Table 6 General outlines of normal-phase high performance liquid chromatography systems for the separation of alkaloids

Stationary phase	*Mobile phase*		
Silica gel	Dichloromethane, Chloroform, Diethyl/isopropyl ether, Tetrahydrofuran, or Ethyl acetate	Methanol or Isopropanol	Ammonia, Diethylamine or Triethylamine (*c.* 1% of the mobile phase)

phosphate or citrate buffer, pH *c.* 4, containing perchlorate, acetate or chloride as the ion pairing agent. High loadability and different selectivity compared with column chromatography are important features of countercurrent chromatography.

See also: **III/Alkaloids:** Gas Chromatography; Thin Layer (Planar) Chromatography. **Natural Products:** High-Speed Countercurrent Chromatography.

Further Reading

Baerheim Svendsen A and Verpoorte R (1983) *Chromatography of alkaloids. Part A: Thin-layer chromatography*. Amsterdam: Elsevier Science Publishers.

Manske RHF and Holmes HL (eds) *The Alkaloids*, Volume 1–5 (1950–1995), Manske RHF (ed.) Volume 6–16 (1955–1977), Manske RHF and Rodrigo R (eds) Volume 17–20 (1979–1981), Brossi A (ed.) Volume 21–40 (1983–1992), Cordell GA (ed.) Volume 40– (1992–) New York: Academic Press.

Cordell GA (1981) *Introduction to Alkaloids. A Biogenetic Approach*. New York: John Wiley.

Glasby JS (1975) *Encyclopedia of Alkaloids*, vols 1 and 2. New York: Plenum Press.

Hesse M (1974) *Progress in Mass Spectrometry*, vol. 1, parts 1 and 2. *Mass Spectrometry of Indole Alkaloids*. Weinheim, Verlag Chemie.

Hesse M and Bernhard HO (1975) *Progress in Mass Spectrometry*, vol. 3. *Mass Spectrometry of Alkaloids*. Weinheim: Verlag Chemie.

Pelletier SW (ed.) (1983) *Alkaloids: Chemical and Biological Perspectives*, vols 1–6. New York: John Wiley.

Popl M, Fähnrich J and Tatar V (1990) *Chromatographic Analysis of Alkaloids*. New York: Marcel Dekker.

Sangster AW and Stuart KL (1965) Ultra-violet spectra of alkaloids. *Chemical Reviews* 65: 69–130.

Southon IW and Buckingham J (1989). *Dictionary of Alkaloids*. London: Chapman & Hall.

Verpoorte R and Baerheim Svendsen A (1984) *Chromatography of alkaloids. Part B: Gas-liquid chromatography and high-performance liquid chromatography*. Journal of Chromatography Library. Volume 23B. Amsterdam: Elsevier Science Publishers.

Verpoorte R (1986) Methods for the structure elucidation of alkaloids. *Journal of Natural Products* 49: 1–25.

Thin-Layer (Planar) Chromatography

J. Flieger, Medical Academy, Lublin, Poland

Introduction

In 1938, Izmailow and Schraiber pioneered the thin-layer chromatography (TLC) method for the analysis of plant material containing alkaloids. The subject matter of their scientific research was an extract of a plant rich in tropane alkaloids. Later on, the method was developed by Bekesy, who applied it to the separation of ergot alkaloids. Since then, numerous papers exploring the detection, isolation and quantitative determination of alkaloids by TLC have been published. It has been stated that no other method has delivered so much information on alkaloids.

From the chemical point of view, alkaloids form a very diverse group of organic nitrogen compounds of a basic character (with the exception of some derivatives of purine and colchicine). They have tertiary or quaternary amino groups in their molecules and only a few contain secondary amino groups. Considering the fact that analytical problems connected with alkaloids are mostly concerned with their physicochemical properties, they are commonly divided according to the type of chemical structure into tropane, quinoline, indole, diterpene and others. Another useful classification is based on botanical groups (e.g. tobacco, lupine, ergot, strychnos, vinca and catharanthus alkaloids), and this is

especially valuable as far as chemotaxonomical studies are concerned.

In early work, alkaloids were predominantly isolated from the natural plant material. TLC was then used for qualitative and quantitative analysis of plants and the study of the biosynthesis of alkaloids. Because of their powerful physiological properties alkaloids have become important therapeutic compounds and many of them have been prepared synthetically or by partial synthesis. As a consequence, many derivatives have been formed that do not occur in nature. TLC is particularly well suited for checking the processes of synthesis as well as for establishing the progress of reactions and finally testing of products in pharmaceutical preparations. The importance of alkaloids is also fundamental in toxicological analysis; many are used as narcotics and hallucinogenic drugs, as doping substances and as poisons. The presence of alkaloids in drugs of abuse and their metabolites in biological fluids such as urine and blood has also been tested by means of TLC.

Preparation of Samples

Various sample preparation procedures have been developed for pharmaceutical formulations, plant and biological materials. Due to the fact that, in most of them, alkaloids occur as salts together with complex mixtures of nonalkaloid compounds such as inorganic salts or substances of lipophilic character, their pre-separation by a suitable extraction procedure is necessary.

While in the case of the analysis of solutions, alkalified (or acidified) samples and extraction with an organic solvent such as chloroform or diethyl ether is usually sufficient, isolating alkaloids from a plant material is a multistage process and may be conducted using several methods.

Most often preparative isolation is carried out by liquid–liquid extraction. Plant material with a high liquid content should be initially extracted with light petroleum or water containing diluted hydrochloric acid to remove lipids. The release of alkaloidal bases occurs under the influence of the addition of a mineral base, commonly ammonia. Then they are extracted by means of water-immiscible organic solvents or water–alcohol mixtures.

For efficient extraction in the above cases described, alkaloids should be present in the extractable form in at least 95%, so pH adjustment of the sample to $pH = pK_a + 2$ is sufficient.

Further purification is achieved by re-extracting alkaloids from organic solvents into an aqueous phase of the opposite pH, where the alkaloids are present as salts.

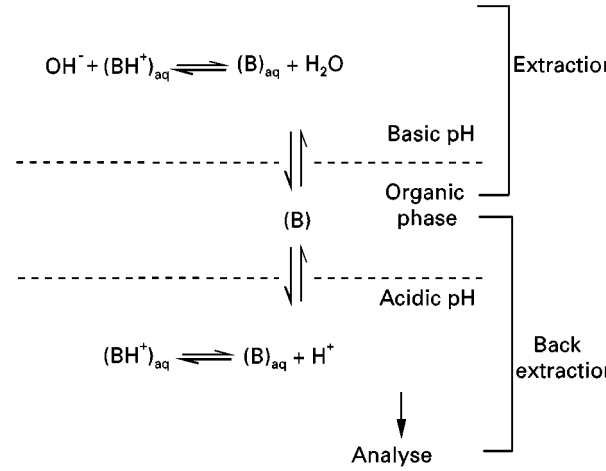

Figure 1 Scheme for the back-extraction procedure of a basic drug (B) (after Adamovics JA (1990) *Chromatographic Analysis of Pharmaceuticals*. New York and Basel: Marcel Dekker, Inc.)

This back-extraction procedure for basic compounds (B) is shown schematically in **Figure 1**.

A liquid extraction technique used to increase extraction efficiency and selectivity is an ion pair extraction originally used to extract strychnine from syrup.

Purification of crude plant extracts from non alkaloidal compounds may be carried out by precipitating the alkaloids with picric acid, Reinecke's salt or Mayer's reagent or by using ion exchange or a small adsorption column. Solid-phase extraction (SPE) is gaining in popularity. Specific sorption conditions under which alkaloids are strongly retained lead to preconcentration of free bases (on aluminium oxide), their salts (on phosphoric acid impregnated silica) or as an ionic form (on ion exchangers).

It should be emphasized that, in the case of silica gel, quaternary alkaloids are more strongly retained than ternary ones with an aqueous buffer–methanol mobile phase. Such differences also create the possibility of separating these two groups of alkaloids.

One of the latest methods of isolating groups of alkaloids from solid samples is supercritical fluid extraction (SFE). The method increases the efficiency of extraction and shortens the overall time of analysis.

While considering the problems of extraction, isolation and purification of alkaloids, one should be cautious about the possibility of undesirable reactions and artefact formation. One reason may be impurities present in the solvents applied. Thus, peroxides (in ethers) cause oxidation, ethyl chloroformate (in chloroform) forms ethylcarbamates of alkaloids; halogen-containing compounds; bromochloromethane and dichloromethane (in chloroform) cause quaternization of tertiary alkaloids, while cyanogen

chloride (in dichloromethane) is the cause of nitrilation of primary and secondary amines. Decomposition may also be caused by a photochemical reaction, especially in chloroform solutions. Finally there may be a reaction with a solvent itself, mainly with chloroform, but also with ketones or strong alkali. The fact that the chloroform used as a component of the mobile phase may present a quenching effect should also be emphasized.

Development Techniques

Adsorbents used in TLC may be either commercial products or home-made plates (now seldom employed). High quality chromatograms can be achieved with HPTLC plates which were introduced in the 1980s.

Plates may be developed in a linear, circular or anticircular mode. The most common technique in TLC of alkaloids is ascending, single, one-dimensional development in tanks saturated with the vapour of the solvent system.

