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Review Article

The impact of chirality on the analysis of alkaloids in plant

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Abstract

Most of the alkaloids are chiral compounds and are clinically administered as the racemic mixture, even though its enantiomers have been known to exert different pharmacological activity. The determination of the enantiomeric composition of alkaloid-containing plants is subject to severe attention from pharmacological and toxicological points of view. This review gives an overview of the chiral analysis of alkaloids that were used in theoretical studies and applications for plants in recent years.

Keywords

chiral alkaloids, high performance liquid chromatography, capillary electrophoresis, chiral selectors, chiral separations

Introduction

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 (Inaki et al. 2016). Living organisms show variation in biological responses to one of a couple of enantiomers in medicines due to the chirality (Ariens et al. 1983). Almost half of the drugs in use are chiral. It is well known that the pharmacological effect is restricted in most of the cases to one of the enantiomers (eutomer) (Ariens et al. 1983). Nonetheless, only about 25% of drugs are administered as pure enantiomers. In pharmacokinetic and pharmacodynamic studies, the chiral separation of drug enantiomers plays a very important role (Eichelbaum et al. 1996). For enantiomer separation on analytical scale a great variety of methods based on chromatographic techniques such as HPLC, GC, SFC, TLC have been developed during the past three decades (Gübitz et al. 2001).

The separation techniques most widely employed to perform chiral analysis have been those based on chromatographic and electrophoretic principles, such as Thin-layer Chromatography (TLC), High Pressure Liquid Chromatography (HPLC), Gas Chromatography (GC), Supercritical Fluid Chromatography (SFC), and Capillary Electrophoresis (CE). Up to date, HPLC has been by far the most used technique to achieve chiral separations (55% of the publications related to chiral analysis), followed by CE (22%) and GC (15%), while SFC and TLC have been the less employed (5 and 3%, respectively) (Fig. 1, data

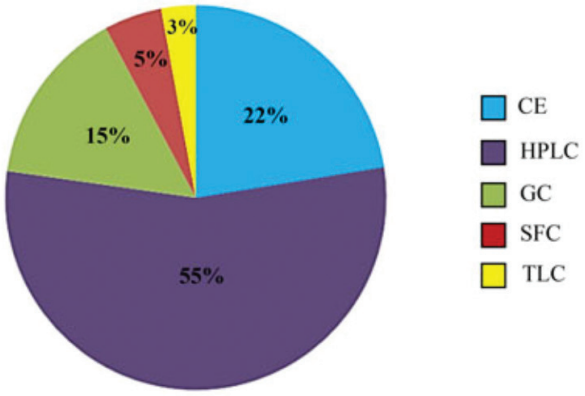

Figure 1. Classification of the number of published articles related to the enantiomeric separation of chiral compounds according to the different chromatographic and electrophoretic separation techniques used. Data obtained from SciFinder Scholar up to June 2019.

obtained from SciFinder Scholar up to June 2019). More recently, HPLC and CE have also been shown to be useful techniques for this purpose. Liquid chromatographic resolution of enantiomers on chiral stationary phases (CSPs) is recognized as the most accurate and convenient tools to determinate the enantiomeric composition of chiral compounds including chiral drugs (Scriba 2019). Besides, capillary electrophoresis (CE) has been increasingly employed for the chiral separation of pharmaceutical agents and drugs. CE provides a number of merits such as short analysis time, high resolution power and low operational cost. Enantio-separations are generally performed by adding a chiral selector to the running buffer. Various additives acting as chiral selectors have been reported in the literature, such as cyclodextrins (CDs), crown ethers, proteins, antibiotics, bile salts and chiral micelles (Bressolle et al. 1996; Mateus et al. 2000).

Alkaloids are the secondary metabolites that are important because of their therapeutic properties. On the basis of their biosynthetic precursor and heterocyclic ring system, the compounds have been classified into various categories which include indole, piperidine, tropane, purine, pyrrolizidine, imidazole, quinolizidine, iso-quinoline and pyrrolidine alkaloids. Alkaloids are able to prevent the onset of various degenerative diseases by free radical scavenging or binding with the oxidative reaction catalyst (Thawabteh et al. 2019). In the last years, an increasing interest has been observed towards bioactive phytochemical substances, especially in alkaloids. Most alkaloids are chiral compounds and are clinically administered as the racemic mixture, although its enantiomers have been shown to exert different pharmacological activity, such as sagunarine, galanthamine, ephedrine, camptothecin (S-form), and vincamine. Numerous studies have indicated that the pharmacological effects of (R) – nicotine are qualitatively similar to but quantitatively less potent than those of (S)- nicotine (Benowitz et al. 2009). It was suggested that (S) – and (R) – nicotine may bind to different subtypes of nicotine acetylcholine receptors each other (Fowler et al. 2008). Galanthamine which is found in Galanthus, and Narcissus, has been approved for the pharmacological treatment of Alzheimer's disease. S- Galantamine is the active ingredient in drug while R-enantiomer is inactive (Mucke 2015). Camptothecin is well known as anticancer factor, only the (S)-enantiomers of CPTs exhibit antitumor activity (Li et al. 2017). The determination of the enantiomeric composition of alkaloid-containing plants is subject to severe attention from pharmacological and toxicological points of view. Here, we would discuss the techniques of chiral alkaloid analysis including HPLC, CE and sample preparation for multiple sample matrices. Then, we focus on the application of them in determination of chiral alkaloids in plants. We hope our study may overview of the chiral analysis of alkaloids for plants in recent years.

Techniques of chiral alkaloid analysis

High performance liquid chromatography

The chiral separation of alkaloids in natural product was conducted by direct method which is based on diastereomer formation by using a chiral stationary phase (CSP) (Haginaka 2002). 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 1). 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 (Lämmerhofer 2010; Kalíková et al. 2011; Scriba 2016;

Table 1. Application of nine major types of CSP and their commercial CSP (Aboul-Enein HY et al. 2003).

