TLC of Alkaloids on Cyanopropyl Bonded Stationary Phases. Part II. Connection with RP18 and Silica Plates

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Abstract

Some standards of the alkaloids and synthetic or natural mixtures are separated by two-dimensional thin-layer chromatography (TLC) on different adsorbent layers. Normal- and reversed-phase systems are used to obtain significant differences in the separation selectivity. Optimization of the one-dimensional TLC separation of the alkaloids' standards is performed on cyanopropyl-silica, RP18W, and silica layers in various eluents containing (besides diluent and modifier) silanol blockers, such as diethyl amine or ammonia. The most selective systems are used for the separation of the alkaloids' mixtures by two-dimensional TLC with an adsorbent gradient method. The mixtures of alkaloids or plant extracts (*Chelidonium majus, Fumaria officinalis, or Glaucium flavum*) are chromatographed in system I; the plates are connected with the plate pre-coated with various adsorbent, and partly separated fractions are transferred to the second layer and developed in system II. CN-silica–RP18W and CN-silica–silica are used as the connected layers. The alkaloids are identified by R_F values of standards, and the components of plant extracts are identified in both systems, and by the comparison of UV spectra obtained in diode array detector denistometry.

Introduction

Multidimensional techniques make it possible to separate very complex mixtures (e.g., plant extracts). Multidimensional chromatography, which can be easily carried out with a gas chromatography technique, is a difficult task when liquid chromatography (LC) has to be used. This is because mobile phase components play an important role in the chromatographic system and are difficult to be switched into another one. Multidimensional high-performance liquid chromatography can be carried out when the same mobile phase flows through each column, or when the solvent fraction being rechromatographed is compatibile with the mobile phase used for the next column. For example, an ion-exchange packing can be used with RP packings when it is used in the ion-exchange system buffer as a mobile phase. Similarly, RP cyano packing can be used with C8 or C18 when aqueous mobile phases (weaker in the first column) give differences in the selectivity.

Thin-layer chromatography (TLC) offers the possibility of multidimensional separation: two-dimensional separation by the use of the same stationary phase with different eluent systems (1–6) or by the use of a gradient stationary phase (7–11). As far as the eluent system is concerned, there are no limits in the application of various mobile phases because the solvents can be evaporated from the layer after the development in the first direction. Both methods: the use of the same layer and various eluent systems; and the use of two different layers and various eluent systems; can make use of various selectivities to receive complete separation in the two-dimensional process. The greatest differences are obtained by the combination of a normal-phase system with the adsorption mechanism of separation, and the reversed-phase system with the partition mechanism of separation. Two-dimensional TLC with adsorbent gradient is an effective method for the separation of a large group of substances such, as some natural mixtures (e.g., plant extracts).

The alkaloids are a group of substances which are very important in clinical toxicological and pharmaceutical investigations because of their wide spectrum of biological activity. Therefore, it is necessary to analyze these organic electrolytes. Because alkaloids appear in solutions in two forms (ionized and unionized), they are difficult objects in the chromatographic separation. Ionic samples, especially bases, can interact with free surface silanols of the adsorbent surface, which leads to an increased retention, band tailing, and unrepeatable separation (1). The interactions of alkaloids with underivatized silanol groups can be reduced by various mobile phase additives such as silanol blockers (short chain amines or ammonia).

The aim of this work was to separate the selected alkaloids by the use of adsorbent gradient of C18—cyanopropyl silica or silica—cyanopropyl silica layers with non-aqueous or aqueous

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eluent systems. The most selective systems were applied for the separation of isoquinoline alkaloids from the herbs of *Chelidonium majus*, *Fumaria officinalis*, and *Glaucium flavum*.

Experimental

Thin-layer chromatography was performed on 10 × 10 cm glass plates precoated with CN F_{254}, silica gel Si 60 F_{254}, or RP18W, produced by Merck (Darmstadt, Germany).

