# Alkaloids, Pharmaceutical Analysis of

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*Although alkaloids are naturally occurring bases, additional generalizations are difficult because they include a wide range of structurally dissimilar compounds. They vary greatly in their chemical and physical properties as well as in their distribution in nature. In some cases certain alkaloids are associated with only a single species of plant, whereas others are more widely distributed between biological groupings of plants, and yet others are found in a wide range of unrelated plants. Likewise, the concentration of a particular compound may be highly localized within a given region of one plant and found predominantly in a different region of another plant. In some cases the levels of an alkaloid may be relatively high and its isolation as a natural product may be economically feasible, whereas in other cases the levels may be extremely low and less commercially desirable. The medicinal use of alkaloids in the form of crude plant extracts has been known for several thousand years and today there are hundreds that have been isolated and characterized. However, very few of them are accepted therapeutically and many of these fit into the broad categories of cinchona, ergot, opium, rauwolfia, tropane, vinca and xanthine alkaloids.*

## 1 INTRODUCTION

### 1.1 General Information

As a major class of compounds, alkaloids are naturally occurring bases with a wide range of structures, chemical and physical properties, and pharmacological activities. The use of alkaloids as medical agents in the form of crude plant powders and extracts predates the modern pharmaceutical industry by almost four millennia and in some instances prior to modern science, mass poisonings have resulted from their inadvertent usage. This was especially prevalent in Europe during the Middle Ages.

Historically, a primary source of many alkaloids has been flowering plants, although they may be found throughout nature, such as the ergot alkaloids in the grain fungus *Claviceps purpurea*. The first crude alkaloid extract to be studied chemically was opium, which is derived from the latex of the poppy *Papaver somniferum*.[1] Although the initial characterization work on opium that led to the isolation of morphine was carried out in the early 1800s, its use as an analgesic and its narcotic properties had been known for centuries before this.

There are often complex relationships between the alkaloids and their occurrence in nature. In some cases, certain alkaloids such as morphine are associated with only a single species of plant, whereas others like *l*-hyoscyamine are more widely distributed between biological groupings of plants, and yet others (e.g. nicotine) are found in a wide range of unrelated plants.[2] Likewise, the concentration of a given compound may be highly localized within a given area of a particular plant, such as in its leaves, bark, or roots, and this same alkaloid, if present in a different plant, may be found predominantly in another region. In some cases the levels of an alkaloid may be relatively high and its isolation as a natural product may be economically feasible, whereas in other cases the levels may be extremely low and less commercially desirable. A more in-depth discussion of the above items may be found elsewhere.[1–3]

Although today there are several thousand alkaloids that have been isolated and identified structurally, this article will focus only on a relatively small number that are considered to be pharmaceutically more important in terms of their accepted therapeutic value. Some of these compounds are among the earliest alkaloids identified for their medicinal effects, such as morphine, brucine, caffeine, quinine, cinchonine and colchincine.

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### 1.2 Common Properties

Although most alkaloids are crystalline colorless solids, some of the more complex conjugated compounds such as berberine may be colored or, like quinine, fluorescent. A common chemical feature of the alkaloids are that they are bases, which is the basis for many of the commonly used colorimetric methods and thin-layer chromatography (TLC) spray reagents. These are based on the reaction of either organic (e.g. ninhydrin) or inorganic (e.g. Mayer's and Dragendorff's) reagents with the alkaloid to form highly colored products. Beyond this, additional generalizations are more difficult. Structurally, the basic nitrogen or nitrogens (which vary greatly in number and basicity) may be found in a variety of structural environments and hence the equilibrium properties and hydrophobic characteristics vary dramatically between alkaloids.

Illustrated in Figures 1–7 are some of the significant structural differences between a number of the more common pharmaceutically important alkaloids. Nicotine, which is distributed throughout the plant kingdom, is a relatively small molecule with two heterocyclic nitrogens. Similarly caffeine, another widely distributed small molecule, contains four heterocyclic nitrogens but it also contains two additional carbonyl functionalities. Because of their widespread presence and usage, there have been numerous methods developed for assaying both nicotine and caffeine in their natural states and in a host of different formulations and products (Gilpin and Pachla[4–7] and past biannual reviews in this series). Other alkaloids such as berberine, colchicine, and morphine contain only a single nitrogen but are either structurally more complex and/or contain other polar functionalities. For example, the two hydroxyl groups on morphine have a significant influence on its chromatographic properties. Because of these significantly different structural features, the overall ease of analyzing alkaloids and their pharmacological activity vary greatly.

As a result of their basicity, many alkaloids are thermally and photolytically labile, especially in the presence of oxygen. Common breakdown products are the corresponding N-oxides. In the case where the alkaloids contain other reactive groups they may undergo a variety of other reactions and rearrangements. Hydrolysis is often common. As such, during stability testing it is important to evaluate the pharmaceutical products for these likely decomposition candidates. In many cases some of these products, as well as other naturally occurring minor alkaloids and related impurities, may be extremely difficult or impossible to distinguish from the target analyte using simple nonseparation-based analytical procedures. Although details concerning the chemical reactivity of the various classes of alkaloids are presented below, more extensive treatments of this topic may be found elsewhere.[1–3] Commonly, alkaloids, like other organic bases, are stabilized via conversion to their corresponding inorganic or organic salts (i.e. hydrochloride or citrate salts).

