n*-hexane (15). Some antidepressants were analyzed on CN plates in systems containing as mobile phases mixture of acetonitrile, ether, hexane or petroleum ether; diethyl ether, acetonitrile and ethyl methyl ketone (17).

Psychotropic drugs, which are basic compounds, caused a number of analytical problems – the peaks on chromatograms are asymmetric, the efficiency of systems is low, the reproducibility and

* Corresponding author: e-mail: monika.hajnos@umlub.pl; phone +48 81535 7378

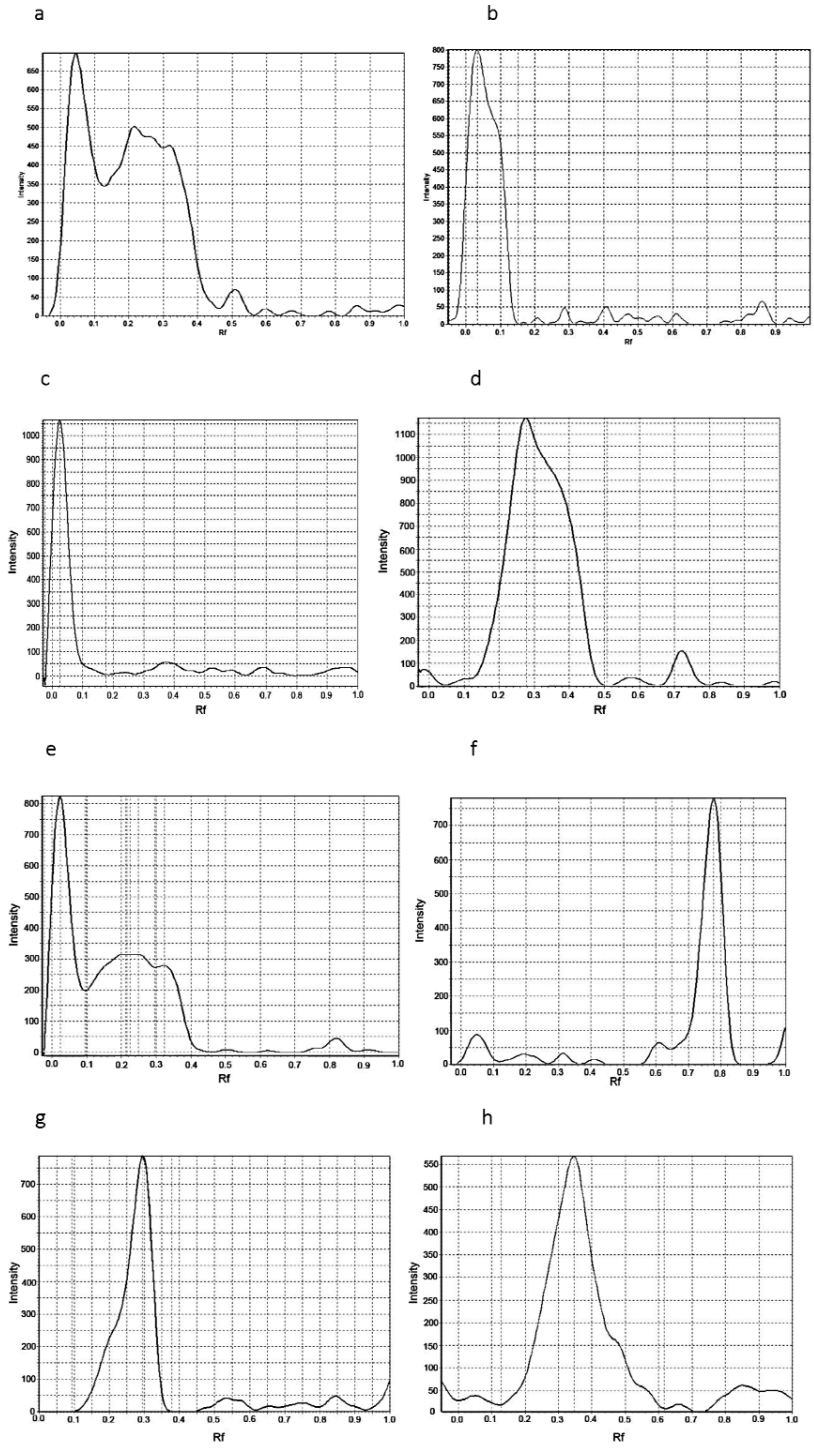


Figure 1. Densitograms of quetiapine chromatographed on HPTLC plates in different systems: a. C18/MeOH–H_2O (60 : 40, v/v); b. CN/MeOH–H_2O (60 : 40, v/v); c. C18/MeOH–H_2O (70 : 30, v/v) buffered with acetate buffer at pH 3.5; d. CN/MeOH–H_2O (70 : 30, v/v) buffered with acetate buffer at pH 3.5; e. C18/MeOH–H_2O (70 : 30, v/v) buffered with ammonium buffer at pH 8.3; f. CN/MeOH–H_2O (70 : 30, v/v) buffered with ammonium buffer at pH 8.3; g. C18/MeOH–H_2O (70 : 30v/v) containing 1% acetic acid; h. CN/MeOH–H_2O (70 : 30, v/v) containing 1% acetic acid.

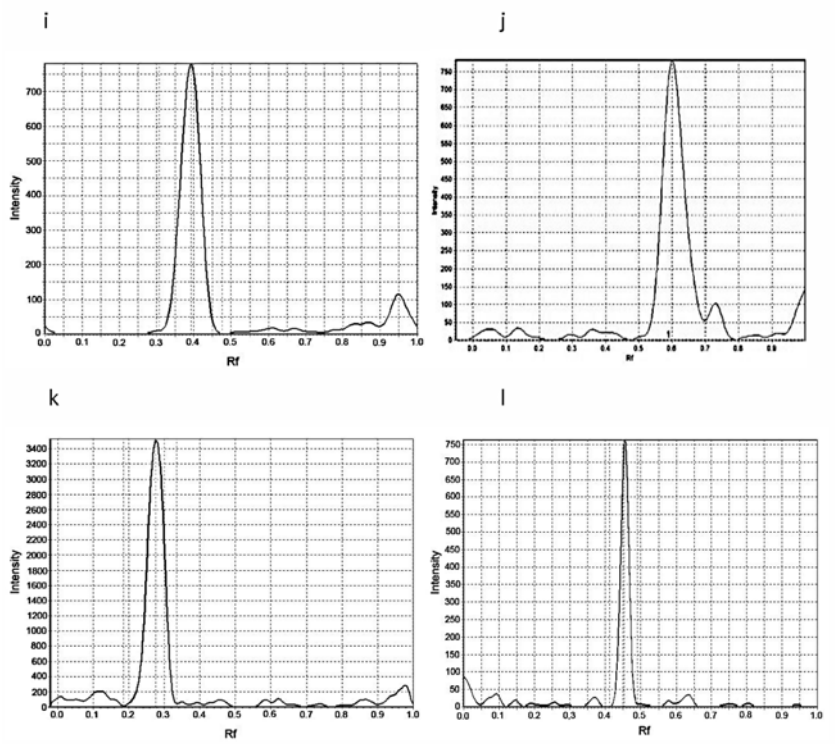


Figure 1. cont.: i. C18/MeOH–H_2O (80 : 20, v/v) containing 1% ammonia; j. CN/MeOH–H_2O (80 : 20, v/v) containing 1% ammonia; k. C18/MeOH–H_2O (80 : 20, v/v) buffered with acetate buffer at pH 3.5 containing 0.05M DEA; l. CN/MeOH–H_2O (80 : 20, v/v) buffered

selectivity of the separation can often be unsatisfactory. This is caused by the partial dissociation of these compounds in aqueous solutions and the different interactions of neutral and ionic form with the stationary phase residual silanols. For this reason, optimization of the chromatographic system for obtaining correct results is necessary.

The aim of this paper was an investigation of retention behavior of selected psychotropic drugs in normal-phase and reversed-phase systems on different chemically bonded stationary phases with various eluents. Influence of various composition of mobile phases on retention, peak shape, efficiency and separation selectivity of these drugs on C18, CN and diol stationary phases was investigated. Systems with the best parameters and highest selectivity were used for the analysis of psychotropic drug standards' mixtures. The application of the most selective systems in 2D-TLC separations of standards' mixture of investigated drugs has been also presented.