The mixture of the alkaloid standards or extracts was applied 1 cm from the edge of the plate. The plate was developed in the first dimension using nonaqueous eluent. After drying at room temperature for 1 h, the plate was cut into narrow strips (2 × 10 cm) containing a partly separated mixture of alkaloids. The strips were connected between two glass plates by the use of clips. Next, alkaloids were transferred in a vertical chamber to the second plate, using methanol with 2% acetic acid, to a distance of 1 cm. The narrow strip was then detached and the plate with alkaloids was dried at room temperature for 24 h. The plate was developed in the second dimension in a DS chamber (Chromdes, Lublin, Poland) using the second mobile phase.

The following mobile phases were applied: (*i*) For SiO_2-layers, nonaqueous eluents containing acetone, diisopropyl ether, diethylamine (DEA); or methanol, acetone, diisopropyl ether-containing DEA. For details about system optimization, see reference 12. (*ii*) For C18-layers, aqueous eluents containing methanol and acetate buffer at pH 3.5 + DEA; or acetonitrile + aqueous ammonia. For details about system optimization, see the literature (13). (*iii*) For CN-layers, nonaqueous eluents containing methanol, diisopropyl ether and 2% aqueous ammonia. For details about system optimization, see the literature (14).

Solvents and reagents were analytical grade from Merck (Darmastadt, Germany).

In some cases, multiple development techniques were applied. Unidimensional multiple development (UMD) consists of the development of the plate with the same eluent on the whole distance of the plate with drying of the plate after each development. Double development is marked as 2× in Table I and in figure captions. Sometimes eluents of various compositions were used for the development on the whole distance of the plate with drying of the plate after each development. In such a situation, both mobile phases are marked as I and II in Table I and in figure captions.

The location of the spots was determined under UV light (λ = 254 nm). Plates were scanned by a CAMAG TLC REPROSTAR 3 with computer program Videostore, by densitometer CAMAG TLC SCANNER 3 with computer program CATS 4, or with diode-array spectrophotometer (J&M Aalen, Germany TLC-DAD scanner) working in the range 191–612 nm. The systems used in the experiments and the values of the retardation factor (R_F) for the investigated alkaloids are listed in Table I. R_F coefficients (average from three measurements) were predicted with a standard error $\sigma \leq 4 \times 10^{-4}$).

Table I. R_F Values of Investigated Alkaloids in Applied Chromatographic Systems

		CN	SiO_2			RP 18	
Name of alkaloids	Abbreviation	Eluent: 2 × 10% MeOH / $(iPr)_2O$ + 2% NH_3	25% MeOH + 25% acetone + $(iPr)_2O$ + 0.1 M DEA	I. 5% acetone + $(iPr)_2O$ + 0.1 M DEA II. 5% acetone + 5% MeOH + $(iPr)_2O$ + 0.1 M DEA	I. 5% acetone + $(iPr)_2O$ + 0.1 M DEA II. 10% acetone + 10% MeOH + $(iPr)_2O$ + 0.1M DEA	2 × 80% MeOH + H_2O + 0.05M DEA	80% MeCN + H_2O + 2% NH_3
Boldine	Bo	0.52				0.75	
Berberine	Be	0	0.19			0.12	
Emetine	Em	0.32				0.45	
Glaucine	G	0.58		0.23	0.3	0.57	0.37
Caffeine	Caff	0.48				0.87	
Colchicine	Co	0.22				0.86	
Chelerythrine	Chlr	0.84	0.68	0.71	0.74	0.57	0.65
Chelidonine	Chld	0.46	0.33	0.41	0.54	0.51	0.63
Papaverine	P	0.45				0.78	
Protopine	Pr	0.48	0.25	0.25	0.33	0.37	0.37
Sanquinarine	S	0.77	0.77	0.62	0.64	0.53	0.43
Tubocurarine	T	0				0.29	
Atropine	A	0.38				0.51	
Brucine	Br	0.19				0.43	
Cinchonine	C	0.47				0.43	
Quinine	Q	0.52				0.41	
Strychnine	St	0.31				0.37	

