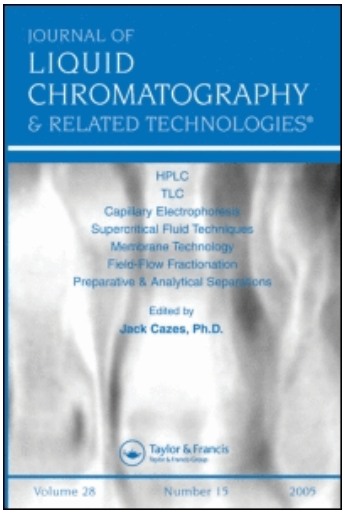


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analysis of Selected Anti-Depressive Drugs by High Performance Thin-Layer Chromatography

Anna Petruczynik[a]; Mariusz Brończyk[a]; Tomasz Tuzimski[b]; Monika Waksmundzka-Hajnos[a]
[a] Department of Inorganic Chemistry, Medical University of Lublin, Lublin, Poland [b] Department of Physical Chemistry, Medical University of Lublin, Lublin, Poland

To cite this Article Petruczynik, Anna , Brończyk, Mariusz , Tuzimski, Tomasz and Waksmundzka-Hajnos, Monika(2008) 'Analysis of Selected Anti-Depressive Drugs by High Performance Thin-Layer Chromatography', Journal of Liquid Chromatography & Related Technologies, 31: 13, 1913 — 1924
To link to this Article: DOI: 10.1080/10826070802194831
URL: http://dx.doi.org/10.1080/10826070802194831

Journal of Liquid Chromatography & Related Technologies®, 31: 1913–1924, 2008

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802194831

Analysis of Selected Anti-Depressive Drugs by High Performance Thin-Layer Chromatography

Anna Petruczynik,[1] Mariusz Brończyk,[1] Tomasz Tuzimski,[2] and Monika Waksmundzka-Hajnos[1]

[1]Department of Inorganic Chemistry, Medical University of Lublin, Lublin, Poland
[2]Department of Physical Chemistry, Medical University of Lublin, Lublin, Poland

Abstract: The retention behavior of 11 anti-depressive basic drugs was investigated on silica layers with non-aqueous mobile phases containing polar modifier – methanol, medium polar diluent – diisopropyl ether and aqueous ammonia or diethyl amine. The effect of ammonia or amine concentration and modifier concentration on retention, separation selectivity, and efficiency was examined. Investigated compounds were also chromatographed in RP systems on RP18 and CN-silica layers by use of aqueous eluents with various additives. The best results were obtained with addition of ammonia, playing the role of silanol blocker, which causes elimination of ion-exchange interaction of basic analytes with surface residual silanols.

On the basis of these investigations, systems for extraction from human serum and quantitative determination of amitriptiline and doxepin were selected. Cyano SPE columns conditioned and pre-eluted with methanol-water (1:1) and eluted with methanol-water (8:2) containing 5% of formic acid were used for sample preparation with high recoveries of both drugs. RP-18 plates eluted with 70% methanol in water + 1% ammonia were used for quantitative analysis by a calibration curve method.

Keywords: Amitriptyline, Doxepin, NP, Quantitative analysis, Retention behavior, RP systems, TCA, Tricyclic antidepressants

Correspondence: Monika Waksmundzka-Hajnos, Department of Inorganic Chemistry, Medical University of Lublin, 20-081, Lublin, Poland. E-mail: monika.hajnos@am.lublin.pl

INTRODUCTION

Tricyclic antidepressants (TCA) are most often applied drugs in therapy of mental illness of depressive character. Derivatives of dibenzodiazepine, dibenzcycloheptadiene and dibenzoxepine are widely known. The above mentioned drugs used by a healthy person causes psychic hypofunction and a slight sedative effect. However, for the mentally ill, these drugs cancel depressive symptoms, act as psychomotor stimulants, and cancel feelings of anxiety and mental stress.[1,2]

Tricyclic antidepressants are one of the most toxic groups among antidepressive drugs and, in case of poisoning, are serious health and life hazards.[2,3] The sickness dose is often the lethal dose and is only 3–4 times higher than maximum therapeutic dose. It is 500–4,000 mg of amitriptyline for adults and 375–500 mg for children.[2] The typical poisoning picture develops 2–6 h after medicine reception because these drugs are easy digestible from the alimentary tract.

