Thin-Layer Chromatography of Alkaloids on Cyanopropyl Bonded Stationary Phases. Part I.

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Abstract

Selected alkaloids are chromatographed on cyanopropyl–silica thin layers using various nonaqueous and aqueous eluents. Because of the strong retention of these basic compounds, nonaqueous eluents containing medium polar diluents, strongly polar modifiers, and silanol blockers (ammonia or diethylamine) are required for separation. Likewise, aqueous eluents containing modifiers (acetonitrile, methanol, and tetrahydrofuran), buffered aqueous solutions at pH 2–8, ion-pair reagents [octane sulfonic acid sodium salt, sodium dodecyl sulphate, and bis-(2-ethylhexyl)phosphoric acid], or silanol blockers (ammonia, tetrabutyl ammonium chloride, and diethyl amine) are investigated. The separation selectivity as well as spot symmetry and efficiency system in the applied eluent systems are analyzed. The most selective and efficient systems are used in two-dimensional separations of isoquinoline alkaloids' mixture and the plant extracts *Chelidonium majus, Fumaria officinalis,* and *Glaucium flavum*. Two-dimensional thin-layer chromatography on cyanopropyl layer with diode array detection densitometry enables the separation and identification of some alkaloids in plant extracts.

Introduction

Surface-modified sorbents, especially polar-bonded stationary phases, have increased in importance as stationary phases for both thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). This can often be attributed to the extended range of selectivity, which is possible when such phases are used in combination with simple eluents. Surface-modified sorbents have moderate polarity and can be used for both normal phase (NP) and reversed-phase (RP) chromatography. This makes it possible to use the layers of polar bonded stationary phases for two-dimensional separations when various retention mechanisms [adsorption in NP systems (1) and partition in RP (2)] responsible for the unique separation selectivities for application in difficult separations.

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In the last ten years the cyanopropyl (CN)-bonded stationary phases have been used in NP systems for phytochemical analysis by high-performance (HP) TLC to separate furocoumarins (3), anthraquinone aglycones (4), flavonoids (5,6), alkaloids (7,8), dyestuffs and plant pigments (9), and catechines (10). CN-plates were also used in biochemical analysis for the separation of nucleosides (11), nucleotides and bases (12), steroid hormones (9,13) and cholic acid derivatives (14), in pharmaceutical analysis for the separation of vitamins (9), benzodiazepine derivatives (15,16), nitrosamines and amines (17), and in environmental analysis to separate herbicides (18), pesticides (9,19,20), and phenols (21).

In planar chromatography, especially on plates with polar bonded phases (CN-, Diol-, and NH_2-silica), two-dimensional separations can be realized using NP adsorption systems with non-aqueous eluents and RP partition systems with aqueous eluents, which allows better or full separation of complex mixtures. The method can be applied during the analysis of complex plant mixtures, allowing selected compounds to be identified by retention coefficients in two directions and by the comparison of spectra of extract components and standards.

The aim of this work was to present the optimization of the separation of the selected alkaloids on CN-silica layers by use of various eluents in NP and RP systems to obtain sufficient selectivity of separation, system efficiency, and spot symmetry. The application of the most selective systems in 2D-TLC separations of standards' mixture of isoquinoline alkaloids and alkaloid extracts from herbs of *Chelidonium majus, Fumaria officinalis,* and *Glaucium flavum* has been presented. 2D-TLC connected with densitometry or diode array detection (DAD)-densitometry is a useful method for the identification of alkaloids in plant extracts.

Experimental

TLC was performed on 10 × 10-cm glass CN F_{254} HPTLC precoated plates (E. Merck, Darmstadt, Germany) in horizontal

Teflon chambers with an eluent distributor (DS, Chromdes, Lublin, Poland). Samples (2 µL) of 2.5% (w/v) solution in methanol were spotted, and plates were developed face-down to the distance of 8 cm from the origin at ambient temperature. Binary mixtures of polar modifier: methanol (MeOH), ethyl acetate (AcOEt), and ethyl methyl ketone (EtMeCO), in diisopropyl ether (iPr_2O) as diluent were used as mobile phases. The additives to non-aqueous eluents, such as diethylamine (DEA) and ammonia, were applied. Aqueous eluents consisted of the modifier, methanol (MeOH), acetonitrile (MeCN), or tetrahydrofuran (THF), and acetate or phosphate buffers at various pHs. Ion-pair reagents [such as: sodium dodecyl sulphate

Table I. Values of Retardation Factor (R_f), Asymmetry Factor (A_S), and Theoretical Plate Number Per Meter (N/m) For Investigated Alkaloids Obtained on CN Plates in Eluent Systems Containing MeOH–(iPr)2O (20:80) and Ammonia.