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

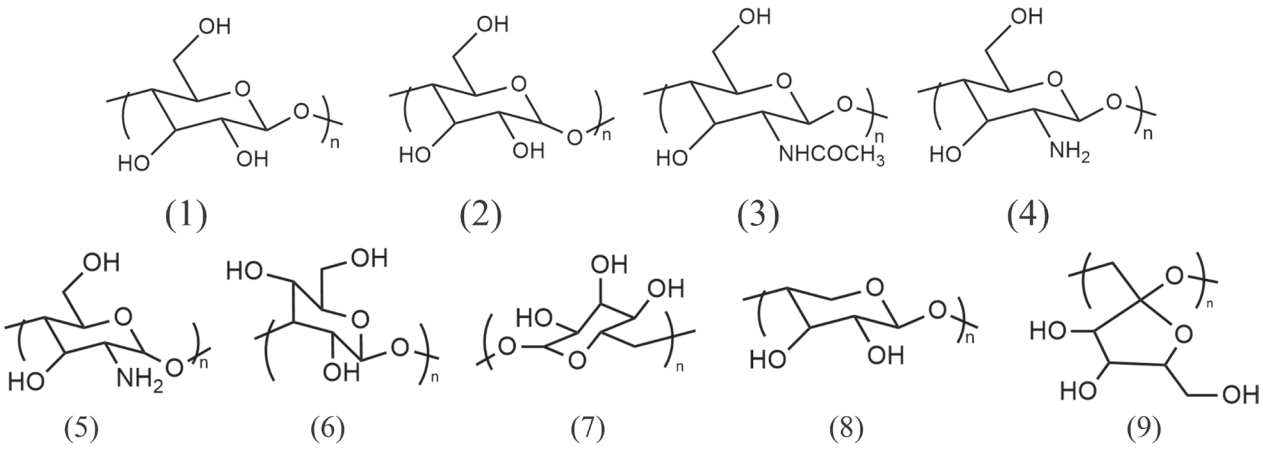

Figure 2. Structures of the various kinds of polysaccharides: (**1**) Cellulose; (**2**) Amylose; (**3**) Chitin; (**4**) Chitosan; (**5**) Galatosamine; (**6**) Curdlan; (**7**) Dextran; (**8**) Xylan; (**9**) Inulin.

Fernandes et al. 2017). There are more than a hundred commercially available CSPs that have been developed over the past thirty years, (Ali et al. 2017) not to mention the even larger number of "home-made" CSPs. HPLC CSPs are classified by Lammerhofer into nine major types according to the interactions between CSPs and analytes (Lammerhofer et al. 2010).

Polysaccharide selectors have a long tradition in enantioselective liquid chromatography. In 1973, Hesse and Hagel introduced microcrystalline cellulose triacetate (MCTA) as a polymeric selector material (without supporting matrix) for enantioselective liquid chromatography. While MCTA exhibits widely applicable enantio-recognition and favorable loading capacities for preparative separations, it suffers from poor pressure stability, slow separations, and low chromatographic efficiency. A solution to the mechanical stability problem of MCTA was proposed by Okamoto and co-workers in 1984. The cellulose derivatives were coated at about 20 wt% onto the surface of macro-porous silica beads (100 or 400 nm pore size). These materials exhibited considerably improved mechanical stability and much better efficiencies, and permitted HPLC enantiomer separations. Such coated polysaccharide-based CSPs were state-of-the-art for several decades (Lammerhofer et al. 2010).

Until now, they have prepared about 200 kinds of polysaccharide derivatives based on different polysaccharides including cellulose, amylose, chitin, chitosan, galactosamine, curdlan, dextran, xylan, and inulin (Fig. 2) (Aboul-Enein et al. 2003). These derivatives have been coated on a macro-porous silica gel as CSPs, and their chiral recognitions were then evaluated by HPLC.

The enantioselectivity and the elution order of the various enantiomers differed among these polysaccharides depending on the sugar units, linkage position, and linkage type. Among them, the derivatives of cellulose and amylose usually exhibit higher recognition abilities than the others, although it depends on the structure of a specific racemate. Triesters and tricarbamate are the most useful and successful cellulose and amylose's derivatives. 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) (Aboul-Enein et al. 2003). The application of coated polysaccharide-derived CSPs has been reviewed in Table 2 including the names of 20 CSPs and their most frequent applications. 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 (Lammerhofer et al. 2010).

Capillary electrophoresis

Chiral analysis is one of the main fields of the chemical quality control of pharmaceuticals in which CE plays a pivotal role (Pellati et al. 2007). Because of the versatility and the high separation power, capillary electrophoresis (CE) meets the requirements for the herbal drugs's quality control. The wide opportunity for selectivity tuning allows the analysis of molecules with a wide range of polarity and molecular weight (Gotti 2011). CE is considered a miniaturized and environmentally friendly technique, as it requires very small volumes of sample and solvents (Xie et al. 2010). Moreover, HPLC chromatographic chiral columns are quite expensive, while CE does not require any column. Additionally, CE provides high efficiency, versatility, simplicity, and feasibility enabling fast method development and high success rate, since the type and the concentration of the chiral selector can be easily optimize (Fejos et al. 2020), which is impossible in HPLC where, in general, chiral stationary phases (CSP) are usually used for chiral analysis. Also, different chiral selectors can be combined in CE, whereas this is a difficult approach in chromatographic techniques (Saz et al. 2016).

A particular mode of fast capillary zone electrophoresis (CZE) is non-aqueous capillary electrophoresis (NACE), which employ non aqueous buffer system. Using organic solvents instead of water not only helps increase the hydrophobic analytes solubility but also improves selectivity. Actually, NACE widened the set of physicochemical

Table 2. Various Polysaccharide-Based Commercial CSP (Aboul-Enein et al. 2003; Caccamese et al. 2006; Zhao et al. 2009).