Diagnostics of poisoning by tricyclic antidepressants has special significance and chromatographic methods play, in this field, a primary role.[2,4–9] Because these compounds posess nitrogen atom in heterocyclic ring and/or in amine group they are difficult object of analysis. It is caused by the appearance of drug molecules in solution as ionized and unionized forms which can interact with silanol groups of the silica matrix by ion-exchange processes and by hydrogen bond interactions. Silica is the most widely used material in chromatography. Silica supports are still superior to other supports in terms of efficiency, rigidity, and performance. Also, in the case of bonded stationary phases, nonpolar or polar ligands are bonded to the silica matrix. However, there are several problems with silica-based materials: severe peak tailing in the chromatography of basic compounds, irreproducibility for the same chemistry layers, and limited pH stability. Besides the reversed phase retention mechanism, an ion-exchange retention mechanism also occurs which often results in poor symmetry of peaks, irreproducible retention and worse separation.[10] Asymmetric peaks can be explained in terms of kinetic phenomena, i.e., when the kinetics of mass transfer of one type of column site is slower than the other.[11] For basic solutes, the kinetics of the ion-exchange interaction with silanol groups may be slower than those with the alkyl ligands, giving rise to peak tailing.[12]

Silanol interaction can be reduced by several methods. In RP systems, buffered aqueous phases at low pH (2.0–3.5)[13] or at high pH ($>$7.0 when ionisation of some bases is suppressed)[14] can be applied. Usually, addition of ion-pairing reagents to the mobile phase[10,15,16] or silanol blockers (ammonia or short chain amines), which cause increase of the system efficiency and improvement of peak symmetry, is

necessary. In normal phase systems, strongly polar modifiers, medium polar diluents, as well as basic additives are often applied.

The aim of this paper was investigation of retention behavior of selected tricyclic antidepressants in various chromatographic systems, i.e., in normal-phase and reversed-phase systems, and search of the effect of kind and concentration of modifier and kind and concentration of silanol blockers. The aim of the paper was also choice of conditions for extraction of amitriptyline and doxepin from serum and their quantitative and qualitative determination by HPTLC + densitometry.

EXPERIMENTAL

HPTLC was performed on 10 × 10 cm glass plates precoated with 0.2 mm layer of RP-18WF_{254}s, CN F_{254}s, and SiO_2 60 F_{254} produced by E. Merck (Darmstadt, Germany). Plates were conditioned in eluent vapours about 10 min and developed face down in horizontal DS-chambers (Chromdes, Lublin, Poland). Solvents: methanol (MeOH), diisopropyl ether (iPr_2O), diethylamine (DEA), and aqueous ammonia 25% were of analytical grade produced by Polish Reagents (Gliwice, Poland). Bidistilled water was used as a component of aqueous solutions.

Investigated drugs and their producers are listed in Table 1. Drug standards were dissolved in 80% MeOH in water + 5% HCOOH. Solutes for chromatographic experiments had a concentration of 0.1%. Solutes were applied to the layer by use of a Desaga Sarsted-Gruppe AS-30 applicator (Desaga, Heidelberg, Germany).

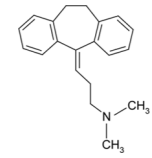
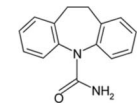
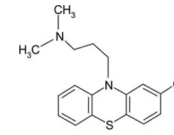
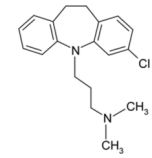
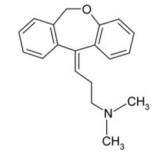
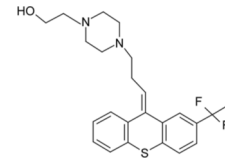
Spots of investigated compounds were localized in UV light by use of a UV lamp (Cabinet, Camag, Muttenz, Switzerland) and scanned by Videoscanner TLC Camag Reprostar 3 with Videostore computer program (Camag, Muttenz, Switzerland).