Preconditioning the plate with the vapours, thus preventing demixing of the mobile-phase components, can also be performed in sandwich-type chambers produced by Camag (Vario-KS) and Chromdes (DS). In some cases, especially where compounds differ in polarity, repeated development of the plate with the same solvent or solvents of increasing strength or the continuous development technique has some advantages. In other cases, programmed multiple development with the same solvent may be successfully applied. Also useful is two-dimensional development, which is especially valuable for separating a greater number of alkaloids in a given section of the plant.

Great differences in the polarity of alkaloid molecules make gradient elution advantageous. This technique may be developed in both glass chambers and in horizontal chambers as well as with overpressured layer chromatography.

Worth noticing is one technique related to TLC – thin-layer electrophoresis, which has been used as a two-dimensional combination with TLC for the separation of ergot alkaloids.

Separation Methods

It is obvious that the kind of adsorbent used and solvent system composition determine the separation mechanism occurring in the chromatographic process. The adsorbent also determines the method of sample preparation. Thus, for adsorption and partition chromatography, alkaloids are mostly applied as bases in organic polar solvents; for ion exchange sorbents they are applied in the form of salts in aqueous solution.

Choosing the optimal chemical character of the stationary and mobile phase is especially important in the case of alkaloids because of the ionization ability of their molecules. Dissociation of bases in aqueous solution can be expressed by the following equation:

$$B + H_2O \Leftrightarrow BH^+ + OH^-$$

or, in the case of the conjugated acid BH^+, by:

$$BH^+ \Leftrightarrow B + H^+$$

with a dissociation constant (acidic) K_a.

The dependence of the molar ratio of nondissociated molecules [B] to the total concentration of an alkaloid [B] + [BH^+] on the pH of the mobile phase is shown in the curves presented in **Figure 2**. The pK_a values of chosen alkaloids are summarized in **Table 1**.

For TLC of alkaloids, numerous chromatographic systems have been reported. Some are presented in **Table 2**, together with their practical applications.

Adsorption Chromatography

Silica gel is the most frequently used solid-phase in adsorption chromatography. The weakly acidic properties of its surface may be the reason for the chemisorption of alkaloids, especially when neutral nonpolar solvents are used.

Tailing of spots may occur and the danger in using a neutral mobile phase is the formation of double spots, resulting from partial deprotonation of molecules if alkaloids are applied as salts. This is why

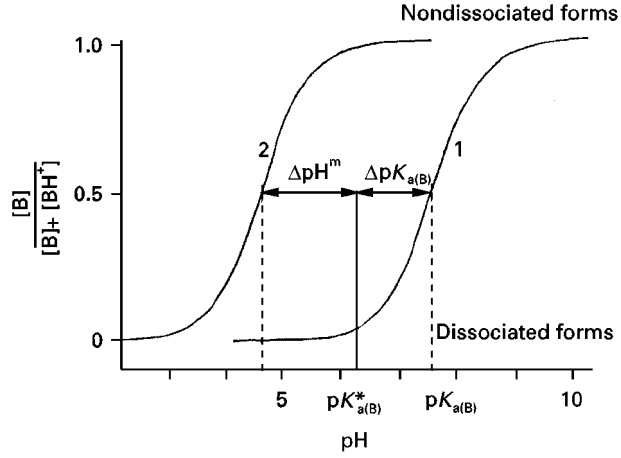

Figure 2 Dependence of the degree of dissociation of an alkaloid (B) on pH of buffer in mixed solvent ($\Delta pK_{a(B)} < 0$ and $\Delta pH_m > 0$). 1, solution in water; 2, solution in mixed solvent $pK^*_{a(B)} = pK_a$ in 50% (w/w) methanol (after Popl M, Fähnrich J and Tatar V (1990) *Chromatographic Analysis of Alkaloids. Chromatographic Science*. New York and Basel: Marcel Dekker, Inc.).

Table 1 Values of pK_a for the dissociation of alkaloids in water

Alkaloid	pK_a	*Alkaloid*	pK_a
Aconitine	8.35	Methylecgonine	9.16
Arecaidine	9.07	Morphine	8.21
Arecoline	7.41	Narceine	3.30
Atropine	9.85	α-Narcotine	6.37
Benzoylecgonine	11.80	Nicotine	8.02
Berberine	11.73		($pK_{a2} = 3.12$)
Brucine	8.16	Nicotyrine	4.76
	($pK_{a2} = 2.50$)	Papaverine	6.40
Caffeine	1.00	*d,l*-Pelletierine	9.40
Cinchonidine	8.40	Pilocarpine	6.87
	($pK_{a2} = 4.17$)	Piperine	1.98
Cinchonine	8.35	Protopine	5.99
	($pK_{a2} = 4.28$)	Quinidine	8.77
Cocaine	8.39		($pK_{a2} = 4.20$)
Codeine	8.21	Quinine	8.34
Colchicine	1.85		($pK_{a2} = 4.30$)
d-Coniine	10.90	Retronecine	8.88
Cytisine	8.12	1-Scopolamine	7.55
	($pK_{a2} = 1.20$)	Solanine	7.54
Emetine	8.43	Sparteine	11.96
	($pK_{a2} = 7.56$)		($pK_{a2} = 4.80$)
Ergometrine	6.73	Strychnine	8.26
Harmine	7.61		($pK_{a2} = 2.50$)
Heliotridine	10.55	Thebaine	8.15
Heroine	7.60	Theobromine	1.00
1-Hyoscyamine	9.65	Theophylline	1.00
		Tropacocaine	9.88
Isopilocarpine	7.18	Tropine	10.33
		Yohimbine	7.45
			($pK_{a2} = 3.00$)

Reproduced with permission from Popl *et al.* (1990).

silica gel is most often used in combination with basic mobile phases or the gel is impregnated with basic buffers or basic compounds (KOH, NaOH, $NaHCO_3$). Colchicine is the exception to these rules and, because of its neutral character, can be analysed in neutral solvent systems in combination with silica gel plates.

There are fewer applications using alumina. Basic alumina is most often used. The weakly basic character of the surface allows the use of neutral solvent systems as mobile phases. Depending on the nature of the alkaloids examined, neutral or acidic alumina may sometimes be more suitable.

As presented in detail in **Table 2**, solvent systems used in adsorption chromatography are either binary or ternary mixtures of chloroform, benzene, ethyl acetate and others. Alkalification of the mobile phase is achieved by the addition of ammonia, diethylamine, triethylamine or triethanolamine. Very interesting methods for choosing a suitable solvent were proposed in the late 1960s, and were based on the weighted average values of dielectric constants, and by the introduction of homogenous azeotropic mixtures (methanol–chloroform–methyl acetate, methanol–acetone-chloroform, methanol–benzene). When choosing the proper solvent strength, especially in complex eluent mixtures used for the analysis of alkaloids, the x_e, x_d, x_n parameters developed by Snyder are useful. They refer to the possibility of a solvent acting as a proton acceptor, proton donor or the one exhibiting strong dipole interaction. All possible compositions of quaternary, ternary and binary solvent mixtures have been described by the Prisma model. It may be applied either in normal or reversed-phase systems with the aim of optimizing the conditions of separation.

Pseudo-reversed-phase Chromatography

Chromatographic systems composed of silica gel and buffered aqueous organic mobile phases have been successfully used in recent years to isolate and separate alkaloids. The retention mechanism occurring here, described as pseudo-reversed phase, is fairly complex. An important role is played by the hydrophobic

Table 2 Examples of the most popular chromatographic systems for TLC of the main alkaloid groups

Compounds separated	*Applications*	*Adsorbent*	*Solvent system*
Phenylethylamine derivatives			
Ephedrine and its derivatives	Qualitative identification of ephedrine	Silica gel	Butanol–acetic acid–water (6 : 3 : 1)
		Silanized silica gel	1 mol L^{-1} acetic acid–3% potassium chloride
		Silanized silica gel impregnated with anionic and cationic detergents	1 mol L^{-1} acetic acid–methanol (80 : 20)
	Determination of ephedrine in Herba Ephedrae	Silica gel	Isopropyl ether–acetone–tetrahydrofuran (15 : 3 : 2)
	Determination of ephedrine in bulk drugs	Silica gel	*n*-Butanol–water–formic acid (7 : 2 : 1)
Colchicine and related compounds	Determination of colchicine in bulk drugs, dragees (BP)	Silica gel Aluminium oxide	Chloroform–acetone–ammonium hydroxide (5 : 4 : 0.2 or 25 : 20 : 0.4)
	Analysis of colchicine in tablets and plant material: *Colchicum autumnale* seeds, *Iphigena indica*	Silica gel	Chloroform–methanol (80 : 0.5)
	Separation of colchicine and 3-demethylocolchicine, demecolcine in Turkish *Colchicum* and *Merendera* species	Silica gel	Benzene–ethyl acetate–butylamine (5 : 4 : 1 or 7 : 2 : 1)
Imidazole alkaloids			
Pilocarpine	Qualitative identification of pilocarpine	Silica gel	Chloroform–acetone–diethylamine (5 : 4 : 1) Chloroform–acetone–water (5 : 4 : 1)
	Determination of pilocarpine in ocular system	Aluminium oxide Silica gel	Chloroform Methanol–1% potassium dihydrogen phosphate (pH 6 : 9 : 1)
	Determination of pilocarpine nitrate in bulk drugs (EP)	Silica gel	Chloroform–methanol–ammonium hydroxide (85 : 14 : 1)
	Separation of pilocarpine, isopilocarpine, pilocarpic acid and isopilocarpic acid in eye drops	Silica gel	Ethanol–chloroform–28% ammonium hydroxide (53 : 30 : 17)
Indole alkaloids			
Strychnos alkaloids	Determination of strychnine in biological specimens	Silica gel	Dichloromethane–methanol–water–formic acid–diethanolamine (72.3 : 25 : 2.5 : 0.1 : 0.1)
	TLC analysis of strychnine and brucine in plant extract from *Strychnos nux vomica*	Silica gel	Methanol–4 mol L^{-1} ammonium hydroxide (9 : 1)
	Separation of strychnine and brucine	Aluminium oxide	Benzene-ethanol (9 : 1 or 8 : 2)
Yohimbine type Rauwolfia alkaloids and related bases	Determination of reserpine in *Rauwolfia serpentina* and *R. cubana* stem bark	Silica gel Cellulose	Chloroform–methanol (19 : 1 or 9 : 1) Ethyl acetate–cyclohexane–diethylamine (210 : 90 : 1) Butanol–acetic acid–water (60 : 15 : 25)
	Isolation of alkaloids from *Mitragyna speciosa*	RP-18	Methanol–water (4 : 2)
	TLC analysis of extract from *Uncaria*	Silica gel	Chloroform–acetone (5 : 4) Ethyl acetate–isopropanol–ammonium hydroxide (100 : 2 : 1)
	Determination of serpentine and ajmalicine in *Catharanthus roseus*	Silica gel	Chloroform–methanol (9 : 1) Ethyl acetate–methanol (3 : 1) Chloroform–acetone–diethylamine (5 : 4 : 1)
	TLC analysis of ajmaline steroisomers, vincine, vincamine	Silica gel	Acetone–petrol ether–diethylamine (2 : 7 : 1) Hexane–chloroform–methanol (5 : 1 : 1)