Trade name	Chemical name	Applications
Cellulose CSPs		
Chiralcel OB	Cellulose trisbenzoate	Small aliphatic and aromatic compounds
Chiralcel OB-H[b]	Cellulose trisbenzoate	Small aliphatic and aromatic compounds
Chiralcel OJ	Cellulose tris(4-methyl benzoate)	Aryl methyl esters, aryl methoxy esters
Chiralcel OJ-R[b]	Cellulose tris(4-methyl benzoate)	Aryl methyl esters, aryl methoxy esters
Chiralcel CMB	Cellulose tris(3-methylbenzoate)	Aryl esters and arylalkoxy 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
Chiralcel CTA	Cellulose triacetate, microcrystalline	Amides, biaryl compounds
Chiralcel OK	Cellulose triscinnamate	Aromatic 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 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 × 0.46 cm, particle size 10 m, except as noted,
[b] Column size 25 cm × 0.46 cm, particle size 5 μm.
[c] Column size 15 cm × 0.46 cm, particle size 10 μm.
[d] Column size 15 cm × 46 cm, particle size 5 μm.

characteristics of the solvents, which affect the electrophoretic characteristic by their influence on solute–solvent, solute–additive and ion–ion interactions. Most importantly, pKa values of basic analytes in organic solvents are notable different from those in water allowing separations which are difficult to be obtained in water. Moreover, NACE can be ideally suited for online coupling with mass spectrometry thanks to the high volatility and low surface tension of many organic solvents. A chiral microemulsion electrokinetic chromatography method has been developed to split up the enantiomers of the phenethylamines ephedrine, N-methylephedrine, norephedrine, pseudoephedrine, adrenaline (epinephrine), 2-amino-1-phenylethanol, diethylnorephedrine, and 2-(dibutylamino)-1-phenyl-1-propanol, respectively. The separations were achieved using an oil-in-water microemulsion consisting of the oil-component ethyl acetate, the surfactant sodium dodecylsulfate, the cosurfactant 1-butanol, the organic modifier propan-2-ol and 20 mM phosphate buffer pH 2.5 as aqueous phase. For enantio-separation sulphated -cyclodextrin was added. The developed method was successfully applied to impurity analysis (Borst et al. 2010). Electrokinetic chromatography (EKC), and in particular micellar electrokinetic chromatography (MEKC) has been introduced in 1984 by Terabe and proved to be not only the method of choice in analysis of neutral compounds but also one of the most versatile separation approach among the electromigration methods. In MEKC a surfactant (often sodium dodecyl sulfate, SDS) is introduced into the background electrolyte (BGE) at concentration above the critical micelle concentration in order to generate a micellar pseudo-stationary phase. Separation mechanism is a combination of chromatographic partitioning of solutes between pseudo-stationary phase and continuous phase and the electrophoretic mechanism.

In MEKC, the separation selectivity can be modulated not only by variation of BGE type, pH and concentration, but also by benefit of the proper surfactant selected with the optimal concentration. Because phyto-markers to be simultaneously analyzed in plant materials are often acidic, basic and neutral compounds, MEKC is widely applied. The polymeric chiral surfactant as a pseudo-stationary phase or chiral selector in MEKC proved to be a powerful and useful technique for the simultaneous quantitative analysis of chiral alkaloids with high sensitivity.

Microemulsion electrokinetic chromatography (MEEKC), which is similar to MEKC, is used as separation environment with pseudo-stationary phases represented by microemulsions. Typically, the microemulsions are composed of nanometer-sized oil droplets suspended in an aqueous buffer (oil-in-water microemulsions, O/W). These systems are stabilized by using surfactant i.e. SDS and a co-surfactant - a short-chain alcohol such as butanol. The oil droplets are collected by dispersing n-octane or other types of hydrophobic solvents (Hoffmann et al. 2009). Capillary electrochromatography (CEC), likes other electrokinetic techniques, combines the charged solutes electromigration with chromatographic partition; but then, the latter event is different to MEKC and MEEKC, the establishment between a liquid mobile phase and a solid stationary phase is packed in a fused-silica capillary (small particles, ~3 μm) which is fixed by silica sintering frits. CEC can also be used in monolithic stationary phases formed by the in situ (in capillary) polymerization of monomers. The mobile phase in CEC is driven through the stationary phase by electroosmotic flow (EOF), can leads to high separation efficiency by assuming a flat and homogeneous profile.

The analytical separation of chiral analytes is one of the most popular applications in capillary electrophoresis

Table 3. Summary of different modern techniques features.

Methods	Extraction characteristic	Advantages	Disadvantages	Major factors affecting the technique efficiencies	Method applications
UAE (Ultrasound assisted extraction)	– Using of acoustic waves in kilohertz range – Accelerating both mass transfer and solvent penetration	– High extraction efficiency and good reproducibility – Low solvent consumption – Low cost – Environmental friendliness (Samar et al. 2018)	– Heat generation leading to the degradation and racemization of chiral compounds (Samar et al. 2018; Dey et al. 2020)	Extraction solvent, liquid to solid ratio, temperature, extraction time, ultrasonic power and frequency	– Opium alkaloids from papaver plants (Fakhari et al. 2010) – Tropane alkaloids from Radix physochlainae (Yohannes et al. 2019)
MAE (Microwave assisted extraction)	– Utilizing electromagnetic radiations with a frequency ranging from 0.3–300 GHz. – Heating dielectric mateirals and improving solvent penetration	– Low solvent consumption. – Simultaneous extract many samples – Short extraction time (Samar et al. 2018; Soib et al. 2020)	– Nonhomogeneous heating distribution – Overheating of extraction solvent (Dey et al. 2020)	Microwave power, sample size, solid to liquid ratio, extraction time, solvent and sample nature	– Berberine and palmitine from Rhizoma coptidis (Xiong et al. 2016) – Atropine and scopolamine from Solanaceae family plants (Ciechomska et al. 2018)
SFE (Supercritical fluid extraction)	– Using pressurized fluids called as supercritical fluids (mainly CO_2) as extraction solvents (King et al. 1989)	– High flexibility and selectivity (Da Silva et al. 2016) – Extraction solvents can be removed easily from extracts (Nowak et al. 2016) – Environmental friendliness (Klein-Júnior et al. 2016)	– Complexity of system configuration – Requirement for a personal training program to operate the instrument (Herrero et al. 2006)	Pressure, temperature, Modifier (methanol, ethanol and water), flow of carbon dioxide (Klein-Júnior et al. 2016)	– dl-tetrahydropalmatine from *Corydalis yanhusu* (Liu et al. 2008) – Evodiamine and rutaecarpine from *Evodia rutaecarpa* (Liu et al. 2010)
PSE (Pressurized solvent extraction)	– Utilizing pressurize solvents – Enhancing transport capacity of solvents and mass transfer rates (Kaufmann et al. 2002)	– Using an extensive range of solvents – Low solvent consumption – Short extraction time – Automated instruments – Performing an oxygen- and light – free extraction condition (Samar et al. 2018)	– Using expensive laboratory equipment – Requirement for a personal training program to operate the instrument (Samar et al. 2018)	Solvents nature, temperature, Pressure (Klein-Júnior et al. 2016)	– Quaternary alkaloids from *Macleaya microcarpa* (Urbanová et al. 2011) – Galanthamine and lycorine from Amaryllidaceae plants (Mroczek et al. 2009)

