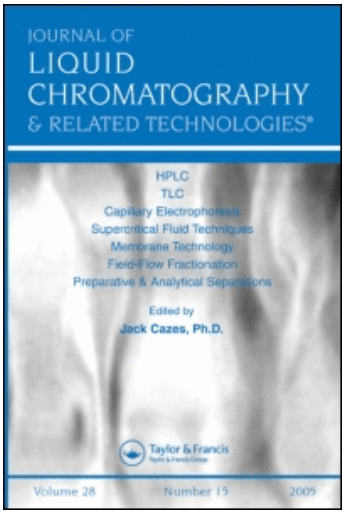

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Retention Behaviour of Selected Alkaloids on Bonded Stationary Phases by HPLC

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Retention Behaviour of Selected Alkaloids on Bonded Stationary Phases by HPLC

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ABSTRACT

Retention of selected alkaloids on polar bonded stationary phases such as diol-, NH_2-silica in normal-phase systems and diol-, CN-silica in reversed phase systems is determined by use of HPLC. Mixtures of 2-propanol and *n*-heptane are used as non-aqueous eluents; aqueous buffered (at pH about 8) solutions of methanol or acetonitrile were used in RP systems. Obtained results are presented graphically as retention—eluent composition relationships, as log k vs. log k correlation diagrams, and by chromatographic spectrum. The selectivity of separation in NP and RP systems has been discussed. In RP systems, retention on diol and CN-phases is compared with the results obtained on C18 and silica phases.

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The most selective systems for the separation of particular groups of alkaloids are chosen.

Key Words: Polar bonded stationary phases; Alkaloids; Selectivity; RP systems; NP systems; HPLC; Retention behaviour.

INTRODUCTION

Heterocyclic bases, as pharmacologically active compounds, widely used as pharmaceuticals and synthesised as secondary metabolites in plants, are the subject of scientific interests. Therefore, it is necessary to analyse these organic electrolytes.

Since heterocyclic bases appear in solutions as ionised and unionised forms, they are difficult subject of chromatographic separation. Polar adsorbents, especially silica, are widely used for the separation of alkaloids and basic drugs mainly by TLC and HPTLC techniques. Because of strong interactions of basic nitrogen with surface silanols, solvents with high eluent strength are used as mobile phases, i.e., mixtures of highly polar solvents, including alcohols (MeOH, EtOH) and aqueous ammonia solution, or ethylenediamine as ionisation suppressing agents.[1–12] However, in most cases the addition of toxic solvents as chloroform, benzene, toluene is necessary.[4–10,12] Thin layer chromatography is mostly used for qualitative analysis of extracts or fractions from preparative processes,[4–8] more rarely for quantitative determination.[10,12]

RP-HPLC, using eluents as buffered aqueous organic modifiers, is the method recommended for screening of plant material, where chromone,[1] polhydroxy,[2] cinchona,[3] and other[13–17] alkaloids are present. However, ionic samples, especially basic compounds, can interact with underivatised free silanols of silica-based alkyl bonded columns. It appears that retention occurs by an ion-exchange process that involves protonated bases and ionised silanols. This case leads to increased retention, band tailing, and column-to-column irreproducibility. It is generally desirable to minimise this silanol effect by using a higher buffer concentration, or by incorporation of amines into mobile phases.[12,18–20] If the RPC method development is unable to provide an adequate separation due to poor band spacing, anionic pairing reagents (sulphonic acids, alkyl sulphonates) are employed.[21–26] However, IP systems are difficult for application in routine analysis, because of slow column equilibration and appearance of artifactual peaks.

The aim of this paper is the search of simplified procedures for the separation of selected alkaloids by HPLC. For this purpose, the selectivity of separation of some isoquinolines and other alkaloids in various

chromatographic systems was analysed. Sorbents with polar bonded stationary phases, such as diol-silica and aminopropyl-silica with non-aqueous eluents, and diol-silica and cyanopropyl-silica with aqueous eluents, were applied. Comparative selectivity of separation in reversed-phase on silica columns, and on C-18 columns was examined. In the analysis of alkaloids in plant extracts, the use of polar bonded stationary phases is rarely reported; CN-silica in reversed-phase systems by HPLC[26,27] and NH_2-silica in normal-phase systems by OPLC has been earlier quoted.[28]

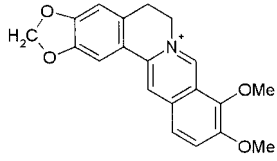
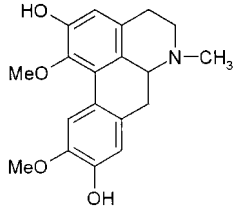
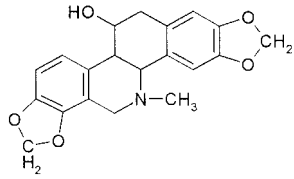
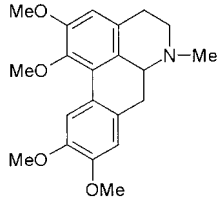
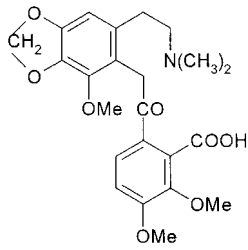
EXPERIMENTAL

The analysis was carried out using liquid chromatograph LC-10 AT_{VP} Shimadzu equipped with a column, UV-VIS SPD-10AV_{VP} Shimadzu detector, and Rheodyne 20 μL injector. The following columns were used in the experiments: SUPELCOSIL™ LC-18 $150 \times 4.6\,mm^2$, $d_p = 5\,\mu m$; SUPELCOSIL™ LC-CN $150 \times 4.6\,mm^2$, $d_p = 5\,\mu m$; SUPELCOSIL™ LC-NH2 $150 \times 4.6\,mm^2$, $d_p = 5\,\mu m$; SUPELCOSIL™ LC-DIOL $250 \times 4.6\,mm^2$, $d_p = 5\,\mu m$; and SUPELCOSIL™ LC-SI $150 \times 4.6\,mm^2$, $d_p = 5\,\mu m$, purchased from Supelco (Bellefonte, PA). Peaks were detected at $\lambda = 254\,nm$. Solvents such as 2-propanol, *n*-heptane, methanol, acetonitrile of grade for chromatography, were purchased from E. Merck (Darmstadt, Germany). The pH of phosphate buffers used in experiments in $0.01\,M\,L^{-1}$ concentrations, were measured in aqueous solutions. Standards of alkaloids purchased from Sigma–Aldrich (Poznań, Poland) are listed in Table 1.