Name of alkaloids		20% MeOH + $(iPr)_2O$ + 0.5% NH_3			20% MeOH + $(iPr)_2O$ + 1% NH_3			20% MeOH + $(iPr)_2O$ + 2% NH_3			20% MeOH + $(iPr)_2O$ +5% NH_3		
		R_f	A_S	N/m	R_f	A_S	N/m	R_f	A_S	N/m	R_f	A_S	N/m
Boldine	Bo	*			0.50	0.73	2490	0.53	0.85	2780	0.46	0.89	3420
Berberine	Be	0.0			0.0			0.0			0.0		
Emetine	Em	*			0.34	0.50	1580	0.41	0.54	2310	0.41	0.75	3620
Glaucine	G	*			0.51	0.73	2810	0.55	0.73	2990	0.50	0.78	4040
Codeine	Cd	0.34	0.56	1140	0.39	0.57	1510	0.44	0.75	2280	0.38	1.29	2900
Laudanosine	L	*			0.19	0.23	270	0.29	0.36	1000	*		
Narceine	Nc	0.53	1.00	9830	0.55	0.89	4990	0.58	0.88	6560	0.55	1.00	13870
Noscapine	No	0.54	1.00	4840	0.56	0.84	3670	0.59	0.74	4680	0.56	1.08	7450
Papaverine	P	*			*			0.44	0.65	1580	0.43	0.64	1710
Protopine	Pr	*			*			*			*		
Tubocurarine	T	0.0			0.0			0.0			0.0		
Atropine	A	*			*			*			*		
Brucine	Br	0.09	0.47	50	0.16	0.30	170	0.26	0.52	790	0.27	1.22	960
Cinchonine	C	0.15	0.27	120	0.24	0.21	410	0.48	0.20	3660	0.53	0.40	1620
Quinine	Q	*			0.23	0.42	290	0.49	0.30	2800	0.57	0.72	5200
Homatropine	Ho	*			*			0.25	0.54	560	0.38	0.292570	
Yohimbine	Y	*			0.60	0.96	3530	0.47	0.67	6700	*		
Caffeine	Caff	0.42	1.61	2050	0.46	0.64	2410	0.44	1.32	2380	0.42	0.94	2860
Novocaine	N	*			*			*			*		
Scopolamine	Sc	0.0			0.34	0.35	1680	0.23	0.40	940	0.34	0.55	2280

* Very wide spot.

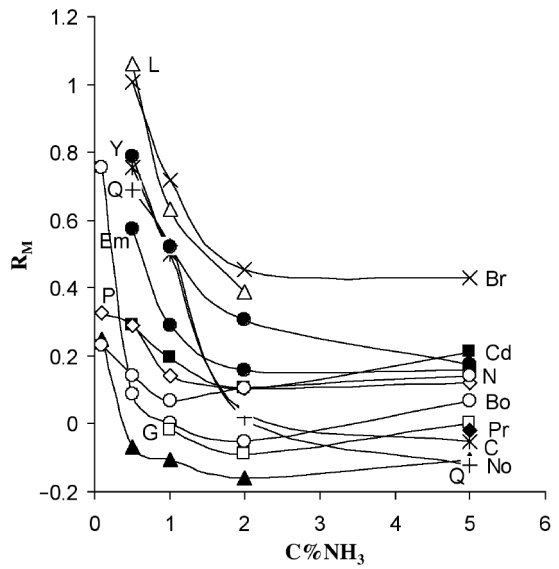

Figure 1. Dependence of R_M versus ammonia concentration in the mobile phase for investigated alkaloids. System: CN-silica–MeOH–iPr2O (20:80). See Table I for abbreviations of alkaloids.

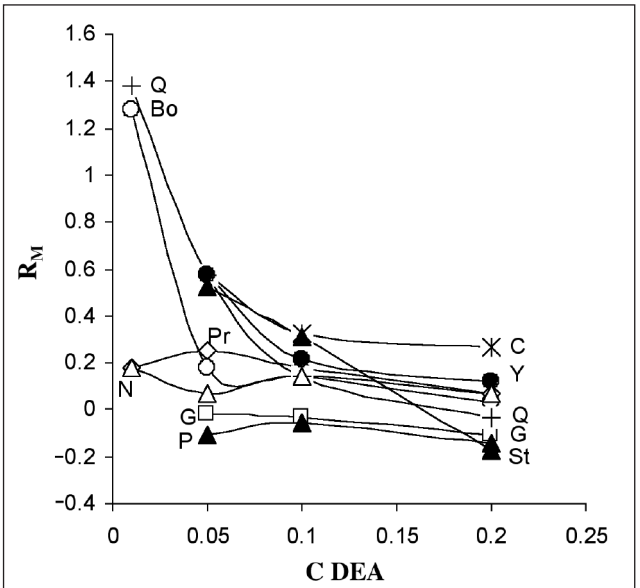

Figure 2. Dependence of R_M versus DEA concentration in the mobile phase for investigated alkaloids. System: CN-silica–MeOH–iPr2O (20:80). See Table I for abbreviations of alkaloids.

(SDS), octane sulfonic acid sodium salt (OSA-Na), or bis-(2-ethylhexyl)phosphoric acid (HDEHP)] or silanol blockers [such as ammonia, tetrabutyl ammonium chloride (TBA-Cl), or diethyl amine (DEA)] were added to some aqueous eluents. Solvents and reagents were analytical grade from Merck. The location of the spots was determined under UV light (λ = 254 nm). Plates were scanned by CAMAG TLC REPROSTAR 3 with computer program Videostore, by densitometer CAMAG TLC SCANNER 3 with computer program CATS 4, or with a diode-array spectrophotometer (J&M Aalen, Aalen, Germany) TLC-DAD scanner working in the range 191–612 nm. The investigated compounds are listed in Table I.

Plant extracts were obtained by the percolation of ground plant material with 1% aqueous acetic acid. Extracts were evaporated in vacuum evaporator under reduced pressure, and dry residues were dissolved in MeOH.