(CE), commonly achieved by adding chiral selectors, most frequently cyclodextrins (CDs) to the background electrolyte (BGE). These popular selectors are composed of (1,4)-linked α-D-glucopyranose units forming a truncated cone shape and contain a hydrophilic outer surface surrounding a rather lipophilic cavity (Saz et al. 2016).

Sample preparation for chiral alkaloids

It is worth mentioning due to the different complexity of the matrices in natural pant, different sample preparation procedures have been evaluated including ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), pressurized solvent extraction (PSE), and supercritical fluid extraction (SFE) (see in Table 3).

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 (Vinatoru 2001). Then, the cavitation bubbles are formed, grow, oscillate and collapse giving rise to smaller bubbles. 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 accelerates both the mass transfer of alkaloids into solution and the solvent penetration (Shang et al. 2016).

The UAE procedure is optimized with regard to extraction solvent, liquid to solid ratio, extraction time and temperature for the plant sample (Esclapez et al. 2011). Besides, the ultrasonic power and frequency (given in Watt and Kilohertz, respectively) can be adjusted easily to enhance extraction yields. Mostly, the increase of ultrasonic power and frequency can lead to a higher yield, as demonstrated for the extraction of opium alkaloids from papaver plants (Fakhari et al. 2010) and tropane alkaloids from Radix physochlainae (Yohannes et al. 2019). However, in some cases, excessively high ultrasonic power may decrease the alkaloid contents in the extract due to degradation as described for the extraction of tropane alkaloids from Radix physochlainae in over 90 W, which should be ascribed to the thermal effect and the unstable ether/ester bonds in the structures of the target alkaloids beginning to break (Yohannes et al. 2019). In addition, extraction solvent and temperature are two most important factors affecting the efficiency of the UAE procedure. Basically, extraction solvent polarity should be appropriate with the polarity of target alkaloid to dissolve easily. Besides, solvent can also affect the cavitation process. In fact, high viscosity and high surface tension lead to difficulties in this process (Klein-Júnior et al. 2016). High temperatures can facilitate extraction due to the increased solubility of alkaloids in extraction solvent. Moreover, at higher temperature, the liquid viscosity and density decreased, resulting in increased mass transfer. Furthermore, cavitation bubbles within the fluid formed more easily due to the decreased solvent viscosity, and thus the tensile strength of the liquid was reduced (Klein-Júnior et al. 2016). Nevertheless, the disadvantage of too high temperature is the degradation and racemization of chiral alkaloids as reported for

the extraction of evodiamine enantiomers from Evodiae fructus, in which S-(+)-evodiamine start to convert to its R-(−)-form at 40 °C partly (Nguyen et al. 2013). For this reason, temperature should be optimized as it can be a factor playing a dual role in the extraction process.

Microwave assisted extraction (MAE)

The MAE process utilizes the electromagnetic radiations with a frequency ranging from 0.3–300 GHz, that induces ion migration and dipole rotation resulting in the heating of dielectric materials and accelerating the penetration of extraction solvent into the matrix (Delazar et al. 2012). Besides, a higher dielectric constant gives higher thermal energy release and faster heating, so the effectiveness of MAE is depended on the dielectric properties of both extraction solvent and sample matrix (Chan et al. 2011). Therefore, only specific solvents having high dipole moment as water, methanol and ethanol can interact and be used for extraction solvent in MAE. In addition, the moisture of sample is also an important factor affecting the efficiency of the MAE procedure. Water contained in plant matrices absorbs microwave radiation creating pockets of localized heating which promotes the plant cell walls rupture leading to enhanced release of alkaloids into the solvent and the increase of extraction yield. Basically, there will be a higher extraction yields expectation for higher water content matrices (Klein-Júnior et al. 2016).

For MAE optimization, several factors can affect the extraction yield, including sample size, solid to liquid ratio, extraction time, solvent nature and microwave power should be modified (Christen et al. 2008). In general, higher microwave power may lead to higher extraction yields. However, in some cases the alkaloid contents can reach a plateau and even decrease at higher powers as reported for the extraction of alkaloids from *Stephania cepharantha*, in which the yields of cycleanine, isotetrandrine and cepharanthine decline significantly when the power increases to 150 W. Probably, the high power causes rapid local heating, resulting in degradation of the target compound (Liu et al. 2016). In the extraction of bioactive alkaloids (sinoacutine, palmatine, isocorydine and L-tetrahydropalmatine) from *Stephania sinica*, no significant differences ($p > 0.05$) in alkaloid yields are observed under different microwave powers (150, 300 and 400 W) (Xie et al. 2014). As pointed out above, solid to liquid ratio is also important for MAE efficiency. Logically, the yield of alkaloids increased when the solid to liquid ratio rose. However, once reaching the maximum value, the yield of alkaloids may decline as the percentage of solvent increases, which was demonstrated for the extraction of the bioactive alkaloids (liensinine, dauricine, isoliensinine, neferine and nuciferine) from *Nelumbo nucifera* (Xiong et al. 2016). Aiming to reduce the potential of localized sample overheating which leads to degradation of alkaloids, the extraction solvents must have a high dissipation factor, so organic solvent-water mixtures are usually used as demonstrated for the extraction of major alkaloids (berberine and palmitine) from Rhizoma coptidis, in which ethanol 50% is used as extraction solvent (Teng et al. 2013). In the extraction of the bioactive alkaloids (sinoacutine, palmatine, isocorydine and l-tetrahydropalmatine) from Stephania sinica, the yields of all alkaloids rose when the ethanol concentration increased from 20% to 40%. Then, after reaching a maximum between 40% and 80% ethanol, the extraction yields decreased as the ethanol concentration further increases up to 95%. As the ethanol concentration increases from 20% to 40%, the solubility of alkaloids increased according to the theory of polarity. However, at higher ethanol concentrations (above 80%), less amounts of water present may result in a drop of microwave energy absorption, and the poorer endothermic capacity leads to a lower yield of alkaloids (Xie et al. 2014).