### 1.3 Trends in Analytical Methodology

For over two decades (Gilpin and Pachla[4–7] and past biannual reviews in this series) separation-based procedures have been, and continue to be, the most often used methods for assaying alkaloids and their formulated products. This is consistent with the same trends in other areas of pharmaceutical analysis and often is essential in developing stability-indicating and purity-profiling methods. During this time a variety of techniques have been employed, ranging from simple screening procedures based on the use of an initial thin-layer separation (Table 1[8,9]) in combination with a colorimetric spray reagent (e.g. iodoplatinate, Dragendorff–Munier & Macheboeuf, iodine–potassium iodide reagents[10]) to more elaborate sample pretreatment and work-up procedures in combination with either an isocratic or a gradient elution high-performance liquid chromatography (HPLC) separation. In the latter

**Table 1** TLC separation of some common alkaloids according to retention factor ($R_f$) values

| Compound | $R_f$ values for A[a] | $R_f$ values for B[a] | $R_f$ values for C[a] |
|---|---|---|---|
| Ajmaline | 0 | 0.1 | 0.5 |
| Atropine | 0.1 | 0.2 | 0.4 |
| Brucine | 0.2 | 0.2 | 0.4 |
| Chinchonine | 0.3 | 0.2 | 0.4 |
| Cocaine | 0.6 | 0.6 | 0.7 |
| Codeine | 0.3 | 0.2 | 0.4 |
| Colchicine | 0 | 0 | 0.5 |
| Dihydrocodeine | 0.3 | 0.2 | 0.4 |
| Dihydromorphinone | 0.1 | 0.1 | 0.2 |
| Emetine | 0.5 | 0.4 | 0.7 |
| Ergotamine | 0 | 0 | 0.2 |
| Homatropine | 0.2 | 0.2 | 0.4 |
| Morphine | 0 | 0 | 0.1 |
| Narcotine | 0.6 | 0.5 | 0.7 |
| Papaverine | 0.5 | 0.4 | 0.7 |
| Pilocarpine | 0.1 | 0.1 | 0.4 |
| Quinidine | 0.2 | 0.1 | 0.3 |
| Quinine | 0.2 | 0.1 | 0.2 |
| Reserpine | 0.5 | 0.2 | 0.7 |
| Scopolamine | 0.3 | 0.2 | 0.6 |
| Serpentine | 0 | 0 | 0.2 |
| Strychnine | 0.4 | 0.3 | 0.5 |
| Thebaine | 0.5 | 0.5 | 0.7 |
| Yohimbine | 0.4 | 0.2 | 0.6 |

[a] A = Benzene–ethylacetate–diethylamine (70:20:10); B = chloroform–cyclohexane–diethylamine (40:50:10); C = acetone–chloroform–diethylamine (40:50:10).

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instance, assays based on reversed-phase conditions are the most commonly used methods and the most often used eluent additives are simple buffers that are added to control the protonation/deprotonation of the basic nitrogen(s) and hence their retention properties. An in-depth discussion of the influence of eluent pH on solute retention is considered in the article **Eluent Additives and the Optimization of High-performance Liquid Chromatography Procedures** in this publication.

For many of the alkaloids, one of the more commonly encountered problems in developing reliable reversed-phase assays is peak tailing. This problem often is exacerbated when more than one nitrogen is present in the alkaloid and/or the alkaloid contains other polar subsitutents. Peak tailing is the result of residual silanol groups that are present on the reversed-phase packing. Because silica is an amorphous material, the number and distribution of these groups change depending on the synthetic route that is employed to produce the silica,[11,12] which in turn dramatically influences the nature and performance of the reversed-phase packings.[13] To the practicing chromatographer, this problem manifests itself as manufacturer-to-manufacturer and batch-to-batch differences in column performance for a given stationary phase. Additionally, this problem is exacerbated for solutes that have polar functional groups that can interact strongly with residual silanols such as amines and heterocyclics, which are common structural features of alkaloids. In order to minimize the residual silanol problems, one of four approaches are generally used: postreaction end-capping; preparation of sterically blocking phases; electronic manipulation of the attached surface groups; and the use of mobile phase additives. The first three of these approaches are controlled by the manufacturer and are important considerations when purchasing a column, especially for strongly interacting solutes like some of the alkaloids. Although the performance of commercially available bonded phases has improved dramatically, there are still many reversed-phase applications where residual silanol activity leads to unacceptable chromatograms in terms of severely tailing peaks, and as columns age the problem of exposed silanol groups increases even for high-performance bonded phases. Many of these unwanted effects can be eliminated or at least minimized through the use of secondary mobile phase additives. Such problems usually can be addressed by the addition of compounds to the eluent that dynamically modify the surface by a competitive sorption mechanism and hence act to suppress undesirable interactions that can arise between basic solutes and residual silanols. The agents used to do this are strongly sorbing compounds (i.e. molecules that contain a polar head group and a nonpolar tail) that do not interfere with detection. The most commonly used compounds to mask silanol activity and hence to improve peak symmetry are alkylamines,[14] however, in a few cases other compounds (e.g. perfluororalkyl surfactants) have been employed.[15] Alkylamines also are used to enhance the performance of normal-phase separations both in terms of HPLC procedures and in terms of routine TLC screening methods (Table 1).

## 2 GENERAL INFORMATION AND ANALYTICAL METHODS

### 2.1 Cinchona Alkaloids

The medicinal value of this group of alkaloids has been known since the 17th century, when crude extracts from cinchona bark, a plant species indigenous to the Andes, were first used for the treatment of malaria.[1,2] Although there have been more than two dozen cinchona alkaloids that have been isolated and identified, four of the pharmaceutically more important compounds are quinine, quinidine, cinchonidine and cinchonine. The structures of these are given in Figure 1. They are made up of two parts: a quinoline nucleus and a quinuclidine moiety. Of these, quinine and quinidine are the primary alkaloids of various species of *Cinchona* and *Remijia* and are present at levels of 1–4% and 0.3–3%, respectively.[16]

Upon oxidation, quinine, quinidine, cinchonidine and cinchonine are converted to the corresponding ketones and they undergo acetylation to form O-acetyl derivatives that reconvert to the starting material on hydrolysis. The vinylic group is susceptible to acid attack and rearrangements. In the case of quinine and quinidine, this tendency is greater than it is for the methoxy group. Greater details