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

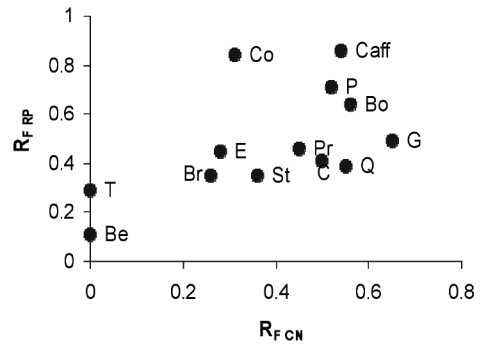

Figure 1. Correlation diagram of R_F values obtained in systems: I direction: CN/10% metanol + diisopropyl ether + 2% ammonia; II direction: RP 18W/80% methanol + acetate buffer at pH 3.5 + 0.05 ML^{-1} diethylamine.

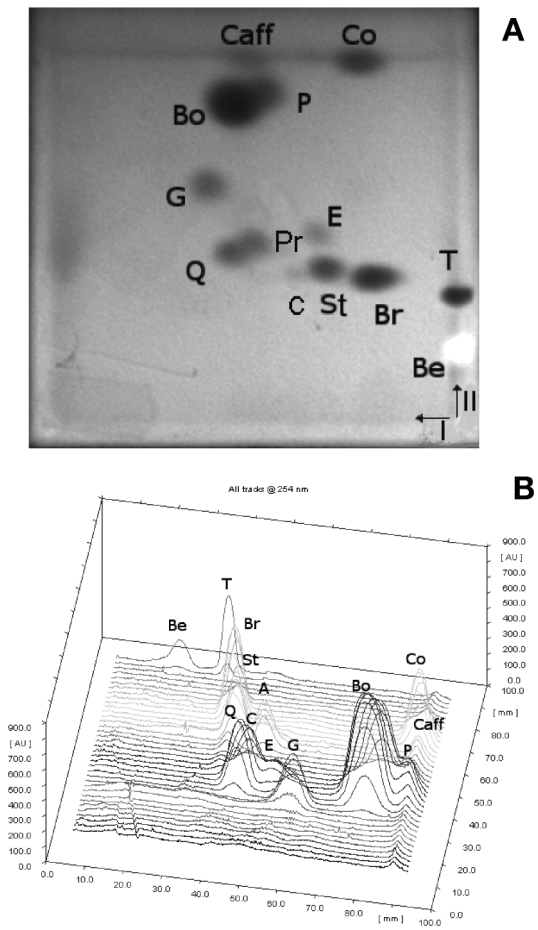

Figure 2. Videoscans of chromatogram of alkaloid standards scanned at 254 nm and 366 nm. Systems: I direction: CN/10% metanol + diisopropyl ether + 2% ammonia; II direction: RP 18W/80% methanol + acetate buffer at pH 3.5 + 0.05 ML^{-1} diethylamine (A); Densitogram of the plate scanned at 254 nm (B).

Results and Discussion

Alkaloid standards were chromatographed on various adsorbents: C18, silica, and CN-silica in different eluent systems. Preliminary experiments were performed by a one-dimensional TLC method on different plates. Good results were obtained in eluent systems containing a methanol buffer mixture with diethylamine as the mobile phase on C18 plates (13). On the silica layer, the most symmetrical spots and a sufficient separation selectivity were obtained by the use of aqueous and