RESULTS AND DISCUSSION

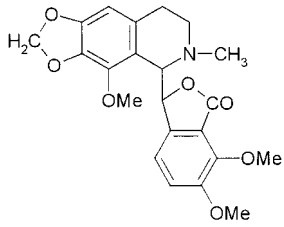
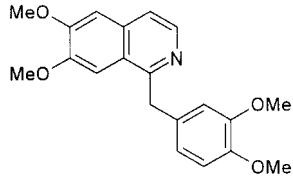
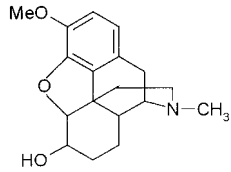
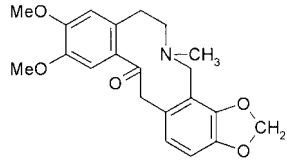
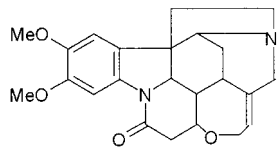
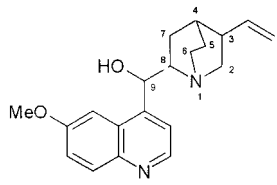
In the normal-phase system, alkaloids were chromatographed on aminopropyl and diol phases by use of 2-propanol–*n*-heptane mixtures as eluents. Alkaloids were strongly retained on polar bonded stationary phases' surfaces and need mobile phases with high eluent strength. The results obtained for solutes on aminopropyl phases are presented in Figs. 1(A) and (B), as retention–modifier concentration ($\log k$ vs. ϕ) relationships. The retention of alkaloids can be decreased several times by the change of modifier concentration from 40% to 80%. Moreover, the change of mobile phase concentration changes the selectivity of separation. For example, isoquinoline alkaloids, such as santonine, protopine, and boldine (Sa, Pr, B) eluted almost together when 80% 2-propanol in *n*-heptane as eluent was used, are better separated when lower concentration of modifier (40% or 60%) was applied. Whereas,

Table 1. List of investigated alkaloids.

Abbreviation	Name of alkaloid	Chemical structure	pK_a
Be	Berberine		>10
B	Boldine		6.62
Chld	Chelidonine		>10
G	Glaucine		6.4
Nc	Narceine		9.3

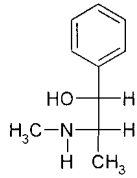
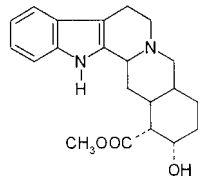
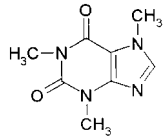
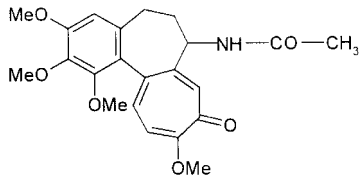
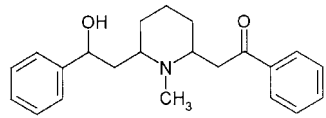
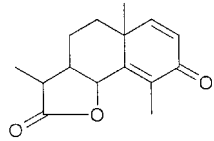
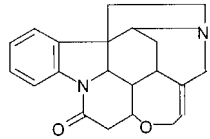
(*continued*)

Table 1. Continued.

Abbreviation	Name of alkaloid	Chemical structure	pK_a
N	Narcotine		7.8
P	Papaverine		8.07
Pa	Paracodine		—
Pr	Protopine		8.28
Br	Brucine		8.28
Q	Quinine		I = 5.1 II = 9.7

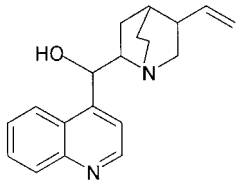
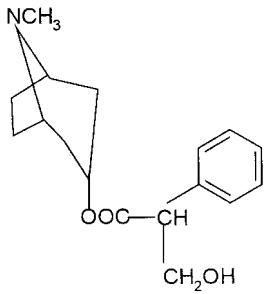
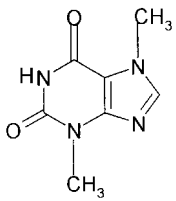
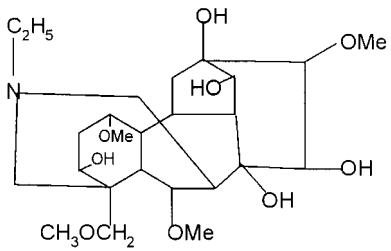
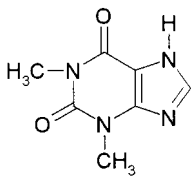
(*continued*)

Table 1. Continued.

Abbreviation	Name of alkaloid	Chemical structure	pK_a
E	Ephedrine		9.96
Y	Yohimbine		6.7
Caf	Caffeine		14
Co	Colchicine		12.35
L	Lobeline		8.03
Sa	Santonine		—
St	Strychnine		8.26

(*continued*)

Table 1. Continued.

Abbreviation	Name of alkaloid	Chemical structure	pK_a
C	Cinchonine		6.8
At	Atropine		11.7
Tb	Theobromine		13.3
A	Aconitine		7.5
T	Theophylline		8.77