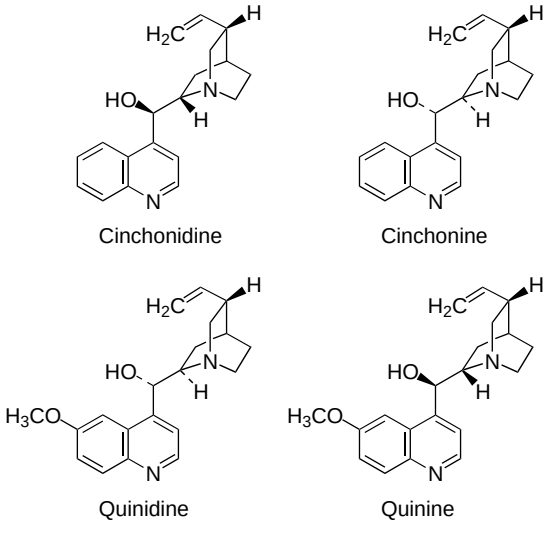


**Figure 1** Common cinchona alkaloids.

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concerning the chemical reactivity and related properties of these compounds may be found elsewhere.[1,2]

Typically, the cinchona alkaloids are white solids that form sparingly water-soluble mono-salts or highly water-soluble bis-salts. One of the more distinctive spectral features is their fluorescence in acidic media, which has been used in their direct spectrofluorimetric determination as well as by HPLC in combination with fluorimetric detection. Each of these alkaloids has two sites of protonation with respective p$K_1$ and p$K_2$ values in the 5.1–5.8 and 9.7–10.0 ranges.[16] When assayed using reversed-phase HPLC conditions, these are the structural features that must be appropriately controlled in order to obtain optimum separation performance.

A representative listing of some of the many methods published for the more common cinchona alkaloids is presented in Table 2.[17–40]

**Table 2** Analytical procedures for cinchona alkaloids

| Analyte | Technique | Refs. |
|---|---|---|
| (+)- and (−)-Cinchonine | LC normal-phase conditions | 17 |
| Bisbenzylisoquinoline | LC reversed-phase conditions | 18 |
| Cinchoncaine · HCl and 2-hydroxyquinoline-4-carboxylic acid diethylaminoamide | LC and first-derivative spectroscopy | 19 |
| Isoquinolines | LC reversed-phase conditions | 20, 21 |
| Quinidine and its dihydroxy and dimethoxy derivatives | LC and diode array detection of cinchona bark extracts | 22 |
| Quinidine and quinine | FIA using a chemi-luminescence reaction | 23 |
| Quinidine and quinine | AA as metal complexes | 24, 25 |
| Quinidine | Electrochemical and sensor | 26 |
| Quinidine and quinine | Isotachophoresis | 27 |
| Quinidine and quinine | Spectrophotometric | 28, 29 |
| Quinidine | Spectrofluorimetric | 30 |
| Quinine | FIA | 31 |
| Quinine | Electrochemical and sensor | 32 |
| General | LC reversed-phase conditions | 33–36 |
| General | LC reversed-phase conditions/thermospray MS | 37, 38 |
| General | Spectrophotometric | 39 |
| General | Titrimetry | 40 |

AA, Atomic absorption; FIA, flow injection analysis; LC, liquid chromatography; MS, mass spectrometry.

## 2.2 Ergot Alkaloids

Historically, the medical value of some of the ergot alkaloids has been known for over 3000 years. They are the oldest known mycotoxins and are found in the *Claviceps purpurea*, a filamentous fungus that grows on rye and other gramineaceous crop plants. During the Middle Ages in Europe, ergot poisoning through their vasconstriction and/or hallucination actions was a common occurrence. In a single epidemic in AD 944 it has been reported that about 20 000 people in France died from ingesting ergot-infested flour.[1]

A common structural feature of many of the ergot alkaloids is their tetracyclic ergoline nucleus, as illustrated

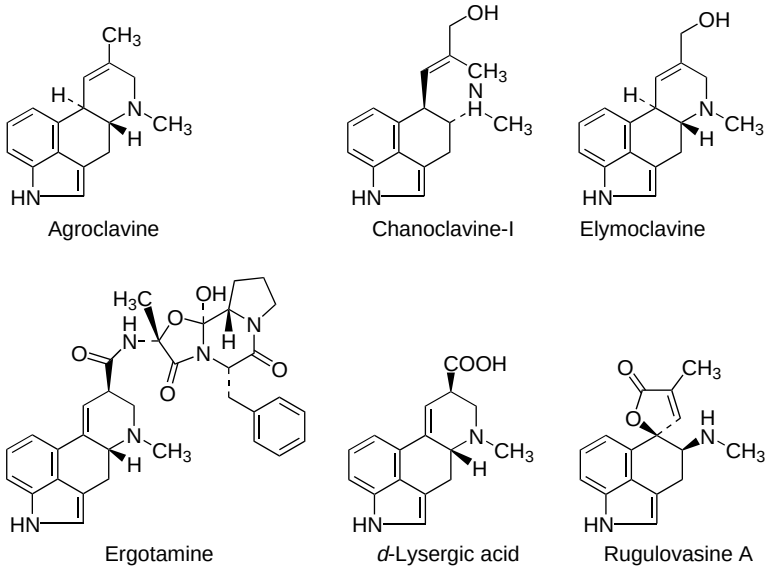


**Figure 2** Common ergot alkaloids.

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in Figure 2. Although all contain an indole structure, some of the clavine compounds, for example, chanoclavine-I and -II and rugulovasine A and B, may contain fewer rings and some of the more complex compounds, for example, the peptide alkaloid ergotamine, contain additional ring systems. The ergot alkaloids can be divided into four subgroups: the clavine alkaloids; the lysergic acid derivatives; the lysergic acid amides; and the ergot peptide alkaloids. Some of the more common compounds are chanoclavine-I (a precursor of agroclavine and elymoclavine), ergonovine, ergotamine and *d*-lysergic acid.