Table 2 *Continued*

Compounds separated	*Applications*	*Adsorbent*	*Solvent system*
Ergot alkaloids	Determination of ergotamine tartrate in bulk drugs and	Silica gel	Dimethylformamide–ether–chloroform–ethanol (15 : 70 : 10 : 5)
	dihydroergotamine mesylate (USPXXI, EP,BP)	Silica gel	Chloroform–ethanol (9 : 1)
	Qualitative identification of hallucinogen ergot alkaloids from *Ipomoea Tricolor Cav*	Silica gel	Ethanol–tetrahydrofuran–ethyl acetate (1 : 1 : 8) Water–ethanol–ether (5 : 35 : 60) Acetonitrile–ethanol–toluene (85 : 10 : 5)
	Quantitative analysis of ergot alkaloids: lysergol, ergometrine, agroclavine, ergotamine, ergocristine, ergotaminine, ergocristinine	Silica gel (circular U-RPC)	Water–ethanol–ether (1 : 7 : 12) Acetonitrile–ethanol–toulene (17 : 2 : 1)
	Qualitative identification of ergot alkaloids	Silica gel	Stepwise gradient elution: **1** Chloroform–diethylamine (12 stages, 7 steps) **2** Chloroform–acetone–diethylamine (11 stages, 5 steps)
Pyridine and piperidine alkaloids			
Tobacco alkaloids	Rapid TLC identification of cytisine and nicotine	Silica gel	Dichloromethane–methanol–10% ammonium hydroxide (83 : 15 : 2)
	Determination of nicotine, nornicotine, anabasine, nicotyrine, 2,2-dipiridyl	Silica gel (OPLC)	Ethyl acetate–methanol–water (12 : 35 : 3)
Tropane alkaloids	Quantitative determination of atropine in Chinese medicine	Silica gel	Chloroform–acetone–methanol–ammonium hydroxide (70 : 10 : 15 : 1)
	Determination of atropine in pharmaceutical preparations: bulk drugs and injections (USPXXI)	Silica gel	Chloroform–diethylamine (9 : 1)
	Qualitative identification of atropine, scopolamine, tubocurarine in African arrow poison	Silica gel	Chloroform–cyclohexane–diethylamine (3 : 6 : 1)
	TLC analysis of *Belladonna* tinctura (atropine, scopolamine)	Silica gel with micro-crystalline cellulose (5 : 2)	Chloroform–acetone–methanol–ammonium hydroxide (73 : 10 : 15 : 2)
	Analysis of *Hyoscyamus* extract	Silica gel	Methanol-ammonium hydroxide (98 : 2) Chloroform–butylamine (9 : 1) Ethyl acetate–formic acid–ammonium hydroxide (10% : 83 : 15 : 2) Water–methanol–sodium acetate buffer (0.2 mol L^{-1} aqueous: 28 : 12 : 60 : 1)
Pseudotropine alkaloids	Determination of cocaine and local anaesthetics	Silica gel	Two-dimensional: **1** Cyclohexane–benzene–diethylamine (75 : 15 : 10) **2** Chloroform–methanol (8 : 1)
	Identification of alkaloids in *Erythroxylium hypericifolium* leaves	Aluminium oxide	Chloroform–ethanol (1 : 1) Butanol–ethanol (95 : 1)
Quinoline alkaloids			
Cinchona alkaloids	Quantitative analysis of 17 cinchona alkaloids	Silica gel	Chloroform–acetone–methanol–25% ammonium hydroxide (60 : 20 : 20 : 1)
	TLC analysis of cinchona alkaloids as pure substances	Silica gel	Chloroform–diethylamine (9 : 1) Chloroform–methanol–ammonium hydroxide (25% : 85 : 14 : 1) Kerosene–acetone–diethylamine (23 : 9 : 9)

Table 2 *Continued*

Compounds separated	*Applications*	*Adsorbent*	*Solvent system*
			Toluene–diethyl ether–diethylamine (20 : 12 : 5)
	Determination of quinidine and dihydroquinidine in serum	Silica gel	Ethyl acetate–ethanol–*n*-butanol–ammonium hydroxide (56 : 28 : 4 : 0.5)
	Preparative TLC quinoline alkaloids from *Orixa japonica* stems	RP-18 Silica gel	Methanol–water (2 : 1) Benzene–ethyl acetate (4 : 1)
	Determination of quinine hydrochloride, quinidine sulfate in bulk drugs (EP, BP)	Silica gel	Diethylamine–ether–toluene (10 : 24 : 40)
	Determination of cinchonine in bulk drugs	Silica gel sprayed with 0.1 mol L^{-1} methanolic potassium hydroxide	Ammonium hydroxide–methanol (1.5 : 100)
Isoquinoline alkaloids			
Protoberberine and protopine alkaloids	Determination of berberine in biological matrix	Silica gel	Ethyl acetate–methyl acetate–methanol–water (27 : 23 : 6 : 5)
	Separation of berberine in presence of quaternary alkaloids in plant extracts	Silica gel (OPLC)	Ethyl acetate–tetrahydrofuran–acetic acid (6 : 2 : 2)
	Quantitative analysis and qualitative identification of protoberberine alkaloids	Silica gel	Two-step development in twin trough chamber: **1** Ethyl acetate-methanol–ammonium hydroxide (10 : 10 : 1) **2** Benzene–ethyl acetate–isopropanol–methanol–water (20 : 10 : 5 : 5 : 1) Second trough containing 5 mL conc. NH_3
	Quantitative analysis of berberine in capsule	Silica gel	Ethyl acetate–acetone–formic acid–water (20 : 17 : 4 : 2)
	TLC analysis of protopine and allocryptopine from Turkish *Papaver curviscapum*	Silica gel	Benzene–ethanol–ammonium hydroxide (8 : 2 : 0.03) Benzene–acetone–methanol (7 : 2 : 1) Toluene–acetone–methanol–ammonium hydroxide (45 : 45 : 7 : 3)
	Determination of berberine in bulk drugs	Silica gel sprayed with 0.1 mol L^{-1} methanolic potassium hydroxide	Ammonium hydroxide–methanol (1.5 : 100)
	Determination of sanguinarine, chelidonine, protopine, allocryptopine in *Chelidonium maius*	Silica gel	Toluene–methanol–diethylamine (60 : 5 : 2) saturated with formamide
Morphine alkaloids	Analysis of morphine alkaloids in opium	Silica gel	Benzene–ethanol (17 : 1 or 9 : 1) Benzene–dioxane–ethanol–ammonium hydroxide (50 : 40 : 5 : 5) Toluene–acetone–ethanol (96%)–ammonium hydroxide (25%) (45 : 45 : 7 : 3) Hexane–chloroform–diethylamine (50 : 30 : 7) Ethyl acetate–methanol–ammonium hydroxide (85 : 10 : 5 or 75 : 20 : 5)
	Determination of morphine and semisynthetic derivatives	Silica gel	Chloroform–triethanolamine (95 : 5) Chloroform–methanol–water (7 : 5 : 1) Butanol–ammonium hydroxide–water–methanol (20 : 1 : 4 : 2)