EXPERIMENTAL

HPTLC was performed on 10 × 10 cm glass plates precoated with 0.2 mm layer of RP-18F_{254}s, CN F_{254}s, and diol F_{254} produced by E. Merck (Darmstadt, Germany). Plates were developed face down to a distance of 9 cm from the origin at 20 ± 1°C in horizontal DS-chambers (Chromdes, Lublin, Poland).

Solvents: methanol (MeOH), acetonitrile (MeCN), tetrahydrofuran (THF), methyl ethyl ketone (MEK), diisopropyl ether (iPr_2O), diethylamine (DEA), and aqueous ammonia 25% were of HPLC grade produced by E. Merck. Acetic acid was of analytical grade produced by Polish Reagents (Gliwice, Poland). Bidistilled water was used as a component of aqueous solutions.

Photos of the plate were taken by the digital camera Kodak EasyShare C913. The treatment of the photographs was performed using the computer programme Sorbfil TLC.

Asymmetry factor (A_S) was calculated by the computer programme at 10% of peak hight, theoretical plate number (N) was calculated from chromatograms using equation:

$$N = 16(\frac{z}{w})^2$$

where: z – substance migration distance; w – peak width at base.

RESULTS AND DISCUSSION

The retention of 10 psychotropic drugs (Table 1) was investigated on RP18 plates with aqueous eluents, CN and diol plates by the use of aqueous and nonaqueous eluents.

The first experiments were performed on RP18 and CN plates with a mobile phase containing acetonitrile or methanol and water only. In mobile phases containing organic modifier and water, investigated psychotropic drugs – weak organic bases – are present in the ionized and neutral forms, which interact differently with active sites of the stationary phases. The ionized form interacts strongly with ionized free silanols, which causes tailing, and low efficiency of the chromatographic system. For this reason, poor spots shape, low system efficiency and separation selectivity were obtained. Figure 1a shows peaks obtained for quetiapine on RP18 and CN layers in eluent system containing mixture of methanol and water with a very bad shape.

In order to obtain better spot shapes, higher efficiency and improved separation selectivity, mobile phases containing different additives (such as: acetic acid, ammonia, buffers at different pH or diethylamine) were applied. The application of mobile phases containing organic modifier and acetate buffer at pH 3.5 on RP18 and CN adsorbents, where the silanol ionization was partially suppressed, caused an improvement of spot shapes, but they were still asymmetric for most of investigated compounds and some compounds were still strongly retained on RP18 plates e.g., quetiapine (Fig. 1b). The use of a mobile phase containing a buffer at pH 8.3 (Fig. 1c), when the dissociation of the analytes is partially suppressed, does not cause significant improvement of peak shapes and increase the system efficiency for most investigated drugs in comparison to a system containing the acidic buffer.

In the next series of experiments, the retention, peak symmetry, theoretical plate number on both tested adsorbents in systems containing aqueous mixtures of methanol or acetonitrile with addition of 1% acetic acid as mobile phase were examined (Fig. 1d). In this systems, a slightly better peak shapes and an increase of theoretical plate number, compared with a system with acidic buffer can be observed, however, the asymmetry factor for all investigated drugs was not in the optimal range of A_S values ($0.8 < A_S < 1.5$).

A significant improvement of the spot shapes and an increase of theoretical plate number was obtained on C18 and CN adsorbents in the eluent system with addition of 1% ammonia. The addition of ammonia caused suppression of basic analytes' dissociation and blocking of free silanols. Good results were obtained by use both stationary phases and MeOH or MeCN as organic modifiers, but the most symmetric spots and highest system efficiency were obtained for mobile phases with ammonia on RP18 layers, when MeOH was used as organic modifier. Figure 1e depicts the peaks obtained for quetiapine on both adsorbents in this eluent system. Most symmetric spots were also obtained for olanzapine and lamotrigine.