The most commercially important ergot compounds belong to the peptide subgroup. Typically these compounds must be protected from air oxidation, light, and heat. They hydrolyze to form lysergic acid, proline, a second amino acid, an $\alpha$-keto acid and ammonia. In the case of ergotamine, a compound used for its antimigraine properties, the hydrolysis products are lysergic acid, proline, L-phenylalanine, pyruvic acid and ammonia.[1] The ergot alkaloids form colored products with sulfuric acid and a characteristic blue product with *p*-dimethylaminobenzaldehyde. In many cases a double bond is present at either the 8,9-position (e.g. agroclavine, chanoclavine-I and -II, and paspalic acid) or the 9,10-position (e.g. ergotamine, penniclavine, setoclavine, and *d*-lysergic acid) (Figure 2). These differences can be distinguished in their respective ultraviolet (UV) spectra where $\lambda_{max}$ is at 284 nm for the indole structure (i.e. the $\Delta^{8,9}$ compounds) and at 318 nm for the 4-vinyl indole structure (i.e. the $\Delta^{9,10}$ compounds). This difference in UV properties between the two types of structures for the $\Delta^{8,9}$ and $\Delta^{9,10}$ alkaloids can be used in combination with variable or dual-wavelength detection to impart additional specificity to HPLC-based methods.

A representative listing of some of the methods published for the more common ergot alkaloids is presented in Table 3.[41–48]

### 2.3 Opium Alkaloids

The opium alkaloids have been studied more than any other group.[16] They are derived from the latex of a single species of the poppy *Papaver somniferum* and consist of several closely related compounds, including codeine, morphine, neopine, oripavine, and thebaine. Of these, morphine is the most abundant. A second related group of morphinandienone bases include sinomenine and hasubanonine, metaphenine, and protometaphenine. These latter alkaloids are found in Japanese *Sinomenium* and *Stephania* plants.[1] The structural difference between these two groups of compounds is shown in Figure 3.

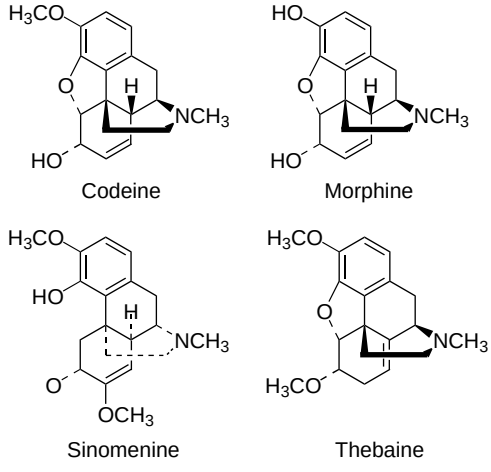


**Figure 3** Common opium alkaloids.

**Table 3** Analytical procedures for ergot alkaloids

| Analyte | Technique | Ref. |
|---|---|---|
| Ergonotamine maleate and tartrate | FIA ampometric detection with Kel-F graphite composite electrode | 41 |
| Ergonotamine maleate and tartrate | LC fluorescence detection | 42 |
| Ergot epimers | CE | 43 |
| General | MS | 44 |
| General | NMR | 45 |
| General | TLC | 46 |
| General | CZE enantiomeric separation using cyclodextrins | 47 |
| General | LC reversed-phase conditions with eluent additives | 48 |

CE, Capillary electrophoresis; NMR, nuclear magnetic resonance; CZE, capillary zone electrophoresis.

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The first compound to be isolated in pure form from crude opium extracts was morphine, by Serturner in 1805, although 2 years prior to this Derosne had reported the separation of a mixture of morphine and noscapine. The presence of these compounds occurs in nature at levels of 4–21% and 4–8%, respectively.[1] Subsequently, codeine was isolated in 1833. This latter compound also can be produced easily via O-methylation of the phenolic group in morphine. Likewise, codeine can be oxidized at this same position to form the corresponding ketone, codeinone, which also results from the acid hydrolysis of thebaine.[3] In general the morphinandienones can undergo two types of acid-catalyzed rearrangements, forming either aporpines or dibenzazonines. The synthesis and various reaction pathways of this group of compounds are well established.[1–3]

Typically the morphinandienones have UV maxima at 235–240 and 275–280 nm. However, for sinomenine, where the double bond at the 4,5-position is missing, the $\lambda_{max}$ is at 232 and 265 nm. The mass spectrometric,