Purification

Due to the complexity of sample matrices (as plant and biological samples) and the low concentration of existed alkaloids, samples should be purified and enriched right after extraction step to facilitate the identification and/or quantification process. Practically, the most popular clean-up methods utilized for sample purification of alkaloids are liquid-liquid extraction (LLE) and solid phase extraction (SPE). Besides, new techniques such as Liquid Membrane Extraction (LME) and Solid-Phase Micro Extraction (SPME) have also been developed based on LLE and SPE, respectively.

Liquid-liquid extraction (LLE)

Actually, liquid-liquid extraction (LLE) is primarily used to extract and purify alkaloids from crude plant samples. This method is based on the relative solubility of compounds between two immiscible solvents. Alkaloids have polarity varying between pH which depends on its pKa, the solubility in specific solvent are also affected by pH (Fattorusso et al. 2008). In the acid solutions (pH is lower than its pKa), alkaloids 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 (as lipids, resins, carotenoids and chlorophylls). Aiming to eliminate hydrophilic interferences, the extracted aqueous layer should be alkalinized which leads to the alkaloids becoming non-polarity and can be easily extracted from aqueous to organic solvents (Boyaci et al. 2015). Moreover, samples with a high concentration of nonpolar compounds should be preferably extracted with water containing acids. In contrary, organic solvent immiscible with water should be used as extraction solvent to extract alkaloid from samples containing a large number of water soluble compounds (as phenolic acids, flavonoids, etc.) (Petruczynik 2012). For enrichment, new solvents which is suitable for analytical instrument can be created by using the reconstituting organic solvents layer after being colleted and vaporized.

In the extraction of (±)-ammodendrine, (±)-anabasine, (±)-coniine and (±)-nornicotine from *Lupinus sulphureus*

Table 4. Extraction conditions from various sample matrices using SPE.

Sample matrices	Analytes	SPE sorbent types	Loading solvents	Washing solvents	Elution solvents	Ref.
Rauwolfia serpentina	Indole alkaloids	SCX	Acidified MeOH	MeOH	5% NH_4OH /MeOH	(Sheludko et al. 1999)
Solanaceous hairy roots	Tropane alkaloids	C_{18}	MeOH : 30 mM phosphate buffer pH 8 (25:75)	1) MeOH : 30 mM phosphate buffer pH 8 (25:75) 2) H_2O	1) 0.2% TFA/H_2O 2) MeOH : 0.2% TFA/H_2O (98 : 2)	(Kursinszki et al. 2005)
Stephania cepharantha	Isoquinoline alkaloids	SCX	0.01 M HCl	1) H2O 2) MeOH	MeOH : 25% NH_4OH (97.5 : 2.5)	(Liu et al. 2016)
Human hair	Nicotine and its metabolites	SCX	Acidified aqueous solution	1) 2% FA 2) MeOH	1) 5% NH4OH/MeOH 2) $CHCl_3$: isopropOH : NH_4OH (78 : 20 : 2)	(Miller et al. 2011)
Mice plasma	Piperine analogues	C_{18}	Diluted (1:4, v/v) plasma samples	H_2O	MeOH	(Sachin et al. 2010)
Chinese medicinal prescriptions	Caffeine	C_{18}	H_2O	H_2O	CH_2Cl_2	(Ku et al. 1999)
Senecio leucophyllus	Pyrrolizidine alkaloids	C_{18}	Acidified H_2O and acidified MeOH	H_2O	25% MeOH	(Hosch et al. 1996)
Rat serum	Pyrrolizidine alkaloids	C_{18}	Phosphate buffer pH 8.1	H_2O	1% NH_4OH/MeOH	(Xiong et al. 2009)
Dog plasma	Quinolizidine alkaloids	Poly(styrene-divinylbenzene)	Diluted (1:1, v/v) plasma samples	2% ACN	ACN	(Liu et al. 2006)
Evodiae fructus	Evodiamine Rutaecarpine	C_{18}	MeOH	30% isopropOH	ACN	(Nguyen et al. 2013)

and *Lupinus formosus*, the extraction solvent was a mixture of chloroform and HCl 1M solution. The target alkaloids were extracted to acid solutions while other more hydrophobic interferences were distributed in chloroform and removed. After that, the pH of the aqueous layer was adjusted to 9.0–9.5 with concentrated ammonium hydroxide. Then, the alkaloids were extracted to chloroform layers and more hydrophilic interferences in basic aqueous layer were removed (Lee et al. 2008). Due to the sensitivity to strong acidic and basis conditions of tropane alkaloids, organic acid and ammonia solutions is primarily used to acidify/alkalize extraction solvent as reported for the extraction of l-hyoscyamine and scopolamine from thorn apple's leaves. In this study, 1% tartaric acid in methanol was used as extraction solvent at 90 ± 5 °C on heating mantle for 15 min (Mroczek et al. 2006).

The major drawback of this method is requirement for repetitive extraction causing time consuming and solvent wasting. Moreover, the formation of emulsions is difficult to break and may be effect to the extraction yields (Klein-Júnior et al. 2016).

Solid phase extraction (SPE)

Aiming to overcome the disadvantages of LLE method, solid phase extraction (SPE) has been developed and applied in sample preparation since the 1970s. In this method, sorbent phase loading the extract onto will retain alkaloids. Then, interferences in extract are washed away and the analytes is eluted by suitable solvents (Thurman et al. 1998). The SPE process has five stages including conditioning, loading, washing and elution step. Practically, SPE is utilized to purify and enrich alkaloids from both biological and crude plant samples (Table 4).

