Chapter

Chiral Alkaloid Analysis

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Abstract

Alkaloids are distributed in plant kingdom and play important role in protection, germination as well as plant growth stimulants. Most of them are chiral compounds and are clinically administered as the racemic mixture, even though its enantiomers have been known to exert different pharmacological activity. Liquid chromatography using chiral stationary phases (CSP) proved to be an essential tool with a wide range of applications, including analysis of the stereochemistry of natural compounds. This review gives an overview of chiral separation alkaloids that were used in theoretical studies and/or applications in recent years. It shows the possibilities of polysaccharide CSPs have now also been established as the first-choice of chiral phases for enantiomer separation.

Keywords: chiral alkaloid, sample preparation, enantiomer separation, chiral stationary phase

1. Introduction

Over the centuries, humans have depended on nature for their essential needs of food supplies, shelters, apparel, transport means, fertilizers, flavors and fragrances, and the last not but least, medicines. Sophisticated traditional medicine systems have been generated by the plants over thousands of years. Moreover, plants maintain the significant sources of modern remedies for humanity. Additionally, according to WHO, 80% of the world's population—primarily those of developing countries rely on plant-derived medicines for their healthcare [1]. People continue to consider nature as a source of potential chemotherapeutic agents. Over 50% of clinical drugs all over the world are the product of natural plants and their derivatives. While more than 25% of the total are extracted from higher plants [2].

History of pharmacy was for centuries identical with the history of pharmacognosy, or study of materia medica, which were obtained from natural sources—mostly plants but minerals, animals, and fungi. Chirality is one of the universal phenomena in nature. For instance, chiral biomolecules such as amino acids, sugars, proteins and nucleic acids have created living organisms. In natural surroundings, these biomolecules are present in one of the two possible enantiomeric forms, e.g., amino acids in the L-form and sugars in the D-form. Living organisms show variation in biological responses to one of a couple of enantiomers in medicines due to the chirality [3].

A range of chemicals that accurate enzymatic metamorphosis defines stereochemical configurations. Consequently, there is a certain chirality in most organic

compounds in nature. It is important to emphasize that some phytochemicals exist in only one enantiomeric form, while others the optical rotation of the metabolite can be different [4].

2. Background on chiral alkaloid

Alkaloids are cyclic organic compounds that contain nitrogen in a negative oxidation state. They are generally distributed in flora and are an essential role in plant protection, sprouting and stimulating plant growth. Alkaloids-containing plants are often used as traditional medicines and these compounds usually have marked pharmacological activity [5]. Over 21,000 alkaloids have been identified, which thus constitute the largest group among the nitrogen-containing secondary metabolites [6]. Alkaloids are significantly pharmaceutical, e.g. morphine as pain relief medicines, codeine as an antitussive in cough medicines, colchicine in the treatment of gout and familial Mediterranean fever (FMF), Quinine as an anti-malarial and a muscle relaxant, Quinidine, as an antiarrhythmic agent to prevent ventricular arrhythmias and L-hyoscyamine (in the form of its racemic mixture known as atropine) as antimuscarinic; i.e., as an antagonist of muscarinic acetylcholine receptors [7].

The first isolations of alkaloids in the nineteenth-century new investigation into medicine of several alkaloid-containing drugs and were accidental with the advent of the separation process for the extraction of drugs. In 1803, the French apothecary Derosne probably isolated narcotine. Several years later, the Hanoverian apothecary Sertürner further investigated opium (1806) and isolated morphine (1816) [7].

Based on their structures, alkaloids are divided into several subgroups: non-heterocyclic alkaloids and heterocyclic alkaloids, which are again divided into 7 major groups according to their basic ring structure [8]. Families reported to be rich in alkaloids are: Liliaceae, Amaryllidaceae, Apocynaceae, Berberidaceae, Leguminosae, Papaveraceae, Ranunculaceae, Rubiaceae and Solanaceae [9]. Most of alkaloids are chiral compounds and are clinically administered as the racemic mixture, although its enantiomers have been shown to exert different pharmacological activity.

2.1 Non-heterocyclic alkaloids

Phenylethylamine alkaloids in medicinal herbs (i.e. Citrus species and *Ephedra sinica*) are used ubiquitously for their effects on the metabolic process of humans by stimulating lipolysis and thus supporting to reduce the fat mass in obese people. Particularly, Ephedra Herba (Ma Huang) contain several alkaloids such as (1R, 2S)-(−)-ephedrine, (1S, 2S)-(+)-pseudoephedrine, (1R, 2S)-(−)-norephedrine, (1S, 2S)-(+)-norpseudoephedrine, (1R,2S)-(−)-N-methylephedrine, and (1S, 2S)-(+)-N-methylpseudoephedrine [10]. Each of these six compounds also has an enantiomer that does not occur naturally in the plant [11, 12]. Separation and quantification of optical isomers of ephedrine-type alkaloids are important since ephedrine-type alkaloids in natural have been found to be strengthened with inexpensive (racemic) synthetic similarity, and these enantiomers could exhibit important differences in pharmacological activities. To diminish essential public health risk, adulteration of Ephedra products could be discovered by the presence of both enantiomers, such as naturally occurring (−)-ephedrine and synthetic (+)-ephedrine in the samples [13].

In the case of C. urantium alkaloids, synephrine has also effect on human metabolism that could help to reduce fat mass in obese people, since it stimulates lipolysis, raises the metabolic rate and promotes the oxidation of fat through increased thermogenesis [14]. Synephrine is a chiral compound and is clinically administered as the racemic mixture, although its enantiomers have been

illustrated to apply different pharmacological activity on α- and β-adrenoreceptors. Particularly, (R)-(−)-synephrine is from 1 to 2 orders of magnitude more active than its (S)-(+)-counterpart (**Figure 1**) [15].

2.2 Heterocyclic alkaloids

2.2.1 Tropane alkaloids

Solanaceae contain mainly tropane alkaloids such as atropine, anisodamine and scopolamine; these plants are extensively used both in traditional medicine and as sources for the extraction of the pharmacologically important (parasympatolytic and anti-cholinergic) alkaloids [10]. Atropine is existed in racemic mixture of (S)-hyoscyamine and (R)-hyoscyamine. (S)-hyoscyamine is original in plants and (R)-hyoscyamine forms under alkaline conditions. (S)-hyoscyamine functions competitive antagonist of muscarinic receptors, thereby inhibiting the parasympathetic activities of acetylcholine on the salivary and sweat glands, as well as gastrointestinal tract, while the (R)-hyoscyamine is mostly inactive. Atropine, which is more often applied than (S)-hyoscyamine, exhibits approximately half of the pharmacological activity of (S)-hyoscyamine. In reverse, Scopolamine is mostly applied as pure enantiomer, e.g. (S)-scopolamine bromide [16].

Anisodamine, a tropane alkaloid isolated from Solanaceae family (Scopolia tangutica Maxim.). In China for decades, Anisodamine is an effective cholinoceptor antagonist and has been used as a spasmolytic drug to effect on smooth muscle by feature of its weaker side effect on the central nervous system than atropine. This kind of alkaloids have biological characteristic including cholinoceptor agonists and antagonists, like most chiral drugs, depend strongly on their stereochemistry. The effectiveness differences among four isomers of anisodamine racemic on muscarinic receptors have been perceived (**Figure 2**) [17].

2.2.2 Aconitine alkaloids

Aconitum plants (Ranuncolaceae) are generally distributed across Asia and North America. In the Chinese Pharmacopeia, two species of them, A. carmichaeli Dexb. and A. kusnezoffiiare were listed. Aconitine and the congener mesaconitine and hypaconitine (**Figure 3**) are the important diester-diterpene alkaloids of aconitum plants. Although they have toxic effects on human health, they can also be used at low doses because their pharmacological effects such as anti-inflammatory and anti-pain are effectively [10].

2.2.3 Quinolizidine alkaloids

In the legume alkaloids, the largest single group is quinolizidine alkaloids. In distribution to species in the more primitive tribes of the Papilionoideae, they appear to be restricted. Because of their toxicity in humans and animals as

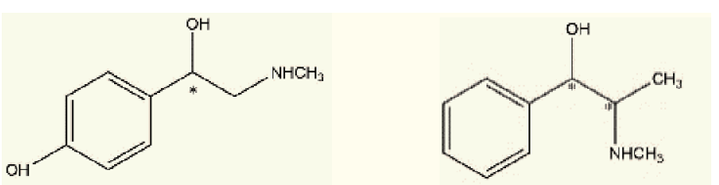


Figure 1.
*Molecular structure of synephrine and ephedrine alkaloids (*chiral center).*

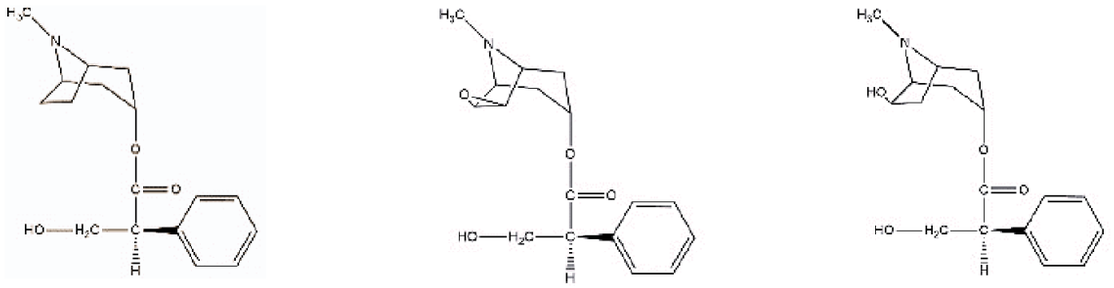
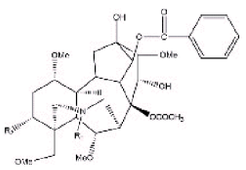


Figure 2.
Molecular structure of (S)-hyoscyamine, (S)-scopolamine, and anisodamine.