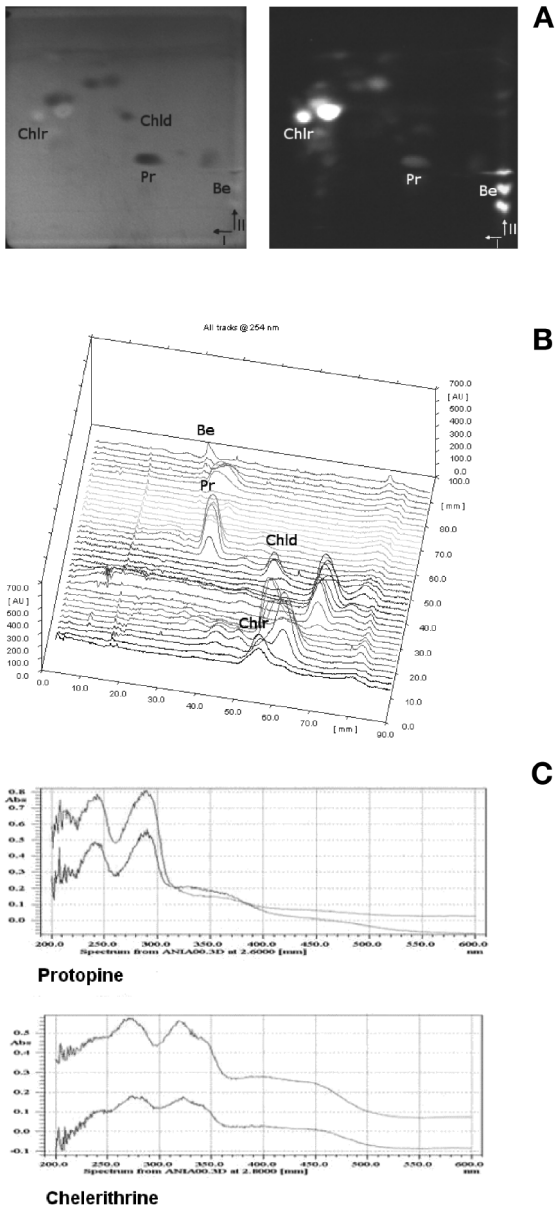

Figure 3. Videoscans of chromatogram of *Chelidonium majus* herb extract scanned at 254 nm and 366 nm. Systems: I direction: CN/10% metanol + diisopropyl ether + 2% ammonia; II direction: RP 18W/80% methanol + acetate buffer at pH 3.5 + 0.05 ML^{-1} diethylamine (A); Densitogram of the plate scanned at 254 nm (B); Spectra of extract components and alkaloid standards (C).

nonaqueous eluent systems with the addition of diethylamine (12). The best results on cyanopropyl silica layer were obtained using methanol–water–ammonia or methanol–diisopropyl ether–ammonia mixtures as mobile phases (14). The retention parameters of the selected alkaloids are presented in Table I. In all systems, the applied spots were symmetrical, but the separation selectivity of the investigated alkaloids was not satisfactory. In one system, only partial separation of alkaloids was possible.

On the basis of the results obtained, the choice of orthogonal systems of varying selectivity was possible for the separation of the synthetic and natural alkaloid mixtures by the 2D-TLC method. The correlation of R_F values obtained on C18 and cyanopropyl silica layers is presented in Figure 1. The dispersion of the points indicates the differences in the retention parameters obtained on both adsorbent layers. Such correla-