The chemical structures of alkaloids always have secondary or tertiary amine groups, the strong cation exchange (SCX) sorbents are an ideal choice, in which washing solvent will be aqueous solution and organic solvent to seperate and eliminate both hydrophilic compounds and hydrophobic compounds from plant matrices. After that, alkaloids will be deprotonated for elution by alkalized solvents which has pH at 2 units above pKa of analytes and evaporated to enrich sample (Thurman et al. 1998). Due to the effectiveness of SCX sorbents in the elimination of hydrophobic interferences, this sorbent tend to be suitable for chiral reversed-phase HPLC. In other to enrich alkaloids (cycleanine, isotetrandrine and cepharanthine) extracted from Stephania cepharantha and remove matrix compounds, several solid phases including C_{18}, PEP (polydivinylbenzene), SCX and C_8/SCX were evaluated. The authors observed that SCX and C_8/SCX provides selective extraction, reproducible results and a clean extract. In contrary, C_{18} and PEP failed to provide satisfactory alkaloid yields due to the decreased alkaloid solubility in alkalized aqueous loading solvent (Liu et al. 2016).

If the analytes are unstable in strong alkaline solutions, the weak cation exchange (WCX) will be used 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 values of analytes's pKa to maintain the ionized state of both sorbent and analytes. Finally, the alkaloids will be eluted by the acidic solutions (Ezel et al. 2015).

Besides, the C_{18} and C_8 sorbents are also applied to extract aromatic alkaloids and eliminate hydrophilic interferences from matrices as reported for the purification and enrichment of evodiamine and rutaecarpine from Evodiae fructus. In this study, SPE C_{18} was utilized with methanol as loading solvent. Matrix interferences were removed by 30% isopropanol in water. Then, the alkaloids were eluted by acetonitrile (Nguyen et al. 2013). In addition, the C_{18}

Table 5. Summary of CSPs, mobile phase compositions, and applications in analysis of chiral alkaloids.

Alkaloid groups	Analyte	Plant	Columns	Separation conditions	Ref.
Tropane alkaloids	(-) and (+) hyoscyamine	Solanaceaes seeds	Chirobiotic V, Chiralpak-AY3	Ethanol, 0.1% DEA	(Marín-Sáez et al. 2016)
	(+)-(3R,6R)- and (−)-(3S,6S)-3α,6β-tropanediol	Erythroxylaceae species	Chiralpak AD-H	n-hexane and 2-propanol (9:1) with 0.1% of diethylamine	(Muñoz et al. 2016)
Aconitine alkaloids	(R)-nicotine; (S)-nicotine; anabasine, and anatabine	Tobacco	Chiralpak AGP	NH_4OH- methanol (90:10)	(Ji et al. 2019)
Isoquinoline alkaloids	Mucroniferanine A	*Corydalis mucronifera*	Chiralpak AD-H	n-hexane–2-propanol (70:30)	(Zhang et al. 2018)
	(±) Zanthonitidine A	*Zanthoxylum nitidum*	Daicel Chiralpak ID	EtOH– TFA, 100:0.1	(Zhao et al. 2018)
Pyrrolizidine alkaloids	intermedine and lycopsamine	*Symphytum uplandicum*	Chiralpak IA	ACN/methanol (80:20) and methanol/methyl-t-butyl ether (90:10)	(Pawar et al. 2010)
Indole alkaloids	(12S, 22S)-Dihydroxyisoechinulin A (2) and (12R/S)- Neoechinulin A	*Cannabis sativa* L	Chiralpak AS-H column	Hexane/ isopropanol/diethylamine (4:1:0.05)	(Xiaoli et al. 2016)
	dihydrocarneamide A and iso-notoamide B	*Paecilomyces variotii*	Phenomenex-Chirex-3126 column	MeCN–H_2O (5:95).	(Zhang et al. 2015)

sorbents are usually applied to eliminate polar impurities from aqueous matrices which would exert a negative impact on chiral alkaloids analysis by normal-phase HPLC and CE. In some case, the reverse phase and strong anion exchange (SAX) sorbents could be used for eliminating hydrophobic and anion interferences by loading unretained alkaloids through cartridges as reported for the extraction of nicotine alkaloid enantiomers from cigarettes (Kodama et al. 2009). Table 4 lists examples of protocols that were developed using SPE for extraction and purification alkaloids from various sample matrices.

Application in chiral alkaloids

A variety of chromatographic and electrophoretic separation techniques have been employed for the qualitative and quantitative determination of alkaloids (Pellati et al. 2007). CE is rapid, efficient, versatile, and low cost, whereas HPLC is well established, accurate, sensitive, reproducible, and robust (Suntornsuk 2010). These methods allow the analyte determination which are still mixing with other compounds. This section provides an overview of application of enantioselective chromatographic and electrophoretic methods for the determination of chiral alkaloids in herbal products.

Enantioselective chromatographic methods

Analytical applications, including analyte, separation conditions and CSPs, are summarized in Table 5. A review sheds light on the most novel examples of chiral alkaloids separations on CSPs bring back efficient analyses was prepared.

New column materials also improved the ability of tropane alkaloids enantiomer separation. Separation of (R, S)-hyoscyamine was achieved using chiral stationary phase with immobilizing α-1-acid glycoprotein (Chiral AGP) (Soares et al. 2009), alternatively, enantiomer separation of atropine could be achieved by a chirobiotic V column packed with vancomycin as chiral selector (Aehle et al. 2010). The 6β-hydroxyl derivative of (S)-hyoscyamine called anisodamine was separated from the mixture including synthetic enantiomer and diastereomers by a Chiralpak AD-H column as chiral stationary phase, combined with an amylose derivative as a chiral selector (Yang et al. 2007). Satropane, 3α-paramethylbenzenesulfonyloxy-6β-acetoxy-tropane, could be resolved in lesatropane (3S, 6S-isomer) and desatropane (3R, 6R-isomer). A novel muscarinic agonist called lesatropane is in under preclinical development in China for the treatment of primary glaucoma as a single enantiomer drug. The separation of lesatropane from desatropane was conducted by both Chiralpak AD-H and Chiralpak AS-RH column (Yang et al. 2011).