Alkaloid	R1	R2
Aconitine	C2H5	OH
Mesaconitine	CH3	OH
Hypaconitine	CH3	H

Figure 3.
Molecular structure of aconitine, mesaconitine and hypaconitine.

components of poisonous plants, these compounds become important. In contrast, some of them are potentially useful in pharmacological activities [18]. Radix sophorae flavescentis (*Sophora flavescens*) is frequently used in Traditional Chinese medicine for treating acute hepatitis and jaundice; it was found that quinolizidine alkaloids were the main constituents of this herbal drug such as matrine, sophoridine, sophocarpine, lehmannine, sophoranol, oxy-matrine, oxysophocarpine which have some chiral center [10, 19]. In natural, they exist as an isomer, sophocarpine (**Figure 4**) is an example. The naturally (−)-sophocarpine isolated from the root of the Chinese medicinal herb *Sophora flavescens* Ait. (Fam. Leguminosae) [20].

2.2.4 Isoquinoline alkaloids

Bis-benzylisoquinoline alkaloids have fascinated by the significant pharmacological impacts; especially, protoberberines are a structural class of organic cations (quaternary ammonium alkaloids) mostly distributed in Ranuncolaceae (e.g., Rhizoma coptidis), Berberidaceae (e.g. Cortex berberdis), Papaveraceae (e.g. Herba chelidoni) and Rutaceae (e.g. Cortex phellodendri) [10]. The most considered chiral isoquinoline alkaloids are tetrahydroprotoberberine backbone structure such as tetrahydropalmatine (THP), tetrahydroberberine (THB), and corydaline [21]. (DL)-THP and (DL)-THB are highly abundant in C. yanhusuo and a variety of Corydalis plants. (L)-THP can also be isolated from Stephania plants [22]. Tetrahydropalmatine is one of the active ingredients isolated from Rhizoma Corydalis (yanhusuo), a traditional Chinese medicine that has been used for the treatment of chest pain, epigastric pain, dysmenorrhea, traumatic swelling, and pain for thousands of years [23]. The analgesic activity of (−)-THP is much higher than that of (+)-THP. Clinically, THP is used as the racemic mixture (**Figure 5**) [22].

Amaryllidaceae alkaloids are an important class of iso-quinoline derivatives; among them galanthamine, that is found in Galanthus and Narcissus species, has been approved for the pharmacological treatment of Alzheimer's disease [24]. There are several chiral centers in this molecule, but only one S-enantiomer responsible for Alzheimer's disease, other stereoisomers considered as impurity (**Figure 6**) [25].

Morphinane alkaloids (opium alkaloids) such as morphine, codeine, thebaine, papaverine and narcotine belong to isoquinoline derivatives and show a broad

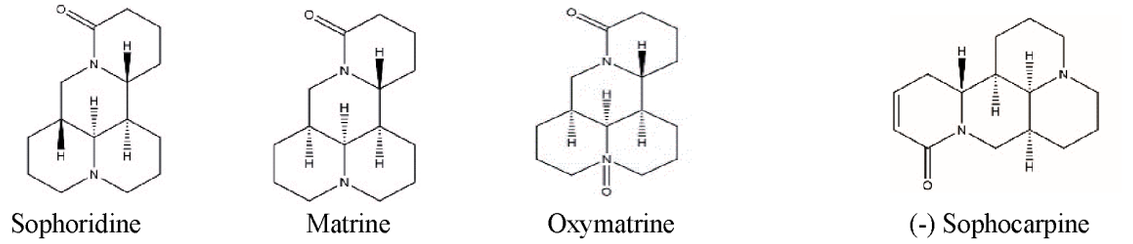


Figure 4.
Molecular structures of four quinolizidine alkaloids.

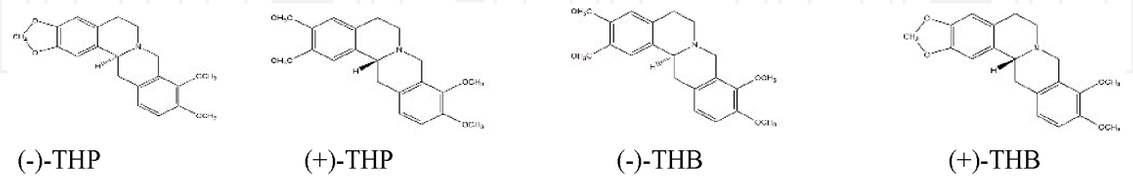


Figure 5.
Molecular structures of the enantiomers of tetrahydropalmatine (THP), and tetrahydro-berberine (THB).

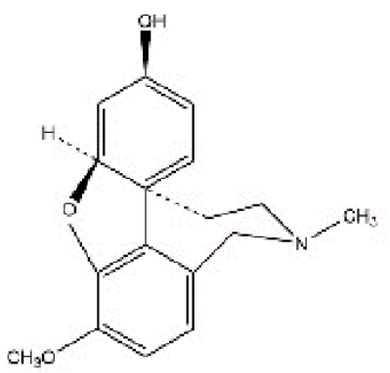


Figure 6.
Molecular structures of galanthamine.

range of pharmacological activities; their major application is in analgesia, sedation and cough depression [26]. Although opiate alkaloids have an important place in medicine, the illegal trafficking and abuse of heroin (the diacetyl derivative of morphine) has become a widespread problem [27]. Opium, the exudates from *Papaver somniferum*, contains more than 30 alkaloids and is the raw material for extraction; also, the dried heads of *P. somniferum*, so-called poppy straw, is used as a source of morphine and thebaine. **Figure 7** show the molecular structure of opium alkaloids, most of them have multi chiral center.

2.2.5 Pyrrolizidine alkaloids

Although these alkaloids have at present no great medicinal significance, they are important in that they constitute the poisonous hepatotoxic constituents of plants of the genus Senecio (Compositae), well-known for their toxicity to livestock [28]. Pyrrolizidine alkaloids are found in a variety of plant species growing wide world such as Gynura segetum that belongs to the Compositae family and Senecio and Tussilago genera [10]. The majority of naturally occurring pyrrolizidine alkaloids (PA) are hepatotoxic causing liver damage and in some cases liver cancer. Toxic PAs are often responsible for serious health problems through direct consumption of PA-containing herbal teas, herbal medicines, and herbal dietary supplements [29].

The most important pyrrolizidine alkaloids senecionine, seneciphylline, retrorsine and senkirkine, contain the 4-azabicyclo [3.3.0] octane system with senecionine and seneciphylline differing only for the presence in the latter of the C_{13}-C_{23} double bond (**Figure 8**) [30].

2.2.6 Indole alkaloids

Indole alkaloids constitute a wide class of natural products most of them pharmacologically important and characterized by very different activities [31]. In the recent years, attention has been focused on the biological activity of yohimbine which is a monoterpenoid indole alkaloid (**Figure 9**). It displayed the treatment of erectile functional disturbance and anxiogenic [32]. Hydroindole alkaloids such as mesembrine and congeners (mesembrenone, Δ_7 mesembrenone, mesembranol and its stereoisomer epimesembranol) have been isolated from Sceletium species used for the psychoactive effects [33].

The vinca alkaloids were isolated from the Madagascar periwinkle, Catharantus roseus G. Don., which included a class of about 130 terpenoid indole alkaloids [32]. In early 1965, people obviously know their clinical quality. And this group of compounds has been taken advantage of as an anticancer servant for more than 40 years and is a symbol of the compound that gives the trend to drug development [34, 35]. Among these base (+)-vincamine exhibits a valuable therapeutic activity in cerebral insufficiencies. Due to the presence of three stereogenic centers eight stereoisomers (four enantiomeric pairs) are in fact possible (**Figure 10**) [36].

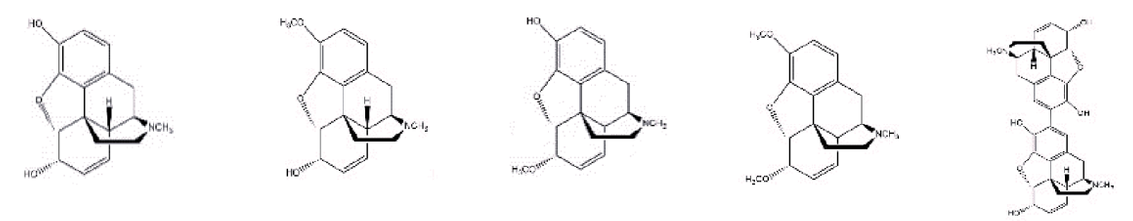


Figure 7.
Molecular structures of: (1) morphine; (2) codeine; (3) oripavine; (4) thebaine; (5) pseudomorphine.

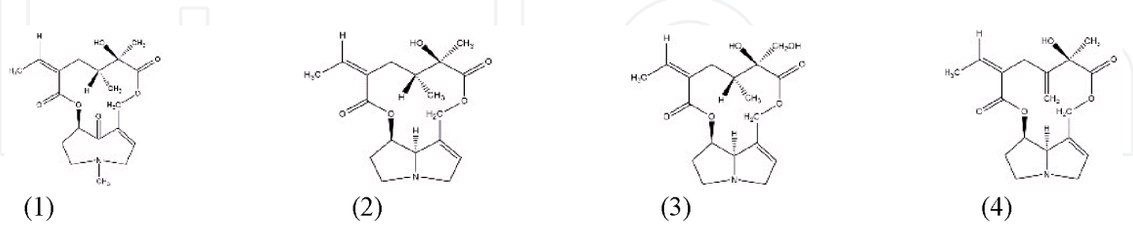


Figure 8.
Molecular structures of four toxic PAs. (1) senkirkine; (2) senecionine; (3) retrorsine; (4) seneciphylline.

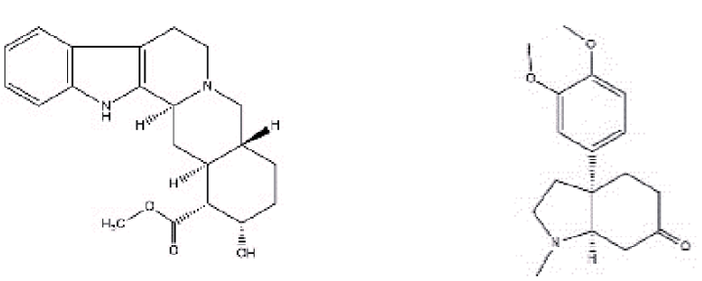


Figure 9.
Molecular structure of yohimbine and mesembrine.