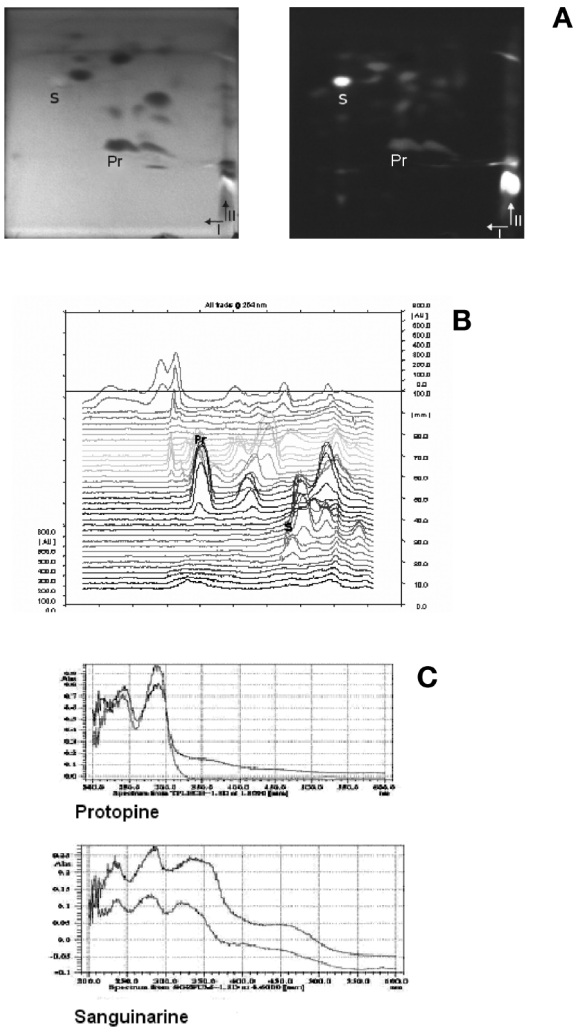

Figure 4. Videoscans of chromatogram of *Fumaria officinalis* herb extract scanned at 254 nm and 366 nm. Systems: I direction: CN/10% metanol + diisopropyl ether + 2% ammonia; II direction: RP 18W/80% methanol + acetate buffer at pH 3.5 + 0.05 ML^{-1} diethylamine (A); Densitogram of the plate scanned at 254 nm (B); Spectra of extract components and alkaloid standards (C).

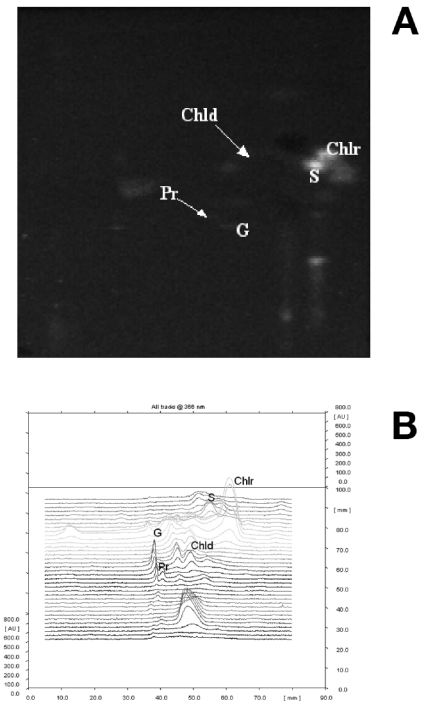

Figure 5. Videoscan of chromatogram of *Glaucium flavum* herb extract scanned at 366 nm. Systems: I direction: CN/10% metanol + diisopropyl ether + 2% ammonia; II direction: RP 18W/80% acetonitrile + water + 2% ammonia (A); Densitogram of the plate scanned at 254 nm (B).