Further improvement of efficiency, peak symmetry, and separation selectivity for psychotropic drugs on both adsorbents was obtained when DEA was added to the mobile phase. In Figure 1f, densitogram obtained for quetiapine on RP18 plate in eluent containing DEA additive is presented. DEA, as strong base, interacts with ionized silanols, blocking the interactions of these groups with the compounds analyzed. This explains why considerable improvement in peak symmetry, system efficiency, and often separation selectivity was observed in chromatographic systems containing amines.

The retention of psychotropic drugs on CN stationary phase as a function of DEA concentration (0.005–0.05 M) is presented in Figure 2. The increase of DEA concentration initially causes an increase of retention of most investigated drugs and then retention decreases, as an effect of silanols' blocking. Significant differences in retention for most investigated compounds were observed in range 0.005 – 0.02 M of DEA concentration. Further increasing of DEA concentration from 0.02 – 0.05 M does not cause distinct differences in retention. With the change of DEA concentration, the changes in separation selectivity can be also observed.

The increase of DEA concentration causes also the significant improvement of system efficiency and spot symmetry. In the system with 0.005 M of DEA $N/m > 5000$ were obtained only for venlafaxine and lamotrigine, while in the system with 0.04 or 0.05 M DEA, 6 or 7 drugs have $N/m > 5000$ and for other investigated compounds $N/m > 1000$. In most cases, increasing the concentration of diethylamine results in improvment of peak symmetry. In the concentration of 0.005 M DEA, only six investigated drugs have the acceptable asymmetry factors. In chromatographic system with mobile phases containing 0.03–0.05 M DEA, symmetry of spots was acceptable for all investigated compounds and for nine of compounds it was excellent ($0.9 > A_S > 1.2$). In the next series of experiments, the effects of composition of nonaqueous mobile phases on retention, spot shape and system efficiency on CN and diol

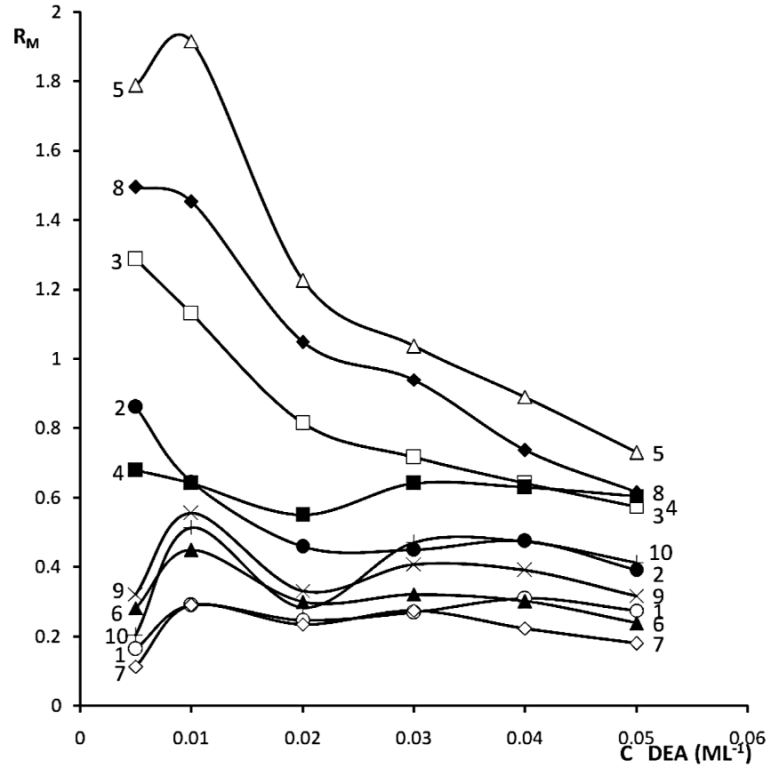


Figure 2. Relationships between R_M and DEA concentration in mobile phase for psychotropic drugs. System: CN, MeOH–H_2O (80 : 20, v/v) buffered with acetate buffer at pH 3.5 containing 0.005 – 0.05 M DEA