2.2.7 Miscellaneous alkaloids

Steroidal alkaloids including verticine and verticinone are distinguished by cholestane carbon skeleton (isosteroid alkaloids) with a hexacyclic benzo [7, 8] fluoreno [2,1-b] quinolizine nucleus (**Figure 11**). These compounds have been isolated from plants from Liliaceae family typically Bulbus fritillariae used as a traditional medicine in Japanese, Turkish, Pakistani, and south-east Asian folk medicines [10]. Pharmacological studies demonstrate that verticine and verticinone in Bulbus Fritillariaeare the primary active ingredients responsible for the antitussive activity [37].

Stemona, belonging to Stemonaceae family, is known in the folk medicine of Southeast Asia, China, and Japan since its Phyto-preparations (primary the roots) are used to treat diseases about bronchitis, pertussis and tuberculosis. Interestingly many alkaloids, structurally defined as pyrido[1,2-α]azepines, have been recognized in this plant species and are considered the important pharmacological activity. All the Stemona alkaloids are polycyclic and contain multiple stereocenters [38]. Up to now, there are about 139 Stemona alkaloids which the scientist isolated (**Figure 12**).

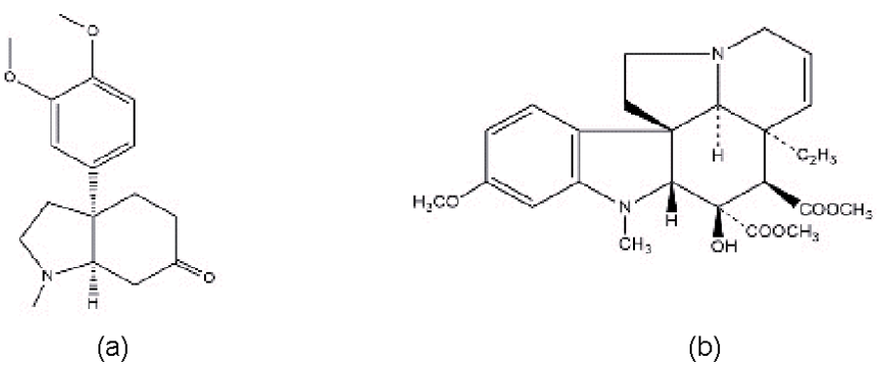


Figure 10.
Molecular structure of major vinca alkaloids isolated from Catharanthus roseus*: (+)-Catharanthine (A) and (−)-Vindoline (B).*

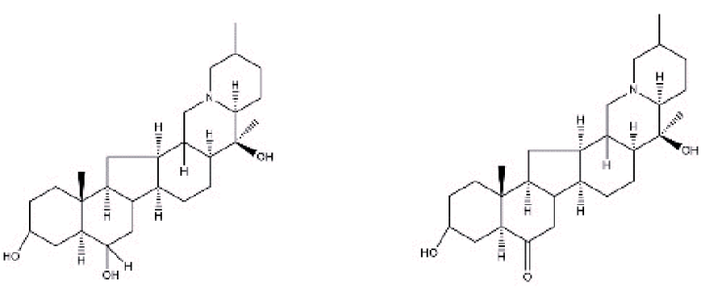


Figure 11.
Molecular structures of verticine and verticinone.

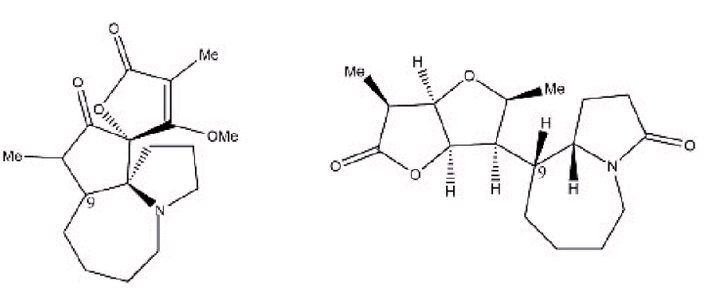


Figure 12.
Molecular structure of stemonamine and parvistemoline.

3. Techniques of extraction and purification

Analytical methods usually contain several steps, such as sampling, sample preparation, isolation, and quantification. Remarkably sample preparation just recently is concentration as an important analytical step. According to Wen *et al*. [39, 40], the main objective of sample preparation are removal of interferences, and preconcentration of the analytes that is considered a bottleneck of analytical processes. In this chapter, we will provide the current state of the art in sample preparation for analyzing alkaloids in herbal matrices, focusing on extraction, clean-up steps and purification.

3.1 Extraction

3.1.1 Ultrasound assisted extraction (UAE)

Ultrasound Assisted Extraction (UAE) technique is based on the using of acoustic waves in the kilohertz range spreading in liquid medium. These waves created by the ultrasound produces regions of compression and rarefaction in the molecules. Then, the cavitation bubbles are formed and collapse giving rise to smaller bubbles that could act as new cavitation nuclei or simply get dissolved. When the bubbles collapse at the surface of the herbal material, a shockwave having very high temperature and pressure is induced, resulting in plant cell disruption which enhances both the mass transfer of alkaloids into solution and the solvent penetration [41]. In addition, the swelling of plant materials can be enhanced by ultrasound, leading to improving of solvent penetration which increases the extraction yield [42]. The UAE procedure is optimized with regard to extraction solvent, temperature and liquid to solid ratio for the plant sample [41]. UAE has some advantageous properties including high extraction efficiency, good reproducibility, low solvent consumption, low cost and environmental friendliness. However, the major disadvantage of UAE is generating heat, leading to the degradation of thermo labile products and racemization of chiral compounds [43]. To avoid such types of drawback, extraction is carried on under an ice bath to reduce the temperature [44]. **Table 1** lists examples of protocols that were developed using MAE from various plants.

3.1.2 Microwave assisted extraction (MAE)

The MAE technique uses the electromagnetic radiations with a frequency range of 0.3–300 GHz, that stimulates ion migration and dipole rotation leading to the heating of dielectric materials and the penetration of extraction solvent into the matrix [49]. The released thermal energy is increased gradually with higher dielectric constant, so the effectiveness of MAE is depended on the dielectric properties of both extraction solvent and sample matrix [50]. Therefore, only specific solvents which have high dipole moment as water, methanol and ethanol can be used for extraction solvent in MAE and the moisture of plant sample is an important factor in the extraction efficiency [51]. Basically, higher water content matrices will be expected to give higher extraction yields. Water contained in plant matrices absorbs microwave radiation creating pockets of localized heating in the sample. This heat promotes the plant cell walls rupture which enhances the release of alkaloids into the solvent and the increase of extraction yield [50]. In addition to having a high dielectric constant, the extraction solvents must have a high dissipation factor to reduce the potential of localized sample overheating resulting degradation of alkaloids in plants [43]. Therefore, organic solvent-water mixtures, polar

Plant	Compounds	Solvent	Solvent: biomass (mL: g)	Temp (°C)	Time duration (min)	Ref.
Macleaya cordata	Protopine Allocryptopine Sanguinarine Chelerythrine Dihydrochelerythrine Dihydrosanguinarine	1-hexyl-3-methylimidazolium tetrafluoroborate ([C6MIM][BF4]) aqueous solution	500: 1	80	15	[45]
Catha edulis	Norephedrine Cathine Cathinone	0.1 N HCl	600: 1	—	45	[46]
Ipomoea genera	Ergot alkaloid (ergine, ergometrine, lysergic acid α-hydroxyethylamide) Penniclavine Chanoclavine Lysergol	70% methanol	100: 1	60	30	[47]
Carica papaya	Carpaine Pseudocarpaine Dehydrocarpaine I Dehydrocarpaine II	100% methanol	100: 7.5	—	20	[48]

Table 1.
Extraction conditions from various plants using UAE.

organic solvents and water are usually used as extraction solvent. Moreover, other parameters relating to extraction performance as sample size, sonication power, solid to liquid ratio, extraction time and microwave power should be modified for MAE procedure optimization [34]. The main advantages of MAE are the low solvent consumption, the ability to extract many samples simultaneously and the short extraction time [43, 52]. The major drawbacks of MAE are nonhomogeneous heating distribution and overheating of extract which may cause racemization or thermal degradation of chiral alkaloids [44]. **Table 2** lists examples of protocols that were developed using MAE from various plants.

3.1.3 Supercritical fluid extraction (SFE)

The SFE process utilizes pressurized fluids (mainly CO_2) as extraction solvents. In this technique, a fluid is heated and compressed to reach above critical point of it's creating the fluid having physicochemical properties of both liquid and gas states called supercritical fluid [58]. Specifically, supercritical fluid has a density similar to liquid (0.3–0.8 g/cm^3), a viscosity similar to gas (10^{-4} – 10^{-3} g/s.cm) and a diffusion coefficient that is intermediate between liquid and gas [59]. Therefore, supercritical fluid has higher transport capacity which facilitate to fluid diffusion through plant materials in comparison to traditional extraction solvents [43]. In addition, the density is related to polarity property of fluid which directly impact in solubility of compounds in extraction solvent. This parameter can be modified by controlling temperature and/or pressure so the flexibility and selectivity of the technique is enhanced, enabling selective extraction of different compounds from the plant matrix [60]. Carbon dioxide is commonly used in SFE because it has ideal properties including low critical temperature (31.3°C) and can be easily remove from extracts [51]. However, carbon dioxide is less effective in extraction of polar compounds from matrix because of its low polarity property. Aiming to extract

Plant	Compounds	Solvent	Solvent: biomass (mL: g)	Tem (°C)	Time duration	Micro wave power (W)	Ref.
Peganum harmala	Vasicine, Harmalin Harmine	80% ethanol	30: 1	80	8 min	600	[53]
Stephania sinica	Sinoacutine Palmatine Isocorydine L-tetrahydro palmatine	65% ethanol	24: 1	60	90 s	150	[54]
Lotus plumule	Liensinine Dauricine Isoliensinine Neferine Nuciferine	65% methanol	—	—	4 min	200	[55]
Corydalis decumbens	Protopine Palmatine Allocryptopine Jatrorrhizine Tetrahydro palmatine Corypalmine Bicuculline	90% methanol	20: 1	40	5 min	—	[56]
Menispermum dauricum	Bianfugedine, Menisporphine, 6-O-demethyl menisporphine Bianfugecine Dauriporphine Dauriporphinoline	70% ethanol	20: 1	60	11 min	—	[57]

Table 2.
Extraction conditions from various plants using MAE.

more polar alkaloids, the modifiers such as methanol, ethanol or water are added to extend the range of the solvating strength [43, 47]. For optimization of SFE procedure, these parameters such as pressure, temperature, modifier, flow of carbon dioxide and modifier [51]. Besides, the extraction time also effects on extraction yield, since an inadequate extraction time can result in incomplete extraction, while too long extraction time can cause the degradation of compounds. The major drawback of SFE are the complexity of system configuration and the requirement for a personal training program to operate the instrument [61].