Determination of Amitriptyline and Doxepin in Human Serum

Human serum FFP of group A was defrozen and filtered by use of a Filter Disc (Quant) grade 390 (J. T. Baker, Philipsburg, USA). For fortification of serum samples, solutes of drugs of 0.05% concentration in water were prepared by dissolution of dose of tablets and filtration by Filter Disc (Quant) grade 390 produced by (J. T. Baker, Philipsburg, USA). Forty mL of serum was mixed with 1.6 mL of aqueous solution of each drug separately.

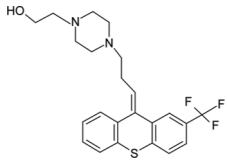
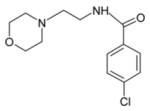
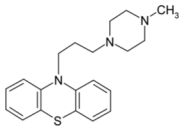
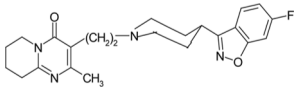
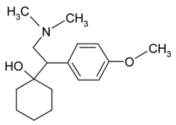
Bakerbond SPE Cyano columns and SPE chamber – Baker SPE-12G (J. T. Baker, Philipsburg, USA) were used for solid-phase extraction. SPE columns were conditioned by elution of 5 mL of

Table 1. Chemical structures of investigated compounds

Name of compounds	Symbol	Producer	Chemical formula
Amitriptyline	A	Polfa, Poland	
Amizepin	Am	Polpharma, Poland	
Chloropromazine	Ch	Polfa, Poland	
Clomipramine	C	A.W.D., Germany	
Doxepin	D	Pliva, Poland	
Flupentixole	F	Wyeth-Ledeler Pharma, Austria	

(continued)

Table 1. Continued

Name of compounds	Symbol	Producer	Chemical formula
Haloperidol	H	Polfa, Poland	
Moclobenid	M	Roche, Switzerland	
Perazine	P	Lobor, Poland	
Risperidone	R	Jansen Pharm., Belgium	
Wenlafaxine	W	H. Lundech, Denmark	

methanol-water solution (1:1). The whole portion of serum, fortified with the investigated drug, was introduced to the column. The column was pre-washed with 5 mL of methanol-water solution (1:1). The extracted substance was eluted with 5 mL of mobile phase: 80% methanol in water +5% formic acid. The mobile phase velocity was 1 mL·min^{-1}. Ten μL of eluate was introduced to the RP-18 layer and chromatographed by use of 70% aqueous methanol containing 1% ammonia. Chromatograms were scanned and peak surface areas of the extracted substances and standards were determined by DAD scanner (J&M, Aalen, Germany). Quantification was performed by a calibration curve method. UV spectra of amitriptyline and doxepin were taken directly from the layer by the same scanner.

RESULTS AND DISCUSSION

The first part of our work was a search of retention behavior of investigated drugs (see Table 1) in various chromatographic systems. Investigated substances were strongly retained on a silica layer and the use of methanol-medium polar diluent (dichloromethane or diisopropyl ether) mixtures of high eluent strength was necessary, but even a 70% solution of methanol in diisopropyl ether did not give satisfactory results. The obtained spots were wide and asymmetric. Because of this, the use of aqueous ammonia or diethyl amine as mobile phase additive was decided. Figure 1 presents R_M vs ammonia concentration and shows a significant decrease of retention with the increased concentration of ammonia. The use of ammonia causes, also, an increase of system efficiency – spots were compact and symmetric, but, simultaneously, separation selectivity became worse. A similar effect was observed when diethylamine was the mobile phase additive. With the increasing concentration of DEA, retention of analytes and selectivity decreased (see Figure 2), but system efficiency increased. Since the retention can be also controlled by the change of modifier concentration, when the required minimum of DEA (0.1 M/L) was the mobile phase component, the retention by different modifier concentrations was examined. Figure 3 shows R_M vs methanol concentration plots. Increase of methanol concentration causes, not only retention decreases, but also changes the separation selectivity. For example, flupentixole (F) and wenlafaxine (W) are not separated by lower methanol concentrations, but were well separated by mobile phase containing 60–70% of methanol.