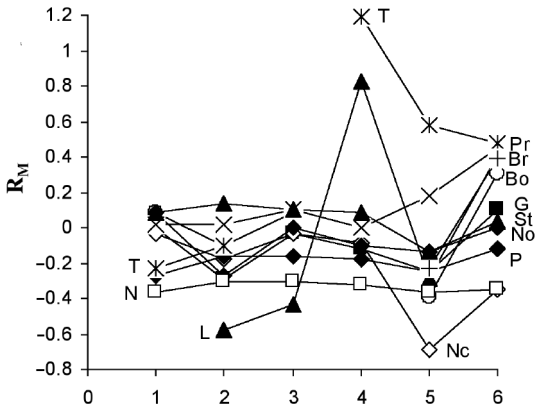

Figure 3. Graphical comparison of R_M values obtained for alkaloids in the chromatographic systems of CN-silica with eluents: 1, 80% MeOH–aqueous buffer at pH 3.3 + 0.01M OSA; 2, 80% MeOH–aqueous buffer at pH 3.3 + 0.01M SDS; 3, 80% MeOH–aqueous buffer at pH 3.3 + 0.01M of HDEHP; 4, 80% MeOH–water + 0.01M TBA-Cl; 80% MeOH–water + 2% ammonia; 5, 80% MeOH–water + 0.01M DEA. See Table I for abbreviations of alkaloids.

Results and Discussion

The retention of alkaloids (12 isoquinoline alkaloids and 12

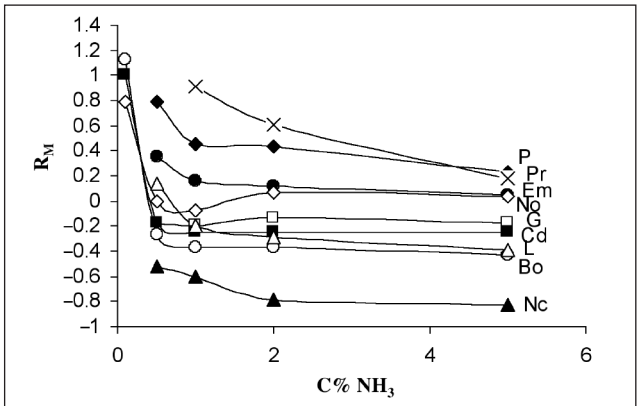

Figure 4. Dependence of R_M versus ammonia concentration in the mobile phase for investigated alkaloids. System: CN-silica– MeOH–water (70:30). See Table I for abbreviations of alkaloids.

Table II. Values of Retardation Factor (R_f), Asymmetry Factor (A_S), and Theoretical Plate Number Per Meter (N/m) For Investigated Alkaloids Obtained on CN Plates in Eluent Systems Containing MeOH–$(iPr)_2O$ (20:80) and DEA

Name of alkaloids	20% MeOH + $(iPr)_2O$ + 0.01 ML^{-1} DEA			20% MeOH + $(iPr)_2O$ + .0.05 ML^{-1} DEA			20% MeOH + $(iPr)_2O$ + 0.1 ML^{-1} DEA			20% MeOH + $(iPr)_2O$ + 0.2 ML^{-1} DEA		
	R_f	A_S	N/m	R_f	A_S	N/m	R_f	A_S	N/m	R_f	A_S	N/m
Boldine	*			0.4	0.38	1370	*			*		
Berberine	0.0			0.0			0.0			0.0		
Emetine	*			0.25	0.83	2410	0.37	0.5	17000	0.47	0.59	3600
Glaucine	0.0			0.51	0.67	2980	0.52	0.75	3610	0.56	0.72	5630
Codeine	0.0			0.34	0.58	1010	0.39	1.78	6030	0.48	0.78	4430
Laudanosine	0.0			0.0			0.0			0.0		
Narceine	*			0.55	1.00	7240	0.53	0.87	6820	0.57	0.92	13030
Noscapine	*			0.56	0.89	4560	0.53	0.94	4180	0.58	0.75	8960
Papaverine	*			*			0.40	0.84	1900	0.46	0.58	1840
Protopine	*			*			*			*		
Tubocurarine	0.0			0.0			0.0			0.0		
Atropine	*			0.22	1.13	2680	0.38	1.00	3140	*		
Brucine	0.0			0.21	1.00	5610	0.32	0.78	1880	0.35	0.85	1660
Cinchonine	*			0.21	1.00	3590	0.42	0.58	5400	0.52	0.57	9530
Quinine	*			0.22	0.75	3080	0.42	0.75	4270	0.53	0.64	8640
Homatropine	0.0			0.21	1.00	7060	0.38	0.69	5670	0.43	0.42	3980
Yohimbine	*			0.38	0.29	1040	*			*		
Caffeine	0.40	2.13	1570	*			*			*		
Novocaine	*			*			*			*		
Scopolamine	*			*			*			0.44	0.81	3860

* Very wide spot.