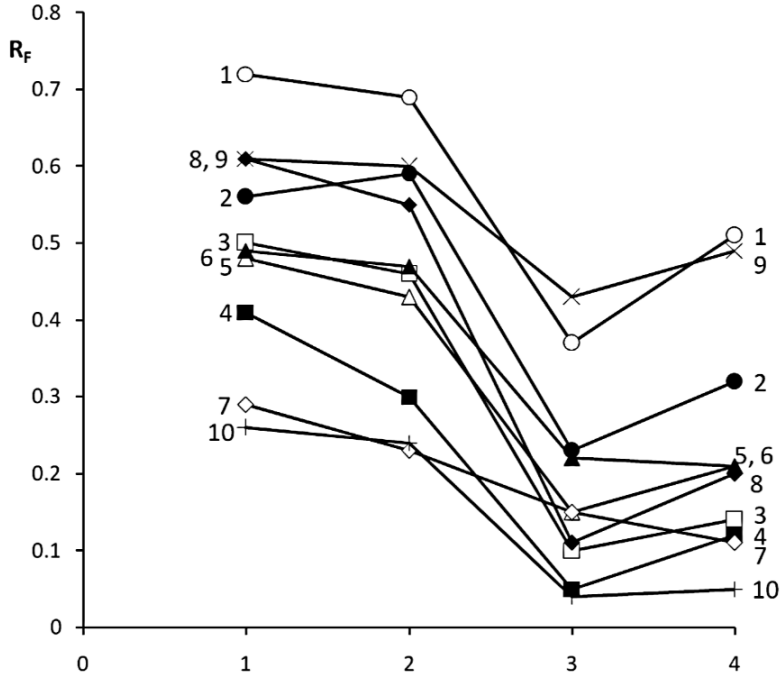


Figure 3. Graphical comparison of R_F values obtained for psychotropic drugs in chromatographic systems: 1. CN; 10% MeOH + 5% MEK + iPr_2O +1% ammonia; 2. CN; 15% MeOH + iPr_2O +1% ammonia; 3. Diol; 10% MeOH + 5% MEK + iPr_2O +1% ammonia; 4. CN; 15% MeOH + iPr_2O +1% ammonia

Table 1. Structure of the investigated compounds.

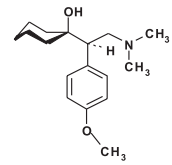
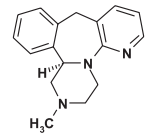
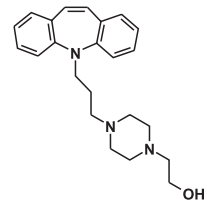
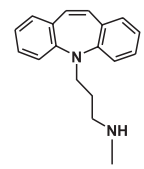
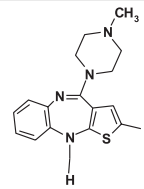
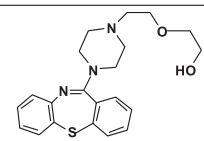
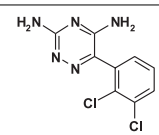
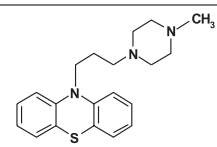
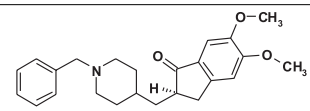
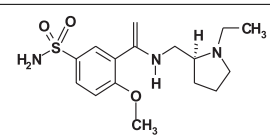
	Name of compound	Structure
1	Venlafaxine	
2	Mitrazapine	
3	Opipramole	
4	Desipramine	
5	Olanzapine	
6	Quetiapine	
7	Lamotrigine	
8	Perazine	
9	Donazepil	
10	Sulpiride	

Table 2. Asymmetry factor (A_S) and theoretical plate number (N/m) values for investigated psychotropic drugs obtained on CN or diol plates in different nonaqueous eluent systems.