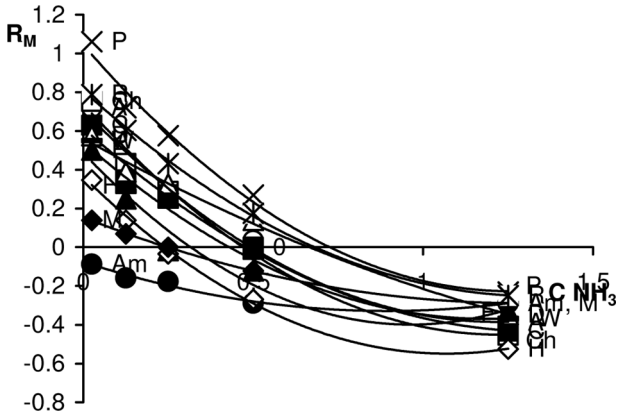


Figure 1. Plots of R_M vs. C% concentration of ammonia in the mobile phase. System: SiO_2/50% MeOH + iPr_2O + NH_3. Symbols: see Table 1.

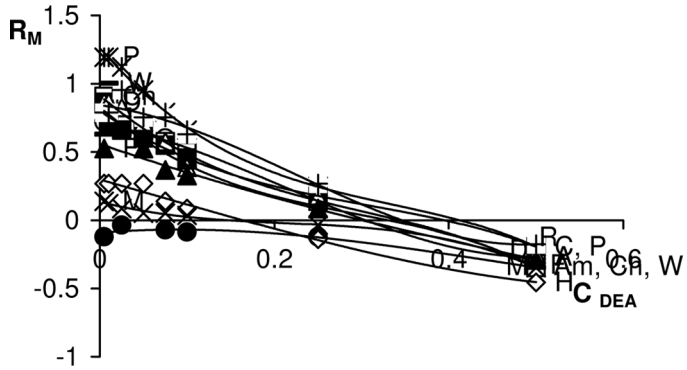


Figure 2. Plots of R_M vs. C (M/L) of DEA in the mobile phase. System: SiO_2/50% MeOH + iPr_2O + DEA. Symbols: see Table 1.

However, chloropromazine (Ch) and amizepin (Am) are better separated by lower concentrations of methanol and are eluted together with 60–70% of methanol in diisopropyl ether (see Figure 3). The best eluent systems for separation of the investigated drugs on silica was 50% MeOH in diisopropyl ether with 0.05 M DEA.

The next system used was a cyanopropyl layer with nonaqueous and aqueous eluents. Making and attempting to use NP. systems did not give satisfactory results – drugs were strongly retained near the starting line. Aqueous mobile phases also had too low an eluent strength in high ranges. Even 50% methanol in water with the addition of formic acid did

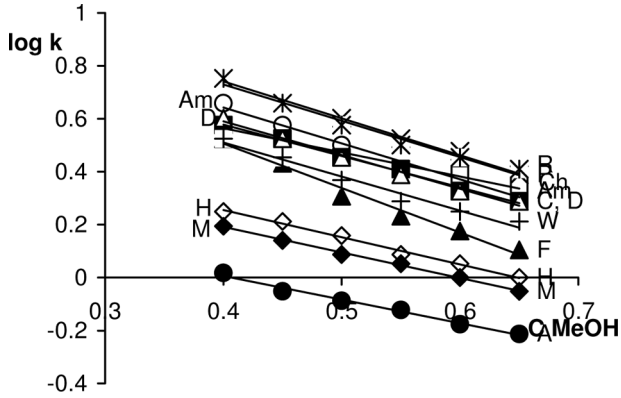


Figure 3. Plots of R_M vs. C (volume fraction) of MeOH in the mobile phase. System: SiO_2/MeOH + iPr_2O + DEA. Symbols: see Table 1.

not move substances from the starting line. Only 80% aqueous methanol containing 5% formic acid was sufficient for the elution of investigated substances, but their separation selectivity was poor, i.e., they eluted in a narrow range from 05–0.7 R_F unit.