Table 2 *Continued*

Compounds separated	*Applications*	*Adsorbent*	*Solvent system*
	Determination of Dabsyl derivatives of morphine in urine	Silica gel	Chlororoloform–ethanol–triethanolamine (30 : 2 : 0.05)
Isoquinoline bases	Determination of papaverine, codeine, eupaverine	RP-18 (IP-TLC)	Water–acetone (20 : 80, 100 : 0) with 0.1 mol L^{-1} of ion reagent–sodium alkylsulfonate
	Determination of emetine and tubocurarine	Silica gel	Ethyl acetate–isopropanol–ammonium hydroxide (25% : 9 : 7 : 2)
	TLC analysis of emetine hydrochloride in bulk drugs (USPXXI, BP)	Silica gel	Chloroform–diethylamine (9 : 1)
	TLC analysis of codeine in bulk drugs (EP)	Silica gel	Ammonium hydroxide–cyclohexane–ethanol (6 : 30 : 72)
	TLC analysis of papaverine hydrochloride in bulk drugs (EP)	Silica gel	Diethylamine–ethyl acetate–toluene (1 : 2 : 7)
	Determination of codeine, chlorpheniramine, phenylephrine, paracetamol (acetaminophen) in syrup and tablets	Silica gel	Butanol–methanol–toluene–water–acetic acid (3 : 4 : 1 : 2 : 0.1)
Benzylisoquinoline alkaloids	Determination of alkaloids in *Anisocycla cymosa* roots and plant extract	Silica gel	Chloroform–methanol–diethylamine–ammonium hydroxide (8 : 2 : 2 : 0.5) Benzene–acetone–ammonium hydroxide (15 : 15 : 1)
	Determination of bisbenzylisoquinoline alkaloids in *A. jollyana* leaves	Silica gel	Chloroform–toluene–methanol–acetone–ethyl acetate–ammonium hydroxide (270 : 30 : 80 : 30 : 3)
		Aluminium oxide	Toluene–chloroform–methanol–ammonium hydroxide (100 : 150 : 40 : 3)
Aporphine alkaloids	Analysis in plant material	Silica gel	Cyclohexane–ethyl acetate (3 : 2) Cyclohexane–acetone (9 : 1) Petrol ether–acetone (7 : 3) Chloroform–methanol (9 : 1)
Various isoquionoline alkaloids	Determination of cocaine, heroin and local anaesthetics in street drugs	Silica gel	Benzene–chloroform–triethanolamine (9 : 9 : 4) Ethyl acetate–isopropanol–28% ammonium hydroxide (40 : 30 : 3)
	Analysis of major drugs of abuse in urine	Silica gel	Ethyl acetate–cyclohexane–methanol–ammonium hydroxide (conc.)–water (70 : 15 : 8 : 2 : 0.5) Ethyl acetate–cyclohexane (50 : 60)
Diterpene and steroidal alkaloids			
Diterpene	Determination of aconitine nitrate in bulk drugs	Silica gel spray 0.1 mol L^{-1} potassium hydroxide methanol	Ammonium hydroxide–methanol (1.5 : 100)
	Determination of aconitine, 3-deoxyaconitine, mesaconitine in *Wutou* and *Aconitum*	Silica gel	Cyclohexane–ethyl acetate–ethylenediamine (8 : 1 : 1)
		Aluminium oxide (neutral)	Gradient elution: hexane, hexane–diethyl ether (25 : 75), diethyl ether, diethyl ether–methanol
	Isolation of norditerpenoid alkaloids from extract of roots of *Delphinum tatsienense*	Silica gel (centrifugal TLC)	Diethyl ether–75% methanol–0.3% diethylamine
		Silica gel (preparative TLC)	Diethyl ether–5% methanol
	TLC of 8 diterpenoid alkaloids from *Aconitum septentrionale*	Silica gel	Hexane–chloroform (6 : 4)
		Aluminium oxide (centrifugal TLC)	Chloroform–methanol (8 : 2 or 97 : 3) Gradient of hexane, ether and methanol

Table 2 *Continued*

Compounds separated	*Applications*	*Adsorbent*	*Solvent system*
Steroidal alkaloids	Isolate ecdysteroids from the herba of *Siline tatarica*	Silica gel (droplet countercurrent chromatography)	Ethyl acetate–methanol–ammonium hydroxide (17 : 5 : 3) Dichloromethane–ethanol (17 : 3) Chloroform–methanol–acetone (6 : 2 : 1) Methanol–water (13 : 7)
Veratrum alkaloids	Determination of veratrum alkaloids jervine, veratroylzygadenine, rubijervine, isorubijervine, veromine in *Veratrum* root and tincture	Silica gel	Benzene–ethanol–diethylamine (80 : 16 : 4)
		Aluminium oxide	Benzene–ethanol (95 : 5)
Solanum alkaloids	Determination of solanum alkaloids (solanidine) from spiked milk and α-solasonine, α, β-solamargine from *Solanum ptycanthum*	Silica gel	Methanol–chloroform–1% ammonium hydroxide (2 : 2 : 1)
Miscellaneous heterocyclic systems			
Pyrrolizidine alkaloids	TLC analysis in plant material	Silica gel	Dichloromethane–methanol–ammonium hydroxide (85 : 15 : 2 or 75 : 23 : 2 or 79 : 20 : 1) Chloroform–methanol (4 : 1)
		Silica gel impregnated with 0.1 mol L^{-1} NaOH	Chloroform–methanol–ammonium hydroxide (60 : 10 : 1 or 17 : 38 : 0.25)
Lupin alkaloids	TLC of lupanine and 7-hydroxylupanine from *Cytisophyllum seccilifolium*	Silica gel	Chloroform–methanol–28% ammonium hydroxide (85 : 14 : 1)
Carbazole alkaloids		Silica gel	Benzene–chloroform (1 : 1)
Xanthine alkaloids	Qualitative identification and preparative TLC of alkaloids from *Bosistoa floydi* leafs	Silica gel	Chloroform–ethyl acetate (3 : 2)
Purine bases	Determination of purine bases in urine	Silica gel	Two-dimensional: **1** Chloroform–methanol (4 : 1) **2** Butanol–chloroform–acetone–ammonium hydroxide (4 : 3 : 3 : 1)
	Determination of caffeine, theophylline and 15 drugs in Chinese herbal preparations	Silica gel	Dichloromethane–methanol–water (183 : 27 : 5) Ethyl acetate–toluene–dimethylformamide–formic acid (75 : 70 : 4 : 2) Dichloromethane–methanol (183 : 27)
	Determination of caffeine and theobromine in bulk drugs (EP)	Silica gel	Ammonium hydroxide–acetone–chloroform–butanol (1 : 3 : 3 : 4)
	Determination of theophylline in capsules (USPXXI) in tablets with ephedrine hydrochloride and phenobarbital (USPXXI, EP)	Cellulose Silica gel	Methanol–water Chloroform–acetone–methanol–ammonium hydroxide (50 : 10 : 10 : 1)
Quinolizidine	Qualitative identification	Silica gel	Chloroform–cyclohexane–butylamine (5 : 4 : 1)
		Aluminium oxide	1.5% Methanol in chloroform

BP, British Pharmacopoeia; EP, European Pharmacopoeia, USPXXI, The United States Pharmacopeia, Twenty-first Revision.

interactions of siloxane groups with the non-polar fragments of the separated alkaloids, as well as by ion exchange interactions. In the retention of alkaloids a dominant role is played by the ion exchange mechanism which is due to the weak cation exchange properties of silica gel at pH = 2–8 and the fact that aromatic amines chromatographed in an aqueous mobile phase are weakly protonized at pH = $pK_a - 1$. The selectivity of such systems depends then, primarily, on the pH of the mobile phase but

also on the kind of organic modifier, which is usually methanol or acetonitrile.

Reversed-phase Chromatography

Nonpolar adsorbents have rarely been applied in TLC of alkaloids, perhaps because of the low efficiency of such systems, which is caused by the interaction of alkaloid molecules with silanol groups present on the adsorbent surface in addition to the hydrocarbon chains. In reversed-phase chromatography on silanized silica gel, alkaloids as easily ionized compounds require specific conditions of analysis such as suppression of dissociation, ion suppression or the application of specific ion pair reagents.

The suppression of dissociation is achieved with a mobile phase of a suitable pH ($pH \geq pK_a$) for the specific solvent, in accordance with the curve shown in **Figure 2**.

Reversed-phase conditions may also be obtained by impregnating silica gel with paraffin or silicone oil. Additionally, chemically bonded reversed phases with polar groups (cyano- and amino-layers) have been employed. Their properties depend on the kind of compounds to be separated and on the composition of the mobile phase.

Ion Pair Chromatography

The use of ion pair chromatography (IP-TLC) of alkaloids may be carried out on normal and reversed phases. This technique has been applied to analyse basic drugs, including alkaloids, on silica gel using normal-phase systems. The best results are obtained by applying chloride and bromide as counterions of at least $0.1\,mol\,L^{-1}$ concentration in the spreading slurry or in the solvent.

Reversed-phase IP-TLC is far more widely used. The counterion reagents which may be present in the mobile phase and serve for impregnation in the nonpolar stationary phase may be di-(2-ethylhexyl) orthophosphoric acid (HDEHP), camphoric and camphorosulfonic acids, sodium dodecylsulfate and simple hydrophilic anionic reagents such as perchloric acid, oxalic acid and trichloroacetic acid. The acidic environment of the mobile phase ensures ionization of the acidic counterions and enables the creation of an ion pair with the alkaloids protonized under these conditions. The behaviour of some isoquinoline bases using RP-18 plates and alkylsulfonates as counterions has also been investigated.

Although retention and separation selectivity in IP-TLC depend on many factors, optimization of such chromatographic systems is basically concerned with pH changes, concentration and the chain length of the counterion or the concentration of organic modifier in the mobile phase.