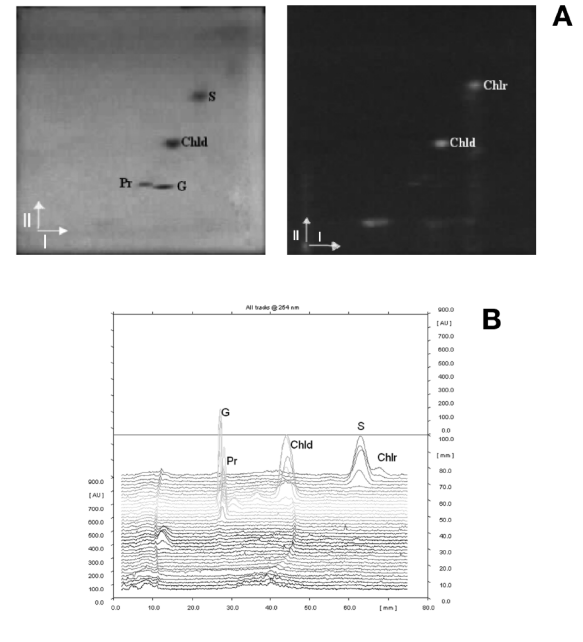

Figure 6. Videoscans of chromatogram of alkaloid standards scanned at 254 nm and 366 nm. Systems: I direction: CN/10% methanol + diisopropyl ether + 2% ammonia; II direction: SiO_2/1. 5% acetone + diisopropyl ether + 0.1 ML^{-1} diethylamine; 2. 5% acetone 5% methanol diisopropyl ether + 0.1 ML^{-1} diethylamine (A); Densitogram of the plate scanned at 254 nm (B).

tion diagrams are very useful for planning the separation of complex mixtures, and the selectivity differences can be employed in practical applications. For example, as it is seen in the correlation diagram presented in Figure 1, such partly separated fractions as: G, (Q + Bo + Caff), (C + P), Pr, (St + Co), (E + Br), and (Be + T) can be obtained on CN-silica. These partly separated fractions can be fully separated on a C18 layer. The data obtained from the graphical correlation were put into practice for the separation of a standard mixture containing 13 alkaloids by the 2D-TLC method. Figures 2A and 2B present videoscans and densitograms of the plate obtained at λ = 254 nm after the separation of the mixture; the first development was performed on a CN-silica plate with the mobile phase containing methanol–diisopropyl ether and ammonia, and the development in perpendicular direction on RP18W plate with the mobile phase containing a mixture of methanol–buffer–diethylamine. All investigated alkaloids were well-separated, and the spots were compact and symmetrical.