alkaloids from the other groups) was investigated on CN-silica plates by the use of non-aqueous and aqueous eluents. Alkaloids were strongly retained on cyanopropyl phases, and the use of strongly polar eluents was necessary. Binary mixtures of diisopropyl ether as diluent and MeOH, AcOEt, or EtMeCO as modifiers were applied. With most solvent combinations, the spots were wide and asymmetric. The smallest spots were obtained with the systems containing 20% MeOH in iPr_2O, but the system efficiency was still unsatisfactory. It was decided to use ion-suppressing agents for heterocyclic bases, which are also silanol blockers, to reduce ion-exchange processes, aqueous ammonia, or short-chain aliphatic amines. They also improved system efficiency. The retention parameters, asymmetry factors, and theoretical plate number as a function of the ammonia concentration in the eluent containing 20% MeOH in iPr_2O are presented in Table I. Figure 1 shows the dependencies of R_M as a function of ammonia concentration in the mobile phase. With the increase of the concentration of ammonia (up to 5% of ammonia), system efficiency increases when for 13 alkaloids N/m reaches a value higher than 1,000. Moreover, with the increasing concentration of ammonia, the retention of alkaloids decreases due to blocking of surface silanols and increases at a higher concentration of ammonia (2% in most cases). The best selectivity of separation was achieved by low concentrations of ammonia (0.1–0.5%). However, in such conditions, spots of alkaloids were still wide and asymmetric. With the increasing concentration of ammonia, the symmetry of spots improves, but only for six compounds did it reach acceptable values (0.8–1.5).

The influence of the addition of DEA to nonaqueous mobile phase on the retention of alkaloids was also investigated. Figure 2 shows R_M versus amine concentration relationships. Increasing concentration of DEA in the mobile phase causes the decrease of retention of the investigated compounds. Simultaneously, it increases the efficiency of systems, which is shown by the increasing theoretical plate number (see Table II).

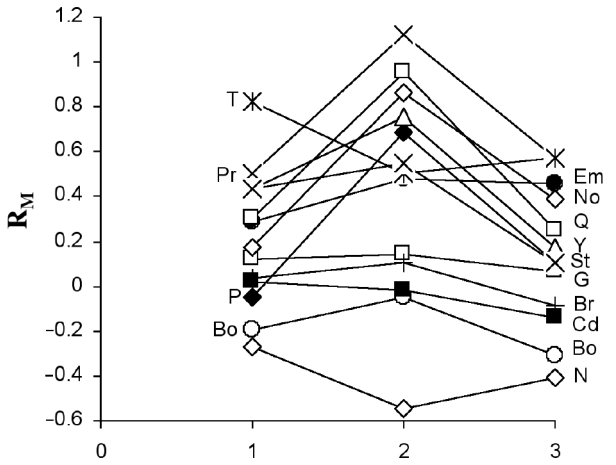

Figure 5. Graphical comparison of R_M values obtained for alkaloids in chromatographic systems of CN-silica with eluents: 1, 50% MeCN–water + 2% ammonia; 2, 30% THF–water + 2% ammonia; 3, 60% MeOH–water + 2% ammonia. See Table I for abbreviations of alkaloids.

Table III. Values of Retardation Factor (R_f), Asymmetry Factor (A_S), and Theoretical Plate Number Per Meter (N/m) For Investigated Alkaloids Obtained on CN Plates in Eluent Systems Containing MeOH–H_2O (70:30) and Ammonia

Name of alkaloids	70% MeOH + H_2O + 0.1% NH_3			70% MeOH + H_2O + 0.5% NH_3			70% MeOH + H_2O + 1% NH_3			70% MeOH + H_2O + 2% NH_3			70% MeOH + H_2O + 5% NH_3		
	R_f	A_s	N/m	R_f	A_s	N/m	R_f	A_S	N/m	R_f	A_S	N/m	R_f	A_S	N/m
Boldine	0.07	0.82	40	0.65	0.50	4540	0.70	0.60	7210	0.70	0.79	7210	0.73	2.00	7880
Berberine	0.0			0.0			0.0			0.0			0.0		
Emetine	*			0.31	0.50	830	0.41	0.73	2130	0.43	0.67	1830	0.47	0.82	2350
Glaucine	0.0			0.60	0.43	3530	0.61	0.33	3680	0.58	0.44	2920	0.60	0.38	3530
Codeine	0.09	0.46	50	0.60	0.54	4100	0.64	0.50	5610	0.64	0.60	4430	0.64	0.55	6110
Laudanosine	*			*			*			*			*		
Narceine	0.10	0.54	160	0.50	1.07	6230	0.53	1.07	6820	0.46	1.19	3760	0.48	1.33	5080
Noscapine	*			0.50	1.27	3450	0.54	0.77	3640	0.46	1.22	2150	0.48	1.38	2110
Papaverine	*			*			*			0.53	0.35	1380	0.54	0.67	1660
Protopine	0.0			0.14	0.63	490	0.26	0.40	2100	0.27	0.88	1970	0.37	0.53	3360
Tubocurarine	*			*			0.11	0.44	70	0.20	0.62	320	0.40	0.64	1690
Atropine	0.0			0.19	0.60	710	*			0.46	0.38	3420	*		
Brucine	*			0.29	0.70	2960	0.56	0.32	2360	0.58	0.46	2920	0.59	0.52	2630
Cinchonine	*			022	0.29	880	0.48	0.13	2470	0.53	0.20	2460	*		
Quinine	*			0.19	0.21	490	0.44	0.17	2200	0.49	0.28	2410	0.55	0.39	2440
Homatropine	*			*			*			*			0.54	0.50	3950
Yohimbine	*			0.20	0.50	640	0.51	0.45	2810	0.66	0.50	5020	0.55	0.80	3220
Caffeine	0.66	1.00	8480	0.70	0.89	9530	0.72	0.78	11300	0.74	0.80	9660	0.73	0.72	10420
Novocaine	*			*			0.67	0.69	10990	0.71	0.82	15580	*		
Scopolamine	0.0			0.15	0.70	1240	0.48	0.50	4430	0.49	0.54	3580	0.54	0.55	5690

* Very wide spot.