Partition Chromatography

In the past, partition chromatography conducted on paper was a perfect model for establishing optimum extraction systems for alkaloid isolation. In paper chromatography, the system allowing partition conditions is mainly composed of cellulose with an aqueous solvent or an aqueous buffer solution of pH 3–7, depending on the nature of the alkaloids. Silica gel combined with an aqueous phase or a water-saturated organic solvent also allows for the domination of the partition mechanism, thanks to deactivation of the surface silanol groups. The aqueous phases in such systems are often alkalized with aqueous ammonium hydroxide or acidified with hydrochloric acid.

Partition conditions, similar to paper chromatography, may be obtained by impregnating cellulose or silica gel with a solution of formamide in ethanol and using mobile phases immiscible with the stationary phase, such as chloroform, benzene, cyclohexane or their mixtures.

Ion Exchange Chromatography

Ion exchange techniques are applied for both crude fractionation and separation and determination of alkaloids.

The typical ion exchange sorbents used for TLC of alkaloids have been as follows: anion exchangers AG 1-X4 and Cellex D, and cation exchangers with cellulose (alginic acid and sodium carboxymethylcellulose), paraffin (Rexyn 102) and polystyrene (Dowex 50-X4) matrices.

While choosing the best eluent for ion exchange chromatography, pH values should be carefully considered. They are closely correlated with the number of charges in the alkaloid molecules and at the same time decide the retention values. The trends for most alkaloids fit the type of curves shown in **Figure 3**.

One of the popular adsorbents which may function as an ion exchanger is aluminium oxide (AI_2O_3) with an aqueous mobile phase. Depending on the kind of aluminium oxide used, a cation- or anion-exchanging mechanism may occur. Thus, in aqueous alcoholic solution basic alumina functions as a cation exchanger (I), but acidic alumina acts as an anion exchanger (II). With neutral alumina, both types of reactions may take place depending on the conditions used:

$$\text{(I)}\quad \text{Al–O–Na} + (\text{BH})^+\text{Cl}^- \rightarrow \text{Al–OH} + \text{B} + \text{Na}^+ + \text{Cl}^-$$

$$\text{(II)}\quad \text{Al–Cl} + \text{BH}^+\text{OH}^- \rightarrow \text{Al–OH} + (\text{BH})^+\text{Cl}^-$$

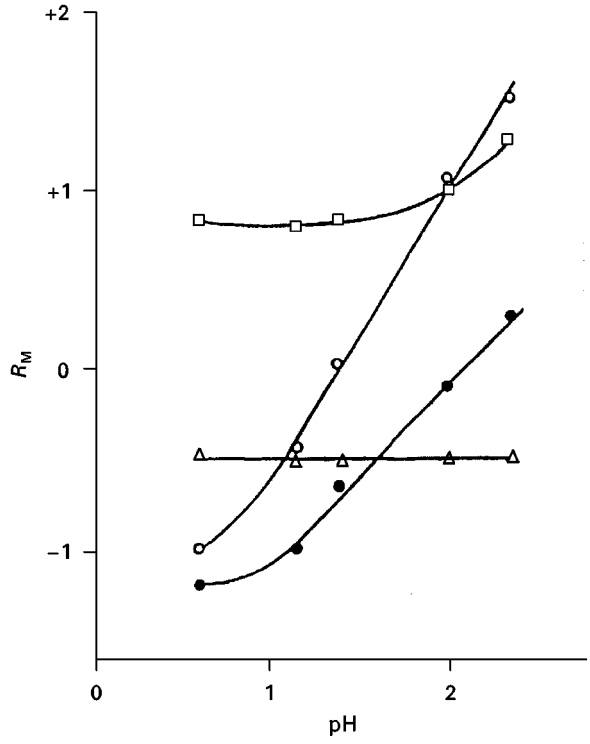

Figure 3 R_M versus pH curves for some alkaloids on alginic acid thin layers (after Lepri L, Desideri PG and Lepori M (1976) Chromatographic Behaviour of Alkaloids of Thin Layer of Cation Exchangers. Journal of Chromatography 123, 175. Amsterdam: Elsevier).

Adsorbents Impregnated with Metal Salts

The use of silica gel and aluminium oxide impregnated with metal salts (cadmium and zinc nitrate) for the separation of some alkaloids has been studied.

For steroid alkaloids, the impregnation of the stationary phase with silver salts – so-called argentation TLC – has been applied. This technique is based on the formation of π-complexes with the separated compounds during the chromatographic process.

Detection of Alkaloids

Only a few alkaloids are directly visible on the chromatogram as coloured spots and visualization methods have to be applied to detect them. In order to detect the compounds under UV light, fluorescing indicators are added to the adsorbent.

Alkaloids become visible in short wavelength UV light ($\lambda = 254$ nm), where they appear as dark zones on a fluorescent background. This is considered to be a nonselective method of detection because, on the layer containing a fluorescent indicator, the emission is quenched in regions where all aromatic organic compounds absorb the UV light with which the plates are irradiated.

Some alkaloids, such as indoles, quinolines, isoquinolines and purines, cause pronounced quenching of fluorescence, but some (e.g. tropine alkaloids) only weakly quench UV light. Sometimes compounds can be detected under a UV lamp due to their own luminescence. Excitation is usually performed using long wavelength radiation of $\lambda = 365$ nm. Alkaloids absorb radiation and then usually emit it in the visible region of the spectrum, where they appear as bright-coloured luminous zones of blue, blue-green or violet, for example, Rauwolfiae radix, Chinae cortex, Ipecacuanhae radix, Boldo folium, and of yellow, e.g. colchicine, sanguinarinae, berberine.

Other methods of physical detection make the most of the chemical properties of alkaloids. As basic compounds, these properties can be detected using pH indicators applied to the chromatogram by dipping it or spraying it with 0.01–1% indicator solutions.

Bromocresol Green with pH transition from 3.8 to 5.4 is applied for many alkaloids; Bromocresol Purple (pH = 5.2–6.8) is predominantly applied for ephedrine.

Another nonselective detection method for alkaloids as lipophilic substances is the treatment of a chromatogram with iodine vapour or dipping into or spraying with 0.5–1% iodine solutions. Molecular iodine is enriched in the chromatogram zones and colours them brown. Emetine and cephaeline, the two major alkaloids of ipecacuanha, begin to glow after treatment with iodine vapour. In this case, the molecular iodine which acts as a quencher must be removed by heating, before the yellow (emetine) and blue (cephaeline) fluorescent zones become visible.

Although the methods described are usually fairly sensitive and allow a detection limit of less than 1 μg, sometimes they are insufficient. That is why they have to be supplemented by specific reactions with a number of detection reagents (**Table 3**).

The most popular reagents which react with tertiary and quaternary nitrogen atoms present in alkaloid molecules are Dragendorff's reagent and potassium iodoplatinate. Alkaloids containing primary and secondary amino groups treated with dimethyl sulfate give quaternary nitrogen atoms, permitting effective detection with these reagents too.

Dragendorff's and iodoplatinate reagents exists in various modifications. The replacement of water in these reagents by acetic acid or ethyl acetate, diethyl ether–methanol or hydrochloric acid increases the sensitivity of the reaction and significantly improves the sharpness of spots. Spraying 10% sodium nitrate solution after the use of Dragendorff's reagent causes

Table 3 Selection of detection reagents for postchromatographic derivatization of alkaloids

Reagent	*Substances detected*	*Reaction*	*Method*	*Result*
Ammonia vapour	Alkaloids, e.g. morphine, heroin, 6-mono-acetylmorphine	Morphine and heroin form fluorescent oxidation products	Heat the chromatogram in the drying cupboard to 110–120°C for 25 min and place it for 15 min in a twin-trough chamber, whose second trough contains 10 mL of 25% ammonia solution. Then immerse for 2 s in a solution of liquid paraffin–*n*-hexane (1 : 2)	Morphine, 6-monoacetylmorphine and heroin appear as blue fluorescent zones on a dark background under UV light ($\lambda = 365$ nm). In each case the detection limits are 2 ng of substance per chromatographic zone. The fluorimetric determination is carried out in UV light $\lambda_{exc} = 313$ nm, $\lambda_{fl} = 390$ nm
Formaldehyde reagent (1,2-naphthoquinone-4-sulfonic acid)–perchloric acid	Alkaloids, e.g. codeine, morphine, heroin, 6-mono-acetylmorphine	The reaction mechanism has not been elucidated. It is possible that formaldehyde reacts by oxidation, as in Marquis reaction	Dry the chromatogram in a stream of warm air for 5 min, immerse in the reagent solution for 4 s and heat to 70°C for *c.* 10 min	Morphine alkaloids yield blue chromatogram zones on a pale blue background. The detection limits are 10–20 ng of substance per chromatogram zone. The absorption photometric analysis can be performed at reflectance $\lambda = 610$ nm
2-Methoxy-2, 4-diphenyl-3(2H)-furanone (MDPF)	Alkaloids from *Colchicum autumnale* (Colchicine)	MDPF reacts directly with primary amines to form fluorescent products	Free the chromatogram from mobile phase in a stream of warm air (45 min), immerse in the reagent solution for 4 s and then heat to 110°C for 20 min	Colchicine appears as a yellow fluorescent zone on a dark background in UV light (365 nm). The detection limit is 10 ng per chromatogram zone. The fluorimetric analysis is carried out with excitation at $\lambda_{exc} = 313$ nm, and evaluation at $\lambda_{fl} > 390$ nm
2,4-Dinitrophenyl-hydrazine	Alkaloids	Reagent reacts with carbonyl groups with the elimination of water to yield hydrazone and with aldoses or ketoses to yield coloured osazones	Immerse the chromatogram in the dipping solution for 2 s or spray and then dry in a stream of warm air (10–20 min at 110°C)	Substances yield yellow to orange-yellow chromatogram zones on an almost colourless background
2,6-Dichloro-quinone–4-chloroimide	Isoquinoline alkaloids	Reagent reacts with phenols or anilines which are not substituted in the *p*-position	Dry the chromatogram for 5 min in a stream of warm air, immerse in the dipping solution for 5 s and then heat to 110°C for 2 min	Cephealine produces a blue colour immediately on immersion, while emetine only does so on heating. On storage this colour slowly changes to brown (background light brown). The detection limits are *c.* 10 ng per chromatogram zone. The absorption photometric analysis was made at $\lambda = 550$ nm
o-Phthal-aldehyde (OPT, OPA)	Ergot alkaloids	In the presence of 2-mercaptoethanol, *o*-phthalaldehyde reacts with primary amines to yield fluorescent isoindole derivatives	Immerse the dried chromatogram for 1 s in the reagent solution and then heat to 40–50°C in the dry cupboard for 10 min	Substance zones are produced that mainly yield blue (or yellow) fluorescence under long wavelength light ($\lambda = 365$ nm)
Phosphomolybdic acid	Morphine	Morphine can be oxidized with phosphomolybdic acid, whereby a portion of the Mo(VI) is reduced to Mo(IV) which forms blue-grey oxides with the remaining Mo(VI)	Dry the chromatogram in a stream of warm air and immerse for 2–3 s in the reagent solution, or spray the layer with it	Blue zones appear on a yellow background immediately or after a few minutes