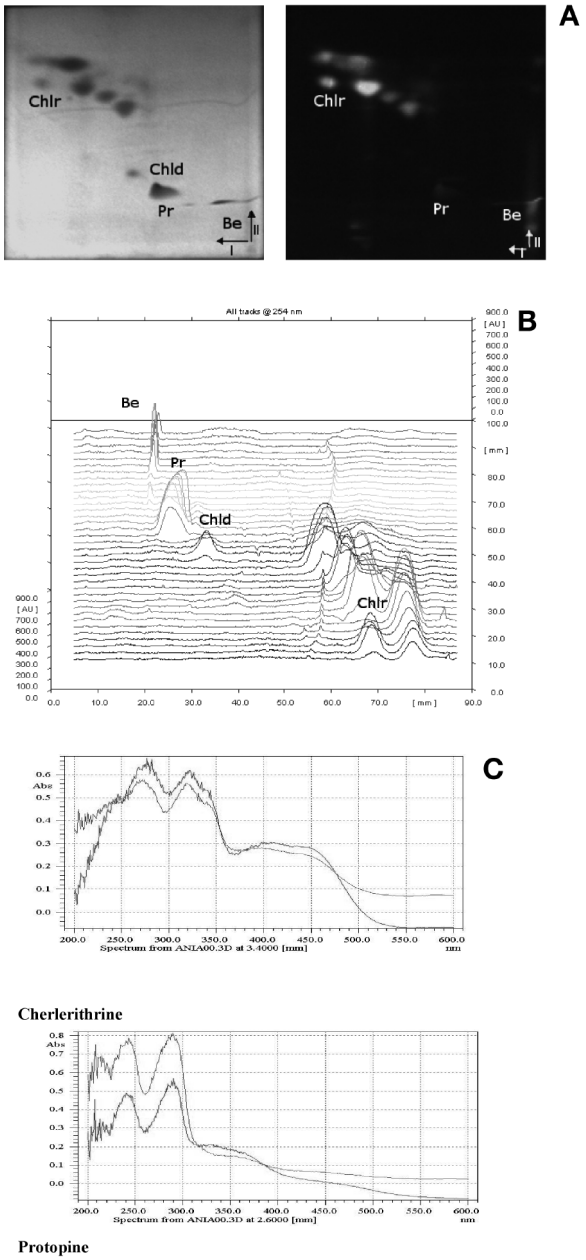

Figure 7. Videoscans of chromatogram of *Chelidonium majus* herb extract scanned at 254 nm and 366 nm. Systems: I direction: CN/10% metanol + diisopropyl ether + 2% ammonia; II direction: SiO_2/1. 25% methanol 25% acetone diisopropyl ether + 0.1 ML^{-1} diethylamine (A); Densitogram of the plate scanned at 254 nm (B); Spectra of extract components and alkaloid standards (C).

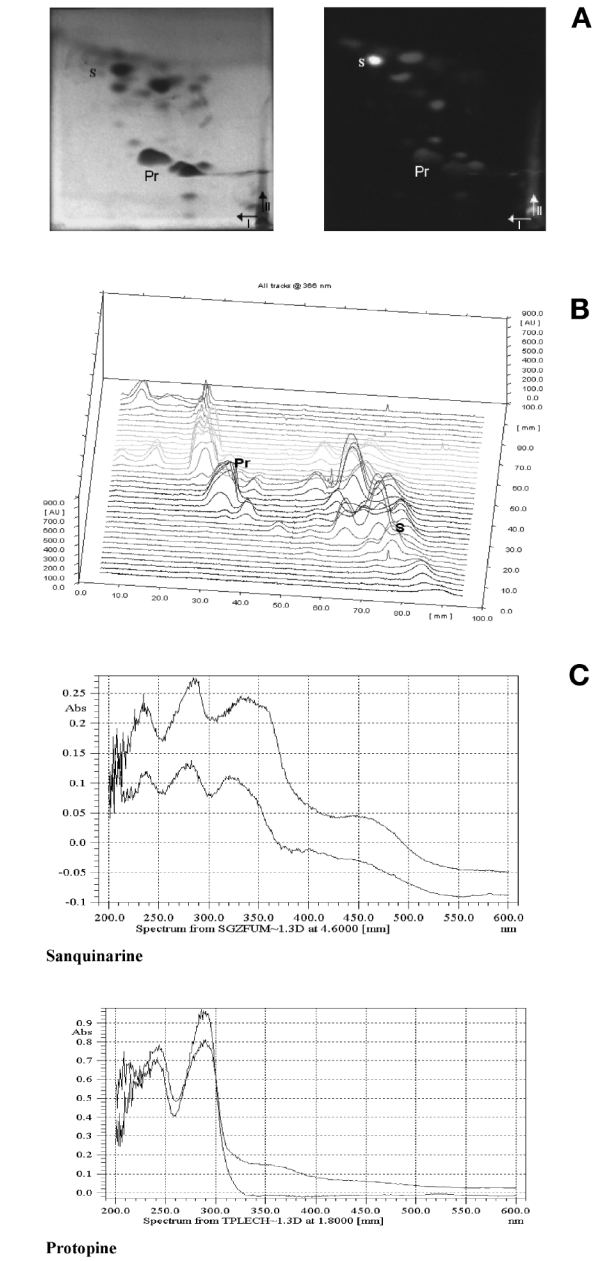

Figure 8. Videoscans of chromatogram of *Fumaria officinalis* herb extract scanned at 254 nm and 366 nm. Systems: I direction: CN/10% metanol diisopropyl ether + 2% ammonia; II direction: SiO_2/25% methanol 25% acetone diisopropyl ether + 0.1 ML^{-1} diethylamine (A); Densitogram of the plate scanned at 254 nm (B); Spectra of extract components and alkaloid standards (C).