However, the selectivity of separation gets worse with the increasing amine concentration. When eluents with the highest amine concentration were used, the symmetry of spots got worse (see Table II). In the concentration of 0.2M DEA, only four have the acceptable asymmetry factors (0.8–1.5), and in the concentration of 0.05M, four compounds have the correct values (0.9–1.2) of asymmetry factor (noscapine, brucine, cynchonine, and homatropine). Using an eluent with 0.05M, DEA system efficiency was also acceptable, and for 14 alkaloids, the theoretical plate number was higher than 1000 per meter. In summary, in NP systems, the best results of separation were obtained with the mobile phase composed with MeOH, iPr_2O, and DEA or aqueous ammonia.

The next step of these experiments was the optimization of the retention of alkaloids in RP systems on HPTLC-CN plates with aqueous mobile phases. With the use of aqueous MeOH mobile phases, alkaloids were strongly retained on the surface, and spots were wide and tailing. The satisfactory results were obtained only for caffeine ($A_S = 1, N = 17400$). To improve the results, the composition of the mobile phase was optimized by changing the buffer pH and/or ion-pair reagents and concentration. Changes in pH did not improve the system efficiency. In most cases, spots were still wide and tailing when basic or acidic buffers were used as eluent components. Symmetrical spot ($A_S = 1$) and high system efficiency (about 9000 theoretical plates per meter) was obtained only for Narceine at pH values of both 2 and 8. In order to obtain further changes of retention, selectivity, and efficiency, eluents containing anionic ion-pair reagents were used. In Figure 3 the diagram of retention presents the values of R_M for investigated alkaloids obtained in systems consisting of 80% MeOH, buffer at pH 3.3, and 0.01M/L of various ion-pair reagents. It is clearly seen that the change of the kind of ion-pair reagent causes the changes in retention and separation selectivity of the investigated alkaloids, especially for the isoquinoline group. The changes of elution sequence can often be observed, for example, for protopine and noscapine or noscapine and papaverine. However, only for few alkaloids were system efficiency and spot symmetry satisfactory. For example, the correct asymmetry factors and theoretical plate number up to 1000 were obtained only for narceine, caffeine, and scopolamine with the HDEHP as the ion-pair reagent or similarly, with the use of dodecyl sulfate.

Better results were obtained with eluent systems containing aqueous ammonia. Figure 4 shows the dependencies of R_M coefficients of the investigated compounds as a function of ammonia concentration in the mobile phase (70% MeOH in water). The increase of ammonia concentration initially causes a decrease of retention of alkaloids. Further increasing of ammonia concentration from 2–5% does not cause distinct differences in retention. In some cases, the increase of retention can be observed. It proves the dual effect of ammonia as the surface silanol blocker and as ion-supressing agent of alkaloids. With the change of ammonia concentration, the changes in separation selectivity can be observed. Furthermore, the increase of ammonia concentration causes the improvement of the system efficiency and spot symmetry (see Table III). In the system with 0.1% of ammonia, the sufficient efficiency was

Table IV. Values of Retardation Factor (R_f), Asymmetry Factor (A_S), and Theoretical Plate Number Per Meter (N/m) For Investigated Alkaloids Obtained on CN Plates in Eluent Systems Containing MeOH–H_2O (70:30) and Various Additives

Name of alkaloids	80% MeOH + b. pH 3.5 + 0.01 ML^{-1} OSA-Na			80% MeOH + b. pH 3.5 + 0.01 ML^{-1}TBA-Cl			80% MeOH + b. pH 3.5 + 0.01 ML^{-1} DEA			60% MeOH + H_2O + 2 % NH_3		
	R_f	A_S	N/m	R_f	A_S	N/m	R_f	A_S	N/m	R_f	A_S	N/m
Boldine	*			*			0.33	1.00	980	0.67	1.16	5690
Berberine	0.47	0.24	310	*			0.0			0.0		
Emetine	*			*			0.29	0.86	6380	0.26	0.68	540
Glaucine	0.50	0.46	690	0.57	0.48	860	0.44	0.88	1220	0.46	0.35	1520
Codeine	*			*			0.33	1.312	1060	0.58	0.43	3300
Laudanosine	*			*			0.28	0.57	6030	*		
Narceine	0.37	0.43	2200	0.46	0.29	1870	0.54	0.94	5160	0.30	1.31	1380
Noscapine	0.45	0.39	690	0.56	0.32	990	0.50	0.53	1720	0.29	1.61	520
Papaverine	*			0.60	0.34	940	0.57	0.69	1620	0.44	0.88	1190
Protopine	*			*			0.26	0.33	21450	0.21	0.45	1110
Tubocurarine	*			*			0.25	1.00	7880	0.21	0.38	370
Atropine	*			*			0.29	1.50	4430	*		
Brucine	*			*			0.29	1.43	3990	0.55	0.28	2290
Cinchonine	*			*			0.28	0.86	6030	*		
Quinine	*			*			0.28	0.75	7450	0.36	0.29	1240
Homatropine	*			*			0.28	0.67	12310	0.39	0.35	1310
Yohimbine	*			*			0.65	0.62	4600	0.41	1.33	2310
Caffeine	0.70	0.89	7880	0.68	0.94	9000	0.69	0.88	12900	0.72	0.72	12450
Novocaine	*			*			0.69	0.63	16570	0.64	0.75	9970
Scopolamine	0.45	0.46	830	0.45	0.30	640	0.48	0.58	4580	0.44	0.42	3720

* Very wide spot.

obtained only for narceine and caffeine, though in the system with 2% ammonia, 18 alkaloids have over one thousand theoretical plates per meter. Unfortunately, the spot symmetry was still unsatisfactory (only for five alkaloids), and the asymmetry factor was in acceptable limits ($0.8 < A_S < 1.5$).