3.1.4 Pressurized solvent extraction (PSE)

PSE process uses the pressurize solvents to enhance transport capacity of solvents and mass transfer rates which leads to improve extraction performance. In this technique, extraction solvents are heated at/or above the solvent's boiling points to decrease viscosity while keeping its in liquid state thanks to an elevated pressure [62]. Therefore, the extraction process is enhanced kinetic which leads to decreasing both the extraction time and solvent consumption. Similar to SFE, these parameters such as solvents nature, temperature and pressure should be modified to optimize PSE procedure [51]. Logically, higher temperature would be expected to give higher alkaloid extraction yields. However, excessive temperature may cause

degradation and racemization of chiral alkaloids. The main advantages of PSE are utilizing an extensive range of solvents (except strong acids/bases), low solvent consumption, short extraction time, automated instruments and performing an oxygen- and light – free extraction condition. Besides, the major drawbacks of PSE are similar to SFE such as using expensive laboratory equipment and requirement for a professional training to operate instrument [43].

3.2 Purification

Due to the alkaloids usually exist in plants at low concentration and the complication of plant matrices, samples should be purified and enriched which facilitate to identify and/or quantify process right after extraction step. Liquid–liquid extraction (LLE) and solid phase extraction (SLE) are popular clean-up methods utilized for sample preparation of alkaloids. Moreover, other techniques based on LLE and SPE methods, such as Liquid Membrane Extraction (LME) and Solid-Phase Micro Extraction (SPME) have also been developed.

3.2.1 Liquid–liquid extraction (LLE)

Liquid–Liquid Extraction (LLE) is the most simple and traditional clean-up method. LLE method is based on the relative solubility of compounds between two immiscible solvents. The alkaloids have polarity varying between pH, so the solubility of alkaloids in specific solvent are also affected by pH [34]. In the acid solutions (pH is lower than pKa of alkaloids), the analytes are protonated which leads to better water solubility so this aqueous phase can be washed with less polar organic solvents such as ethyl acetate, n-hexane and diethyl ether to eliminate hydrophobic interferences. After that, this aqueous layer is alkalinized which leads to the alkaloids becoming non-polarity and can be extracted by organic solvents to eliminate hydrophilic interferences [63]. For enrichment, the organic solvents layer can be collected, vaporized and reconstituted into new solvents which is suitable for analytical instrument. The main disadvantages of this method are requirement for repetitive extraction causing time consuming and solvent wasting [51].

3.2.2 Solid phase extraction (SPE)

To overcome the drawback of LLE method, solid phase extraction (SPE) has been developed and applied in sample preparation since the 1970s. In this method, extract is loaded onto a sorbent phase which will retain alkaloids. Then, interferences in extract are washed away and the analytes is eluted by suitable solvents [64]. In fact, cartridge is the most popular SPE type due to its convenience. The SPE procedure have five step including conditioning, loading, washing and elution step. Several factors can affect to the extraction efficiency such as concentration of analytes in solvent, loading solvent nature, sorbent types, particle size, volume used for loading – washing – eluting, flow rate and elution solvent. Each factor has specified role which depends on the affinity of analytes and solid phase. Because the chemical structures of alkaloids always have secondary or tertiary amine groups, the strong cation exchange (SCX) sorbents are an ideal choice for sample preparation. When using these sorbents, washing solvent will be water and organic solvent to eliminate both hydrophilic and hydrophobic from plant matrices. After that, alkaloids will be deprotonated for elution by alkalized solvent which has pH at 2 units above pKa of analytes and evaporated to enrich sample [64].

If the analytes are unstable in strong alkaline solutions, the weak cation exchange (WCX) will be use instead. The WCX sorbent has carboxylic acid as

functional group which has pKa value about 4.8, so these sorbents should be conditioned by solutions having pH above 6.8 for sorbent ionization. In addition, the loading and washing solvent pH should be adjusted at the value above 6.8 and below 2 value of analytes's pKa to maintain the ionized state of both sorbent and analytes. Finally, the alkaloids will be eluted by the acidic solutions [63]. Besides, the C_{18} and C_8 sorbents are also applied to extract aromatic alkaloids and eliminate hydrophilic interferences from matrices. In some case, those sorbents could be used for eliminating hydrophobic interferences by loading unretained alkaloids through cartridges [64].

4. High performance liquid chromatography in chiral separation

High performance liquid chromatography (HPLC) is currently the most widely used chromatographic enantio-separation technique [65]. HPLC has become one of the most common modern chemical analysis techniques because of its versatility, efficiency, stability, reproducibility and sensitivity. With these advantages, HPLC continues to be one of the best choices for chiral analysis and separation. Basically, chiral separation by HPLC techniques included direct and indirect methods. The indirect method is based on diastereomer formation by the derivatization reaction of analytes and a chiral reagent, then the separation of diastereomeric derivatives is performed by using a column having an achiral stationary phase. In addition, the direct method uses a chiral stationary phase for chiral separation or forms diastereomer by using a chiral mobile phase additive (CMPA).

HPLC using CSPs has demonstrated to be extremely useful, accurate, versatile, and it has been a widely used technique in diverse fields and applications, emphasizing (**Table 3**). The CSP mode is generally the most straightforward and convenient means for chromatographic enantiomer separation; it is the method of choice for both analytical and preparative applications [66–69]. A hundred CSPs have been developed and commercialized thirty years ago [70]. Besides, the larger number of CSPs are made in laboratory for specialized separation. CSPs are divided into nine major types by Snyder basing on the interaction mechanism between stationary phase and analytes [71].

- Macromolecular selectors of semisynthetic origin (polysaccharides)
- Macromolecular selectors of synthetic origin (poly(meth)acrylamides), (poly-tartramides)
- Macromolecular selectors of natural origin (proteins)
- Macrocyclic oligomeric or intermediate-sized selectors (cyclodextrins, macrocyclic antibiotics, chiral crown ethers)
- Synthetic, neutral entities of low molecular weight (Pirkle-type phases, brush-type CSPs)
- Synthetic, ionic entities of low molecular weight that provide for ion exchange
- Chelating selectors for chiral ligand-exchange chromatography.

Type	CSP	Typical column trade name	Application
I	Polysaccharide	AD, OD, OJ, AS, IA, IB, IC	Alkaloids, tropines, amines, beta blockers, aryl methyl esters, aryl methoxy esters
II	Synthetic-Polymer CSPs	Kromasil CHI-DMB and CHI-TBB	Acidic, neutral, and basic compounds
III	Protein Phases	Chiral HSA, Chiral AGP, Ultron ES-OVM, Chiral CBH	Benzodiazepine, Warfarin and oxazepam, beta blockers
IV	Cyclodextrin	Cyclobond I, II, III	Beta blockers
V	Macrocyclic Antibiotic	Chirobiotic V, T, R, TAG; vancomycin	Polar compounds such as underivatized amino acids
VI	Chiral Crown-Ether	ChiroSil RCA(+); SCA(−); ChiralHyun-CR-1	Amino acids, amino acid esters, amino alcohols
VII	Donor-Acceptor Phases	Whelk-O 1, ULMO, Sumichiral 2500, Sumichiral OA 4900	Amides, epoxides, esters, ureas, carbamates, ethers, aziridines, phosphonates, aldehydes,ketones, carboxylic acids, alcohols
VIII	Chiral Ion-Exchangers	Chiralpak QN-AX; Chiralpak QD-AX	Chiral carboxylic, sulfonic, phosphonic, and phosphoric acids
IX	Chiral Ligand-Exchange	Chiralpak MA+, Nucleosil Chiral-1	Amino acids

Table 3.
Application of nine major types of CSP and their commercial CSP [72].

4.1 Type I polysaccharide-derived CSPs in HPLC

Polysaccharide-derived CSPs are widely used in enantio-separation of a large number of chiral compounds [71]. The development of polysaccharide-derived CSPs has continued for about three decades. It can be roughly divided into two stages (i) the coated CSPs stage, and (ii) the immobilized CSPs stage.

4.1.1 Coated polysaccharide-derived CSPs

Polysaccharide selectors have been used for enantioselective liquid chromatography technique for a long time. In 1973, a polymeric selector (without supporting matrix) was introduced by Hesse and Hagel named as microcrystalline cellulose triacetate (MCTA) used for enantioselective liquid chromatography. MCTA are widely applied in enantio-recognition and preparative separations due to its ideal loading capacities. However, this material has major disadvantages including poor pressure stability, slow separations, and low chromatographic efficiency. To overcome the mechanical stability problem of MCTA, a solution was found by Okamoto and co-workers in 1984, in which the surface of macro-porous silica beads (100 or 400 nm pore size) was coated by the cellulose derivatives at about 20 wt%. Thanks to this coating, the mechanical stability of this material was remarkably improved resulting in better efficiencies qualified for HPLC enantiomer separations. Such coated polysaccharide-based CSPs were the highest level of polymeric selector developments for several decades [71].

Nowadays, about 200 kinds of polysaccharide derivatives were introduced by using different polysaccharides which includes cellulose, amylose, chitin, chitosan, galactosamine, curdlan, dextran, xylan, and inulin [72] (**Figure 13**).