Chiral separation of isoquinoline alkaloid has also achieved by using chiral stationary phase, for example with tetrahydropalmatine (THP) which was analyzed by chiral high-performance liquid chromatography (HPLC) on a Chiralcel. Quantification of OJ column by UV at 230 nm. The method was used to determine the pharmacokinetics of THP enantiomers in rats and dogs after oral administration of racemic THP or (-)-THP (Hong et al. 2005). Another report discusses sanguinarine derivatives. Chiral determination of benzophenanthridine alkaloids of Hylomecon that are extracted with methanol was proceeded by Chiralcel OD (4.6 × 250 mm) column with mobile phase is isopropanol-hexane-diethylamine (20/80/0.1, v/v/v) (Kang et al. 2003). 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 × 250 mm) column; 50% ethanol as mobile phase; wavelength 230 nm (Ma et al. 2008). Cularinoids are a group of about 60 compounds of isoquinoline alkaloids. The HPLC enantiomeric separation of the racemic cularinoid alkaloids N-p-methoxy-1,α-dihydroaristoyagonine and 4',5'demethoxy-1,α-dihydroaristoyagonine was accomplished by five chiral stationary phases (CSPs), in which there is a polysaccharide-derived CSP called Chiralpak AD, leading to the good enantioselectivity and resolution factor (Caccamese et al. 2006).

Indole derivatives are popular in chiral synthesis, chemical asymmetric catalysis, biological and medicinal chemistry. Lately, there were reports about the enantiomeric separation of several chiral plant growth regulators and related compounds, such as 3-(3-indolyl)-butyric

Table 6. Overview of approaches for enantioseparation of chiral alkaloids by CE and CEC.

Chiral alkaloids	Chiral selector	Background electrolyte (BGE)	Ref.
Ephedra alkaloids	DM-β-CD (5%)	Tris/phosphoric acid, pH 2.5, 5% tetrabutylammonium chloride	(Jiang et al. 2007)
Amphetamine, methamphetamine, ephedrine, pseudoephedrine and norephedrine	(+)-(18-crown-6)-tetracarboxylic acid and/or carboxymethyl-ß-cyclodextri	Acetic acid (pH 2.5 and 2.8)	(Taschwer et al. 2014)
Tropane alkaloids	HDMS-β-CD, HDAS-β-CD, or sulfated β -CD (varying concentrations)	Sodium borate, pH 9.2, 0.8% octane, 6.6% 1-butanol, 2.0% SDS	(Bitar et al. 2007)
(S)-hyoscyamine, (R)-hyoscyamine	sulfated β-cyclodextrin	35 mM sodium dihydrogen phosphate solution pH 8.5	(Heine et al. 2003)
Cocaine and its stereoisomers	sulfated β-cyclodextrin	10 mmol L(-1) phosphate buffer, 10% methanol at pH 3	(Cabovska et al. 2003)
Phenethylamines ephedrine, pseudoephedrine, and methylephedrine	β-cyclodextrin	125 mmol/L tetrabutylammonium L-argininate in a 75 mmol/L phosphate buffer pH 1.5	(Wahl et al. 2018)
Quinine, quinidine, cinchonine, and cinchonidine	hydroxypropyl-β- cyclodextrin	25 mM ammonium acetate buffer (pH = 5)	(Zhang et al. 2014)
dl-tetrahydropalmatine and (RS)-tetrahydroberberine	(2-hydroxy)propyl-β-cyclodextrin	deionized water 0.1 M NaOH and the BGE	(Yan et al. 2020)
Vincamine, vinpocetine and vincadifformine	Methylated-β-cyclodextrin (2-hydroxy)propyl- β -CD	15 mM NaOH (pH 2.5-H_3PO_4)	(Sohajda et al. 2010)

acid, abscisic acid and structurally related molecules including a variety of substituted tryptophan compounds. Recently, Zhao et al., reported that a coated and immobilized chiral stationary phases were suitable for the separation of indole derivatives; however, the coated CSP possesses a higher resolving power than the immobilized one (Zhao et al. 2009). A biogenetically interesting indole alkaloid was found in the leaves of *Nitraria tangutorum* in 1999 named Tangutorine. 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 C2-symmetric chiral selector (Kromasil CHI- (DMB) (Putkonen et al. 2003). Chiralpak AD and Chiralcel OD as chiral stationary phases were used for the HPLC enantiomeric separation of racemic indole alkaloids tacamonine, 17α-hydroxytacamonine, deethyleburnamonine, and vindeburnol (Caccamese et al. 2001).

Enantioselective electrophoretic methods

One of the most popular applications in capillary electrophoresis (CE) is the analytical scale separation of chiral analytes which is commonly succeeded by adding chiral selectors, most frequently cyclodextrins (CDs) to the background electrolyte (BGE). Since decades, cyclodextrins (CDs) are one of the most powerful selectors in chiral capillary electrophoresis for the enantio-separation of diverse organic compounds. A wide range of different CD derivatives (such as methylated, sulfated, carboxymethylated, sulfobutylated ones) were available as randomly substituted derivatives in order to meet the urgent need of market (Zhu et al. 2016). CDs still continue to be the most frequently used chiral selectors in CE as can be seen from the large number of applications even in the relative short period of time covered by this review (Table 6). An increasing number of publications has appeared describing CD-meditated capillary EKC enantioseparations in nonaqueous CE (NACE) with organic solvent-based electrolytes. Organic solvents often offer different selectivities compared to aqueous BGEs.