No.	CN 10% MOH + 5% MEK + iPr_2O + 1% ammonia		CN 15% MOH + iPr_2O + 1% ammonia		Diol 10% MOH + 5% MEK + iPr_2O + 1% ammonia		Diol 15% MOH + iPr_2O + 1% ammonia	
	As	N/m	As	N/m	As	N/m	As	N/m
1	0.94	20530	1	52900	0.58	9360	0.55	10940
2	1.07	34840	1.08	50520	1.1	16330	0.71	15750
3	0.91	21950	0.92	30710	0.91	3670	0.83	3870
4	0.93	18680	0.85	10000	0.89	920	0.91	2840
5	0.86	25600	0.92	16980	0.9	9070	0.91	8710
6	0.92	41680	1	38350	0.91	13770	0.91	8160
7	0.92	13730	0.83	10450	0.91	5920	1	3440
8	0.92	26460	0.86	27780	0.82	3740	0.83	6940
9	1	54000	1.08	40000	0.92	36520	1	32940
10	1	7510	1	9400	0.71	1260	1	1110

stationary phases were examined. The investigated psychotropic drugs were strongly retained on cyanopropyl and diol phases, when nonaqueous eluents were used, and the use of strongly polar modifiers and diluents of medium polarity was necessary. With most binary solvent combinations such as MeOH, ethyl acetate (AcOEt), ethyl methyl ketone (EMK) as modifiers and dichloromethane or diisopropyl ether (iPr_2O) as diluents, the spots were wide and asymmetric.

For this reason, the use of DEA or ammonia, to reduce ion-exchange processes, was necessary. In the systems with addition of DEA to mobile phase containing mixture of MeOH and dichloromethane or iPr_2O, a slight improvement of peak shapes was observed, but they were still asymmetric. Similar results in chromatographic systems with DEA was obtained on diol layers. The addition of ammonia to mobile phase containing MeOH and dichloromethane on both stationary phases also does not improve symmetry of spots and system efficiency.

A significant improvement of peak symmetry, efficiency and separation selectivity for the compounds was achieved when ammonia was added to mobile phase containing MeOH or its mixture with MEK and iPr_2O on both CN and diol plates. The use of mixture of MeOH and MEK resulted in different separation selectivity compared to system containing only MeOH as a modifier. Large differences in separation selectivity of investigated drugs were obtained on both layers, e.g., in eluent containing MeOH, MEK and ammonia. Venlaflaxine and sulpiride or olanzapine and lamotrigine are eluted together on diol but are well separated on CN, while opipramol, olanzapine and quetiapine or perazine and donazepil are eluted together on CN but separated on diol stationary phase (Fig. 3).

A_S and N/m values for the psychotropic drugs chromatographed on CN and diol plates with nonaqueous mobile phases containing ammonia are presented in Table 2. The better spot shapes and especially greater system efficiency for most investigated compounds were obtained on CN plates. On CN stationary phase spots symmetry was acceptable for all investigated drugs, whereas when diol plates were used for eight drugs in two tested nonaqueous eluent systems with addition of ammonia the results are acceptable. On CN plate with eluent conatining MEK for nine psychotropic drugs excellent A_S values were obtained. On CN plates in both eluents N/m > 10 000 were for nine, on diol plates only for three investigated compounds.

Good spot shape and system efficiency for investigated drugs were obtained in several chromatographic systems such as: C18 stationary phase with eluents containing mixture of MeOH, water and ammonia or MeOH, buffer pH at 3.5 and DEA; CN stationary phase with aqueous eluent containing MeOH, buffer pH at 3.5 and DEA or with nonaqueous eluents containing mixture of MeOH, iPr_2O and ammonia or MeOH, MEK, iPr_2O and ammonia; diol stationary phase with nonaqueous eluents contain-