Table 3 *Continued*

Reagent	*Substances detected*	*Reaction*	*Method*	*Result*
Trichloroacetic acid	Alkaloids from, e.g. *Veratrum colchicum*	The reaction mechanism has not yet been elucidated	Dry the chromatogram in a stream of cold air and immerse for 1 s in the reagent solution or spray with it and then heat at 120°C for 10 min	Light blue fluorescent zones appear mainly under long wavelength UV light ($\lambda = 365$ nm). The fluorescence can be stabilized and intensified by dipping the plate into a solution of liquid paraffin–*n*-hexane (1 : 2)
Sulfuric acid	Alkaloids	The reaction mechanism has not yet been elucidated	Dry the chromatogram in a stream of warm air for 10 min, immerse in the dipping solution for 1–2 s or spray with the spray solution, dry in a stream of warm air and then heat to 95–140°C for 1–20 min	Under long wavelength UV light ($\lambda = 365$ nm) characteristic substance-specific yellow, green, red and blue fluorescent chromatogram zones usually appear, and are often recognizable in visible light
7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-chloride reagent)	Alkaloids	NBD reacts with nucleophilic compounds to yield the corresponding 7-substituted 4-nitrobenzofurazan derivatives	Dry the chromatograms. Immerse in dipping solution of sodium acetate in methanol–water for 1 s. Dry in a stream of warm air and dip after cooling in NBD-chloride reagent in ethanol and then heat to 100°C for 2–3 min. Alternatively the chromatogram can be sprayed with the appropriate spray solutions	Under UV light ($\lambda = 365$ nm) the chromatogram zones fluoresce greenish-yellow, olive brown or violet. The plate background also fluoresces, but appreciably less. The detection limits are 100–800 ng substance per chromatogram zone
tert-Butyl hypochlorite	Alkaloids	The reaction mechanism has not yet been elucidated	Dry the chromatogram, immerse in dipping solution of reagent in carbon tetrachloride or cyclohexane for 1 s (or spray or expose to its vapours) then immerse in dipping solution of chloroform, paraffin oil and triethanolamine (6 : 1 : 1) for 1 s and dry in hot air	The analysed compounds appear in long wavelength UV light (365 nm), yellow to violet fluorescent zones, on a dark background. The detection limit for morphine is 10 ng and for papaverine 1 ng per chromatogram zone
Formaldehyde-sulfuric acid (Marquis reagent)	Alkaloids, e.g. morphine, codeine, heroin, 6-monoacetylmorphine	Morphine reacts with formaldehyde in acidic solution to yield a cyclic ketoalcohol, which is transformed into the coloured oxonium or carbenium ion in acidic conditions	Dry the chromatogram in a stream of warm air for 5 min, immerse in the dipping solution for 6 s and heat to 110°C for 20 min	Morphine alkaloids yield reddish chromatogram zones (codeine yielded blue on a pale pink background). If a quantitative fluorimetric analysis is to follow, the chromatogram is exposed to ammonia vapour for 20 min and immersed for 2 s in 20% dioctyl sulfosuccinate in chloroform. After drying, morphine alkaloids appear as pink to red flourescent zones on a blue fluorescent background under UV light ($\lambda = 365$ nm). The fluorimetric analysis is carried out at $\lambda_{exc} = 313$ nm, $\lambda_{fl} = 560$ nm

Table 3 *Continued*

Reagent	*Substances detected*	*Reaction*	*Method*	*Result*
Iron (III) chloride-perchloric acid (FCPA reagent)	Indole alkaloids, e.g. from *Rauwolfia, Tabernaemontana, Mitragyna, Strychnos, Synclisia, Cinchona*	The reaction mechanism has not yet been elucidated	Free the chromatogram from mobile phase in a stream of warm air (45 min), immerse in the dipping solution for 4 s. Dry and heat to 110°C for 20 min	Variously coloured chromatogram zones are produced on a colourless background. For instance, strychnine appears as a red and brucine as a yellow chromatogram zone on a colourless background. The detection limit for both is 10 ng per chromatogram zone. The light absorption in reflectance was measured at $\lambda = 450$ nm
Hydrochloric acid vapour	Alkaloids, e.g. papaverubines	The reaction mechanism has not yet been elucidated	Free the chromatogram from mobile phase (first in a stream of cold air for a few minutes, than at 100°C for 5 min), place in the free trough of the prepared twin-trough chamber for 5 min and then evaluate	Alkaloids are visible after irradiation with unfiltered UV light from a mercury lamp

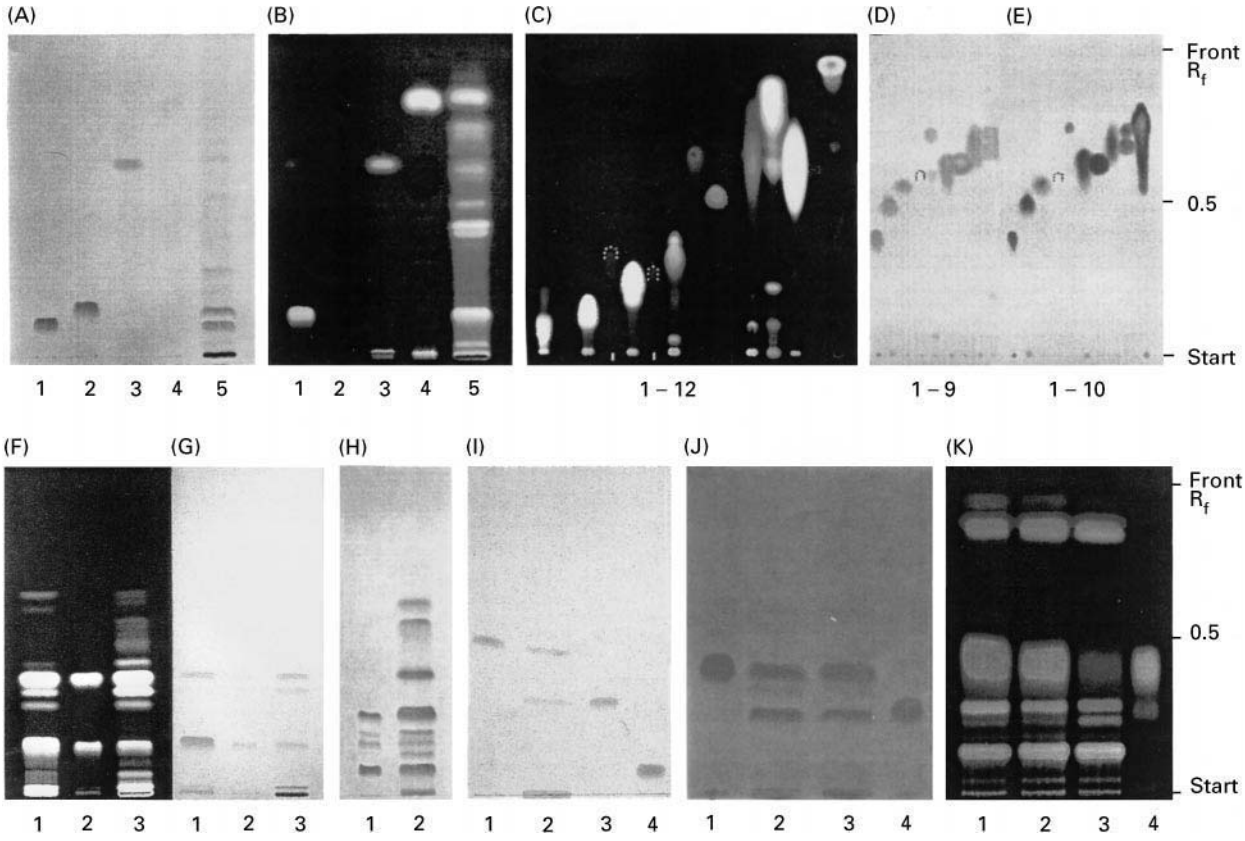

Figure 4 (See Colour Plate 54). The chromatograms of the separated alkaloids developed on silica gel or alumina in solvent systems 1–4, detected with different reagents. Solvent systems: 1, toluene–ethyl acetate–diethylamine (70 : 20 : 10); 2, chloroform–diethylamine (90 : 10); 3, toluene–chloroform–ethanol (28.5 : 57 : 14.5); 4, 1-propanol–water–formic acid (90 : 9 : 1). For identification of compounds, reagents used and obtained results, see Table 4. (Reproduced with permission from Wagner H and Bladt S (1996) *Plant Drug Analysis. Thin-layer Chromatography Atlas.* Berlin: Springer.)