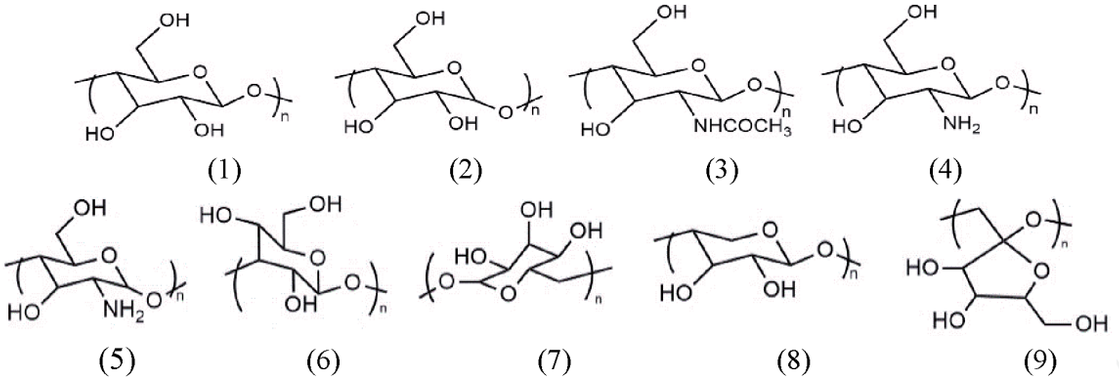


Figure 13.
Structures of the various kinds of polysaccharides: (1) cellulose; (2) amylose; (3) chitin; (4) chitosan; (5) Galatosamine; (6) Curdlan; (7) dextran; (8) Xylan; (9) inulin.

These materials have been coated on a surface of macro-porous silica gel to create CSPs and followed by the evaluation of chiral recognitions on HPLC.

Each of coated polysaccharide-based CSPs exhibit the different enantioselectivity and elution order of the various enantiomers due to the structural differences of CSPs including sugar units, linkage position, and linkage type. In particular, the derivatives of cellulose and amylose usually perform higher recognition abilities than the others, though this property also depends on the structure of a specific racemate. The most useful and successful derivatives of cellulose and amylose are triesters and tricarbamate. It has been claimed by Aboul-Enein and Ali that for the resolution of about 500 test racemates, about 80% of them have been successfully resolved on only two kinds of polysaccharide derivative-based CSPs (cellulose and amylose tris (3,5-diphenylcarbamate) CSPs) [72]. More specifically, three famous commercially available CSPs, CHIRALCEL OD, OJ, and CHIRALPAK AD, have fully or partially resolved 70% racemates among over 100 racemates tested [71].

A current strategy introduced by Snyder for chiral separation method development includes trial-and-error experiments of various polysaccharide-type CSPs under multiple respective mobile-phase conditions using fully automated column- and solvent-switching. Nowadays, more and more studies have focused on developing a more efficient screening procedure to enhance the chance for success and shorten the experiment time: the most favorable CSP in the normal phase mode is

Chiralpak AD – –– > Chiralcel OD – – – – > Chiralcel OJ

The separation efficiency of column should be tested before conducting screening experiment if serial instead of parallel screening is utilized. The application of coated polysaccharide-derived CSPs has been reviewed in **Table 4** including the names of CSPs and their most frequent applications.

4.1.2 Immobilized polysaccharide-derived CSPs

The coated CSPs are formed by coating the polysaccharide derivatives onto surface of silica gel. Due to the weak linkages and interactions between the polysaccharide derivatives (chiral selector) and silicagel (substrate), a number of organic solvents including chloroform, dichloromethane, tetrahydrofuran and ethyl acetate which can dissolve or swell the chiral selector are not allowed

Trade name	Chemical name	Applications
Cellulose CSPs		
Chiralcel OB	Cellulose trisbenzoate	Small aliphatic and aromatic compounds
Chiralcel OJ	Cellulose tris(4-methyl benzoate)	Aryl methyl esters, aryl methoxy esters
Chiralcel OC	Cellulose trisphenylcarbamate	Cyclopentanones
Chiralcel OD	Cellulose tris(3,5-dimethylphenylcarbamate)	Alkaloids, amines, β -adrenergic blockers
Chiralcel OD-H[b]	Cellulose tris(3,5-dimethylphenylcarbamate)	Alkaloids, amines, β -adrenergic blockers
Chiralcel OD-R[c]	Cellulose tris(3,5-dimethylphenylcarbamate)	Alkaloids, amines, β -adrenergic blockers
Chiralcel OD-RH[d]	Cellulose tris(3,5-dimethylphenylcarbamate)	Alkaloids, amines, β -adrenergic blockers
Chiralcel OF	Cellulose tris(4-chlorophenylcarbamate)	β -Lactams, dihydroxypryidines, alkaloids
Chiralcel OG	Cellulose tris(4-methylphenylcarbamate)	β -Lactams, alkaloids
Chiralcel OA	Cellulose triacetate on silica gel	Small aliphatie compounds
Amylose CSPs		
Chiralpak AD	Amylose tris(3,5-dimethylphenylcarbamate)	Alkaloids, tropines, amines, β -adrenergic blockers
Chiralpak AD-R[a]	Amylose tris(3,5-dimethylphenylcarbamate)	Alkaloids, tropines, amines, β -adrenergic blockers
Chiralpak AD-RH[b]	Amylose tris(3,5-dimethylphenylcarbamate)	Alkaloids, tropines, amines, β -adrenergic blockers
Chiralpak AD-H	Amylose tris-(3,5-dimethylphenylcarbamate)	Alkaloids
Chiralpak AR	Amylose tris(R)-1-phenylethylcarbamate	Alkaloids, tropines, amines
Chiralpak AS	Amylose tris(S)-1-methylphenylcarbamate	Alkaloids, tropines, amines

[a]*Columns supplied by Daicel Chemical Industries, Tokyo, Japan, Dimension are column size 25 cm X 0,46 cm, particle size 10 μm, except as noted.*
[b]*Column size 25 cm X 0,46 cm, particle size 5 μm.*
[c]*Column size 15 cm X 0,46 cm, particle size 10 μm.*
[d]*Column size 15 cm X 46 cm, particle size 5 μm.*

Table 4.
Various polysaccharide-based commercial CSP [72, 90, 91, 93].

using as mobile phase components. Besides, the mixtures of alkanes (n-pentane, n-hexane or n-heptane) and alcohols (2-propanol (IPA), ethanol or methanol (n-pentane, n-hexane or n-heptane)) and alcohols (2-propanol (IPA), ethanol or methanol) are favorable mobile phase solvents used in normal phase mode. The addition of "prohibited solvents" may lead to better separation and greater solubility of racemic analytes than the standard solvent combinations so the performance of separation method is improved. Besides, the better solubility of racemic analytes also facilitates preparative separations. In addition, the spectroscopic techniques used for chiral recognition should ideally be in the "prohibited solvents". As a result, chemical immobilization of polysaccharide derivatives becomes an interesting research topic to overcome the drawbacks of the coated

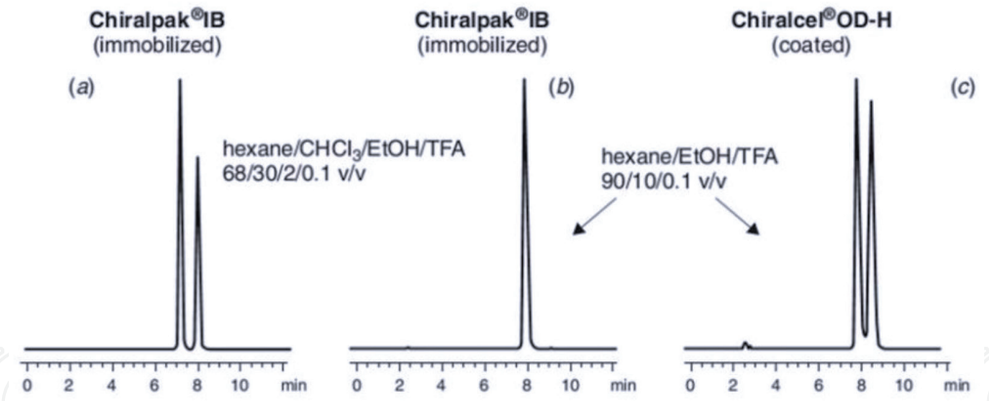


Figure 14.
Effect of column type (immobilized vs. coated polysaccharide-based CSP) and mobile phase on enantioselectivity. Enantiomer separations of N-benzyloxycarbonyl-phenylalanine (a–c) with Chiralpak IB (immobilized) and Chiralcel OD (coated). Flowrate: 1 mL/min; temperature: 25°C; UV detection at 230 nm. Note that the mobile phases used in (a) is forbidden mobile phases for the coated version Chiralcel OD [71].

CSPs (see in **Figure 14**). In 2005, Daicel and Chiral Technologies introduced a set of three immobilized polysaccharide CSPs as [71]:

- Chiralpak®IA (immobilized version of Chiralpak AD)
- Chiralpak®IB (immobilized Chiralcel OD) [61]
- Chiralpak®IC based on the cellulose tris (3,5-dichlorophenylcarbamate) selector that is not available in coated form.

Finally, it should be noted that polysaccharide CSPs have now also been established as the first-choice of chiral phases for enantiomer separation.

5. Analytical methods of chiral alkaloids in medicinal plants

Analytical applications, including CSPs, separation conditions and analyte, are summarized in **Table 5**. A review focused mainly on the latest examples of chiral alkaloids separations on CSPs for efficient analyses was prepared.

Novel column materials also improved enantiomer separation of tropane alkaloids. Separation of (R, S)-hyoscyamine was achieved using chiral stationary phase with immobilizing α-1-acid glycoprotein (Chiral AGP®) [83], alternatively, enantiomer separation of atropine could be achieved by a chirobiotic V column packed with vancomycin as chiral selector [84]. Anisodamine, the 6β-hydroxyl derivative of (S)-hyoscyamine was separated from its synthetic enantiomer and diastereomers by a Chiralpak AD-H column as chiral stationary phase, which uses an amylose derivative as chiral selector [85]. Satropane, 3α-paramethylbenzenesulfonyloxy-6β-acetoxy-tropane, could be resolved in 3S, 6S-isomer named lesatropane and 3R, 6R-isomer named desatropane. Lesatropane as a novel muscarinic agonist is being under preclinical development in China as a single enantiomer drug for the treatment of primary glaucoma. The separation of lesatropane from desatropane was conducted by both Chiralpak AD-H and Chiralpak AS-RH column [86].