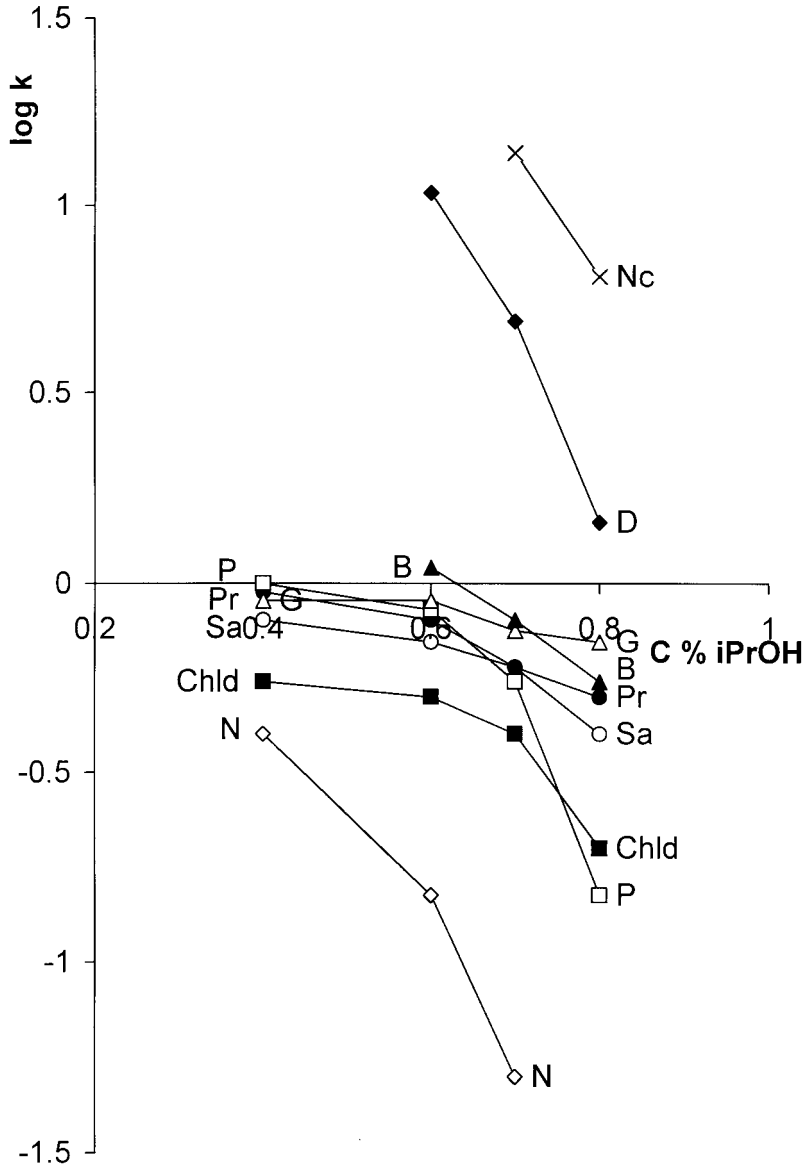


Figure 1. (A and B) Plots of log k vs. volume fraction of 2-propanol (ϕ) obtained for investigated alkaloids in system: NH_2-silica/2-propanol–n-heptane. Abbreviations, see Table 1.

(continued)

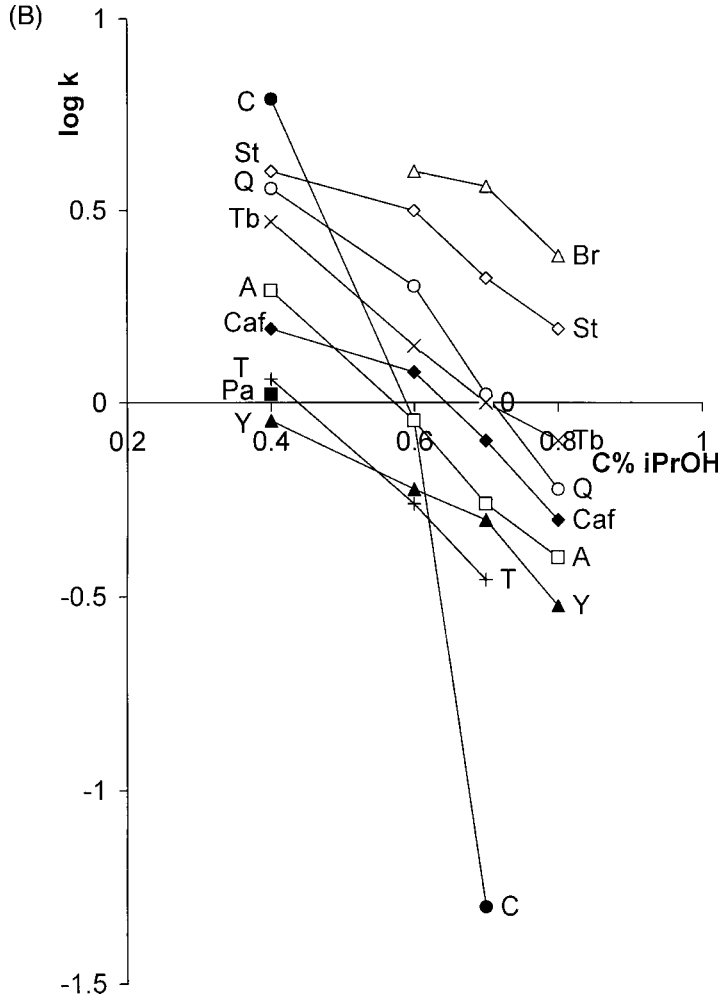


Figure 1. Continued.

papaverine, protopine, and glaucine (P, Pr, G) eluted together at lower concentration of modifier in *n*-heptane are better separated when 80% of 2-propanol in *n*-heptane was used. Similar conclusions can be drawn from Fig. 1(B). The change of modifier concentration causes alteration of retention and selectivity of separation—the changes in the sequence of elution can be observed.

Clearer changes in separation selectivity can be still obtained by the change of stationary phase. Figure 2 presents a correlation diagram of retention factors (log k values) obtained on aminopropyl and diol phases by use of 2-propanol + n-heptane mixtures as mobile phases. The points are strongly dispersed, which indicates the differences in separation selectivity. For example, the group of alkaloids containing dionine, brucine, strychnine, theobromine, cinchonine, and theophylline (D, Br, St, Tb, C, T) eluted in narrow range on diol-silica, are sufficiently well separated on the aminopropyl phase. Similar observations can be noticed for quinine, caffeine, boldine,