**Table 4** Analytical procedures for opium alkaloids

| Analyte | Technique | Refs. |
|---|---|---|
| 6-Acetylmorphine, diamorphine and morphine | LC to study hydrolysis of dimorphine | 49 |
| Apomorphine | LC reversed-phase conditions with $C_{18}$ column | 50 |
| Apomorphine | Analytical profile | 51 |
| Hydromorphone and morphine | LC | 52 |
| Codeine | LC reversed-phase conditions with $C_{18}$ column | 53 |
| Codeine | FIA using spectrofluorimetric detection | 54 |
| Codeine | LC reversed-phase conditons | 55 |
| Codeine | Spectrophotometric | 56, 57 |
| Codeine | Isotachophoresis | 58 |
| Codeine and byproducts | CE analytes in Kodynal, Ipecarin, Spasmoveralgin, and Alganon formulations | 59 |
| Codeine, morphine, papaverine and thebaine | TLC using spectrodensitometry | 60 |
| Codeine, morphine, noscarpine, papaverine and thebaine | LC reversed-phase gradient conditons using a base-deactivated $C_{18}$ with 1-heptanesulfonic acid as the eluent modifier | 61 |
| Codeine and related alkaloids | LC reversed-phase conditons | 62–64 |
| Codeine and related alkaloids | TLC/HPTLC/OPLC | 65 |
| Codeine and related alkaloids | Differential pulse polarography | 66 |
| Ethylmorphine | LC reversed-phase conditions with $C_{18}$ column | 67 |
| Hydrocordone | IR chromatographic isolation/IR identification | 68 |
| Morphine | Review of the use of biosensors | 69 |
| Morphine | LC reversed-phase gradient conditions using a phenyl column to assay ipecac formulations | 70 |
| Morphine | FIA using chemiluminescence detection | 71, 72 |
| Morphine | LC reversed-phase conditions | 73 |
| Morphine | NIR reflectance | 74 |
| Noscapine | TLC/HPTLC/OPLC | 75 |
| Noscapine | LC | 76 |
| Papaverine | AA by indirect measurement | 77 |
| Papaverine | LC reversed-phase conditons with $C_{18}$ column | 78 |
| Papaverine | Electrochemical | 79–81 |
| Papaverine | TLC using spectrodensitometry detection | 82 |
| Papaverine | Colorimetric or UV | 83–86 |
| General | CE using guest–host, nonaqueous, or micellar conditions | 87–89 |
| General | LC review | 90 |
| General | LC reversed-phase conditions | 91–97 |
| General | SFC using a packed column | 98 |

HPTLC, High-performance thin-layer chromatography; IR, infrared; NIR, near-infrared; OPLC, overpressured layer chromatography; SFC, supercritical fluid chromatography.

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spectroscopic and chromatographic properties of the opium alkaloids have been studied by numerous investigators and they are generally easily analyzed via a variety of techniques. A representative listing of some of the many methods published for the more common opium alkaloids is presented in Table 4.[49–98]

## 2.4 Rauwolfia Alkaloids

Although approximately 150 species of plants belong to the *Rauwolfia* genus, *R. serpentina*, a plant found in India, is the most important member. However, other species such as *R. vomitoria* and *R. tetraphylla*, which are found respectively in Africa and Central America, have become alternative sources. Likewise, synthetically produced reserpine now competes favorable in price with the natural product.[1] The medicinal use of extracts from this family of plants, like the ergot alkaloids, has been known for about 3000 years but the major active compound reserpine, which occurs at about the 1% level, was not isolated and identified until 1952. Other minor (i.e. at about the 0.1% range) alkaloids that belong to this class are ajmalicine, ajmaline, rescinnamine, reserpiline, and yohimbine (see Figure 4).

Like the ergot alkaloids, a common structural feature of the rauwolfia alkaloids is the indole nucleus, as illustrated in Figure 4. Alkaline hydrolysis of reserpine, the principal alkaloid of this class, produces reserpic acid, 3,4,5-trimethoxybenzoic acid, and methanol. Vigorous oxidation of the resulting reserpic acid leads to loss of the indole structure via production of 4-methoxy-*N*-oxalylanthranilic acid.[2]

**Table 5** Analytical procedures for rauwolfia alkaloids

| Analyte | Technique | Refs. |
|---|---|---|
| Ajmaline | Radioimmunoassay | 99 |
| 3,4-Dihydroreserpine, isoreserpine, reserpine and 3,4,5,6-tetra-hydroserpine | LC | 100 |
| 3,4-Dihydroreserpine, isoreserpine, reserpine and 3,4,5,6-tetra-hydroserpine | Electrochemical by differential pulse polarography | 101 |
| Rescinnamine | LC normal-phase conditions and fluorescence detection | 102 |
| Reserpiline and reserpine | Spectrofluorimetric, differences in fluorescence excitation and emission spectra used | 103 |
| Reserpine | Colorimetric | 104 |
| Reserpine | LC | 105–107 |
| Reserpine | Electrochemical | 108 |
| Reserpine | HPLC/TLC | 109 |
| Reserpine | Spectrofluorimetric | 110 |
| Yohimbine | Review of the interaction of analyte with microcrystalline vs carboxymethylcellulose | 111 |
| Yohimbine | Spectrofluorimetric based on the oxidation of the analyte with Ce(IV) | 112 |
| General | LC reversed-phase conditions in combination with thermospray MS | 113, 114 |
| General | GC/MS | 115 |

GC/MS, Gas chromatography/mass spectrometry.