Chiral separation of isoquinoline alkaloid has also achieved by using chiral stationary phase. For example, the determination of tetrahydropalmatine (THP) was performed by using a Chiralcel OJ column with quantification by UV at

Columns	Analyte	Plant	Separation conditions	Ref.
CHIRALPAK AS-H column	(12S,22S)-Dihydroxyisoechinulin A (2) and (12R/S)-Neoechinulin A	Cannabis sativ L	Hexane/isopropanol/diethylamine (4:1:0.05)	[73]
CHIRALPAK AD-H column	Mucroniferanine A	Corydalis mucronifera	n-hexane–2-propanol (70:30)	[74]
	(±)-homocrepidine A	Dendrobium crepidatum	n-hexane/2-propanol (95:5)	[75]
	(+)-(3R,6R)- and (−)-(3S,6S)-3α,6β-tropanediol	Erythroxylaceae species	n-hexane and 2-propanol (9:1) with 0.1% of diethylamine	[76]
Chirobiotic V, Chiralpak-AY3 column	(−) and (+) hyoscyamine	Solanaceaes seeds	Ethanol, 0.1% DEA	[77]
Chirex 3019 chiral column	S-(−)-canadine and R-(+)-canadine	*Hydrastis Canadensis* L.	Hexan:DCE:EtOH:TFA (75:40:7:0.1)	[78]
CHIRALPAK AGP column	(R)-nicotine; (S)-nicotine; anabasine, and anatabine	Tobacco	NH_4OH- methanol (90:10)	[79]
A Phenomenex Lux Cellulose-2 chiral column	(+)- and (−)-5-hydroxyl-8-oxyberberine	Coptis chinensis	CH_3CN:H2O (40:60)	[80]
Chiralpak IA column	intermedine and lycopsamine	Symphytum uplandicum	ACN/methanol (80:20) and methanol/methyl-t-butyl ether (90:10)	[81]
Phenomenex-Chirex-3126 column	dihydrocarneamide A and iso-notoamide B	Paecilomyces variotii	MeCN–H_2O (5:95).	[82]

Table 5.
Summary of CSPs, mobile phase compositions, and applications.

230 nm. This developed method was used for determining the pharmacokinetics of THP enantiomers in rats and dogs after oral administration [87]. Another report of isoquinoline alkaloid group is sanguinarine derivatives. Chiral determination of Benzophenanthridine alkaloids from methanol extracts of Hylomecon species was conducted by Chiralcel OD (4.6 x 250 mm) column with mixture of isopropanol-hexane-diethylamine (20/80/0.1, v/v/v) as a mobile phase [88]. The stereochemistry of L-isocorypalmine and the D/L ratio of tetrahydropalmatine, stylopine, and corydaline were established unambiguously by using a chiral Chiralcel OD (4.6 x 250 mm) column; 50% ethanol as mobile phase; wavelength 230 nm [89]. Cularinoids are a group of isoquinoline alkaloids consisting of about 60 members. The HPLC enantiomeric separation of the racemic cularinoid alkaloids N-p-methoxy-1,α-dihydroaristoyagonine and 4′,5'demethoxy-1,α-dihydroaristoyagonine was accomplished using five chiral stationary phases (CSPs), the good enantioselectivity and resolution factor obtained with a polysaccharide-derived CSP (Chiralpak AD) [90].

Indole derivatives are widely used in chiral synthesis, chemical asymmetric catalysis, biological and medicinal chemistry. Recently, the enantiomeric separation of several chiral plant growth regulators and related compounds, such as 3-(3-indolyl)-butyric acid, abscisic acid and structurally related molecules including a variety of substituted tryptophan compounds was reported. Chiral stationary phases such as coated and immobilized were suitable for the separation of indole derivatives; however, the coated CSP possesses a higher resolving power than the immobilized one [91]. Tangutorine, a biogenetically interesting indole alkaloid, was found in the leaves of Nitraria tangutorum in 1999. It was separated from its synthetic enantiomer by chiral stationary phases two polysaccharide-derived CSP (Chiralcel OD and Chiralpak AD) and a network polymer incorporating a bifunctional C_2-symmetric chiral selector (Kromasil CHI-DMB) [92]. The HPLC enantiomeric separation of racemic indole alkaloids tacamonine, 17α-hydroxytacamonine, deethyleburnamonine, and vindeburnol was accomplished using Chiralpak AD and Chiralcel OD as chiral stationary phases [93].

The enantiomers of homocamptothecin (hCPT) derivatives which constitute a promising series of potent anticancer agents targeting DNA topoisomerase I were separated by using the combination of two silica-based normal phase column including Chiralcel OD-H (celluloses tris-3,5-dimethylphenylcarbamateand) Chiralcel OJ (celluloses tris-methylbenzoate) or Chiralpak AD (amyloses tris-3,5-dimethylphenylcarbamate) and Chiralpak AS (amyloses tris-(S)-1-phenylethyl-car-bamate) [94]. A method for the simultaneous determination of eight Cinchona alkaloids (quinine, quinidine, cinchonine, cinchonidine, and their corresponding dihydro analogs) using a novel strong cation-exchange-type chiral stationary phase (cSCX) column in HPLC has been developed and exemplarily applied to impurity profiling of a commercial alkaloid sample [95].

6. Conclusion

Most of alkaloids are chiral compounds and are clinically administered as the racemic mixture, although its enantiomers have been shown to exert different pharmacological activity. The complication of sample matrices and low concentration of chiral alkaloids are major challenges for analytical processes. To improve the performance of analytical procedure, we provide the current state of the art in sample preparation focusing on extraction and purification to remove interferences and enrich analyte concentrations. HPLC using CSPs has demonstrated to be extremely useful, accurate, versatile, its mode is generally the most straightforward

and convenient means for chromatographic enantiomer separation. The development of CSPs for HPLC is a continuous and challenger issue covering various types of CSPs.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014 Jan 10;4:177. doi: 10.3389/fphar.2013.00177.

[2] Ravindra Kumar Pandey, Shiv Shankar Shukla and *et al*., Fingerprinting Analysis and Quality Control Methods of Herbal Medicines, ISBN 9781138036949, Published July 1, 2018 by CRC Press.

[3] Nguyen LA, He H, Pham-Huy C. Chiral drugs: an overview. Int J Biomed Sci. 2006 Jun;2(2):85-100. PMID: 23674971; PMCID: PMC3614593.

[4] Pellati F., and Benvenuti S., Chromatographic and electrophoretic methods for the analysis of phenetylamine alkaloids in Citrus aurantium. J. Chromatogr. A, 1161, 71-88 (2007).

[5] Gurib-Fakim, A., Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of medicine, 27, 1-93 (2006).

[6] Wink, M. (2013). Evolution of secondary metabolites in legumes (Fabaceae). South African Journal of Botany, 89. doi: 10.1016/j.sajb.2013.06.006

[7] Evans, W. C., *Pharmacognosy: Part 5* Pharmacopoeial and related drugs of biological origin, chapter alkaloids, 353-414, 16th edition (2009).

[8] Lesley Braun, Marc Cohen. Herbs and Natural Supplements. 4th ed. Volume 2. Churchill Livingstone Australia November 2014. p 1324.ISBN: 9780729581738.

[9] Amedeo Amedei', Elena Niccolai. Natural Products Analysis: Instrumentation, Methods, and Applications. Chapter 4: Plant and Marine Sources: Biological Activity of Natural Products and Therapeutic Use. p 53. 2014. DOI:10.1002/9781118876015.

[10] Gotti R., Capillary electrophoresis of phytochemical substances in herbal drugs and medicinal plants. J. Pharm. Biomed. Anal. 55, 775-801 (2011).

[11] Karen W. Phinney, Toshihide Ihara, Lane C. Sander, Determination of ephedrine alkaloid stereoisomers in dietary supplements by capillary electrophoresis. J. Chromatogr. A., 1077, 90-97 (2005).

[12] McCooeye, M., Ding, L., Gardner, G. J., Fraser, C. A., Lam, J., Sturgeon, R. E., and Mester, Z., Separation and Quantitation of the Stereoisomers of Ephedra Alkaloids in Natural Health Products Using Flow Injection-Electrospray Ionization-High Field Asymmetric Waveform Ion Mobility Spectrometry-Mass Spectrometry. Anal. Chem., 75, 2538-2542 (2003).

[13] Wang, M., Marriott, P. J., Chan, W-H., Lee, A. W. M., Huie, C. W., Enantiomeric separation and quantification of ephedrine-type alkaloids in herbal materials by comprehensive two-dimensional gas chromatography. J. Chromatogr. A, 1112, 361-368 (2006).

[14] Stohs SJ, Preuss HG, Shara M. A review of the human clinical studies involving Citrus aurantium (bitter orange) extract and its primary protoalkaloid p-synephrine. Int J Med Sci. 2012;9(7):527-538.

[15] Stohs SJ, Preuss HG, Shara M. A review of the receptor-binding properties of p-synephrine as related to its pharmacological effects. Oxid Med Cell Longev. 2011;2011:482973.

[16] Kohnen-Johannsen KL, Kayser O. Tropane Alkaloids: Chemistry, Pharmacology, Biosynthesis and Production. Molecules. 2019;24(4):796. 2019 Feb 22.

[17] Du N, Liu Y, Zhang X, Wang J, Zhao J, He J, Zhou H, Mei L, Liang X. Discovery of new muscarinic acetylcholine receptor antagonists from Scopolia tangutica. Sci Rep. 2017 Apr 7;7:46067.

[18] D. Cardoso, R.T. Pennington, *et al*., Reconstructing the deep-branching relationships of the papilionoid legumes, South African Journal of Botany. Volume 89. 2013. pp 58-75. https://doi.org/10.1016/j.sajb.2013.05.001.

[19] Liang N, Kong DZ, Nikolova D, Jakobsen JC, Gluud C, Liu JP. Radix Sophorae flavescentis versus antiviral drugs for chronic hepatitis B. Cochrane Database Syst Rev. 2018 Aug 23;2018(8):CD013106. doi: 10.1002/14651858.

[20] Zheng K, Li C, Shan X, Liu H, Fan W, Wang Z. A study on isolation of chemical constituents from Sophora flavescens Ait. and their anti-glioma effects. Afr J Tradit Complement Altern Med. 2013 Nov 2;11(1):156-160. doi: 10.4314/ajtcam.v11i1.24.

[21] Xiao W, Shen G, Zhuang X, Ran X, Zhu M, Li H. Characterization of human metabolism and disposition of levo-tetrahydropalmatine: Qualitative and quantitative determination of oxidative and conjugated metabolites. J Pharm Biomed Anal. 2016 Sep 5;128:371-381.