RP systems with RP-18 HPTLC plates were also optimized for separation of the investigated compounds. Because of the basic character of the drugs, they were strongly retained on the layer with asymmetric spots. The use of buffered mobile phases at pH = 3.5 or at pH = 7.8 did not give satisfactory results – spots were elongated and separation selectivity was insufficient. The ion-pair additives (HDEHP, PSA-Na) did not give improvement of spot symmetry or separation selectivity. The use of aqueous ammonia improved separation selectivity and system efficiency. Figure 4 shows dependencies of retention vs. ammonia concentration. Increase of ammonia concentration from 0.2–2% causes a decrease of retention, but selectivity of the separation is still sufficient and peak profiles are significantly more symmetric and narrow. The best selectivity was obtained with 1% ammonia and such additive was used to elute at various methanol concentrations (see Figure 5). As is seen, the best separation selectivity was obtained with 70% methanol in water + 1% ammonia. To obtain better separation selectivity, the plate was multiple developed with the same eluent for the same distance (usually triply developed).[17] The Videoscann of the plate with the standards and mixture of selected drugs separated on RP-18 layer in above eluent system is presented in Figure 6. It is seen that the spots are compact, symmetric, and well separated.

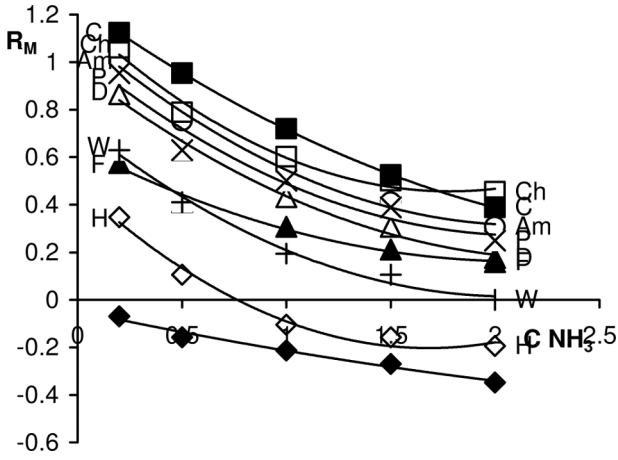


Figure 4. Plots of R_M vs. C% concentration of ammonia in the mobile phase. System: RP-18/70% MeOH + water + NH_3. Symbols: see Table 1.

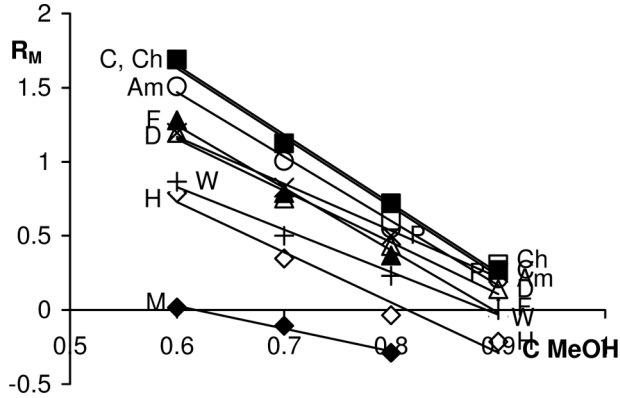


Figure 5. Plots of R_M vs. C (volume fraction) of MeOH in the mobile phase. System: RP-18/70% MeOH + water + 1% of ammonia. Symbols: see Table 1.