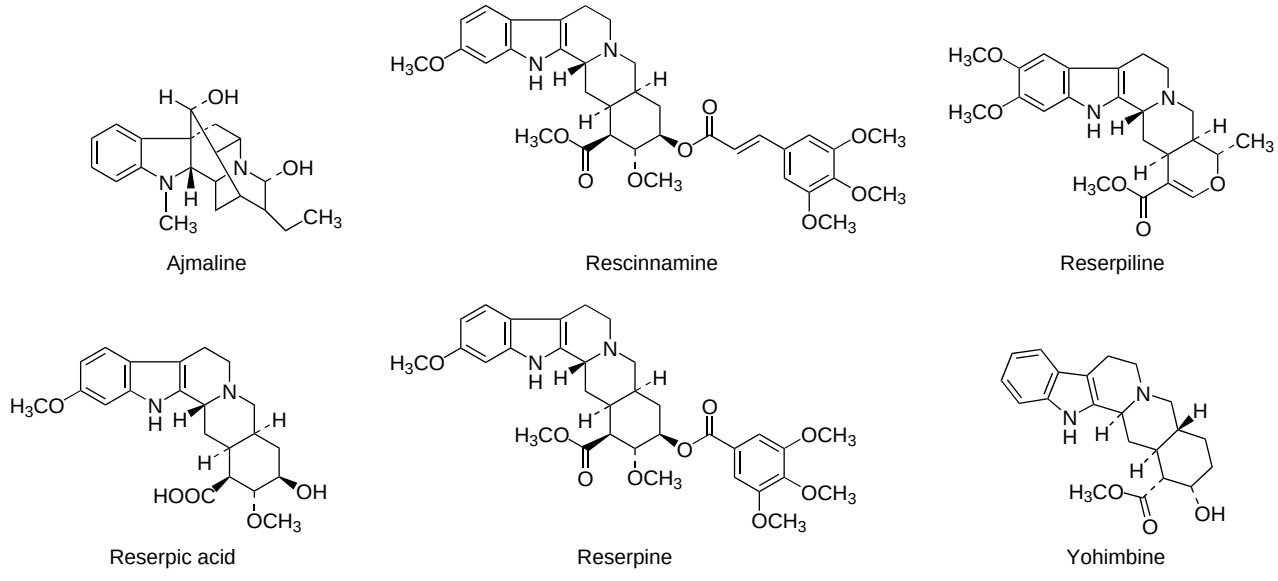


**Figure 4** Common rauwolfia alkaloids.

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A representative listing of some of the methods published for the more common rauwolfia alkaloids is presented in Table 5.[99–115]

### 2.5 Tropane Alkaloids

Tropane alkaloids occur in a variety of *Erythroxylaceae*, *Solanaceae* and *Convolvulaceae* plants, which include *Atropa belladona*, *Datura stramonium*, *Erythroxylon coca*, and *Hyosyamus niger*. The most common compounds in this group are cocaine, *l*-hyoscyamine and its racemized form atropine, hyoscine, scopolamine, and meteloidine (Figure 5). The early use of the tropane alkaloids can be traced to the 16th century both in Europe and South America, where crude preparation of *Atropa belladonna* and dried coca leaves were used, respectively, as medical aids. However, their isolation of the active alkaloids was not until the 19th century. Geiger first prepared *l*-hyoscyamine in 1883 and Wohler prepared cocaine in 1862.

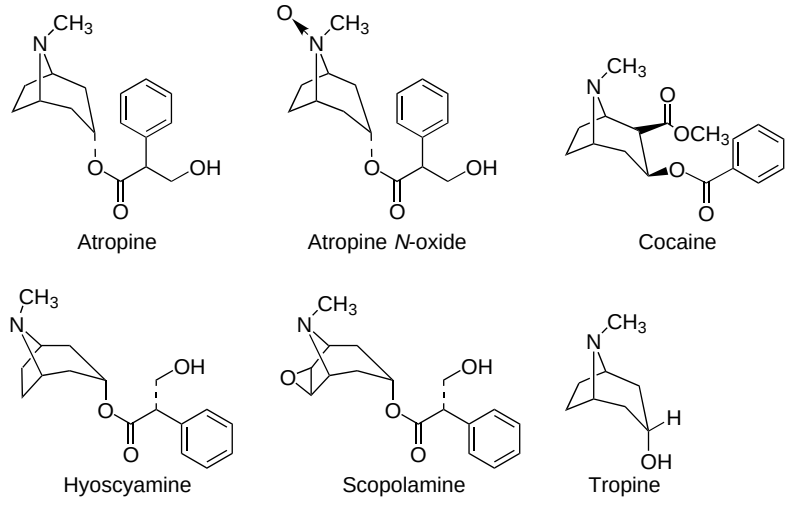


**Figure 5** Common tropane alkaloids.

**Table 6** Analytical procedures for tropane alkaloids

| Analyte | Technique | Refs. |
|---|---|---|
| Atropine | LC reversed-phase conditions using cyano column | 116 |
| Atropine | Electrochemical and sensor | 117 |
| Atropine | LC ion-pairing reagent/column switching made it possible to determine analyte in complex preparations of other gastrointestinal drugs | 118 |
| Atropine | LC chiral reagents added to mobile phase to resolve isomers | 119, 120 |
| Atropine | LC ion-pairing reagent | 121 |
| Atropine | LC reversed-phase conditions | 122 |
| Atropine analogs | LC reversed-phase conditions | 123 |
| Atropine, cocaine, homatropine and scopolamine | LC enantiometric separation using β-cyclodextrin-bonded phase | 124 |
| Atropine, homatropine and scopolamine | CE | 125 |
| Coca leaves | Reversed-phase LC/GC comparison: GC better for resolving cocaine and related products; reversed-phase LC faster and more convenient | 126 |
| Cocaine | Review of the use of biosensors | 69 |
| Cocaine | AA by indirect measurement | 77 |
| Cocaine | Reversed-phase LC esterified with optically pure 2-octanol prior to RPLC | 127 |
| Scopolamine | Electrochemical and sensor | 128 |
| Scopolamine | LC comparison of interaction of analyte with microcrystalline cellulose vs sodium carboxymethylcellulose | 129 |
| General | TLC/HPTLC/OPLC | 130 |
| General | LC reversed-phase conditions | 131–134 |
| General | LC enantiometric separation using β-cyclodextrin-bonded phase | 135 |

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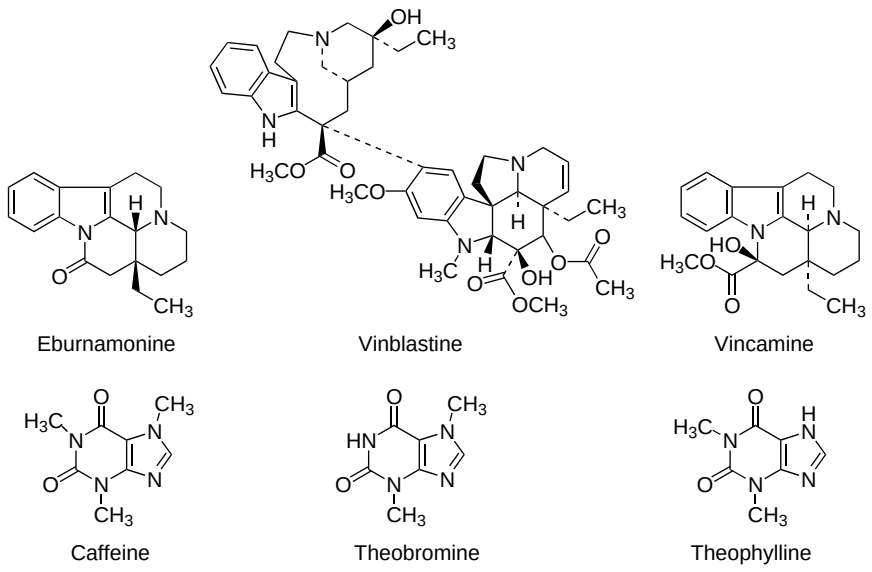