A CZE method using dimethyl--cyclodextrin (DM--CD) as modifier with tetrabutylammonium chloride (TBAC) as addition has been developed for the chiral separation of ()-ephedrine, ()-pseudoephedrine, ()-N-methylephedrine, and ()-norephedrine in Ephedra sinica and its medicinal preparation (Jiang et al. 2007). Medicinal herbs containing phenylethylamine alkaloids including Citrus species and Ephedra sinica are widely used because of their effects on human metabolism especially thanks to their lipolysis stimulation, therefore promoting the fat mass reduction in obesity treatment. Specifically, ephedra extracts such as Ephedra Herba (Ma Huang) contain alkaloids such as: (1R,2S)-(−)-ephedrine, (1S,2S)-(+)-pseudoephedrine, (1R,2S)-(−)-norephedrine, (1S,2S)-(+)-norpseudoephedrine, (1R,2S)-(−)-N-methylephedrine, and (1S,2S)-(+)-N-methylpseudoephedrine (Gotti 2011). The U.S. Food and Drug Administration (FDA) ban dietary supplements containing ephedra depending on the dosage due to the possible adverse effects while using these products. In addition, the National Institute of Standards and Technology (NIST) issued standard reference materials with certified values for ephedra alkaloids, synephrine and caffeine. Chiral analysis provides a useful tool in order to characterize Ephedra sinica and its medicinal phytopreparations because only (1R,2S)-(−)- ephedrine, (1S,2S)-(+)-pseudoephedrine enantiomers are found in nature. Simple CZE analysis of ephedrine and pseudoephedrine in herbal drugs (tablets) was carried out in a 25 mM triethanolamine phosphate buffer (pH 2.5) in condition of reversed EOF. Highly sulphated -cyclodextrin was added as the chiral additive to obtain the enantioselectivity in need as well as separate ephedrine and pseudoephedrine enantiomers from other herbal drug ingredients (Amini et al. 2006). A chiral system using three neutral cyclodextrins, namely -CD, hydroxypropyl--cyclodextrin (HP--CD) and Heptakis (2,6-di-O-methyl)--cyclodextrin (DM-CD) under acidic conditions was achieved the simultaneous enantio-resolution of octopamine and synephrine enantiomers in Citrus species dietary supplements_ a mixtures with the presence of tyramine and N-methyl tyramine. Particularly, another application of chiral analysis was the thermal racemization

evaluation of l-synephrine. Although information on the kinetics of the conversion was not available, the research showed that the real samples extraction process did not have any consequences about chiral artifacts become racemic of synephrine effectively at relatively high temperature (i.e. 1000C) (Avula et al. 2005). Three CE-UV methods developed by Phinney and co-workers that allowed the enantioseparation of ephedrine and pseudoephedrine by using chiral selectors including neutral HP--CD, DM--CD and charged sulfated CD. The combination of negatively charged sulfated -CD (2.8%) and DM--CD (1.2%) under acidic conditions (pH 2.5) provided the best separation of the two couples of enantiomers. NIST is developing the standard reference materials (SRMs) containing ephedra by using each of three CE method to analyze. The SRM samples are suitable in product adulteration detection by its potential in specific stereoisomers identification, although they only found the naturally occurring enantiomers (−)-ephedrine and (+)-pseudoephedrine (Phinney et al. 2005).

Tropane alkaloids are obtained from Solanaceae, e.g., *Atropa belladonna*, *Hyoscyamus niger*, and *Datura stramonium* (Aehle et al. 2010). The first pure compounds were atropine isolated from *Atropa belladonna* and hyoscyamine from *Hyoscyamus niger* (Kodama et al. 2009). Today, atropine" is defined as the racemic mixture of (S)- hyoscyamine and (R) hyoscyamine. (S)-hyoscyamine is found in plants while (R)-hyoscyamine can be formed under alkaline conditions. (S)-hyoscyamine is a strong acetylcholine-inhibitor as a muscarine receptor- blocker, while the (R)-hyoscyamine is mostly inactive. Although atropine exhibits approximately half of the (S)-hyoscyamine pharmacological activity, it is still prefered to (S)-hyoscyamine. Baseline separation of atropine in sulfated--cyclodextrin enabled the quantitation of 0.5% (R)-hyoscyamine (45 μg/ml) in (S)-hyoscyamine (Heine et al. 2003). The same sulfated -cyclodextrin proved useful for separation of cocaine enantiomers and diastereomers, i.e. (-)-cocaine (= (2R, 3S)-cocaine, the active enantiomer formed in coca plants), (+)-cocaine, (-)-pseudococaine, and (+)-pseudococaine (Cabovska et al. 2003).

Enantio-separations with high resolution and short migration times of all tropane alkaloids were achieved by using heptakis (2, 3-di-O-methyl-6-O-sulfo)-β-CD and sulfated β-CD in the microemulsion BGE and were superior to corresponding CD modified CE methods (Yaser et al. 2007). The enantiomeric separation of three vinca alkaloid enantiomers (vincamine, vinpocetine and vincadifformine) has achieved in an aqueous capillary electrophoresis (CE) system using Methylated-β-cyclodextrin and (2-hydroxy) propyl- β -CD of CD (Sohajda et al. 2010).

Camptothecins (CPT) are valuable quinoline alkaloids present in *Camptotheca acuminata*, *Nothapodytes foetida*, *Ophiorrhiza pumila* and *Tabernaemontana heyneana*. It occurs in different plant parts such as the roots, twigs, and leaves. It consists of a pentacyclic ring structure that includes a pyrrole quinoline moiety and one asymmetric center within the -hydroxy lactone. Only the (S)-enantiomers of CPTs exhibit antitumor activity (Caccamese et al. 2006). The interest towards these compounds is grown because of the demonstrated activity in the treatment of colorectal and ovarian cancer. Camptothecin used for the clinical activities as well as for the downstream synthesis of the derivatives is currently obtained from natural sources. The main issue in analytical methods development in order to determine camptothecin in plant materials is the poor water solubility. The enantiomeric resolution of homocamptothecin derivatives was obtained using highly S--CD as chiral selectors at acidic pH (Goossens et al. 2006).

Conclusion

In general, the enantiomeric separation of a pure chiral alkaloids developed on pure compounds whereas there were only a few methods reporting the simultaneous chiral separation of different alkaloids on plant matrix. It is worth to mention, chiral capillary electrophoretic determination of alkaloids focusing on enantiomeric purity. Therefore, the development of multicomponent methods to achieve the simultaneous enantiomeric separation of different kinds of alkaloids is still a challenge. In this sense, the challenges for the next years in this field should focus on the development of sample preparation to achieve the simultaneous enantiomeric separation of alkaloids belonging to different chemical families, as well as their environmentally friendly extraction from different matrices by using solid phase extraction techniques with low consumption of time, samples and solvents.

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