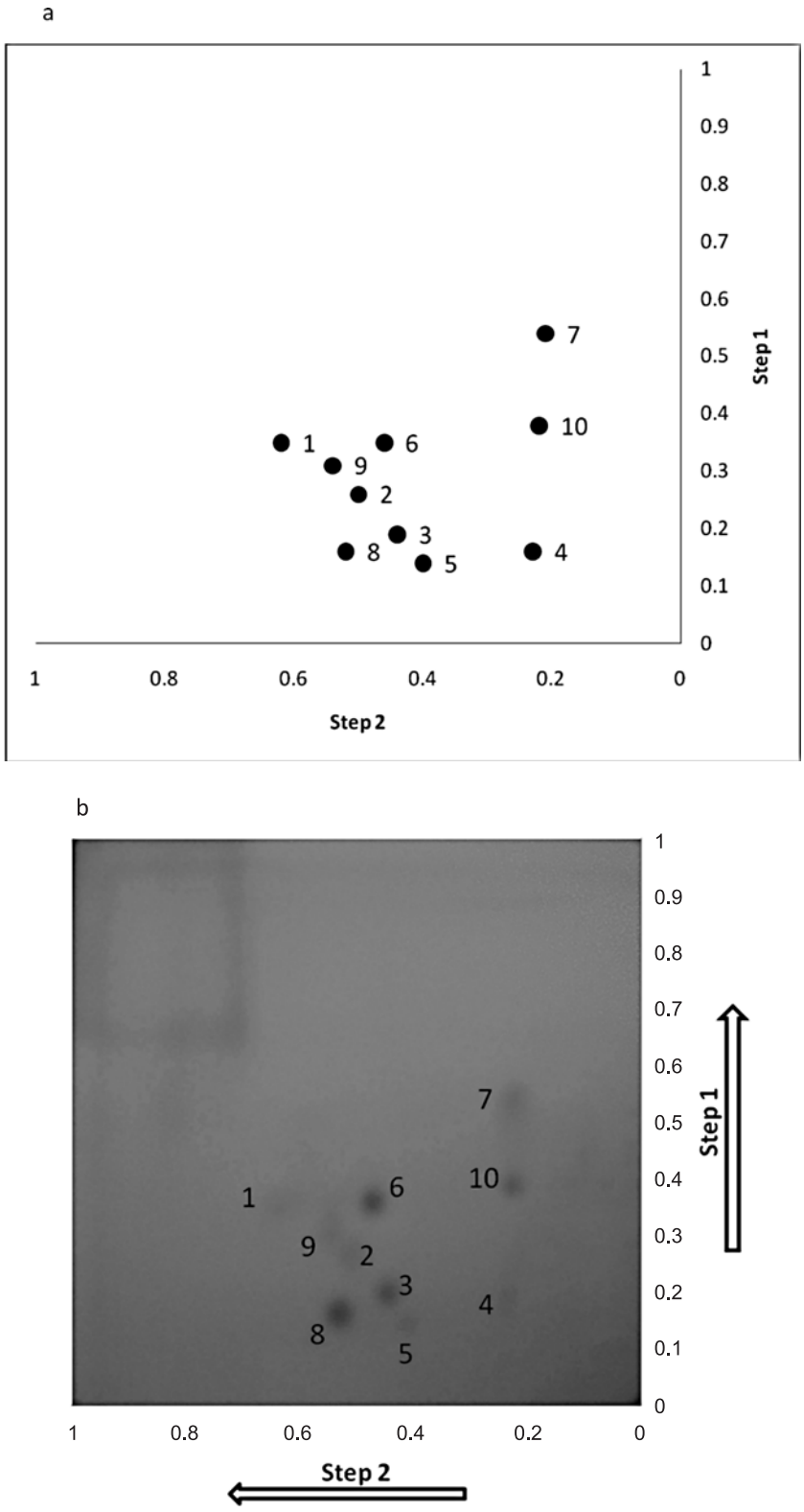


Figure 4. a. Correlation diagram of R_F values obtained in systems: I direction: CN; 80% methanol + acetate buffer at pH 3.5 + 0.05 M DEA; II direction: CN; 10% MeOH + 5% MEK + iPr_2O +1% ammonia

b. Photo of the 2D-TLC chromatogram of psychotropic drug standards at λ = 254 nm obtained in systems: I direction: CN; 80% methanol + acetate buffer at pH 3.5 + 0.05 M DEA; II direction: CN; 10% MeOH + 5% MEK + iPr_2O +1% ammonia

ing mixture of MeOH, iPr_2O and ammonia or MeOH, MEK, iPr_2O and ammonia, but in all systems the mixture of the investigated drugs was not completely separated. It means, that the identification of all investigated drugs in one run by 1D-HPTLC in examined systems is impossible. On the basis of the results, optimal eluent systems for the analysis of drugs on CN-layer by two-dimensional TLC (2D-TLC) were chosen.