**Figure 6** Common vinca and xanthine alkaloids.

A significant structural feature of the tropane alkaloids are that they are esters of an organic acid that is attached at the 3-position in either an $\alpha$- or $\beta$-configuration to the central tropane structure. Very gentle neutral hydrolysis of these compounds produces tropine and the corresponding organic acid. In the case of *l*-hyoscyamine, the most widespread alkaloid of *Solanaceae* plants, the hydrolysis products are tropine and *s*-(−)-tropic acid. In the presence of more vigorous thermal and acidic conditions, tropine can undergo additional reactions to form a variety of products, and oxidation of tropine leads to both equatorial and axial N-oxide isomers. This is illustrated in Figure 5 for atropine.

A representative listing of some of the many methods published for the more common tropane alkaloids is presented in Table 6.[116–135]

### 2.6 Vinca Alkaloids

There are approximately 100 alkaloids that are present in six species of the genus *Vinca*. This group of plants are found throughout western Asia and the Mediterranean region of Europe. The most important species are *Vinca major* and *Vinca minor*, and the more common compounds include apovincamine, eburnamenine, hervine, reserpinine, sarpagine, (−)-tabersonine, vincadine, vincamajine, and vincamine. Of these, vincamine is the most important alkaloid and may be found at levels up to 2–3%. However, at least half of the vincamine currently used is partially synthesized from tabersonine, and numerous derivatives of vincamine have been prepared.[1]

A typical feature of the *Vinca* alkaloids is the eburna nucleus, which results in a characteristic mass spectrometric pattern. The major fragmentation pathway occurs via Diels–Alder reaction in the C-ring. The radicals produced can undergo two reaction schemes resulting in two major fragments with *m*/*z* differences of 41. For example, in the case of apovincamine, ions are observed at 308 and 267, and for eburnamenine, ions are observed at 249 and 208. However, besides the eburna nucleus, the remaining structural features vary widely between the different alkaloids, as illustrated by eburnamonine, vinblastine and vincamine in Figure 6.

**Table 7** Analytical procedures for vinca alkaloids

| Analyte | Technique | Ref. |
|---|---|---|
| Catharanthine, vinblastine, vincristine and vindoline | LC | 136 |
| Vinblastine and degradation products | LC reversed-phase conditions/MS field desorption and chemical ionization | 137 |
| Vinblastine, vincristine and indole impurities | LC | 138 |
| Vinblastine sulfate | Reviews 131 references and deals with the synthesis, physical properties, stability, and analytical methodology | 139 |
| Vinblastine sulfate | LC reversed-phase conditions | 140 |
| Vinblastine sulfate | LC reversed-phase conditions and column comparison of $\alpha$-acid glycoprotein versus human serum albumin | 141 |

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**Table 8** Analytical procedures for xanthine alkaloids

| Analyte | Technique | Refs. |
|---|---|---|
| Caffeine | LC reversed-phase conditions with $C_{18}$ column | 53 |
| Caffeine | AA or voltametric complex formed with molybdophosphate | 142 |
| Caffeine | Oxidimetric titration | 143 |
| Caffeine | UV derivative spectrometry | 144 |
| Caffeine and Analogs | LC and micellar electrokinetic capillary chromatography | 145 |
| Caffeine and Theophylline | Luminescence as function of pH and presence of a heavy atom such as iodine | 146 |
| 8-Chlorotheophylline | Electrochemical | 147–150 |
| Diprophylline | Colorimetric | 151 |
| Etoffyline and theophylline | Reversed-phase LC $C_{18}$ column | 152 |
| Theophylline | LC reversed-phase conditions with $C_{18}$ column | 153–155 |
| Theophylline | LC normal-phase conditions | 156 |
| Theophylline | Electrochemical | 157 |
| Theophylline | NIR to study dissolution rate, film coating thickness and hardness | 158 |
| Theophylline | CE | 159 |
| Theophylline | UV and colorimetric | 160 |
| Theophylline | Biosensor nafion film containing theophylline oxidase and a ferricytochrome *c* cofactor | 161 |
| Theophylline | LC dansyl chloride derivative to enhance detection | 162 |
| Theophylline | Colorimetric after treatment with 4-nitroaniline | 163 |
| Theophylline | Fluoresence at 615 nm and excitation at 300 nm after treatment with europium(III) | 164 |
| Theophylline | Potentiometric | 165 |
| Theophylline | Stopped-flow fluorimetry based on measuring kinetics of reaction with Ce(IV) | 166 |
| Theophylline | TLC | 167 |
| Theophylline | Chromatography | 168 |
| Theophylline | Luminescence at room temperature | 169, 170 |
| Theophylline analogs | LC reversed-phase conditions | 171 |
| General | HPTLC | 172, 173 |
| General | LC review | 174–178 |
| General | IR | 179 |
| General | Luminescence | 180 |