The separation of multicomponent mixtures such as some plant extracts is a difficult task, and one-dimensional chromatography often does not lead to a satisfactory separation of their components. The application of the 2D-TLC technique, especially with an adsorbent gradient, offers the possibility of improving the separation selectivity and allows qualitative analysis of the components of plant extracts. Figures 3 and 4 show videoscans and densitograms of two-dimensional chromatograms of plant extracts separated in the same conditions as the standard mixture. The position of the spots on the plate and the comparison of the UV spectra of the extracts' components and the standards obtained from DAD densitometer enabled us to identify some alkaloids in the plant material. In this way, chlerithrine, protopine berberine, and chelidonine (Figures 3A, 3B, and 3C), as well as protopine and sanguinarine (Figures 4A, 4B, and 4C) were identified in *Chelidonium majus* and *Fumaria officinalis*, respectively. The next videoscans and densitograms present the separation of a *Glaucium flavum* extract in the modified system with acetonitrile as a modifier of an aqueous eluent (80% MeCN–water–2% ammonia [Figures 5A and 5B]). A total separation of *Glaucium flavum* alkaloids was obtained and glaucine, protopine, sanguinarine, chlidonine, and chelerithrine were identified.

Other experiments were performed by the use of 2D-TLC with a solid-phase gradient when the separation in the first direction was performed on a cyanopropyl layer with a non-aqueous mobile phase and the separation in the second direction was performed on a silica layer with a non-aqueous eluent. The spots of the alkaloids were compact, symmetrical, and the differences in the separation selectivity enabled the separation of the investigated alkaloids (see Figures 6A and 6B). Similar systems were used for the separation of plant extracts. On the basis of the position of spots on the plate and the UV spectra, the following alkaloids were identified in the examined herbs: chelidonine, chelerithrine, protopine, and berberine in *Chelidonium majus* (Figures 7A, 7B, and 7C); protopine and sanguinarine in *Fumaria officinalis* (Figures 8A, 8B, and 8C); and glaucine, protopine, chelidonine, chelerithrine, and sanguinarine in *Glaucium flavum* (Figures 9A and 9B).

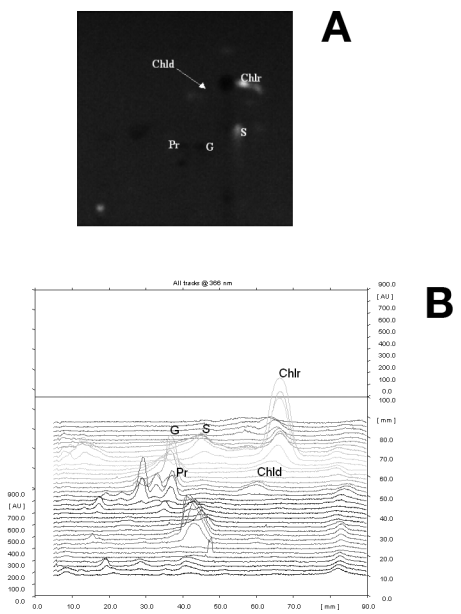

Figure 9. Videoscans of chromatogram of Glaucium flavum herb extract scanned at 254 nm 366 nm and. Systems: I direction: CN/10% metanol + diisopropyl ether + 2% ammonia; II direction: SiO_2/1. 5% acetone + diisopropyl ether + 0.1 ML^{-1} diethylamine; 2. 5% acetone + 5% methanol + diisopropyl ether + 0.1 ML^{-1} diethylamine (A); Densitogram of the plate scanned at 254 nm (B).

Conclusions

On the basis of the retention data of the investigated alkaloids, the systems of orthogonal selectivity were chosen for two-dimensional separation of the alkaloids' mixture as well as plant extracts. The systems were used with adsorbent gradient: CN-silica–MeOH + iPr_2O + ammonia – C18–MeOH (or MeCN) + water + DEA; CN-silica–MeOH + iPr_2O + ammonia – SiO_2/MeOH + acetone + iPr_2O + DEA.

The 2D-TLC method allows the possibility of identifying the plant extract components on the basis of the location of spots on the layer, by the UV spectra of the components and standards obtained by the use of the DAD scanner.

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