The correlation of R_F values obtained on CN layer in aqueous mobile phase containing 80% MeOH + 20% acetic buffer pH 3.5 + 0.05 M DEA and nonaqueous mobile phase containing 10% MeOH + 5% MEK + iPr_2O + 1% ammonia is presented in Figure 4a. The dispersion of points indicates the differences of the retention parameters obtained in both eluent systems. These correlations are very useful for planning the two-dimensional separation of complex mixtures, and the selectivity differences can be employed in practical applications. The data obtained from the correlation were put into practice for the separation of a mixture containing all investigated psychotropic drugs by the 2D-TLC method. Figure 4b presents the chromatogram of psychotropic drugs separated on CN plate in the same eluent systems. By the use of these selected mobile phase systems with their differing selectivities, the identification of the psychotropic drug is possible by the R_F values obtained for each drug in two eluent systems.

CONCLUSIONS

On RP18 and CN stationary phases, for mobile phases containing organic modifier and water, the investigated psychotropic drugs, which occur as neutral and ionic forms, give highly asymmetric spots, and system efficiency is poor. An addition of buffer to the mobile phase, at acidic or basic pH, leads to slight improvement of spot shape and system efficiency. An addition of acetic acid to the aqueous mobile phases results in further improvement in spot shape, but they are still asymmetric. The best efficiency and most symmetrical spots were obtained for aqueous mobile phase systems by use of mobile phases with addition of DEA.

On CN and diol plates in NP systems, the best results for the separation of psychotropic drugs were obtained with the mobile phase composed of MeOH, MEK, iPr_2O and aqueous ammonia.

On the basis of the retention data of the investigated drugs, obtained on CN stationary phase, the systems of orthogonal selectivity were chosen for two-dimensional thin-layer chromatography of the psychotropic drugs mixture.

REFERENCES

1. Sasaki C., Shinozuka T., Murakami C., Irie W., Maeda K., Watanabe T., Nakamaru N. et al.: Forensic Sci. Int. 227, 90 (2013).
2. de la Torre C.S., Martinez M.A., Almarza E.: Forensic Sci. Int. 155, 193 (2005).
3. Choong E., Rudaz S., Kottelat A., Guillarme D., Veuthey J.-L., Eap C.B.: J. Pharm. Biomed. Anal. 50, 1000 (2009).
4. Xiong C., Ruan J., Cai Y., Tang Y.: J. Pharm. Biomed. Anal. 49, 572 (2009).
5. Sabbioni C., Bugamelli F., Varani G., Mercolini L., Musenga A., Saracino M.A., Fanali S., Raggi M.A.: J. Pharm. Biomed. Anal. 36, 351 (2004).
6. Shirvi V.D., Channabasavaraj K.P., Kumar G.V., Mani T.T.: J. Planar Chromatogr. 23, 369 (2010).
7. Potale L.V., Khodke A.S., Patole, M.C. Damle S.M.: J. Planar Chromatogr. 25, 72 (2012).
8. Dhaneshwar S.R., Patre N.G., Mahadik M.V.: Acta Chromatogr. 21, 83 (2009).
9. Ramesh B., Narayana P.S., Reddy A.S., Devi P.S.: J. Planar Chromatogr. 24, 160 (2011).
10. Shah D.A., Nakrani R.S., Baldania S.L., Chhalotiya U.K., Bhatt K.K.: J. Planar Chromatogr. 25, 174 (2012).
11. Patel S.K., Patel N.J., Patel P.U., Patel D.B., Prajapati A.M., Patel S.A.: J. Planar Chromatogr. 22, 121 (2009).
12. Skibiński R., Komsta Ł., Kosztyła I.: J. Planar Chromatogr. 21, 289 (2008).
13. Ahrens B., Blankenhorn D., Spangenberg B.: J. Chromatogr. B 772, 11 (2002).
14. Tantawy M.A., Hassan N.Y., Elragehy N.A., Abdelkawy M.: J. Adv. Res. 4, 173 (2013).
15. Skibiński R., Misztal G., Komsta Ł., Korólczyk A.: J. Planar Chromatogr. 19, 73 (2006).
16. Skibiński R., Komsta Ł., Hopkała H., Suchodolska I.: Anal. Chim. Acta 590, 195 (2007).
17. Oztunc A., Onal A., Erturk S.: J. Chromatogr. B 774, 149 (2002).

Received: 18. 01. 2014