The kind of modifier also influences the retention and separation selectivity. Eluents with the addition of 2% ammonia were used. The selectivity of separation can be observed on the diagram presented in Figure 5. From the diagram, the most selective systems for the separation of a particular group of alkaloids can be chosen. For example, protopine and tubocurarine can be better separated when ACN is used as a modifier; yohimbine and brucine are better separated when MeOH or ACN is used as a modifier. The change of organic modifier also causes marked changes in the system efficiency and the differences in spot symmetry. For example, in the system with MeOH, over 5000 theoretical plates per meter were obtained for glaucine, whereas with THF only approximately 700 was obtained. In the system with MeOH, the system efficiency for quinine was high (over 5000 theoretical plates per meter) and low with eluents containing acetonitrile or THF (only about a few hundred per meter). The symmetry of spots was best (0.8–1.5) in the system with MeOH for most of the investigated alkaloids. Thus, the system with MeOH proved to be the most effective, and for 17 alkaloids, the theoretical plate number was higher than 1,000 (for ACN, only 6 alkaloids and for THF only 3 alkaloids have such theoretical plate number). Because of that, systems containing 80% MeOH with various additives (aqueous ammonia, DEA, and TBA-Cl) were tested in further investigations. The results are presented in Table IV. The use of TBA-Cl does not give satisfactory results. In most cases, spots were wide and tailing. By far, the best results were obtained with 0.1M DEA. The improvement of the system efficiency for almost all the investigated alkaloids was also obtained. Twenty alkaloids exceed 1,000 theoretical plates per meter and some (protopine, homatropine, caffeine, and novocaine) exceed even 10,000. Most of the spots were also symmetric.

On the basis of the results, optimal eluent systems for the separation of alkaloids' mixture on CN-layer by the method of two-dimensional TLC were chosen. Figures 6 (See page 5A) and 7 present the separation of alkaloid standards' mixture obtained on CN-layer and aqueous eluent: 60% MeOH–water–2% ammonia and nonaqueous one: 10% MeOH–iPr_2O–2% ammonia. By the use of these systems with their differing selectivities, the separation of 14 out of 15 isoquinoline alkaloids with symmetric spots has been obtained. Aqueous mobile phase was used as the first direction eluent and non-aqueous mobile phase was used as the second direction eluent. In the same systems by 2D-TLC natural mixtures, the extracts obtained from *Chelidonium majus* and *Fumaria officinalis* have been separated. By comparison of the retention and UV spectra (obtained by use of DAD densitometry) of standards and mixture components, the following alkaloids were identified: in

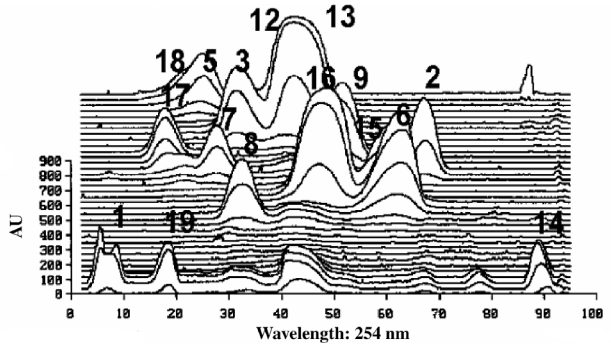

Figure 7. Densitogram of the plate scanned at 254 nm. Numbers indicate the following alkaloids: 1, berberine; 2, boldine; 3, chelidonine; 5, chelerithrine; 6, codeine; 7, dionine; 8, emetine; 9, glaucine; 12, unidentified peak; 13, noscapine; 14, narceine; 15, papaverine; 16, paracodine; 17, protopine; 18, sanguinarine; and 19, tubocurarine.

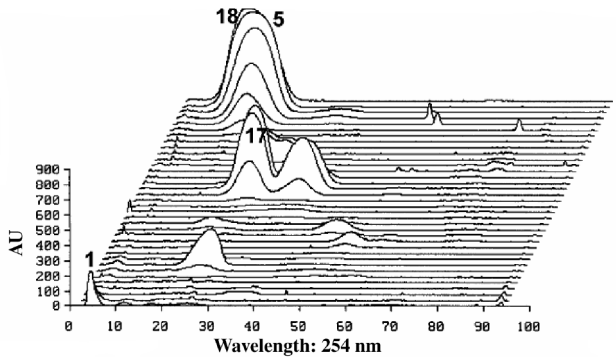

Figure 9. Densitogram of the plate scanned at 254 nm. Peak numbers are the same as in Figure 7.