[22] Wang JB, Mantsch JR. L-tetrahydropalamatine: a potential new medication for the treatment of cocaine addiction. Future Med Chem. 2012 Feb;4(2):177-186. doi: 10.4155/fmc.11.166.

[23] Wu H, Waldbauer K, Tang L, Xie L, McKinnon R, Zehl M, Yang H, Xu H, Kopp B. Influence of vinegar and wine processing on the alkaloid content and composition of the traditional Chinese medicine Corydalis Rhizoma (Yanhusuo). Molecules. 2014 Aug 4;19(8):11487-11504.

[24] Heinrich M. Galanthamine from Galanthus and other Amaryllidaceae--chemistry and biology based on traditional use. Alkaloids Chem Biol. 2010;68:157-165. doi: 10.1016/s1099-4831(10)06804-5.

[25] Smith SW. Chiral toxicology: it's the same thing...only different. Toxicol Sci. 2009 Jul;110(1):4-30. doi: 10.1093/toxsci/kfp097. Epub 2009 May 4.

[26] Carlin MG, Dean JR, Ames JM. Opium Alkaloids in Harvested and Thermally Processed Poppy Seeds. Front Chem. 2020 Aug 27;8:737.

[27] Hindson, B. J., Francis, P. S., Purcell, S. D., Barnett, N. W., Determination of opiate alkaloids in process liquors using capillary electrophoresis. J. Pharm. Biomed. Anal., 43, 1164-1168 (2007).

[28] Molyneux RJ, Gardner DL, Colegate SM, Edgar JA. Pyrrolizidine alkaloid toxicity in livestock: a paradigm for human poisoning? Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2011 Mar;28(3):293-307.

[29] Li N, Xia Q, Ruan J, Fu PP, Lin G. Hepatotoxicity and tumorigenicity induced by metabolic activation of pyrrolizidine alkaloids in herbs. Curr Drug Metab. 2011 Nov;12(9):823-834.

[30] Chen T, Mei N, Fu PP. Genotoxicity of pyrrolizidine alkaloids. J Appl Toxicol. 2010 Apr;30(3):183-96. doi: 10.1002/jat.1504. PMID: 20112250; PMCID: PMC6376482.

[31] Hamid HA, Ramli AN, Yusoff MM. Indole Alkaloids from Plants as Potential Leads for Antidepressant Drugs: A Mini Review. Front Pharmacol. 2017 Feb 28;8:96. doi: 10.3389/fphar.2017.00096.

[32] Almagro L, Fernández-Pérez F, Pedreño MA. Indole alkaloids from Catharanthus roseus: bioproduction and their effect on human health. Molecules. 2015 Feb 12;20(2):2973-3000.

[33] Patnala, S., and Kanfer, I., HPLC Analysis of Mesembrine-Type Alkaloids in Sceletium Plant Material Used as an African Traditional Medicine. J. Pharm. Pharmaceut. Sci., 13(4), 558-570 (2010).

[34] Fattorusso, E., and Taglialatela-Scafati, O., Modern alkaloids, Chapter 1: Bioactive Alkaloids: Structure and Biology (2008).

[35] Verma, A., Laakso, I., Seppänen-Laakso, T., Huhtikangas, A., and Riekkola, M.-L., A Simplified Procedure for Indole Alkaloid Extraction from Catharanthus roseus Combined with a Semi-synthetic Production Process for Vinblastine. Molecules, 12, 1307-1315 (2007).

[36] Abdel-Salam, O.M.E., Hamdy, S.M., Seadawy, S.A.M. *et al*. Effect of piracetam, vincamine, vinpocetine, and donepezil on oxidative stress and neurodegeneration induced by aluminum chloride in rats. Comp Clin Pathol 25, 305-318 (2016). https://doi.org/10.1007/s00580-015-2182-0.

[37] Gao, Y., Xu, Y., Han, B., Li, J., and Xiang, Q., Sensitive determination of verticine and verticinone inBulbus Fritillariaeby ionic liquid assisted capillary electrophoresis–electrochemiluminescence system. Talanta, 80, 448-453 (2009).

[38] Liu XY, Wang FP. Recent Advances in the Synthesis of Stemona Alkaloids. Nat Prod Commun. 2015 Jun;10(6):1093-1102.

[39] Y. Wen, L. Chen, J. Li, D. Liu, L. Chen, Recent advances in solid-phase sorbents for sample preparation prior to chromatographic analysis, Trends Anal. Chem. 59 (2014) 26-41.

[40] Shang X, Guo X, Li B, Pan H, Zhang J, Zhang Y, Miao X. Microwave-assisted extraction of three bioactive alkaloids from Peganum harmala L. and their acaricidal activity against Psoroptes cuniculi in vitro. J Ethnopharmacol. 2016 Nov 4;192:350-361. doi: 10.1016/j.jep.2016.07.057.

[41] Esclapez, M.D., García-Pérez, J.V., Mulet, A. *et al.* Ultrasound-Assisted Extraction of Natural Products. Food Eng Rev 3, 108 (2011). https://doi.org/10.1007/s12393-011-9036-6.

[42] Vinatoru M. An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrason Sonochem. 2001 Jul;8(3):303-313. doi: 10.1016/s1350-4177(01)00071-2.

[43] Samar Al Jitan, Saeed A. Alkhoori, Lina F. Yousef. Chapter 13 - Phenolic Acids From Plants: Extraction and Application to Human Health. Studies in Natural Products Chemistry, Elsevier,Volume 58, 2018, pages 389-417, https://doi.org/10.1016/B978-0-444-64056-7.00013-1.

[44] Dey P, Kundu A, Kumar A, Gupta M, Lee BM, Bhakta T, Dash S, Kim HS. Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). Recent Advances in Natural Products Analysis. 2020:505-567. doi: 10.1016/B978-0-12-816455-6.00015-9.

[45] Linqiu Li, Mingyuan Huang, Junli Shao, Bokun Lin, Qing Shen. Rapid determination of alkaloids in Macleaya cordata using ionic liquid

extraction followed by multiple reaction monitoring UPLC–MS/MS analysis. Journal of Pharmaceutical and Biomedical Analysis. Volume 135. 2017. Pages 61-66.

[46] Atlabachew M, Chandravanshi BS, Redi-Abshiro M. Preparative HPLC for large scale isolation, and salting-out assisted liquid-liquid extraction based method for HPLC-DAD determination of khat (Catha edulis Forsk) alkaloids. Chem Cent J. 2017 Oct 17;11(1):107. doi: 10.1186/s13065-017-0337-6.

[47] Nowak J, Woźniakiewicz M, Klepacki P, Sowa A, Kościelniak P. Identification and determination of ergot alkaloids in Morning Glory cultivars. Anal Bioanal Chem. 2016 May;408(12):3093-3102. doi: 10.1007/s00216-016-9322-5.

[48] Soib HH, Ismail HF, Husin F, Abu Bakar MH, Yaakob H, Sarmidi MR. Bioassay-Guided Different Extraction Techniques of Carica papaya (Linn.) Leaves on In Vitro Wound-Healing Activities. Molecules. 2020 Jan 24;25(3):517. doi: 10.3390/molecules25030517.

[49] Delazar A, Nahar L, Hamedeyazdan S, Sarker SD. Microwave-assisted extraction in natural products isolation. Methods Mol Biol. 2012;864:89-115. doi: 10.1007/978-1-61779-624-1_5.

[50] Chan CH, Yusoff R, Ngoh GC, Kung FW. Microwave-assisted extractions of active ingredients from plants. J Chromatogr A. 2011 Sep 16;1218(37):6213-6225. doi: 10.1016/j.chroma.2011.07.040. Epub 2011 Jul 23.

[51] Luiz Carlos Klein-Júnior, Yvan Vander Heyden, Amélia Teresinha Henriques. Enlarging the bottleneck in the analysis of alkaloids: A review on sample preparation in herbal matrices. TrAC Trends in Analytical Chemistry, Volume 80, 2016, Pages 66-82.

[52] Kopp T, Abdel-Tawab M, Mizaikoff B. Extracting and Analyzing Pyrrolizidine Alkaloids in Medicinal Plants: A Review. Toxins (Basel). 2020 May 13;12(5):320. doi: 10.3390/toxins12050320.

[53] Shang X, Guo X, Li B, Pan H, Zhang J, Zhang Y, Miao X. Microwave-assisted extraction of three bioactive alkaloids from Peganum harmala L. and their acaricidal activity against Psoroptes cuniculi in vitro. J Ethnopharmacol. 2016 Nov 4;192:350-361. doi: 10.1016/j.jep.2016.07.057.

[54] Dao-Tao Xie, Ya-Qin Wang, Yun Kang, Qiu-Fen Hu, Na-Ya Su, Jian-Ming Huang, Chun-Tao Che, Ji-Xian Guo. Microwave-assisted extraction of bioactive alkaloids from Stephania sinica. Separation and Purification Technology. Volume 130. 2014. Pages 173-181.

[55] Xiong W, Chen X, Lv G, Hu D, Zhao J, Li S. Optimization of microwave-assisted extraction of bioactive alkaloids from lotus plumule using response surface methodology. J Pharm Anal. 2016 Dec;6(6):382-388.

[56] Mao Z, Di X, Zhang J, Wang X, Liu Y, Di X. Rapid and cost-effective method for the simultaneous quantification of seven alkaloids in Corydalis decumbens by microwave-assisted extraction and capillary electrophoresis. J Sep Sci. 2017 Jul;40(14):3008-3014. doi: 10.1002/jssc.201700051.

[57] Wei J, Chen J, Liang X, Guo X. Microwave-assisted extraction in combination with HPLC-UV for quantitative analysis of six bioactive oxoisoaporphine alkaloids in Menispermum dauricum DC. Biomed Chromatogr. 2016 Feb;30(2):241-248. doi: 10.1002/bmc.3541.

[58] Jerry W. King, Fundamentals and Applications of Supercritical Fluid

Extraction in Chromatographic Science, Journal of Chromatographic Science, Volume 27, Issue 7, July 1989, Pages 355-364.

[59] Ernesto Reverchon, Iolanda De Marco. Supercritical fluid extraction and fractionation of natural matter. The Journal of Supercritical Fluids,Volume 38, Issue 2, 2006, Pages 146-166. https://doi.org/10.1016/j.supflu.2006.03.020.