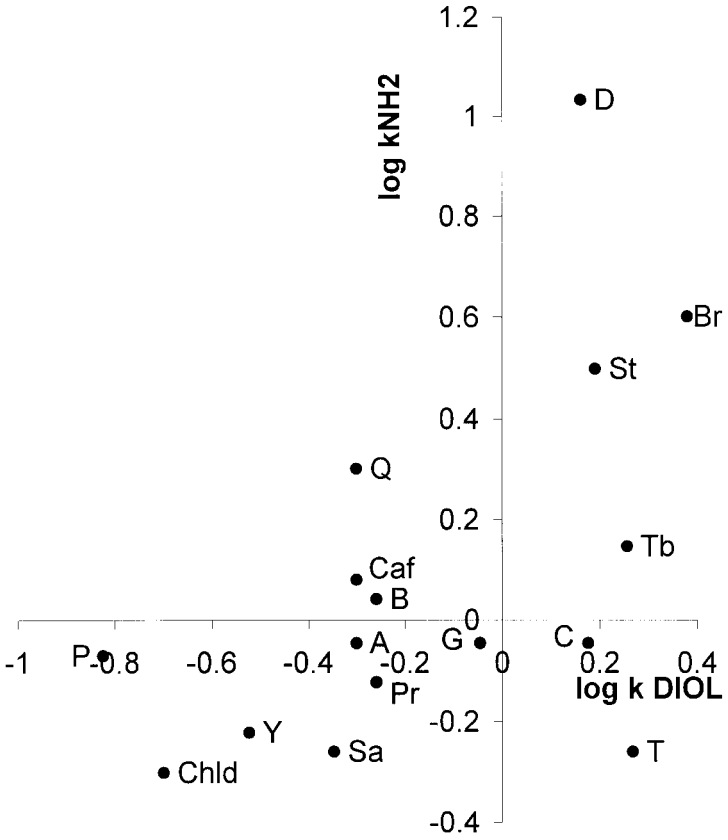


Figure 2. Correlations between log k values of alkaloids on NH_2-silica and diol columns; mobile phase: 2-propanol–n-heptane 60% and 80%, respectively. Abbreviations, see Table 1.

aconitine, and santonine (Q, Caf, B, A, Pr, Sa), which are better separated on the aminopropyl phase. Contemporaneously, there is a group of alkaloids: cinchonine, glaucine, aconitine, papaverine (C, G, A, P), or theophylline, santonine, and chelidonine (T, Sa, Chld), which are eluted in narrow range on the aminopropyl phase, but are sufficiently well separated on diol-silica. A cyanopropyl phase cannot be used for the separation of alkaloids in normal- phase systems because of strong retention of these compounds on its surface.

Polar bonded stationary phases can also be applied for the separation of alkaloids in reversed phase systems. Figures 3(A) and (B) present retention–modifier concentration relationships, obtained for investigated alkaloids on diol phases eluted with buffered (at pH 7.85) aqueous solutions of methanol. It is clearly seen, that relationships log k vs. ϕ are, in most cases, linear. By the change of methanol concentration in aqueous eluent, retention coefficients (k) can be adjusted to the optimal range. In some cases, changes in separation selectivity by the change of eluent concentration can be noticed. For example, narceine and santonine (Nc, Sa) or narcotine and papaverine (N, P), eluted almost together by use of 20% methanol in water, are better separated when 50% methanol in water (buffered at pH 7.58) is used. Whereas, aconitine, yohimbine, and paracodine (A, Y, Pa), eluted together at higher modifier concentrations, are sufficiently well separated when lower concentrations of methanol in aqueous mobile phases are used. In a few cases, variation of elution order by change of modifier concentration can be observed [see Figs. 3(A) and (B)]. Similar conclusions can be drawn from Fig. 4, where retention–modifier concentration relationships for isoquinoline alkaloids chromatographed on cyanopropyl phase in reversed phase systems are presented. The change in separation selectivity with the change of acetonitrile concentration can be noticed: for example, boldine, chelidonine, and santonine (B, Chld, Sa), eluted in narrow range at 60% aqueous acetonitrile (at pH 7.85), are better separated at lower concentrations of modifier.

The selectivity of separation can be distinctly varied by the change of stationary phase. Figure 5 shows the correlation diagram for investigated alkaloids eluted with aqueous buffered methanol from diol and C18 stationary phases. The correlation points are dispersed, which bespeaks differences in selectivity on diol-silica and C18 stationary phases. For example, strychnine, quinidine, glaucine, and brucine (St, Q, G, Br), protopine, chelidonine, and papaverine (Pr, Chld, P), or aconitine, narcotine, narceine (A, N, Nc) eluted in narrow range on diol phase are sufficiently well separated on C18 phase. There are however, groups of alkaloids better separated on diol-phase in RP systems, for example, boldine, papaverine, and narceine (B, P, Nc), brucine, chelidonine, and narcotine (Br, Chld, N), or glaucine, protopine, and dionine

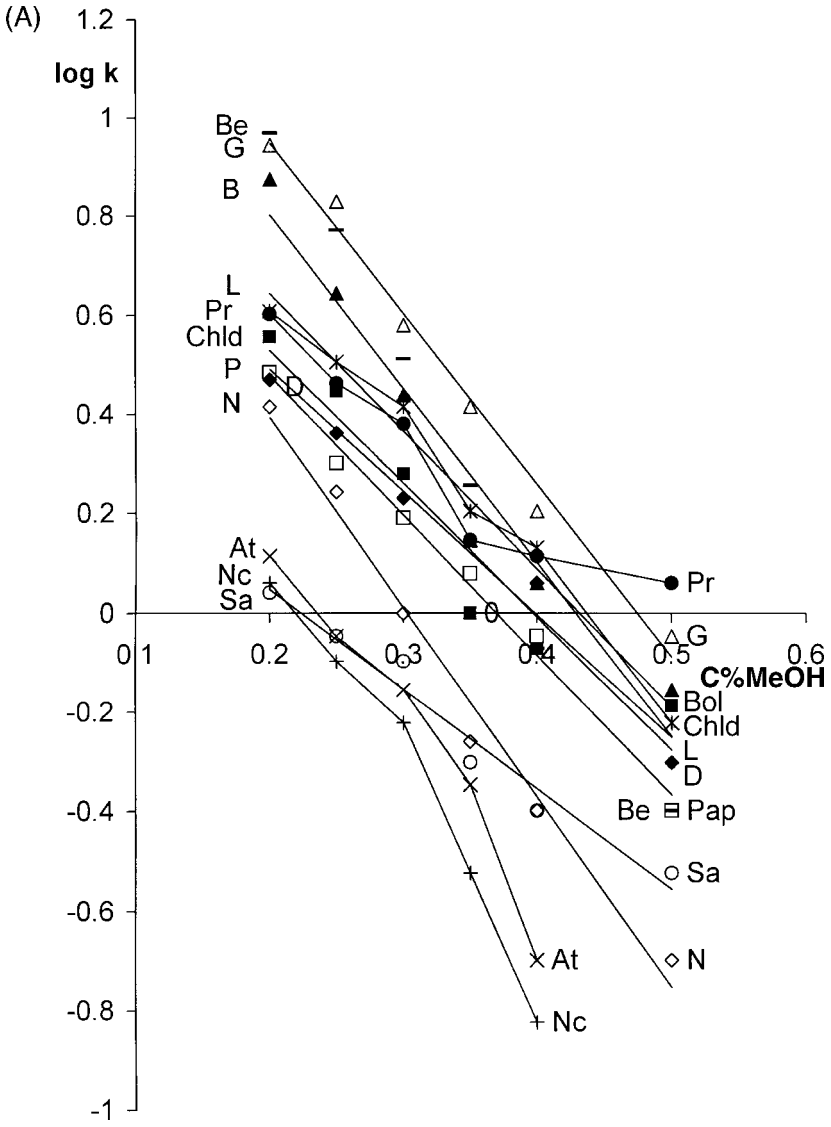


Figure 3. (A and B) Plots of log k vs. volume fraction of methanol (ϕ) obtained for investigated alkaloids in system: diol-silica/methanol–water + phosphate buffer at pH 7.58. Abbreviations, see Table 1.