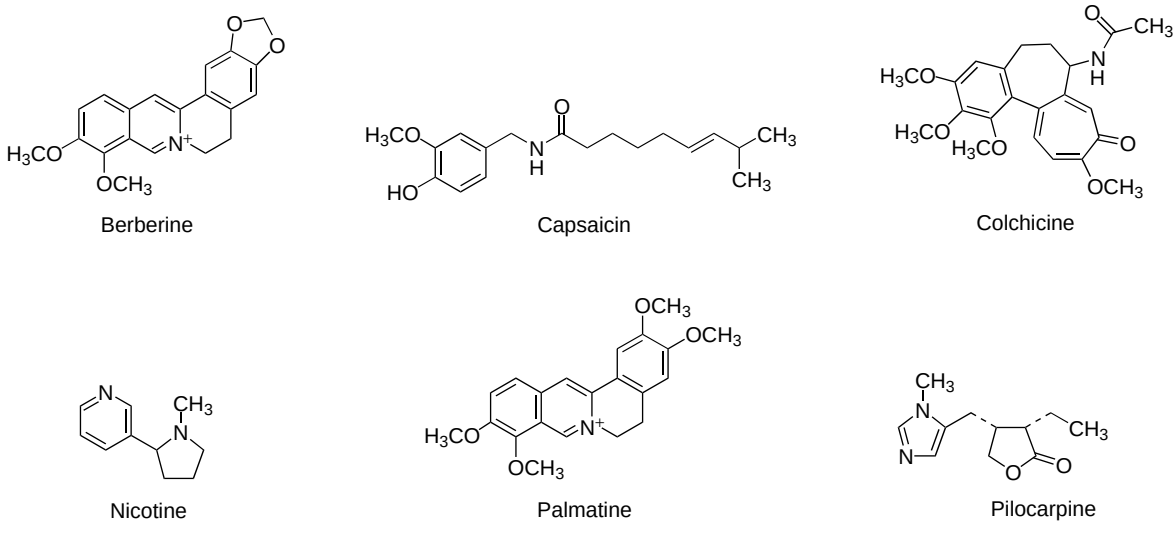


**Figure 7** Common miscellaneous alkaloids.

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**Table 9** Analytical procedures for miscellaneous alkaloids

| Analyte | Technique | Refs. |
|---|---|---|
| 9-Acridone | LC | 181 |
| Campothecin | LC to study photodecomposition | 182 |
| Berberine | LC | 183 |
| Berberine | Electrochemical | 184, 185 |
| Berberine | Spectrofluorimetric | 186, 187 |
| Berberine | FIA/spectrofluorimetric | 188 |
| Berberine | Spectrophotometric | 180–191 |
| Capsaicin analogs | LC | 192 |
| Catharanthine | Radioimmunoassay | 193 |
| Chelidonine protopine | TLC | 194 |
| *Chelidonium majus* alkaloids | LC | 195 |
| Colchine | stripping voltametry | 196 |
| Indole alkaloids from *Catharanthus roseus* | LC reversed-phase conditions and thermospray MS | 197 |
| Indole Alkaloids in suspension culture *Tabernaemontana divaricata* | LC reversed-phase conditions | 198 |
| Pilocarpine, degradation products and impurities | LC reversed-phase conditions | 199–205 |
| Pilocarpine | LC normal-phase conditions | 206 |
| Pilocarpine | LC $\beta$-cyclodextrin column | 207 |
| Pilocarpine | TLC | 208 |
| Pilocarpine | Spectrophotometric | 209 |
| Pilocarpine | AA by indirect measurement of mercury complex | 210 |
| Protoberberine | TLC/densitometric detection | 211 |
| Tetrahydrojatrorrhizing and tetrahydroprotoberberine | LC using a cellulose tris(phenylcarbamate) column | 212 |
| *Ephedrae herba* | LC | 213 |
| *Senecio vulgaris* | LC and NMR to study the interaction of analyte with microcrystalline vs carboxymethylcellulose | 214 |
| General | CE influence of structure on electrophoretic mobility | 215 |
| General | LC normal-phase conditions using a polyol-derivatized silica column | 216 |
| General | LC reversed-phase conditions using cross-linked cyclodextrin columns | 217 |
| General | Counter-current chromatography influence of pH and ion-pair formation | 218 |
| General | Potentiometric titration | 219 |
| General | Colorimetric with 2,6-dichlorophenolindophenol | 220 |

This makes the prediction of chromatographic properties much more unlikely compared to alkaloids with more definable structural changes in terms of their effect on retention (e.g. the common opium alkaloids shown in Figure 3).

A representative listing of some of the methods published for the more common vinca alkaloids is presented in Table 7.[136–141]

## 2.7 Xanthine Alkaloids

The xanthine alkaloids are found throughout nature and the most common compounds in this group are caffeine, theophylline and theobromine. They share a number of pharmacological properties, including central nervous system, cardiac, respiratory stimulant, and smooth-muscle relaxant.

The central structural feature of the xanthine alkaloids is their purine nucleus (lower row of compounds in Figure 6). These three compounds are easily analyzed by a variety of methods, as summarized in Table 8, including many reversed-phase HPLC assays.[53,142–180]

## 2.8 Miscellaneous Alkaloids

There are a wide variety of other alkaloids that vary widely in terms of their source of origin, structure, and pharmacological activities. Because of space limitations it is not possible to consider each of these in a more in-depth discussion, but some of the more important are given in Figure 7 and methods for these as well as other alkaloids are given in Table 9.[181–223]

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## ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| AA | Atomic Absorption |
| CE | Capillary Electrophoresis |
| CZE | Capillary Zone Electrophoresis |
| FIA | Flow Injection Analysis |
| GC/MS | Gas Chromatography/Mass Spectrometry |
| HPLC | High-performance Liquid Chromatography |
| HPTLC | High-performance Thin-layer Chromatography |
| IR | Infrared |
| LC | Liquid Chromatography |
| MS | Mass Spectrometry |
| NIR | Near-infrared |
| NMR | Nuclear Magnetic Resonance |
| OPLC | Overpressured Layer Chromatography |
| SFC | Supercritical Fluid Chromatography |
| TLC | Thin-layer Chromatography |
| UV | Ultraviolet |

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