Table 4 Symbols used in Figure 4

Symbol	*Detection*	*Solvent system*	*Reference compounds*	*Result*
A	Marquis reagent → vis	1	Morphine (1), codeine (2), papaverine (3), noscapine (4), opium extract (5)	Morphine and codeine are immediately stained violet; papaverine: weak violet; noscapine: weak yellow brown
B	Natural products, polyethylene glycol reagent (NP/PEG) → UV 365 nm	1		Morphine, papaverine, noscapine give a blue fluorescence in UV 365 nm; codeine does not fluoresce
C	Sulfuric acid reagent → UV 365 nm	1	Serpentine (1), quinine (2), cinchonine (3), quinidine (4), cinchonidine (5), cephaaeline (6), emetine (7), yohimbine (8), noscapine (9), hydrastine (10), berberine (11), sanguinarine (12)	The fluorescence of quinine and quinidine is a radial blue; cinchonine and cinchonidine: deep violet, berberine and sanguinarine: bright yellow
D	Dragendorff reagent → vis		Strychnine (1), yohimbine (2), physostigmine (3), nicotine (4),	Alkaloids give orange-brown, stable colours
E	Dragendorff reagent followed by sodium nitrite → vis	1	veratrine (5), emetine (6), papaverine (7), lobeline (8), aconitine (9), narcotine (10)	The zones become dark brown
F	Iodine/$CHCl_3$ reagent → UV 365 nm	1	*Cephaelis accuminata* (1), cephaeline: $R_f \sim 0.2$; emetine:	Cephaeline fluoresces bright blue and emetine: yellow-white
G	→ vis	1	$R_f \sim 0.4$ (2). *Cephaelis ipecacuanha* (3)	Cephaeline gives red and emetine weak yellow zones
H	10% H_2SO_4 followed by iodoplatinate reagent → vis	2	China alkaloid mixture (1) *Cinchona succirubra* (2)	The violet-brown zone of quinine is followed by the grey-violet zone of cinchonidine, a weak red-violet zone of quinidine and brown-red cinchonine (1) In *Cinchona succirubra* extract additionally three red-violet zones appear in the R_f range 0.4–0.6 (2)
I	van URK reagent → vis	3	Ergocristine (1), *Secale cornutum* (2), ergotamine (3), ergometrine (4)	Secale alkaloids appear as blue zones in the R_f range of 0.05–0.4
J	UV 254 nm	1	Strychnine (1), *Strychni semen* (2), *Ignatii semen* (3), brucine (4)	Strychnine and brucine are characterized in UV 254 nm by their strong quenching zones
K	UV 365 nm	4	*Chelidonii herba* different trade samples (1–3), sanguinarine (4)	The major alkaloid coptisin at $R_f \sim 0.15$ (bright-yellow) is followed by berberine, chelerythrine, sanguinarine (broad yellow) and chelidonine (weak yellow-green) in the R_f range of 0.75–0.85

the colour of alkaloid zones to be intensified or stabilized and increases the sensitivity to 0.01–0.1 μg.

Modification, where a chromatogram is sprayed with 10% sulfuric acid after the use of Dragendorff's reagent, also causes an increase in the sensitivity of the reaction. Potassium iodoplatinate reagent gives preliminary identification, due to the fact that different colours are obtained with different alkaloids.

Table 5 Examples of prechromatographic derivatization of alkaloids

Prechromatographic derivatization	*Reagent used*	*Special applications*
Oxidation	10% Chromic acid in glacial acetic acid Potassium dichromate Dehydration by heating the applied sample on silica layer	Strychnine and brucine
Reduction	Sodium borohydride solution	Not specified
Iodination	Iodine vapour saturated chamber (18 h)	Quinoline, isoquinoline, indole alkaloids
Nitration	Concentrated nitric acid	Brucine
Dansylation	Dansyl chloride and twice bigger volume of 8% sodium bicarbonate solution	Morphine, 6-monoacetylmorphine, morphine-6-nicotinate

Table 6 Systematic analysis of alkaloids on TLC plates

Chemical skeleton	*Plant drug*	*Botanical origin*	*Major alkaloid*	*Fluorescence in UV light (366 nm)*	*Colour with iodoplatinate reagent*	*hR_F values*							
						S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Tropane	Fol. Belladonnae Rad. Belladonnae	*Atropa belladonna* L., Solanaceae	Atropine		Violet-blue	38	40	16	5	12	0	10	17
	Fol. Hyoscyami	*Hyoscyamus niger* L., Solanaceae	Homatropine		Violet-blue	37	45	15	5	23	4	24	15
	Fol. Stramonii	*Datura stramonium* L., Solanaceae	Apoatropine		Violet-blue	54	67	40	20	26	15	40	16
	Rad. Scopoliae	*Scopolia carniolica* Jacq. Solanaceae	Scopolamine		Violet	56	60	19	3	34	30	0	52
			Scopoline		White	60	90	44	20	44	46	50	37
	Fol. Duboisiae	*Duboisia myoporoides* R. Br., Solanaceae	Tropacocaine		Violet	65	90	56	34	45	58	78	35
	Fol. Cocae	*Erythroxylon coca* Lamarck Erythroxylaceae	Cocaine		Violet	73	90	65	36	58	84	77	62
Indole	Semen Calabaris	*Physostigma venenosum* Balfour Papilionaceae	Physostigmine		Pink	65	>90	32	4	44	59	50	46
	Rad. Rauwolfiae	*Rauwolfia serpentina*	Reserpine	Green-yellow	White	72	80	20	0	46	63	35	69
	Rad. Serpentinae	Bentham, Apocynaceae	Serpentinine	Dark brown	Red-brown	24	15	0	0	4	0	0	0
	Semen Strychni	*Strychnos nux vomica* L., Loganiaceae	Serpentine	Yellow-green	Yellow-brown	53	56	8	0	10	0	3	12
			Ajmaline	Blue	Beige	47	42	12	3	30	6	13	56
			Strychnine		Yellow	53	76	28	5	38	57	60	22
	Cortex	Pausinystalia	Brucine		Violet-brown	42	63	18	0	19	50	54	12
	Yohimbehe	Yohimbe Pierre, Rubiaceae	Yohimbine	Green-blue	Light yellow	63	62	18	3	37	33	15	60
			Ergocristinine	Violet-blue	Light brown	61	57	13	0	20	0	27	70
	Secole cornutum	*Claviceps purpurea* Tulasne Clavicipitaceae	Ergotamine	Violet-blue	Pink	24	16	0	0	3	10	5	59
			Ergometrine	Violet-blue	White	14	6	0	0	2	3	0	64
			Ergometrinine	Violet-blue	Violet-blue	42	25	3	0	8	12	10	62
			Ergocristine	Violet-blue	Beige-light brown	51	38	14	5	13	46	15	70
			Ergotaminine	Violet-blue	Pink	24	51	0	0	14	42	15	68
			Dihydroergotamine	Violet-blue	Brownish	21	12	0	0	3	7	0	61
			Dihydroergocristine	Violet-blue	Brownish	12	30	3	0	7	15	7	69
Isoquinoline	Opium	*Papaver somniferum* L., Papaveraceae	Thebaine		Red-brown	65	90	51	16	50	71	76	40
			Narceine		Deep-blue	3	0	0	0	3	0	0	0
			Morphine		Deep-blue	10	8	0	0	3	3	0	34
			Papaverine	Yellowish	Yellow	67	90	42	3	47	85	84	70
			Codeine		Pink-violet	38	53	16	4	26	12	27	35
			Noscapine	Blue	Light-yellow	72	90	51	10	57	81	79	72
			Hydrastinine	Steel blue	Violet-blue	66	90	58	41	50	0	25	0
			Dihydromorphinone		Brownish yellow	24	23	8	1	11	5	8	16
			Dihydrocodeine	Blue	Violet-blue	38	54	18	6	28	10	30	25
			Dihydrocodeinone		Violet	51	65	21	4	30	48	43	18
	Fol. Boldo	*Peumus boldus* Monimiaceae	*Boldine*	Violet	Beige	16	16	3	0	5	24	6	58
Quinoline	Cortex Chinae	*Cinchona Succirubra, Pavon,* Rubiceae	Quinidine	Blue	Light yellow	34	40	15	0	25	12	18	50
			Quinine	Blue	Yellow-white	19	26	7	0	17	9	18	43
			Cinchonine		Beige-brown	38	44	17	7	27	0	22	40
Imidazole	Fol. Jaborandi	*Pilocarpus microphyllus Stapf e.a.;* Rutaceae	Pilocarpine		Light brown	41	52	9	0	13	32	25	55