(continued)

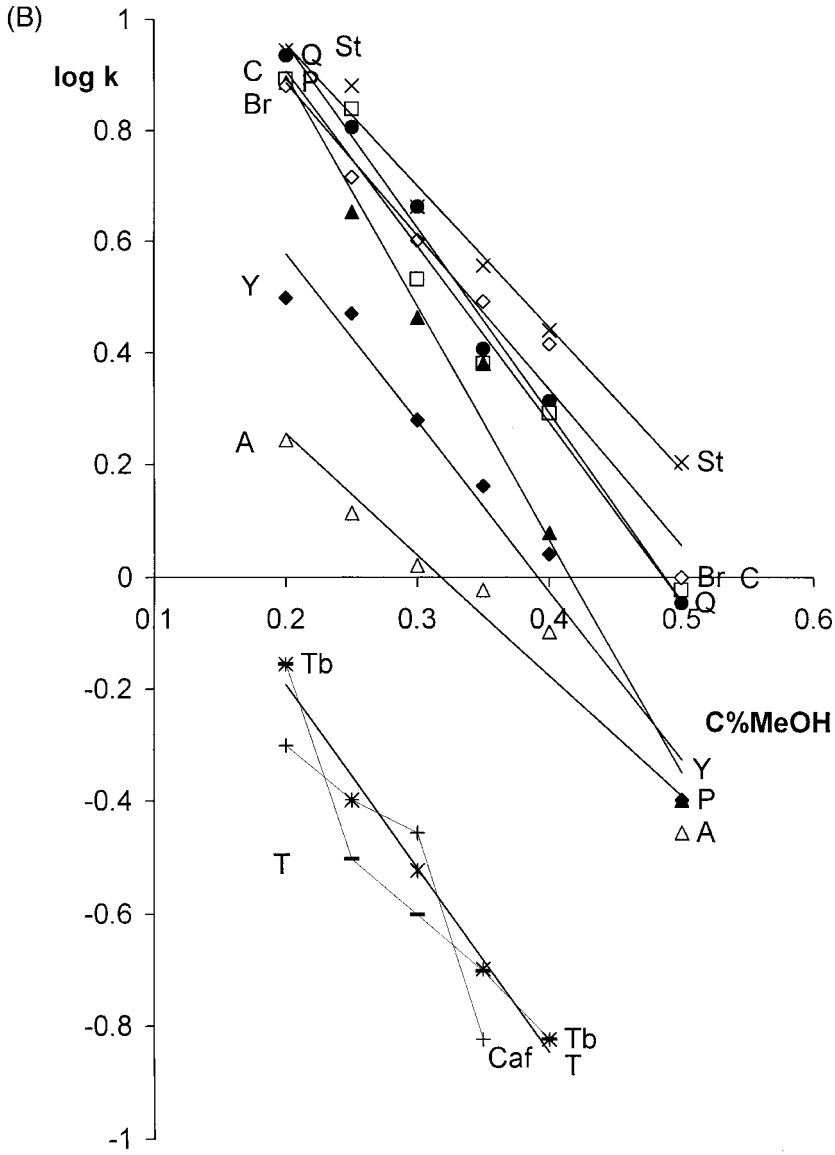


Figure 3. Continued.

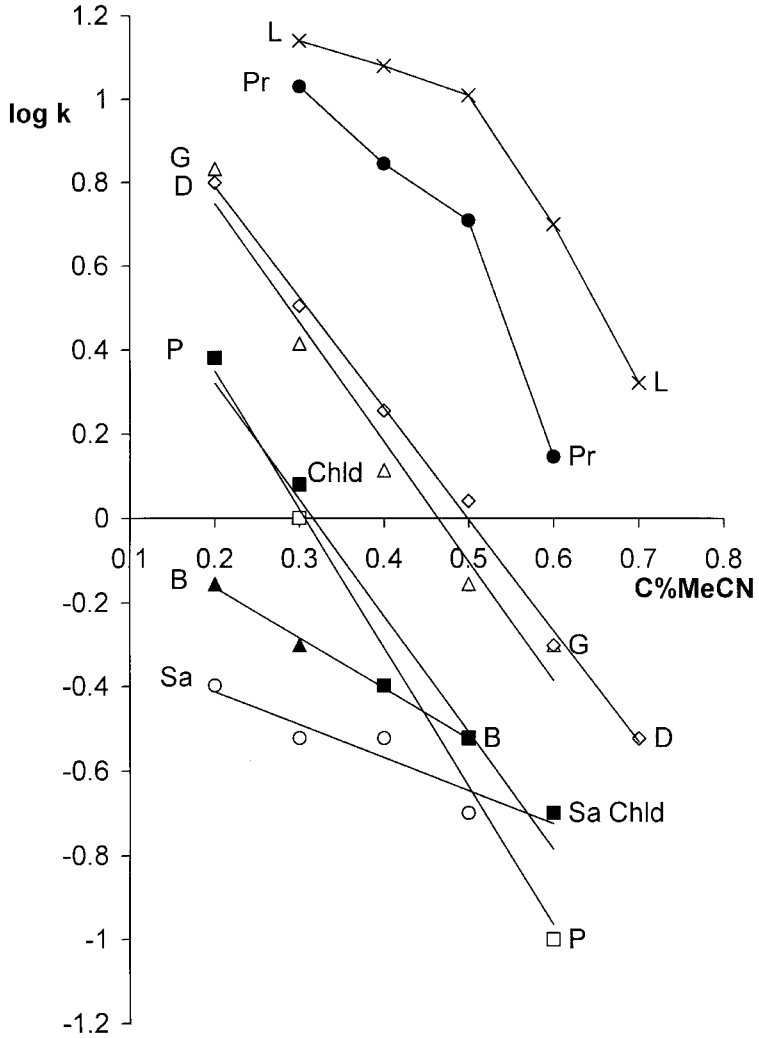


Figure 4. Plots of log k vs. volume fraction of acetonitrile (ϕ) obtained for investigated alkaloids in system: CN-silica/acetonitrile–water + phosphate buffer at pH 7.85. Abbreviations, see Table 1.