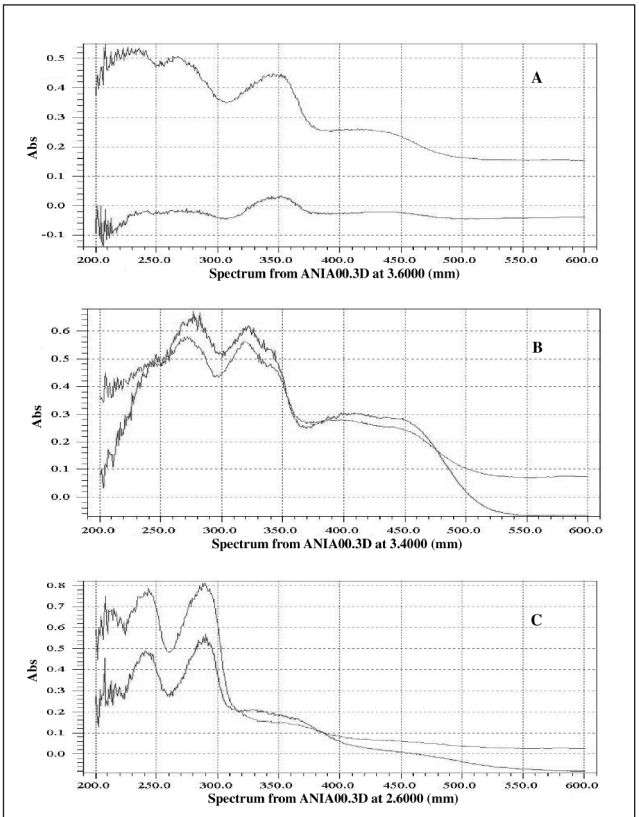

Figure 10. Spectra of extract components and alkaloid standards: berberine (A), chelerythrine (B), and protopine (C).

Chelidonium majus—sanguinarine (S), protopine (Pr), chelerythrine (Chlr), berberine (Be) [see Figures 8 (see page 5A), 9, and 10)], in *Fumaria* officinalis—Pr and S [see Figure 11 (see page 5A), 12, and 13]. Figures 14 (see page 5A) and 15 present the separation of the selected alkaloid standards in systems: I direction 10% MeOH—in diisopropyl ether + 2% ammonia and II direction eluent: 70% MeOH in water + 0.1 M DEA. The separation of *Glaucium flavum* alkaloids was possible in these eluent systems (see Figure 14 and 15 page 5A) and the alkaloids [Pr, S, Chld, Glaucine (G), and Chlr] were identified [Figure 16 and 17 (see page 6A)].

Conclusion

On CN-silica plates in NP systems, the best results for the separation of alkaloids were obtained with the mobile phase composed of MeOH, iPr_2O, and DEA or aqueous ammonia. On CN-silica plates in RP systems, pH changes do not improve the system efficiency.

The change of the ion-pair reagent in aqueous eluent causes changes in retention and separation selectivity of the investigated alkaloids, especially from the isoquinoline group, but only for a few alkaloids were system efficiency and spot symmetry satisfactory.

In aqueous eluent systems with 2% ammonia, theoretical plates per meter of over 1000 were obtained for 18 out of 24 alkaloids, but the spot symmetry was still unsatisfactory.

The change of organic modifier also causes marked changes in the system efficiency as well as differences in spot symmetry, and the best results were obtained with MeOH as aqueous eluent modifier.

The best results were obtained with 0.1M DEA as the additive to aqueous MeOH. For most of the investigated alkaloids, improved system efficiency and symmetric spots were also obtained.

Optimal eluent systems for the separation of alkaloids' mixture from the isoquinoline group on CN-layer by the method of 2D-TLC were as follows: aqueous eluent, 60% MeOH + water + 2% ammonia (or 0.1M DEA) and nonaqueous one, 80% iPr_2O + 2% ammonia.

Isoquinoline alkaloids present in extracts from *Chelidonium majus, Fumaria officinalis* and *Glaucium flavum* herbs can be separated and identified on CN-silica layer by 2D-TLC separations with DAD-densitometry.

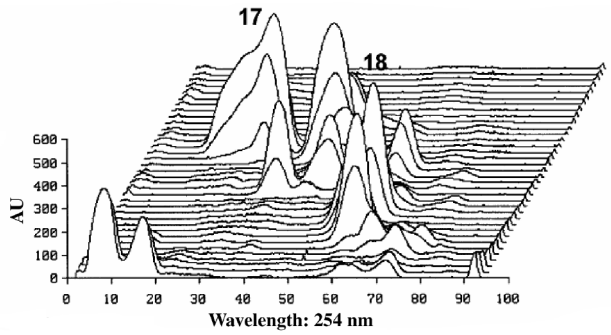

Figure 12. Densitogram of the plate scanned at 254 nm. Numbers are the same as in Figure 7.

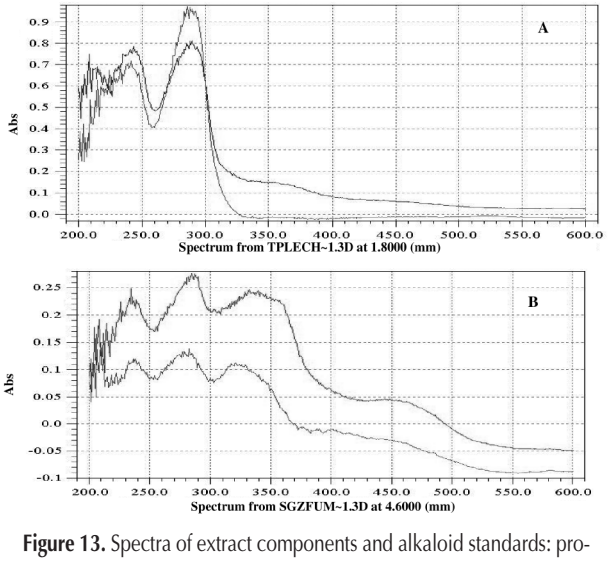

Figure 13. Spectra of extract components and alkaloid standards: protopine (A) and sanguinarine (B).