Taking into account the above results, two systems were tested for solid-phase extraction of two common drugs used in therapy – amitriptyline and doxepin in human serum: SPE C_{18} columns and SPE Cyano columns. Human serum FFP of group A was defrozen and filtered. Samples of serum were fortified with the investigated compounds. Because of better recoveries, SPE cyano columns were

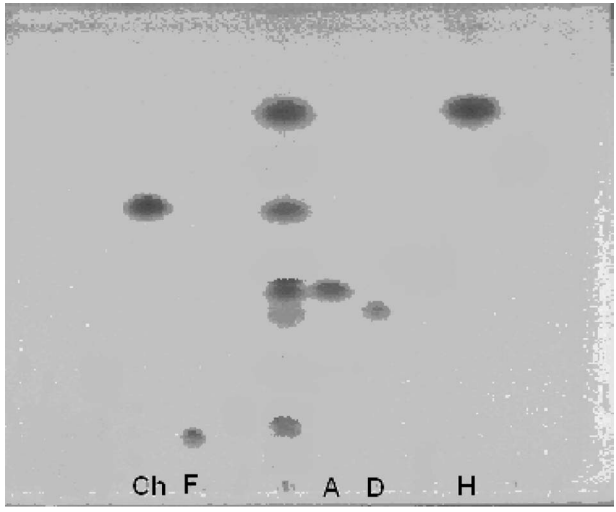


Figure 6. Videoscann of selected drugs and their mixture on RP-18 layer. Eluent: 70% MeOH + water + 1% of ammonia.

Table 2. Parameters of calibration curve for amitripthyline and doxepin

Compound	Equation of calibration curve	r	Range of linearity	LOD	LOQ
A	$y = 2.6125\,x + 0.1151$	0.9984	0.08–0.20 mg/ml	0.01931	0.05851
D	$y = 2.9357\,x + 0.042$	0.9993	0.06–0.18 mg/ml	0.01957	0.05929

applied. Moreover, since the investigated substances were strongly retained on cyano phases in a wide range of mobile phase concentrations, purification of serum samples was easily possible. Fortified serum samples were introduced to the SPE columns conditioned with 50% MeOH + water and washed with the same mobile phase. Drugs were eluted with the mobile phase containing 80% MeOH-water +5% formic acid. In this solution, basic drugs forming cations were better dissolved in aqueous media.

The quantitative analysis of amitriptyline and doxepin was performed by a calibration curve method. HPTLC system RP-18 layers/70% methanol in water + 1% ammonia was selected also for quantitative analysis of amitriptyline and doxepin in human serum.

Samples were applied to the layer by autosampler; plates were conditioned in eluent vapour. The following recoveries were determined: 83.7% for amitriptyline and 82.5% for doxepin. Extracted drugs were chromatographed in the above system. Table 2 presents parameters of the calibration curves for both drugs. The use of the DAD scanner permitted the search of identity of isolated compounds by comparison of their spectra with the spectra of standards.[18] Figures 6a and b compare spectra of amitryptiline and doxepin isolated from serum with the spectra of standards.

CONCLUSIONS

Tricyclic antidepressants – drugs of basic character – indicate strong retention and high diffusion of spots on RP-18 and cyanopropyl layers with eluents consisting of organic modifier-water or aqueous buffer solution.

Mobile phase additives such as diethylamine or aqueous ammonia, playing the role of silanol blockers, enable one to obtain compact and symmetric spots of basic drugs.

Systematic optimization of retention behavior of the investigated drugs enables a choice the best system for solid-phase extraction of amitriptyline and doxepin from human serum – SPE Cyano columns

conditioned with methanol water (1:1) and eluted with 80% methanol in water containing 5% of formic acid.

For quantitative purposes, the best system was RP-18 with 70% methanol in water containing 1% of ammonia.