(G, Pr, D) are better separated on the diol stationary phase. A similar correlation diagram presents selectivity differences on cyanopropyl and C18 phases for investigated compounds when acetonitrile aqueous buffered mobile phases were used. In this case, CN-silica seems to be most selective: strychnine,

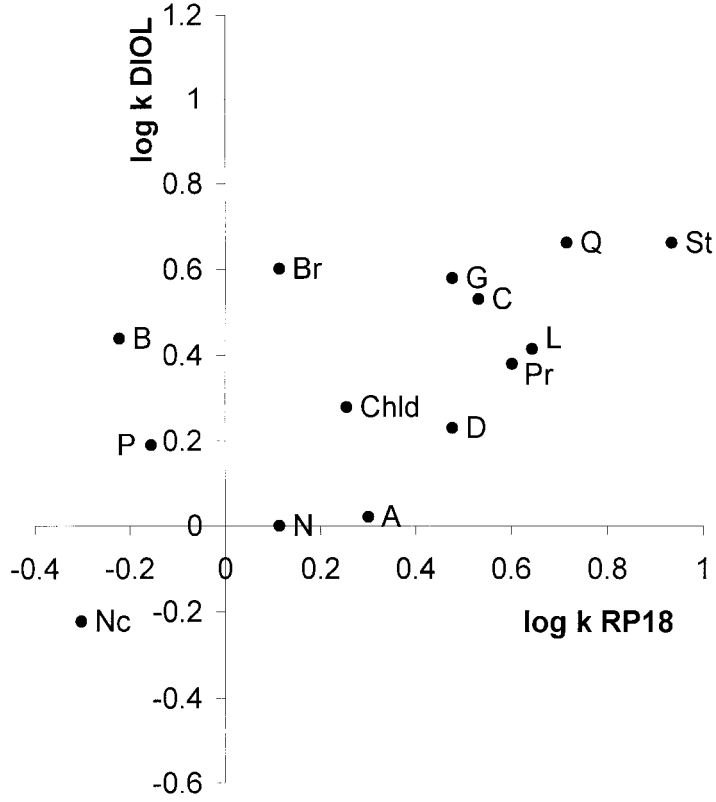


Figure 5. Correlations between log *k* values of alkaloids on diol and C18 columns; mobile phase: methanol + water (at pH 7.58), 30% and 70%, respectively. Abbreviations, see Table 1.

quinidine, dionine, protopine, chelidonine (St, Q, D, Pr, Chld), or glaucine, yohimbine, aconitine, emetine, colchicine are eluted practically together on C18, and better separated when CN-silica as stationary phase was used (see Fig. 6). The changes in separation selectivity in reversed phase systems by using aqueous buffered methanol eluents and various stationary phases, are presented in Fig. 7. The differences in separation selectivity are noticeable. The high separation selectivity on silica in pseudo-reversed phases[29] should be mentioned.

Figure 8 presents correlation diagrams for retention coefficients obtained on diol-silica by use of RP (aqueous buffered methanol) and NP (2-propanol +

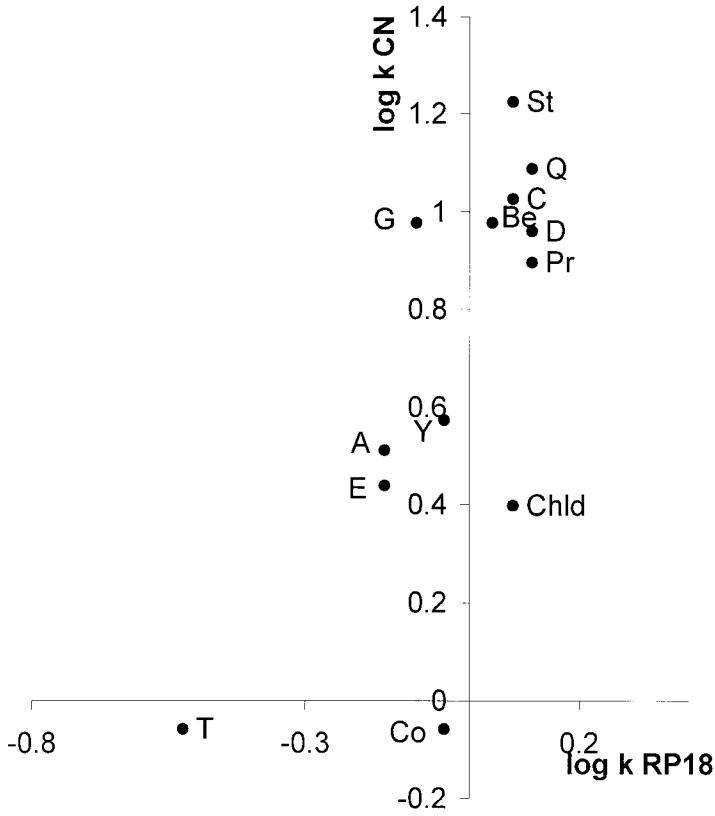


Figure 6. Correlations between log *k* values of alkaloids on CN-silica and C18 columns; mobile phase: acetonitrile + water (at pH 7.85), 25% and 70%, respectively. Abbreviations, see Table 1.

n-heptane) systems. Points are strongly dispersed, which bespeaks selectivity differences of both systems. The use of aqueous eluents permits the separation of the following groups: quinine, boldine, aconitine, santonine, and caffeine (Q, B, Pr, A, Sa, Caf), or strychnine, cynchonine, dionine, theobromine, and theophylline (St, C, D, Tb, T), which are poorly separated in non-aqueous systems. However, such alkaloids as theophylline and caffenine (T, Caf), dionine, protopine, yohimbine, chelidonine (D, Pr, Y. Chld), and cinchonine, glaucine and quinine (C, G, Q) are better separated on diol phase with non-aqueous eluent.