[60] Rui P.F.F. da Silva, Teresa A.P. Rocha-Santos, Armando C. Duarte. Supercritical fluid extraction of bioactive compounds. TrAC Trends in Analytical Chemistry. Volume 76, 2016, Pages 40-51. https://doi.org/10.1016/j.trac.2015.11.013.

[61] Miguel Herrero, Alejandro Cifuentes, Elena Ibañez. Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. Food Chemistry, Volume 98, Issue 1, 2006, Pages 136-148. https://doi.org/10.1016/j.foodchem.2005.05.058.

[62] Kaufmann B, Christen P. Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. Phytochem Anal. 2002 Mar-Apr;13(2):105-113. doi: 10.1002/pca.631.

[63] Ezel Boyacı, Ángel Rodríguez-Lafuente, Krzysztof Gorynski, Fatemeh Mirnaghi, Érica A. Souza-Silva, Dietmar Hein, Janusz Pawliszyn. Sample preparation with solid phase microextraction and exhaustive extraction approaches: Comparison for challenging cases, Analytica Chimica Acta, Volume 873, 2015. Pages 14-30. https://doi.org/10.1016/j.aca.2014.12.051.

[64] Thurman, E. M., & Mills, M. S. (1998). Solid-phase extraction: Principles and practice. New York: Wiley. Pp. 1-48.

[65] Teixeira J, Tiritan ME, Pinto MMM, Fernandes C. Chiral Stationary Phases for Liquid Chromatography: Recent Developments. Molecules. 2019 Feb 28;24(5):865. doi: 10.3390/molecules24050865.

[66] Lämmerhofer, M. Chiral recognition by enantioselective liquid chromatography: Mechanisms and modern chiral stationary phases. J. Chromatogr. A 2010, 1217, 814-856, doi:10.1016/j.chroma.2009.10.022.

[67] Fernandes, C.; Tiritan, M.E.; Cravo, S.; Phyo, Y.Z.; Kijjoa, A.; Silva, A.M.S.; Cass, Q.B.; Pinto, M.M.M. Newchiral stationary phases based on xanthone derivatives for liquid chromatography. Chirality 2017, 29, 430-442, doi:10.1002/chir.22706.

[68] Scriba, G.K.E. Chiral recognition in separation science—An update. J. Chromatogr. A 2016, 1467, 56-78, doi:10.1016/jchroma.2016.05.061.

[69] Kalíková, K.; Riesová, M.; Tesařová, E. Recent chiral selectors for separation in HPLC and CE. Cent. Eur. J. Chem. 2011, 10, 450-471, doi:10.2478/s11532-011-0142-3.

[70] Ali I, Suhail M, Asnin L. Chiral separation of quinolones by liquid chromatography and capillary electrophoresis. J Sep Sci. 2017 Jul;40(14):2863-2882. doi: 10.1002/jssc.201700200.

[71] Snyder, L. R., Kirkland, J. J., and Dolan, J. W., Introduction to modern liquid chromatography, 3rd ed., 666-715 (2010).

[72] Aboul-Enein, H. Y., and Ali, I., Chiral Separations by Liquid Chromatography and Related Technologies, 90, chapter 2 (2003).

[73] Xiaoli Yan, Yuefang Zhou, Jiajing Tang, Mei Ji, Hongxiang Lou, Peihong Fan. Diketopiperazine indole alkaloids

from hemp seed. Phytochemistry Letters,Volume 18, 2016, Pages 77-82. https://doi.org/10.1016/j.phytol.2016.09.001

[74] Zhang J, Zhang QY, Tu PF, Xu FC, Liang H. Mucroniferanines A-G, Isoquinoline Alkaloids from Corydalis mucronifera. J Nat Prod. 2018 Feb 23;81(2):364-370. doi: 10.1021/acs.jnatprod.7b00847.

[75] Hu Y, Zhang C, Zhao X, Wang Y, Feng D, Zhang M, Xie H. (±)-Homocrepidine A, a Pair of Anti-inflammatory Enantiomeric Octahydroindolizine Alkaloid Dimers from Dendrobium crepidatum. J Nat Prod. 2016 Jan 22;79(1):252-256. doi: 10.1021/acs.jnatprod.5b00801.

[76] Muñoz, Marcelo & González, Natalia & Joseph-Nathan, Pedro. (2016). Enantiomeric High-performance liquid chromatography resolution and absolute configuration of 6β-benzoyloxy-3α-tropanol. Journal of Separation Science. 39. 10.1002/jssc.201600061.

[77] Jesús Marín-Sáez, Roberto Romero-González, Antonia Garrido Frenich, Enantiomeric determination and evaluation of the racemization process of atropine in Solanaceae seeds and contaminated samples by high performance liquid chromatography-tandem mass spectrometry. Journal of Chromatography A, Volume 1474, 2016, Pages 79-84.

[78] Francisco García Sánchez, Aurora Navas Díaz, Ignacio Medina Lama, Alfonso Aguilar & Manuel Algarra (2014). HPLC enantioseparation of the alkaloid canadine and determination of enantiomeric purity with chiral/photometric and achiral/polarimetric detection, Journal of Liquid Chromatography & Related Technologies, 37:1, 26-38, DOI: 10.1080/10826076.2012.733996.

[79] Huihua, Ji & Wu, Ying & Fannin, Franklin & Bush, Lowell. (2019). Determination of tobacco alkaloid enantiomers using reversed phase UPLC/MS/MS. Heliyon. 5. e01719. 10.1016/j.heliyon.2019.e01719.

[80] Wang, Lei & Zhang, Sheng-Yuan & Chen, Liang & Huang, Xiao-Jun & Zhang, Qing-Wen & Jiang, ren-wang & Yao, Fen & Ye, Wen-Cai. (2014). New enantiomeric isoquinoline alkaloids from Coptis chinensis. Phytochemistry Letters. 7. 89-92. 10.1016/j.phytol.2013.10.007.

[81] Pawar, Rahul & Grundel, Erich & Mazzola, Eugene & White, Kevin & Krynitsky, Alexander & Rader, Jeanne. (2010). Chiral stationary phases for separation of intermedine and lycopsamine enantiomers from Symphytum uplandicum. Journal of separation science. 33. 200-5. 10.1002/jssc.200900611.

[82] Peng Zhang, Xiao-Ming Li, Jia-Ning Wang, Xin Li, Bin-Gui Wang. Prenylated indole alkaloids from the marine-derived fungus Paecilomyces variotii. Chinese Chemical Letters, Volume 26, Issue 3, 2015, Pages 313-316.

[83] Soares, R., Singh, A. K., Kedor-Hackmann, E. R. M., and Santoro, M. I. R. M., Determination of Atropine Enantiomers in Ophthalmic Solutions by Liquid Chromatography Using a Chiral AGP® Column. J. AOAC. INT., 92, 1663-1672 (2009).

[84] Aehle, E., and Drager, B., Tropane alkaloid analysis by chromatographic and electrophoretic techniques: An update. J. Chromatogr. B., 878, 1391-1406 (2010).

[85] Yang, L.-M., Xie, Y.-F., Chen, H.-Z., and Lu, Y., Diastereomeric and enantiomeric high-performance liquid chromatographic separation of synthetic anisodamine. J. Pharm. Biomed. Anal., 43, 905-909 (2007).

[86] Yang, L.M., Xie, Y. F., Gu, Z. H., Wang, A. L., Chen, H. Z., and Lu, Y., Development and Validation of a Reversed-Phase HPLC Method for Determination of Lesatropane and Enantiomeric Impurity. Chirality, 23, 581-584 (2011).

[87] Hong, Z., Fan, G., Chai, Y., Yin, X., and Wu, Y., Stereoselective Pharmacokinetics of Tetrahydropalmatine after Oral Administration of (−)-Enantiomer and the Racemate. Chirality, 17, 293-296 (2005).

[88] Kang, J. S., Pham, H. L., Lim, H. M., Kim, Y. H., and Blaschke, G., Achiral and chiral determination of Benzophenanthridine alkaloids from methanol extracts of Hylomecon species by HPLC. Arch. Pharm. Res., 26, 114-119 (2003).

[89] Ma, Z-Z., Xu, W., Jensen, N. H., Roth, B. L., Liu-Chen, L-Y., and Lee, D. Y. W., Isoquinoline Alkaloids Isolated from Corydalis yanhusuo and Their Binding Affinities at the Dopamine D1 Receptor. Molecules, 13, 2303-2312 (2008).

[90] Caccamese, S., Scivoli, G., Bianca, S., L'opez-Romero, J. M., and Ortiz-L'opez, F. J., Chiral high-performance liquid chromatographic separation and circular dichroism spectra of the enantiomers of cytotoxic aristocularine alkaloids. J. Chromatogr. A, 1129, 140-144 (2006).

[91] Zhao, L., Ming, Y., Zhang, H. L., and Jiang, S., Enantioselective Separation of Indole Derivatives by Liquid Chromatography Using Immobilized Cellulose (3,5-Dimethylphenylcarbamate) Chiral Stationary Phase. J. Anal. Chem. 64, 795-805 (2009).

[92] Putkonen, T., Tolvanen, A., Jokela, R., Caccamese, S., and Parrinello, N., Total synthesis of (±)-tangutorine and chiral HPLC separation of enantiomers. Tetrahedron, 59, 8589-8595 (2003).

[93] Caccamese, S., Principato, G., Jokela, R., Tolvanen, A., and Belle, D., Chiral HPLC Separation and CD Spectra of the Enantiomers of the Alkaloid Tacamonine and Related Compounds. Chirality, 13, 691-693 (2001).

[94] Goossens, J. F., Foulon, C., Bailly, C., Bigg, D. C. H., Bonte, J. P., and Vaccher, C., Chiral Resolution of Enantiomers of Homocamptothecin Derivatives, Antitumor Topoisomerase I Inhibitors, Using High Performance Liquid Chromatography on Polysaccharide-Based Chiral Stationary Phases. Chromatographia, 59, 305-313 (2004).

[95] Jasiewicz, B., and Wyrzykiewicz, E., Mass Spectrometry of Bis-quinolizidine Alkaloids: Differentiation of Stereoisomers and Metamers Using ESI and FAB Mass Spectrometry. Spectroscopy Letters, 42, 49-57 (2009).