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References

1. M. Waksmundzka-Hajnos, A. Petruczynik, and A. Hawrył. Comparison of chromatographic properties of cyanopropyl-, diol- and aminopropyl- polar-bonded stationary phases by the retention of model compounds in normal-phase liquid chromatography systems. *J. Chromatogr. A* **919:** 39–50 (2001).
2. D.H. Marchand, K. Croes, J.W. Dolan, and L.R. Snyder. Column selectivity in reversed-phase liquid chromatography. VII. Cyanopropyl columns. *J. Chromatogr. A* **1062:** 57–64 (2005).
3. M. Waksmundzka-Hajnos, A. Petruczynik, M.L. Hajnos, T. Tuzimski, A. Hawrył, and A. Bogucka-Kocka. Two dimensional thin-layer chromatography of selected coumarins. *J. Chromatogr. Sci.* **44:** 510–17 (2006).
4. Sz. Nyiredy and Z. Fater. Automatic mobile phase optimization, using the "PRISMA" model, for the TLC separation of apolar compounds. *J. Planar Chromatogr.* **8:** 341–45 (1995).
5. I. Fecka and W. Cisowski. TLC determination of tannins and flavonoids in extracts from some Erodium species using chemically modified stationary phases. *J. Planar Chromatogr.* **15:** 429–32 (2002).
6. I. Fecka, A. Kowalczyk, and W. Cisowski. Optimization of the separation of flavonoid glycosides and rosmarinic acid from Mentha piperita on HPTLC plates. *J. Planar Chromatogr.* **17:** 22–25 (2004).
7. M. Waksmundzka-Hajnos and A. Petruczynik. Retention behavior of some alkaloids in thin layer chromatography with bonded stationary phases and binary mobile phases. *J. Planar Chromatogr.* **14:** 364–69 (2001).
8. M. Glensk, B. Zbikowska, and W. Cisowski. TLC separation of

Uncaria tomentosa alkaloids on chemically modified stationary phases. *J. Planar Chromatogr.* **17:** 14–17 (2004).
9. M.H. Daurade, L.E. Vagueresse, and M. Bounias. Separation, quantification, spectral properties and stability of photosynthetic pigments on CN-coated HPTLC plates. *Chromatographia* **31:** 5–10 (1991).
10. I. Fecka, W. Cisowski, and M. Luczkiewicz. Determination of catechin and epicatechin in an extract from Uncaria tomentosa bark by TLC with chemically modified stationary phases. *J. Planar Chromatogr.* **14:** 405–408 (2001).
11. T. Cserhati. Retention behaviour of some synthetic nucleosides on CN, diol and NH2 precoated high-performance thin-layer chromatographic plates. *J. Chromatogr. Sci.* **29:** 210–16 (1991).
12. W. Morden and I.D.Wilson. Separation of nucleotides and bases by high-performance thin layer chromatography with identification by tandem mass spectrometry. *J. Planar Chromatogr.* **8:** 98–102 (1995)
13. M. Bathori, A. Hunyadi, G. Janicsak, and I. Mathe. TLC of ecdysteroids with four mobile phases and three stationary phases. *J. Planar Chromatogr.* **17:** 335–41 (2004).
14. S.B. Gaica, D.M. Opsenica, B.A. Solaja, Z.Lj. Esic, and D.M. Milokovic-Opsenica. The retention behaviour of some cholic acid derivatives on different adsorbents. *J. Planar Chromatogr.* **15:** 299–305 (2002).
15. T. Cserhati and H.E. Hauck. Retention characteristics of CN, NH2 and diol precoated high-performance thin-layer chromatographic plates in the adsorption and reversed-phase separation of some benzodiazepine derivatives. *J. Chromatogr.* **514:** 45–55 (1990).
16 T. Cserhati and S. Olajos. Separation of some benzodiazepine derivatives on CN nano-TLC plates. *Fresenius'. J. Anal. Chem.* **337:** 60 (1990).
17. M.T. Matyska, A.M. Siouffi, and N. Volpe. Computer-aided optimization of a sample clean-up procedure-application to nitrosamines and amines. *J. Planar Chromatogr.* **8:** 39–46 (1995).
18. T. Tuzimski and A. Bartosiewicz. Correlation of retention parameters of pesticides In norma land RP systems and their utilization for the separation of a mixture of ten urea herbicides and fungicides by two-dimensional TLC on cyanopropyl-bonded polar stationary phase and two-adsorbent-layer Multi-K plate. *Chromatographia* **58:** 781–88 (2003).
19. T. Tuzimski. Two-dimensional TLC with adsorbent gradients of the type silica-octadecyl silica and silica-cyanopropyl for separation of mixtures of pesticides. *J. Planar Chromatogr.* **18:** 349–357 (2005).
20. T. Tuzimski. Separation of a mixture of eighteen pesticides by two-dimensional thin-layer chromatography on a cyanopropyl-bonded polar stationary phase. *J. Planar Chromatogr.* **17:** 328–34 (2004).
21. H. Majstorovic, D. Ratkov-Zebeljan, Z. Lj. Tesic, and D.M. Milkovic-Opsenica. Interpretation of the mechanisms of chromatographic separation on CN-silica. Part II. TLC of some phenols. *J. Planar Chromatogr.* **17:** 9–13 (2004).

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