REFERENCES

1. Brandys, J. *Toxicology (in polish)*; Jagiellonian University: Cracow, Poland, 1999.
2. Zając, M.; Pawełczyk, E. *Drug Chemistry (in polish)*; Medical University of Poznañ: Poland, 2000.
3. Tomaszewski, Ch.; Rungle, J.; Gibbs, M.; Colucciello, S.; Price, M. Evaluation of a rapid beside toxicology screen in patients suspected of drug toxicity. J. Emerg. Med. **2005**, *28*, 389–394.
4. Kostowski, W.; Herman Z.S. *Pharmacology (in polish)*. PZWL: Warsaw, Poland, 2005.
5. Wiater, I.; Madej, K.; Parczewski, A.; Kala, M. Optimum TLC system for identification of phenothiazines and tri- and tetracycline antidepressants. Microchim. Acta **1998**, *129*, 121–126.
6. Maślanka, A.; Krzek, J. Desitometric high-performance thin-layer chromatography – identification and quantitative analysis of psychotropic drugs. J. Assoc. Off. Anal. Chem. **2005**, *88*, 70–79.
7. Shirke, P.; Patel, M.D.; Tirodkar, V.B.; Tamhane, V.; Sethi, P.D. Estimation of amitriptyline hydrochloride and chlordiazepoxide in combined dosage form by HPTLC. East. Pharmacist **1994**, *37*, 179–180.
8. Karpińska J.; Starczewska, B. Simultaneous LC determination of some antidepressants combined with neuroleptics. J. Pharm. Biomem. Anal. **2002**, *29*, 519–525.
9. Yoshida, H.; Hidaka, K.; Ishida, J.; Yoshikumi, K.; Nohta, H.; Yamaguchi, M. Highly selective and sensitive determination of tricyclic antidepressants in human plasma using high-performance liquid chromatography with post = column tris(2, 2′-bipirydyl) ruthenium (III) chemiluminescence detection. Anal. Chim. Acta **2000**, *413*, 137–145.
10. Snyder, L.R.; Kirkland, J.J.; Glajch J.L. *Practical HPLC method Development*. J. Wiley: New York, 1997.
11. Fornstedt, T.; Zhong, G.; Guichon, G. Peak tailing and mass transfer kinetics in linear chromatography. J. Chromatogr. A **1996**, *741*, 1–12.
12. Vervoort, R.J.M.; Debets, A.J.J.; Claessen, H.A.; Cramers, C.A.G.; Jong, J. Optimisation and characterisation of silica-based reversed-phase liquid chromatographic systems for the analysis of basic pharmaceuticals. J. Chromatogr. A **2000**, *897*, 1–22.
13. McCalley, D.V. Comparative evaluation of bonded-silica reversed-phase columns for high-performance liquid chromatography using strongly basic compounds and alternative organic modifiers buffered at acid pH. J. Chromatogr. A **1997**, *769*, 169–178.

14. Sellergren, B.; Zander, A.; Renner, T.; Swietlow, A. Rapid method for analysis of nicotine and nicotine-related substances in chewing gum formulations. J. Chromatogr. A **1998**, *829*, 143–152.
15. Marchais, S.; Vermeulem, E.S.; Semple, J.; Sundell, S.; Wikström, H.V. Ion-pair formation of hydroquinine by chromatography. Anal. Chim. Acta **2001**, *426*, 85–93.
16. LoBrutto, R.; Jones, A.; Kazakievich, Y.V. Effect of counter-anion concentration on retention in high-performance liquid chromatography of protonated basic analytes. J. Chromatogr. A **2001**, *913*, 189–196.
17. Szabady, B. The Different Modes of Development, in *Planar Chromatography. A Retrospective View for the Third Millennium*; Nyiredy, Sz., Ed.; Springer: Budapest, 2001.
18. Ahrens, B.; Blankenhorn, D.; Spangenberg, B. Advanced fibre optical scanning in thin-layer chromatography for drug identification. J. Chromatogr. B **2002**, *772*, 11–18.

Received December 17, 2007
Accepted January 30, 2008
Manuscript 6311G