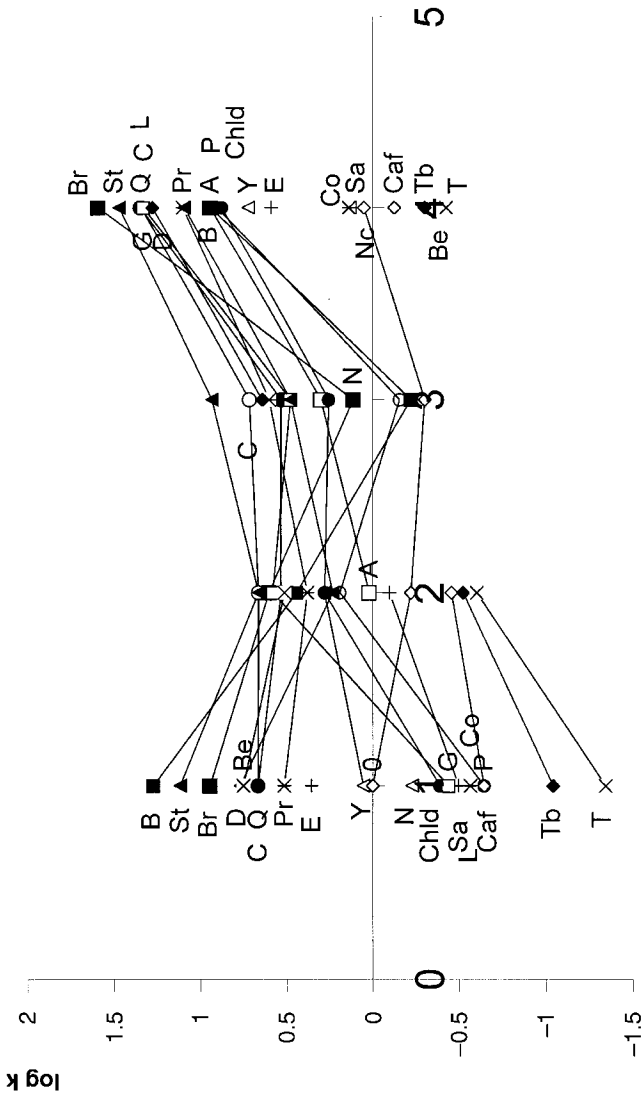


Figure 7. Graphical comparison of log k values obtained for investigated alkaloids (see Table 1) in following chromatographic systems: 1, silica; 2, diol-silica; 3, C18; 4, CN-silica. Mobile phase: methanol in water (at pH 7.58) in concentrations 40%, 30%, 70%, and 25%, respectively.

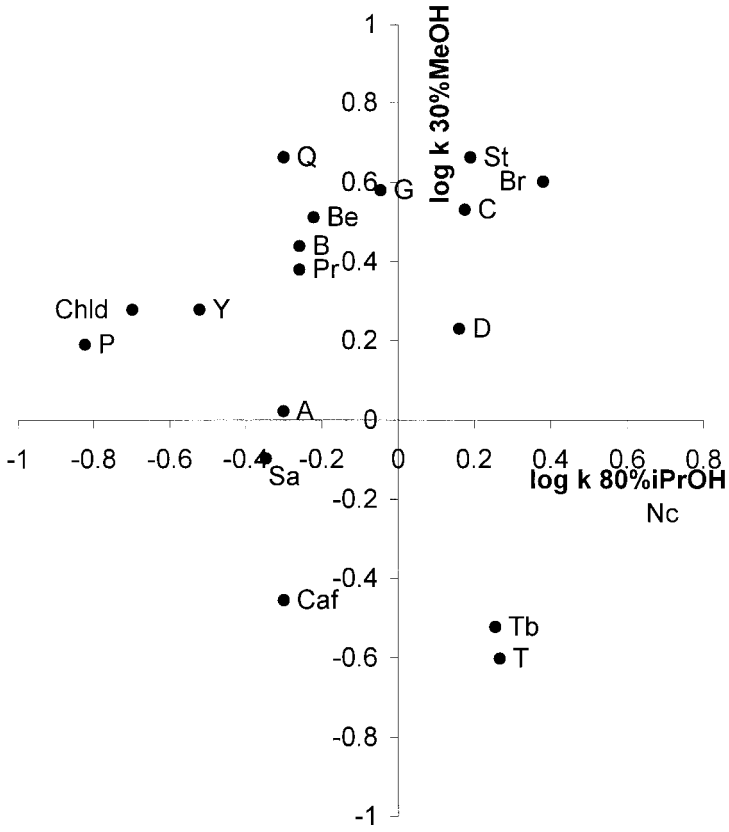


Figure 8. Correlations between log k values of alkaloids on diol-silica column; mobile phases: methanol + water (at pH 7.58) and 80% *i*PrOH in *n*-heptane. Abbreviations, see Table 1.

CONCLUSIONS

NH_2-silica and diol phases can be used for the separation of alkaloids in normal-phase systems with the mobile phases of high eluent strength. Retention and separation selectivity can be regulated by change of modifier (2-propanol) concentration and/or a kind of stationary phase.

Diol and CN-silica can be applied to the separation of alkaloids in reversed-phase systems with aqueous buffered methanol or acetonitrile solutions. Retention and separation selectivity can be regulated by the change of modifier kind and concentration.

The differences in separation selectivity of alkaloids on polar bonded stationary phases, C18 and silica, can be applied in scheduling of multidimensional liquid separations.