Table 6 *Continued*

Chemical skeleton	*Plant drug*	*Botanical origin*	*Major alkaloid*	*Fluorescence in UV light (366 nm)*	*Colour with iodoplatinate reagent*	*hR_F values*							
						S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Pyridine	Semen Arecae	*Areca catechu* L., Palmae	Arecoline		Violet	66	90	56	34	48	0	0	0
	Herba Lobeliae	*Lobelia inflata* L., Lobeliaceae	Lobeline		Red-brown	68	90	48	14	48	55	60	55
Quinolizidine		*Sarothamnus Scoparius;* Leguminosae	Sparteine		Violet	70	90	68	68	55	0	55	5
Dihydroindole	Fol. Catharanti	*Catharantus roseus* Apocynaceae	Aspidospermine		White	65	90	54	20	49	50	60	65
Aporphine	Rhizoma Corydalidis	*Corydalis cava* L. Schweigg et Koerte Papaveracae, Fumariaceae	Bulbocapnine	Blue	White	65	>90	35	7	54	78	70	48
Isoquinoline	Rad. Ipecacuanhae	*Cephaelis ipecacuanha* Rubiaceae	Emetine	Blue	Red-brown	67	90	40	6	45	38	58	50
			Cephaeline	Violet-blue	White	56	63	19	2	23	25	17	37
Miscellaneous alkaloids													
Derivatives of diterpene	Aconiti Tuber	*Aconitum napellus* L., Ranunculaceae	Aconitine		Red-browm	68	>90	35	3	49	36	60	65
Xanthine	Herba Ephedrae	*Ephedra sinica* Stapf. Ephedraceae	Ephedrine		Light-grey	47	41	4	0	4	11	0	57
Colchicine	Semen Colchici	*Colchicum autumnale* L, Liliaceae	Colchicine		Light brown								

TLC systems

S_1, Silica gel G, activated: chloroform–acetone-diethylamine (5 : 4 : 1).
S_2, Silica gel G, activated: chloroform–diethylamine (9 : 1).
S_3, Silica gel G, activated: cyclohexane–chloroform–diethylamine (5 : 4 : 1).
S_4, Silica gel G, activated: cyclohexane–diethylamine (9 : 1).
S_5, Silica gel G, activated: benzene–ethyl acetate–diethylamine (7 : 2 : 1).
S_6, Aluminium oxide G, activated: chloroform.
S_7, Aluminium oxide G, activated: cyclohexane–chloroform (3 : 7) + 0.05 diethylamine.
S_8, Silica gel G, impregnated with 0.1 mol L^{-1} sodium hydroxide, activated: methanol.
(Reproduced with permission from Svendsen AB and Verpoorte R (1983) *Chromatography of Alkaloids.* Journal of Chromatography Library. Amsterdam: Elsevier.)

For particular alkaloids, specific reagents can be used; for instance, Marqui's reagent (formaldehyde–sulfuric acid) or Fröhde's reagent (sulfomolybdic acid–sulfuric acid) for morphine. König's reaction can be used to detect nicotine and related alkaloids; Wachtmeister's reagent (*bis*-diazatized benzidine-sulfuric acid) is applied for alkaloids belonging to the protoberberine and protopine group.

The Vitaly reaction is specific for the tropane alkaloids, and reaction with 4-dimethylaminobenzaldehyde for indole alkaloids. Some examples of applications of different reagents are illustrated in **Figure 4** and **Table 4**.

The use of π-acceptor reagents producing colour spots (TCNQ: 7,7,8,8-tetracyano-quinodimenthane; TNF: 2,4,7-trinitrofluorenone; TetNF: 2,4,5,7-tetranitro-9-fluorenone; DDQ: 2,3-dichloro-5,6-dicyanoquinone; DNFB: 2,4-dinitrofluorobenzene) for the detection of alkaloids has been employed.

Initial derivatization during sample preparation or *in situ* on the layer after the application of the sample is called prechromatographic derivatization and comprises oxidation, reduction, iodination, nitration and dansylation (**Table 5**).

Starting chromatographic separation with sample derivatization allows better-quality results to be obtained, especially as far as reproducibility and

lowering the detection limits are concerned. Morphine as a dansyl derivative is an example of fluorescence stabilization and intensity augmentation as a result of treatment of the chromatogram with a 20% solution of liquid paraffin in *n*-hexane.

A similar phenomenon is observed for codeine, morphine, monoacetylmorphine and heroin with the aid of hydrophilic liquids, such as a 20% solution of dioctyl sulfasuccinate in ethanol as a fluorescence intensifier.

Enhanced sensitivity can be achieved by impregnating the layer, by adding the reagent to the solvent or by spraying the plate after development. In addition to the reagents mentioned above, fluorescence intensifiers such as triethanolamine, glycerol and Triton X-100 are quite popular.

Identification and Quantification

The forte of TLC is qualitative analysis. It is possible to identify unknown alkaloids owing to the large amount of R_F data available from the literature and the ability to perform a chemical reaction using a wide spectrum of different reagents *in situ*. For some alkaloid drugs, a compilation of TLC data has been elaborated and stored in computer-based information systems.

Many authors make an identification based on R_F values in a number of chromatographic systems. One scheme has been described in which the analysis of a series of alkaloids by eight TLC systems, combined with observations under UV light ($\lambda = 366$ nm) and colour reactions with iodoplatinate reagent (**Table 6**) is used.

For precise identification, UV or infrared spectra after elution have become indispensable. Together with the melting point and optical rotation values, they are sufficient for the identification and comparison of isolated pure substances. Other spectral methods such as nuclear magnetic resonance or mass spectrometry have frequently been used to identify alkaloids.

Although quantitative determination in TLC is more difficult and requires more effort, it is becoming increasingly important nowadays. There exist two forms of quantitative analysis: direct and indirect. The first method is based on the elution of spots with a suitable solvent and determination in solution, followed by spectrophotometric, fluorometric or acid–base potentiometric titration. The second possibility utilizes adsorption of UV and visible radiation or luminescence of alkaloids, and is performed by the means of photodensitometry, densitometry and fluorimetry *in situ*. This latter technique requires the use of an optical scanner, which is a relatively expensive piece of equipment.

See Colour Plate 54.

See also: **II/Chromatography: Thin-Layer (Planar):** Layers; Modes of Development: Forced Flow, Overpressured Layer Chromatography and Centrifugal; Spray Reagents. **III/Alkaloids:** Gas Chromatography; Impregnation Techniques: Thin-Layer (Planar) Chromatography; Liquid Chromatography.

Further Reading

Adamovics JA (1990) *Chromatographic Analysis of Pharmaceuticals*. New York: Marcel Dekker.

Bieganowska ML and Petruczynik A (1994) Thin-layer reversed-phase chromatography of some alkaloids in ion-association systems. *Chemia Analityczna* 39: 139.

Camag Bibliography Service Thin-layer Chromatography. Cumulative CD, Version 1.00. Camag 1997.

Deyl A, Macek K and Janak J (1975) *Liquid Column Chromatography. A Survey of Modern Techniques and Applications*. Amsterdam: Elsevier.

Golkiewicz W, Gadzikowska M, Kuczyński J and Jusiak L (1993) Micropreparative chromatography of some quaternary alkaloids from the roots of *Chelidonium majus* L. *Journal of Planar Chromatography* 6: 382.

Jork H, Funk W, Fischer W and Wimmer H (1990) *Thin-layer Chromatography, Reagents and Detection Methods*. Weinheim: VCH.

Lepri L, Desideri PG and Lepori M (1976) Chromatographic behaviour of alkaloids of thin layer of cation exchangers. *Journal of Chromatography* 123: 175.

Niederwiesser A and Pataki G (1972) *Progress in Thin Layer Chromatography and Related Methods*. Michigan: Ann Arbor Science.

Popl M, Fähnrich J and Tatar V (1990) *Chromatograpic Analysis of Alkaloids. Chromatographic Science*. New York: Marcel Dekker.

Rönsch H and Schreiber K (1967) Analytische und präparative Dünnschichtchromatographische Trennung von 5α-gesättigten BZW. Δ^5-ungesättigten Steroidalkaloiden und–sapogeninen an silbernitrat-haltigen Adsorptionsschichten. *Journal of Chromatography* 30: 149.

Smith RM (1996) Supercritical fluid extraction of natural products. *LC/GC International, the Magazine of Separation Science,* Vol. 9, p. 8. Chester, UK: Advanstar Communications.

Soczewiński E and Flieger J (1996) Thin Layer Chromatography of Alkaloids. Journal of Planar Chromatography. 9, 107.

Svendsen AB (1989) Thin layer chromatography of alkaloids. *Journal of Planar Chromatography* 2: 8.

Svendsen AB and Verpoorte R (1983) *Chromatography of Alkaloids*. Amsterdam: Elsevier.

Touchstone JC (1992) *Practice of Thin Layer Chromatography*. New York: John Wiley.

Wagner H and Bladt S (1996) *Plant Drug Analysis. Thin Layer Chromatography Atlas*. Berlin: Springer.