REFERENCES

1. Houghton, P.J. Chromatography of the chromone and flavonoid alkaloids. J. Chromatogr. A **2002**, *967*, 75–84.
2. Molyneux, R.J.; Gardner, D.R.; James, L.F.; Colegate, S.M. Polyhydroxy alkaloids: chromatographic analysis. J. Chromatogr. A **2002**, *967*, 57–74.
3. McCalley, D.V. Analysis of the Cinchona alkaloids by high performance liquid chromatography and other separation techniques. J. Chromatogr. A. **2002**, *967*, 1–19.
4. Friedman, M.; Kozuke, N.; Harden, L.A. Preparation and characterization of acid hydrolysis products of the tomato glycoalkaloid. J. Agric. Food Chem. **1998**, *46*, 2096–2101.
5. El Shazly, A.; El Dominaty, M.; Witte, L.; Wink, M. Pyrrolizidine alkaloids in members of the Boraginaceae from Sinai (Egypt). Biochem. Systematics Ecol. **1998**, *26*, 619–636.
6. Zwickenpflug, W.; Meger, M.; Richter, E. Occurrence of the tobacco alkaloid myosmine in nuts and nut products of *Arachis hypogea* and *Corylus avellana*. J. Agric. Food Chem. **1998**, *46*, 2703–2706.
7. Ma, X.Q.; Jiang, S.H.; Zhu, D.Y. Alkaloid patterns in Huperzia and some related genera of Lycopodiaceae Sensu Lato occurring in China and their contribution to classification. Biochem. Systematics Ecol. **1998**, *26*, 723–728.
8. Yuan, L.M.; Zi, M.; Ai, P.; Chen, X.X.; Li, Z.Y.; Fu, R.N.; Zhang, T.Y. Versatile two-phase solvent system for alkaloid separation by high-speed counter-current chromatography. J. Chromatogr. A **2001**, *927*, 92–96.
9. Cardoso, C.A.L.; Vilegas, W.; Honda, N.K. Qualitative determination of indole alkaloids, triterpenoids and steroids of *Tabernaemontana hilariana*. J. Chromatogr. A **1998**, *808*, 264–268.
10. Yang, F.; Quan, J.; Zhang, T.; Ito, Y. Preparative separation of alkaloids from the root of *Sophora flavescens* Ait by pH-zone-refining counter-current chromatography. J. Chromatogr. A **1998**, *822*, 316–320.
11. Then, M.; Szentmihalyi, K.; Sarközi, A. Effect of sample handling on alkaloid and mineral content of aqueous extracts of greater celandine (*Chelidonium majus* L.). J. Chromatogr. A **2000**, *889*, 6974.
12. Verotta, L.; Peterlongo, F.; Elisabetsky, E.; Amador, T.A.; Nunes, D.S. High performance liquid chromatography–diode array detection–tandem

mass spectrometry analyses of the alkaloid extracts of Amazon Psychotria species. J. Chromatogr. A **1999**, *841*, 165–176.
13. Sun, S.W.; Kuo, C.H.; Lee, S.S.; Chen, C.K. Determination of bisbenzylisoquinoline alkaloids by high performance liquid chromatography (II). J. Chromatogr. A **2000**, *891*, 189–194.
14. Bajad, S.; Johri, R.K.; Singh, K.; Singh, J.; Bedi, K.L. Sample high performance liquid chromatography method for the simultaneous determination of ketoconazole and piperine in rat plasma and hepatocyle culture. J. Chromatogr. A **2002**, *949*, 43–47.
15. Rosso, A.; Zuccaro, S. Determination of alkaloids from the colchicine family by reversed-phase high performance liquid chromatography. J. Chromatogr. A **1998**, *825*, 96–101.
16. Tikhomiroff, C.; Jolicoeur, M. Screening of *Catharanthus roseus* secondary metabolites by high performance liquid chromatography. J. Chromatogr. A **2002**, *955*, 87–93.
17. Valverde, J.; Tamayo, G.; Hesse, M. β-Carboline monoterpenoid glucosides from *Palicourea adusta*. Phytochemistry **1999**, *52* (8), 1485–1489.
18. Yang, F.; Ito, Y. Preparative separation of lappaconitine, ranaconitine, *N*-deacetylloppaconitine and *N*-deacetylranaconitine from crude alkaloids of sample *Aconitum sinomontanum* Nokai by high-speed countercurrent chromatography. J. Chromatogr. A **2002**, *943*, 219–225.
19. Yang, F.; Ito, Y. pH-Zone-refining counter-current chromatography of lappaconitine from *Aconitum sinomontanum* Nokai I. Separation of prepurified extract. J. Chromatogr. A **2001**, *923*, 281–285.
20. Tesarova, E.; Zaruba, K.; Flieger, M. Enantioseparation of semisynthetic ergot alkaloids on vancomycin and teicoplanin stationary phases. J. Chromatogr. A **1999**, *844*, 137–147.
21. Fabre, N.; Claparols, C.; Richelme, S.; Angelin, M.L.; Fouraste, I. Direct characterization of isoquinoline alkaloids in a crude plant extract by ion-pair liquid chromatography–electrospray ionization tandem mass spectrometry example of *Eschscholtzia californica*. J. Chromatogr. A **2000**, *904*, 35–46.
22. Sun, S.W.; Lee, S.S.; Wu, A.C.; Chen, C.K. Determination of bisbenzylisoquinoline alkaloids by high performance liquid chromatography. J. Chromatogr. A **1998**, *799*, 337–342.
23. Ciolino, L.A.; Turner, J.A.; McCauley, H.A.; Smallwood, A.W.; Yi, T.Y. Optimization study for the reversed-phase ion-pair liquid chromatographic determination of nicotine in commercial tobacco products. J. Chromatogr. A **1999**, *852*, 451–463.
24. Mroczek, T.; Glowniak, K. Simultaneous determination of N-oxides and free bases of pyrrolizidine alkaloids by cation-exchange solid-phase extraction and ion-pair high performance liquid chromatography. J. Chromatogr. A **2002**, *949*, 249–262.

25. Musshoff, F.; Schmidt, P.; Dettmeyer, R.; Priemer, F.; Wittig, H.; Madea, B. A systematic regional study of dopamine and dopamine-derived salsolinol and norsalsolinol levels in human brain areas. Forensic Sci. Int. **1999**, *105*, 1–11.
26. Petruczynik, A.; Gadzikowska, M.; Waksmundzka-Hajnos, M. Optimization of the separation of some *Chelidonium majus* L. alkaloids by reversed phase high performance liquid chromatography using cyanopropyl bonded stationary phase. Acta Pol. Pharm. **2002**, *59*, 61–64.
27. Lau, O.W.; Mok, C.S. High performance liquid chromatographic determination of atropine and atropine-like alkaloids in pharmaceutical preparations with indirect conductometric detection. J. Chromatogr. A **1997**, *766*, 270–276.
28. Nagy-Turak, A.; Vegh, Z. Extraction and in situ densitometric determination of alkaloids from *Catharantus roseus* by means of overpressured layer chromatography on amino-bonded silica layers. I. Optimization and validation of the separation system. J. Chromatogr. **1994**, *668*, 501–507.
29. Golkiewicz, W.; Gadzikowska, M. Isolation of some quaternary alkaloids from the extract of roots of *Chelidonium majus* L. by column and thin layer chromatography. Chromatographia **1999**, *50*, 52–56.

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